The Association between Early-Onset Sepsis and Neonatal Encephalopathy

Kathleen P. Car[1], Firdose Nakwa[1], Fatima Solomon[2,3] Sithembiso C. Velaphi[1], Cally J. Tann[4,5,6], Alane Izu[2,3], Sanjay G Lala[1,7], Shabir A. Madhi[2,3*], Ziyaad Dangor[1,2,3*]

Affiliations: [1]Department of Paediatrics, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa. [2] South African Medical Research Council: Vaccines and Infectious Diseases Analytical Research Unit, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa. [3]Department of Science and Technology/National Research Foundation: Vaccine Preventable Diseases, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa [4] Department of Infectious Disease Epidemiology, School of Hygiene and Tropical Medicine, London, UK. [5] Medical Research Council/Uganda Virus Research Institute and London School of Hygiene and Tropical Medicine Uganda Research Unit, Entebbe, Uganda. [6] Institute for Women's Health, University College London, London, UK. [7]Perinatal HIV Research Unit, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa

*SAM and ZD contributed equally to manuscript as co-senior authors.

Address correspondence to:
Ziyaad Dangor
Department of Paediatrics, University of Witwatersrand,
26 Chris Hani Road, Soweto
Email: ziyaad.dangor@wits.ac.za
(Tel) +27 83 471 0933
(Fax) +27 86 765 6326

Running Title: Early-onset sepsis and neonatal encephalopathy

Conflict of interest: All authors have no conflicts of interest to disclose

Funding Source: No funding was secured for this study.

Financial Disclosure: SAM is funded in part by National Research Foundation/ Department of Science and Technology: South African Research Chair Initiative in Vaccine Preventable Diseases and South Africa Medical Research Council. ZD is in part funded by the Carnegie Corporation of New York (Grant number B8749). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Abbreviations:

aORs:	Adjusted odds ratios
CHBAH:	Chris Hani Baragwaneth Academic Hospital
CI:	Confidence Interval
CFR:	Case Fatality Rate
CoNS:	Coagulase-negative staphylococci
CRP:	C-reactive protein
CSF:	Cerebrospinal Fluid
DHIS:	District Health Information System
EOS:	Early-onset sepsis
GBS:	Group B Streptococcus, *Streptococcus Agalactiae*
HIE:	Hypoxic Ischeamic Encephalopathy
HIV:	Human immunodeficiency virus
HREC:	Human Research Ethics committee
IAP:	Intrapartum Antibiotic Prophylaxis
ICD-10:	International Classification of Diseases, Tenth revision
IQR:	Interquartile Range
LMIC:	Low middle income country
NE:	Neonatal Encephalopathy
NHLS:	National Health Laboratory Service
PCR:	Polymerase Chain Reaction
TOBY:	Total Body Hypothermia Study
UK:	United Kingdom
VIDA:	Vaccines and Infectious Diseases Analytical Research Unit

Abstract:

Objective: We evaluated the association between early-onset sepsis and neonatal encephalopathy in a low-middle-income setting.

Methods: We undertook a retrospective study in newborns with gestational age ≥35 weeks and/or birth weight ≥2 500 grams, diagnosed with neonatal encephalopathy. Early-onset sepsis was defined as culture-confirmed sepsis or probable sepsis.

R**esults:** Of 10 182 hospitalized newborns, 1 027 (10.1%) were diagnosed with neonatal encephalopathy, of whom 52 (5.1%) had culture-confirmed and 129 (12.5%) probable sepsis. The case fatality rate for culture-confirmed sepsis associated neonatal encephalopathy was 3-fold higher compared to neonatal encephalopathy without sepsis (30.8% vs. 10.5%, p<0.001). Predictors of mortality for culture-confirmed sepsis associated neonatal encephalopathy included severe neonatal encephalopathy (aOR 6.51, 95%CI: 1.03-41.44) and seizures (aOR 10.64, 95%CI: 1.05-107.39).

Conclusion: In this setting, 5% of neonatal encephalopathy cases was associated with culture-confirmed sepsis and a high case fatality rate.

Introduction

The year-on-year decline in neonatal mortality rates (19 per 1 000 live births) from 2000 to 2015, has lagged behind the reduction in death rates in children 1-59 months of age (43 per 1 000 live births), resulting in newborns now contributing up to 45% of all under-5 childhood deaths(1). In South Africa, the estimated neonatal mortality rate in 2017 was 12 per 1 000 live births and accounted for 32% of under-five childhood deaths (2,3). Using post-mortem minimally invasive tissue sampling coupled with ante-mortem clinical information, we recently reported complications of prematurity (52.9%), complications of intrapartum events (15.0%) and infection (9.8%) being the leading causes of neonatal deaths in our setting (2).

Neonatal encephalopathy (NE) is characterized by disturbed neurologic function, manifesting as reduced level of consciousness or seizures, difficulty with initiating and maintaining respiration, and depression of tone and reflexes in an infant born at or beyond 35 weeks of gestation(4). Globally, the incidence of neonatal encephalopathy is estimated at 3.0 (95%CI: 2.7-3.3) per 1 000 live births(5); being in the causal pathway of approximately 23% neonatal deaths (99% occurring in low and middle income countries; LMICs)(6). Although pathogenesis of neonatal encephalopathy is multifactorial, it is commonly attributed to hypoxic ischemic encephalopathy (HIE), which has an incidence of 9-13 per 1 000 live births in South Africa(7). Early-onset sepsis (EOS), including intra-uterine infection, and placental inflammation/ infection are suspected risk factors for neonatal encephalopathy in LMICs (8–14). It is postulated that infection may cause the immature brain to be more susceptible to perinatal events, thereby contributing to pathogenesis of neonatal encephalopathy (15–17). In a study from Uganda, infants with neonatal encephalopathy (n=202) had an eight-fold greater likelihood of bacteraemia(13). In a recent systematic review of few studies (n=17),

Group B *streptococcus* (GBS) sepsis was associated with 0.58% (95% CI: 0.18 - 0.98) of all neonatal encephalopathy cases (18).

The aim of this study was to investigate the association between EOS and neonatal encephalopathy in a low-middle income setting in South Africa, and analyse for predictors of death in newborns with EOS and neonatal encephalopathy.

Methods

A retrospective descriptive study was undertaken from 1st January 2016 to 30th June 2018 at the neonatal unit of Chris Hani-Baragwanath Academic Hospital (CHBAH), a public secondary-tertiary care hospital in Soweto, South Africa. Although South Africa is classified as an upper-middle income country, Soweto is a low to middle-income setting where access to health care for pregnant women and children <6 years of age is free of charge(19). The neonatal unit admits approximately 4 000 newborns annually, from a birth cohort of approximately 28 000 live births (20 000 at CHBAH and 8 000 at surrounding community health centres). It is also a referral centre for newborns requiring critical care, including whole body cooling, from a local district hospital (Bheki Mlangeni District Hospital) where approximately 3 000 births occur annually. Newborns at CHBAH with neonatal encephalopathy are graded using Sarnat staging - stage-1 as mild neonatal encephalopathy, stage-2 as moderate neonatal encephalopathy, and stage-3 as severe neonatal encephalopathy(20). Criteria for therapeutic hypothermia is based on modified TOBY criteria(21), the discretion of the attending-physician and available resources at CHBAH. In all newborns with neonatal encephalopathy, a full blood count and blood culture are drawn at birth, a C-reactive protein (CRP) at 24 - 48 hours of life and cerebrospinal fluid (CSF) culture within 72 hours of life.

