



RESEARCH ARTICLE

REVIEWED Detection of pathogens associated with early-onset neonatal sepsis in cord blood at birth using quantitative PCR [version 3; peer review: 2 approved]

Christina W. Obiero ^{1,2}, Wilson Gumbi³, Stella Mwakio ¹, Hope Mwangudzah ¹, Anna C. Seale ^{1,4,5}, Mami Taniuchi⁶, Jie Liu⁶, Eric Houpt⁶, James A. Berkley ^{1,7,8}

¹Clinical research, KEMRI-Wellcome Trust Research Programme, Kilifi, Kenya

²Global health, Academic Medical Center of the University of Amsterdam, Amsterdam, The Netherlands

³Bioscience department, KEMRI-Wellcome Trust Research Programme, Kilifi, Kenya

⁴Epidemiology and Population Health, London School of Hygiene & Tropical Medicine, London, UK

⁵College of Health and Medical Sciences, Haramaya University, Harar, Ethiopia

⁶Division of Infectious Diseases and International Health, University of Virginia, Virginia, USA

⁷Centre for Tropical Medicine, University of Oxford, Oxford, UK

⁸The Childhood Acute Illness & Nutrition (CHAIN) Network, Nairobi, Kenya

V3 First published: 05 Jan 2022, 7:3
<https://doi.org/10.12688/wellcomeopenres.17386.1>
 Second version: 11 May 2022, 7:3
<https://doi.org/10.12688/wellcomeopenres.17386.2>
 Latest published: 08 Nov 2022, 7:3
<https://doi.org/10.12688/wellcomeopenres.17386.3>

Abstract

Background: Early onset neonatal sepsis (EONS) typically begins prior to, during or soon after birth and may be rapidly fatal. There is paucity of data on the aetiology of EONS in sub-Saharan Africa due to limited diagnostic capacity in this region, despite the associated significant mortality and long-term neurological impairment.

Methods: We compared pathogens detected in cord blood samples between neonates admitted to hospital with possible serious bacterial infection (pSBI) in the first 48 hours of life (cases) and neonates remaining well (controls). Cord blood was systematically collected at Kilifi County Hospital (KCH) from 2011-2016, and later tested for 21 bacterial, viral and protozoal targets using multiplex PCR via TaqMan Array Cards (TAC).

Results: Among 603 cases (101 [17%] of whom died), 179 (30%) tested positive for ≥ 1 target and 37 (6.1%) tested positive for multiple targets. *Klebsiella oxytoca*, *Escherichia coli/Shigella* spp., *Pseudomonas aeruginosa*, and *Streptococcus pyogenes* were commonest. Among 300 controls, 79 (26%) tested positive for ≥ 1 target, 11 (3.7%) were positive for multiple targets, and *K. oxytoca* and *P. aeruginosa* were most common. Cumulative odds ratios across controls: cases (survived): cases (died) were *E. coli/Shigella* spp. 2.6 (95%CI 1.6-4.4); *E.*

Open Peer Review

Approval Status

	1	2
version 3		
(revision)		
08 Nov 2022		view
version 2		
(revision)		
11 May 2022		view
version 1		
05 Jan 2022	view	

1. Kari A. Neemann , University of Nebraska Medical Center, Omaha, USA

2. Kirsty Le Doare , St George's University of London, London, UK

Any reports and responses or comments on the article can be found at the end of the article.

faecalis 4.0 (95%CI 1.1-15); *S. agalactiae* 4.5 (95%CI 1.6-13); *Ureaplasma* spp. 2.9 (95%CI 1.3-6.4); Enterovirus 9.1 (95%CI 2.3-37); and *Plasmodium* spp. 2.9 (95%CI 1.4-6.2). Excluding *K. oxytoca* and *P. aeruginosa* as likely contaminants, aetiology was attributed in 9.4% (95%CI 5.1-13) cases using TAC. Leading pathogen attributions by TAC were *E. coli/Shigella* spp. (3.5% (95%CI 1.7-5.3)) and *Ureaplasma* spp. (1.7% (95%CI 0.5-3.0)).

Conclusions: Cord blood sample may be useful in describing EONS pathogens at birth, but more specific tests are needed for individual diagnosis. Careful sampling of cord blood using aseptic techniques is crucial to minimize contamination. In addition to culturable bacteria, *Ureaplasma* and Enterovirus were causes of EONS.

Keywords

Neonate, sepsis, molecular, aetiology, PCR

Corresponding author: Christina W. Obiero (cobiero@kemri-wellcome.org)

Author roles: **Obiero CW:** Conceptualization, Data Curation, Formal Analysis, Funding Acquisition, Investigation, Methodology, Project Administration, Visualization, Writing – Original Draft Preparation, Writing – Review & Editing; **Gumbi W:** Investigation, Validation, Writing – Review & Editing; **Mwakio S:** Resources, Writing – Review & Editing; **Mwangudzah H:** Resources, Writing – Review & Editing; **Seale AC:** Conceptualization, Funding Acquisition, Methodology, Writing – Review & Editing; **Taniuchi M:** Investigation, Resources, Software, Validation, Writing – Review & Editing; **Liu J:** Investigation, Resources, Software, Validation, Writing – Review & Editing; **Haupt E:** Investigation, Resources, Software, Validation, Writing – Review & Editing; **Berkley JA:** Conceptualization, Formal Analysis, Funding Acquisition, Methodology, Resources, Supervision, Validation, Writing – Review & Editing

Competing interests: No competing interests were disclosed.

Grant information: This work was supported by Wellcome [203077; 205184, to AC]; the UNICEF/UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases (TDR) under grant OXF-TDR01; CWO is supported by the Drugs for Neglected Diseases initiative [OXF-DND02]. EH is supported in-part by the National Institutes of Health [K24 AI102972]. JAB is supported by the Bill & Melinda Gates Foundation within the Childhood Acute Illness and Nutrition (CHAIN) Network [OPP1131320] and by the MRC/DfID/Wellcome Joint Global Health Trials scheme [MR/M007367/1].

The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Copyright: © 2022 Obiero CW *et al.* This is an open access article distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

How to cite this article: Obiero CW, Gumbi W, Mwakio S *et al.* **Detection of pathogens associated with early-onset neonatal sepsis in cord blood at birth using quantitative PCR [version 3; peer review: 2 approved]** Wellcome Open Research 2022, 7:3 <https://doi.org/10.12688/wellcomeopenres.17386.3>

First published: 05 Jan 2022, 7:3 <https://doi.org/10.12688/wellcomeopenres.17386.1>

REVISED Amendments from Version 2

In response to feedback from the reviewer, we have updated the article by clarifying the following: (i) sensitivity analysis of samples not included in our analysis was not done but may have been useful in assessing potential bias arising from how we selected our cases and controls; (ii) some pathogens were fastidious/unculturable and detected by TAC only. In addition, we acknowledge the shift from terms such as “sub-Saharan Africa” and “developing countries” in preference for “African countries” and “resource-limited settings.” We used the term “sub-Saharan Africa” to refer to previous studies done in this region where our study site is located.

Any further responses from the reviewers can be found at the end of the article

Introduction

Forty-one percent of global neonatal deaths occur in sub-Saharan Africa¹ and the risk of dying is highest in the first week of life². Infection is a leading cause of neonatal mortality, accounting for ~37% of deaths in sub-Saharan Africa³, and is associated with long-term neurological impairment⁴. Early-onset neonatal sepsis (EONS) is often due to maternal transmission of pathogens⁵ prior to, during, or soon after birth, and can be rapidly fatal. Neonatal sepsis lacks a consensus definition and the reference point for EONS is variable, based on the timing of onset of symptoms or sampling of a positive culture⁶, i.e., occurring within the first 48 hours^{7,8}, 72 hours⁹ or seven days¹⁰ of life. Most research conducted in developing countries has focused on culturable bacterial pathogens, with *Klebsiella* spp., *Escherichia coli* and *Staphylococcus aureus* identified as leading causes of EONS^{11,12}. Group B Streptococcus (GBS) has been variably implicated in EONS, and may be underestimated due to its rapid fatality and surveillance methodology^{13–15}. There are limited published data on viruses such as Herpes Simplex Virus (HSV)¹⁶ and Cytomegalovirus (CMV)¹⁷ as causes of EONS in this setting.

Blood culture is the gold standard diagnostic test for EONS, despite low sensitivity^{18,19}. One to two millilitres of blood volume is recommended to improve microorganism recovery²⁰, but smaller volumes are often obtained from sick neonates. Intrapartum antimicrobials²¹, presence of fastidious organisms and culture contamination may also contribute to low culture yields. Lack of availability of microbiology facilities, lengthy turn-around times²² and high rates of culture-negative sepsis contribute to antibiotic consumption¹⁹, exacerbating antimicrobial resistance²³, affecting the gut microbiota²⁴, and potentially missing important non-culturable organisms.

Nucleic acid amplification techniques can detect a broad range of pathogens²⁵ with up to 90% sensitivity and 93% specificity compared to microbial culture in some studies²². Recently, a custom TaqMan Array Card (TAC) approach based on quantitative reverse-transcription polymerase chain reaction (RT-qPCR)²⁶ was applied to neonatal blood and respiratory samples in South Asia and South Africa^{27,28}. Causal attribution of organisms identified in blood and respiratory samples in EONS using latent class modelling was 23% in South Asia²⁸ and 27% in South Africa²⁷. Bacteria were predominant and *Ureaplasma*

spp. was identified as a significant pathogen in these studies. However, healthy controls were not sampled in identical circumstances to cases in South Asia (cases were recruited from study health facilities while controls were identified from the community using an automated algorithm; controls were older than cases at sample collection)²⁸ whilst in South Africa both cases and controls were recruited from the study hospital²⁷.

Cord blood provides a potential opportunity for early pathogen detection prior to the clinical onset of infection, and with adequate sample volumes²⁹. Biomarkers in cord blood may correlate with peripheral blood parameters including total and differential white blood cell counts³⁰, and acute phase reactants such as C-reactive protein, serum amyloid A, haptoglobin³¹, interleukin-6 and procalcitonin³². Culture, PCR and sequencing have identified pathogenic bacteria and correlate with acute phase reactants in cord blood^{33,34}. However, cord blood contamination may easily occur^{29,33,35}.

We hypothesized that pathogens detected in cord blood using a molecular technique would be associated with subsequent admission and death with suspected EONS. In a nested case control study of cord blood samples systematically collected at birth, we selected neonates hospitalized within 48 hours of life with possible serious bacterial infection (pSBI) and a random set of neonates who were sampled identically and remained well.

Materials and methods

Study design and participants

We performed a retrospective case-control study of cord blood samples obtained at delivery at Kilifi County Hospital (KCH) within a systematic clinical surveillance of maternal and neonatal adverse events (clinicaltrials.gov NCT01757028)³⁶. KCH serves a mostly rural population along the Kenyan coast. About half of all admissions to the neonatal ward are from the KCH maternity department, where there are ~4000 deliveries per year. Hospital deliveries and neonatal admissions have increased since maternity user fees were abolished (Free Maternity Service policy, 2013)³⁷. Maternal clinical data and cord blood samples were obtained and analysed during the surveillance (clinicaltrials.gov NCT01757028)³⁶ and stored for future research. Data were collected using a standardized maternal admission record³⁶. Cord blood samples were obtained by trained clinicians using standard aseptic techniques and universal safety precautions. After delivery of the neonate and the placenta, the umbilical cord was swabbed using 70% isopropyl alcohol and spirit, double clamped and cut. Approximately 10 ml of venous cord blood was collected using either a sterile 18-gauge needle (preferred) or 5Fr gastric tube and a syringe into ethylenediamine tetra-acetic acid (EDTA) tubes (BD Diagnostics, USA), centrifuged within an hour of collection; plasma and cell pellet aliquots were then frozen separately at -80°C.

