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REVIEW

Early-Onset Neonatal Sepsis in Low- and Middle-Income Countries: Current Challenges and Future Opportunities

Kirsty Sands[1,2], Owen B Spiller[2], Kathryn Thomson[1,2], Edward AR Portal[2], Kenneth C Iregbu[3], Timothy R Walsh[1,2]

[1]Ineos Institute of Antimicrobial Research, Department of Zoology, University of Oxford, Oxford, UK; [2]Division of Infection and Immunity, Cardiff University, Cardiff, UK; [3]National Hospital Abuja, Abuja, Nigeria

Correspondence: Kirsty Sands, Email kirsty.sands@zoo.ox.ac.uk

Abstract: Neonatal sepsis is defined as a systemic infection within the first 28 days of life, with early-onset sepsis (EOS) occurring within the first 72h, although the definition of EOS varies in literature. Whilst the global incidence has dramatically reduced over the last decade, neonatal sepsis remains an important cause of neonatal mortality, highest in low- and middle-income countries (LMICs). Symptoms at the onset of neonatal sepsis can be subtle, and therefore EOS is often difficult to diagnose from clinical presentation and laboratory testing and blood cultures are not always conclusive or accessible, especially in resource limited countries. Although the World Health Organisation (WHO) currently advocates a ß-lactam, and gentamicin for first line treatment, availability and cost influence the empirical antibiotic therapy administered. Antibiotic treatment of neonatal sepsis in LMICs is highly variable, partially caused by factors such as cost of antibiotics (and who pays for them) and access to certain antibiotics. Antimicrobial resistance (AMR) has increased considerably over the past decade and this review discusses current microbiology data available in the context of the diagnosis, and treatment for EOS. Importantly, this review highlights a large variability in data availability, methodology, availability of diagnostics, and aetiology of sepsis pathogens.

Keywords: early-onset neonatal sepsis, low- and middle-income countries, antimicrobial resistance, diagnosis, treatment

Introduction

Neonatal sepsis, the immune systemic dysregulation arising from a bloodstream infection in neonates less than 28-days old, remains a leading cause of neonatal morbidity and mortality.[1–4] A recent meta-analysis estimated the global incidence of neonatal sepsis to be 2824 per 100,000 live births,[5] significantly higher than estimates reported in 2018 at 2202 per 100,000 live births.[6] Global estimates are largely based on data from high-income countries (HICs), however morbidity and mortality following neonatal sepsis is greatest in low- and middle-income countries (LMICs),[2,4,7–9] where data is scarce. Whilst published systematic reviews on the global incidence of neonatal sepsis include some available data from LMICs, primarily India, Ethiopia and Bangladesh, there are several African, Asian, and South American countries with very little to no data accessible. Moreover, there are very few studies that incorporate incidence data from more than one location/hospital from the same country, and therefore it is likely the true burden of neonatal sepsis in LMICs is under characterised, warranting additional multinational epidemiological studies.

Neonatal sepsis can be caused by bacteria, fungi, viruses, and parasites, however bacterial sepsis is reported most frequently.[1,4,7] The epidemiology of neonatal sepsis ultimately varies across geographies and studies have shown that the bacterial pathogens dominant in LMICs are different to those causing neonatal sepsis in HICs. In LMICs, Gram-negative bacteria such as *Klebsiella pneumoniae, Acinetobacter baumannii* and *Escherichia coli* in addition to *Staphylococcus aureus* are the most frequently reported cause of bacterial neonatal sepsis,[1,4,7] whereas in HICs, *Streptococci agalactiae* (Group-B *Streptococcus* (GBS)) and *E. coli* are most prevalent.[10–12]

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Traditionally, neonatal sepsis is categorised according to onset, with Early Onset Sepsis (EOS) and Late Onset Sepsis (LOS) occurring within 72h and >72h after birth respectively.[2,11] The place of birth, whether the neonate is born within the hospital (ie "inborn"), or the neonate was admitted to hospital with signs of sepsis (ie "outborn"), are often used when analysing the epidemiology and risk factors of neonatal sepsis.[13–15] Data from previous studies suggests that EOS correlates to maternal gut microbiome colonisation with vertical transmission between the mother and neonate.[16–19]

The purpose of this narrative review was to collate published data on EOS including available epidemiology and microbiology data, and data on antimicrobial resistance (AMR) profiles of pathogens causing neonatal sepsis. The diversity of bacterial species identified and rates of AMR, the difficulties in diagnosis, and which antibiotics are currently being used and investigated in LMICs are discussed.

