Severe Infection and Mortality in Kenyan Newborns

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A dissertation submitted in partial fulfillment of the requirements for the degree of

Doctor of Philosophy

University of Washington

2018

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Program Authorized to Offer Degree:

Public Health - Epidemiology

University of Washington

Abstract

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This dissertation addresses severe neonatal infection and neonatal mortality in high-burden settings. Worldwide, almost half of all deaths in children under 5 years of age occur in the neonatal period (days 0-27 of life). Complications of prematurity, intrapartum events and severe infections cause the majority of these deaths, despite known effective interventions. The risk of neonatal mortality in Kenya is more than 23 times as high as the risk in the lowest mortality countries. We conducted a prospective cohort study of 380 pairs of mothers and newborns identified from two referral-level facilities in rural Western Kenya within 96 hours of life. Pairs were eligible if either the mother had an intrapartum risk factors for peripartum infection, or the newborn had a clinical signs of possible severe illness (pSBI), or both. Among high-risk newborns with risk factors for or clinical signs of severe illness [Early-onset neonatal sepsis (EOS) study population], we: 1) Determined prevalence and correlates of neonatal bacteremia; 2) Determined incidence and risk factors for 7-day mortality; and 3) Determined the performance of World Health Organization (WHO) empiric illness algorithms in predicting mortality and identified a novel set of prognostic factors to improve mortality prediction. The prevalence of bacterial blood stream infection as identified by blood culture was low, but antibiotic treatment coverage was high. Despite low prevalence of bacteremia, high coverage of WHO-recommended antimicrobial therapy, and receipt of hospital care, 7-day mortality risk was high. Signs of probable severe bacterial infection (pSBI) in the WHO Integrated Management of Childhood Illness (IMCI) were strongly associated with mortality, and WHO empiric algorithms for identifying and classifying severe illness in young infants performed well in predicting mortality. Among high-risk newborns who were not exclusively low birthweight, in addition to 4 of the 7 IMCI signs (poor feeding, fast breathing, only moves when stimulated, fever), least area shrinkage and selection operator regression determined that apnea and low birthweight were important prognostic factors for mortality. Strategies to prevent mortality among a population of high-risk newborns for whom currently-recommended interventions are insufficient to prevent death are urgently needed.

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Acknowledgements

Thank you to my incredible committee Grace John-Stewart, Judd Walson, Ali Rowhani-Rahbar, Maneesh Batra, and Patrick Heagerty for their dedicated mentorship, patience, constructive criticism, encouragement, pragmatic perspectives and wise advice. I appreciate Grace for her insightful observations about what I needed and required as a mentee, and for pushing me in ways that often made me uncomfortable. I appreciate Judd for his infectious energy, universally positive attitude and approach, and for introducing and fostering incredible opportunities which have opened academic and professional doors that have changed my life. His belief in me and my capacity helped give me confidence to pursue new challenges, and his open style provided a venue for candid and invaluable discussion. Ali taught me what it means to be a "true" epidemiologist and has helped me grow and develop a passion for epidemiology as a skill and profession which I didn't yet appreciate when I entered this program. I am thankful to him for modeling how an epidemiologist approaches research and problems, and for fostering in me the desire to do the same. Maneesh's warm mentorship style and expertise in neonatal medicine helped me learn technical material in an environment that focused on the application and meaning of the work. I appreciate Patrick's calm mentorship, patience for teaching a non-biostatistician, ability to communicate technical concepts in a way I could digest, and his often helpful "fatherly advice". Thank you to the many colleagues in Kenya who welcomed me into their offices, communities and homes. I would like to acknowledge Jackie Naulika, who coordinated the field work for the studies in this dissertation, for her important contribution. Her hard work and passion, her often differing perspective, and her friendship and humor made the work possible, enriching and fun. I am incredibly indebted to my colleagues, cohort members and friends in the Epidemiology PhD program, Pediatric Infectious Disease Group, Global WACh, Club D and SWiG for encouragement, comradery, friendship, advice and support. Finally, thank you to my family: Jackie and Chuck Levine, Julia and Blair Davis and Jeremy Levine. I am thankful for their unconditional support and encouragement and for providing a venue from time-to-time in which I was discouraged from discussing anything dissertation-related; this was often the break and perspective I needed to stay healthy, humble and grounded. I am especially thankful to my nephews Briggan, Baylor and Max for their intoxicating spirit and for helping me appreciate the vulnerability of infants and the incomparable and incredible joy, laughter and love they bring into the world when they are healthy and strong.

Introduction

An estimated 2.7 million neonatal deaths (deaths in first 27 days of life) occurred in 2015, accounting for almost half of all deaths in children under 5 years[1]. Neonatal mortality risk is highest in the region of sub-Saharan Africa (SSA) and South Asia (SA)[2]. In Kenya, the neonatal mortality rate (NMR) is 23 per 1000 live births, more than 20 times the rate in countries with the lower risk[3]. The vulnerable neonatal period is also a time of substantial opportunity to prevent mortality. The majority of neonatal deaths could be prevented by known interventions [4,5]. However, in resource-constrained settings, delivery of high-quality, skilled care necessary to save newborn lives remains challenging. The three most common causes of newborn death globally are complications of prematurity, severe invasive infections including sepsis, pneumonia and meningitis, and intrapartum events (previously referred to as birth asphyxia)[6,7]. The majority of these deaths, approximately 80%, could be prevented with known interventions and simple strategies[2,3,8].

Newborn problems and severe illnesses are difficult to differentiate; clinical presentation is similar for multiple syndromes and illnesses[9-11]. Even in well-resourced settings, available diagnostics are insensitive and non-specific for diagnosis of the primary causes of newborn mortality and even these imperfect gold-standard methods for detecting, diagnosing and confirming or ruling out causes of illness are often unavailable in resource-constrained settings. In high-burden settings, clinical empiric algorithms are often used to detect and classify severe illness to inform clinical management[12,13].

Severe infections account for approximately a third of newborn deaths globally[6,7,14]. The incidence of severe infection is highest in the sub-Saharan Africa and south Asia regions, where case-fatalities are also the highest[11,15]. Estimates of the burden and incidence vary widely in different settings, and etiologies are not well described[16,17,11].

Invasive blood stream infection accompanied by diagnostic or clinical indication of systemic illness originating within the first 72 hours of life, early-onset sepsis (EOS), is difficult to diagnose. A combination of clinical evaluation, laboratory results and non-specific biomarkers of infection and inflammation are used to classify “clinically suspected” cases from “culture-proven” bacteremia, based on isolation of a pathogenic

organism from blood culture[18,19]. In low-resource settings identification of possible severe bacterial infections (pSBI) including sepsis, pneumonia, and meningitis in newborns is based on the presence of clinical features predictive of mortality and easily identified by health workers with limited training, for referral to health facility for care or antibiotic treatment in the community if referral is not possible[20,21]. But identification and confirmation of severe infection is challenging. Misclassification may result in unnecessary or inappropriate antibiotic treatment, and failure to identify and treat other causes of illness. Even among newborns with signs of pSBI, isolation of a pathogenic organism is rare; 10.4% of newborns with clinical signs of infection in days 0-6 of life had a probable or definite pathogen identified in culture in a large multi-site study[22]. Identification and differentiation of newborns who will benefit from antibiotic therapy, supportive care and management remains difficult. Characterization of common etiologic agents, and their antibiotic susceptibility patterns, will inform empiric antibiotic treatment regimens where blood culture and more advanced DNA-based diagnostics and antibiotic susceptibility testing is rarely available. Yet large etiology studies have focused primarily on older newborns in community-based settings, and a recent multi-site study was limited to SA, where the distribution of common etiologies likely differs from among younger newborns in facility settings in SSA[22,23].

Early-identification of newborns at highest risk of mortality who require targeted interventions to prevent death remains challenging. Case-fatality for pSBI in SSA is estimated to be highest in the world, at 14.1% (95 % CI: 7.2 – 21.0%)[11]. Newborns in health facility are an accessible population who may benefit from simple, low-cost interventions to prevent morbidity and mortality, but predictors of severe morbidity and mortality among neonates with pSBI are not well described[24]. In over-burdened health facilities, access to high-quality skilled care at and immediately following birth is often limited by cost, inadequate human resources, limited infrastructure, lack of equipment and supplies. Maternal and intrapartum characteristics already routinely collected may help differentiate high-risk neonates who would benefit from careful observation, targeted prophylaxis, early treatment, or more frequent follow-up. Simple, low-cost facility-based interventions can save newborn lives. Intrapartum antibiotic prophylaxis, expeditious antibiotic therapy and supportive care for suspected infection[25,26] and essential newborn care and basic resuscitation for newborns that fail to spontaneously breath at birth can prevent mortality from infection and intrapartum

events[8,27,28]. Yet capacity to provide high-quality case management for newborns who require advanced care is often lacking[2,27-29 30].

This dissertation addressed risk factors and causes of severe infection and neonatal mortality in high-burden settings in Kenya, to inform approaches to mortality prevention in settings where the risk of death is high.

We conducted a prospective cohort study of 380 pairs of mothers and newborns identified from postnatal and newborn units at two referral-level facilities in rural Western Kenya within 96 hours of newborn life. Pairs were eligible if either the mother had an intrapartum risk factors for peripartum infection, or the newborn had clinical signs of possible severe illness, or both. In this high-risk facility-based population, we described prevalence of severe infection and incidence of mortality, determined risk factors for severe infection and mortality, and developed a novel newborn mortality risk prediction tool.

Chapter 1 is a cross-sectional analysis of the prevalence and etiology of bacterial blood stream infections (bacteremia) among young infants in two health facilities in Kenya. We estimate the prevalence of definite/likely bacteremia and of possible bacteremia, describe the etiology of infection and antibiotic susceptibility of bacterial isolates, summarize treatment characteristics at enrollment, and assess correlates of bacteremia.

In Chapter 2 we describe the in-hospital and post-discharge incidence of 7-day mortality among high-risk newborns using a prospective cohort study design and determine intrapartum and newborn risk factors for mortality.

In Chapter 3 we evaluate the predictive performance of the World Health Organization empiric algorithms for severe illness identification and classification in predicting newborn mortality risk, among a population of newborns in referral-level facilities who are not exclusively very low birthweight. We use least area shrinkage and selection criteria (lasso) machine-learning methods to identify the most important prognostic factors to develop a novel tool to attempt to improve mortality risk prediction in low-resource health facility settings

This dissertation elucidates key gaps and key opportunities for addressing neonatal mortality in low-resource settings.

Chapter 1: Prevalence and Correlates of Bacteremia in High-risk Newborns

Title:

Bacteremia uncommon in high-risk Kenyan neonates with suspected infection

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ABSTRACT

Background: Early-onset neonatal infections are an important cause of newborn mortality, but the distribution of common etiologies and risk factors for bacteremia in young infants are not well described in sub-Saharan Africa. We aimed to describe prevalence, etiology and correlates of neonatal bacteremia in two Kenyan health facilities.

Methods: We conducted a cross-sectional study of newborns less than 96 hours old with intrapartum risk factors or clinical signs of illness or probable severe bacterial infection (pSBI) in the first 72 hours of life, enrolled from two referral-level facilities. At enrollment, clinical and treatment history were ascertained from maternal interview and patient file abstraction and trained study nurses conducted comprehensive newborns physical exams. Bacterial blood culture was conducted, and antibiotic susceptibility testing performed on bacterial isolates. Generalized linear model log-binomial regression was used to determine correlates for bacteremia.

Results: Three hundred seventy-nine newborns were enrolled and had blood culture results available, of whom 73% had intrapartum risk factors and 70% had clinical signs of neonatal infection at enrollment. The median gestational age was 38 weeks (IQR: 34, 39) and 46.4% were enrolled within 24 hours of birth. Most infants received antibiotics at or before enrollment (82.3%, n=312), and antibiotic administration prior to blood collection for culture was common (77.3%, n=293). Median blood volume for culture was 2 mL (min: 0.5, max: 3.0). Six infants had a definite or likely pathogen detected (prevalence: 1.6%, 95% CI: 0.6-3.4%). There were 21 newborns with a possible pathogen identified [prevalence definite/likely or possible pathogen: 7.1%, (95% CI: 4.7-10.2%, n=27). All newborns with definite/likely bacteremia had at least one sign of pSBI compared with 70.7% among newborns without bacteremia. Newborns with definite/likely bacteremia had higher prevalence of maternal fever than newborns without bacteremia [16.4% versus 0.3%, prevalence ratio 31.7 (95% CI: 6.2, 162.8), p=0.0373], however, precision and stability of this estimate was limited due to low statistical power.

Conclusions: Blood culture infrequently detected pathogens among high-risk newborns. New methods to detect or rule out severe invasive infections in high-risk newborns would be useful to guide clinical management in resource-limited settings.

BACKGROUND

Severe neonatal infections cause approximately a third of newborn deaths globally[6,7]. Bacterial blood stream infection (bacteremia) leads to severe systemic illness, multi-organ system dysfunction and death if untreated. Survivors of neonatal severe invasive infection experience developmental delay, disability and severe neurocognitive deficient. The incidence of severe invasive infections in the neonatal period are highest in sub-Saharan Africa (SSA) and South Asia (SA), but estimates vary widely: from 2% and 15%, and the epidemiology and etiology of early-onset blood stream infection in young infants are not well described in these settings [11,15].

Neonatal bacteremia can occur following delivery up to 28 days of life. Early-onset infection, infections originating in the first 72 hours of life, are commonly due to vertical transmission of infection from the mother in utero, during, or shortly after delivery. Postnatal acquisition commonly originates from contact with the mother, other contacts, medical staff, or from contact with fomites and infected equipment within the medical care setting. The distribution of causal pathogens during these specific periods is not well described in SSA. Etiologies may differ in this setting and few studies include robust data on the etiology of early-onset infection[9,17,22,31,32].

Bacterial blood culture is the gold-standard diagnostic in well-resourced settings, and is used to guide antimicrobial treatment regimens and duration, but is rarely available in low-resource settings[33]. Identification of possible severe bacterial infections (pSBI) in resource-constrained settings is commonly based on empiric algorithms[13,20,34]. Large etiology studies have focused primarily on older newborns in community-based settings. A recent multi-country community-based study was limited to South Africa, where the distribution of common etiologies likely differs from among younger newborns in facility settings in SSA[22,23]. Characterization of common etiologic agents, and their antibiotic susceptibility patterns can inform empiric antibiotic treatment regimens in settings where culture in not available.

Deaths and severe morbidity due to newborn infections are preventable. Infection-control practices and clean delivery practices, antibiotic prophylaxis for high-risk women and newborns and appropriate antibiotic therapy and case-management for sick newborns can prevent deaths from infection[2,8,25,26,35-42]. These approaches to prevent and treat severe neonatal infection are often not available in the highest

mortality settings. The case-fatality of pSBI in SSA is estimated to be highest in the world, at 14.1% (95% CI: 7.2 – 21.0%)[11]. Maternal risk-based screening and targeted intrapartum antibiotic prophylaxis have resulted in substantial declines in early-onset infections in well-resourced settings but are uncommon in low-resource settings[35,43-45]. Risk factors for severe invasive infection including preterm birth, low birthweight and severe intrapartum events inform clinical management in well-resourced settings, but their influence is less well understood in low-resource settings in which the prevalence of infectious illness such as HIV, malaria and tuberculosis are higher. Studies in South Africa indicate maternal HIV infection may influence maternal colonization, newborn colonization, clearance and infection, but results are inconsistent and have not been replicated in other settings[46-49]. Empiric treatment recommendations that consider host-related characteristics and the local epidemiology of infectious illness are lacking in high-burden settings[34,50]. Identification of maternal, intrapartum and newborn characteristics associated with bacteremia in this population may help to inform targeted antibiotic prophylaxis, early-treatment or vigilant observation for high-risk newborns.

We aimed to determine prevalence and correlates of early-onset neonatal bacteremia among high-risk newborns in two Kenyan health facilities.

METHODS

Screening and enrollment

We conducted a cross-sectional study between April, 2015 and March, 2016 at Homa Bay Country and Kisii Teaching and Referral Hospitals in rural Western Kenya. Methods for this cohort have been previously described (*Levine et al. 2018, under review*). Mother/neonate dyads were screened and identified from maternity, postnatal, newborn units and outpatient child health wards within 96 hours of life. Screening occurred before labor if the mother was in the first stage and was capable of providing informed consent, or as soon as possible after delivery. Newborns were screened after delivery or at presentation to facility. We included a purposive sample of newborns with clinical signs of infection or risk factors for infection to ensure adequate occurrence of blood stream infection, which is rare in the general population. Newborns with risk factors for but no signs of illness were included to prevent missing possible cases, since

determination of infection is difficult and progression to severe disseminated disease occurs quickly. Neonates with signs of possible severe illness in the first 72 hours of life (history of poor feeding, fast breathing >60 breaths/minute when calm, chest indrawing, fever ≥ 37.5 °C, axial or ≥ 38 °C, rectal, hypothermia < 35.5 °C, only moves when stimulated or no movement, history of convulsions, 5-minute Apgar score score ≤6, lethargy, apneic attacks, severe jaundice) or intrapartum risk factors for peripartum infection (maternal fever 72 hours prior to delivery (≥ 38.0°C), delivery <37 weeks gestation, maternal history of tachycardia (>100 beats/min), fetal distress (meconium stained liquor, fetal tachycardia), uterine/abdominal tenderness 72 hours prior to delivery, prolonged rupture of membranes (>18 hours), foul smelling amniotic fluid/vaginal discharge, clinical chorioamnionitis, obstructed labor), were eligible. Newborns for whom the biological mother was absent or incapacitated were excluded, as were those with weight at enrollment less than 1000 g, among whom it would not be possible to obtain adequate blood volume for culture. Newborns who were referred or transferred from other facilities or were delivered out-of-hospital were eligible if they presented within 4 days of birth.

All mothers provided written or witnessed and documented informed consent for participation of themselves and their newborns, in Gusii, Duolo, Kiswahili, or English. The study was approved by the Institutional Review Board at the University of Washington and the Kenya Medical Research Institute Scientific Ethics Review Unit.

Data and sample collection and processing

At enrollment, clinical and treatment history were ascertained from maternal interview and patient medical file abstraction, and trained study nurses conducted comprehensive newborns physical exams. Mothers were assessed for possible TB infection and exposure in pregnancy, based on WHO screening guidelines modified for pregnancy.

Patient records and maternal self-report was used to determine maternal HIV status, and all women with negative or unknown status were tested using antibody rapid tests (Determine® (Abbott Laboratories, North Chicago, IL, USA) and/or Unigold® (Trinity Biotech, Bray, Ireland)). Immediately following enrollment, 1-3 mL of whole blood was aseptically collected in VersaTREK REDOX direct draw blood-culture media from neonates for bacterial blood culture and samples were shipped within 24 hours to the lancet laboratory

in Nairobi for processing. Samples were incubated using a VersaTREK 528 system for 5 to 7 days where gas production and gas consumption was monitored, signaling need for further bacterial identification. Positive cultures were gram stained and sub-cultured onto blood, chocolate, and MacConkey agar plates and an optochin disc was placed onto the blood agar plate to detect pneumococci. Antibiotic susceptibility testing was conducted on bacterial isolates.

Non-research personnel at the medical facilities provided clinical management for participants, but study-related laboratory results were communicated to managing physicians as appropriate to inform patient care.

Statistical analysis

Each positive blood culture case was evaluated by an expert study team that included an infectious disease pediatrician and a neonatologist, to classify the isolate as a definite/likely pathogen, possible pathogen, or contaminant, and disagreement was settled via discussion and consensus. Criteria for assessment included gestational age at delivery, birthweight, newborn presentation (with or without clinical signs of pSBI), age at symptom presentation, time in hospital prior to sample ascertainment, history of indwelling catheter or invasive procedure, receipt and timing of intrapartum and/or newborn antibiotics, resolution of symptoms and/or vital status at 7 days, time to positive growth detection, volume and burden of isolate growth. Definite/likely bacteremia was defined as isolation of ≥1 definite or likely pathogen[51,52] from a normally sterile site (blood). Possible bacteremia was defined as isolation of ≥ organism considered as a possible pathogen in early newborn infection, or possible contaminant. No bacteremia was defined by a blood culture in which no organism was identified.

The prevalence of definite/likely bacteremia was calculated as the proportion of newborns meeting stated criteria from among all newborns with viable blood samples. A second category of definite/likely or possible bacteremia was defined as the proportion of newborns with either definite/likely or possible bacteremia, from among all those with viable blood samples.

The presence of any signs of probable severe bacterial infection (pSBI) was defined as one or more of the following signs documented in patient files or observed at physical exam conducted at enrollment by study nurse: history of poor feeding, fast breathing >60 breaths/minute when calm, chest indrawing, fever

≥ 37.5 °C, axial or ≥ 38 °C, rectal, hypothermia < 35.5 °C, only moves when stimulated or no movement, history of convulsions. The presence of any intrapartum risk factors for peripartum infection was defined as any one of the following documented in patient files at enrollment: maternal fever 72 hours prior to delivery (≥ 38.0°C), delivery <37 weeks gestation, maternal history of tachycardia (>100 beats/min), fetal distress (meconium stained liquor, fetal tachycardia), uterine/abdominal tenderness 72 hours prior to delivery, prolonged rupture of membranes (>18 hours), foul smelling amniotic fluid/vaginal discharge, clinical chorioamnionitis, obstructed or prolonged labor.

Descriptive statistics including frequencies and proportions, medians and interquartile ranges were used to summarize characteristics of the study population at enrollment, including maternal, intrapartum and newborn characteristics, treatment history, distribution and antibiotic susceptibility of bacterial isolates, with 95% confidence intervals (CI) estimated from the binomial distribution using exact methods.

Correlates of definite/likely bacteremia and of definite/likely or possible bacteremia were assessed among those with complete data available for each specific exposure of interest; observations with missing data were excluded from analysis for that factor. Generalized linear log-binomial regression (log-binomial GLM) was used to estimate prevalence ratios and 95% CIs comparing the prevalence of definite/likely bacteremia and of possible *or* definite/likely bacteremia among exposure groups of interest. Both outcome groups (definite/likely bacteremia and possible or definite/likely bacteremia) were compared to a reference group with a negative blood culture. When there were no observations in a comparison group, leading to lack of separation, we report prevalence difference rather than prevalence ratio as the measure of association, calculated with log binomial GLM as described above. Models for the association between maternal HIV infection and bacteremia and infant pSBI symptoms and bacteremia were adjusted for enrollment site based on *a priori* decisions from causal modeling. All other analyses were crude Fisher's exact tests were used for all hypothesis tests, at an accepted alpha level of 0.05. We did not adjust for multiple comparisons in these exploratory analyses.