Neonates born between March 2011 and March 2016 (Figure 1) who were resident of the Kilifi Health and Demographic Surveillance System (KHDSS)³⁸ and had cord blood samples available were considered for this analysis. Cases were defined as neonates hospitalized within 48 hours of life with one or more features of possible serious bacterial infection (pSBI): history of difficulty feeding, history of convulsions, movement

Herpesvirus (PhHV) and artificial construct containing the region targeted by the MS2 PCR assay were added to each sample during lysate preparation to evaluate extraction and amplification efficiency. For each batch of extractions, a blank (about 2.5 ml of nuclease-free water) was processed through the complete protocol, and later assayed to rule out contamination during the extraction and amplification processes. A positive target in the blank would invalidate positive results for that target in the same batch of TNA extractions. Testing of TNA on TAC was done either on the same day or the day following extraction.

Detection of targets using TAC RT-qPCR

Real-time reverse transcription PCR assays were performed using a custom TAC (Thermo Fisher, CA, USA) on a QuantStudio 7 Flex instrument (Life Technologies, USA) to detect 16 bacterial, four viral and one protozoal targets (Figure 1)^{42,43}. The choice of targets was based on previous studies on neonatal sepsis²⁸. Organisms such as CoNS that have been previously shown to be clinically insignificant in our setting⁴⁴ were not included in the TAC panel. The uidA gene detects both *E. coli* and *Shigella* species, hence were included as a single target on the TAC cards. Primers and probes were adapted from published assays to detect acute febrile illness^{42,45} and sepsis⁴³ optimized for the universal cycling conditions on the card. Positive controls were plasmids for DNA and *in vitro* transcripts for RNA. Cards were designed, quality-controlled, and validated at the University of Virginia who provided onsite training.

For each experiment, 25 µL of TaqMan Fast Virus one-step master mix (4444434, Applied Biosystems, Thermo Fisher Scientific) was mixed with 75 µL of TNA extract or nuclease-free water (for no template control [NTC]) to make a 100 µL PCR reaction mix. Each 100 µL PCR reaction + sample mix was then transferred into the fill port of TAC after which the TAC was then centrifuged to ensure complete filling of the reaction wells, sealed and run. The reactions included a reverse transcription at 50°C for 10 minutes, initial denaturation at 95°C for 20 seconds, then 40 three-second cycles of 95°C, and 60°C for 30 seconds. Up to eight samples were tested per card, blinded to case-control status, with one NTC included in every 10 cards to check for reagent contamination. Analysis utilized QuantStudio Real-Time PCR Software version 1.2 (Applied Biosystems, Thermo Fisher). Results were quality-checked by examining target amplification plots. Baseline adjustment for targets or reaction wells with irregular amplification was done when a false amplification curve was generated or an inaccurate threshold cycle (Ct) value was yielded. Upon review of the reaction fluorescence curves for each target, we set the cut off threshold cycle (Ct) value for all targets at <40. Samples were deemed positive when any of the duplicate reactions yielded amplification curves that crossed the threshold as defined and controls were valid. We repeated *Ureaplasma* spp. testing using singleplex PCR on 261 samples which had parallel positive blanks on TAC, and excluded TAC results from four samples for which repeat singleplex PCR was not possible due to depletion of TNA.

Statistical analysis

Characteristics associated with case status were investigated using backward stepwise logistic regression retaining variables with $P < 0.1$. We initially estimated the odds ratio for all cases (survived and died) versus controls. Then, since several organisms of potential public health relevance were not detected at all in controls and could not be meaningfully analysed in this way, we estimated the cumulative odds of pSBI across ordered groups of controls: cases-survived: cases-died using ordinal logistic regression which can accommodate zero values. We tested the proportional odds assumption to confirm that the relationship between each pair of outcome variable (controls, cases-survived, and cases-died) were similar prior to performing ordinal logistic regression. We estimated the attribution fraction (AF) among cases with “punafcc” in STATA v15 (StataCorp, TX, USA)⁴⁶.

Results

Of 15,409 deliveries during the study period, 604 cases and 300 controls were selected (Figure 2). One case was subsequently excluded due to sample inhibition to amplification. Thus, 603 cases comprising 502 EONS survivors and 101 EONS deaths (58 [57%] and 74 [73%] of whom died within 24 and 48 hours after birth respectively) were included. Admissions on day 0, 1 and 2 of life among EONS cases were as follows: 256 (51%), 184 (37%) and 62 (12%) respectively in 502 survivors, compared with 93 (92%), 7 (6.9%) and 1 (1.0%) in 101 deaths ($P < 0.001$).

Compared with controls, pSBI cases were more likely to be born of mothers who were nulliparous (odds ratio [OR] 1.7, 95% confidence interval [CI] 1.2-2.3) or presented with drainage of liquor (OR 2.0, 95% CI 1.3-3.1), vaginal bleeding (OR 4.8, 95% CI 2.1-11) or oedema (OR 3.0, 95% CI 1.3-6.9) (Table 1). Admission with pSBI was not associated with maternal fever, prolonged rupture of membranes (PROM) or abnormal urinalysis at admission (prior to delivery).

Among newborns, assessment of appearance, pulse, grimace, activity, and respiration (APGAR) score <9 at 5 minutes (OR 15, 95% CI 6.2-35), resuscitation at birth (OR 3.6, 95% CI 1.8-7.3) and gestation of <32 weeks (OR 2.9, 95% CI 1.1-7.7) were associated with a pSBI case status (Table 2). Newborn mid-upper arm circumference (MUAC) (OR 0.77, 95% CI 0.64-0.94 per cm) and head circumference (OR 0.90, 95% CI 0.81-0.99 per cm) were also associated with pSBI, but birth weight was not associated with pSBI (OR 1.2, 95% CI 0.73-2.0) in this adjusted model.

Among 502 EONS survivors and 101 EONS deaths, 141 (28%) and 38 (38%) respectively tested positive for at least one TAC target, whilst 30 (6.0%) and 7 (6.9%) tested positive for multiple targets. The most frequent organisms detected were *K. oxytoca*, *E. coli/Shigella* spp., *P. aeruginosa*, and *S. pyogenes* (Table 3). Among 300 controls, 79 (26%) tested positive for at least one target, led by *K. oxytoca* and *P. aeruginosa*, and 11 (3.7%) were positive for multiple targets. *L. monocytogenes* and

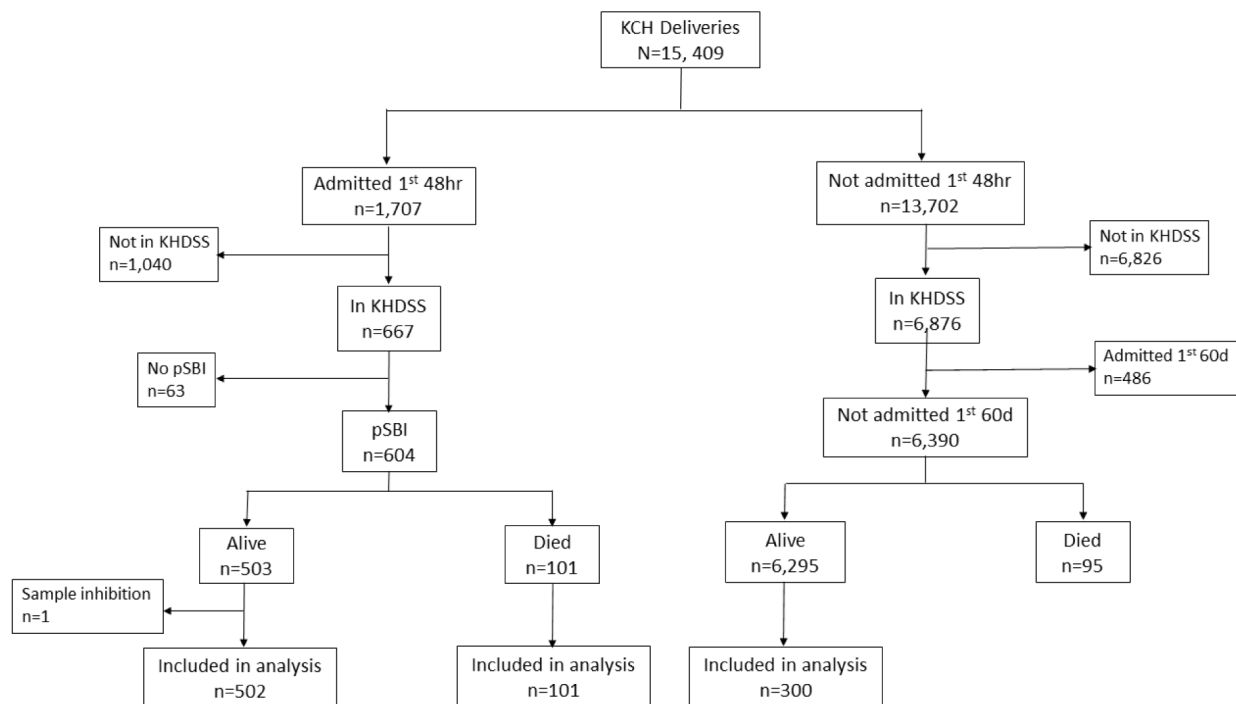


Figure 2. Study Participant Flow. Selection of cases and controls from a cohort of 15,409 deliveries at Kilifi County Hospital (KCH) between March 2011 and March 2016. Cases were hospitalised within the first 48 hours of life, resident of the Kilifi Health Demographic Surveillance System (KHDSS) and presented with one or more of the WHO-defined criteria for possible serious bacterial infection (pSBI). Controls were resident of the KHDSS and not hospitalised within the first 60 days of life.

Table 1. Maternal characteristics.