Data Collection

Only published articles in English published between 1990–2021 were searched. PubMed, Google Scholar, SCOPUS, Cochrane library and Science Direct were utilised for the search parameters. PubMed was the primary search engine and searches on the 20th of September 2021 revealed 4636 hits for "neonatal sepsis", of which 464 hits were for "early onset neonatal sepsis", 195 hits for "neonatal sepsis" + "developing countries" and 35 hits for "neonatal sepsis" + "low and middle income". The term "neonatal bloodstream infection" was also searched, producing 20 additional hits. Data was screened from articles and reports from searches on the following: "neonatal sepsis" + "diagnosis" + "developing", "neonatal sepsis" + "treatment" + "developing" and "neonatal sepsis" + "antimicrobial resistance"+ "developing" and "bloodstream infections" + "neonatal" + "early" and "bloodstream infections" + "low and middle income" PubMed, Google Scholar and Science Direct were used to search for "early onset neonatal sepsis" + "[LMICs] country".

Epidemiology of Neonatal Sepsis in LMICs: A Summary

Most of the literature define EOS being within the first 72h of life, especially for pre-term neonates,[20] however there are studies that use the value of the metric at either 48h,[21,22] or <7 days,[23] especially for term neonates.[20,24] Neonates born at term have a lower risk of neonatal sepsis compared to premature (<37 weeks gestational age), very low birth weight (VLBW, <1500g), or extremely low birth weight (ELBW) neonates.[25–27] A lack of clarity with neonatal sepsis definitions may bias and cloud data comparisons.[28,29] Fleischmann et al 2021 performed a systematic review on the global incidence and mortality of neonatal sepsis and called for a "harmonisation of neonatal sepsis definitions",[5] mirrored in the STROBE-NI extension in 2016.[30] With no universal gold standard in the definition of neonatal sepsis, it can be difficult to evaluate the true burden of EOS, and whether a category from birth to a particular timepoint of suspected/clinical sepsis is most appropriate. The division of EOS and LOS does not necessarily capture relevant clinical epidemiological context such as the birthplace/cohort and/or environmental exposures and therefore epidemiology data (inborn and outborn) is also necessary to best inform clinical practice and antibiotic treatment.

The true incidence of neonatal sepsis in LMICs is difficult to quantify and reported incidence of EOS will likely be influenced by the diagnostic parameters used, including accessibility to microbiology facilities and blood cultures. Single site studies often vary in terms of the cohorts included in the study, making it difficult to extrapolate to a country or regional level. For example, referral hospitals, larger hospitals, or hospitals with a large catchment area may have higher rates of neonatal sepsis due to a larger patient population, compared to small district hospitals.[31] Many global estimates are limited to inclusion of data from a relatively small number of countries, not an accurate representation of global incidence, more a biased reflection on available data, with scarcity of data from a large quantity of LMICs. However, from the data available, the incidence of EOS (and all neonatal sepsis; some studies include pooled metrics and/or variations in the definition of EOS) is considerably lower in HICs compared to LMICs; the NeonIN study collated data from 30 neonatal units in the UK and reported an EOS incidence of 0.7 cases per 1000 live births,[24] similar to EOS rates reported from studies in America between 0.5–0.8 cases per 1000 live births.[32–35] Comparably, a study from Norway reported EOS incidence of 0.97/1000 live births between 1996–2018.[36] Whereas, a study in Greece reported a weighted EOS incidence rate of 1.8/1000 live births,[37] indicating some variability in the HIC data. A higher EOS was similarly reported in a large study among preterm infants conducted in China (upper-middle income) between 2015–2018 (n=27,532 enrolled infants) with an incidence of 9.7/1000 live births. It is worth noting that, for this study the cohort

of infants (all preterms, whereas other data may be aggregated) may also be a contributing factor to a higher reported EOS,[38] highlighting variability in the EOS literature overall.

Epidemiology of EOS in LMICs in Africa

Appropriate studies included in a review by Seale et al in 2009 reported the incidence of EOS for data collected from multiple African based studies (Malawi, South Africa, Tanzania, Kenya, Nigeria, Ethiopia and Zimbabwe) as high as 21/1000 live births in a study from Zimbabwe,[39] however, acknowledge these studies are single site based studies, often with different cohorts of neonates (inborns and admissions).[31] A single site study from Soweto in South Africa reported an EOS incidence (data collected 2013–2014) of 39.3/1000 live births, with a culture proven sepsis incidence at 3.2/1000 live births, higher than reported by Ballot et al in 2012 with 0.93/1000 EOS (data collected 2009–2010).[40] Whereas a single site study from Nigeria conducted between 2013–2014 reported a higher incidence of EOS at 8.8/1000 live births.[41] In 2020, a systematic review by Khalil et al collated published data from the Middle East from 2000 onwards and included data on middle-income and high-income categories, as per the United Nations classification with a pooled EOS largely falling within the ranges described above, between 0.6–15.7/1000 live births.[42] However this was only possible to calculate for 12/33 studies included in the review.

