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Beyond Early- and Late-onset Neonatal Sepsis Definitions

What are the Current Causes of Neonatal Sepsis Globally? A Systematic Review and Meta-analysis of the Evidence

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Abstract: Sepsis remains a leading cause of neonatal mortality, particularly in low- and lower-middle-income countries (LLMIC). In the context of rising antimicrobial resistance, the etiology of neonatal sepsis is evolving, potentially making currently-recommended empirical treatment guidelines less effective. We performed a systematic review and meta-analysis to evaluate the contemporary bacterial pathogens responsible for early-onset sepsis (EOS) and late-onset neonatal sepsis (LOS) to ascertain if historical classifications—that guide empirical therapy recommendations based on assumptions around causative pathogens—may be outdated. We analyzed 48 articles incorporating 757,427 blood and cerebrospinal fluid samples collected from 311,359 neonates across 25 countries, to evaluate 4347 significant bacteria in a random-effects meta-analysis. This revealed gram-negative bacteria were now the predominant cause of both EOS (53%, 2301/4347) and LOS (71%, 2765/3894) globally. In LLMICs, the predominant cause of EOS was *Klebsiella* spp. (31.7%, 95% CI: 24.1–39.7%) followed by *Staphylococcus aureus* (17.5%, 95% CI: 8.5 to 28.4%), in marked contrast to the *Streptococcus agalactiae* burden seen in high-income healthcare settings. Our results reveal clear evidence that the current definitions of EOS and LOS sepsis are outdated, particularly in LLMICs. These outdated definitions may be guiding inappropriate empirical antibiotic prescribing that inadequately covers the causative pathogens responsible for neonatal sepsis globally. Harmonizing sepsis definitions across neonates, children and adults will enable a more acurate comparison of the epidemiology of sepsis in each age group and will enhance knowledge regarding the true morbidity and mortality burden of neonatal sepsis.

Key Words: neonatal sepsis, early-onset sepsis, late-onset sepsis, bacteremia, antimicrobial resistance

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The global child mortality rate is declining, yet mortality remains high for the vulnerable neonatal population, with 47% of deaths in children under 5 occurring in the first 28 days of life.[1,2] The third greatest contributor to neonatal mortality is neonatal infection, including sepsis and meningitis, resulting in up to 570,000 sepsis-attributable deaths each year.[3,4]

The United Nations Sustainable Development Goals have called for the end of preventable child deaths by 2030.[5] The success of this goal is highly dependent on the effective treatment and prevention of neonatal infections. In the context of burgeoning global antimicrobial resistance (AMR), understanding the evolving epidemiology and etiology of neonatal sepsis is paramount.[6]

It has long been considered that early-onset neonatal sepsis (EOS)—that is, sepsis occurring within the first 72 hours after birth—is predominantly caused by vertical transmission of bacteria (typically *Streptococcus agalactiae* and *Escherichia coli*) from mother to infant, either *in utero* or during birth[7,8] Conversely, late-onset neonatal sepsis (LOS), occurring at >72 hours of life, is considered to be acquired by nosocomial or community sources of infection.[7,8] However, recent studies have reported a potential epidemiological shift in the causative pathogens responsible for EOS, with an increase in the proportion of multidrug-resistant (MDR) Gram-negative bacteria responsible for systemic neonatal infections evident from the first day of life, accompanied by concerning AMR profiles previously evident in infants with hospital-acquired LOS.[9,10]

The World Health Organization (WHO) suggests the early administration of empirical antibiotics following a clinical diagnosis of neonatal sepsis, which is not defined in its timing of acquisition in their guidelines (by EOS or LOS),[11] but rather recommends ampicillin/benzylpenicillin and gentamicin as first-line treatment regimens, and third-generation cephalosporins as an alternative agent, for treating neonatal sepsis.[11] However, these empirical recommendations are based on data on the presumed causative pathogens of neonatal sepsis that are largely derived from high-income countries (HIC).[12] However, with 98% of the neonatal sepsis mortality burden arising from low- and middle-income countries (LMICs),[13] and with mounting evidence to suggest differing etiologies of the bacterial pathogens causing neonatal infection between high- and low-resourced healthcare settings,[14,15] improving the understanding of the causative pathogens driving neonatal sepsis is essential.

A growing body of evidence suggests the WHO-recommended neonatal sepsis antibiotic regimens may be becoming redundant, as the contemporary causative bacteria responsible for neonatal sepsis are increasingly less susceptible to the current empirical antibiotic regimens in many settings.[13,16] Recent epidemiological studies across multiple LMICs suggest poor coverage is provided by the currently-recommended empirical regimens, resulting in high rates of divergent empirical antibiotic prescribing across clinical settings, which may propagate AMR.[13,17,18]

An increasing prevalence of AMR is evident in the Gram-negative bacteria causative of neonatal sepsis, with rates of

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nonsusceptibility of up to 97% (to ampicillin) and 70% (to gentamicin) reported.[9,12,15,18,19] In fact, 570,000 sepsis-attributable deaths occur in neonates each year, contributed by a lack of efficacy to currently available and recommended antibiotics.[3,4] Consequently, many physicians, particularly in LMICs (where the burden of AMR and neonatal mortality is highest),[13] are no longer following empirical therapeutic recommendations due to the realization that current empirical treatment guidelines are unlikely to be efficacious.[15,17] Despite this, there is a concerning dearth of quality evidence to support new empirical regimens in neonates, and this warrants global attention.