Newborns with physician-diagnosed neonatal encephalopathy were identified from an electronic database (administered by the Vaccines and Infectious Diseases Analytical Research Unit; VIDA) using ICD-10 codes pertaining to neonatal encephalopathy, HIE, birth asphyxia, and intrauterine hypoxia (Supplementary Table 1). We excluded newborns that did not strictly meet the case definition of neonatal encephalopathy (i.e.: <35 weeks gestation age)(4). In children among whom gestational age was not recorded in the database, birth weight ≥2500 grams was used as a proxy for gestational age ≥35 weeks. Laboratory results were sourced from the VIDA database or the National Health Laboratory Service (NHLS) Trakcare® system.

Culture-confirmed sepsis was defined as culture of pathogenic bacteria (excluding contaminants) on blood and/or CSF sampled within 72 hours of life. Bacteria considered as contaminants were: *Coagulase-negative staphylococci* (CoNS), *Micrococcus* species, *Bacillus* species, *Corynebacterium* species and *Streptococcus viridans*. The inclusion of "probable sepsis" was based on the low sensitivity (~8%) of blood culture in newborns with invasive bacterial disease(18). We defined probable sepsis as a CRP >32 mg/L (based on a Ugandan study) and/or an immature to total neutrophil ratio ≥0.3(13,22). We chose this high CRP threshold to be predictive of probable sepsis as CRP values can be elevated in newborns with neonatal encephalopathy in the absence of sepsis, and further altered by the use of therapeutic hypothermia (23–27). Importantly, there is no consensus on which CRP value is predictive of sepsis in newborns with encephalopathy(24,26–28). "Overall" sepsis was considered a composite of culture-confirmed or probable sepsis.

Statistical Analysis

Incidence was calculated as the number of cases per 1 000 live births (based on the District Health Information System; DHIS) for Soweto and surrounding areas. Neonatal encephalopathy cases were stratified into, culture-confirmed sepsis, probable sepsis, overall sepsis (confirmed + probable), and no sepsis. Categorical variables were reported as frequencies and proportions, and comparisons done using the Chi-squared or Fisher's exact test. Continuous variables were reported as medians and compared using the Mann-Whitney U test. To determine predictors of mortality in cases with culture-confirmed sepsis associated neonatal encephalopathy, we undertook a multivariable logistic regression analysis reporting adjusted odds ratios (aORs) with 95% confidence intervals; we included variables that were significant in the univariate analysis.

Data were analysed using STATA version 13.1 and differences with p-value <0.05 were considered statistically significant. The study was approved by the University of Witwatersrand Human Research Ethics Committee (HREC number: M181058).

Results

During the observation-period, 1 027 (10.1%) of 10,182 hospitalized newborns were diagnosed with neonatal encephalopathy, including 495 (48.2%), 396 (38.6%) and 110 (10.7%) neonates with mild, moderate and severe neonatal encephalopathy (Figure 1). The overall incidence of neonatal encephalopathy was 13.0 per 1000 live births (95% CI: 12.2-13.8).

Overall, 181 (17.6%) of the neonatal encephalopathy cases had sepsis, including 52 (5.1%) neonates with culture-confirmed sepsis and 129 (12.5%) with probable sepsis. Of the 52

culture-confirmed cases with neonatal encephalopathy, bacteria was cultured from blood only, CSF only, and blood and CSF in 39 (75%), 7 (13.5%) and 6 (11.5%) cases, respectively. Clinical characteristics were similar between newborns with culture-confirmed sepsis associated neonatal encephalopathy compared to newborns without sepsis, however, those with culture-confirmed sepsis had higher CRP (33 mg/L vs 3 mg/L, p<0.001, aOR: 1.11; 95%CI: 1.08-1.14; p<0.001) and case fatality rate (CFR) (30.8% vs. 10.5%, p<0.001, aOR: 2.26; 95%CI: 0.85-6.01; p=0.102) albeit not being significant in a multivariable analysis (Table 1 and Supplementary Table 2). Clinical characteristics were also mostly similar when comparing newborns with probable sepsis with newborns without sepsis; and comparing newborns with overall sepsis (confirmed + probable) with newborns without sepsis (Supplementary Tables 3 and 4).

The incidence of culture-confirmed and probable sepsis associated neonatal encephalopathy were 0.7 (95% CI: 0.5-0.9) and 1.6 (95% CI: 1.4-1.9), respectively (Supplementary Table 5). Group B *streptococcus* was the most commonly identified organism (n=17, 32.7%), for an overall incidence of 0.22 (95% CI: 0.13-0.35) (Figure 2, Supplementary Table 5). The most commonly cultured gram-negative organisms associated with neonatal encephalopathy were *Klebsiella pneumoniae* (n=10, 19.2%) and *E. coli* (n=5, 9.6%), with an incidence of 0.13 (95% CI: 0.04-0.23) and 0.06 (95% CI: 0.02-0.15), respectively (Figure 2, Supplementary Table 5). Most of the GBS associated neonatal encephalopathy cases in our study had moderate-severe encephalopathy (65%), and of these, 73% were managed with therapeutic hypothermia.

In a multivariable regression analysis comparing survivors of culture-confirmed sepsis associated neonatal encephalopathy with those that demised, severe neonatal encephalopathy

and seizures were associated with a 6.51 (95% CI: 1.03-41.44; p=0.047) and a 10.64 (95% CI: 1.05-107.39; p=0.045) increased odds of death respectively (Table 2). Overall, culture-confirmed sepsis associated neonatal encephalopathy due to gram-negative organisms had 4.1-fold (95%CI:1.5 – 11.0) higher CFR (54.6%) than gram-positive organisms (13.3%; p=0.001). Similarly, in overall sepsis (confirmed + probable) associated neonatal encephalopathy, cases with severe neonatal encephalopathy (aOR 7.54, 95% CI: 2.41-23.56, p=0.001) and seizures (aOR 3.60 95% CI: 1.25-10.38, p=0.018) were associated with increased odds of death (Supplementary Table 6).