Epidemiology of EOS in LMICs in South Asia

Although a systematic review from Sankar in 2019 reported the incidence of neonatal sepsis from five South Asian countries, the majority of data included was from India and did not distinguish between EOS and LOS, therefore data was not included herein.[43] A study published in 2013 from Bangladesh reported a high EOS incidence percentage of 76.8/1000 live births (reported as 12.8% of n=600).[23] A lower rate of 24/1000 live births was reported in a single site study in a tertiary care hospital in North India between 1995–2006.[44] Although a relatively small sample size, recent data from Bhutan revealed a higher rate of EOS compared to LOS at 54.5% and 45.5% respectively, and an incidence of culture-proven sepsis at 19/1000 live births.[27]

Epidemiology of EOS in LMICs in South America

A study in Brazil reported EOS rates comparable to HICs at 4.0/1000 live births and confirmed EOS at 0.31/1000 live births,[45] although the sample size was limited to n=46, and the authors acknowledged that all neonates enrolled to the study were >35 weeks gestational age; a distinction many studies do not make in their analysis. Lower figures were however also reported by Freitas and Romero (2017), with an EOS incidence in Brazil of 1.7/1000 live births,[46] and a recent publication from Suriname reported a similar EOS incidence rate of 1.37/1000 live births, for data collected between 2017–2018.[47] Examining the available incidence data may suggest there is a lower rate of EOS in South America compared to Asia and Africa. There is, however, a scarcity of data from many South American countries and where data does exist, these are often small single site studies therefore these data and conclusions must be interpreted with caution.

Epidemiology of Neonatal Sepsis in LMICs: Multi-Site Studies

Over the last five years there have been a few multi-site or multinational studies that combine epidemiology and microbiology data presenting incidence and mortality data for neonatal sepsis in LMICs for example BARNARDS,[1] NeoAMR,[48] and, DeNIS.[4] Although these studies may not always differentiate the incidence between EOS and LOS, and therefore incidence data has not been included within this review for comparison, they can allow for a standardised (definitions and equipment across study networks) comparison and statistical analysis of collected data within each network. Whilst these studies offer a larger data comparison, they do often show concordance with single site studies, indicating that data, even with small sample sizes, are clinically insightful (particularly from countries with data available) and most conclude high rates of neonatal sepsis in LMICs.

Epidemiology of Neonatal Sepsis: Community Level and Rural Data

Community level and rural data form a large current gap in the literature. For example, for many African, Asian and South American countries, the percentage of home births, perhaps related to culture, religion and cost, is higher compared to HICs.[49,50] For practical, logistical, and cultural reasons, the extent of neonatal infection in home births is not currently well known.[50] A review of neonatal infections in community-based studies published in 2009 noted a large incidence range of clinical sepsis, however the authors acknowledged that intermittent availability of blood cultures, negative blood cultures and access to tertiary healthcare facilities can explain such variation.[51] Furthermore, they noted that blood culture and incidence data from countries with the largest rates of home births was lacking, mirrored by more recent pooled studies with Okomo et al (2019) systemic review of African literature on the aetiology of invasive bacterial infection in neonates where they reported almost all of 151 studies included were hospital based.[7] The WHO, on behalf of the Partnership for Maternal Newborn and Child Health, stated "Almost 60% of African women give birth without a skilled attendant – 18 million a year at home".[52] To truly understand the global burden of neonatal sepsis, microbiology epidemiology studies reaching into rural communities and outside of tertiary hospitals offer valuable insight and will be essential. Due to access to healthcare restrictions for rural based mothers and neonates,[53] they are not well represented in the current tertiary level neonatal admissions datasets.