Characteristic	Cases (n=603)	Controls (n=300)	Univariable Odds Ratio (95% CI) ^a	P value ^a	Multivariable Odds Ratio (95% CI) ^b	P value ^b
Age, years	26 (21-32)	26 (21-32)	1.0 (0.9 to 1.0)	0.593	-	-
Weight, kg	60 (53-70)	60 (54-67)	1.0 (0.9 to 1.0)	0.632	-	-
Height, cm	156 (151-160)	156 (151-160)	0.9 (0.9 to 1.0)	0.283	-	-
MUAC, cm	26 (24-28)	25 (24-28)	1.0 (0.9 to 1.1)	0.326	-	-
Marital status						
Married	543 (90)	264 (88)	1.0	-	-	-
Single	38 (6.3)	24 (8.0)	0.8 (0.5 to 1.3)	0.335	-	-
Divorced	2 (0.3)	2 (0.7)	0.5 (0.1 to 3.5)	0.472	-	-
Widowed	3 (0.5)	1 (0.3)	1.5 (0.2 to 14)	0.744	-	-
Missing	17 (2.8)	9 (3.0)	0.9 (0.4 to 2.1)	0.839	-	-
Education Level						
None	105 (17)	58 (19)	1.0	-	-	-
Primary	360 (60)	178 (59)	1.1 (0.8 to 1.6)	0.555	-	-
Secondary	86 (14)	36 (12)	1.3 (0.8 to 2.2)	0.281	-	-

Characteristic	Cases (n=603)	Controls (n=300)	Univariable Odds Ratio (95% CI) ^a	P value ^a	Multivariable Odds Ratio (95% CI) ^b	P value ^b
Higher	34 (5.6)	12 (4.0)	1.6 (0.8 to 3.3)	0.230	-	-
Missing	18 (3.0)	16 (5.3)	0.6 (0.3 to 1.3)	0.211	-	-
Nulliparous						
No	383 (63)	207 (69)	1.0	-	1.0	-
Yes	185 (31)	65 (22)	1.5 (1.1 to 2.1)	0.010	1.7 (1.2 to 2.3)	0.004
Missing	35 (6.0)	28 (9.0)	0.7 (0.4 to 1.1)	0.143	0.7 (0.4 to 1.3)	0.304
<i>Presenting complaints</i>						
History of fever						
No	584 (97)	294 (98)	1.0	-	-	-
Yes	10 (1.7)	1 (0.3)	5.0 (0.6 to 40)	0.124	-	-
Missing	9 (1.5)	5 (1.7)	0.9 (0.3 to 2.7)	0.861	-	-
Drainage of liquor						
No	489 (81)	266 (89)	1.0	-	1.0	-
Yes	109 (18)	30 (10)	2.0 (1.3 to 3.0)	0.002	2.0 (1.3 to 3.1)	0.002
Missing	5 (0.8)	4 (1.3)	0.7 (0.2 to 2.6)	0.568	0.0 (0.0 to 0.0)	0.975
Ruptured membranes						
No	401 (66)	210 (70)	1.0	-	-	-
Yes	172 (29)	77 (26)	1.2 (0.9 to 1.6)	0.331	-	-
Missing	30 (5.0)	13 (4.3)	1.2 (0.6 to 2.4)	0.581	-	-
PROM >18h						
No	471 (78)	240 (80)	1.0	-	-	-
Yes	27 (4.5)	9 (3.0)	1.5 (0.7 to 3.3)	0.280	-	-
Missing	105 (17)	51 (17)	1.0 (0.7 to 1.5)	0.799	-	-
Vaginal bleeding						
No	539 (89)	287 (96)	1.0	-	1.0	-
Yes	61 (10)	7 (2.3)	4.6 (2.1 to 10)	<0.001	4.8 (2.1 to 11)	<0.001
Missing	3 (1.0)	6 (2.0)	0.3 (0.1 to 1.1)	0.063	0.0 (0.0 to 0.0)	0.993
Dysuria						
No	577 (96)	287 (96)	1.0	-	-	-
Yes	21 (3.5)	8 (2.7)	1.3 (0.6 to 3.0)	0.527	-	-
Missing	5 (0.8)	5 (1.7)	0.5 (0.1 to 1.7)	0.273	-	-
Decreased foetal movements						
No	576 (96)	293 (98)	1.0	-	1.0	-
Yes	23 (3.8)	1 (0.3)	11.7 (1.6 to 87)	0.016	6.0 (0.8 to 47)	0.085
Missing	4 (0.7)	6 (2.0)	0.3 (0.1 to 1.2)	0.096	0.0 (0.0 to 0.0)	0.993

Characteristic	Cases (n=603)	Controls (n=300)	Univariable Odds Ratio (95% CI) ^a	P value ^a	Multivariable Odds Ratio (95% CI) ^b	P value ^b
<i>Admission examination</i>						
Emergency signs ^c						
No	500 (83)	271 (90)	1.0	-		
Yes	98 (16)	27 (9.0)	2.0 (1.3 to 3.1)	0.003	1.6 (1.0 to 2.5)	0.058
Missing	5 (0.8)	2 (0.7)	1.4 (0.3 to 7.0)	0.718	0.0 (0.0 to 0.0)	0.976
Temperature, °C						
36-38	483 (80)	252 (84)	1.0	-	-	-
>38	7 (1.2)	0 (0.0)	1.0	-	-	-
<36	47 (7.8)	17 (5.7)	1.4 (0.8 to 2.6)	0.212	-	-
Missing	66 (11)	31 (10.3)	1.1 (0.7 to 1.7)	0.649	-	-
Oedema						
No	551 (91)	287 (96)	1.0	-	1.0	-
Yes	48 (8.0)	7 (2.3)	3.6 (1.6 to 8.0)	0.002	3.0 (1.3 to 6.9)	0.009
Missing	4 (0.7)	6 (2.0)	0.3 (0.1 to 1.2)	0.103	1.0	-
Positive nitrite and/or leucocytes (2+/3+) ^d						
No	417 (69)	214 (71)	1.0	-	-	-
Yes	108 (18)	46 (15)	1.2 (0.8 to 1.8)	0.339	-	-
Missing	78 (13)	40 (13)	1.0 (0.7 to 1.5)	0.997	-	-
Antibiotics in the last 4 weeks						
No	539 (89)	274 (91)	1.0	-	-	-
Yes	61 (10)	26 (8.7)	1.2 (0.7 to 1.9)	0.473	-	-
Missing	3 (1)	0 (0)	1.0	-	-	-

Data are N (%) or median (IQR)

Abbreviations: CI, confidence interval; kg, kilogram; cm, centimetre; MUAC, mid-upper arm circumference; PROM, prolonged rupture of membranes; °C, degree Celsius.

^aUnivariable logistic model for all cases vs. controls

^bMultivariable logistic model for all cases vs. controls, including variables with P<0.1

^cDanger signs at triage suggesting need for emergency care (include airway not patent, respiratory rate >30 or <10 breaths/minute, systolic blood pressure >160 or <90 mmHg, diastolic blood pressure >90 mmHg, heart rate <40 or >120 beats/minute, unconscious or alert only to pain, other obstetric emergencies (including imminent delivery) requiring immediate intervention)

^dUrinalysis results at admission

M. tuberculosis were not detected in either cases or controls. CMV was the commonest virus detected (19/603 (3.2%) cases versus 8/300 (2.7%) controls, $p=0.7$) and co-detection of CMV with a bacterial target was found in four cases and two controls.

We observed four patterns of target detection by TAC (Figure 3):

i) Group 1: detected in a low proportion (<5.0%) in both cases (12/603 [2.0%]) and controls (6/300 [2.0%]), ((HSV 1,

HSV 2, *S. aureus*, *Salmonella enterica*, *S. pneumoniae*, and *A. baumannii*);

ii) Group 2: detected in a low proportion (<5.0%) in cases (27/603 [4.5%]) but none in controls (0/300 [0.0%]), (*H. influenzae*, *N. meningitidis*, *E. faecalis*, Enterovirus, *Plasmodium* spp., and *S. agalactiae*);

iii) Group 3: detected in a high proportion ($\geq 5.0\%$) in both cases (123/603 [20%]) and controls (70/300 [23%]), (CMV, *S. pyogenes*, *P. aeruginosa*, and *K. oxytoca*); and

Table 2. Neonatal birth characteristics.

Characteristic	Cases (n=603)	Controls (n=300)	Univariable Odds Ratios (95% CI) ^a	P value ^a	Multivariable Odds Ratios (95% CI) ^b	P value ^b
Weight, kg	2.7 (1.9-3.2)	3 (2.7-3.3)	0.4 (0.3 to 0.5)	<0.001	1.2 (0.7 to 2.0)	0.454
Length, cm	47.5 (43.0-49.5)	48.5 (47.0-50.0)	0.9 (0.8 to 0.9)	<0.001	1.0 (0.9 to 1.1)	0.814
MUAC, cm	10.0 (8.2-10.7)	10.5 (9.8-11.2)	0.6 (0.5 to 0.7)	<0.001	0.8 (0.6 to 0.9)	0.010
Head circumference, cm	33.3 (31.0-34.9)	34.0 (33.0-35.0)	0.8 (0.8 to 0.9)	<0.001	0.9 (0.8 to 0.9)	0.036
Sex						
Male	345 (57)	151 (50)	1.0	-	1.0	-
Female	258 (43)	149 (50)	0.8 (0.6 to 1.0)	0.051	0.8 (0.6 to 1.1)	0.177
Gestation, weeks						
≥37	373 (62)	247 (82)	1.0	-	1.0	-
≥32 to <37	122 (20)	44 (15)	1.8 (1.3 to 2.7)	0.002	1.2 (0.8 to 1.9)	0.457
<32	88 (15)	6 (2.0)	9.7 (4.2 to 23)	<0.001	2.9 (1.1 to 7.7)	0.035
Missing	20 (3.3)	3 (1.0)	4.4 (1.3 to 15)	0.017	3.0 (0.8 to 11)	0.096
Mode of delivery						
Vaginal	441 (73)	205 (68)	1.0	-	-	-
Caesarean section	162 (27)	91 (30)	0.8 (0.6 to 1.1)	0.225	-	-
Missing	0 (0.0)	4 (1.3)	1.0	-	-	-
Resuscitated at birth ^c						
No	415 (69)	287 (96)	1.0	-	1.0	-
Yes	186 (31)	11 (4.0)	11.7 (6.2 to 22)	<0.001	3.7 (1.8 to 7.3)	<0.001
Missing	2 (0.3)	2 (0.7)	0.7 (0.1 to 4.9)	0.713	1.0	-
APGAR Score at 5 minutes						
≥9	352 (58)	290 (97)	1.0	-	1.0	-
<9	229 (38)	6 (2.0)	31.4 (14 to 72)	<0.001	15 (6.2 to 35)	<0.001
Missing	22 (3.7)	4 (1.3)	4.5 (1.5 to 13)	0.006	8.8 (1.1 to 70)	0.039

Data are N (%) or median (IQR)

Abbreviations: CI, confidence interval; kg, kilogram; cm, centimetre; MUAC, mid-upper arm circumference; APGAR, appearance, pulse, grimace, activity, and respiration.

^aUnivariable logistic model for all cases vs. controls

^bMultivariable logistic model for all cases vs. controls, including variables with P<0.1

^cResuscitation using bag mask ventilation with oxygen and/or cardiopulmonary resuscitation

- iv) Group 4: detected in a high proportion (≥5%) in cases (47/603 [7.8%]) and low proportion (<5.0%) in controls (6/300 [2.0%]) (*K. pneumoniae*, *Ureaplasma* spp. and *E. coli/Shigella* spp.).

Upon examining cumulative odds, detection of any bacterial, viral or protozoal target was not associated with pSBI and death (OR 1.3, 95% CI 1.0-1.7) (Table 3). However, the high

proportions of *K. oxytoca* and *P. aeruginosa* in both cases and controls suggested contamination or clinically insignificant traces of DNA in blood. Excluding these organisms, detection of any target was associated with pSBI and death (OR 2.1, 95% CI 1.4-2.8).

E. coli/Shigella spp. (P <0.001), *E. faecalis* (P =0.034), *S. agalactiae* (P =0.004), *Ureaplasma* spp. (P =0.010), Enterovirus

Table 3. TaqMan results.