Discussion

The overall burden (per 1 000 live births) of neonatal encephalopathy in our low-middle income setting (13.0; 95% CI: 12.2-13.8) was four-fold greater than global estimates (3.0; 95% CI 2.7-3.3)(5). Contributing to this, was a higher burden of culture-confirmed sepsis associated neonatal encephalopathy (0.7; 95% CI: 0.5-0.9 per 1 000 live births) in our settings than previously described (11,13,29). One-third of culture-confirmed sepsis cases were caused by GBS, and other dominant pathogens were *Klebsiella pneumoniae* (19%) and *E. coli* (10%). Newborns with severe neonatal encephalopathy and culture-confirmed sepsis had an approximately seven-fold increased odds of death.

The prevalence of culture-confirmed sepsis associated neonatal encephalopathy in our setting (5.1%; 95% CI: 3.8-6.6%) was similar to that reported in a systematic review (5.1%)(30), albeit, higher than reported in Uganda (3.5%), The Netherlands (1.3%) and California (1.5%)(8,11,29). While limited data is available on mortality in newborns with culture-confirmed sepsis associated neonatal encephalopathy, our CFR (30.8%) was similar to other

low income countries (32.6%)(13) but greater than reported in high income countries (14%-21%)(11,29).

There is lack of consistency among studies trying to ascertain pathogens associated with neonatal encephalopathy. In the absence of molecular techniques, blood cultures are used to detect pathogenic bacteria but have low sensitivity (31). In a recent study, the use of molecular PCR techniques, resulted in a 70% increase in the incidence of early-onset GBS disease (1.91 per 1 000 live births) compared to blood culture-only based incidence (1.12 per 1 000 live births)(31). The incidence of GBS-associated neonatal encephalopathy in our study (0.22; 95%CI; 0.13-0.35) was 12-times higher than reported in the UK (0.019; 95% CI: 0.019-0.02)(18). Furthermore, neonatal encephalopathy attributed to GBS sepsis in our study (1.7%, 95% CI: 1.0-2.6) was more than two-fold greater than global estimates (0.58%; 95% CI; 0.18-0.98%) reported in a recent meta-analysis that analysed data from 17 studies (14 from high income countries)(18). The CFR (17.7%) for GBS-associated neonatal encephalopathy in our study was, however, similar to global estimates (21%)(18).

Intrapartum antibiotic prophylaxis (IAP) has been shown to significantly reduce the incidence of Early Onset GBS disease worldwide (32) and while risk factor based IAP policies are in place at a National level in South Africa, implementation and coverage are as low as 26% (33). These findings further emphasise the need for GBS preventative strategies, including possibly maternal immunization with a GBS vaccine that could reduce the risk of in-utero GBS infection that may drive the pathogenesis of neonatal encephalopathy.

Because of the low sensitivity of culture to detect sepsis in newborns, many studies have used a composite of confirmed and probable sepsis. The association of overall sepsis (confirmed +

probable) with neonatal encephalopathy in our study (17.6%, 95%CI: 15.3-20.1) was higher than reported in high-income settings such as The Netherlands (3.9%) and California (11.3%)(11,29) that used similar case definition to that of ours. The CFR of overall sepsis associated neonatal encephalopathy in our study was lower than reported in low-income and resource setting of Uganda (44.4%)(13), which could be explained by the limited health care services available to mothers and children with no therapeutic hypothermia and no mechanical ventilation being offered in Uganda(13). Although our study was not designed to evaluate the effectiveness of therapeutic hypothermia, we did not identify any association between therapeutic hypothermia and death in neonates with culture-confirmed or probable sepsis. Therapeutic hypothermia has, however, been associated with neuro-protection in 60% of newborns with sepsis-associated perinatal asphyxia in Belgium(29), which has also been observed in murine models following gram-positive intrauterine infections(34).

Limitations of this retrospective study include that diagnosis of neonatal encephalopathy was based on individual physician diagnosis, hence, the possibility of misclassification. Also, we cannot systematically exclude underlying severe structural brain abnormalities that may not have been investigated for. Furthermore, diagnosing sepsis in newborns is difficult, and ancillary laboratory markers of infection/inflammation may be elevated in neonatal encephalopathy and affected by therapeutic hypothermia(23–27). To overcome this limitation, we used a higher CRP threshold as a marker of sepsis, hence, may have underestimated the burden of probable sepsis associated neonatal encephalopathy.

In conclusion, we report a high incidence and CFR of culture-confirmed sepsis associated neonatal encephalopathy in South Africa. We also showed a high burden of GBS-associated

neonatal encephalopathy which impart supports the need for maternal vaccination particularly where intrapartum antibiotic prophylaxis is unlikely to be effective(35).

Conflict of interest: All authors have no conflicts of interest to disclose

Funding Source: No funding was secured for this study.

Financial Disclosure: SAM is funded in part by National Research Foundation/ Department of Science and Technology: South African Research Chair Initiative in Vaccine Preventable Diseases and South Africa Medical Research Council. ZD is in part funded by the Carnegie Corporation of New York (Grant number B8749). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Authors Contributions:

Kathleen P. Car, Firdose Nakwa, Shabir A. Madhi and Ziyaad Dangor conceptualized and designed the study, designed the data collection instruments, carried out analyses, drafted the initial manuscript, and reviewed and revised the manuscript.

Sithembiso C. Velaphi and Cally J. Tann assisted with the study design and analysis, and critically reviewed the manuscript for important intellectual content.

Fatima Solomon, Alane Izu and Sanjay G Lala designed the data collection instruments, collected data, and reviewed and revised the manuscript.

Fatima Solomon, Alane Izu and Shabir A. Madhi maintain the database used in the study, and critically reviewed the manuscript for important intellectual content.

All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

Acknowledgements
We are thankful to the VIDA and CHBAH Neonatal Department for their help and access to databases. The electronic database upon which this study was partly based, is funded and managed by VIDA on behalf of the Department of Paediatrics at CHBAH

References

1. Liu L, Oza S, Hogan D, Chu Y, Perin J, Zhu J, et al. Global, regional, and national causes of under-5 mortality in 2000-15: an updated systematic analysis with implications for the Sustainable Development Goals. *Lancet.* 2016 Dec 17;388(10063):3027-3035.
2. Madhi SA, Pathirana J, Baillie V, Izu A, Bassat Q, Blau DM, et al. Unraveling Specific Causes of Neonatal Mortality Using Minimally Invasive Tissue Sampling : An Observational Study. *Clin Infect Dis*. 2019 Oct 9;69(Suppl 4):S351-S360.
3. Dorrington RE, Bradshaw D, Laubscher R, Nannan N. Rapid mortality surveillance report 2016. Cape Town: South African Medical Research Council,2018.
4. American College of Obstetricians and Gynecologists' Task Force on Neonatal Encephalopathy. Executive summary: Neonatal encephalopathy and neurologic outcome, second edition. *Pediatrics*. 2014;133(5):e1482-8.
5. Kurinczuk JJ, White-Koning M, Badawi N. Epidemiology of neonatal encephalopathy and hypoxic-ischaemic encephalopathy. *Early Hum Dev*. 2010;86(6):329-338.
6. Lawn JE, Cousens S, Zupan J. Neonatal Survival 1 4 million neonatal deaths : When ? Where?Why? *Lancet*. 2005:9-18.
7. Bruckmann EK, Velaphi S. Intrapartum asphyxia and hypoxic ischaemic encephalopathy in a public hospital : Incidence and predictors of poor outcome. *S Afr Med J.* 2015;105(4):298-303
8. Tann CJ, Nkurunziza P, Nakakeeto M, Oweka J, Kurinczuk JJ, Were J, et al. Prevalence of bloodstream pathogens is higher in neonatal encephalopathy cases vs. controls using a novel panel of real-time PCR assays. *PLoS One*. 2014;9(5).
9. Badawi N, Kurinczuk JJ, Keogh JM, Alessandri LM, O'Sullivan F, Burton PR, et al. Antepartum risk factors for newborn encephalopathy: the Western Australian case-

control study. *BMJ*. 1998;317(7172):1549-1553.