Neonatal Mortality Following Sepsis

Neonatal mortality following sepsis is higher in LMICs compared to HICs, however wide ranges are reported in the literature. Although the incidence of mortality is declining, the neonatal (<1 month) reduction is slower than the post-neonatal (1–59 months) mortality reduction.[54] An analysis published in 2015 from the South Asian Association for Regional Cooperation (SAARC) reported that 39% of global neonatal mortality and stillbirths occurred in South Asia, and that Pakistan, India and Afghanistan have made slower progress in mortality reduction figures.[54] In 2012 Seale et al published a pooled estimate for case-fatality risk associated with a neonatal possible severe bacterial infection (including sepsis amongst other serious infections) of 9.8% from sub-Saharan Africa, South Asia and Latin America.[55] A 2-year surveillance in Suriname reported an in-hospital mortality rate of 25.9% (with an EOS 1.37/,1000 live births), with the authors noting a high proportion of Gram-negative bacterial EOS in the fatal cases.[47] A high mortality rate associated with EOS was similarly reported by Ogundare et al (2019) in a Nigerian hospital with a mortality rate of 32.1% of EOS cases (56 EOS cases).[56]

Bacterial Species Causing EOS

Multiple bacterial species isolated from blood cultures including Gram-positive and Gram-negative bacteria, have been reported to cause EOS in LMICs in the literature. The aetiology of EOS is extremely diverse and complex in LMICs. Whilst it is known that *K. pneumoniae* and *E. coli* colonize the gut,[7,57] and studies have, by molecular typing, suggested transmission of Gram-negative bacteria from mothers to their infants,[58] further studies are needed to understand the pathogenesis and to monitor the aetiology of EOS in LMICs. Improving infrastructure, resources and equipment in LMICs laboratories will maximise isolate capture from blood cultures,[59] especially for *Streptococci* and mollicutes, allow species level identification and continued surveillance of antimicrobial resistance (AMR).

It is also likely that exogenous factors such as the hospital environment, medical procedures, previous antibiotics and the timing of blood cultures/diagnostic parameters also influence the reported bacterial cause of EOS. Although ascending infection and prolonged rupture of membranes (PROM) may necessitate delivery via caesarean section,[60] neonates who go on to develop EOS born via caesarean section may not been exposed to the mother's microbiome during vaginal delivery and may have acquired the pathogen elsewhere. Available data from LMICs suggest a large overlap in the bacterial species causing EOS and LOS, compared to a discrepancy of species causing EOS and LOS found in data from HICs.[4,43] Mukherjee et al (2021) discuss complications in differentiating the microbiology for EOS and LOS in LMICs stating that limited infection prevention control (IPC) and hygienic practices, may, regardless of the time of onset, result in all infections being hospital acquired.[57] Previous data suggests VLBW neonates requiring prolonged hospitalisation are more susceptible to LOS, related to limited IPC interventions resulting in nosocomial outbreaks, particularly in neonatal intensive care units (NICU).[61–66] *Klebsiella, Serratia* and *Acinetobacter* bacterial species that can readily

colonise hospital surfaces may therefore act as reservoirs for both EOS and LOS.[1,66] Conventional microbiology techniques may be a limiting factor in accurate species level identification and the microbiology of EOS in LMICs is likely to be more diverse than current data alludes to.

Bacterial Diversity of EOS in Africa

There is a wealth of data in Africa supporting that Staphylococci is dominant in EOS. For example a multisite study including sites in Ghana and South Africa (also Pakistan, India, Bangladesh and Bolivia) reported *S. aureus* as the most common bacterial species identified across all age groups for infants (0–6 days as representative of EOS).[67] *S. aureus* is commonly reported from inborn and outborn EOS in Nigerian studies,[41,56,59] and across smaller studies over the past decade in Africa.[68] Gram-positive organisms were also found to be significantly more prevalent in EOS compared to LOS for studies in both Ghana between 2010–2013,[22] and Uganda in 2010.[69] A 10-year review of neonatal bloodstream infections in Kenya also revealed Gram-positive bacteria, both *S. aureus* and CoNS as the major EOS organisms.[70] Similarly, a prospective study conducted between 2011–2012 in Egypt, three Egypt-based NICUs neonatal intensive care units (NICU) reported CoNS as the most dominant bacterial species in EOS.[71] Staaden et al in 2021 (South Africa) discussed the increase and role of CoNS in neonatal sepsis,[72] as in EOS sepsis they found Staphylococci (both *S. aureus* and CoNS) to be the second most prevalent species. Although recommended to confirm likely infection, not all studies stated whether a second blood culture was performed upon the initial identification of CoNS.[73] A sub-Saharan systematic review by Okomo et al (2019) excluded CoNS from their analysis of bacterial pathogens;[7] however, they acknowledged that many published studies will inevitably either miss reporting real pathogens or include data as contaminants/not clinically relevant bacteria. Additionally, Okomo et al's 2019 (does not differentiate EOS and LOS data) African systematic review reported that for East and West Africa *S. aureus* was the most frequent pathogen whereas in central and Southern Africa, *Klebsiella spp*. was predominant.[7]