	Controls (n=300)	Cases (n=603)	Cases survived (n=502)	Cases died (n=101)	Cases vs Controls odds ratio (95% CI) ^a	Cases (died) vs. Cases (survived) vs Controls cumulative odds ratio (95% CI) ^b	Attributable Fraction among cases (95% CI) ^c
Bacteria							
<i>Acinetobacter baumannii</i>	1 (0.3)	3 (0.5)	3 (0.6)	0 (0)	1.5 (0.2 to 14)	1.0 (0.3 to 3.6)	0
<i>Escherichia coli/Shigella spp.</i>	4 (1.3)	34 (5.6)	27 (5.4)	7 (6.9)	4.4 (1.6 to 13)	2.6 (1.6 to 4.4)	3.5 (1.7 to 5.3)
<i>Enterococcus faecalis</i>	0 (0)	4 (0.7)	3 (0.6)	1 (1.0)	-	4.0 (1.1 to 15)	0.5 (0.0 to 1.0)
<i>Haemophilus influenzae</i>	0 (0)	1 (0.2)	0 (0)	1 (1.0)	-	-	0.2 (0.0 to 0.5)
<i>Klebsiella oxytoca</i>	39 (13)	58 (9.6)	47 (9.4)	11 (11)	0.7 (0.5 to 1.1)	0.8 (0.5 to 1.2)	0
<i>Klebsiella pneumoniae</i>	1 (0.3)	9 (1.5)	7 (1.4)	2 (2.0)	4.5 (0.6 to 36)	2.7 (1.0 to 7.3)	0.9 (0.0 to 1.8)
<i>Listeria monocytogenes</i>	0 (0)	0 (0)	0 (0)	0 (0)	-	-	0
<i>Mycobacterium tuberculosis</i>	0 (0)	0 (0)	0 (0)	0 (0)	-	-	0
<i>Neisseria meningitidis</i>	0 (0)	3 (0.5)	2 (0.4)	1 (1.0)	-	5.2 (1.0 to 28)	0.4 (0.0 to 0.9)
<i>Pseudomonas aeruginosa</i>	21 (7.0)	32 (5.3)	24 (4.8)	8 (7.9)	0.7 (0.4 to 1.3)	0.9 (0.5 to 1.6)	0
<i>Streptococcus agalactiae</i>	0 (0)	7 (1.2)	5 (1.0)	2 (2.0)	-	4.5 (1.6 to 13)	0.9 (0.2 to 1.6)
<i>Staphylococcus aureus</i>	1 (0.3)	1 (0.2)	1 (0.2)	0 (0)	0.5 (0.0 to 8.0)	0.4 (0.0 to 4.9)	0
<i>Streptococcus pneumoniae</i>	1 (0.3)	3 (0.5)	3 (0.6)	0 (0)	1.5 (0.2 to 14)	1.0 (0.3 to 3.6)	0
<i>Streptococcus pyogenes</i>	9 (3.0)	20 (3.3)	20 (4.0)	0 (0)	1.1 (0.5 to 2.5)	0.8 (0.5 to 1.4)	0
<i>Salmonella spp.</i>	1 (0.3)	3 (0.5)	2 (0.4)	1 (1.0)	1.5 (0.2 to 14)	2.0 (0.2 to 20)	0.2 (0.0 to 1.0)
<i>Ureaplasma spp.</i>	2 (0.7)	16 (2.7)	12 (2.4)	4 (4.0)	4.1 (0.9 to 18)	2.9 (1.3 to 6.4)	1.7 (0.5 to 3.0)
Any bacteria	72 (24)	156 (26)	123 (25)	33 (33)	1.1 (0.8 to 1.5)	1.2 (0.9 to 1.6)	4.5 (0.0 to 11)
Any bacteria excluding <i>K. oxytoca</i> and <i>P. aeruginosa</i>	20 (6.7)	89 (15)	70 (14)	19 (19)	2.4 (1.5 to 4.0)	2.1 (1.5 to 3.1)	7.9 (4.3 to 11)
Viruses							
Cytomegalovirus	8 (2.7)	19 (3.2)	15 (3.0)	4 (4.0)	1.2 (0.5 to 2.7)	1.3 (0.6 to 2.7)	0.6 (0.0 to 2.7)
Enterovirus	0 (0)	6 (1.0)	3 (0.6)	3 (3.0)	-	9.1 (2.3 to 37)	0.9 (0.2 to 1.6)
Herpes Simplex Virus 1	1 (0.3)	1 (0.2)	1 (0.2)	0 (0)	0.5 (0.0 to 8.0)	0.4 (0.0 to 4.9)	0
Herpes Simplex Virus 2	1 (0.3)	1 (0.2)	1 (0.2)	0 (0)	0.5 (0.0 to 8.0)	0.4 (0.0 to 4.9)	0
Any viruses	10 (3.3)	27 (4.5)	20 (4.0)	7 (6.9)	1.4 (0.6 to 2.8)	1.6 (0.8 to 3.2)	1.6 (0.0 to 3.9)
Protozoa							
<i>Plasmodium spp.</i>	0 (0)	7 (1.2)	6 (1.2)	1 (1.0)	-	2.9 (1.4 to 6.2)	0.8 (0.1 to 1.4)
Any bacteria, viruses and protozoa	79 (26)	179 (30)	141 (28)	38 (38)	1.2 (0.9 to 1.6)	1.3 (1.0 to 1.7)	6.6 (0.0 to 14)
Any bacteria (excluding <i>K. oxytoca</i> and <i>P. aeruginosa</i>), viruses and protozoa	30 (10)	115 (19)	91 (18)	24 (24)	2.1 (1.4 to 3.3)	2.0 (1.4 to 2.8)	9.4 (5.1 to 13)

^aOrdinary logistic regression^bOrdinal logistic regression^cAttributable fraction is calculated from cumulative odds ratio

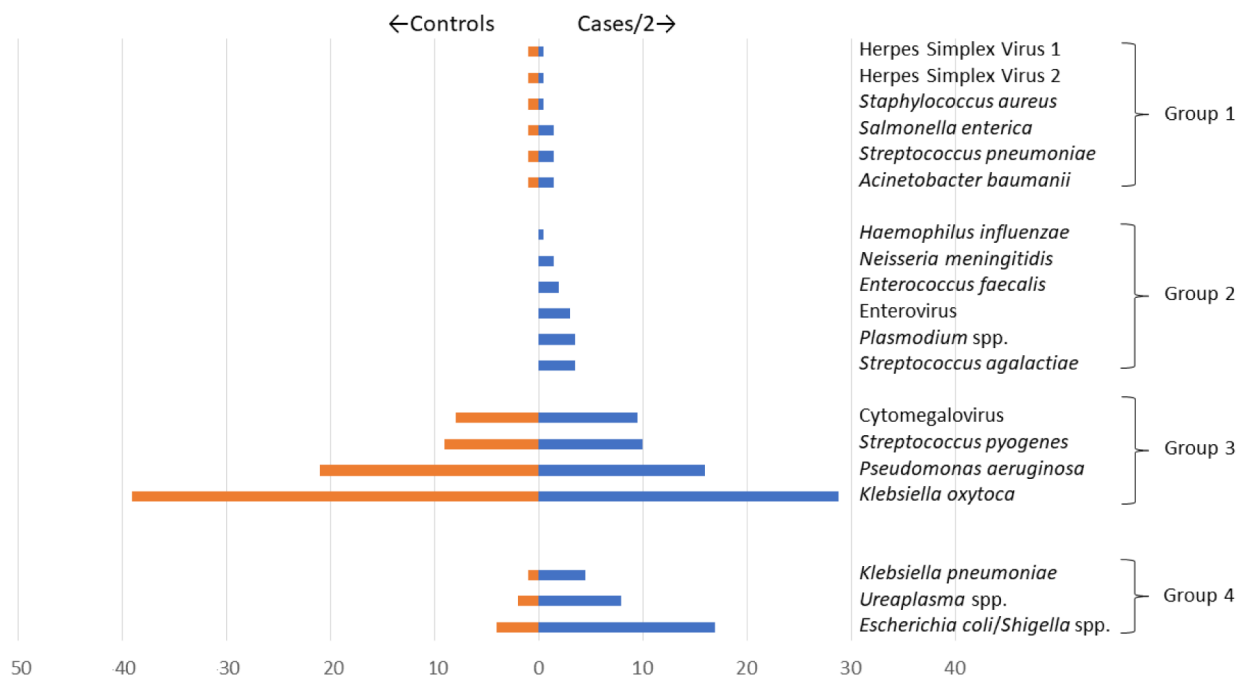


Figure 3. Patterns of detection of Taqman PCR Targets. Organisms included in the TAC were detected in 4 distinct groups: Group 1 (Herpes Simplex Virus 1, Herpes Simplex Virus 2, *Staphylococcus aureus*, *Salmonella enterica*, *Streptococcus pneumoniae*, and *Acinetobacter baumannii*); Group 2 (*Hemophilus influenzae*, *Neisseria meningitidis*, *Enterococcus faecalis*, Enterovirus, *Plasmodium* spp., and *Streptococcus agalactiae*); Group 3 (Cytomegalovirus, *Streptococcus pyogenes*, *Pseudomonas aeruginosa*, and *Klebsiella oxytoca*); and Group 4 (*Klebsiella pneumoniae*, *Ureaplasma* spp. and *Escherichia coli/Shigella* spp.). Weighting of cases (represented as cases/2) was done to allow for improved accuracy in assessing the distribution of organisms tested, comparing cases to controls, since cases (n=603) were ~twice more than controls (n=300). Weighting was done by calculating the number of eligible cases/controls divided by the number of enrolled cases/controls i.e. the inverse of the sampling fraction for cases/controls.

($P = 0.002$), and *Plasmodium* spp. ($P = 0.004$) were associated with pSBI and death (Table 3). *K. pneumoniae* ($P = 0.050$) and *N. meningitidis* ($P = 0.054$) had P values of borderline significance.

Overall, 6.6% (95% CI 0-14) of all pSBI cases were attributed to the bacterial, viral or protozoal targets. Excluding *K. oxytoca* and *P. aeruginosa* as likely contaminants, 9.4% (95% CI 5.1-13) of cases were attributed to the tested targets. Overall, 4.5% (95% CI 0-11) and 1.6% (95% CI 0-3.9) of pSBI cases were attributed to bacterial and viral targets respectively. The leading attributed pathogens were *E. coli/Shigella* spp. (AF 3.5%, 95% CI 1.7-5.3) and *Ureaplasma* spp. (AF 1.7, 95% CI 0.5-3.0). *E. faecalis*, *H. influenzae*, *K. pneumoniae*, *N. meningitidis*, *S. agalactiae*, *Salmonella enterica*, CMV, Enterovirus and *Plasmodium* spp. were each attributed to less than 1% of the pSBI cases.

A total of 11 (1.8%) of 603 cases had presumed pathogens isolated from blood culture at admission and *S. aureus* (n=5) was the most common isolate (Table 4). The OR for a positive admission blood culture for death among admitted pSBI cases was 1.1 (95% CI 0.24-5.2) and 0.2% (95% CI 0-3.2) of pSBI were attributed to the pathogens identified through blood culture.

Discussion

We used a novel approach to identify causes of EONS by investigating stored cord blood samples collected at birth with a custom TAC, spatially multiplexed PCR to interrogate the presence of multiple pathogens. This is the first study evaluating diagnostic performance using cord blood in an African setting. These samples were obtained at delivery prior to admission with signs of pSBI. Approximately 60% of 603 EONS cases and 92% of all deaths were admitted on day 0 of life. A total of 58 of 101 (57%) deaths occurred within the first 24 hours of life, after cord blood samples had been obtained. This underscores the importance of prompt diagnosis for targeted treatment and makes a cord blood approach potentially attractive in epidemiological studies, and possibly for managing 'at risk' neonates since cord blood could be collected, stored and tested at a later stage if the newborn develops signs of pSBI.

E. coli/Shigella spp. and *Ureaplasma* spp. had the highest causal attribution in our results, supporting the latter as an important pathogen in this setting. *Ureaplasma* spp. are associated with maternal colonization and adverse pregnancy outcomes⁴⁷. Appropriate treatment of high-risk neonates is needed given the emerging resistance of *Ureaplasma* spp. to macrolides. Two healthy controls and 16 pSBI cases, of whom 12 survived,

Table 4. Admission blood culture results among cases and corresponding TAC PCR results.