Analyses were conducted in Stata 14.2 (StataCorp. 2015. Stata Statistical Software: Release 14. College Station, TX: StataCorp LP.)

RESULTS

Overall, 577 newborn/mother pairs were screened between April 2015 and March 2016, of whom 400 met preliminary eligibility criteria. Twenty pairs were determined not to meet eligibility criteria and were excluded from this analysis. One newborn died before blood for culture was obtained, leaving a final analytic population of 379 newborns; 142 (37.5%) from Homa Bay County Hospital and 237 (62.5%) from Kisii Teaching and Referral Hospital (Table 1). Newborns ranged from 1 hour to 89 hours of age at enrollment, and 46.4% (n=176) were less than 24 hours old. Fifteen percent (n=57) of newborns were exposed to HIV. The median gestational age was 38 weeks (IQR: 34, 39) and 39.9% of newborns for whom gestational age estimates were available were premature (<37 weeks completed gestation) (n=144). The median birthweight was 2.9 kg (IQR: 1.9, 3.5, min: 1.0, max: 4.8 kg). Seventy-one percent (n=269) of newborns presented with at least one sign of pSBI at enrollment and 73% (n=277) had at least one intrapartum risk factor for severe infection.

The majority of newborns in the study population received antibiotics at or before enrollment (82.3%, n=312), and antibiotic administration prior to ascertainment of blood for bacterial culture was common (77.3%, n=293). Almost all antibiotic regimens administered included the WHO-recommended combination therapy of gentamicin and penicillin for pSBI (n=307, 98.4% of those administered antibiotics), with or without additional antimicrobials (Supplementary Table S1)[12,13]. The median volume of blood collected for bacterial culture was 2.0 mL and ranged from 0.5 - 3.0 mL.

The prevalence of bacteremia with a definite or likely pathogen was 1.6% (95% CI: 0.6-3.4%, n=6); and with a possible pathogen was 5.5% (95% CI: 3.5-8.3%, n=21), [prevalence definite/likely or possible pathogen: 7.1%, (95% CI: 4.7-10.2%, n=27)]. Pathogens included those commonly acquired via vertical transmission, postnatal exposure and in nosocomial infections. Definite/likely pathogens included *Enterococcus faecalis* (n=1), *Escherichia coli* (n=1), *Klebsiella pneumoniae* (n=2), *Enterobacter cloacae* (n=1), *Enterobacter aerogenes* (n=1). Coagulase-negative *staphylococci* species, a possible pathogen or possible contaminant, were the most commonly identified isolates (n=19). Other possible pathogens or contaminants included *Bacillus spp*. (n=1) and yeast (n=1). There were no newborns with multiple isolates. The distribution and antibiotic susceptibility of bacterial isolates are summarized in Supplementary Table S2. Antibiotic resistance to WHO-recommended first-line antimicrobial therapies was common; 66.7% (n=4)

of isolates considered definite/likely pathogens were resistant to both antimicrobials in the combination therapy, and 83.3% (n=5) were resistant to at least one of the two antibiotics (Supplementary Table S1).

All newborns with definite/likely bacteremia presented with at least one sign of pSBI (n=6, prevalence: 100.0%) and received antimicrobial therapy at or prior to enrollment (n=6, prevalence: 100%). Among those without bacteremia, 70.7% (n=249) had at least one sign of pSBI. Of those with definite/likely bacteremia, 83.3% (n=5) were administered the WHO-recommended antibiotic therapy (Table S1).

The prevalence of definite/likely bacteremia and of possible or definite/likely bacteremia among groups defined by maternal factors, intrapartum factors, newborns and treatment characteristics, and prevalence ratios comparing the prevalence of bacteremia by exposure group category, are provided in Table 1. Maternal fever prior to delivery was associated with definite/likely bacteremia, with 1 infant (16.3%) of those with define/likely bacteremia with maternal fever pre-delivery versus 1 (0.3%) of those without bacteremia (PR: 31.7, 95% CI: 6.2, 162.8, p=0.0373), but the small number of events limited precision and stability of the estimate. None of the other intrapartum or newborn factors assessed were associated with definite/likely bacteremia or with definite/likely or possible bacteremia in the study population.

DISCUSSION

Among high-risk neonates with risk factors for early infection or clinical presentation consistent with possible severe illness in two referral-level Kenyan facilities, the prevalence of culture-confirmed bacteremia was low.

It is possible that some newborns had invasive infections which were missed by blood culture. Bacterial blood culture is known to be insensitive in young infants due to small sample volumes and low pathogen burden[53]. Although our culture methodology was approved for blood volumes as low at 0.5 mL, some samples may not have been of sufficient size to yield bacterial growth. Additionally, although we attempted to collect samples prior to antimicrobial treatment, most infants had received antibiotics prior to blood collection, further limiting opportunities for detection. More sensitive DNA-based detection methods or use of biomarkers of inflammation and systemic infection may improve upon the sensitivity of culture and enable better estimates of bacteremia. Isolates classified as possible pathogens could have been possible contaminants or could be responsible for causing illness. Classification of isolates as contaminants or

pathogens is difficult in newborn infection, and there is no accepted guideline on classification. The distribution of pathogens that cause illness differ by age of the newborn, hospital/community setting, and geographic location. To assess the sensitivity of our results to our classification assumptions, we determined correlates of bacteremia assuming all possible pathogens were indicative of blood stream infection, and assuming all possible pathogens were contaminants.

The burden of bloodstream infection may also have been lower than expected in our study population. We used inclusion criteria for possible severe illness which was more broad than the WHO algorithms for pSBI[13]. Symptoms of newborn infections are non-specific and similar for sepsis, pneumonia, and meningitis, and non-infectious syndromes such as complications of prematurity and of intrapartum events[10,12]. Cerebrospinal fluid assessment to diagnosis meningitis and X-ray or other diagnostics for pneumonia were not conducted as part of routine care in this setting, thus we were unable to detect or rule out other types of severe invasive infection. It is possible many newborns in our population were experiencing other illnesses or newborn problems.

Maternal fever was associated with definite/likely bacteremia, although this association was imprecise and unstable due to low statistical power. Fever is a strong indication of intra-amniotic and uterine infection, which is also one of the most prominent risk factors for neonatal bacterial infection[54-57]. Prompt and appropriate intrapartum antibiotic therapy can prevent severe infectious morbidity and mortality in both the women and the newborn[35,58]. Newborns born to women with suspected or known infection should be observed for signs of early infection, particularly if the women did not receive intrapartum antibiotics. Systematic documentation of condition of pregnancy and delivery, and communication between obstetric and newborn care providers is essential to care management of the mother/newborn dyad.

Almost all bacterial isolates were resistant to one or both WHO-recommended empiric antimicrobials for sick young infants[12,13]. In light of the high pre-culture antibiotic exposure in the study population, the distribution of antimicrobial resistance and etiology are unlikely to represent pathogen distribution more widely. However, considering the small number of isolates, the occurrence of resistant organisms does indicate that agents resistant to recommended therapies are not uncommon in early-infant

infections in the region. It should also be noted that despite resistance, all newborns with pathogens isolated survived; recommended therapies may continue to provide some benefit despite resistance.

Due to the rare occurrence of positive blood culture with a non-likely contaminant, our estimates had poor precision and we lacked adequate statistical power to test for associations between intrapartum, maternal and newborn characteristics and severe invasive infection. Larger studies in which the outcomes of interest are more common are needed to estimate such associations with adequate precision. To better describe the causes, etiology and antibiotic susceptibility of blood stream infections in young infants in sub-Saharan Africa, large, prospective cohort studies are needed.

Causes of newborn illness and mortality are difficult to determine and classify even in well-resourced settings, and global estimates of the burden of severe illness and distribution of causes of newborn death in low-resource settings vary widely in different settings, across, time, with different estimation methods. Future studies to describe the distribution of causes and burden of severe infections will help inform appropriate strategies to address important causes of newborn death[23].

CONCLUSION

Blood culture infrequently detected pathogens among high-risk newborns. New methods to detect or rule out severe invasive infections in high-risk newborns would be useful to guide clinical management in resource-limited settings. Data on common etiologies and their antibiotic resistance patterns could help inform empiric management in similar sub-Saharan African settings.

TABLES

Table 1. Correlates of early-neonatal bacteremia in Kenyan neonates (Homa Bay and Kisii, Kenya) (N=379)

Characteristic	No bacteremia n = 352 Frequency (%)	Definite/Likely bacteremia [a] n = 6 Frequency (%)	Prevalence Ratio or Difference (95 % CI)	Possible or definite/likely bacteremia [b] n = 27 Frequency (%)	Prevalence Ratio or Difference (95 % CI)
Enrollment Site					
Kisii	223 (63.4)	5 (83.3)	Ref.	14 (51.9)	Ref.
Homa Bay	129 (36.7)	1 (16.7)	0.4 (0.0, 3.0)	13 (48.2)	1.5 (0.8, 3.2)
Maternal					
Maternal age <20	62 (17.6)	1 (16.7)	1.1 (0.1, 9.8)	5 (18.5)	1.1 (0.4, 2.8)
35+	14 (4.0)	1 (16.7)	4.7 (0.6, 39.2)	2 (7.4)	1.9 (0.5, 7.2)
Nulliparous	143 (40.6)	2 (33.3)	0.7 (0.1, 4.0)	12 (44.4)	1.2 (0.6, 2.4)
Mother TB suspect or high TB risk [c]	8 (2.3)	0 (0.0)	-1.7 (- 3.0, -0.4)	3 (11.1)	**4.2 (1.5, 11.8)**
Mother HIV positive	50 (14.2)	1 (16.7)	3.0 (0.3, 35.3) [d]	7 (25.9)	1.7 (0.7, 4.4) [d]
Intrapartum					
Out-of-hospital birth	11(3.1)	0 (0.0)	-1.7 (-3.1, -0.4)*	0 (0.0)	-7.4 (-10.0, -4.7)*
Fetal tachycardia at/during delivery [e]	35 (11.2)	0 (0.0)	-2.1 (-3.8, -0.4)*	4 (17.4)	1.6 (0.6, 4.5)
Maternal fever prior to delivery [g]	1 (0.3)	1 (16.7)	**31.7 (6.2, 162.8)** [f]	1 (4.4)	7.6 (1.8, 32.2) [f]
Maternal abdominal/uterine tenderness prior to delivery [h]	20 (6.4)	0 (0.0)	-2.0 (-3.6, -0.4)*	0 (0.0)	-7.3 (-10.1, -4.4)*
Maternal tachycardia [i]	17 (5.4)	0 (0.0)	-2.0 (-3.5, -0.4)*	1 (5.6)	0.7 (0.1, 5.2)
Prolonged rupture of membrane (PROM) [j]	53 (16.8)	0 (0.0)	-1.9 (-3.5, -0.2)*	6 (25.0)	1.6 (0.7, 3.8)
Clinical chorioamnionitis [k]	6 (1.92)	0 (0.0)	-1.9 (-3.5, -0.4)*	1 (5.6)	2.0 (0.3, 13.1)
Obstructed or prolonged labor [l]	78 (24.2)	0 (0.0)	-2.4 (-4.3, -0.5)*	8 (32.0)	1.4 (0.6, 3.2)
Foul-smelling amniotic fluid/vaginal discharge [m]	14 (4.5)	0 (0.0)	-2.4 (-4.3, -0.5)*	0 (0.0)	-7.7 (-10.6, -4.8)*
Meconium-stained liquor [n]	41 (13.1)	0 (0.0)	-2.2 (-3.9, -0.5)*	4 (16.0)	1.2 (.5, 3.4)
Received intrapartum antibiotics	12 (3.4)	0 (0.0)	-1.7 (-3.1, -0.4)*	0 (0.0)	-7.4 (10.0, -4.7)*
Caesarean section (w/out vaginal attempt)	123 (34.9)	1 (16.7)	0.4 (0.0, 3.2)	8 (32.0)	0.8 (0.4, 1.8)
Multiple birth	45 (12.8)	2 (33.3)	3.3 (0.6, 17.6)	5 (18.5)	1.5 (0.6, 3.8)
Newborn					
Male	203 (57.7)	4 (66.7)	1.5 (0.3, 7.9)	18 (66.7)	1.4 (0.7, 3.1)
Preterm (< 37 weeks) [o]	133 (39.6)	2 (33.3)	0.8 (0.1, 4.1)	11 (44.0)	1.2 (0.55, 2.5)
Low or very low birthweight (<2500 g)	128 (36.4)	3 (50.0)	1.7 (0.4, 8.5)	12 (44.4)	1.4 (0.7, 2.8)
Age <24 hours	167 (47.4)	1 (16.7)	0.2 (0.0, 2.0)	9 (33.3)	0.6 (0.3, 1.3)
Presented with any pSBI signs [p]	249 (70.7)	6 (100.0)	0.2 (0.5, 4.2)*	20 (74.1)	1.3 (0.6, 3.1) [d]

Treatment					
Antibiotics prior to blood	275 (78.1)	4 (66.7)	0.6 (01, 3.0)	18 (77.8)	0.6 (0.3, 1.3)
Antibiotics at/prior to enrollment (before or after sample collection)	291 (82.7)	6 (100.0)	2.0 (0.4, 3.6)*	21 (66.7)	0.8 (0.3, 1.8)

Ref: Reference category. Bold indicates p-value for Fisher's exact test <0.05. TB: Tuberculosis
No bacteremia defined as no isolates identified from bacterial blood culture
Prevalence ratio estimates and 95% CI from generalized linear model (GLM) log-binomial regression, comparing no bacteremia to definite/likely bacteremia, and no bacteremia to definite/likely bacteremia or possible bacteremia. Columns do not total 100% because definite/likely is a sub-group of definite/likely/possible.
p-values are for 2-sided Fisher's exact test
*Prevalence difference
[a] Positive blood culture with a definite or likely pathogen
[b] Positive blood culture with a definite or likely pathogen or possible pathogen
[c] Persistent cough, persistent night sweats, recent weight loss, prior tb diagnosis, recent tb treatment, household member with tb or on tb treatment during pregnancy
[d] Adjusted for enrollment site
[e] Fetal tachycardia n = 335
[f] Maternal fever no bacteremia vs. definite/likely bacteremia: p = 1.00 no bacteremia vs. definite/likely or possible bacteremia: 0.0363;
[g] Maternal fever n = 336; [h] Maternal abdominal/uterine tenderness n = 336;[i] Maternal tachycardia n = 341;[i] PROM n = 340;[k] Clinical chorioamnionitis n = 336;[l] Obstructed or prolonged labor n = 347; [m] Foul-smelling amniotic or vaginal fluid n = 339; [n] Meconium-stained liquor n= 338;[o] Gestational age/preterm n= 361; [p] History of poor feeding, fast breathing, chest indrawing, hyperthermia ≥37.5 axial or ≥38 rectal, hypothermia <35.5, only moves when stimulated/no movement, history of convulsions
q maternal tb suspect/high tb risk no bacteremia vs. definite/likely/possible p = 0.04
out-of-hospital birth no bacteremia vs. definite/likely: p = 1.00, no bacteremia vs. definite/likely/possible: p = 1.00
maternal tachycardia no bacteremia vs. definite/likely: p = 1.00, no bacteremia vs. definite/likely/possible: p = 1.00
maternal uterine/abdominal tenderness no bacteremia vs. definite/likely: p=1.00, no bacteremia vs. definite/likely/possible: p = 0.3801
foul-smelling fluid/vaginal discharge no bacteremia vs. definite/likely: p=0.3420, no bacteremia vs. definite/likely/possible: p = 0.6108
fetal tachycardia no bacteremia vs. definite/likely: p =1.00
intrapartum antibiotics no bacteremia vs. definite/likely: p = 1.00, no bacteremia vs. definite/likely/possible: p = 1.00
PROM no bacteremia vs. definite/likely: p = 0.5953
chorioamnionitis no bacteremia vs. definite/likely: p = 1.00
meconium-stained liquor no bacteremia vs. definite/likely: p = 1.00
presented with any pSBI signs: no bacteremia vs. definite/likely p = 0.1879
any antibiotics prior to enrollment no bacteremia vs. definite/likely: p = 0.5949

SUPPLEMENTARY MATERIAL

Table S1. Prevalence of antibiotic treatments among newborns with suspected or confirmed bacteremia in Kisii and Homa Bay Kenya (N=379)

Antibiotic treatment regimen and delivery mode	Not bacteremia n=352 Frequency (%)	Definite/likely bacteremia n=6 Frequency (%)	Possible, definite or likely bacteremia n=27 Frequency (%)
Gentamicin and penicillin	287 (81.5)	5 (83.3)	20 (74.1)
+ Ampicillin	1 (0.3)	0 (0.0)	0 (0.0)
+ Ampliclox	2 (0.6)	0 (0.0)	0 (0.0)
+Metronidazole/Flagyl	5 (1.4)	0 (0.0)	0 (0.0)
+ Cephalexin/Keflex/Keftab	2 (0.6)	0 (0.0)	0 (0.0)
Penicillin alone	1 (0.3)	0 (0.0)	0 (0.0)

Table S2. Neonatal blood culture isolates and antibiotic resistance (Homa Bay, and Kisii, Kenya) (N = 379)

Class			Penicillins		Aminogycosides	Beta-lactamase inhibitors	Cephalosporins	Quinolones	Other
Antibiotics	**N (%)**	**penicillin & gentamicin**[a]	ampicillin	ampi/ amoxicillin	genta-micin	amoxicillin clavulanic acid	ceftriaxone	ciprofloxacin	Cotrim-oxazole
Definite or likely pathogens	6 (1.6)					% Resistant			
Enterococcus faecalis	1	**0/1**	0/1	N/T	0/1	0/1	N/T	N/T	N/T
Escherichia coli	1	**0/1**	N/T	1/1	0/1	1/1	0/1	1/1	1/1
Klebsiella pneumoniae	2	**2/2**	N/T	2/2	2/2	2/2	2/2	0/2	2/2
Enterobacter cloacae	1	**1/1**	N/T	1/1	1/1	1/1	1/1	0/1	1/1
Enterobacter aerogenes	1	**1/1**	N/T	1/1	1/1	1/1	1/1	0/1	1/1
Possible pathogens	21 (5.5)								
Coagulase-negative *Staphylococcus*	19								
Bacillus spp.	1								
Yeast	1								
Total	27 (7.1)								

N/T = not tested

[a] First-line WHO-recommended antibiotic therapy

Chapter 2: Incidence of and Risk Factors for Newborn Mortality Among High-Risk Newborns

Title:

Risk factors for early mortality among Kenyan newborns with suspected severe infection: a prospective cohort study

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ABSTRACT

Background: We determined incidence and risk factors for mortality among high-risk Kenyan newborns.

Methods: A prospective cohort study was conducted in two health facilities in western Kenya. Newborns with clinical signs of severe illness or intrapartum risk factors for infection were enrolled within 96 hours of birth and followed for 7 days.

Results: Among 380 newborns, the cumulative mortality within 7 days was 5.8% (95% CI: 3.7%-8.6%). The prevalence of a positive blood culture with a definite/likely pathogen was 1.6% (95% CI: 0.6%-3.4%), among whom no deaths occurred. In the higher mortality site (mortality 12.0%), 94% of deaths occurred in newborns with ≥1 Integrated Management of Childhood Illness (IMCI) severe disease sign, all of whom had received WHO-recommended antibiotic regimens. Multiple IMCI signs were associated with mortality: fast breathing (RR: 4.7, 95% CI: 2.0-11.4); chest indrawing (RR: 3.4, 95% CI: 1.5-8.1); poor feeding (RR: 11.9, 95% CI: 2.8-49.9); hypothermia (RR: 4.8, 95% CI: 1.8-12.8); movement only with stimulation/no movement (RR: 4.5, 95% CI: 2.0-10.5); and convulsions (RR: 4.0, 95% CI: 1.7-9.4). The presence of ≥1 IMCI sign was associated with a 12-fold mortality risk (RR: 12.4, 95% CI: 1.7-91.0), as compared with no signs.

Conclusions: Mortality was substantial among newborns with signs of or risk factors for severe infection, despite low prevalence of culture-confirmed bacteremia and high antibiotic treatment coverage. IMCI signs identify newborns at excess mortality risk, even in a high-risk facility-based population. Blood culture may not add substantially to informing risk among high-risk newborns in resource-constrained settings.

BACKGROUND

2.7 million neonatal deaths (first 28 days of life) occurred globally in 2015, accounting for almost half of all deaths in children under 5 years[1,59]. Approximately 20% of neonatal deaths are attributable to severe infections and could be prevented[6]. As almost two-thirds of neonatal deaths occur in the first 7 days of life, the early neonatal period is a time of vulnerability during which there is opportunity for substantial impact[1].

In well-resourced settings, pregnancy and intrapartum risk-based screening for causes of early newborn infection, including Group B *streptococcal* colonization, maternal genitourinary tract and uterine infection and prolonged rupture of membranes (PROM), identify women for prophylactic intrapartum antibiotics and newborns who require close observation or antibiotics[19,44,45,53,60,61]. Such factors are less routinely available to inform management in low-resource settings[9,54,55]. Symptoms of severe invasive infection in infants are non-specific and advanced diagnostic and laboratory capacity is often unavailable, making detection difficult [11,18,53]. Syndromic algorithms such as the WHO Integrated Management of Childhood Illness (IMCI) and WHO Pocket book of Hospital Care for Children are therefore primarily used to inform clinical management in many high mortality areas [12,13]. The IMCI specifies criteria for severe disease and probable severe bacterial infection (pSBI) in young infants in community and first-level health facilities, based on a gold standard of physician diagnosis of probable severe infection, and indicates recommended therapy.

We aimed to determine mortality incidence and risk factors among newborns with intrapartum risk factors for severe infection and/or clinical signs of pSBI in referral-level health facilities in Kenya and to determine if pSBI criteria are associated with mortality risk in the first few days of life.

MATERIALS AND METHODS

Setting, population, enrollment

We conducted a prospective cohort study of high-risk mother/newborn pairs at Homabay County and Kisii Teaching and Referral (KTRH) government hospitals in rural Western Kenya where the regional neonatal mortality rate is 19 deaths/1,000 live births [62]. Homabay Hospital is a county-level facility where approximately 3,000 deliveries occur annually. The newborn unit has 10 designated beds and an average

of 228 annual admissions, supervised by a consultant pediatrician and pediatric nurse. The area is holoendemic for malaria, and has the highest adult HIV prevalence in the country, 26%[63]. KTRH is a provincial-level referral facility with approximately 8,000 deliveries per year. The newborn unit holds 15 designated beds and an average of 157 newborn admissions per year, supervised by two consultant pediatricians. Malaria is less common in the region and the adult HIV prevalence is 4.7%[63].