10. Ellis M, Manandhar N, Manandhar DS, Costello AM. Risk factors for neonatal encephalopathy in Kathmandu, Nepal, a developing country: unmatched case-control study. *BMJ*. 2000;320(7244):1229-1236.
11. Jenster M, Bonifacio SL, Ruel T, Rogers EE, Tam EW, Partridge JC, et al. Maternal or neonatal infection: Association with neonatal encephalopathy outcomes. *Pediatr Res*. 2014;76(1):93-99.
12. Nelson KB, Penn AA. Is infection a factor in neonatal encephalopathy? *Arch Dis Child Fetal Neonatal Ed*. 2015;100(1):F8-F10.
13. Tann CJ, Nakakeeto M, Willey BA, Sewegaba M, Webb EL, Oke I, et al. Perinatal risk factors for neonatal encephalopathy : an unmatched case-control study. *Arch Dis Child Fetal Neonatal Ed.* 2018 May;103(3):F250-F256.
14. Pauliah SS, Shankaran S, Wade A, Cady EB, Thayyil S. Therapeutic Hypothermia for Neonatal Encephalopathy in Low- and Middle-Income Countries : A Systematic Review and Meta-Analysis. *PLoS One*. 2013;8(3):e58834.
15. Hagberg H, Mallard C, Ferriero DM, Vannucci SJ, Levison SW, Vexler ZS, et al. The role of inflammation in perinatal brain injury. *Nat Rev Neurol*. 2015;11(4):192-208.
16. Fleiss B, Tann CJ, Degos V, Sigaut S, Van Steenwinckel J, Schang AL, et al. Inflammation-induced sensitization of the brain in term infants. *Dev Med Child Neurol*. 2015;57(S3):17-28.
17. Peebles DM, Wyatt JS. Synergy between antenatal exposure to infection and intrapartum events in causation of perinatal brain injury at term. BJOG: An International Journal of Obstetrics & Gynaecology. 2002;109:737-739.
18. Tann CJ, Martinello KA, Sadoo S, Lawn JE, Seale AC, Vega-Poblete M, et al. Neonatal Encephalopathy with Group B Streptococcal Disease Worldwide: Systematic

Review, Investigator Group Datasets, and Meta-analysis. *Clin Infect Dis*. 2017; 6;65(suppl_2):S173-S189.

19. Leatt A, Shung-King M, Monson J. Healing inequalities: The free health care policy. *South African Child Gauge*. 2006:51-56.
20. Sarnat, HB.; Sarnat M. Neonatal Encephalopathy Following Fetal Distress: A Clinical and Electroencephalographic Study. *Arch Neurol*. 1976;33(10):696-705
21. Azzopardi D, Brocklehurst P, Edwards D, Halliday H, Levene M, Thoresen M, et al. The TOBY study. Whole body hypothermia for the treatment of perinatal asphyxial encephalopathy: A randomised controlled trial. *BMC Pediatr*. 2008;8(17).
22. Polin RA. Management of Neonates With Suspected or Proven Early-Onset Bacterial Sepsis. *Pediatrics*. 2012;129(5):1006-1015.
23. Sanka S, Muniraman H, Gardner D, Pawaletz A, Jennings C, Vayalakkad A, et al. 1100 C-Reactive Protein Concentrations in Neonates with Hypoxic-Ischaemic Encephalopathy and Effect of Total Body Hypothermia. *Arch Dis Child* 2012;97:A315;
24. Xanthou M, Fotopoulos S, Mouchtouri A, Lipsou N, Zika I, Sarafidou J. Inflammatory mediators in perinatal asphyxia and infection. *Acta Paediatr Suppl.* 2002;91(438):92-7.
25. Shang Y, Mu L, Guo X, Li Y, Wang L, Yang W, et al. Clinical significance of interleukin-6, tumor necrosis factor-α and high-sensitivity C-reactive protein in neonates with hypoxic-ischemic encephalopathy. *Exp Ther Med*. 2014;8(4):1259-1262.
26. Rath S, Narasimhan R, Lumsden C. C-reactive protein (CRP) responses in neonates with hypoxic ischaemic encephalopathy. *Arch Dis Child Fetal Neonatal Ed*. 2014;99(2):F172.
27. Chakkarapani E, Davis J, Thoresen M. Therapeutic hypothermia delays the C-reactive protein response and suppresses white blood cell and platelet count in infants with

neonatal encephalopathy. *Arch Dis Child Fetal Neonatal Ed*. 2014;99(6):F458-63.

28. Hofer N, Zacharias E, Müller W, Resch B. An update on the use of C-reactive protein in early-Onset neonatal sepsis: Current insights and new tasks. *Neonatology*. 2012;102(1):25-36.

29. Hakobyan M, Dijkman KP, Laroche S, Naulaers G, Rijken M, Steiner K, et al. Outcome of Infants with Therapeutic Hypothermia after Perinatal Asphyxia and Early-Onset Sepsis. *Neonatology* 2019;115:127–133.

30. Jacobs SE, Berg M, Hunt R, Tarnow-Mordi WO, Inder TE, Davis PG. Cooling for newborns with hypoxic ischaemic encephalopathy. *Cochrane Database Syst Rev Art*. 2013;(1):3-5.

31. Velaphi SC, Westercamp M, Moleleki M, Pondo T, Dangor Z, Wolter N, et al. Surveillance for incidence and etiology of early-onset neonatal sepsis in Soweto, South Africa. *PLoS One*. 2019;14(4):e0214077.

32. Le Doare K, O'Driscoll M, Turner K, Seedat F, Russell NJ, Seale AC, et al. Intrapartum Antibiotic Chemoprophylaxis Policies for the Prevention of Group B Streptococcal Disease Worldwide: Systematic Review. *Clin Infect Dis.* 2017;65(December):S143–51.

33. Price CA, Green-Thompson L, Mammen VG, Madhi SA, Lala SG, Dangor Z. Knowledge gaps among South African healthcare providers regarding the prevention of neonatal group B streptococcal disease. *PLoS One* 13(10): e0205157.