Organisms	Cases (n=603)	
	Blood culture	TAC cord blood
Presumed significant organisms		
<i>Acinetobacter</i> spp.	2	0
<i>Klebsiella pneumoniae</i>	1	0
<i>Pantoea</i> spp.*	1	0
<i>Staphylococcus aureus</i>	5	1 (<i>K. oxytoca</i> + <i>P. aeruginosa</i>)
<i>Streptococcus</i> Group B	1	1 (CMV)
<i>Streptococcus</i> Group G*	1	1 (<i>K. oxytoca</i>)
Total	11	3

Columns show number of neonates with either positive culture or TAC (TAC organisms are indicated within the brackets).

Abbreviations: CMV, cytomegalovirus.

*These organisms were not included on the TAC.

tested positive for *Ureaplasma* spp. in our study. Although the clear association of *Ureaplasma* spp. with sepsis and mortality indicates pathogenicity, asymptomatic presentation or recovery in ill neonates without targeted antimicrobials has been reported and is not unusual⁴⁸. Additionally, the culture-independent molecular method identified non-culturable organisms such as Enterovirus, which is shown to cause serious sepsis-like illness in neonates in other settings⁴⁹. However, despite the use of sensitive molecular assays, 90% of pSBI cases still had unknown aetiology on cord blood analysis.

The overall causal attribution of 6.6% (95% CI 0-14) increased to 9.4% (95% CI 5.1-13) with exclusion of *K. oxytoca* and *P. aeruginosa* in our study. As expected, the attributable proportion was lower than in the Sepsis Aetiology in Neonates in South Africa study (SANISA [27%, 95% CI 23-32])²⁷ and the Aetiology of Neonatal Infection in South Asia study (ANISA [23%, 95% CI 19-26])²⁸ since much of the latter study's attribution went to detection of RSV in respiratory samples, which this study did not examine. Although SANISA (n=27) and ANISA (n=28) were large prospective studies and tested more targets by TAC than we did (n=21), they also failed to attribute aetiology to a large proportion of pSBI cases. There were also differences in pSBI case definitions (SANISA used a predefined set of clinical and laboratory criteria²⁷, while ANISA used WHO clinical criteria but excluded tachypnoea²⁸) and differences in selection and sampling of controls (SANISA sampled healthy neonates at study hospital²⁷ while ANISA used an automated algorithm triggered at the first postnatal visit to select randomly registered controls²⁸). Our pSBI definition was based on the WHO Young Infants Clinical Signs study which derived a decision rule (presence of ≥ 1 sign: history of difficulty feeding, history of convulsions, movement only when

stimulated, respiratory rate of ≥ 60 breaths/min, severe chest indrawing, and a temperature of $\geq 37.5^\circ\text{C}$, or $\leq 35.5^\circ\text{C}$) predicting severe illness in neonates aged 0–6 days with 87% sensitivity and 74% specificity³⁹. The performance of these signs in distinguishing neonates with sepsis from those without sepsis has not been adequately investigated. Current WHO⁵⁰ and Kenya national paediatric guidelines⁵¹ for empiric antimicrobials in neonates suspected to have sepsis are based on this limited evidence. Neonates with sepsis often present with subtle and non-specific clinical signs that overlap with those seen in other non-infectious diagnoses⁵². Thus, our case definition may have resulted in the inclusion of neonates who did not have true sepsis, contributing to low attribution rates. Development and use of a highly sensitive and specific consensus definition for neonatal sepsis is critically needed in clinical practice and research⁶.

Bacterial organisms (25% bacterial compared to 4.1% viral targets detected) were predominant in our study, similar to SANISA²⁷ and ANISA²⁸ results from blood samples. Thirty percent of cord blood samples of pSBI cases in our study tested positive for at least one target by TAC, compared to blood samples in SANISA (37%)²⁷ and ANISA (12%)²⁸. We identified multiple targets in 7% of pSBI cases compared to 11% cases in SANISA²⁷ and 1% in ANISA²⁸. At least one target was positive in 28% of healthy controls in our study compared to 20% in SANISA²⁷. Thus, background positivity of cord blood among healthy neonates in our study was greater than in SANISA. All cases and controls were first selected based on the presence or absence of pSBI. All 604 pSBI cases who resided in the KHDSS had cord blood samples available for testing and were included in this analysis. 300 controls were randomly selected from a subset of 6,295 neonates who were resident of the KHDSS, remained well during the first 60 days of life,

and had cord blood samples available for testing. Therefore, we ensured that cases and controls had an equal chance of being selected in respective groups, with controls derived from similar circumstances to cases for optimal group comparison, unlike in ANISA where controls were recruited from the community. Although the cases had a lower gestation age and were generally smaller than the controls based on the anthropometric measurements, we believe that bias risk was minimal since we would expect sick neonates to present with known underlying risk factors of infection, such as prematurity.

K. oxytoca and *P. aeruginosa* were identified in large numbers in both cases and controls. This could be due to environmental contamination of laboratory materials or reagents, which has been widely reported for *K. oxytoca*⁵³, contamination of the specimen by gut flora or skin commensals post-delivery⁵⁴, as was reported in SANISA²⁷, or true subclinical detection of circulating non-viable genetic material, or low copies of organisms insufficient to cause disease. Overall causal attribution increased with exclusion of *K. oxytoca* and *P. aeruginosa*, suggesting non-significance of these bacteria in EONS. Cord blood sample contamination has been reported in studies evaluating the diagnostic use of cord blood cultures, by comparing results obtained to peripheral venous blood cultures^{29,33,35}. Although cord blood provides a non-invasive alternative to peripheral blood sampling with better culture yields^{30,55}, the risk of contamination cannot be ignored. Careful aseptic techniques and training of clinical staff are imperative to optimize sample collection and may improve the validity of results. Aseptic techniques were used during cord blood sample collection to minimise sample contamination, since identification of pathogens associated with adverse maternal and perinatal outcomes was planned³⁶, including a recently published study on the association of flavivirus exposure with congenital microcephaly⁵⁶. In addition, cord blood analysis using PCR has mostly focused on vertically transmitted viruses^{57–59}, and more research on cord blood testing using molecular diagnostics is needed to better understand the clinical significance of detected organisms. Detection of organisms known to cause permanent neurodevelopmental sequelae in asymptomatic congenital infection such as CMV⁶⁰ (eight healthy controls in our study) may inform management. However, we did not follow up these infants for post-discharge outcomes in this retrospective analysis. Nonetheless, the PCR detections for *E. coli/Shigella* spp., *E. faecalis*, *K. pneumoniae*, *N. meningitidis*, *S. agalactiae*, *Ureaplasma* spp., and *Plasmodium* spp. had clear directional association across controls, surviving cases, and cases who died.

A limitation of this study was that we could not rigorously compare cord blood PCR to cord blood culture since we did not have paired specimens. However, studies such as ANISA²⁸ and SANISA²⁷ which performed blood TAC PCR and culture in parallel reported discordance of results between the two tests. In our study, blood culture was performed on later specimens at ward admission. Although blood culture is the gold standard test for sepsis, culture-negative neonatal sepsis is common¹⁹, and this is evident in the low positivity rate among the pSBI cases in our study. In addition, the tests differed

in the volumes of blood used for processing (0.1 ml equivalent per PCR reaction versus ~2ml for culture⁶¹) and timing of testing (immediately for culture, stored for ~5 years for TAC). Low burden of infection at the limit of detection, different sampling timepoints, and decreased *S. agalactiae* sampling sensitivity due to antisepsis measures associated with caesarean section delivery¹⁴, may have contributed to failure to detect *S. agalactiae* by cord blood TAC, in a pSBI case from whom *S. agalactiae* was isolated from admission blood culture five hours after delivery. In addition, some of the pathogens were fastidious/unculturable and were only detected by TAC. Low aetiological attribution by culture among pSBI cases underscores the need for better diagnostics as bacteraemia was associated with an increased likelihood of case fatality. Sensitivity analysis of neonates excluded in our study despite having available cord blood samples, some of which were of low volumes would have been useful in checking for any selection bias in our case-control selection, and in comparing our PCR results.

Maternal variables at delivery can aid prompt initiation of antimicrobials. Intrapartum fever (temperature $\geq 38^{\circ}\text{C}$), chorioamnionitis, pre-labour rupture of membranes ≥ 18 hours, preterm pre-labour rupture of membranes, PROM ≥ 18 hours, maternal GBS colonization or bacteriuria, multiparity, and poor intrapartum and postpartum infection control practices have previously been shown to predispose neonates to infection^{5,62,63}. We lacked complete data on intrapartum antibiotic use and were unable to assess its impact on pathogen identification. In addition to an immunological immaturity⁶⁴, prematurity, low birth weight, complicated or instrument-assisted delivery, and low APGAR scores, contribute to an increased risk of admission with EONS. Although not the primary aim of our study, we observed that being identified as very preterm (<32 weeks) as well as head circumference and MUAC, which are associated with maturity⁶⁵, were associated with EONS. However, low birth weight was not associated with EONS in our study.

Although TAC provided epidemiological data on potential causes of EONS in our setting, including the role of nonculturable organisms such as *Ureaplasma* spp. and Enterovirus, 90% of pSBI cases lacked epidemiological attribution. The presence of presumed contaminants in both cases and controls was only discernible on a population basis rather than from an individual's results. Thus, despite allowing for customization of a panel of pathogen targets, requirement of small blood volumes, and rapid pathogen detection, TAC in its current form may have a limited role in individual diagnosis in clinical practice, particularly in settings like ours where associated costs of setting up and using this platform will be prohibitive. Further research using this technology alongside highly specific diagnostic methods is needed to better understand the aetiology, distribution and determinants of disease. In addition, our study was limited by use of archived samples and retrospective analysis of data. Future prospective studies using specific definitions of EONS alongside paired cord blood and peripheral blood cultures are needed to better understand the performance of TAC in detection of pathogens associated with EONS.

In conclusion, we were able to identify organisms associated with subsequent EONS and death using cord blood at birth and an identically sampled comparator group of healthy neonates in sub-Saharan Africa. Further prospective research on the clinical utility of cord blood in our setting is needed alongside development and use of rapid and specific point-of-care diagnostics, that will guide prompt management in seriously ill neonates. Robust evidence of the causes of EONS is vital, given the potential for prevention and targeted treatment strategies such as maternal immunization and intrapartum antibiotic prophylaxis⁶⁶, including oral azithromycin for reduction of bacterial carriage and risk of EONS⁶⁷. Coverage for *Ureaplasma* spp. in at-risk neonates should be considered when updating antimicrobial guidelines given the strength of combined data from three studies (ours, SANISA and ANISA) and the potential adverse outcomes associated with this organism⁶⁸.

Data availability

Underlying data

Harvard Dataverse: Replication Data for: Detection of pathogens associated with early-onset neonatal sepsis in cord blood at birth using quantitative PCR, <https://doi.org/10.7910/DVN/FXKGRB>⁶⁹

This project contains the following underlying data:

- Maternal variables-1.tab

- Neonatal variables-1.tab
- PCR Ct values-1.tab

Extended data

Harvard Dataverse: Replication Data for: Detection of pathogens associated with early-onset neonatal sepsis in cord blood at birth using quantitative PCR, <https://doi.org/10.7910/DVN/FXKGRB>⁶⁹

This project contains the following extended data:

- CObiero_Detection of pathogens in cord blood_Codebook.pdf
- CObiero_Detection of pathogens in cord blood_readme.txt
- Detection of pathogens at birth_Extended data.pdf

Data are available under the terms of the [Creative Commons Attribution 4.0 International license](#) (CC-BY 4.0).