Pairs were eligible if either a maternal intrapartum risk factor for infection[9,18,19,33,53,56,57,64] was present, or if the newborn had a clinical sign suggestive of pSBI in the first 72 hours of life, based on IMCI criteria for suspected "very severe disease" in young infants, the WHO Pocket Book of Hospital Care For Children, and standard clinical practice in Kenya (Table 1)[12,13].

Potential participants were identified either after delivery or at facility presentation if referred or transferred, within 96 hours of delivery. Intrapartum risk factors were considered present if any factor was documented in patient records. Newborn clinical features were considered present if any feature was documented in records, identified during enrollment physical exam (chest indrawing, only moves when stimulated, fast breathing, abnormal temperature), or reported by caregiver (convulsions, poor feeding).

Participants provided informed consent in their language of choice (Gusii, Kiswahili, Dholuo, English).

Data and sample collection and processing

At enrollment, pregnancy, delivery, and postnatal medical history was abstracted from medical records and mothers were interviewed to collect sociodemographic information and clinical history using a standardized questionnaire. Incomplete or unavailable records were supplemented with maternal self-report. Study nurses conducted newborn physical exams. Results of newborn lumbar puncture to evaluate cerebrospinal fluid for meningitis, X-ray for pneumonia, and complete haemogram, were abstracted if conducted as part of routine care.

Mothers underwent rapid diagnostic testing for malaria (First Response Malaria Antigen Detection Card Test® (Premier Medical Corporation Pvt. Ltd., Maharashtra, India)). Maternal HIV status was defined

based on medical records and self-report. If a woman did not have a negative HIV test documented within a month, rapid antibody tests were conducted (Determine® (Abbott Laboratories, North Chicago, IL, USA) and/or Unigold® (Trinity Biotech, Bray, Ireland)).

Neonates born to HIV-infected mothers were considered HIV-exposed and had dried blood spots (DBS) collected (≥24 hours of age) for early-infant diagnosis conducted by CDC HIV-R lab in Kisumu, Kenya, using standard DNA-PCR methods (Roche *HIV-1 DNA test*® (Roche Molecular Systems, Branchburg, NJ, USA)).

Immediately following enrollment, 1-3 mL of whole blood was aseptically collected in VersaTREK REDOX direct draw blood-culture media from neonates for bacterial blood culture, conducted by Lancet Laboratories in Nairobi using the VersaTREK automated microbial detection system. Media were incubated in aerobic culture for up to 7 days for bacterial growth. Positive cultures were further evaluated for speciation and antibiotic sensitivity. Confirmed bloodstream infection was defined as isolation of ≥1 definite or likely pathogen from a normally sterile site (blood)[51,52].

Neonates were followed up at 3 and 7 days post-enrollment on the ward or via phone if discharged to ascertain vital status. Patient tracing was conducted for discharged participants who could not be reached by phone. Death was defined as death within 7 days of enrollment, from caregiver report or facility record.

Clinical management was provided by non-research health facility personnel, but clinically-relevant laboratory results and observations were communicated as appropriate to inform treatment.

Data were collected on paper-based case report forms and entered into RedCap, a web-based database system (http://project-redcap.org)(Institute of Translational Health Science (ITHS) grant support (UL1TR000423 from NCRR/NIH)). Statistical analyses were conducted using Stata 14.2 (StataCorp. 2015. Stata Statistical Software: Release 14. College Station, TX: StataCorp LP.)

The University of Washington Institutional Review Board and Kenya Medical Research Institute Scientific Ethics Review Unit provided ethical approval.

Statistical analysis

Demographic, health history and clinical characteristics were described using descriptive statistics.

The cumulative incidence of mortality in 7 days was estimated overall and by site, with 95% confidence intervals (CI) estimated from the binomial distribution using exact methods. Kaplan-Meier methods were used to compare survival distributions by site, eligibility criteria and pSBI signs, and equality of survivorship was tested using the non-parametric log-rank test.

To determine mortality risk factors, we calculated crude relative risks (RR) and risk differences (RD) with 95% CIs from contingency tables, stratified by enrollment site, using exact methods from the binomial distribution. Hypothesis tests were based on Fisher's exact test for small-sample estimates with alpha level of 5%. All analyses were exploratory. We did not adjust for multiple comparisons.

RESULTS

Population characteristics

Four hundred pairs were enrolled. Twenty pairs were eligible based only on the presence of maternal HIV infection without other eligibility criterion present, and were excluded from this analysis, leaving a study population of 380, of whom 378 (99.5%) completed follow-up (Figure 1). Participant characteristics are provided in Table 2. 97.1% of deliveries occurred in a health facility. Half (51.8%) of mothers completed primary school or less and the vast majority (95.3%) used a pit latrine or open defecation for toileting. Overall, 15.0% of women were HIV positive, though this differed by site: 34.5% in Homabay and 3.4% in Kisii.

Clinical characteristics and outcomes

Approximately half (47.6%) of the participants had both intrapartum risk factors and newborn clinical signs at enrollment, and a quarter each were enrolled based on only intrapartum (25.5%) or newborn factors (26.8%). The median age at enrollment was 25 hours (IQR: 13-42). A substantial proportion of newborns were low birthweight (37.1%) or preterm (40.1%) Most newborns (73.2%) had ≥1pSBI sign.

The cumulative 7-day mortality was 5.8% (95% CI: 3.7%-8.6%, n=22). Mortality risk was higher in Homabay (12.0%, 95% CI: 7.1%-18.5%) than Kisii (2.1%, 95% CI: 0.7%-4.9%) (Figure 2a). Most deaths

occurred early; 14 within one day. All deaths occurred in facility prior to discharge. All newborns who died had received antibiotics at or before enrollment and the regimens among those who died all included, at a minimum, WHO/IMCI-recommended combinations of intramuscular gentamycin and penicillin for pSBI (data not shown)[13].

Culture-confirmed bacteremia prevalence was low (1.6%, 95% CI: 0.6%-3.4%), among whom no newborns died. Pathogen distribution and risk factors are described elsewhere (*Naulikha J., Levine GA. et al, in process*). No newborns were HIV DNA-positive.

Mortality risk was higher in those with an intrapartum risk factor and newborn clinical sign than in those with only an intrapartum or newborn factor, and was higher with each additional pSBI sign present (Figures 2b-3).

Mortality risk factors

Mortality risk factors in Homa Bay are presented in Tables 3-4.

Distributions of demographic and clinical characteristics and mortality risk differed by site (Table 2, Figure 2a), thus, analyses were conducted by site. We lacked sufficient statistical power in Kisii, but present exploratory estimates in Supplementary Data (Tables S1, S2).

In general, the proportion of newborns who died was larger in those with each pSBI sign than those without each sign and survival distributions differed between those with and without each sign (Figure 4). Six of seven pSBI signs were associated with mortality: fast breathing (RR: 4.7, 95% CI: 2.0-11.4), chest indrawing (RR: 3.4, 95% CI: 1.5-8.1), poor feeding (RR: 11.9, 95% CI: 2.8-49.9), hyperthermia (RR: 3.4, 95% CI: 1.4-8.4), movement only with stimulation/no movement (RR: 4.5, 95% CI: 2.0-10.5), and convulsions (RR: 4.0, 95% CI: 1.7-9.4). Each of the above signs was associated with more than a 20% difference in the absolute risk of death, compared with those without that sign. The presence of any pSBI sign was associated with a 12-fold mortality risk (95% CI: 1.7-91.0) (Figure 5, Table 3).

No intrapartum risk factors for peripartum infection were associated with mortality. Among newborns with only an intrapartum factor but no newborn infection signs, there were no deaths. Mortality risk was similar among HIV-exposed and unexposed (RR: 0.8, 95% CI: 0.3-2.1). No demographic or

household characteristics were associated with mortality. Low birthweight infants had a 3.3-fold risk of death compared with normal weight (95% CI: 1.3, 8.6). Those who were never breastfed and those who received antibiotics were more likely to die, likely due to confounding by illness severity. Due to the rarity of positive blood culture and lack of HIV acquisition, we were unable to determine precise estimates of risk for these exposures (Table 4).

DISCUSSION

In this facility-based population of newborns with intrapartum risk factors or clinical signs of severe infection, mortality was substantial. The majority of deaths happened within 24 hours of enrollment, and within a health facility. Most newborns received antibiotics and all newborns who died had received WHO-recommended antimicrobial therapy[13]. The peripartum period provides an important opportunity for impact with an accessible, high-risk population.

The high mortality despite recommended antibiotic therapy indicates an important gap and priority area for the development and application of interventions to prevent newborn mortality in facilities. Facility-based childbirth and postnatal care coverage have improved in many settings, but interventions to prevent mortality among small and sick newborns and to improve care quality are crucial for improving survival[4,65-67]. Among newborns with possible severe illness, antibiotics may be insufficient to prevent mortality or may not be appropriately tailored to relevant pathogens. Additional case-management and supportive care including feeding support, respiratory and breathing support, thermal and fluid management is also essential for especially vulnerable newborns[2,8,26,37,68].

We found that IMCI pSBI signs were strongly associated with mortality, even among this high-risk population. The IMCI are intended for community and primary health-levels to identify newborns for urgent antibiotic therapy or hospital referral[20]. Although our population had a broader set of inclusion factors within the context of referral-level care, where the distribution of risk factors and of mortality was likely substantially higher than the general population, the criteria identified newborns at the highest mortality risk. The excess risk associated with IMCI signs likely substantially underestimates the true excess risk when applied in a general population. The estimates were determined based on a reference population which included

newborns with other clinical factors indicative of severe illness, among whom the mortality risk was substantially higher than in a general population. Additionally, care providers were aware of the identification of clinical features which could have influenced care and resulted in lower mortality, which would falsely attenuate the true association.

Positive blood culture with a definite/likely pathogen was not associated with mortality in this study, although we were limited by low prevalence of culture positivity. The rare isolate recovery may be due to the high prevalence of pre-culture antibiotics and to small blood volumes, which reduce the utility of blood culture in young infants[69,70]. Detection methods for disseminated infections with focal points other than the bloodstream, and more sensitive pathogen detection methods such as PCR, were not routine practices, thus we were unable to detect meningitis and pneumonia and may have missed identifying some pathogens in the bloodstream. Given that most deaths occurred very early, processing and reporting times for culture may further limit utility in informing therapy. All newborns with positive cultures had already received antibiotics before results were available.

Although newborn antibiotics were common, intrapartum antibiotics were rare. Improving intrapartum antibiotic coverage for suspected or confirmed intraamniotic and peripartum infections has the dual benefit of treating infection in the woman and preventing newborn transmission[71,72]. Preventing postnatal delays in antibiotic administration when indicated may also prevent newborn infection or disseminated disease.

Breastfeeding was less common among newborns who succumbed to illness. Newborns who are less severely ill likely have more opportunity to breastfeed than the more severely ill, who may be separated from their mothers for treatment or procedures that interfere with access and ability to breastfeed. These findings support current recommendations to encourage breastfeeding in newborn units so that high-risk newborns can also benefit from the important protective effects of breastfeeding[73].

In the absence of pSBI signs, intrapartum risk factors were not associated with mortality. However, newborns with both intrapartum factors *and* clinical features had the highest mortality, suggesting the importance of treatment continuity and vigilant observation of especially high-risk dyads throughout the perinatal period.

In addition to infection, other causes of death present with similar clinical features and cause a large proportion of early neonatal deaths and may explain some deaths in our population [6,11]. There is a paucity of robust newborn cause-of-death data in low-resource facility-based settings, but accurate mortality attribution data is essential to developing appropriate interventions for this population.

Mortality risk was higher in Homabay than Kisii, despite less common clinical evidence of severe disease. This may reflect differences in populations served by the facilities, and differences in clinical assessment. Homabay is also a lower tier facility with less advanced treatment and management capacity.

Our study had several strengths. It was conducted among a high-risk population in two public health facilities likely generalizable to similar low-resource settings. We included different facility levels in regions of high and moderate HIV and malaria prevalence. We obtained high-quality data on newborn presentation and high follow-up completion.

This study also has limitations. Both neonate and mother needed to have survived long enough to be enrolled, leading to possible selection bias. The denominator was not live-births and the 7-day mortality risk is not an early-neonatal mortality rate. We relied on medical records for pregnancy and delivery history and are unable to determine whether factors that were not documented were not present, not recorded, or not assessed. We anticipate lack of documentation would under-report potential exposures and bias estimates of association towards the null, thus our estimates are likely conservative. We lacked information on treatments received after enrollment and are unable to determine whether duration of antibiotic therapy was consistent with current recommendations and limiting our ability to understand how to optimize clinical care. The small sample size and rarity of exposures and events limited statistical power and impaired precision. Finally, the population included only high-risk newborns, limiting the ability to generalize to unselected newborn populations.

CONCLUSION

Mortality among facility-based newborns with intrapartum risk factors or clinical signs of severe infection was substantial, despite low prevalence of culture-confirmed bacteremia and high antibiotic treatment coverage. In resource-constrained health facilities, IMCI pSBI signs may help identify newborns

at highest risk of death, even among a high-risk population. Blood culture may not add substantially in informing risk in similar settings. As access to and utilization of facility-based care in the perinatal period continues to expand, strategies to enhance care quality and effective interventions to improve survival among small and sick newborns are needed.

Funding

This work was supported by a grant from the Firland Family Foundation [201305]; and an International Pilot Award from the Center for AIDS and STDs at the University of Washington [Parent Award 30AI027757-26].

Conflicts of Interest

All authors declare nothing to disclose.

Acknowledgements

We would like to thank the data collection team members at the study sites and health facility staff at KRTC and Homabay County Hospital for their valuable contribution to this study, and the study subjects and their families for their willingness to participate. We are also thankful to the Global Center for Integrated Health of Women, Adolescents and Children, the Pediatric Infectious Disease Research Group, Kenya Research and Training Center, and the Center for AIDS and STDs at the University of Washington Department of Global Health for support throughout this project. Thank you to Dr. Patricia Pavlinac for her role in conceptualizing this project and proposal development, and to Dr. Günther Fink for his insightful comments on an earlier draft.

TABLES

Table 1. Eligibility criteria

Inclusion: ≥1 of the following intrapartum or neonatal factors:

Intrapartum[9,56,57]

- PROM (>18 hours)
- Foul-smelling amniotic fluid/vaginal discharge
- Fever 72 hours prior to delivery (≥38°C)
- Maternal tachycardia (>100 beats/min)
- Fetal distress (meconium stained liquor, fetal tachycardia)
- Uterine/abdominal tenderness prior to delivery
- Clinical chorioamnionitis
- Obstructed labor
- Delivery <37 completed weeks gestation
- Maternal HIV infection[a]

Neonatal [12,13]

- Fast breathing (>60 breaths/min)[b]
- Severe chest wall indrawing[b]
- History of poor feeding [b]
- Temperature <35.5 °C[b,c]
- Temperature ≥37.5 °C[b,c]
- Moves only when stimulated/no movement [b]
- History of convulsions[b]
- Lethargy[d]
- Severe jaundice[d]
- Apnea[e]
- Five-minute Apgar score <6[e]

Exclusion:

- Mother does not/ is not able to consent
- Infant weight <1 kg (minimum blood for culture can't be collected)
- Infant >96 hours

[a] Excluded as an independent eligibility factor in this analysis
[b] IMCI signs of severe disease in young infants and WHO Pocket Book of Hospital Care for Children danger sign for young infants
[c] Axial temperature ≥37.5 °C/rectal temperature ≥38 °C considered high temperature as per IMCI. WHO Pocket Book threshold is >38 °C.
[d] WHO Pocket Book of Hospital Care for Children sign of newborn pSBI
[e] Signs of newborn pSBI used in standard clinical practice in Kenya

Table 2. Enrollment characteristics of newborns with intrapartum risk factors or clinical signs of severe disease

	Kisii (n = 238)	Homabay (n = 142)	Combined (N = 380)
Characteristic	Freq. n (%) or Median (IQR)	Freq. n (%) or Median (IQR)	Freq. n (%) or Median (IQR)
Demographic and household			
Travel time to study facility ≥1 hour	105 (44.1)	71 (50.0)	176 (46.3)
Maternal age (years)			
<20	36 (15.1)	31 (21.8)	67 (17.6)
20-35	193 (81.1)	104 (73.2)	297 (78.2)
>35	9 (3.8)	7 (4.9)	16 (4.2)
Married	194 (81.9) [a]	113 (79.6)	307 (81.0) [b]
Mother completed primary education or less	152 (63.9)	45 (31.7)	197 (51.8)
Pit latrine or bush toilet	222 (93.3)	140 (98.6)	326 (95.3)
First birth	106 (44.5)	50 (35.2)	224 (59.0)
Attended any ANC	217 (92.0) [c]	138 (97.2)	355 (93.9) [d]
Maternal antibiotics during pregnancy	215 (91.9) [e]	104 (76.5) [f]	319 (83.9) [g]
Mother HIV Positive	8 (3.4)	49 (34.5)	57 (15.0)
Paternal HIV (maternal report)			
Positive	6 (2.5)	31 (21.8)	37 (9.7)
Unknown or decline to state	68 (28.6)	40 (28.2)	108 (28.4)
Maternal malaria diagnosis during pregnancy	15 (6.4) [c]	26 (18.6) [h]	41 (10.8) [i]
Intrapartum			
Cesarean delivery	76 (31.9)	65 (45.8)	141 (37.1)
Maternal malaria RDT+ at enrollment	0 (0.0) [c]	7 (4.9)	7 (1.9)

Delivery in a health facility	233 (97.9)	135 (95.7) [j]	368 (97.1)
Intrapartum antibiotics received	6 (2.5) [c]	6 (4.4) [k]	12 (3.2) [l]
Newborn and postnatal			
Referred or transferred to study site	61 (25.6)	63 (44.4)	124 (32.6)
Male	144 (60.5)	78 (54.9)	222 (58.4)
Preterm birth (<37 weeks)	109 (46.8) [m]	36 (27.9) [n]	145 (40.1) [o]
Birthweight (kg)			
Low or very low (<2.5 kg)	99 (41.6)	42 (29.6)	141 (37.1)
Normal (2.5 – 4 kg)	132 (55.5)	92 (64.8)	224 (60.0)
Macrosomic (>4 kg)	7 (2.9)	8 (5.6)	15 (4.0)
Multiple birth	40 (16.8)	10 (7.0)	50 (13.2)
Age at enrollment (hrs)	23 (12, 37)	30 (17, 46)	25 (13, 42)
IMCI severe illness signs (number) [p]			
0	48 (20.2)	62 (43.7)	110 (29.0)
1	88 (37.0)	28 (19.7)	116 (30.5)
≥2	102 (42.9)	52 (36.6)	154 (40.5)
Newborn antibiotic exposure	233 (98.3)	79 (56.0) [j]	312 (82.5)

Freq.: Frequency; IQR: Interquartile range; ANC: Antenatal care

[a] n = 237 [b] n = 379 [c] n = 236 [d] n = 378 [e] n = 234 [f] n = 136 [g] n = 370 [h] n = 140 [l] n = 376 [j] n = 141 [k] n = 137 [l] n = 373 [m] n = 233 [n] n = 129 [o] n = 362 [p] Includes fast breathing, chest indrawing, poor feeding, axial temperature ≥37.5 °C or ≥38 °C rectal, temperature < 35.5 , only moves when stimulated/no movement, history of convulsions.

Table 3. Clinical presentation and early-neonatal mortality risk among newborns with intrapartum risk factors for infection or signs of severe disease in Homabay, Kenya (n=142)

Factor	Cumulative 7-day Mortality (%) (95% CI) [a]	Relative Risk (95% CI) [b]	Risk Difference (95% CI) [b]	p-value [c]
IMCI severe illness criteria				
Fast breathing	30.3 (15.6, 48.7)	**4.7 (1.9, 11.4)**	**23.9 (7.5, 40.2)**	**0.0008**
Chest indrawing	29.3 (12.6, 51.1)	**3.4 (1.5, 8.1)**	**20.7 (1.8, 39.6)**	**0.0103**
Poor feeding	27.3 (16.1, 41.0)	**11.9 (2.8, 49.9)**	**25.0 (12.8, 37.2)**	**<0.0001**
Hypothermia [d]	22.2 (2.8, 60.0)	2.5 (0.65, 9.9)	13.5 (-14.2, 41.1)	0.2134
Hyperthermia [d]	30.0 (11.9, 54.3)	**3.4 (1.4, 8.4)**	**21.2 (0.5, 42.0)**	**0.0158**
Only moves when stimulated/ no movement	36.8 (16.3, 61.6)	**4.5 (2.0, 10.5)**	**28.7 (6.5, 50.9)**	**0.0022**
History of convulsions	33.3 (14.6, 57.0)	**4.0 (1.7, 9.4)**	**25.1 (4.3, 45.8)**	**0.0043**
≥2 IMCI severe illness signs	28.8 (17.1, 43.1)	**13.0 (3.1, 54.5)**	**26.6 (13.9, 39.3)**	**<0.0001**
0 or 1 IMCI severe illness signs	2.2 (0.3, 7.8)	**Ref.**	Ref.	
≥1 IMCI severe illness	20.0 (11.9, 30.4)	**12.4 (1.7, 91.0)**	**18.4 (9.1, 27.7)**	**0.0005**
No IMCI severe illness signs	1.6 (0.0, 8.7)	Ref.	Ref.	
≥1 intrapartum [e] & ≥1 newborn factor [f]	27.9 (17.1, 40.8)	_ _ _	**27.9 (16.6, 39.1)**	**<0.0001**
Only newborn factor(s) [f]	0.0 (0.0, 19.5) [g]	_ _ _	_ _ _	_ _ _
Only intrapartum factor(s) [e]	0.0 (0.0, 5.6) [g]	Ref.	Ref.	

RR: Relative risk; RD: Risk Difference (Absolute difference in cumulative incidence/proportion); Ref.: Reference. **Bold** indicates *p* <0.05

[a] Binomial exact confidence intervals estimated using stratified analyses. [b] RR and RD estimates and 95% confidence intervals estimated using exact methods and binomial distribution. All estimates are crude. [c] 2-sided Fisher's exact test. [d] RR and RD compare high temperature to normal temperature and low temperature to normal temperature. [e] Includes prolonged rupture of membranes, foul-smelling amniotic/vaginal fluid, fever prior to delivery, maternal tachycardia, abdominal tenderness, obstructed labor, chorioamnionitis, meconium stained liquor, fetal tachycardia, delivery <37 weeks. [f] Includes 7 IMCI

severe illness signs and lethargy, attacks of apnea, 5-minute Apgar score <6, severe jaundice. [g] One-sided 97.5% confidence interval.