34. Falck M, Osredkar D, Maes E, Flatebø T, Wood TR, Sabir H, et al. Hypothermic Neuronal Rescue from Infection-Sensitised Hypoxic-Ischaemic Brain Injury Is Pathogen Dependent. *Dev Neurosci*. 2017;39(1-4):238-247.

35. Vekemans J, Moorthy V, Friede M, Alderson MR, Sobanjo-Ter Meulen A, Baker CJ, et al. Maternal immunization against Group B streptococcus: World Health

Organization research and development technological roadmap and preferred product characteristics. *Vaccine*. 2019;37(50):7391-7393.

Figure 1: Newborns with neonatal encephalopathy at or referred to CHBAH from January 2016 – June 2018

Footnote for Figure 1:

[1]ICD 10 Codes; G93.4 Neonatal Encephalopathy, P21 Birth Asphyxia, P21.0 Severe Birth Asphyxia, P21.1 Mild and Moderate Birth Asphyxia, P21.9 Birth Asphyxia unspecified, P91.6 Hypoxic ischemic injury of the Newborn, P20.0 Intrauterine hypoxia first noted before onset of labour, P20.1 Intrauterine hypoxia first noted during labour and delivery, P20.9 Intrauterine hypoxia, unspecified. [2] EOS diagnosed on Blood Culture or CRP > 32 or I:T ratio ≥ 0.3. Contaminants were considered as *Coagulase-negative staphylococci (CoNS), Micrococcus* species, *Bacillus* species, *Corynebacterium* species *and Streptococcus viridans*

Figure 2: Incidence and organism specific prevalence of culture-confirmed sepsis associated neonatal encephalopathy

Table 1: Clinical and laboratory characteristics of neonates with early-onset sepsis associated neonatal encephalopathy

Footnote for Table 1:

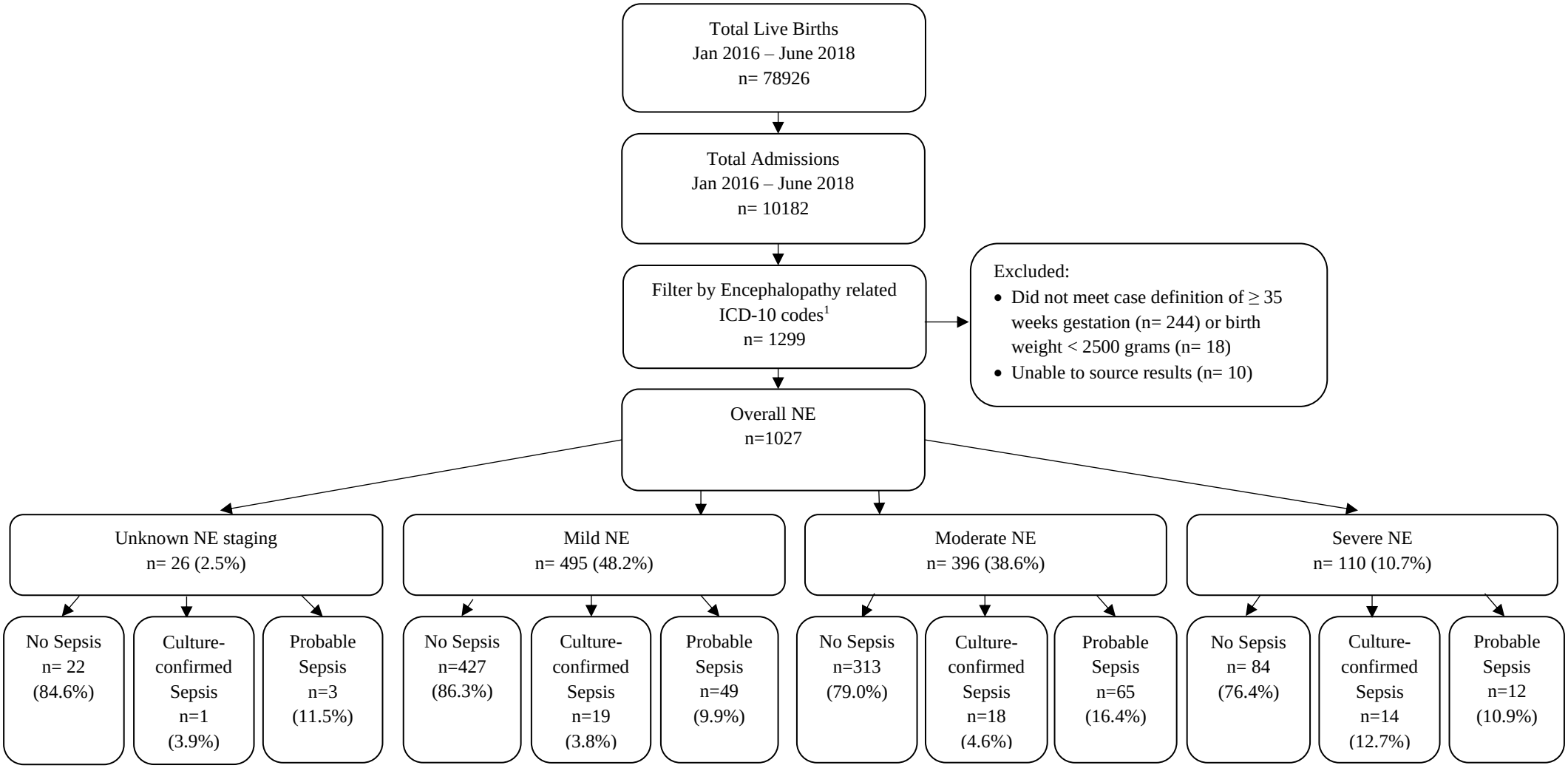
*Statistically significant in multivariable analysis when compared to neonatal encephalopathy without sepsis; [1] Probable sepsis diagnosed as CRP > 32 mg/L or I:T ratio ≥ 0.3; [2] Data was missing in 10 cases; [3] Neonatal encephalopathy graded according to Sarnat Classification; [4] Data was missing in 26 cases;[5] Data was missing in 307 cases; [6] Data was missing in 21 cases; [7] Data was missing in 29 cases.

Table 2: Predictors of mortality in neonates with culture-confirmed sepsis associated neonatal encephalopathy

Footnote for Table 2:

* Comparing Severe NE vs Mild and Moderate [1]Comparing Demised vs Survived using Chi-squared or Wilcoxon rank-sum (Mann-Whitney) test, [2]Adjusted odds ratio with 95% confidence using multivariate logistic regression, [3] Data was missing in 1 case; [4] Neonatal encephalopathy graded according to Sarnat Classification; [5] Data was missing in 1 case; [6] Data was missing in 15 cases.