Acknowledgements

We thank all the children and parents/guardians who contributed to this analysis. This study is published with the permission of the Director of the KWTRP. Surveillance at Kilifi County Hospital was undertaken at the KWTRP and we thank the clinical and nursing staff and all those involved.

References

- Hug L, Alexander M, You D, *et al.*: **National, regional, and global levels and trends in neonatal mortality between 1990 and 2017, with scenario-based projections to 2030: a systematic analysis.** *Lancet Glob Health.* 2019; 7(6): e710–e20.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Baqui AH, Mitra DK, Begum N, *et al.*: **Neonatal mortality within 24 hours of birth in six low- and lower-middle-income countries.** *Bull World Health Organ.* 2016; 94(10): 752–58B.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Alliance for Maternal and Newborn Health Improvement (AMANHI) mortality study group: **Population-based rates, timing, and causes of maternal deaths, stillbirths, and neonatal deaths in south Asia and sub-Saharan Africa: a multi-country prospective cohort study.** *Lancet Glob Health.* 2018; 6(12): e1297–e308.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- 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(Suppl 1): 73–85.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Camacho-Gonzalez A, Spearman PW, Stoll BJ: **Neonatal infectious diseases: evaluation of neonatal sepsis.** *Pediatr Clin North Am.* 2013; 60(2): 367–89.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- McGovern M, Giannoni E, Kuester H, *et al.*: **Challenges in developing a consensus definition of neonatal sepsis.** *Pediatr Res.* 2020; 88(1): 14–26.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Vergnano S, Menson E, Kennea N, *et al.*: **Neonatal infections in England: the NeonIN surveillance network.** *Arch Dis Child Fetal Neonatal Ed.* 2011; 96(1): F9–F14.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Shah P, Yoon EW, Chan P, *et al.*: **The Canadian Neonatal Network Annual Report.** 2016.
[Reference Source](#)
- Stoll BJ, Gordon T, Korones SB, *et al.*: **Early-onset sepsis in very low birth weight neonates: a report from the National Institute of Child Health and Human Development Neonatal Research Network.** *J Pediatr.* 1996; 129(1): 72–80.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Centre for Disease Control and Prevention: **ABCs Report: group B streptococcus, 2017.** 2017.
[Reference Source](#)
- Zaidi AK, Huskins WC, Thaver D, *et al.*: **Hospital-acquired neonatal infections in developing countries.** *Lancet.* 2005; 365(9465): 1175–88.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Zaidi AK, Thaver D, Ali SA, *et al.*: **Pathogens associated with sepsis in newborns and young infants in developing countries.** *Pediatr Infect Dis J.* 2009; 28(1 Suppl): S10–8.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Seale AC, Bianchi-Jassir F, Russell NJ, *et al.*: **Estimates of the Burden of Group B Streptococcal Disease Worldwide for Pregnant Women, Stillbirths, and Children.** *Clin Infect Dis.* 2017; 65(suppl_2): S200–S19.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Seale AC, Koech AC, Sheppard AE, *et al.*: **Maternal colonization with *Streptococcus agalactiae* and associated stillbirth and neonatal disease in coastal Kenya.** *Nat Microbiol.* 2016; 1(7): 16067.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Quan V, Verani JR, Cohen C, *et al.*: **Invasive Group B Streptococcal Disease in South Africa: Importance of Surveillance Methodology.** *PLoS One.* 2016; 11(4): e0152524.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Harris JB, Holmes AP: **Neonatal Herpes Simplex Viral Infections and Acyclovir: An Update.** *J Pediatr Pharmacol Ther.* 2017; 22(2): 88–93.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Yadav SS, Narula G, Narayan S, *et al.*: **Cytomegalovirus infection in six neonates.** *Indian Pediatr.* 2010; 47(2): 174–5.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Cantey JB, Baird SD: **Ending the Culture of Culture-Negative Sepsis in the Neonatal ICU.** *Pediatrics.* 2017; 140(4): e20170044.
[PubMed Abstract](#) | [Publisher Full Text](#)

19. Klingenberg C, Kornelisse RF, Buonocore G, *et al.*: **Culture-Negative Early-Onset Neonatal Sepsis - At the Crossroad Between Efficient Sepsis Care and Antimicrobial Stewardship.** *Front Pediatr.* 2018; **6**: 285.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
20. Schelonka RL, Chai MK, Yoder BA, *et al.*: **Volume of blood required to detect common neonatal pathogens.** *J Pediatr.* 1996; **129**(2): 275–8.
[PubMed Abstract](#) | [Publisher Full Text](#)
21. Viel-Therault I, Fell DB, Grynspan D, *et al.*: **The transplacental passage of commonly used intrapartum antibiotics and its impact on the newborn management: A narrative review.** *Early Hum Dev.* 2019; **135**: 6–10.
[PubMed Abstract](#) | [Publisher Full Text](#)
22. Pammi M, Flores A, Versalovic J, *et al.*: **Molecular assays for the diagnosis of sepsis in neonates.** *Cochrane Database Syst Rev.* 2017; **2**(2): CD011926.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
23. Investigators of the Delhi Neonatal Infection Study (DeNIS) collaboration: **Characterisation and antimicrobial resistance of sepsis pathogens in neonates born in tertiary care centres in Delhi, India: a cohort study.** *Lancet Glob Health.* 2016; **4**(10): e752–60.
[PubMed Abstract](#) | [Publisher Full Text](#)
24. Seedat F, Stinton C, Patterson J, *et al.*: **Adverse events in women and children who have received intrapartum antibiotic prophylaxis treatment: a systematic review.** *BMC Pregnancy Childbirth.* 2017; **17**(1): 247.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
25. Águeda S, Leitão A, Rocha G, *et al.*: **Viral Infections in a Neonatal Intensive Care Unit.** *Pediatr Therapeut.* 2013; **3**(2): 154.
[Publisher Full Text](#)
26. Diaz MH, Waller JL, Napoliello RA, *et al.*: **Optimization of Multiple Pathogen Detection Using the TaqMan Array Card: Application for a Population-Based Study of Neonatal Infection.** *PLoS One.* 2013; **8**(6): e66183.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
27. Velaphi SC, Westercamp M, Moleleki M, *et al.*: **Surveillance for incidence and etiology of early-onset neonatal sepsis in Soweto, South Africa.** *PLoS One.* 2019; **14**(4): e0214077.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
28. Saha SK, Schrag SJ, El Arifeen S, *et al.*: **Causes and incidence of community-acquired serious infections among young children in south Asia (ANISA): an observational cohort study.** *Lancet.* 2018; **392**(10142): 145–59.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
29. Polin JJ, Knox I, Baumgart S, *et al.*: **Use of umbilical cord blood culture for detection of neonatal bacteremia.** *Obstet Gynecol.* 1981; **57**(2): 233–7.
[PubMed Abstract](#)
30. Newberry DM: **Comparison of Placental and Neonatal Admission Complete Blood Cell Count and Blood Cultures.** *Adv Neonatal Care.* 2018; **18**(3): 215–22.
[PubMed Abstract](#) | [Publisher Full Text](#)
31. Mithal LB, Palac HL, Yogev R, *et al.*: **Cord Blood Acute Phase Reactants Predict Early Onset Neonatal Sepsis in Preterm Infants.** *PLoS One.* 2017; **12**(1): e0168677.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
32. Su H, Chang SS, Han CM, *et al.*: **Inflammatory markers in cord blood or maternal serum for early detection of neonatal sepsis-a systemic review and meta-analysis.** *J Perinatol.* 2014; **34**(4): 268–74.
[PubMed Abstract](#) | [Publisher Full Text](#)
33. Herson VC, Block C, McLaughlin JC, *et al.*: **Placental blood sampling: an aid to the diagnosis of neonatal sepsis.** *J Perinatol.* 1998; **18**(2): 135–7.
[PubMed Abstract](#)
34. Mithal LB, Malczynski M, Qi C, *et al.*: **Umbilical Cord Blood Diagnostics for Early Onset Sepsis in Premature Infants: Detection of Bacterial DNA and Systemic Inflammatory Response.** *bioRxiv.* 2017; 200337.
[Publisher Full Text](#)
35. Beeram MR, Loughran C, Cipriani C, *et al.*: **Utilization of umbilical cord blood for the evaluation of group B streptococcal sepsis screening.** *Clin Pediatr (Phila).* 2012; **51**(5): 447–53.
[PubMed Abstract](#) | [Publisher Full Text](#)
36. Seale AC, Barsosio HC, Koeh AC, *et al.*: **Embedding surveillance into clinical care to detect serious adverse events in pregnancy.** *Vaccine.* 2015; **33**(47): 6466–8.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
37. Lang'at E, Mwanri L, Temmerman M: **Effects of implementing free maternity service policy in Kenya: an interrupted time series analysis.** *BMC Health Serv Res.* 2019; **19**(1): 645.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
38. Scott JA, Bauni E, Moisi JC, *et al.*: **Profile: The Kilifi Health and Demographic Surveillance System (KHDSS).** *Int J Epidemiol.* 2012; **41**(3): 650–7.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
39. Young Infants Clinical Signs Study Group: **Clinical signs that predict severe illness in children under age 2 months: a multicentre study.** *Lancet.* 2008; **371**(9607): 135–42.
[PubMed Abstract](#) | [Publisher Full Text](#)
40. 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.
[PubMed Abstract](#) | [Publisher Full Text](#)
41. Obiero CW, Mturi N, Mwarumba S, *et al.*: **Clinical features to distinguish meningitis among young infants at a rural Kenyan hospital.** *Arch Dis Child.* 2021; **106**(2): 130–136.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
42. Liu J, Ochieng C, Wiersma S, *et al.*: **Development of a TaqMan Array Card for Acute-Febrile-Illness Outbreak Investigation and Surveillance of Emerging Pathogens, Including Ebola Virus.** *J Clin Microbiol.* 2016; **54**(1): 49–58.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
43. Moore CC, Jacob ST, Banura P, *et al.*: **Etiology of Sepsis in Uganda Using a Quantitative Polymerase Chain Reaction-based TaqMan Array Card.** *Clin Infect Dis.* 2019; **68**(2): 266–72.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
44. Seale AC, Obiero CW, Jones KD, *et al.*: **Should First-line Empiric Treatment Strategies for Neonates Cover Coagulase-negative Staphylococcal Infections in Kenya?** *Pediatr Infect Dis J.* 2017; **36**(11): 1073–78.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
45. Abade A, Eidex RB, Maro A, *et al.*: **Use of TaqMan Array Cards to Screen Outbreak Specimens for Causes of Febrile Illness in Tanzania.** *Am J Trop Med Hyg.* 2018; **98**(6): 1640–42.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
46. Newson R: **PUNAFCC: Stata module to compute population attributable fractions for case-control and survival studies.** 2017.
[Reference Source](#)
47. Sprong KE, Mabenge M, Wright CA, *et al.*: **Ureaplasma species and preterm birth: current perspectives.** *Crit Rev Microbiol.* 2020; **46**(2): 169–81.
[PubMed Abstract](#) | [Publisher Full Text](#)
48. Viscardi RM, Terrin ML, Magder LS, *et al.*: **Randomised trial of azithromycin to eradicate Ureaplasma in preterm infants.** *Arch Dis Child Fetal Neonatal Ed.* 2020; **105**(6): 615–22.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
49. Chuang YY, Huang YC: **Enteroviral infection in neonates.** *J Microbiol Immunol Infect.* 2019; **52**(6): 851–57.
[PubMed Abstract](#) | [Publisher Full Text](#)
50. WHO Guidelines Approved by the Guidelines Review Committee: **Pocket book of hospital care for children: Guidelines for the management of common childhood illnesses.** Second ed: World Health Organization, 2013; 412.
[PubMed Abstract](#)
51. MOH: **Basic Paediatric Protocols for ages up to 5 years.** Fourth ed, 2016.
[Reference Source](#)
52. Hermansen CL, Mahajan A: **Newborn Respiratory Distress.** *Am Fam Physician.* 2015; **92**(11): 994–1002.
[Reference Source](#)
53. Lowe C, Willey B, O'Shaughnessy A, *et al.*: **Outbreak of extended-spectrum beta-lactamase-producing Klebsiella oxytoca infections associated with contaminated handwashing sinks(1).** *Emerg Infect Dis.* 2012; **18**(8): 1242–1247.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
54. Underwood MA, Sohn K: **The Microbiota of the Extremely Preterm Infant.** *Clin Perinatol.* 2017; **44**(2): 407–427.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
55. Meena R, Meena KK, Athwani V, *et al.*: **Umbilical Cord Blood Culture in Diagnosis of Early Onset Neonatal Sepsis.** *Indian J Pediatr.* 2020; **87**(10): 793–797.
[PubMed Abstract](#) | [Publisher Full Text](#)
56. Barsosio HC, Gitonga JN, Karanja HK, *et al.*: **Congenital microcephaly unrelated to flavivirus exposure in coastal Kenya.** *Wellcome Open Res.* 2019; **4**: 179.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
57. Hou GQ, Chen SS, Lee CP: **Pathogens in maternal blood and fetal cord blood using Q-PCR assay.** *Taiwan J Obstet Gynecol.* 2006; **45**(2): 114–9.
[PubMed Abstract](#) | [Publisher Full Text](#)
58. Theiler RN, Caliendo AM, Pargman S, *et al.*: **Umbilical cord blood screening for cytomegalovirus DNA by quantitative PCR.** *J Clin Virol.* 2006; **37**(4): 313–6.
[PubMed Abstract](#) | [Publisher Full Text](#)
59. Avila EC, Finger-Jardim F, Goncalves CV, *et al.*: **High Incidence of Herpes Simplex Virus-1 in Cord Blood and Placenta Infection of Women in Southern Brazil.** *Rev Bras Ginecol Obstet.* 2020; **42**(1): 5–11.
[PubMed Abstract](#) | [Publisher Full Text](#)
60. Dollard SC, Grosse SD, Ross DS: **New estimates of the prevalence of neurological and sensory sequelae and mortality associated with congenital cytomegalovirus infection.** *Rev Med Virol.* 2007; **17**(5): 355–63.
[PubMed Abstract](#) | [Publisher Full Text](#)
61. Huber S, Hetzer B, Crazzolara R, *et al.*: **The correct blood volume for paediatric blood cultures: a conundrum?** *Clin Microbiol Infect.* 2020; **26**(2): 168–73.
[PubMed Abstract](#) | [Publisher Full Text](#)
62. Schuchat A, Zywicki SS, Dinsmoor MJ, *et al.*: **Risk factors and opportunities for prevention of early-onset neonatal sepsis: a multicenter case-control study.** *Pediatrics.* 2000; **105**(1 Pt 1): 21–6.
[PubMed Abstract](#) | [Publisher Full Text](#)
63. Chan GJ, Lee AC, Baqui AH, *et al.*: **Risk of early-onset neonatal infection with maternal infection or colonization: a global systematic review and meta-analysis.** *PLoS Med.* 2013; **10**(8): e1001502.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)