Klebsiella spp. is elsewhere reported to be one of the dominant species in neonatal sepsis in LMICs.[1,72,74] Pillay et al in 2021 (South Africa), reported *K. pneumoniae* within the three leading causes of EOS, with coagulase-negative staphylococcus (CoNS) and *A. baumannii*.[74] Two years previously however, Velaphi et al (South Africa) in 2019 published data to the contrary revealing GBS and *Ureaplasma* spp. to be the most frequently reported cause of EOS.[75] This study combined data from blood cultures and PCR-based testing, which may account for an increased detection of GBS and mollicute bacteria and the authors of this study highlight that GBS may generally be under-represented in EOS due to diagnostic tool limitations, relying on conventional microbiology alone. Both of these species are particularly fastidious and require specialist media for culture that yield very small colonies that would be lost in mixed bacterial cultures. A systematic review on GBS infection in neonates in LMICs found that although data is lacking for large regions of Africa, Asia and Latin America, GBS neonatal sepsis and infections are problematic in Eastern and Southern Africa.[76] Two single site studies one in central Africa, in the Democratic Republic of Congo (DRC) and one in South Africa also reported GBS as a dominant cause of EOS.[40,77] On the other hand, one of the few EOS studies with microbiology data collected between 2017–2018 also from the DRC, report a predominance of Gram-negative bacteria with *Enterobacter cloacae* complex, *K. pneumoniae* and *Serratia marcescens* as the three most prevalent pahogens,[78] again emphasising bacterial diversity of EOS.

A study including Middle East countries listed *E. coli* in the top three bacterial causes for EOS for both the HICs and middle-income countries with aggregated data into the study. *Klebsiella* species were the most common in the middle-income countries, and their analysis revealed the risk of *Klebsiella* EOS was increased in countries with a lower GDP. Notably, during this study data for Egypt revealed the most common bacterial genera/species causing EOS included *Klebsiella, E. coli, Enterobacter*, and *S. aureus*.[42]

Bacterial Diversity of EOS in South Asia

Interestingly, a study in Pakistan reported the second most frequent bacterial species causing EOS after *S. aureus* to be GBS.[79] GBS was also within the top three bacterial causes for EOS from a tertiary care hospital in Karachi (between 2007–2011).[80] A study in India reports 53% (n=23) of EOS cases were caused by a Gram-negative bacteria, with *Klebsiella* and *Citrobacter* being most frequent. Of the 47% EOS caused by Gram-positive, 40% were *Staphylococci*.[81]

Data from case reports also emphasises bacterial diversity, with a report of septicaemia in a two day old neonate being caused by *Pantoea agglomerans* (formally known as *Enterobacter agglomerans*).[82] The Aetiology of Neonatal Infection in South Asia (ANISA) study between 2011–2014 reported *E. coli, Klebsiella* spp., *S. aureus* and Group A *Streptococcus (Streptococcus pyogenes)* to be the most frequently identified pathogens from blood cultures and reported a higher incidence of Gram-negative organisms among inborn babies compared to outborn.[83] The aetiology of EOS reported in a 2019 systematic review was similar, with *K. pneumoniae, S. aureus* and *E. coli* most dominant, followed by CoNS.[43]

Bacterial Diversity of EOS in South America

A 2-year surveillance in Suriname reported roughly a 50:50 split with Gram-negative and Gram-positive EOS (51.9% Gram-positive EOS), with *S. aureus, Streptococci* including GBS, *E. coli, Klebsiella* and *Serratia marcescens* being amongst the species detected.[47] Two studies in Brazil however, one between 2012–2015 and one between 2016–2019 found a predominance of Gram-positive bacteria (albeit small sample size), particularly GBS.[46] These findings were concordant with earlier data, collected between 1996–1999, with the majority of EOS blood cultures positive for GBS,[84] suggesting Gram-positive causes of EOS in South America are often detected.

Antimicrobial Resistance in Neonatal Sepsis: A Summary

Antimicrobial resistance (AMR) is a global threat to human health, with the burden highest in LMICs.[85–87] Microbiology studies present antimicrobial susceptibility data for blood culture isolates using an array of different laboratory techniques. Due to ease of access and simplicity to determine levels of AMR and classify the bacterial isolate as either sensitive (S) or resistant (R), many hospital laboratories use disc diffusion assays,[70,88,89] or E-tests.[66,90] Facilities with better resources in, other studies report minimum inhibitory concentration data interpreted from automated systems such as the VITEK,[74] or via agar dilution.[1,3] Moreover, different studies follow different reporting guidelines with both CLSI and EUCAST commonly used, amongst others, making data comparison difficult. Whilst there is substantial variability in microbiology laboratory methods, it is clear from the literature that rates of AMR in neonatal sepsis blood culture isolates are increasing.