Figure 1: Newborns with neonatal encephalopathy at or referred to CHBAH from January 2016 – June 2018



[1]ICD 10 Codes; G93.4 Neonatal Encephalopathy, P21 Birth Asphyxia, P21.0 Severe Birth Asphyxia, P21.1 Mild and Moderate Birth Asphyxia, P21.9 Birth Asphyxia unspecified, P91.6 Hypoxic ischemic injury of the Newborn, P20.0 Intrauterine hypoxia first noted before onset of labour, P20.1 Intrauterine hypoxia first noted during labour and delivery, P20.9 Intrauterine hypoxia, unspecified

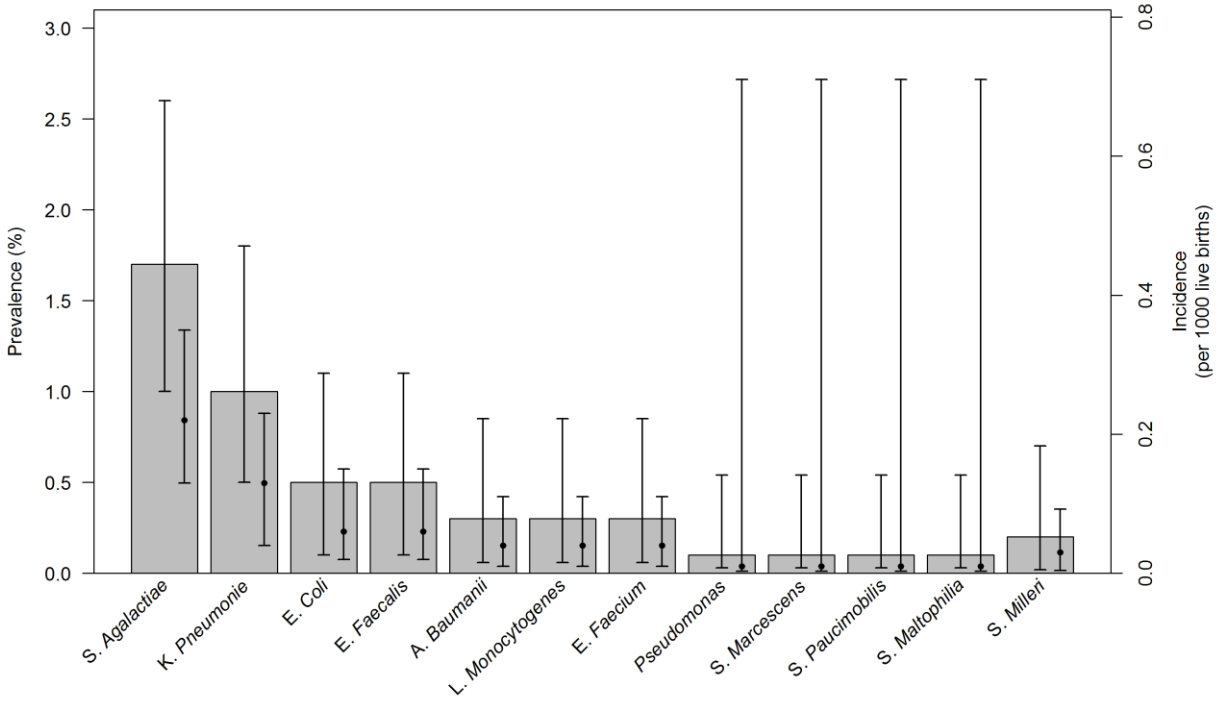


Figure 2: Incidence and organism specific prevalence of culture-confirmed sepsis associated neonatal encephalopathy

Table 1: Clinical and laboratory characteristics of neonates with early-onset sepsis associated neonatal encephalopathy

	Culture-confirmed sepsis n= 52 (%)	Probable sepsis[1] n=129 (%)	Overall sepsis (confirmed + probable) n=181 (%)	No sepsis n=846 (%)
Male sex	25 (48.1)	66 (51.2)	91 (50.3)	408 (48.2)
Median birth weight (IQR)	3043 (2655-3350)	3045 (2790-3485)	3045 (2770-3465)	3082 (2780-3420)
Median gestation[2] (IQR)	39 (37-40)	39 (38-40)	39 (37-40)	39 (38-40)
Normal vaginal delivery	32 (61.5)	77 (59.7)	109 (60.2)	449 (53.1)
HIV-exposed	11 (21.2)	37 (28.7)	48 (26.5)	201 (23.8)
NE Classfication[3]				
Mild NE	19 (36.6)	49 (38.0)	68 (37.6)	427 (50.5)
Moderate NE	18 (34.6)	65 (50.4)	83 (45.9)	313 (37.0)
Severe NE	14 (26.9)	12 (9.3)	26 (14.3)	84 (9.9)
Unknown	1 (1.9)	3 (2.3)	4 (2.2)	22 (2.6)
Therapeutic hypothermia[4]	19 (37.3)	55 (43.7)	74 (41.8)	298 (36.2)
Seizures in hospital[5]	18 (48.7)	32 (33.0)	50 (37.3)	229 (36.2)
Case Fatality Rate	16 (30.8)*	19 (14.7)	35 (19.3)	89 (10.5)
I:T ratio ≥0.3[6]	1 (1.9)	8 (6.3)	9 (5.0)	0 (0.0)
Median CRP[7] (IQR)	33 (10-76)*	57 (41-83)*	55 (35-80)*	3 (1-10)

*Statistically significant in multivariable analysis when compared to neonatal encephalopathy without sepsis; [1] Probable sepsis diagnosed as CRP > 32 mg/L or I:T ratio ≥ 0.3; [2] Data was missing in 10 cases; [3] Neonatal encephalopathy graded according to Sarnat Classification; [4] Data was missing in 26 cases;[5] Data was missing in 307 cases; [6] Data was missing in 21 cases; [7] Data was missing in 29 cases.

Table 2: Predictors of mortality in neonates with culture-confirmed sepsis associated neonatal encephalopathy

	Demised n=16 (%)	Survived n=36 (%)	p value[1]	a OR (95% CI)[2]	p value
Male sex	9 (56.3)	16 (44.4)	0.432	-	-
Median Birth Weight (IQR)	2960 (2695-3178)	3108 (2655-3418)	0.271	-	-
Median Gestation[3] (IQR)	38 (37-40)	38 (37-40)	0.228	-	-
Normal Vaginal Delivery	12 (75.0)	20 (55.6)	0.183	-	-
HIV-exposed	3 (18.8)	8 (22.2)	0.777	-	-
NE Classification[4]					
Mild	2 (12.5)	17 (47.2)	0.007	-	-
Moderate	5 (31.2)	13 (36.1)		-	-
Severe	9 (56.3)	5 (13.9)		6.51 (1.03-41.44)*	0.047
Unknown	0 (0.0)	1 (2.8)		-	-
Therapeutic Hypothermia[5]	5 (31.3)	14 (40.0)	0.549	-	-
Seizures in Hospital[6]	8 (88.9)	10 (35.7)	0.007	10.64 (1.05-107.39)	0.045
Median CRP (IQR)	33 (15-77)	33 (7-76)	0.532	-	-

* Comparing Severe NE vs Mild and Moderate [1]Comparing Demised vs Survived using Chi-squared or Wilcoxon rank-sum (Mann-Whitney) test, [2]Adjusted odds ratio with 95% confidence using multivariate logistic regression, [3] Data was missing in 1 case; [4] Neonatal encephalopathy graded according to Sarnat Classification; [5] Data was missing in 1 case; [6] Data was missing in 15 cases.