Individual newborn clinical features were considered present if documented from any source and absent otherwise. Presence of any newborn clinical feature was classified "yes" if any features were documented from any source, and "no" if all factors were documented as not present *or* were not documented.

Table 4. Risk factors for early-neonatal mortality among newborns with intrapartum risk factors for infection or signs of severe disease in Homabay, Kenya (n=142)

Factor	Cumulative 7-day Mortality (%) (95% CI) [a]	Relative Risk (95% CI) [b]	Risk Difference (95% CI) [b]	p-value [c]
Antenatal				
Maternal age (years)				
<20	12.9 (5.4, 18.1)	1.3 (0.4, 3.7)	2.3 (-10.9, 15.5)	0.7474
20 – 35	10.6 (5.3, 18.9)	Ref.	Ref.	
>35	28.6 (3.7, 71.0)	2.7 (0.7, 9.9)	18.0 (-16.0, 52.0)	0.1898
Mother completed primary education or less	9.3 (4.3, 16.9)	0.5 (0.2, 1.3)	-8.5 (-21.1, 4.1)	0.1216
No antenatal care	25.0(0.6, 80.6)	2.2 (0.4, 12.5)	13.4 (-29.4, 56.2)	0.4030
Father's HIV status (maternal report)				
Known Negative	9.9 (4.1, 19.3)	Ref.	Ref.	
Known Positive	6.5 (0.8, 21.4)	0.7 (0.1, 3.0)	-3.4 (-14.5, 7.7)	0.7189
Unknown status or decline to state	20.0 (9.1, 35.6)	2.0 (0.8, 5.2)	10.1 (-4.1, 24.3)	0.1552
Mother HIV positive	10.2 (3.4, 22.2)	0.8 (0.3, 2.1)	-2.7 (-13.6, 8.2)	0.7882
Parity- First birth	16.0 (7.2, 29.1)	1.6 (0.7, 4.0)	6.2 (-5.6, 18.1)	0.2901
Intrapartum				
Any intrapartum risk factors for severe infection [d]	13.6 (8.3, 20.9)	_ _ _	13.6 (7.6, 19.6) [e]	0.2236
Prolonged rupture of membranes [f g]	14.3 (5.4, 28.5)	1.9 (0.6, 5.9)	6.8 (-5.5, 19.1)	0.3299
Foul-smelling amniotic/vaginal fluid [g h]	0.0(0.0, 41.0)	_ _ _	-12.2 (-18.7, -5.8)	1.0000
Maternal fever [g j]	0.0 (0.0, 97.5) [i]	_ _ _	-8.6 (-13.9, -3.2)	1.0000
Maternal tachycardia [g k]	11.8 (1.5, 36.4)	1.2 (0.3, 5.2)	2.2 (-14.2, 18.6)	0.6752
Uterine tenderness [g l]	15.8 (3.4, 39.6)	2.2 (0.6, 8.0)	8.6 (-8.8, 25.9)	0.3628
Chorioamnionitis [g m]	0.0 (0.0, 52.2) [i]	_ _ _	-10.4 (-16.5, -4.3)	1.0000
Obstructed labor > 18 hrs [g n]	9.7 (3.6, 19.9)	0.8 (0.3, 2.4)	-2.1 (-13.6, 9.4)	0.7662
Meconium stained liquor [g o]	3.6 (0.1, 18.4)	0.3 (0.0, 2.4)	-7.7 (-17.4, 2.1)	0.4483
Fetal tachycardia [g p]	18.2 (7.0, 35.5)	2.4 (0.8, 6.9)	10.6 (-3.8, 25.0)	0.1761
Missing record of ≥3 intrapartum factors [d g]	20.0 (9.1, 35.6)	2.3 (0.9, 5.5)	11.2 (-2.4, 24.7)	0.0847
C-section (w or w/out vaginal attempt)	6.2 (1.7, 15.0)	0.4 (0.1, 1.1)	-10.7 (-20.9, -0.5)	0.0686
Intrapartum antibiotics [g q]	0.0 (0.0, 45.9) [i]	_ _ _	-12.5 (-18.1, -6.9)	1.0000
Maternal malaria RDT+ at enrollment	14.3 (0.4, 57.9)	1.2 (0.2, 7.8)	2.4 (-24.1, 28.9)	1.0000
Newborn/Postnatal				
Male sex	14.1 (7.3, 23.8)	1.5 (0.6, 3.8)	4.7 (- 5.8, 15.3)	0.4448
Delivery out of hospital [r]	16.7 (0.4, 64.1)	1.4 (0.2, 8.9)	4.8 (-25.5, 35.1)	0.5443
Referral or transfer to study facility	15.9 (7.9, 27.3)	1.8 (0.7, 4.4)	7.0 (-4.0, 18.0)	0.2980
Preterm birth (<37 wks) [s]	22.2 (10.1, 39.2)	2.3 (1.0, 5.5)	12.6 (-2.3, 27.4)	0.0806
Birthweight				
Low or very low (<2,500 g)	21.4 (10.3, 36.8)	**3.3 (1.3, 8.6)**	**14.9 (1.5, 28.3)**	**0.0172**
Normal (2,500 g – 4,000 g)	6.5 (2.4, 13.7)	Ref.	Ref.	
Macrosomia (>4,000 g)	25.0 (3.2, 65.1)	3.8 (0.9, 17.0)	18.5 (-12.0, 48.9)	0.1232
Cord cleaning	16.7 (0.4, 64.1)	1.4 (0.2, 1.0)	4.9 (-25.4, 35.2)	0.5415
Never breastfed	43.5 (23.2, 65.5)	**7.4 (3.1, 17.4)**	**37.6 (16.9, 58.3)**	**<0.0001**
Newborn antibiotic exposure [r]	21.5 (13.1, 32.2)	_ _ _	**21.5 (12.5, 30.6)**	**<0.0001**
Positive blood culture w/ definite pathogen	0.0 (0.0, 1.0) [i]	_ _ _	-12.1 (-17.4, - 6.7)	1.0000

RR: Relative risk; RD: Risk Difference (Absolute difference in cumulative incidence/proportion); RDT: Rapid diagnostic test; Ref.: Reference. **Bold** indicates p <0.05

[a] Binomial exact confidence intervals estimated using stratified analyses. [b] RR and RD estimate and 95% confidence intervals from binomial distribution using exact methods. All estimates are crude. [c] 2-sided

Fisher's exact test. [d] Includes prolonged rupture of membranes, foul-smelling amniotic or vaginal fluid, maternal fever prior to delivery, maternal tachycardia, abdominal tender ness, obstructed labor, chorioamnionitis, meconium stained liquor, fetal tachycardia, delivery < 37 weeks. Includes those with and without any newborn clinical factors. [e] Calculated compared to a reference population of those with no intrapartum risk factors (regardless of whether they have newborn clinical factors or not). [f] n = 109 [g] Those with undocumented status on a specific factor excluded from analysis of that factor. [h] n = 105 [l] One-sided 97.5% confidence interval. [j] n=106 [k] n = 111 [l] n = 102 [m] = 101 [n] = 113 [o] n = 108 [p] n = 112 [q] n = 137 [r] n = 141 [s] n = 129

Assessment of individual intrapartum factors was limited to participants for whom presence *or* absence was documented in records. Presence of any intrapartum factors was classified "yes" if any factors were documented, and "no" if all factors were documented as not present *or* were not documented.

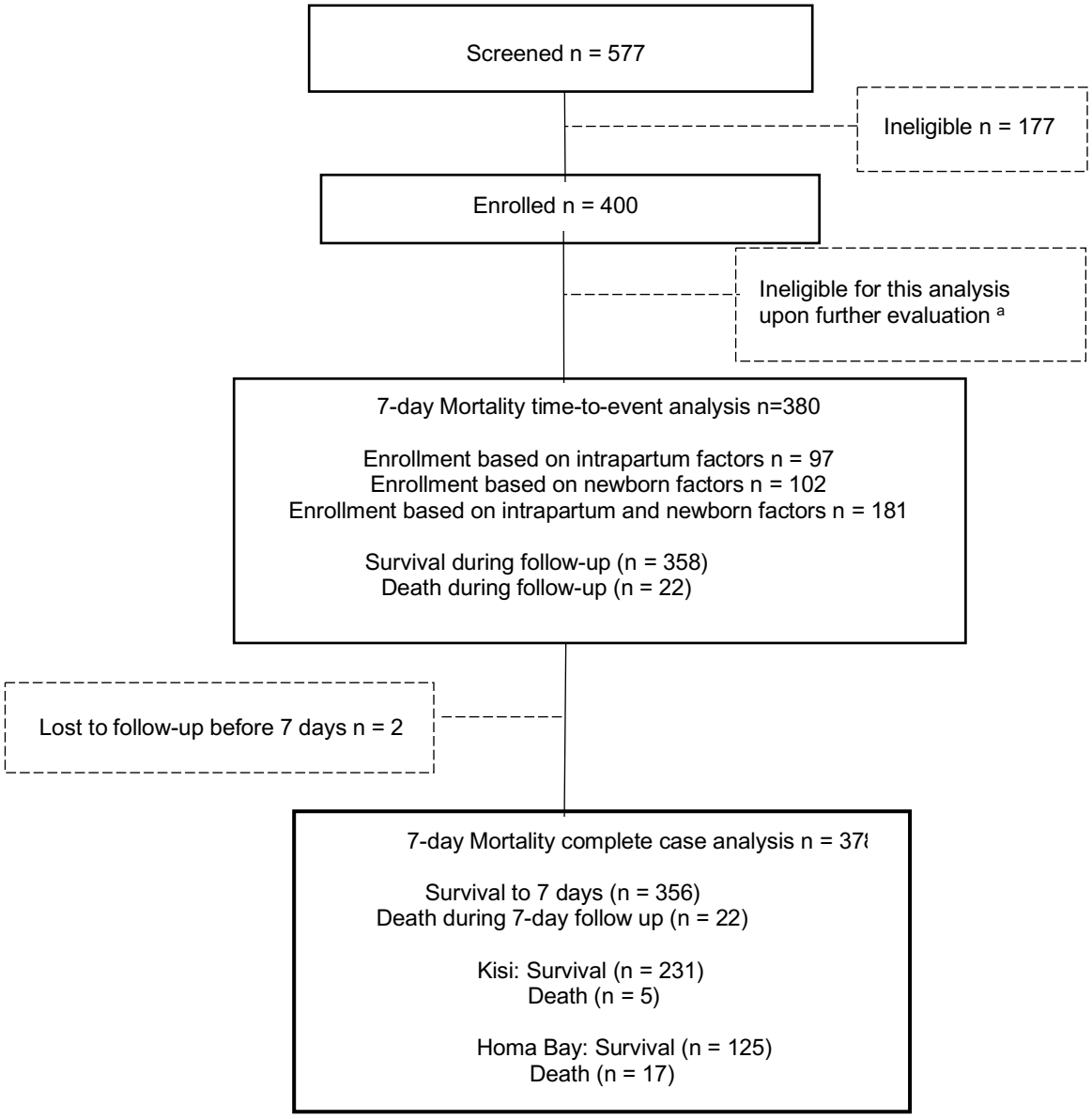

Figure 1. Participant flow diagram of the Early-onset Neonatal Sepsis Study (EOS)

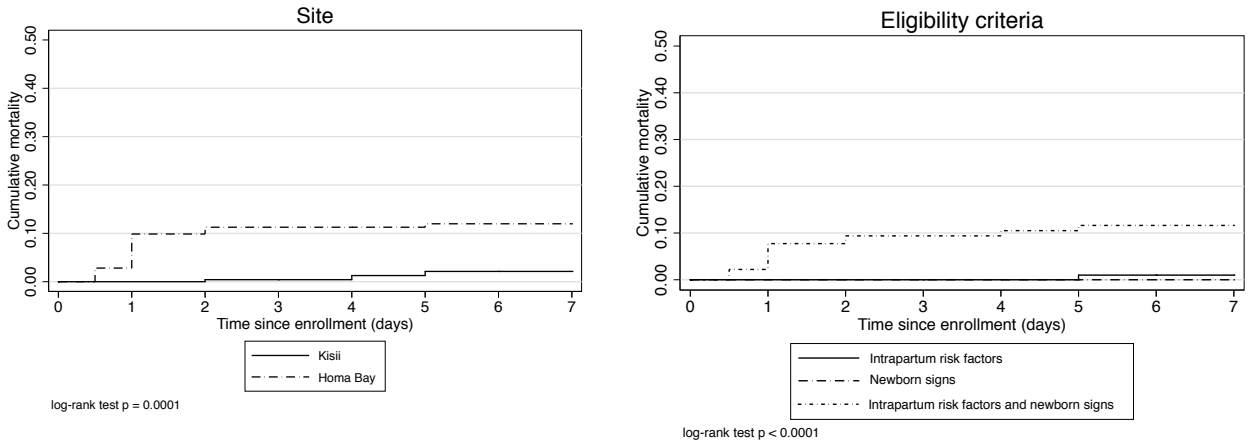

Figure 2. Kaplan-Meier failure estimates of newborn mortality (Kisii and Homa Bay, Kenya) (n=380)

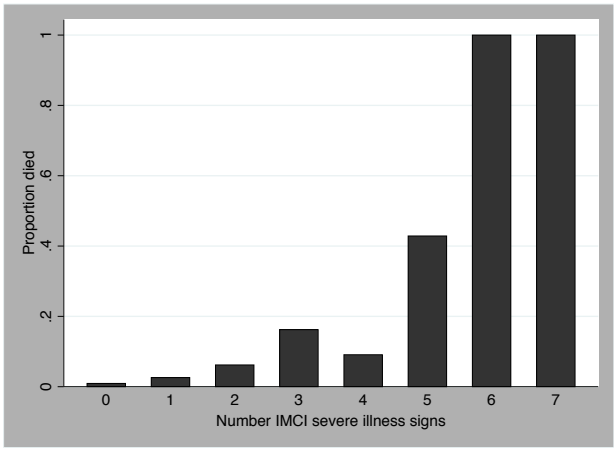

Figure 3. Frequency of IMCI severe illness criteria and 7-day mortality risk among newborns (Kisii and Homa Bay, Kenya) (n= 378)

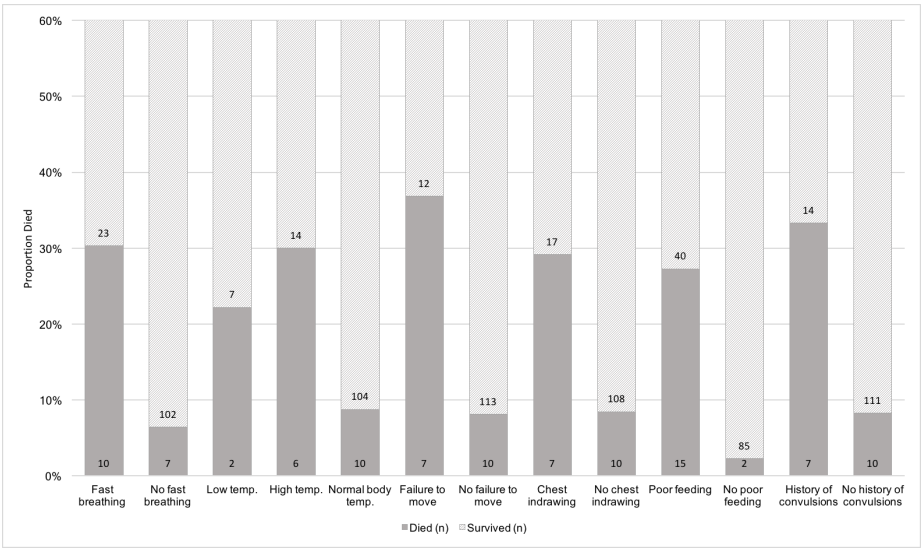

Figure 4. Distribution of IMCI severe illness criteria among newborns who died and those who survived (Kisii and Homa Bay, Kenya) (n=142)

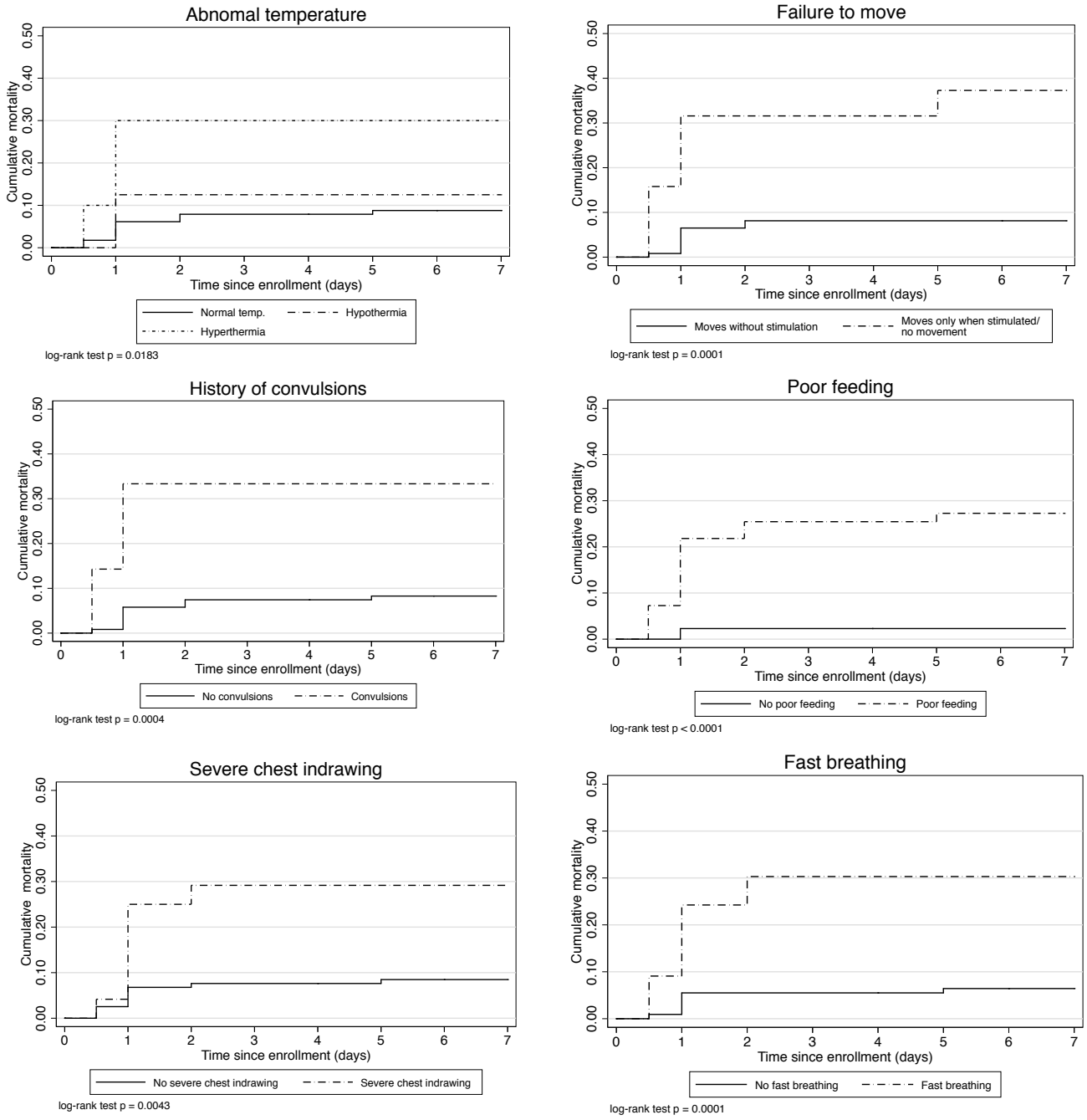

Figure 5a. Kaplan-Meier failure estimates of newborn mortality by IMCI severe illness signs (Homa Bay, Kenya) (n=142)

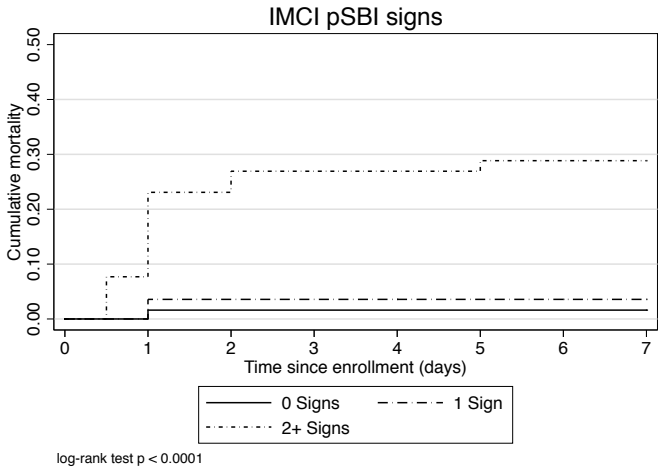

Figure 5b. Kaplan-Meier failure estimates of newborn mortality by number of IMCI severe illness signs (Homa Bay, Kenya) (n=142)

SUPPLEMENTARY MATERIAL

Supplementary Table S1. Clinical presentation and early-neonatal mortality risk among newborns with intrapartum risk factors for infection or signs of severe disease in Kisii, Kenya (n = 236)

Factor	Cumulative 7-day Mortality (%) (95% CI) [a]	Relative Risk (95% CI) [b]	Risk Difference (95% CI) [b]	p-value [c]
IMCI severe illness criteria				
Fast breathing	3.9 (0.5, 13.5)	2.4 (0.4, 14.1)	2.3 (-3.3, 7.9)	0.2954
Chest indrawing	3.6 (1.2, 8.3)	_ _ _	3.6 (0.5, 6.7)	0.0779
Poor feeding	4.8 (0.6, 16.2)	3.1 (0.5, 17.9)	3.2 (-3.5, 9.9)	0.2171
Hypothermia [d]	0.0 (0.0, 23.2) [e]	_ _ _	-2.4 (-4.5, -0.3)	1.0000
Hyperthermia [d]	0.0 (0.0, 24.7) [e]	_ _ _	-2.4 (-4.5, -0.3)	1.0000
Only moves when stimulated	9.1 (1.1, 29.2)	6.5 (1.1, 36.7)	7.7 (-4.4, 19.8)	0.0699
History of convulsions	2.7 (0.9, 6.2)	_ _ _	-2.7 (-5.1, -0.4)	0.5889
≥2 IMCI severe illness sign	2.9 (0.6, 8.4)	2.0 (0.4, 11.6)	1.4 (-2.4, 5.3)	0.6545
0 or 1 IMCI severe illness sign	1.5 (0.2, 5.3)	Ref.	Ref.	
≥1 IMCI severe illness sign	2.6 (0.9, 6.0)	_ _ _	2.0 (0.4, 4.9)	0.5861
No IMCI severe illness sign	0.0 (0.0, 7.7) [e]	Ref.	Ref.	
≥1 intrapartum [f] & ≥1 newborn factor [g]	3.4 (0.9, 8.4)	_ _ _	3.4 (0.1, 6.6)	0.5790
Only newborn factor(s) [g]	1.2 (0.0, 6.4)	_ _ _	1.2 (-1.1, 3.5)	1.0000
Only intrapartum factor(s) [f]	0.0 (0.0, 10.9) [e]	Ref.	Ref.	