64. Fleer A, Gerards LJ, Verhoef J: **Host defence to bacterial infection in the neonate.** *J Hosp Infect.* 1988; **11 Suppl A**: 320–7.
[PubMed Abstract](#) | [Publisher Full Text](#)
65. Thawani R, Dewan P, Faridi MM, *et al.*: **Estimation of gestational age using neonatal anthropometry: a cross-sectional study in India.** *J Health Popul Nutr.* 2013; **31**(4): 523–30.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
66. Boyer KM, Gotoff SP: **Prevention of early-onset neonatal group B streptococcal disease with selective intrapartum chemoprophylaxis.** *N Engl J Med.* 1986; **314**(26): 1665–9.
[PubMed Abstract](#) | [Publisher Full Text](#)
67. Roca A, Oluwalana C, Bojang A, *et al.*: **Oral azithromycin given during labour decreases bacterial carriage in the mothers and their offspring: a double-blind randomized trial.** *Clin Microbiol Infect.* 2016; **22**(6): 565.e1–9.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
68. Viscardi RM: **Ureaplasma species: role in neonatal morbidities and outcomes.** *Arch Dis Child Fetal Neonatal Ed.* 2014; **99**(1): F87–92.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
69. Obiero CW, Gumbi W, Mwakio S, *et al.*: **Replication Data for: Detection of pathogens associated with early-onset neonatal sepsis in cord blood at birth using quantitative PCR.** Harvard Dataverse, V2. 2021.
<http://www.doi.org/10.7910/DVN/FXKGRB>

Open Peer Review

Current Peer Review Status:  

Version 3

Reviewer Report 09 November 2022

<https://doi.org/10.21956/wellcomeopenres.19881.r53199>

© 2022 Le Doare K. This is an open access peer review report distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.



Kirsty Le Doare 

Paediatric Infectious Diseases Research Group and Vaccine Institute, Institute of Infection and Immunity, St George's University of London, London, UK

All changes look appropriate. I maintain my status of 'Approved'.

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Neonatal infection, vaccines, antimicrobial resistance, global health

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Version 2

Reviewer Report 17 May 2022

<https://doi.org/10.21956/wellcomeopenres.19812.r50517>

© 2022 Le Doare K. This is an open access peer review report distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.



Kirsty Le Doare 

Paediatric Infectious Diseases Research Group and Vaccine Institute, Institute of Infection and Immunity, St George's University of London, London, UK

This study from Kenya investigated early-onset sepsis over a 5 year period using the TaqMan array comparing cases and controls. Such studies are much needed as there is very little information

about early-onset aetiology in African countries.

The manuscript is well written and there are only a few minor points to consider:

1. The global health community is moving away from using terms such as “sub-Saharan Africa” and “developing countries” in preference for “African countries” and “resource-limited settings”, as there is a lot of heterogeneity between countries in SSA in terms of development and resources.
2. Methods – Were antibiotics, in labour for example, because of caesarian section monitored? As this could account for the fairly low positivity of results, even by PCR.
3. Methods – How were cases and controls selected? Was any sensitivity analysis undertaken to look for potential bias in case selection?
4. Results – How did the authors account for the fact that the majority of pathogens were not identified by culture?
5. Results – Is it possible to look at the *S. epi* as a potential pathogen if any of the infants were premature or VLBW?
6. Discussion – As with all such studies, relating DNA/RNA to clinical presentation is difficult and the authors have found >1 pathogen in many cases, so it is still difficult to identify causative agents. On the other hand, the fact that the same pathogens appear as have been found in culture but that more fastidious bacteria (ureaplasma) is found on PCR highlights both the need for a better method of assessing sepsis in infants and the need for more prospective studies using molecular methods to identify causation.

Is the work clearly and accurately presented and does it cite the current literature?

Yes

Is the study design appropriate and is the work technically sound?

Yes

Are sufficient details of methods and analysis provided to allow replication by others?

Yes

If applicable, is the statistical analysis and its interpretation appropriate?

Yes

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Neonatal infection, vaccines, antimicrobial resistance, global health

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Author Response 27 May 2022

Christina Obiero, KEMRI-Wellcome Trust Research Programme, Kilifi, Kenya

This study from Kenya investigated early-onset sepsis over a 5-year period using the TaqMan array comparing cases and controls. Such studies are much needed as there is very little information about early-onset aetiology in African countries.

The manuscript is well written and there are only a few minor points to consider:

1. The global health community is moving away from using terms such as “sub-Saharan Africa” and “developing countries” in preference for “African countries” and “resource-limited settings”, as there is a lot of heterogeneity between countries in SSA in terms of development and resources.

Response: Thank you for this important comment. We acknowledge that countries in Africa have different levels of social, economic, environmental, and political development and this plays a role in health service delivery and outcomes. In addition, populations residing in African countries face regional health disparities due to unequitable distribution of resources. In our manuscript, we used the term “sub-Saharan Africa” to refer to data on the aetiology and burden of neonatal sepsis obtained from studies conducted in this region that is distinct from African countries within the Sahara, and other resource-limited settings in Asia and the Middle East.

2. **Methods** – Were antibiotics, in labour for example, because of caesarian section monitored? As this could account for the fairly low positivity of results, even by PCR.

Response: Mothers presenting with signs of infection during labour at Kilifi County Hospital (KCH) are treated with antibiotics. Screening for Group B Streptococcus and peripartum antibiotic prophylaxis are not done at KCH. During the Kilifi Perinatal and Maternal Surveillance (KIPMAT), data on antibiotics used within 4 weeks of labour was collected on a standardised maternal admission record as described in the manuscript. If a mother received antibiotics, clinicians documented whether this was indicated for premature rupture of membranes or not. Data on antibiotic use was not complete as indicated in the discussion section and we were, therefore, unable to assess the impact of antibiotic pretreatment on PCR and culture (cases only) results.

Discussion, page 22: “We lacked complete data on intrapartum antibiotic use and were unable to assess its impact on pathogen identification.”

3. **Methods** – How were cases and controls selected? Was any sensitivity analysis undertaken to look for potential bias in case selection?

Response: Cases and controls were selected from a cohort of 15,409 neonates born at KCH during the study period. Cases included in the analysis were residents of the Kilifi Health and Demographic Surveillance System (KHDSS) and were hospitalised at KCH within the first 48 hours

of life with signs of World Health Organisation-defined possible serious bacterial infection (pSBI). Controls were also KHDSS residents, discharged home well after delivery, and not hospitalised/remained alive during the first 60 days of life. Both cases and controls needed to have adequate volumes of cord blood samples available for analysis. All 604 cases whose cord blood samples were tested had cord blood samples available. In addition to meeting the criteria for being a control, including having cord blood samples available for analysis, the 300 controls in our study were randomly selected from a cohort of 6,295 healthy neonates. Selection bias cannot be completely ruled out especially since those with available cord blood samples were included while some neonates with available cord blood samples were excluded from our analysis. However, we were unable to perform a sensitivity analysis, e.g. testing of samples obtained from neonates who had available samples but were not included in the analysis as this would have required additional diagnostic resources. We have added this as a limitation as shown below:

Discussion, page 22: “Sensitivity analysis of neonates excluded in our study despite having available cord blood samples, some of which were of low volumes would have been useful in checking for any selection bias in our case-control selection, and in comparing our PCR results.”