The use of molecular microbiology techniques and whole genome sequencing, over the past decade has increased our understanding of the dominant mechanisms of AMR in bacteria causing neonatal sepsis.[1,66,90–96] These studies have documented Gram-negative bacteria such as *K. pneumoniae, E. coli*, and *A. baumannii*, which carriage of ESBL antimicrobial resistance genes (ARG) including bla_{TEM}, bla_{SHV} and $bla_{\text{CTX-M-15}}$ and carbapenemase ARGs including $bla_{\text{OXA-23}}$-like, $bla_{\text{OXA-48}}$-like, and bla_{NDM} that confer resistance to a broad range of ß-lactam antibiotics. Moreover, Gram-negative bacteria often co-carry multiple aminoglycoside ARGs, conferring resistance to gentamicin.[1] Similarly, MRSA isolates are resistant to penicillins due to the carriage of bla_{Z} and are often resistant to several other ß-lactams due to the acquisition of bla_{MecA}.[97] Molecular and genomic surveillance of AMR bacteria will improve our understanding of bacterial transmission events, the role of high-risk clones and AMR infections, the role of mobile genetic elements in the spread of AMR in neonatal sepsis and will guide future research for alternative diagnostic and treatment options.

Antimicrobial Resistance in EOS in Africa

Whilst not all AMR data does delineate between EOS and LOS, there are often reports of high resistance to ampicillin and cephalosporins, with high sensitivity to carbapenems, colistin and vancomycin.[70] Ballot et al (2019),[98] reported an increase in multi-drug resistant Enterobacterales (MDRE) in a neonatal unit in South Africa, with an increase from 2.6% in 2013 to 8.9% in 2015 in carbapenem-resistant Enterobacterales (CRE), most notably associated with bla_{NDM}. Data from the Democratic Republic of Congo showed high level Gram-negative resistance (above 50%) to ampicillin, cefotaxime, and gentamicin; all antibiotics used for treatment of EOS.[78] Where *S. aureus* was reported prevalent, rates of methicillin resistant *S. aureus* (MRSA) are usually high (above 30%), whilst remaining largely susceptible to vancomycin.[7,41,43]

Antimicrobial Resistance in EOS in South Asia

High rates of extended-spectrum ß-lactamase (ESBL) producing Gram-negative isolates causing EOS were found in an observational study between 2013–2014 in India.[99] A similar study published in 2015 in Western India reported 28% of *E. coli* and *K. pneumoniae* isolates to be ESBL producers (however this is collated EOS and LOS data), and this study reported high levels of resistance to the commonly used antibiotics at the time: 50–72% resistance to cefotaxime, ampicillin and ceftazidime.[100] A study from Myanmar also revealed high resistance to ampicillin and gentamicin, and notable resistance to the carbapenem antibiotics, reporting ~20% of Gram-negative isolates resistant to meropenem.[25] Similar data is available from Pakistan, India and Nepal, with over 50% of isolates resistant to ampicillin and/or gentamicin, and cephalosporins.[101–103] ANISA reported a higher level of drug susceptibility for first line antibiotics compared to many other studies with 75% susceptible to gentamicin and 41% susceptible to ampicillin or penicillin.[83]

Antimicrobial Resistance in EOS in South America

Comparatively, there are limited studies with EOS data from South America, and available AMR data is scarce. Whilst a study in Brazil reports a high level of sensitivity to first line treatments, including ampicillin sensitivity, the data presented is from a small sample size, with 36 bacterial isolates in total; they report a single *E. coli* isolate resistant to gentamicin.[46] Although there are limited AMR details from the EOS Suriname study, Achten et al in 2021 specifically reported that all bacterial isolates from fatal EOS were sensitive to the local empiric therapy (ampicillin and gentamicin).[47]