RR: Relative risk; RD: Risk Difference (Absolute difference in cumulative incidence/proportion); Ref.: Reference category. **Bold** indicates p <0.05.

[a] Binomial exact confidence intervals estimated using stratified analyses. [b] RR and RD estimate and 95% confidence interval from binomial distribution using exact methods. All estimates are crude. [c] 2-sided Fisher's exact test. [d] RR and RD compare high temperature to normal temperature and low temperature to normal temperature. [e] One-sided 97.5% confidence interval. [f] Includes prolonged rupture of membranes, foul-smelling amniotic/vaginal fluid, fever prior to delivery, maternal tachycardia, abdominal tender ness, obstructed labor, chorioamnionitis, meconium stained liquor, fetal tachycardia, delivery <37 weeks. [g] Includes 7 IMCI severe illness signs and lethargy, attacks of apnea, 5-minute Apgar <6, and severe jaundice.

Individual newborn clinical features were considered present if documented from any source and absent otherwise. Presence of any newborn clinical feature was classified "yes" if any features were documented from any source, and "no" if all factors were documented as not present *or* were not documented.

Supplementary Table S2. Risk factors for early-neonatal mortality among newborns with signs of severe disease or intrapartum risk factors for infection in Kisii, Kenya (n = 236)

Factor	Cumulative 7-day Mortality (%) (95% CI) [a]	Relative Risk (95% CI) [b]	Risk Difference (95% CI) [b]	*p*-value [c]
Antenatal				
Maternal age (years)				
<20	5.6 (0.7, 18.7)	3.5 (0.6, 20.4)	4.0 (-3.7, 12.0)	0.1793
20 – 35	1.6 (0.3, 4.5)	Ref.	Ref.	
>35	0.0 (0.0, 33.6)	_ _ _	-1.6 (-3.4, 0.2)	1.0000
Mother completed primary education or less	4.8 (1.3, 11.8)	7.2 (0.8, 63.7)	4.1 (-0.6, 8.8)	0.0554
No ANC	5.3 (0.1, 26.0)	2.8 (33.3, 24.1)	3.4 (-6.8, 13.6)	0.3477
Father's HIV status (maternal report)				
Known Negative	1.2 (0.2, 4.4)	Ref.	Ref.	
Known Positive	0.0 (0.0, 45.9) [d]	_ _ _	-1.2 (-2.9, 0.5)	1.0000
Unknown status or decline to state	4.5 (0.9, 12.5)	3.6 (0.6, 21.3)	3.3 (-2.0, 8.5)	0.1494
Mother HIV positive	0.0 (0.0, 36.9) [d]	_ _ _	-2.2 (-4.1, -0.3)	1.0000
Parity- First birth	3.8 (1.1, 9.5)	5.0 (0.6, 44.0)	3.1 (-0.9, 7.0)	0.1745
Intrapartum				
Any intrapartum risk factor for infection [e]	2.7 (0.7, 6.6)	2.3 (0.3, 19.8) [f]	1.5 (-2.0, 4.9) [f]	0.6565
Prolonged rupture of membranes [g h]	0.0 (0.0, 19.5) [d]	_ _ _	-2.4 (-4.4, -0.3)	1.0000
Foul-smelling amniotic/vaginal fluid [g i]	0.0 (0.0, 41.0) [d]	_ _ _	-2.2 (-4.1, -0.3)	1.0000
Maternal fever [g j]	0.0 (0.0, 97.5) [d]	_ _ _	-2.2 (-4.1, -0.3)	1.0000
Maternal tachycardia [g j]	0.0 (0.0, 97.5) [d]	_ _ _	-2.2 (-4.1, -0.3)	1.0000
Uterine tenderness [g m]	0.0 (0.0, 97.5) [d]	_ _ _	-2.2 (-4.0, -0.3)	1.0000
Chorioamnionitis [g l]	0.0 (0.0, 84.2) [d]	_ _ _	-10.4 (-16.5, -4.3)	1.0000
Obstructed labor >18 hrs [g m]	4.6 (0.1, 22.8)	2.4 (0.3, 20.5)	2.6 (-6.2, 11.5)	0.3937
Meconium stained liquor [g j]	5.9 (0.2, 28.7)	3.1 (0.4, 26.4)	4.0 (-7.3, 15.3)	0.3224
Fetal tachycardia [g k]	0.0 (0.0, 45.9) [d]	_ _ _	-2.3 (-4.3 -0.3)	1.0000
Missing documentation of ≥3 intrapartum factors [e]	0.0 (0.0, 60.2) [d]	_ _ _	-2.2 (-4.0, -0.3)	1.0000
C-section (w or w/out vaginal attempt)	1.3 (0.0, 7.2)	0.5 (0.1, 4.7)	-1.2 (-4.7, 2.4)	1.0000
Intrapartum antibiotics [l]	0.0 (0.0, 45.9) [d]	_ _ _	-2.2 (-4.1, -0.3)	1.0000
Maternal malaria RDT+ at enrollment [l]	_ _ _	_ _ _	_ _ _	_ _ _
Newborn/Postnatal				
Male sex	2.8 (0.8, 7.0)	2.6 (0.3, 22.5)	1.7 (-1.7, 5.1)	0.6510
Delivery out of hospital	0.0 (0.0, 52.2) [d]	_ _ _	-2.2 (-4.0, -0.3)	1.0000
Referral or transfer to study facility	4.9 (1.0, 13.7)	4.3 (0.7, 25.2)	3.8 (-1.9, 9.4)	0.1104
Preterm birth (<37 wks) [m]	2.8 (0.6, 7.8)	1.7 (0.3, 9.9)	1.1 (-2.7, 4.9)	0.6680
Birthweight				
Low or very low (< 2,500 g)	4.0 (1.1, 10.0)	5.3 (0.6, 46.6)	3.3 (-0.9, 7.4)	0.1680
Normal (2,500 g – 4,000 g)	0.8 (0.0, 4.2) [d]	Ref.	Ref.	
Macrosomia (> 4,000 g)	0.0 (0.0, 45.9) [d]	_ _ _	-0.8 (-2.3, 0.7)	1.0000
Cord cleaning [n]	1.6 (0.2, 5.6)	_ _ _	1.6 (-0.6, 3.7)	1.0000
Never breastfed [o]	1.2 (0.0, 6.3)	0.4 (0.1, 3.8)	-1.5 (-5.0, 1.9)	0.6549
Newborn antibiotic exposure [o]	2.2 (0.7, 5.0)	_ _ _	2.2 (0.3, 4.0)	1.0000
Positive blood culture w/ definite pathogen	1.7 (0.0, 5.2) [d]	_ _ _	-1.7 (-3.4, -0.1)	1.0000

RR: Relative risk; RD: Risk difference (Absolute difference in cumulative incidence/proportion); ANC: Antenatal care; Ref.: Reference; RDT: Rapid diagnostic test. **Bold** indicates $p < 0.05$

[a] Binomial exact confidence intervals estimated using stratified analyses. [b] RR and RD and 95% confidence intervals estimated from binomial distribution using exact methods. All estimates are crude. [c] 2-sided Fisher's exact test. [d] One-sided 97.5% confidence interval. [e] Includes prolonged rupture of membranes, foul-smelling amniotic/vaginal fluid, fever prior to delivery, maternal tachycardia, abdominal tender ness, obstructed labor, chorioamnionitis, meconium stained liquor, fetal tachycardia, delivery <37 weeks. Includes those with and without any newborn clinical factors. [f] RR and RD calculated compared to reference population with no intrapartum risk factors (regardless of whether they have newborn clinical

factors or not). [g] Those with undocumented status on a specific factor excluded from analysis of that factor. [h] n = 230 [i] n = 233 [j] n = 229 [k] n = 222 [l] n = 236 [m] n = 233 [n] n = 159 [o] n = 235

Assessment of individual intrapartum factors was limited to participants for whom presence *or* absence was documented in records. Presence of any intrapartum factors was classified “yes” if any factors were documented, and “no” if all factors were documented as not present *or* were not documented.

Chapter 3: Newborn Mortality Risk Prediction

Title:

WHO Severe Illness Empiric Algorithms Predict Newborn Mortality in Low-Resource Health Facilities

Authors:

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ABSTRACT

Background: Tools to predict newborn mortality risk in low-resource settings are limited and are often designed specifically for very low birthweight (VLBW) or preterm neonates. We determined whether WHO algorithms for severe illness identification can be used to predict mortality in a broad newborn population and to identify additional features to improve prognostic performance.

Methods: We compared diagnostic performance of the Integrated Management of Childhood Illness (IMCI) and WHO Pocket Book of Hospital Care for Children danger signs, to predict mortality risk in newborns enrolled in a prospective cohort study at two referral-level health facilities in Kenya from April 2015–March 2016. Newborn were ≥1,000g, any gestational age, and presented with risk factors for or signs of severe illness. Predictive performance was assessed using area under the receiver operating characteristic curve (AUC) from logistic regression. Multivariable lasso regression with 10-fold cross-validation was used to identify additional prognostic factors.

Results: Among 378 newborns at 2 hospitals in Kenya, the number of IMCI severe illness and WHO Pocket Book danger signs had good diagnostic performance for predicting mortality [AUC:0.79 (95% CI:0.70-0.89), AUC:0.78 (95% CI:0.68-0.88), respectively]. Lasso identified 4/7 IMCI and 4/10 WHO Hospital Book signs (poor feeding, fast breathing, hyperthermia, only moves when stimulated), plus apnea and low birthweight as predictive, with AUC: 0.89 (95% CI:0.82-0.96) for the combination of these signs.

Conclusions: WHO severe illness algorithms were useful mortality risk prediction tools among high-risk newborns. Additional features provided modest improvement in performance but added complexity.

BACKGROUND

Increasing coverage and quality of specialized care in referral-level health facilities for preterm, low birthweight and sick newborns is a key component of global newborn survival strategies[4]. Coverage of essential newborn care and facility-based childbirth have improved in many settings, but overburdened and under-resourced facilities often lack the equipment, infrastructure and human resources necessary to provide high-quality, specialized care and case management for vulnerable newborns[74]. Consequently, neonatal mortality rates remain high in many settings[75,15,76].

Kenya ranks in the bottom fifth of the world for neonatal mortality rate, at 23 per 1,000 live births[14]. The country has struggled to make progress in neonatal mortality reduction, and among small and sick newborns, case-fatality rates are high[77,11,78]. In resource-constrained health-facilities in settings such as Kenya, triage methods to identify newborns at highest risk of death may prevent delays in the delivery of life-saving treatments and ensure that the most vulnerable receive prompt case management. Risk stratification may be useful to identify a population for whom currently-recommended interventions are insufficient to prevent mortality, among whom new interventions and treatment strategies should be targeted and tested.

Illness severity scores and mortality risk prediction tools are one method for risk stratification, yet few newborn risk prediction tools appropriate for use in low-resource settings are available. Tools developed for well-resourced settings, such as the Score for Neonatal Acute Physiology with Perinatal Extension (SNAPPE, SNAPPE II), and the Critical Risk Index for Babies (CRIB) require vital signs and laboratory measures that are rarely available in low-resource settings[79,80]. Even in settings where scoring criteria may be available, risk tools developed and validated in neonatal intensive care units in well-resourced settings would likely fail to perform well in low-resource settings, where the case-mix, availability and norms in treatment and therapy, and skill level of the staffing and care teams would differ substantially. Additionally, such tools were designed primarily for use in adjusting quality and performance measures to account for case-mix and illness severity at the facility level, rather than as a screening or prognostic tool for individual newborns. A recent systematic review of neonatal mortality risk prediction tools identified only one tool designed specifically for use in low-resource settings, the Simplified-Age-Weight risk score (SAW)[81,82]. This and other existing tools potentially suitable for low-resource settings, including the Critical

Risk Index for Babies-II (CRIB II) and a modified CRIB-II that eliminates base-excess as a criterion[83-86], were developed and tested exclusively in very preterm and/or very low birthweight (VLBW) newborns and would fail to discriminate risk among populations which are not exclusively VLBW or very preterm.

Clinical algorithms are widely used in the community and health facility settings in low-resource settings to identify and classify sick young infants who require urgent treatment, admission, or referral to higher-level facilities for specialized case-management[7,18]. These tools may also be useful in discriminating those at highest risk of mortality. The Integrated Management of Childhood Illness (IMCI) 7-sign criteria for "very severe disease" in young infants was developed and validated to determine the signs and symptoms most sensitive to physician-defined severe illness when evaluated by a minimally-trained provider in the community or primary health setting[13,20]. The WHO Pocket Book of Hospital Care for Children (WHO Pocket Book) 10-feature "danger signs" in young infants consist of the same IMCI signs, as well as 3 additional signs which may be more common in populations at higher-level health facilities, or may require more highly-trained staff to discern[12,13]. To the best of our knowledge, the prognostic capacity of these clinical algorithms for mortality risk prediction have not been tested. Furthermore, these tools were designed to identify severe illness, and may not identify newborns who are at risk of death due to other causes.

We aimed to determine the prognostic performance of existing newborn severe illness algorithms, the IMCI signs of severe illness in young infants <60 days, and WHO Pocket Book danger signs for newborns and young infants, for mortality risk prediction. Additionally, we aimed to improve upon existing algorithms by identifying a set of novel features which were the most important prognostic factors in this population.

METHODS

Data collection

We used data from a prospective cohort study to describe the prevalence of and risk factors for early-onset neonatal sepsis (EOS Study) and mortality in Kenya conducted between April 2015 and March 2016, the methods and results of which are described elsewhere (*Levine et. al., under review*). Briefly, mother-newborn dyads were enrolled within 96 hours of childbirth from Homa Bay County Hospital and Kisii Teaching and Referral Hospital (sub-country referral and county-level teaching and referral hospitals,

respectively) both in Western Kenya. Eligibility criteria required the presence of either at least one intrapartum risk factor for severe newborn infection in the women (prolonged rupture of membranes (>18 hours), foul smelling amniotic fluid or vaginal discharge, fever prior to delivery (≥38.0°C), maternal tachycardia (>100 beats/min), fetal distress (meconium stained liquor, fetal tachycardia), uterine/abdominal tenderness 72 hours prior to delivery, clinical chorioamnionitis, obstructed labor, delivery <37 completed weeks gestation), or newborn clinical presentation consistent with possible severe illness within 72 hours of life (refusal to breastfeed/difficulty feeding, low body temperature (<35.5 °C), fever (≥37.5 °C axial or ≥38.0 °C rectal) , tachypnea/fast breathing (>60 breaths/min when calm), severe chest wall in-drawing, history of convulsions, movement only when stimulated or no movement, five-minute Apgar score ≤6, lethargy, apnea, severe jaundice), *or both* intrapartum risk factors and newborn signs of illness. Newborns needed to be ≥1,000 g at enrollment and no eligibility criterion for gestational age (GA) was required. Newborns were followed for 7 days from enrollment in the facility or post-discharge to ascertain vital status. The dataset included 378 newborns with complete follow-up, among whom 22 deaths occurred [cumulative mortality: 5.8% (95% CI: 3.7, 8.7)].

Ethical approval for the EOS study was provided by the University of Washington Institutional Review Board and the Kenya Medical Research Institute Scientific Ethics and Research Committee.

Ascertainment and definition of variables

We assessed the performance of the 7 IMCI severe illness signs in young infants[13,21], the 10 WHO Pocket Book danger signs in newborns and young infants[12], and explored the predictive ability of additional signs of illness or clinical syndromes in young infants from the WHO Pocket Book, and the Young Infant Clinical Signs and Symptoms study (YICSS)[20]. We also evaluated additional newborn characteristics which are well-known risk factors for mortality and are collected as part of routine care, including birthweight, gestational age (GA) and gender.

Newborn features were ascertained from patient charts and medical records, report of the mother and from physical exam conducted by a trained study nurse at enrollment, as follows. Birthweight and GA were ascertained exclusively from patient charts and medical records. Ultrasound was infrequently available and almost all GA estimates were from date of last menstrual period. Chest in-drawing, fast

breathing, movement only with stimulation/no movement, lethargy, and temperature were ascertained from patient charts and were assessed by study nurses at enrollment physical exam. Rectal temperature was used if available, and axillary temperature used otherwise, with 0.5°C added for standardization. History of convulsions and history of difficulty/poor feeding were based on maternal report. All additional newborn clinical features were ascertained exclusively from enrollment physical exam.

Features were classified based on the thresholds or classifications in the relevant guideline or publication[20,12,13]. High and low temperature are discrete danger signs in both algorithms and were considered separately. Drowsy/unconscious was not available in our dataset but lethargy is often included in the cluster of signs lethargic/drowsy or unconsciousness, and was used as a proxy for drowsy/unconscious. Factors identified as present by any of the possible sources were considered present, and factors not documented by any source, or documented as absent by all available sources were considered absent. Clinical features that originated or were documented after enrollment were not considered.

Discrete variables were derived to indicate the presence of each characteristic and clinical feature; presence of "any" IMCI or "any" WHO Pocket Book danger signs; and classifications by threshold values for the minimum number of signs present (e.g. ≥3 IMCI factors; ≥4 IMCI factors etc.). Continuous variables were derived to indicate the number of signs present from each algorithm (IMCI 0-7, WHO Pocket Book 0-10).

The primary outcome was newborn death within 7 days of enrollment either in-hospital or post-discharge, defined by a binary indicator variable.

Statistical methods

Performance of WHO empiric illness algorithms

Prognostic performance measures including sensitivity, specificity, positive and negative predictive value (PPV), (NPV) were estimated for specific factors and for groups of features within algorithms, for predicting the outcome of death, using non-parametric methods with 95% confidence intervals estimated

from the normal distribution. Logistic regression was used to estimate crude and multivariable-adjusted odds ratios of mortality associated with the presence of specific criterion.

Multivariable logistic regression was used to estimate predicted probabilities of mortality associated with the distribution of features within different clinical algorithms, using maximum likelihood methods under the binormal distribution. Model calibrations were assessed using calibration slope and calibration-in-the-large, and discrimination was assessed using discrimination plots of mean model-predicted probabilities by observed mortality outcome, and with discrimination slope[87]. The overall performance of clinical algorithms and individual factors were evaluated using the area under the receiver operating characteristic curve (AUC), Brier Score and Scaled Brier Score, from multivariable logistic regression models to estimate predicted probabilities of mortality[87]. AUC from 10-fold cross-validation within the full data set was estimated to account for over-optimism.

We evaluated the performance of decision rules to classify risk at various thresholds for the minimum number of severe illness signs or criteria present within each algorithm, and determined "optimal" thresholds defined as the largest AUC, based on the Liu method of maximizing the product of sensitivity and specify calculated at each possible threshold[88].

Model derivation and identification of prognostic features

Using non-parametric methods and logistic regression as described above, we estimated the individual diagnostic performance of additional candidate prognostic factors and crude and adjusted odds ratios for mortality associated with each factor. To identify the set of the most important prognostic factors, candidate predictors where restricted to those with a prevalence of approximately ≥5% in our dataset, to support statistical stability and precision in estimates and prevent overfitting and model convergence problems.

Least absolute shrinkage and selection operator (lasso) regression was used to identify factors which independently contributed to mortality prediction from the set of candidate predictors, in multivariable analysis. Lasso is a regularized and penalized regression method for variable selection and estimation which can help prevent common biases of inflated coefficient estimates and model overfitting in small data sets with few events[87,89,90]. A penalty or regularization term λ is estimated or set to penalize and "shrink"

large coefficients, shrinking the absolute size of coefficients and allowing some coefficients to be "shrunk" to zero. A factor with estimated coefficient of zero is interpreted as not contributing to performance and is automatically dropped from the model, resulting in variable selection from among a large number of possible predictors. The final model with a constrained set of coefficients of smaller absolute value minimizes prediction error and maintains parsimony[91].

Multivariable lasso logistic regression models were fit on dichotomous candidate predictor variables to predict the binary outcome of mortality as a function of the combination of possible predictors, using the *glmnet* package in R. An α of 1 was set to estimate the lasso solution, and the optimal regularization parameter λ was estimated via 10-fold cross-validation from 100 different possible values, to minimize the binomial deviance in the model selected. Regression coefficients for predictor variables were extracted from the optimal model fit, and any factor with a non-zero coefficient estimate was interpreted as contributing to mortality prediction. Calibration, discrimination and overall performance were evaluated as described above.

Comparison of risk prediction tools

We compared the performance of each algorithm (IMCI, WHO Pocket Book, novel prognostic factors) to the reference of the simplest algorithm, defined as a model that considered each of the IMCI signs in combination, and additionally assessed whether the performance of the IMCI and WHO algorithms could be improved by adding any additional important prognostic features to the existing criterion in those algorithms. Chi^2 tests of a difference in the AUC from model-predicted probabilities were used for hypothesis testing, with a p-value of <0.05 accepted for all tests. Analyses were exploratory, and we did not adjust for multiple comparisons.

Stata 14.2 (Stata Corp., College Station, Texas) and R version 3.4.4 (March 15, 2018, the R Foundation for Statistical Computing) were used for statistical analyses.