4. Results – How did the authors account for the fact that the majority of pathogens were not identified by culture?

Response: Thank you for this important comment. Only 1.8% of blood cultures done among 603 pSBI cases were positive for known pathogens. None of the controls had cultures done as they were discharged home well soon after birth. Our low culture yield is consistent with that in similar settings. This is because of factors such as poor culture sensitivity, low sample volumes (secondary to difficult venepuncture among neonates), and (maternal) antibiotic pre-treatment. In addition, some of the pathogens detected by TAC are fastidious/unculturable and hence were not identified by culture. Discordance between TAC PCR and culture results was reported in the ANISA and SANISA studies. In our study, TAC PCR and culture samples were collected and tested under different circumstances, as described in the discussion. In addition, we proposed that future studies testing paired cord blood and peripheral venous blood samples using advanced molecular tests and comparing these to culture will be more informative on the aetiology of sepsis and the performance of these tests.

*Discussion, page 22: “A limitation of this study was that we could not rigorously compare cord blood PCR to cord blood culture since we did not have paired specimens. **However, studies such as ANISA and SANISA which performed blood TAC PCR and culture in parallel reported discordance of results between the two tests. In our study, blood culture was performed on later specimens at ward admission. Although blood culture is the gold standard test for sepsis, culture-negative neonatal sepsis is common¹⁹, and this is evident in the low positivity rate among the pSBI cases in our study. In addition, the tests differed in the volumes of blood used for processing (0.1 ml equivalent per PCR reaction versus ~2ml for culture⁶¹) and timing of testing (immediately for culture, stored for ~5 years for TAC). Low burden of infection at the limit of detection, different sampling timepoints, and decreased *S. agalactiae* sampling sensitivity due to antiseptics measures associated with caesarean section delivery¹⁴, may have contributed to failure to detect *S. agalactiae* by cord blood TAC, in a pSBI case from whom *S. agalactiae* was isolated***

from admission blood culture five hours after delivery. **In addition, some of the pathogens were fastidious/unculturable and were only detected by TAC.** Low aetiological attribution by culture among pSBI cases underscores the need for better diagnostics as bacteraemia was associated with an increased likelihood of case fatality."

5. Results – Is it possible to look at the *S. epi* as a potential pathogen if any of the infants were premature or VLBW?

Response: While customising the TaqMan Array Card (TAC), we reviewed the literature and considered organisms shown to cause neonatal sepsis in previous studies as indicated on page 7 (Detection of targets using TAC RT-qPCR). S. epidermidis is a common culture contaminant in different settings including ours. It was the leading admission blood culture contaminant (n=27) among all pSBI cases. We previously examined systematic clinical and microbiologic surveillance data from all neonatal admissions (including premature neonates) to KCH to determine the association of Coagulase-negative Staphylococci (CoNS [including S. epidermidis]) with case fatality and/or prolonged duration of admission among neonates.¹ We found that CoNS had no clinical significance in our setting where long intravascular lines and invasive ventilation are not available, hence not requiring targeted antibiotic treatment. We revised the section describing the selection of TAC targets as follows:

"Detection of targets using TAC RT-qPCR, page 7: "Organisms such as CoNS that have been previously shown to be clinically insignificant in our setting⁴⁴ were not included in the TAC panel."

6. Discussion – As with all such studies, relating DNA/RNA to clinical presentation is difficult and the authors have found >1 pathogen in many cases, so it is still difficult to identify causative agents. On the other hand, the fact that the same pathogens appear as have been found in culture but that more fastidious bacteria (ureaplasma) is found on PCR highlights both the need for a better method of assessing sepsis in infants and the need for more prospective studies using molecular methods to identify causation.

Response: Thank you for this important observation. Aetiological attribution of pathogens to infection is challenging, especially where multiple organisms are detected. This has been shown in several studies (e.g. ANISA and SANISA) investigating the aetiology of infection and using advanced statistical analysis, e.g. Bayesian latent class models. This demonstrates the need for more advanced diagnostics, such as the use of pathogen-specific biomarkers and metagenomics, to better understand the epidemiology of neonatal sepsis, given the large proportion of cases lacking causal attribution following the use of TAC reverse-transcription quantitative polymerase chain reaction.

Reference

1. Seale AC, Obiero CW, Jones KD, et al. Should First-line Empiric Treatment Strategies for Neonates Cover Coagulase-negative Staphylococcal Infections in Kenya? *The Pediatric infectious disease journal* 2017;36(11):1073-78. doi: 10.1097/INF.0000000000001699 [published Online First: 2017/07/22]

Competing Interests: No competing interests to declare.

Version 1

Reviewer Report 19 April 2022

<https://doi.org/10.21956/wellcomeopenres.19223.r49589>

© 2022 Neemann K. This is an open access peer review report distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.



Kari A. Neemann 

Pediatrics, Division of Infectious diseases, Children's Hospital and Medical Center, University of Nebraska Medical Center, Omaha, NE, USA

I would like to commend the authors for preparing a well-cited and thoughtful interpretation of their results. Overall, it is hard what to make of this data in the clinical setting. None of the potential identified pathogens on the cord blood sample were identified by the traditional "gold standard" blood culture which even with its limitations is hard to account for. A large percentage of neonates in both the cases and controls had ≥ 1 typical pathogen identified (*K. oxytoca*, *Streptococcus pyogenes*, and *P. aeruginosa*); organisms which, with the noted exception for *K. oxytoca*, are not typically considered contaminants. I agree with the authors that further prospective studies utilizing paired samples of cord blood multiplex PCR, cord blood culture, and peripheral blood cultures in EONS are needed to fully evaluate the utility of this assay in the clinical setting.

Materials and Methods:

1. "Cases were defined as neonates hospitalized within 48 hours of life with one or more features of pSBI." Please confirm that none of the cases were discharged from the hospital and re-admitted within 48 hours of life.
2. While *Staphylococcus epidermidis* is a common contaminant in most populations, it can represent a true pathogen in neonates though admittedly more so as late-onset sepsis in VLBW infants. Was there any thought to including this as a pathogen?
3. Did every neonate with pSBI have a blood culture obtained? While it is mentioned as routine clinical care it is not clear whether this occurred or not. Try to determine how to interpret the positive blood culture results in the results sections.

Results:

1. 'Table 1. Prolonged rapture of membranes' - change *rapture* to *rupture*.
2. Figure 3. Would label on the right Groups 1-4.

Discussion:

1. I thought the authors did a good job of describing the strengths and limitations of the study and putting this study into the context of the ANISA and SANISA trials.

Is the work clearly and accurately presented and does it cite the current literature?

Yes

Is the study design appropriate and is the work technically sound?

Yes

Are sufficient details of methods and analysis provided to allow replication by others?

Yes

If applicable, is the statistical analysis and its interpretation appropriate?

Yes

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Pediatric Infectious Diseases

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Author Response 03 May 2022

Christina Obiero, KEMRI-Wellcome Trust Research Programme, Kilifi, Kenya

Many thanks for the comments on this manuscript. We have revised the manuscript based on reviewer's comments and responded to specific points as below.

Materials and Methods:

1. "Cases were defined as neonates hospitalized within 48 hours of life with one or more features of pSBI." Please confirm that none of the cases were discharged from the hospital and re-admitted within 48 hours of life.

Response: Thank you for this important comment. All cases included in this analysis were admitted within the first 48 hours of life. None of the pSBI cases were discharged home from the hospital and readmitted within the first 48 hours of life. Unique identifiers are used at our hospital for each admission and laboratory sample, and none of the cases had more than one

episode of admission during the first 60 days of life, including the first 48 hours of life when they developed signs of pSBI and were hospitalised. We have clarified this in the relevant section as shown below:

Study design and participants, Page 6: “None of the cases were readmitted following the initial hospital admission, including during the first 48 hours of life.”

2. While *Staphylococcus epidermidis* is a common contaminant in most populations, it can represent a true pathogen in neonates though admittedly more so as late-onset sepsis in VLBW infants. Was there any thought to including this as a pathogen?

*Response: While customising the TaqMan Array Card (TAC), we reviewed literature and considered organisms shown to cause neonatal sepsis in previous studies as indicated on page 8 (Detection of targets using TAC RT-qPCR). *S. epidermidis* is a common culture contaminant in different settings including ours. It was the leading admission blood culture contaminant (n=27) among all pSBI cases. We previously examined systematic clinical and microbiologic surveillance data from all neonatal admissions to Kilifi County Hospital to determine association of Coagulase-negative Staphylococci (CoNS [including *S. epidermidis*]) with case fatality and/or prolonged duration of admission among neonates and found that CoNS were not clinically significant organisms in our setting where long intravascular lines and invasive ventilation are not available, hence not requiring targeted antibiotic treatment.¹ In addition, our clinical setting lacks factors that often predispose patients to colonization and invasion by CoNS e.g. use of indwelling medical devices. We have revised the section describing the selection of TAC targets as follows:*

Detection of targets using TAC RT-qPCR, page 8: “Organisms such as CoNS that have been previously shown to be clinically insignificant in our setting⁴⁴ were not included in the TAC panel.”

3. Did every neonate with pSBI have a blood culture obtained? While it is mentioned as routine clinical care it is not clear whether this occurred or not. Try to determine how to interpret the positive blood culture results in the results sections.

Response: Thank you for this important comment. All neonates with pSBI had blood culture done at admission for clinical care and as part of ongoing clinical surveillance for bacteraemia on the ward. We have clarified this section as follows:

*Study design and participants, page 6: “Routine laboratory investigations **for all admissions** for clinical care included blood culture (BACTEC Peds Plus/F bottles and BACTEC 9050 instrument, Becton Dickinson, UK) and cerebrospinal fluid (CSF) culture where indicated, as previously described⁴¹.”*

*All 603 pSBI cases included in this analysis had blood cultures done at admission. Eleven (1.8%) neonates had presumed pathogens (led by *Staphylococcus aureus* [n=5]) isolated from blood culture while 37 (6.1%) had contaminants (led by *S. epidermidis* [n=27]). Cord blood samples and admission blood culture samples were obtained at different timepoints and correlation of these two results was not possible in our study as discussed on page 15. In the results section, we indicated that the odds ratio for a positive admission blood culture for death among admitted*

pSBI cases was 1.1 (95% CI 0.24-5.2) and 0.2% (95% CI 0-3.2) of pSBI were attributed to the pathogens identified through blood culture. This means that although bacteraemia was associated with increased mortality among pSBI cases, culture yields were low and provided little information about pathogens causing morbidity and death among these hospitalised neonates. We have updated the discussion section as follows:

Discussion, page 15: ***“Low aetiological attribution by culture among pSBI cases underscores the need for better diagnostics as bacteraemia was associated with an increased likelihood of case fatality.”***

Results:

1. 'Table 1. Prolonged rapture of membranes' - change rapture to rupture.

Response: Thank you for this helpful observation. We have corrected the spelling as required.

2. Figure 3. Would label on the right Groups 1-4.

Response: Thank you for this helpful suggestion. We have labelled the groups on Figure 3 appropriately.

Discussion:

1. I thought the authors did a good job of describing the strengths and limitations of the study and putting this study into the context of the ANISA and SANISA trials.

Response: Many thanks for this comment. Results obtained from our study, ANISA, and SANISA demonstrate the need for more advanced diagnostics such as the use of pathogen-specific biomarkers and metagenomics to better understand the epidemiology of neonatal sepsis in low- and middle-income countries, given the large proportion of cases lacking causal attribution following use of TAC reverse-transcription quantitative polymerase chain reaction.

Reference

1. Seale AC, Obiero CW, Jones KD, et al. Should First-line Empiric Treatment Strategies for Neonates Cover Coagulase-negative Staphylococcal Infections in Kenya? *The Pediatric infectious disease journal* 2017;36(11):1073-78. doi: 10.1097/INF.0000000000001699 [published Online First: 2017/07/22]

Competing Interests: We have no competing interests to declare.