Diagnosis of EOS in LMICs: A Summary

The diagnosis of EOS, especially in pre-term neonates can be difficult as initial clinical symptoms are usually subtle, across different organ systems and may be missed or mis-interpreted as other conditions.[9,73] The most common clinical symptoms of neonatal sepsis overall include temperature instability, presentation of a fever (>38°C), irritability, difficulty breathing/apnea, hypothermia, abdominal distention, tachycardia, lack of feeding, jaundice, and the development of petechiae.[29,73,104] A greater understanding of the pathogenesis and aetiology of EOS neonatal sepsis may guide current and improve future diagnostics. Currently, diagnosing neonatal sepsis, and particularly EOS in LMICs with subtle clinical signs and symptoms amidst clinical characteristics and co-morbidities is challenging. Future research directed towards both understanding the pathogenesis/aetiology of EOS and improving the accuracy of affordable, sustainable rapid diagnostic tools are vital.[104] Machine learning approaches to implement a diagnostic algorithm pooling data on (1) maternal characteristics (PROM), (2) epidemiology, (3) birth characteristics (inborn vs outborn, preterm, VLBW, caesarean section), (4) microbiology and (5) laboratory/biochemical tests also warranting further exploration.[105] Empirical treatment will be administered upon signs of sepsis, and timely and accurate microbiology will facilitate, and guide appropriate antibiotic treatments as required.

Diagnosis of EOS in LMICs: Blood Cultures

The current gold standard diagnosis of neonatal sepsis is a positive blood culture, but it is often substandard and insensitive,[104] and is often recognised as a limited diagnostic method in LMICs studies.[106] Collecting sufficient blood from a neonate is a challenge, with many studies collecting less than the recommended 1mL,[73] and the practical limitations of blood culture analysis, in particular low-level bacteraemia (<10 colony forming units (CFU) /mL) are likely reported as false negatives.[73,107–109] Additionally, limitations in the incubation and laboratory resources may prevent the recovery of certain microorganisms, namely GBS and mollicutes, as previously discussed. Particularly for the diagnosis of EOS, a blood culture and cerebrospinal fluid samples are recommended. If the neonate is suspected of LOS, an additional urine sample may prove valuable in detecting a urinary tract infection,[110] not commonly detected in EOS neonates. The capacity to collect blood cultures and access to a microbiology laboratory will vary and may be especially limited in rural areas, outside the catchment of tertiary hospitals. In addition to resource limitations, and compared to HICs, there are often a greater ratio of patients to staff in LMICs hospitals, especially in NICU where specialist care is paramount. Staffing restraints may further limit diagnostic screening services, both clinically and in the laboratory.

Diagnosis of EOS: Laboratory Tests

When diagnosing EOS, clinicians may utilise laboratory tests in addition to performing blood culture upon clinical presentation of sepsis to facilitate a timely diagnosis. Studies have discussed the benefits of serum assays and biomarkers to aid the diagnosis of sepsis, however access to the assays, requirement for training and equipment, prognostic accuracy of reliability these assays are still a contested topic. A low-cost sustainable serum C-reactive protein (CRP) assay has been shown to be useful in monitoring EOS,[24] although it has limited sensitivity and specificity,[111] particularly during the in early phase of the infection (>12h after presentation of clinical symptoms).[112] There is likely no single biomarker or assay to diagnose neonatal sepsis,[113] and studies have shown diagnostic value for a combined assay of CRP, tumour necrosis factor-α (TNF-α) and interleukin −1 and −6.[114] When considering the implementation of biomarkers in the diagnosis of EOS, a difficulty beyond access and laboratory infrastructure, is the determination of appropriate cut-off values, as there are no universal standards.[29] Furthermore, Iroh Tam and Bendel 2017 discuss future directions for the diagnosis of neonatal sepsis and review the use of PCR and molecular biology,[104] testing of cord blood for both microbial culture and biomarker assessment, and gene expression profiling techniques. Whilst all these techniques offer the ability to improve timely diagnosis of neonatal sepsis, sustainability in many LMICs hospitals will be difficult considering the cold-chain transport logistics, reagent shelf life, constant provision of electricity and cold storage at site.

Treatment of EOS in LMICs

Management and treatment of neonatal sepsis in LMICs is an economic burden as highlighted by Ranjeva et al (2017): calling for a "need for investment in strategies to characterise, diagnose, prevent and manage neonatal sepsis" in sub-Saharan Africa.[115] Early, prolonged and inappropriate antibiotic use may further harm neonates with an increased risk of infection, increased hospitalisation, and an impact on the microbiome.[116,117] "Antibiotic treatment has risks and benefits … [the] administration of antibiotics to uninfected patients mean they only assume the risks".[118] For first line treatment of neonatal sepsis, the WHO currently recommend a ß-lactam antibiotic, primarily ampicillin or cloxacillin for *Staphylococci* infections and gentamicin, with a third-generation cephalosporin for second line treatment.[119] Data presented herein implies that the current WHO empirical treatment guidelines for neonatal sepsis may no longer be effective and this is mirrored with the study by Thomson et al reporting low coverage for ampicillin in combination with gentamicin (multi-site study with EOS and LOS data combined),[3] suggesting that "Ampicillin is now redundant". Whilst many studies report local rates of AMR, most do not include information on the availability of antibiotics at a national or regional level, which will likely influence treatments being prescribed. Additionally, there is a lack of data that analyses antibiotic usage, and if, or to what extent, antibiotic prescription data differs to actual usage data. Further interdisciplinary studies involving hospital and community pharmacies would produce invaluable prescription and usage data and allow contextualisation of AMR data. One important factor, if not the most important factor that may influence choice of antibiotic, is the cost, especially in LMICs where the patient and their family must commonly bear the expense.[3] Ampicillin and gentamicin are amongst the most cost-effective choices for treatment, with cephalosporin and carbapenem antibiotics costing considerably more.[3,120]