RESULTS

Summary of population

The prevalence of IMCI severe illness and WHO Pocket Book danger signs, and mortality risk in those with each sign and in groups defined by the number of signs present are summarized in Table 1. The prevalence of individual IMCI severe illness signs ranged from 6.1% for hypothermia to 25.7% for poor feeding. The additional danger signs in the WHO Pocket Book (lethargic/drowsy/unconscious, grunting, cyanosis) were less common: 6.9% had cyanosis and only 2.7% had grunting. Distributions of the number of danger signs with which newborns presented were similar using each of the two algorithms, due to the relative rarity of the additional WHO Pocket Book signs in this population. Using both algorithms, approximately a quarter of the newborns had no danger signs (prevalence of no IMCI signs: 28.6%, prevalence of no WHO Pocket Book danger signs: 27.0%). Approximately a third of the population had 1 danger sign (prevalence 1 IMCI sign: 30.7%; prevalence 1 WHO Pocket book sign: 26.7%) and about a quarter had 2 danger signs (prevalence 2 IMCI signs: 25.7%; prevalence 2 WHO Pocket book signs: 24.1%). Presenting with ≥4 signs of severe illness based on either algorithm was rare. The cumulative incidence of mortality in 7 days ranged from 3.8% in those with cyanosis (though the rarity of this sign limited precision of the estimate), to 22.0% in those with poor movement. Mortality risks were higher in groups with more severe illness signs present (Table 1).

Performance of WHO empiric illness algorithms for mortality prediction

Table 2a summarizes the prognostic performance of individual IMCI and WHO Pocket Book signs. Poor feeding was the most sensitive individual sign at 77.3%. Sensitivities of the other individual IMCI signs ranged from a 9.1% (hypothermia) to 54.5% (fast breathing and chest in-drawing). The additional Pocket Book signs grunting and cyanosis had low sensitivities: 4.5%, but lethargy was moderately sensitive at 36.4%. The specificities of the majority of signs ranged between approximately 80-95%, with the exception of chest in-drawing, which had a lower specificity at 57.9%.

Models that included each of the individual IMCI and WHO Pocket Book danger signs in combination had strong prognostic performance for mortality prediction and performed similarly: the AUC for a model that considered each of the IMCI factors in combination was 0.85 (95% CI: 0.77, 0.94) and for the WHO Pocket Book was 0.85 (95% CI: 0.77, 0.94), *p*-value for difference in AUC: 0.6451 (Figure 1a, Table 3). Calibration-in-the-large and calibration slope from models considering all the signs in combination

for both algorithms indicated strong model calibration (coefficients for slope close to 1.0 and intercepts close to 0.0). Discrimination plots and discrimination slope indicated strong discrimination for both models (Table 3, Supplementary Figure S3).

Figure 2 depicts the AUC for mortality risk prediction based on the number of signs present using each WHO algorithm, agnostic to the specific signs. The slightly higher temperature threshold for fever and three additional danger signs in the WHO Pocket Book did not result in substantially improved prognostic performance of the WHO Pocket Book when compared with the IMCI severe illness signs in prediction models based on a count of the number of signs present. The AUC for a continuous variable indicating the number of IMCI severe illness signs was 0.79 (95 % CI: 0.70, 0.89) and for the WHO Pocket Book was 0.78 (95 % CI: 0.68, 0.88), *p*-value for difference in AUC: 0.3510.

Table 2b presents performance characteristics for risk classification based on different threshold values for the minimum number of signs present using each WHO algorithm. Classification based on the presence of a minimum of any one sign was extremely sensitive for both algorithms, and the sensitivity was the same regardless of the algorithm used: classification based on a threshold of ≥1 IMCI or ≥1 WHO Pocket Book danger sign was 95.5% sensitive. However, a threshold of ≥1 sign was only 30.1% specific for the IMCI and only 28.4% specific for the WHO Pocket Book. There was no difference in overall performance of the algorithms at this threshold [AUC ≥1 IMCI: 0.63 (95 % CI: 0.58, 0.68); AUC ≥1 WHO Pocket Book: 0.62 (95 % CI: 0.57, 0.67); p-value for difference: 0.0569]. A minimum threshold of ≥1 sign in either algorithm performed well for ruling out mortality risk: approximately 99% of those classified as low-risk based on the absence of any IMCI or WHO Pocket Book signs would be expected to survive in settings with similar mortality risk [NPV ≥1 IMCI: 99.1% (95% CI: 94.9, 100.0); NPV ≥1 WHO Pocket Book: 99.0% (95% CI: 94.7, 100.0)]. However, less than 8% of those classified as high-risk based on the presence of any IMCI or WHO Pocket Book signs would be expected to die in similar mortality risk settings [PPV ≥1 IMCI: 7.8% (95% CI: 4.9, 11.6); PPV ≥1 WHO Pocket Book: 7.6% (95% CI: 4.8, 11.4)].

In both algorithms, a threshold of ≥2 signs was the best prognostic threshold for classification based on the number of signs present, when defined by maximization of AUC, at 0.72 (95% CI: 0.63, 0.80) for the IMCI and 0.71 (95% CI: 0.63, 0.79) for the WHO Pocket Book danger signs, and performed statistically

significantly better than requiring a minimum of ≥1 sign in either algorithm (*p*-value difference in AUC ≥1 vs. ≥2 IMCI: 0.0207; *p*-value for difference in AUC ≥1 vs. ≥2 WHO Pocket Book: 0.0053). However, determination of an optimal threshold depends on the relative prioritization of the sensitivity and specificity of a tool for the intended application. Although ≥2 signs was more specific than ≥1 (specificity ≥2 IMCI: 61.8%; specificity ≥2 WHO Pocket Book: 56.2%), the sensitivity was lower at this threshold; 81.8% for IMCI and 86.4% for the WHO Pocket Book; approximately 15-20% of newborns who died would have been missed using a threshold ≥2 signs in similar mortality settings. The performance of the two algorithms at a threshold of ≥2 signs was not statistically significantly different (*p*-value for difference in AUC ≥2 IMCI vs. ≥2 WHO Pocket Book: 0.8198).

Additional signs of severe illness

Many of the additional candidate predictors were rare in the study population; most factors had a prevalence less than 5% (Supplemental Table S1). Factors that were eligibility criteria in the EOS study (low Apgar score, apnea, lethargy and severe jaundice) were slightly more common. Diagnostic performance for each individual candidate predictor is provided in Supplemental Table S1. Due to low prevalence, point estimates for indicators of performance for many factors are relatively unstable and lack precision.

Derivation and performance of novel risk tool

Each of the 7 IMCI signs of severe illness, all of the WHO Pocket Book danger signs except grunting (prevalence <5%), jaundice, 5-minute Apgar ≤6, apnea, restlessness/irritability, bleeding diathesis, nasal flaring, preterm birth, low birthweight, and male sex had prevalence ≥5% in this dataset and were included in multivariable prediction models as candidate prognostic factors. Due to collinearity with fever based on IMCI criteria, a variable indicating the slightly higher temperature threshold in the WHO Pocket Book was not included. Odds ratios indicating the strength of association with mortality for candidate prognostic factors included in multivariable prediction models are provided in Supplementary Table S2.

Multivariable lasso logistic regression selected poor feeding, fast breathing, temperature ≥38, only moves when stimulated/no movement, apnea, and low birthweight as predictive of mortality. The in-sample

AUC for the predicted probabilities from the final fitted lasso logistic model that included the combination of these factors in the full data ("novel tool") was 0.89 (95% CI: 0.82, 0.96) (Figure 2b.). Calibration-in-the-large and calibration slope indicated strong calibration. Discrimination plots and discrimination slope indicated strong discrimination (Table 3, Supplementary Figure S1).

Comparison of performance of risk prediction algorithms

Estimates of the AUC from 10-fold cross-validation were slightly lower for all multivariable models than the estimates from model development in the full data set, due to over-optimism, but still indicated modest prognostic ability for all algorithms (Table 3).

Models that included the combination of features for the WHO Pocket Book and the novel tool performed similarly with the simpler set of the combination of the 7 IMCI features [*p*-value for difference in AUC IMCI vs. WHO Pocket Book: 0.6451; *p*-value difference in AUC IMCI vs. novel tool: 0.1875], when maximization of the AUC was used to define performance. Models with the additional two features identified in the novel tool (apnea and low birthweight) added to the original IMCI and WHO Pocket Book algorithms failed to perform statistically significantly better than the simple model with the combination of the 7 IMCI signs (*p*-value difference in AUC IMCI vs. IMCI + apnea and low birthweight: 0.1126; *p*-value difference in AUC IMCI vs. WHO Pocketbook + apnea and low birthweight: 0.2036 (Table 3, Figure 2a, 2b).

DISCUSSION

We evaluated whether empiric algorithms for newborn severe illness identification and classification in community and primary health facilities in low-resource settings could be used to discriminate newborns at highest risk of death in referral facilities. We found that the 7-sign IMCI criteria for very severe illness in young infants performed well in predicting mortality among a broad population of newborns in higher-level facilities who were not VLBW but had intrapartum risk factors for or clinical signs of illness. The presence of ≥1 IMCI sign had extremely high sensitivity in discriminating newborns who ultimately experienced treatment failure or succumbed to illness, despite receiving clinical care, but lacked specificity. A threshold of ≥2 IMCI signs had the best overall performance for risk classification based on a

minimum threshold number of IMCI signs and was more specific than classification based on one sign but was less sensitive.

We used modern machine learning methods to identify factors most prognostic for mortality within sparse data and determined that 4 of 7 IMCI signs and 4 of 10 WHO Pocketbook danger signs were among the most prognostic features for newborn mortality risk (difficulty feeding, fast breathing, temperature ≥38 degrees, only moves when stimulated/no movement). Additionally, low birthweight and apnea were strong prognostic features. This novel set of criteria performed well in predicting mortality risk, although the improvement in prognostic capacity over and above that provided by the use of only the simple 7-sign IMCI algorithm was modest.

Two additional important prognostic factors for mortality risk not already included as danger signs in the two existing algorithms were identified: apnea and low birthweight. Although the novel set of criteria performed well in risk prediction, assessment of additional features not currently part of well-established algorithms could introduce complexity with limited benefit. However, birthweight is routinely documented as part of clinical practice in postnatal and newborn units and should routinely be available to clinicians. Our study indicates that consideration of birthweight in parallel with assessment of clinical features of severe illness may provide additional prognostic benefit in mortality prediction. Apnea is a sign of all three of the primary causes of newborn mortality: complications of severe intrapartum events/birth asphyxia including hypoxic-ischemic encephalopathy, severe systematic infection, and complications of prematurity, but was not assessed as a possible predictive factor for severe illness in the YICSS study which informed the criteria included in the IMCI guidelines, potentially because of the challenges in detection by minimally trained providers. It is included in the WHO Pocket Book as a sign of multiple syndromes in young infants, however, and would be assessed as part of more comprehensive diagnostic exams after triage.

The relative trade-off between the sensitivity and specificity of a risk classification tool depends on the population in which the tool is applied and the objective of the end-user. In a population where the prevalence of signs of illness is common, less-than-perfect specificity will result in classification of a large absolute number of newborns as being high-risk who would in fact survive, which may provide only marginal benefit in differentiating newborns who may be the most acutely ill and most urgently require vigilant care

and specialized treatment. The baseline mortality risk and prevalence of clinical risk factors in the population will have substantial impact on the PPV and NPV of any prognostic tool, and the benefit offered by the tool's application. In a population of newborns in which the baseline mortality risk is 5%, using a threshold of ≥1 IMCI or Pocket Book sign would capture the vast majority of newborns who would be expected to die, but few of those classified as "high-risk" would actually be expected to die. A conservative classification scheme which focuses on maximizing sensitivity may not help alleviate the burden to the health system or health facility in a setting where the prevalence of clinical signs of illness is even higher, such as an intensive care unit in a national hospital. A classification aimed at maximizing sensitivity may thus fail to rule out the need for vigilant care or treatment among enough newborns to improve system efficiency or inform resource-allocation. Conversely, in a population with higher overall mortality risk, a larger number of newborns who ultimately succumbed would be "missed" by a less sensitive tool and the probability that a newborn classified as lower risk would survive would be lower.

We explored methods to identify an extremely high-risk population for whom recommended treatments and services may be insufficient to prevent death- among whom new treatment strategies are needed. The majority of the newborns in the study population were already admitted to referral-level newborn care units, and the vast majority of those with signs of probable severe illness had been prescribed the antibiotic regimens based on the WHO IMCI guidelines for care (*Levine et al., 2018 under review*), yet mortality risk was high in the study population. Future studies to test interventions to prevent death in similar identified populations of high-risk newborns are needed.

This study has some notable strengths. We used empiric illness algorithms which are widely adopted and appropriate for health workers with minimal training in low-resource settings and demonstrated that such tools can be used for mortality risk prediction in higher-level health facilities. To the best of our knowledge, our study is the first to develop a mortality risk prediction tool for high-risk newborns in low-resource settings who are not exclusively VLBW or extremely preterm. The Our heterogeneous study population of newborns with risk factors for or signs of clinical illness, who are not exclusively low birthweight or preterm, but among whom low birthweight and preterm were common, is likely broadly representative of newborn populations in postnatal and newborn units in referral-level facilities. Our study also has a number of limitations. The small number of events led to instability in estimates and limited the ability to evaluate

the predictive performance of candidate clinical factors which were rare and may have resulted in unstable or inflated estimates of the effect of rare features when evaluated in multivariable models. Limiting candidate factors to those with prevalence >5% and the use of penalization and shrinkage methods in lasso regression likely prevented some biases from exaggerated coefficient magnitude and model overfitting. We were unable to split our dataset for derivation, testing and validation groups, and lacked a dataset for external validation, thus our estimates of performance are likely overly optimistic. However, the use of cross-validation helped moderate bias due to over-optimism. Future studies to validate these tools in larger external populations are required. EOS study participants were purposively sampled based on a set of clinical characteristics and risk factors, and distribution of the clinical characteristics in the study population are not necessarily a representation of the expected distribution in an unselected newborn population. However, this population is likely representative of newborn units in similar low-resource health facilities; populations among whom the application of similar tools would be recommended.

CONCLUSIONS

WHO clinical algorithms for severe illness detection and classification can be used to identify newborns at highest mortality risk in referral-level health facilities in low-resource settings. Interventions targeting an identified population of critically ill newborns at highest risk of treatment failure and death in referral-level health facility settings are urgently needed.

TABLES AND FIGURES

Table 1. Distribution IMCI severe illness and WHO Pocket Book danger signs and mortality in high-risk newborns (Kisii and Homa Bay, Kenya) (n=378)

	Prev. (%)	Died/n	Cumulative Mortality (95% CI) [a]
IMCI severe illness signs			
Poor feeding	25.7	17/97	17.5 (10.6, 26.6)
Fast breathing	22.2	12/84	14.3 (76, 23.6)
Chest indrawing	42.9	12/162	7.4 (3.9, 12.6)
Hyperthermia (≥37.5 axial/≥38 rectal)	8.7	6/33	18.2 (7.0, 35.5)
Hypothermia (<35.5)	6.1	2/23	8.7 (1.1, 28.0)
Only moves when stimulated/no movement	10.9	9/41	22.0 (10.6, 37.6)
Convulsions	19.3	7/73	9.6 (3.9, 18.8)
Number IMCI severe illness signs			
0 signs	28.6	3/108	0.9 (0.0, 5.1)
1 signs	30.7	3/116	2.6 (0.5, 7.4)
2 signs	25.7	6/97	6.2 (2.3, 13.0)
3 signs	9.8	6/37	16.2 (6.2, 32.0)
4 signs	2.9	1/11	9.1 (0.3, 41.3)
5 signs	1.9	3/7	42.9 (9.9, 81.6)
6 signs	0.3	1/1	100.0 (2.5, 100.0) [b]
7 signs	0.3	1/1	100.0 (2.5, 100.0) [b]
WHO Pocket Book of Hospital Care danger signs [c]			
Hyperthermia (>38)	7.9	6/30	20.0 (7.7, 38.6)
Grunting	2.7	1/10	10.0 (0.3, 44.5) b
Cyanosis	6.9	1/26	3.8 (0.0, 19.6)
Drowsy/unconscious/lethargic [d]	17.2	8/65	12.3 (5.5, 22.8)
Number WHO Pocket Book danger signs			
0 signs	27.0	1/102	1.0 (0.0, 5.3)
1 signs	26.7	2/101	2.0 (0.2, 7.0)
2 signs	24.1	6/91	6.6 (2.5 ,13.8)
3 signs	12.2	5/46	10.9 (3.6, 23.6)
4 signs	5.0	2/19	10.5 (1.3, 33.1)
5 signs	1.6	2/6	33.3 (4.3, 77.7)
6 signs	2.4	2/9	22.2 (2.8, 60.0)
7 signs	0.5	0/2	0.0 (0.0, 84.2) [b]
8 signs	0.5	2/2	100.0 (15.8, 1.0) [b]

Prev.: Prevalence

[a] Estimated 7-day cumulative incidence from stratified analyses using binomial exact distribution

[b] One-sided, 97.5% confidence interval

[c] WHO Pocket Book danger signs also include the IMCI signs poor feeding/history of difficulty feeding, fast breathing, chest indrawing, hypothermia, only moves when stimulated/no movement, history of convulsions

[d] Lethargy used as proxy for drowsy/unconscious

0-7 possible IMCI danger signs. 0-10 possible WHO Pocket Book Danger Signs. Maximum number of WHO Pocket Book signs present in this population was 8.

Table 2a. Performance of individual WHO severe illness [a,b] criterion in predicting mortality among high-risk newborns (Kisii and Homa Bay, Kenya) (n = 378)

Factor	Sens. (%) (95 % CI)	Spec. (%) (95 % CI)	PPV (%) (95 % CI)	NPV (%) (95 % CI)	OR (95 % CI) [c]	p-value
IMCI [a]						
Poor feeding	77.3 (54.6, 92.2)	77.5 (72.8, 81.8)	17.5 (10.6, 26.6)	98.2 (95.9, 99.4)	11.7 (4.2, 32.8)	<0.0001
Fast breathing	54.5 (32.2, 75.6)	79.8 (75.2, 83.8)	14.3 (7.6, 23.6)	96.6 (93.8, 98.4)	4.7 (2.0, 11.4)	0.0005
Chest indrawing	54.5 (32.2, 75.6)	57.9 (52.5, 63.1)	7.4 (3.9, 12.6)	95.4 (91.7, 97.8)	1.6 (0.7, 3.9)	0.2578
Hyperthermia [d]	27.3 (10.7, 50.2)	92.4 (89.2, 94.9)	18.2 (7.0, 35.5)	95.4 (92.6, 97.3)	4.6 (1.6, 12.7)	0.0037
Hypothermia (<35.5)	9.1 (1.1, 29.2)	94.1 (91.1, 96.3)	8.7 (1.1, 28.0)	94.4 (91.4, 96.5)	2.0 (0.4, 9.1)	0.3934
Only moves when stimulated	40.9 (20.7, 63.6)	91.0 (87.5, 93.8)	22.2 (10.6, 37.6)	96.1 (93.5, 97.9)	7.0 (2.8, 17.7)	<0.0001
Convulsions	31.8 (13.9, 54.9)	81.5 (77.0, 85.4)	9.6 (3.9, 18.8)	95.1 (92.0, 97.2)	2.1 (0.8, 5.2)	0.1327
WHO Pocketbook Danger Signs [b]						
Hyperthermia [d]	27.3 (10.7, 50.2)	93.3 (90.1, 95.6)	20.2 (7.7, 38.6)	95.4 (92.6, 97.3)	5.1 (1.8, 14.4)	0.0019
Grunting	4.5 (0.1, 22.8)	97.5 (95.3, 98.8)	10.0 (0.3, 44.5)	94.3 (91.4, 96.4)	1.8 (0.2, 15.2)	0.5729
Cyanosis	4.5 (0.1, 22.8)	93.0 (89.8, 95.4)	3.8 (0.1, 19.6)	94.0 (91.0, 96.3)	0.6 (0.1, 4.9)	0.6587
Drowsy/unconscious/lethargic [e]	36.4 (17.2, 59.3)	84.0 (79.8, 87.6)	12.3 (5.5, 22.8)	95.5 (92.6, 97.5)	3.0 (1.2, 7.5)	0.0185

Table 2b. Performance of the number of WHO severe illness [a, b] criterion present in predicting early neonatal mortality among high-risk newborns (Kisii and Homa Bay, Kenya) (n=378)

Threshold number of signs	Sens. (%) (95 % CI)	Spec. (%) (95 % CI)	PPV (%) (95 % CI)	NPV (%) (95 % CI)	AUC [f] (95 % CI)
IMCI [a]					
≥1 signs	95.5 (77.2, 99.9)	30.1 (25.3, 35.1)	7.8 (4.9, 11.6)	99.1 (94.9, 100.0)	0.63 (0.58, 0.68)
≥2 signs [g]	81.8 (59.7, 94.8)	61.8 (56.5, 66.9)	11.7 (7.1, 17.8)	98.2 (95.5, 99.5)	0.72 (0.63, 0.80)
≥3 signs	54.5 (32.2, 75.6)	87.4 (83.5, 90.6)	21.2 (11.4, 33.9)	96.9 (94.3, 98.5)	0.71 (0.60, 0.82)
≥4 signs	27.3 (10.7, 50.2)	96.1 (93.5, 97.8)	30.0 (11.9, 54.3)	95.5 (92.8, 97.4)	0.62 (0.52, 0.71)
≥5 signs	22.7 (7.8, 45.4)	98.9 (97.1, 99.7)	55.6 (21.2, 86.3)	95.4 (92.7, 97.3)	0.61 (0.52, 0.70)
WHO Pocketbook Danger Signs [b]					
≥1 signs	95.5 (77.2, 99.9)	28.4 (23.7, 33.4)	7.6 (4.8, 11.4)	99.0 (94.7, 100.0)	0.62 (0.57, 0.67)
≥2 signs [g]	86.4 (65.1, 97.1)	56.2 (50.9, 61.4)	10.9 (6.7, 16.4)	98.5 (95.7, 99.7)	0.71 (0.63, 0.79)
≥3 signs	59.1 (36.4, 79.3)	80.1 (75.5, 84.1)	15.5 (8.5, 25.0)	96.9 (94.3, 98.6)	0.70 (0.59, 0.80)
≥4 signs	36.4 (17.2, 59.3)	91.6 (88.2, 94.2)	21.1 (9.6, 37.3)	95.9 (93.2, 97.7)	0.64 (0.54, 0.74)
≥5 signs	27.3 (10.7, 50.2)	96.3 (93.8, 98.0)	31.6 (12.6, 56.6)	95.5 (92.9, 97.4)	0.62 (0.52, 0.71)

Sens.: sensitivity; Spec.: specificity; PPV: positive predictive value; NPV: negative predictive value; AUC: Area under the Receiver Operator Characteristic (ROC) curve

[a] IMCI signs: Poor feeding, fast breathing, chest indrawing, hyperthermia, hypothermia, only moves when stimulated/no movement, convulsions

[b] WHO Pocket Book danger sings include the IMCI signs poor feeding, fast breathing, chest indrawing, hyperthermia (>38), hypothermia, only moves when stimulated/no movement, grunting, cyanosis, drowsy/unconscious.
[C] Odds ratios and 95 % CI from crude logistic regression using maximum likelihood methods. p-value is chi^2 square test that beta coefficient = 0 vs. doesn't =I 0
[d] Hyperthermia classified by IMCI as (≥38) and by WHO Pocket book as (>38)
[e] Lethargy used as proxy for drowsy/unconscious
[f] AUC estimated from logistic regression using maximum likelihood methods
[g] "Optimal" cut-point ≥ 2 signs from empirical estimate with optimal threshold determined by Liu method to maximize product of sensitivity and specificity without adjustment, asymptotic normal confidence interval.
0-7 possible IMCI danger signs. 0-10 possible WHO Pocket Book Danger Signs. Maximum number of Pocket Book signs present in this population was 8.