Supplementary Table 1: ICD-10 codes from a discharge summary database for physician diagnosed neonatal encephalopathy

ICD-10 code	**Overall n=1027 (%)**	**With EOS n=181 (%)**	**Without EOS n= 846 (%)**
P91.6 Hypoxic Ischemic Encephalopathy	801 (77.9)	138 (76.2)	663 (78.4)
G93.4 Neonatal Encephalopathy	195 (19.0)	37 (20.4)	158 (18.7)
P21.1 Mild and Moderate Birth Asphyxia	11 (1.1)	3 (1.7)	8 (1.0)
P21.0 Severe Birth Asphyxia	20 (2.0)	3 (1.7)	17 (2.0)
P21.9 Birth Asphyxia unspecified	0 (0)	0 (0)	0 (0)
P21 Birth Asphyxia	0 (0)	0 (0)	0 (0)
P20.0 Intrauterine hypoxia first noted before onset of labour	0 (0)	0 (0)	0 (0)
P20.1 Intrauterine hypoxia first noted during labour and delivery	0 (0)	0 (0)	0 (0)
P20.9 Intrauterine hypoxia, unspecified	0 (0)	0 (0)	0 (0)

Supplementary Table 2: Clinical and laboratory characteristics of neonates with culture-confirmed sepsis associated neonatal encephalopathy.

	Culture-confirmed sepsis n= 52 (%)	No sepsis n=846 (%)	p value[1]	aOR (95% CI)[2]	p value[3]
Male sex	25 (48.1)	408 (48.2)	0.983	-	-
Median birth weight (IQR)	3043 (2655-3350)	3082 (2780-3420)	0.226	-	-
Median gestation [4] (IQR)	39 (37-40)	39 (38-40)	0.703	-	-
Normal vaginal delivery	32 (61.5)	449 (53.1)	0.235	-	-
HIV-exposed	11 (21.2)	201 (23.8)	0.668	-	-
Classification NE[5]					
Mild	19 (36.5)	427 (50.5)	0.002	-	-
Moderate	18 (34.7)	313 (37.0)		-	-
Severe	14 (26.9)	84 (9.9)		1.39 (0.50-3.83)*	0.527
Unknown	1 (1.9)	22 (2.6)		-	-
Therapeutic hypothermia[6]	19 (37.3)	298 (36.2)	0.875	-	-
Seizures in hospital [7]	18 (48.7)	229 (36.2)	0.128	-	-
Case Fatality Rate	16 (30.8)	89 (10.5)	<0.001	2.26 (0.85-6.01)	0.102
I:T ratio ≥0.3[8]	1 (1.9)	0 (0.0)	0.059	-	
Median CRP[9] (IQR)	33 (10-76)	3 (1-10)	<0.001	1.11 (1.08-1.14)	<0.001

* Comparing Severe NE vs Mild and Moderate; [1]Comparing culture-confirmed sepsis and no sepsis using Chi-squared, Fisher's exact or Wilcoxon rank-sum (Mann-Whitney) test; [2] Logistic regression reporting adjusted odds ratio adjusting for a p value <0.05 in univariate analysis; [3]p value for adjusted odds ratio; [4] Data was available for Culture-confirmed sepsis n=51, No sepsis n=838; [5] Neonatal encephalopathy graded

according to Sarnat Classification; [6] Data was available for Culture-confirmed sepsis n=51, No sepsis n= 824; [7]Data was available for Culture-confirmed sepsis n= 37, No sepsis n=632; [8] Data was available for Culture-confirmed sepsis n= 52, No sepsis n=826; [9] Data was available for Culture-confirmed sepsis n= 52, No sepsis n=817.

Supplementary Table 3: Clinical and laboratory characteristics of neonates with probable sepsis associated neonatal encephalopathy

	Probable sepsis[1] n=129 (%)	No sepsis n=846 (%)	p value[2]	aOR (95% CI)[3]	p value[4]
Male sex	66 (51.2)	408 (48.2)	0.534	-	-
Median birth weight (IQR)	3045 (2790-3485)	3082 (2780-3420)	0.775	-	-
Median gestation[5] (IQR)	39 (38-40)	39 (38-40)	0.170	-	-
Normal vaginal delivery	77 (59.7)	449 (53.1)	0.160	-	-
HIV-exposed	37 (28.7)	201 (23.8)	0.225	-	-
Classification NE[6]					
Mild	427 (50.5)	49 (38.0)	0.031	-	
Moderate	313 (37.0)	65 (50.4)		-	
Severe	84 (9.9)	12 (9.3)		0.37 (0.07 – 2.05)*	0.253
Unknown	22 (2.6)	3 (2.3)			
Therapeutic hypothermia[7]	55 (43.7)	298 (36.2)	0.105	-	-
Seizures in hospital[8]	32 (33.0)	229 (36.2)	0.535	-	-
Case Fatality Rate	19 (14.7)	89 (10.5)	0.156	-	-
I:T ratio ≥0.3[9]	8 (6.3)	0 (0.0)	<0.001	-	-
Median CRP[10] (IQR)	57 (41-83)	3 (1-10)	<0.001	1.29 (1.22-1.38)	<0.001

* Comparing Severe NE vs Mild and Moderate; [1]Probable sepsis diagnosed as CRP >32 mg/L or I:T ratio ≥ 0.3; [2] Comparing probable sepsis vs no sepsis using Chi-squared, Fisher's exact or Wilcoxon rank-sum (Mann-Whitney) test, [3] Logistic regression reporting adjusted odds ratio adjusting for a p value <0.05 in univariate analysis; [4] p value for adjusted odds ratio; [5]Data was available for Probable sepsis n=128, No sepsis n=838; [6] Neonatal encephalopathy graded according to Sarnat Classification; [7] Data was available for Probable sepsis n=126, No sepsis n= 824; [8]

Data was available for Probable sepsis n=97, No sepsis n=632; [9] Data was available for Probable sepsis n=128, No sepsis n=826; [10] Data was available for Probable sepsis n=75, No sepsis n= 817.