In order to further our understanding of prescription and usage patterns, studies capturing clinical data on antibiotic usage, including treatment duration, and how many times, if any, the antibiotic regimes change are needed. If neonates do not respond to treatment within 48h, it is possible (access/cost dependent) that the antibiotic regime will be changed.[121] Importantly, variation in antibiotic dosing guidelines,[122] may lead to varied dosing, and in turn a variation in antibiotic usage and efficacy. Previous studies have documented the benefit of extended-interval dosing (one dose per 24h period) for gentamicin and amikacin,[123–125] the administration of a single dose with comparable efficacy and toxicity risks will likely have logistical and cost advantages (staff to patient, preparation of drug), and may also be more suitable for use in community settings.[126]

Darlow et al suggest a combination of two agents including an aminoglycoside,[120] ß-lactam, and/or phosphoric acid derivative (such as fosfomycin), may improve upon the current empirical guidelines and clinical data from a multi-site study observational study (NeoOBS) is to be published in support of this. Moreover, there is potential of fosfomycin to treat multi-drug resistant infections, particularly those caused by ESBL-producing and carbapenemase-resistant bacteria,[127] and when combined with amikacin may improve treatment in neonatal sepsis.[128] Although fosfomycin has low AMR reported in other multi-site neonatal studies,[1,3] and in wider clinical evaluation;[127] recent microbiology data casts doubt on lasting efficacy due

to the high frequency of induced resistance reported using in vitro assays.[3] Additionally, access to and the related cost (/day) of fosfomycin may preclude wide usage in LMICs. Recent clinical trials advocate the use of Latamoxef for EOS,[129] and the potential for oxacephems (derivatives of cephalosporins) to treat EOS are similarly discussed elsewhere,[120] emphasising ongoing research to offer alternative antibiotics.

Conclusion

The aetiology of EOS reported in the literature is extremely diverse, and limitations in diagnostic microbiology laboratories may result in false negative blood cultures, further limiting the understanding of pathogens causing sepsis. Further epidemiology and microbiology studies, assessing clinical microbiology data in countries where little to no data currently exist are crucial to reveal the incidence of and mortality associated with EOS. Specifically, data that better represents multiple LMICs is needed to understand the incidence of neonatal sepsis in the context of (1) EOS and vertical transmission (2) the diversity of pathogens causing sepsis, (3) AMR and pathogenicity and neonatal outcome, and (4) the role of nosocomial infection and length of hospital stay. Multi-site studies reaching into rural and community hospitals, performing standardised laboratory methodologies will also be essential to understand the aetiology of both Gram-negative and Gram-positive EOS, and in particular, the role of GBS in LMICs. A common theme throughout this review is the unclarity over the true incidence of GBS and whether CoNS in blood cultures is causative or contamination.

Currently, the diagnosis of EOS is suboptimal, however, to improve diagnosis and increase funding in this area of research, a greater understanding of the aetiology in LMICs is essential. Whilst dogma speculates that vertical transmission from the mother to the neonate is a dominant route of infection, an increasing number of studies are supporting a significant role of horizontal environmental transmission occurring within the hospital, in the delivery room, or within hours after birth in home-birth settings, complicating current views. Empirical treatment is still centred around the WHO's guidelines, even though accumulating AMR data, (distinctively higher Gram-negative AMR data in South Asia), indicates these guidelines are becoming increasingly ineffective at resolving sepsis. Evidence-based data generated using innovative approaches involving not only healthcare providers, but policy makers and governmental bodies are needed to in order to reduce neonatal morbidity and mortality in the long term.

Author Contributions

All authors made a significant contribution to the work reported. KS drafted the manuscript. KS, OBS, KT, EP revised the manuscript. KS, OBS, KT, EP, KI and TRW critically reviewed the article and gave final approval of the version to be published.

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