Table 3. Performance of prognostic tools for newborn mortality risk prediction[a]

	Prediction algorithm				
Performance characteristic	IMCI signs [b]	WHO Pocket Book danger signs [c]	Novel tool [d]	IMCI + apnea and low birthweight [e]	WHO Pocket Book + apnea, low birthweight [f]
AIC	142.27	145.12	128.50	134.60	138.59
Log-likelihood	-63.14	-61.56	-57.25	-57.30	-56.29
Calibration					
Calibration-in-the-large	0.00	-0.01	0.00	0.00	0.00
Calibration slope	1.08	1.09	1.00	1.01	1.05
Discrimination					
Discrimination slope	0.18	0.21	0.24	0.24	0.25
AUC (95 % CI)	0.85 (0.77, 0.94)	0.84 (0.73, 0.95)	0.89 (0.82, 0.96)	0.90 (0.84, 0.96)	0.90 (0.82, 0.97)
AUC 10-fold CV	0.76	0.80	0.85	0.82	0.83
Overall performance					
Brier Score	0.0448	0.0429	0.0419	0.0424	0.0413
Scaled Brier	0.1827	0.2173	0.2356	0.2082	0.2465

AUC: Area under the Receiver Operator Characteristic (ROC) curve; MSE: Mean-squared error; CV: cross-validation

[a] Performance characteristics estimated using un-penalized, multivariable logistic regression models that include the combination of each of the signs/factors.

[b] IMCI signs model includes: Poor feeding, fast breathing, chest indrawing, hyperthermia (≥38), hypothermia (<35.5), only moves when stimulated/no movement, convulsions

[c] WHO Pocket Book signs model includes: Poor feeding, fast breathing, chest indrawing, hyperthermia (>38), hypothermia (<35.5), only moves when stimulated/no movement, convulsions, grunting, cyanosis, lethargy/drowsy/unconscious

[d] Novel tool from optimal penalized logistic lasso model includes: Poor feeding, fast breathing, hyperthermia (≥38), only moves when stimulated/no movement, apnea, low birthweight (<2500 g)

[e] Includes 7 IMCI signs plus two additional features in novel tool: apnea and low birthweight

[f] Includes 10 WHO Pocket Book danger signs plus two additional features in novel tool: apnea and low birthweight

Figure 1a. Performance of WHO empiric illness algorithms in mortality risk prediction among high-risk newborns in Homa Bay and Kisii, Kenya (n=378)

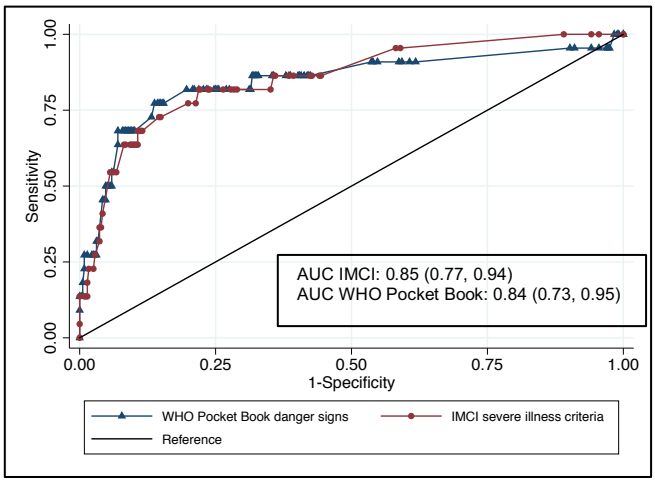
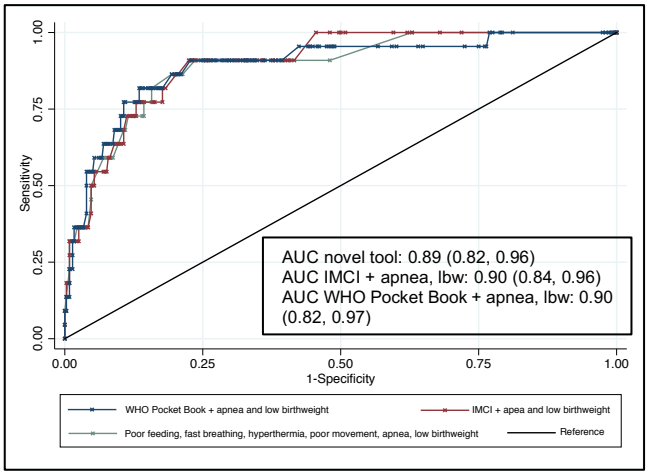
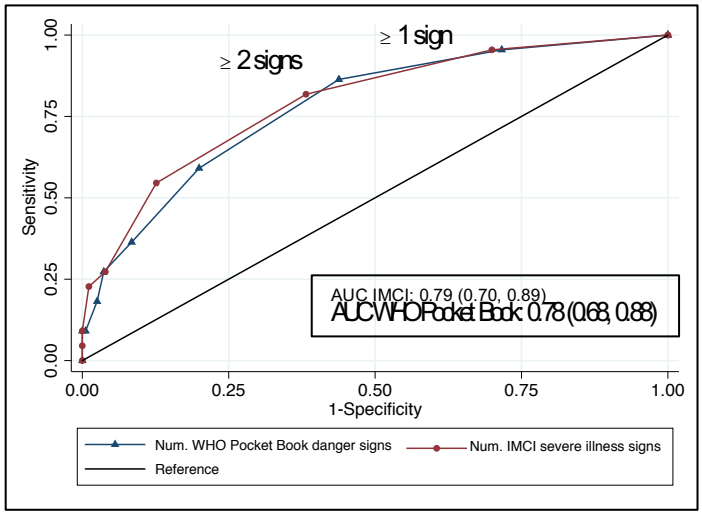


Figure 1b. Performance of novel newborn mortality risk tool alone and in combination with WHO empiric illness algorithms, among high-risk newborns in Homa Bay and Kisii, Kenya (n=378)



AUC: Area under the Receiver Operator Characteristic (ROC) curve; lbw: low birthweight (<2500 g)
IMCI signs of severe illness: Poor feeding, fast breathing, chest indrawing, hyperthermia ≥38, hypothermia, only moves when stimulated/no movement, convulsions
WHO Pocket book danger signs: Poor feeding, fast breathing, chest indrawing, hyperthermia >38, hypothermia, only moves when stimulated/no movement, convulsions, grunting, cyanosis, drowsy/unconscious (lethargy)
Novel tool includes: Poor feeding, fast breathing, hyperthermia ≥38, only moves when stimulated/no movement, apnea, low birthweight (<2500 g)
AUC for combination of signs in each algorithm, estimated from model-predicted probabilities from multivariable logistic regression
p-values for Wald-type chi^2 test for difference in AUC using trapezoidal distribution rule: IMCI vs. WHO Pocket book: p = 0.6451; IMCI vs. Novel tool: *p* = 0.1875; IMCI vs. IMCI + apnea, lbw: p = 0.1126; IMCI vs. WHO + apnea, lbw: p = 0.2036

Figure 2. Performance of the number of signs from WHO empiric illness algorithms for mortality risk prediction among high-risk newborns in Kisii and Homa Bay, Kenya (n=378)



AUC: Area under the receiver operating characteristic curve (ROC)
AUC estimated from model-predicted probabilities from logistic regression
Logistic model with linear variable indicating a count of the number of signs present within each algorithm
IMCI signs include: Poor feeding, fast breathing, chest indrawing, hyperthermia ≥38, hypothermia, only moves when stimulated/no movement, convulsions (0-7 signs possible)
WHO Pocket book danger signs include: Poor feeding, fast breathing, chest indrawing, hyperthermia >38, hypothermia, only moves when stimulated/no movement, convulsions, grunting, cyanosis, drowsy/unconscious (lethargy) (0-10 signs possible)
p-value for Wald-type chi^2 test for difference in AUC using trapezoidal distribution rule: Count of number of IMCI signs vs. count of number of WHO Pocket Book signs p = 0.3510
IMCI Model log-likelihood: -68.45; AIC:140.90
WHO Pocket Book Model log-likelihood: -71.91; AIC: 147.81

SUPPLEMENTARY MATERIAL

Table S1. Performance of additional candidate factors in predicting early neonatal mortality among high-risk newborns in Homa Bay and Kisii, Kenya (n=378)

Factor	Died/ Total with factor	Prevalence	Sensitivity (%)	Specificity (%)	OR (95 % CI) [a]	p-value
5-Min Apgar ≤6 [b]	10/129	**36.1**	47.6 (25.7, 70.2)	64.6 (59.2, 69.7)	1.7 (0.7, 4.0)	0.2630
Apnea	12/53	**14.0**	54.5 (32.2, 75.6)	88.5 (84.7, 91.6)	9.2 (3.7, 22.7)	<0.0001
Jaundice	2/18	**4.8**	9.1 (1.1, 29.2)	95.5 (92.8, 97.4)	2.1 (0.5, 9.9)	0.3366
Capillary refill >3 sec	0/0	0.0	--	--	--	--
Lack of posture in supine	1/14	3.7	4.6 (0.1, 22.8)	96.4 (93.8, 98.0)	1.0 (0.9, 1.1)	0.9367
Restlessness/irritability	1/23	**6.1**	4.6 (0.1, 22.8)	93.8 (90.8, 96.1)	0.7 (0.1, 5.6)	0.7567
High-pitched cry	4/15	4.0	18.2 (5.2, 40.3)	96.9 (94.5, 98.4)	7.0 (2.0, 24.1)	0.0021
Many or severe skin pustules	0/2	0.5	0.0 (0.0, 15.4)	99.4 (98.0, 99.9)	--	--
Palms and soles of feet have red rash, gray patches, blisters or skin peeling	0/10	2.7	0.0 (0.0, 15.4)	97.2 (94.9, 98.6)	--	--
Bleeding diathesis	2/23	**6.1**	9.1 (1.1, 29.2)	94.1 (91.1, 96.3)	1.6 (0.3, 7.3)	0.5468
Anterior fontanelle bulging	0/7	1.9	0.0 (0.0, 15.4)	98.0 (95.9, 99.2)	--	--
Anterior fontanelle sunken	0/4	1.1	0.0 (0.0, 15.4)	98.8 (97.1, 99.7)	--	--
Discharge from ears	0/0	0.0	--	--	--	--
Stiff neck	1/5	1.3	4.6 (0,1, 22.8)	98.9 (97.1, 99.7)	4.2 (0.4, 39.2)	0.2090
Nasal flaring	3/45	**11.9**	13.6 (2.9, 34.9)	88.2 (84.4, 91.4)	1.2 (.3, 4.2)	0.7963
Stridor	0/0	0.0	--	--	--	--
Heart murmur on auscultation	1/3	0.8	4.6 (0.1, 22.8)	99.4 (98.0, 99.9)	8.4 (0.7, 96.7)	0.0869
Abdominal distention	1/2	0.5	4.6 (0.1, 22.8)	99.7 (98.4, 100.0)	16.9 (1.0, 279.8)	0.0483
Umbilicus red or draining pus	0/2	0.5	0.0	99.4 (98.0, 99.9)	--	--
Stiff limbs	1/2	0.5	4.6 (0.1, 22.8)	99.7 (98.4, 100.0)	16.9 (1.0, 279.8)	0.0483
Painful joints, swelling, irritability on movement or touch	1/3	0.8	4.6 (0.1, 22.8)	99.4 (98.0, 99.9)	8.4 (0.7, 96.7)	0.0869
Vomits everything	1/2	0.5	4.6 (0.1, 22.8)	99.7 (98.4, 100.0)	16.9 (1.0, 279.8)	0.0483
Bile stained vomit or fluid up the nasal-gastric tube	1/1	0.3	4.6 (0.1, 22.8)	100.0 (99.0, 100.0)	--	--
Diarrhea	0/0	0.0	--	--	--	--
Blood in stool	0/1	0.3	0.0 (0.0, 15.4)	99.7 (98.4, 100.0)	--	--
Sunken eyes	0/4	1.1	0.0 (0.0, 15.4)	98.9 (97.1, 99.7)	--	--
Slow skin pinch of abdomen	0/4	1.1	0.0 (0.0, 15.4)	98.9 (97.1, 99.7)	--	--
Reduced skin turgor	0/6	1.6	0.0 (0.0, 15.4)	98.3 (96.4, 99.4)	--	--
Preterm birth (< 37 completed weeks gestation) [c]	11/145	**40.3**	50.0 (28.2, 71.8)	60.4 (54.9, 65.6)	1.5 (0.6, 3.6)	0.3402
Low birthweight (<2500 g)	13/141	**37.3**	59.1 (36.4, 79.3)	64.0 (58.8, 69.0)	2.6 (1.1, 6.2)	0.0347
Male sex	15/222	**58.7**	68.2 (45.1, 86.1)	41.9 (36.7, 47.2)	1.5 (0.6, 3.9)	0.3567

Sensitivity, specificity, estimated using non-parametric methods from the exact binomial distribution.

[a] Crude OR and 95% CI estimated from logistic regression using maximum likelihood methods

[b] 357 newborns with 5-Min Apgar score available; [c] 360 newborns for whom gestational age was available

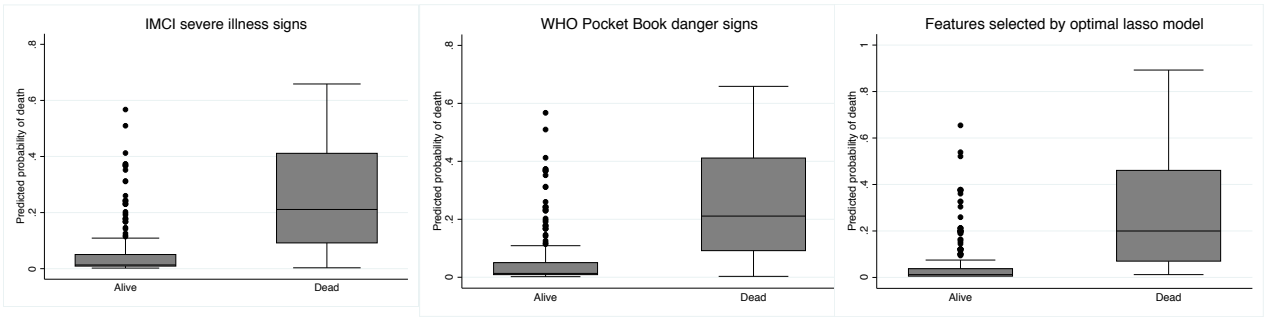
Bold indicates prevalence ≥5% in study population; factor included in multivariable models to identify novel prognostic factors

Table S2. Independent predictors of early neonatal mortality among high-risk newborns in Kisii and Homa Bay, Kenya (n=378)

Factor	OR (95% CI) [a]
IMCI	
Poor feeding	4.9 (1.5, 16.7)
Fast breathing	2.4 (0.8, 7.8)
Chest indrawing	1.3 (0.4, 4.0)
Hyperthermia (≥38)	3.1 (0.7, 14.6)
Hypothermia (<35.5)	0.8 (0.1, 6.0)
Only moves when stimulated/no movement	5.2 (1.3, 20.8)
Convulsions	1.3 (0.3, 6.1)
WHO Pocket Book [b,c]	
Cyanosis	0.9 (0.2, 5.0)
Drowsy/unconscious/lethargic [d]	0.8 (0.2, 3.2)
Additional candidate predictors	
5-Min Apgar ≤6*	1.4 (0.5, 4.6)
Apnea	4.3 (1.4, 13.3)
Severe jaundice	1.9 (0.3, 13.5)
Restless/irritable	0.3 (0.0, 5.1)
Nasal flaring	0.3 (0.0, 3.8)
Bleeding diathesis	3.8 (0.5, 30.8)
Preterm (< 37 completed weeks)	1.1 (0.2, 6.5)
Low Birthweight (<2500 g)	2.7 (0.4, 16.9)
Male sex	1.6 (0.5, 5.1)

[a] Estimates from a multivariable logistic regression model using maximum likelihood methods, from a model including all candidate predictors with prevalence >5% in our data (all candidate variables listed)
[b] WHO Pocket Book indication for fever (>38) not included due to collinearity with IMCI fever (≥38)
[c] Grunting not included due to prevalence <5%
[d] Lethargy used as a proxy for drowsy/unconscious, which was not available in the dataset

Figure S1. Discrimination of IMCI, WHO Pocket Book, and novel risk prediction algorithm for newborn mortality risk prediction in Homa Bay and Kisii, Kenya (n=378)



Plots of mean model-predicted probabilities of death in those observed to die and those who survived. Estimates from models including the combination of features in each algorithm.
IMCI signs include: Poor feeding, fast breathing, chest indrawing, hyperthermia ≥38, hypothermia, only moves when stimulated/no movement, convulsions. WHO Pocket Book danger signs include: Poor feeding, fast breathing, chest indrawing, hyperthermia >38, hypothermia, only moves when stimulated/no movement, convulsions, grunting, cyanosis, drowsy/unconscious (lethargy).
Novel risk prediction tool includes features selected by optimal lasso logistic regression model: Poor feeding, fast breathing, hyperthermia ≥38, only moves when stimulated/no movement, apnea, low birthweight (<2500 g).
Discrimination slope IMCI: 0.18; WHO Pocket Book: 0.21; Novel tool from optimal lasso model: 0.24

Discussion

This dissertation has elucidated key gaps and key opportunities for addressing neonatal mortality in high-burden settings.

In Chapter 1 we found the prevalence of bacteremia as identified by bacterial blood culture was lower than expected among newborns with suspected infection or at high risk of infection; 1.6% for definite/likely bacteremia and 5.5% for possible bacteremia. Other studies in south Asia and sub-Saharan Africa have identified higher prevalence of bacterial isolates using culture in newborns with suspected infection, although eligibility and case definitions, isolate classification methods, age distributions, hospital and community status limit comparability[9,11,22,78,92,93]. Approximately two-thirds of the newborns had clinical signs indicating possible severe infection, but our population also included newborns with other possible neonatal syndromes and those with risk factors for, but no clinical signs of illness. It's possible that blood stream infection was in fact rare in this population, but it's also likely that bacterial culture was an insensitive tool for confirmatory diagnosis and misclassified some newborns who did in fact have bacteremia. More sensitive diagnostic methods for confirmation or to rule out blood stream infection which are feasible in low resource settings are needed to help inform clinical management.

Although bacteremia was rare, in Chapter 2 we report that mortality within 7 days was high in this population of newborns with suspected or at high risk of infection: 5.8% (95% CI: 3.7%-8.6%, n=22) overall, 12.0% (95% CI: 7.1%-18.5%) at the Homa Bay site and 2.1% (95% CI: 0.7%-4.9%) at the Kisii site. Intriguingly, no newborns with confirmed bacteremia died. These results further suggest the limitations of blood culture in informing clinical management among those with suspected infection, but also suggest the need to better understand the distribution and causes of death in newborn units in different settings, to ensure newborns receive adequate and appropriate treatment. The signs of pSBI are non-specific and discrimination between different syndromes remains challenging in young infants[11]. Robust data on causes of death in neonates in low-resource settings are limited, as vital registration systems often don't exist or are incomplete[6,94]. The majority of estimates in low-resource settings come from surveys conducted in the community or from modeling of extrapolated data from individual studies, and may not be representative of causes of death among young infants in health facilities[6,94]. Additional studies to determine causes of death in the facility are essential to developing appropriate interventions.

The high mortality is especially troubling in light of the high coverage of recommended treatments. For young infants with pSBI, the WHO recommends combination 10-day intramuscular gentamycin and penicillin and referral and admission to a higher level health facility for skilled care and case-management, when feasible[13]. All newborns in our study population were receiving care in a referral level facility, and in Chapters 1 and 2, we report that antibiotic treatment coverage consistent with WHO recommendations was extremely high. The high mortality rate in this study suggests that current recommendations may be insufficient to prevent newborn deaths. Additional newborn survival strategies and interventions are needed to ensure the highest risk newborns the care necessary to survive.

We found that the WHO empiric algorithms for identifying possible severe bacterial infection and severe disease in young infants were sensitive not only to identifying newborns with severe infection, but also those at risk of mortality. In Chapter 1 we report that all newborns with definite or likely bacteremia had at least 1 IMCI sign, and none of the newborns with no IMCI signs had definite/likely bacteremia; an absolute difference of 2.4% in the proportion with definite/likely bacteremia (although the rare presentation limited statistical stability of estimates). In Chapter 2 we report that 6 of the 7 Integrated Management of Childhood Illness (IMCI) signs of possible severe bacterial infection (pSBI) in young infants was associated with mortality, and the presence of any one sign was associated with a 12-fold mortality risk (95% CI: 1.7-91.0). This was particularly noteworthy in light of our high-risk population, in which the prevalence of risk factors and of mortality were higher than would be expected in a general population.

The empiric algorithms for identifying and classifying severe illness also performed well in predicting mortality risk. In Chapter 3 we found that a model that considered each of the 7 WHO IMCI criteria for severe illness in young infants had strong risk predictive performance. The addition of the 3 danger signs in the WHO Pocketbook of Hospital Care for Children did not substantially improve predictive performance. Using a simple decision rule based on the presence of any 1 or more IMCI or WHO Hospital Book Danger signs was very sensitive for mortality; 95.5%. However, considering risk based on a minimum threshold of any one sign lacked specificity and would classify a large number of newborns as high risk who would ultimately be expected to survive. A tool that accounted for the risk associated with the presence or absence of each specific danger sign, in combination, ultimately performed the best in mortality risk

prediction. For a chosen level of desired sensitivity to detect mortality risk, a tool which accounts for these features in combination would obtain the highest specificity in ruling out mortality risk.