Supplementary Table 4: Clinical and laboratory characteristics of neonates with overall sepsis (confirmed + probable) associated neonatal encephalopathy

	Overall sepsis (confirmed + probable) n=181 (%)	No sepsis n=846 (%)	p value[1]	aOR (95% CI)[2]	p value[3]
Male sex	91 (50.3)	408 (48.2)	0.617	-	-
Median birth weight (IQR)	3045 (2770-3465)	3082 (2780-3420)	0.738	-	-
Median gestation[4] (IQR)	39 (37-40)	39 (38-40)	0.347	-	-
Normal vaginal delivery	109 (60.2)	449 (53.1)	0.080	-	-
HIV-exposed	48 (26.5)	201 (23.8)	0.432	-	-
Classification NE[5]					
Mild	68 (37.6)	427 (50.5)	0.010	-	-
Moderate	83 (45.9)	313 (37.0)		-	-
Severe	26 (14.3)	84 (9.9)		0.78 (0.30-2.00)*	0.599
Unknown	4 (2.2)	22 (2.6)		-	-
Therapeutic hypothermia[6]	74 (41.8)	298 (36.2)	0.159	-	-
Seizures in hospital[7]	50 (37.3)	229 (36.2)	0.814	-	-
Case Fatality Rate	35 (19.3)	89 (10.5)	0.001	1.95 (0.80-4.76)	0.141
I:T ratio ≥0.3[8]	9 (5.0)	0 (0.0)	<0.001	-	-
Median CRP[9] (IQR)	55 (35-80)	3 (1-10)	<0.001	1.17 (1.14-1.20)	<0.001

* Comparing Severe NE vs Mild and Moderate; [1] Comparing Overall sepsis (confirmed + probable) vs No Sepsis using Chi-squared, Fisher's exact or Wilcoxon rank-sum (Mann-Whitney) test; [2] Logistic regression reporting adjusted odds ratio adjusting for a p value <0.05 in univariate analysis; [3]p value for adjusted odds ratio; [4] Data was available for Overall sepsis (confirmed + probable) n=179, No sepsis n=838; [5] Neonatal

encephalopathy graded according to Sarnat Classification; [6] Data was available for Overall sepsis (confirmed + probable) n=177, No sepsis n= 824; [7] Data was available for Overall sepsis (confirmed + probable) n=134, No sepsis n=632; [8] Data was available for Overall sepsis (confirmed + probable) n=180, No sepsis n=826; [9] Data was available for Overall sepsis (confirmed + probable) n=181, No Sepsis n= 817.

Supplementary Table 5: Prevalence and incidence estimates of neonates with neonatal encephalopathy (NE) stratified by early-onset sepsis (EOS) and organisms cultured.

	Prevalence[1] (95% confidence interval) n=1027	Incidence per 1000 live births[2] (95% confidence interval) n=78926
NE (n=1027)		13.0 (12.2-13.8)
NE with overall sepsis (n= 181)	17.6 (15.3-20.1)	2.3 (2.0-2.7)
NE with culture-confirmed sepsis[3] (n=52)	5.1 (3.8-6.6)	0.7 (0.5-0.9)
NE with probable-sepsis[4] (n=129)	12.5 (10.6-14.7)	1.6 (1.4-1.9)
NE without sepsis (n=846)	82.7 (80.21-84.94)	10.7 (10.00-11.5)
Gram positive bacteria (n=30)	2.92 (1.98-4.14)	0.38 (0.26-0.54)
Gram negative bacteria (n=22)	2.14 (1.35-3.22)	0.28 (0.17-0.42)
Group B Streptococcus (n=17)	1.7 (1.0-2.6)	0.22 (0.13-0.35)
Klebsiella pneumoniae (n=10)	1.0 (0.5-1.8)	0.13 (0.04-0.23)
Escherichia coli (n=5)	0.5 (0.1-1.1)	0.06 (0.02-0.15)
Enterococcus faecalis (n=5)	0.5 (0.1-1.1)	0.06 (0.02-0.15)
Acinetobacter baumannii (n=3)	0.3 (0.06-0.85)	0.04 (0.01-0.11)
Listeria monocytogenes (n=3)	0.3 (0.06-0.85)	0.04 (0.01-0.11)
Enterococcus faecium (n=3)	0.3 (0.06-0.85)	0.04 (0.01-0.11)
Pseudomonas (n=1)	0.1 (0.03-0.54)	0.01 (0.00-0.71)
Serratia marcescens (n=1)	0.1 (0.03-0.54)	0.01 (0.00-0.71)
Sphingomonas paucimobilis (n=1)	0.1 (0.03-0.54)	0.01 (0.00-0.71)
Stenotrophomonas maltophilia (n=1)	0.1 (0.03-0.54)	0.01 (0.00-0.71)
Streptococcus milleri (n=2)	0.2 (0.02-0.70)	0.03 (0.00-0.09)

[1] Prevalence is calculated as the proportion of neonates born with NE; [2] Incidence is calculated as the number of neonates with neonatal encephalopathy and associated sepsis cases per 1 000 live births; [3]Confirmed organism on blood and/or cerebrospinal fluid culture; [4] Probable EOS was a CRP >32mg/L or I:T ratio ≥ 0.3.

Supplementary Table 6: Predictors of mortality in neonates with overall (confirmed + probable) sepsis associated neonatal encephalopathy

	Demised n=35 (%)	Survived n=146 (%)	p value[1]	aOR (95% CI)[2]	p value[3]
Male sex	19 (54.3)	72 (49.3)	0.597	-	-
Median birth weight (IQR)	2940 (2575-3280)	3089 (2790-3485)	0.028	0.99 (0.99-1.00)	0.082
Median gestation[4] (IQR)	38 (37-40)	39 (38-40)	0.055	-	-
Normal vaginal delivery	23 (65.7)	86 (58.9)	0.460	-	-
HIV-exposed	13 (37.1)	35 (24.0)	0.113	-	-
Classification NE[5]					
Mild	2 (5.7)	66 (45.2)	<0.001	-	-
Moderate	17 (48.6)	66 (45.2)		-	-
Severe	16 (45.7)	10 (6.9)		7.54 (2.41 – 23.56) *	0.001
Unknown	0 (0.0)	4 (2.7)		-	-
Therapeutic hypothermia[6]	18 (51.4)	56 (39.4)	0.198	-	-
Seizures in hospital[7]	16 (69.6)	34 (30.6)	<0.001	3.60 (1.25-10.38)	0.018
Culture-confirmed sepsis[8]	16 (45.7)	36 (24.7)	0.013	-	-
Median CRP (IQR)	65 (28-145)	53 (37-76)	0.461	-	-

* Comparing Severe NE vs Mild and Moderate; [1]Comparing Demised vs Survived using Chi-squared or Wilcoxon rank-sum (Mann-Whitney) test, [2]adjusted odds ratio with 95% confidence using multivariate logistic regression; [3]p value for adjusted odds ratio; [4]Data was available for: Demised n= 34, Survived n=145; [5] Neonatal encephalopathy graded according to Sarnat Classification; [6] Data was available for: Demised n= 35, Survived n= 142; [7] Data was available for: Demised n= 23, Survived n=111; [8] Data was available for: Demised n= 35, Survived n= 146.