The IMCI signs were selected based on sensitivity and specificity for identifying severe illness in the community or primary health care level, based on assessment of a minimally trained provider[20]. We used modern machine-learning methods to identify the most important prognostic factors for mortality in this population, based on assessment of more highly trained staff in a higher-level facility. We determined that poor feeding, fast breathing, fever, only moves when stimulated/no movement, all part of the IMCI criteria for severe disease and the WHO Pocket book criteria danger signs, were important prognostic features for mortality. Additionally, apnea and low birthweight, which are not included as danger signs in the existing algorithms, were important prognostic features. The set of these 6 features in combination provided the best predictive performance of the models assessed. But overall, the prognostic performance of this new set of features only minimally improved mortality predication on top of what could be accomplished using existing empiric tools for severe illness detection.

The additional prognostic signs we identified may help predict mortality risk from causes other than severe bacterial infection; the outcome the IMCI algorithm was developed and validated to detect. Apnea is a sign of other common newborn problems including complications of severe intrapartum events and hypoxic-ischemic encephalopathy, systemic infections including sepsis, pneumonia and meningitis, and complications of prematurity[12]; the three most common causes of neonatal mortality[6,7]. Similarly, low birthweight is an important risk factor for mortality and is highly associated with preterm birth and small-for-gestational-age birth and increases the risk of severe invasive infection; primary causes of newborn mortality. Assessment of apnea and consideration of birthweight along with clinical presentation may aid in mortality risk prediction among selected newborn populations in special care newborn units that include not only those with suspected or confirmed severe infection, but also other newborn syndromes and problems.

Among this population of newborns with risk factors for severe infection or signs of clinical infection, the mortality rate was high despite low prevalence of culture-confirmed bacterial blood stream infection. The high mortality is particularly troubling given the high antibiotic treatment coverage and the facility-based setting of deaths. The WHO recommends combination antibiotic treatment and case-management in a

referral-level health facility for newborns with suspected infection in resource-constrained settings[12,13]. Similarly, global newborn mortality reduction strategies recommend improving coverage of facility-based care and management for small and sick newborns, to facilitate the delivery of essential and more comprehensive and skilled care for small and sick newborns[4]. The newborns in this study died despite receiving recommended care for small and sick newborns in resource-constrained settings.

The studies in this dissertation suggest the importance of improving not only access to care in health facilities, but the quality of care delivered during childbirth and in the postnatal period in high-mortality settings. Effective strategies to prevent severe morbidity and newborn mortality are well described, but weak health systems in high-burden countries often lack the key inputs and health system components necessary to ensure high-quality care during childbirth and the postnatal period is available[2-4,8,65,74]. Despite improvements in coverage, quality of care is lagging behind in many high-burden settings, including Kenya[75,77]. Services for safe childbirth and postnatal care and skilled care of sick and small young infants require inputs and resources across the health system, yet facilities often lack the key components necessary: availability of essential medicines and commodities, basic diagnostic and treatment equipment, well-functioning transport and referral systems, adequate skilled human resources, and basic infrastructure[65]. Improving the quality of maternal and newborn care and ensuring these improvements are sustainable is not an easy task, but the lives of nearly 7,000 newborns per day rest on just such progress.

REFERENCES

1. Levels and Trends in Child Mortality- Report 2015 Estimated Developed by the UN Inter-agency Group for Child Mortality Estimation. New York, NY: UN-IGME; 2015.
2. Lawn JE, Blencowe H, Oza S, et al. Every Newborn: progress, priorities, and potential beyond survival. Lancet. 2014;384(9938):189-205.
3. Every Child Alive: The Urgent Need to End Newborn Deaths. Geneva, Switzerland: UNICEF; 2018.
4. Every Newborn: an action plan to end preventable deaths. Geneva, Switzerland: WHO, UNICEF.; 2014.
5. Blencowe H, Cousens S. Addressing the challenge of neonatal mortality. Trop Med Int Health. 2013;18(3):303-312.
6. Oza S, Lawn JE, Hogan DR, Mathers C, Cousens SN. Neonatal cause-of-death estimates for the early and late neonatal periods for 194 countries: 2000-2013. Bull World Health Organ. Vol 93. Switzerland2015.
7. Garces AL, McClure EM, Perez W, et al. The Global Network Neonatal Cause of Death algorithm for low-resource settings. Acta Paediatr. 2017;106(6):904-911.
8. Bhutta ZA, Das JK, Bahl R, et al. Can available interventions end preventable deaths in mothers, newborn babies, and stillbirths, and at what cost? Lancet. 2014;384(9940):347-370.
9. Seale AC, Mwaniki M, Newton CR, Berkley JA. Maternal and early onset neonatal bacterial sepsis: burden and strategies for prevention in sub-Saharan Africa. Lancet Infect Dis. 2009;9(7):428-438.
10. Seale AC, Blencowe H, Zaidi A, et al. Neonatal severe bacterial infection impairment estimates in South Asia, sub-Saharan Africa, and Latin America for 2010. Pediatr Res. 2013;74 Suppl 1:73-85.
11. Seale AC, Blencowe H, Manu AA, et al. Estimates of possible severe bacterial infection in neonates in sub-Saharan Africa, south Asia, and Latin America for 2012: a systematic review and meta-analysis. Lancet Infect Dis. 2014;14(8):731-741.
12. Pocket book of hospital care for children- 2nd ed. Guidelines for the management of common childhood illnesses. 2nd ed. Geneva, Switzerland: World Health Organization; 2013.
13. Integrated Management of Childhood Illness Chart Booklet. Module 2: The sick young infant. March, 2014 ed. Geneva, Switzerland: World Health Organization; 2014.
14. Levels and Trends in Child Mortality: Report 2017, Estimates Developed by the UN Inter-agency Group for Child Mortality Estimation: United Nations Childrens Fund, New York; 2017.
15. Hibberd PL, Hansen NI, Wang ME, et al. Trends in the incidence of possible severe bacterial infection and case fatality rates in rural communities in Sub-Saharan Africa, South Asia and Latin America, 2010-2013: a multicenter prospective cohort study. Reprod Health. 2016;13(1):65.
16. Mulholland EK, Ogunlesi OO, Adegbola RA, et al. Etiology of serious infections in young Gambian infants. Pediatr Infect Dis J. 1999;18(10 Suppl):S35-41.
17. Kabwe M, Tembo J, Chilukutu L, et al. Etiology, Antibiotic Resistance and Risk Factors for Neonatal Sepsis in a Large Referral Center in Zambia. Pediatr Infect Dis J. 2016;35(7):e191-198.
18. Polin RA. Management of neonates with suspected or proven early-onset bacterial sepsis. Pediatrics. 2012;129(5):1006-1015.
19. Brady MT, Polin RA. Prevention and management of infants with suspected or proven neonatal sepsis. Pediatrics. 2013;132(1):166-168.
20. Clinical signs that predict severe illness in children under age 2 months: a multicentre study. Lancet. 2008;371(9607):135-142.

21. Integrated management of childhood illness: caring for newborns and children in the community, adaptation for high HIV or TB settings. Caring for the sick child in the community. A training course for community health workers. Geneva, Switzerland: World Health Organization; 2014.
22. Hamer DH, Darmstadt GL, Carlin JB, et al. Etiology of Bacteremia in Young Infants in Six Countries. Pediatr Infect Dis J. 2014.
23. Islam MS, Baqui AH, Zaidi AK, et al. Infection Surveillance Protocol for a Multicountry Population-based Study in South Asia to Determine the Incidence, Etiology and Risk Factors for Infections Among Young Infants of 0 to 59 Days Old. Pediatr Infect Dis J. 2016;35(5 Suppl 1):S9-15.
24. Wiens MO, Pawluk S, Kissoon N, et al. Pediatric post-discharge mortality in resource poor countries: a systematic review. PLoS One. 2013;8(6):e66698.
25. Blencowe H, Cousens S, Mullany LC, et al. Clean birth and postnatal care practices to reduce neonatal deaths from sepsis and tetanus: a systematic review and Delphi estimation of mortality effect. BMC Public Health. 2011;11 Suppl 3:S11.
26. Zaidi AK, Ganatra HA, Syed S, et al. Effect of case management on neonatal mortality due to sepsis and pneumonia. BMC Public Health. 2011;11 Suppl 3:S13.
27. Lawn JE, Kinney M, Lee AC, et al. Reducing intrapartum-related deaths and disability: can the health system deliver? Int J Gynaecol Obstet. 2009;107 Suppl 1:S123-140, s140-122.
28. Lee AC, Cousens S, Darmstadt GL, et al. Care during labor and birth for the prevention of intrapartum-related neonatal deaths: a systematic review and Delphi estimation of mortality effect. BMC Public Health. 2011;11 Suppl 3:S10.
29. Lawn JE, Lee ACC, Kinney M, et al. Two million intrapartum-related stillbirths and neonatal deaths: Where, why, and what can be done? International Journal of Gynecology & Obstetrics. 2009;107, Supplement:S5-S19.
30. Wall SN, Lee AC, Niermeyer S, et al. Neonatal resuscitation in low-resource settings: what, who, and how to overcome challenges to scale up? Int J Gynaecol Obstet. 2009;107 Suppl 1:S47-62, s63-44.
31. Johri AK, Lata H, Yadav P, et al. Epidemiology of Group B Streptococcus in developing countries. Vaccine. 2013;31 Suppl 4:D43-45.
32. Newton O, English M. Young infant sepsis: aetiology, antibiotic susceptibility and clinical signs. Trans R Soc Trop Med Hyg. 2007;101(10):959-966.
33. Caffrey Osvald E, Prentice P. NICE clinical guideline: antibiotics for the prevention and treatment of early-onset neonatal infection. Arch Dis Child Educ Pract Ed. 2014;99(3):98-100.
34. Obiero CW, Seale AC, Berkley JA. Empiric treatment of neonatal sepsis in developing countries. Pediatr Infect Dis J. 2015.
35. WHO recommendations for prevention and treatment of maternal peripartum infections. In: World Health Organization HRP, ed. Geneva, Switzerland: World Health Organization; 2015.
36. Lassi ZS, Middleton PF, Crowther C, Bhutta ZA. Interventions to Improve Neonatal Health and Later Survival: An Overview of Systematic Reviews. EBioMedicine. 2015;2(8):985-1000.
37. Salam RA, Mansoor T, Mallick D, Lassi ZS, Das JK, Bhutta ZA. Essential childbirth and postnatal interventions for improved maternal and neonatal health. Reprod Health. 2014;11 Suppl 1:S3.
38. Ohlsson A, Shah VS. Intrapartum antibiotics for known maternal Group B streptococcal colonization. Cochrane Database Syst Rev. 2013;1:CD007467.
39. Bang AT, Deshmukh M. Simplified antibiotic regimens for neonatal sepsis--AFRINEST. Lancet. 2015;386(10001):1338-1339.
40. Delair S, Obaro SK. Simplified antibiotic regimens for neonatal sepsis--AFRINEST. Lancet. 2015;386(10001):1337.

41. Dewez JE, Chellani HK, Halim A, van den Broek N. Simplified antibiotic regimens for neonatal sepsis--AFRINEST. Lancet. 2015;386(10001):1337-1338.
42. Mir F, Nisar I, Tikmani SS, et al. Simplified antibiotic regimens for treatment of clinical severe infection in the outpatient setting when referral is not possible for young infants in Pakistan (Simplified Antibiotic Therapy Trial [SATT]): a randomised, open-label, equivalence trial. Lancet Glob Health. 2017;5(2):e177-e185.
43. Weston EJ, Pondo T, Lewis MM, et al. The burden of invasive early-onset neonatal sepsis in the United States, 2005-2008. Pediatr Infect Dis J. 2011;30(11):937-941.
44. Cagno CK, Pettit JM, Weiss BD, et al. Prevention of perinatal group B streptococcal disease: updated CDC guideline
Policy statement-Recommendations for the prevention of perinatal group B streptococcal (GBS) disease
Prevention of perinatal group B streptococcal disease--revised guidelines from CDC, 2010
Intrapartum antibiotics for known maternal Group B streptococcal colonization
Adherence to perinatal group B streptococcal prevention guidelines. Am Fam Physician. Vol 86. United States
England2012.
45. Schrag SJ, Verani JR. Intrapartum antibiotic prophylaxis for the prevention of perinatal group B streptococcal disease: experience in the United States and implications for a potential group B streptococcal vaccine. Vaccine. 2013;31 Suppl 4:D20-26.
46. Cutland CL, Schrag SJ, Zell ER, et al. Maternal HIV infection and vertical transmission of pathogenic bacteria. Pediatrics. 2012;130(3):e581-590.
47. Kwatra G, Adrian PV, Shiri T, Buchmann EJ, Cutland CL, Madhi SA. Serotype-specific acquisition and loss of group B streptococcus recto-vaginal colonization in late pregnancy. PLoS One. 2014;9(6):e98778.
48. Kim SY, Russell LB, Park J, et al. Cost-effectiveness of a potential group B streptococcal vaccine program for pregnant women in South Africa. Vaccine. 2014;32(17):1954-1963.
49. Cutland CL, Schrag SJ, Thigpen MC, et al. Increased Risk for Group B Streptococcus Sepsis in Young Infants Exposed to HIV, Soweto, South Africa, 2004-2008(1). Emerg Infect Dis. 2015;21(4):638-645.
50. Seale AC, Obiero CW, Berkley JA. Rational development of guidelines for management of neonatal sepsis in developing countries. Curr Opin Infect Dis. 2015.
51. Hossain B, Islam MS, Rahman A, et al. Understanding Bacterial Isolates in Blood Culture and Approaches Used to Define Bacteria as Contaminants: A Literature Review. Pediatr Infect Dis J. 2016;35(5 Suppl 1):S45-51.
52. Hossain B, Weber MW, Hamer DH, et al. Classification of Blood Culture Isolates Into Contaminants and Pathogens on the Basis of Clinical and Laboratory Data. Pediatr Infect Dis J. Vol 35. United States2016.
53. Polin RA, Watterberg K, Benitz W, Eichenwald E. The conundrum of early-onset sepsis. Pediatrics. 2014;133(6):1122-1123.
54. Chan GJ, Lee AC, Baqui AH, Tan J, Black RE. Risk of early-onset neonatal infection with maternal infection or colonization: a global systematic review and meta-analysis. PLoS Med. 2013;10(8):e1001502.
55. Chan GJ, Lee AC, Baqui AH, Tan J, Black RE. Prevalence of early-onset neonatal infection among newborns of mothers with bacterial infection or colonization: a systematic review and meta-analysis. BMC Infect Dis. 2015;15:118.
56. Puopolo KM, Draper D, Wi S, et al. Estimating the probability of neonatal early-onset infection on the basis of maternal risk factors. Pediatrics. 2011;128(5):e1155-1163.
57. Puopolo KM, Escobar GJ. Early-onset sepsis: a predictive model based on maternal risk factors. Curr Opin Pediatr. 2013;25(2):161-166.

58. Committee Opinion No. 712: Intrapartum Management of Intraamniotic Infection. Obstet Gynecol. Vol 130. United States2017.
59. Wang H, Liddell C, Coates Mea. Global, regional, and national levels of neonatal, infant and under-five mortality during 1990-2013: a systematic analysis of the Global Burden of Disease Study 2013. Lancet. 2014;384(9947):957-979.
60. Ohlsson A, Shah VS. Intrapartum antibiotics for known maternal Group B streptococcal colonization. Cochrane Database Syst Rev. 2014;6:CD007467.
61. Simonsen KA, Anderson-Berry AL, Delair SF, Davies HD. Early-onset neonatal sepsis. Clin Microbiol Rev. 2014;27(1):21-47.
62. Kenya Demographic and Health Survey, 2014. Rockville, Maryland, USA: Kenya National Bureau of Statistics, Ministry of Health, National AIDS Control Council, Kenya Medical Research Institute, National Council for Population and Development, and The DHS Program, ICF International; 2015.
63. Kenya AIDS Response Progress Report 2016. Nairobi, Kenya: Ministry of Health and National AIDS Control Council; 2016.
64. Mukhopadhyay S, Puopolo KM. Risk assessment in neonatal early onset sepsis. Semin Perinatol. 2012;36(6):408-415.
65. Dickson KE, Kinney MV, Moxon SG, et al. Scaling up quality care for mothers and newborns around the time of birth: an overview of methods and analyses of intervention-specific bottlenecks and solutions. BMC Pregnancy Childbirth. 2015;15 Suppl 2:S1.
66. Das JK, Kumar R, Salam RA, Lassi ZS, Bhutta ZA. Evidence from facility level inputs to improve quality of care for maternal and newborn health: interventions and findings. Reprod Health. 2014;11 Suppl 2:S4.
67. Merali HS, Lipsitz S, Hevelone N, et al. Audit-identified avoidable factors in maternal and perinatal deaths in low resource settings: a systematic review. BMC Pregnancy Childbirth. 2014;14:280.
68. Bhutta ZA, Black RE. Global maternal, newborn, and child health--so near and yet so far. New England Journal of Medicine. 2013;369(23):2226-2235.
69. Schelonka RL, Chai MK, Yoder BA, Hensley D, Brockett RM, Ascher DP. Volume of blood required to detect common neonatal pathogens. J Pediatr. 1996;129(2):275-278.
70. Chiesa C, Panero A, Osborn JF, Simonetti AF, Pacifico L. Diagnosis of neonatal sepsis: a clinical and laboratory challenge. Clin Chem. 2004;50(2):279-287.
71. Chan GJ, Stuart EA, Zaman M, Mahmud AA, Baqui AH, Black RE. The effect of intrapartum antibiotics on early-onset neonatal sepsis in Dhaka, Bangladesh: a propensity score matched analysis. BMC Pediatr. 2014;14:104.
72. Chapman E, Reveiz L, Illanes E, Bonfill Cosp X. Antibiotic regimens for management of intra-amniotic infection. Cochrane Database Syst Rev. 2014;12:Cd010976.
73. Khan J, Vesel L, Bahl R, Martines JC. Timing of breastfeeding initiation and exclusivity of breastfeeding during the first month of life: effects on neonatal mortality and morbidity--a systematic review and meta-analysis. Matern Child Health J. 2015;19(3):468-479.
74. Dickson KE, Simen-Kapeu A, Kinney MV, et al. Every Newborn: health-systems bottlenecks and strategies to accelerate scale-up in countries. Lancet. 2014;384(9941):438-454.
75. Carvajal-Aguirre L, Amouzou A, Mehra V, Ziqi M, Zaka N, Newby H. Gap between contact and content in maternal and newborn care: An analysis of data from 20 countries in sub-Saharan Africa. J Glob Health. 2017;7(2):020501.
76. Katz J, Lee AC, Kozuki N, et al. Mortality risk in preterm and small-for-gestational-age infants in low-income and middle-income countries: a pooled country analysis. Lancet. 2013;382(9890):417-425.
77. Keats EC, Ngugi A, Macharia W, et al. Progress and priorities for reproductive, maternal, newborn, and child health in Kenya: a Countdown to 2015 country case study. Lancet Glob Health. 2017;5(8):e782-e795.

78. Talbert AW, Mwaniki M, Mwarumba S, Newton CR, Berkley JA. Invasive bacterial infections in neonates and young infants born outside hospital admitted to a rural hospital in Kenya. Pediatr Infect Dis J. 2010;29(10):945-949.
79. Richardson DK, Corcoran JD, Escobar GJ, Lee SK. SNAP-II and SNAPPE-II: Simplified newborn illness severity and mortality risk scores. J Pediatr. 2001;138(1):92-100.
80. The CRIB (clinical risk index for babies) score: a tool for assessing initial neonatal risk and comparing performance of neonatal intensive care units. The International Neonatal Network. Lancet. 1993;342(8865):193-198.
81. Rosenberg RE, Ahmed S, Saha SK, et al. Simplified age-weight mortality risk classification for very low birth weight infants in low-resource settings. J Pediatr. 2008;153(4):519-524.
82. Aluvaala J, Collins GS, Maina M, Berkley JA, English M. A systematic review of neonatal treatment intensity scores and their potential application in low-resource setting hospitals for predicting mortality, morbidity and estimating resource use. Syst Rev. 2017;6(1):248.
83. Ezz-Eldin ZM, Hamid TA, Youssef MR, Nabil Hel D. Clinical Risk Index for Babies (CRIB II) Scoring System in Prediction of Mortality in Premature Babies. J Clin Diagn Res. 2015;9(6):Sc08-11.
84. Marete IK, Wasunna AO, Otieno PA. Clinical risk index for babies (CRIB) II score as a predictor of neonatal mortality among low birth weight babies at Kenyatta National Hospital. East Afr Med J. 2011;88(1):18-23.
85. Rastogi PK, Sreenivas V, Kumar N. Validation of CRIB II for prediction of mortality in premature babies. Indian Pediatr. 2010;47(2):145-147.
86. Parry G, Tucker J, Tarnow-Mordi W. CRIB II: an update of the clinical risk index for babies score. Lancet. 2003;361(9371):1789-1791.
87. Steyerberg EW, Vickers AJ, Cook NR, et al. Assessing the performance of prediction models: a framework for traditional and novel measures. Epidemiology. 2010;21(1):128-138.
88. X. L. Classification accuracy and cut point selection. Statistics in Medicine

. . 2012;31(23):2676–2686.
89. Pavlou M, Ambler G, Seaman SR, et al. How to develop a more accurate risk prediction model when there are few events. BMJ. 2015;351:h3868.
90. Pavlou M, Ambler G, Seaman S, De Iorio M, Omar RZ. Review and evaluation of penalised regression methods for risk prediction in low-dimensional data with few events. Stat Med. 2016;35(7):1159-1177.
91. Tibshirani R. Regression Shrinkage and Selection via the Lasso. Journal of the Royal Statistical Society. Series B (Methodological). 1996;58(1):21.
92. Berkley JA, Lowe BS, Mwangi I, et al. Bacteremia among children admitted to a rural hospital in Kenya. N Engl J Med. 2005;352(1):39-47.
93. Aiken AM, Mturi N, Njuguna P, et al. Risk and causes of paediatric hospital-acquired bacteraemia in Kilifi District Hospital, Kenya: a prospective cohort study. Lancet. 2011;378(9808):2021-2027.
94. Liu L, Oza S, Hogan D, et al. Global, regional, and national causes of child mortality in 2000-13, with projections to inform post-2015 priorities: an updated systematic analysis. Lancet. 2014.