To reduce unnecessary deaths in neonates, it is essential that empirical treatment regimens closely align with the contemporary etiology of neonatal sepsis. Based on the available evidence, we hypothesized that the classical bacterial pathogens presumed to cause EOS and LOS are evolving, and current empirical treatment guidelines based upon these definitions may be guiding inefficacious therapy.

As the published evidence suggests the causes of neonatal sepsis may differ between resource-constrained and resource-replete healthcare settings, we further aimed to evaluate differences in the etiology of neonatal sepsis in HICs and low- and lower-middle-income countries (LLMICs). We suggest new strategies for defining the causative bacteria responsible for the burden of neonatal sepsis globally, to emphasize the need to evaluate alternative empirical antibiotic regimens that may better target the contemporary epidemiology of neonatal sepsis, to reduce its unacceptable morbidity and mortality burden globally.

METHODS

We followed preferred reporting items for systematic reviews and meta-analysis guidelines to conduct systematic searches in EMBASE, MEDLINE and Global Health databases using search terms comprised of both MESH terms and keywords relating to EOS and LOS (Material, Supplemental Digital Content 1, http://links.lww.com/INF/F651). Results were limited to human studies with primary data published in English between January 2017 and March 2022 to capture contemporaneous antimicrobial susceptibility profiles and bacteria causative of neonatal sepsis.

Predefined inclusion and exclusion criteria were established to assess study eligibility (Table, Supplemental Digital Content 2, http://links.lww.com/INF/F652). Studies were excluded if they did not clearly define the timing of neonatal sepsis if they analyzed only a single pathogen, or if they included only high-risk populations (for example, very low-birth-weight infants, premature infants <32 weeks' gestation and infants with HIV, tuberculosis or malaria). Small case series (n < 10) were also excluded. Included studies required data to have been collected within the clinical context of suspected neonatal infection and reported data needed to have been collected after 2012, with samples collected from normally sterile sites (blood and/or cerebrospinal fluid).

Abstracts yielded from the above searches were exported and reviewed by authors M.L.H. and P.C.M.W. Full-text articles were sourced, and studies were assessed for quality using GRADE methodology[20] to determine the risk of bias associated with the study. A data extraction tool was used to summarize pathogen data alongside data on study design, patient recruitment methods, publication year, data collection dates and specimen handling and collection methods (Table, Supplemental Digital Content 3, http://links.lww.com/INF/F653).

Meta-analyses of collected data were undertaken to generate and compare pooled estimates of the relative prevalence of pathogens causing EOS and LOS with exact binomial confidence intervals (CIs). Pathogen prevalence for LOS and EOS was additionally calculated after stratifying the income status of the study setting. Analyses were performed in Stata version 16 (Stata Corporation, TX, USA) using the metaprop command.[21] A random-effects model was used to account for the expected heterogeneity between study populations, with weighting completed via DerSimonian and Laird methods.[22] Study heterogeneity between EOS/LOS subgroups was analyzed using the I^2 statistic,[22] and χ^2 tests were used to evaluate the strength of evidence for heterogeneity (with a P value of <0.05 considered statistically significant). Publication bias was assessed using the Luis Furuya–Kanamori index of the Doi plot.[23] A double arcsine transformation of effect size was used for the Doi plot to stabilize the variance of the proportions.

RESULTS

The systematic review of the literature identified 8699 records eligible for inclusion (Fig. 1). Before the screening, 2620 duplicates were removed, leaving 6079 records for review. Title and abstract screening excluded a further 5901 studies (Table, Supplemental Digital Content 4, http://links.lww.com/INF/F654) resulting in 178 for full-text analysis. Investigators were unable to retrieve 2 full-text references after attempting to contact the authors, and these were subsequently excluded. Therefore, 176 full-text studies were assessed for eligibility and ultimately, 48 were included for analysis.

Of the included studies 29 were prospective studies,[13,24–51] and 19 involved retrospective data collection.[52–70] All studies were conducted in hospital settings, with only 1 paper assessing neonates in the community.[38] Six studies provided moderate quality evidence (GRADE level B),[13,28,32,36,38,44] 15 were low quality (GRADE level C)[27,30,31,33,35,41,42,46,48,49,51,53,55,61,68] and 27 were very low quality (GRADE level D).[24–26,29,34,37,40,43,45,47,50,52,54,56–60,62–67,69,70] (Figure, Supplemental Digital Content 5, http://links.lww.com/INF/F655)).

The included articles incorporated 757,427 blood and cerebrospinal fluid samples collected from 311,359 neonates across 25 countries. Pathogens that may be potential contaminants [including coagulase-negative Staphylococci (CoNS), *Streptococcus viridans* and other *Streptococcus* spp., *Stenotrophomonas maltophilia and Burkholderia cepacia*], were removed from the analysis as clinical data was not always included in the retrieved articles. Of the 10,150 significant bacteria identified, 4358 were isolated from neonates with EOS, and 3894 bacterial pathogens were isolated from infants with LOS (Table 1).

The data were unevenly distributed across the WHO regions.[71] Fourteen studies were conducted in World Bank defined[72] HIC,[28,30–32,36,42,44–46,56,58,61,62,64] 7 in upper-middle-income countries (UMICs),[27,43,49,53,60,66,70] and 27 were conducted in LLM ICs.[13,24–26,29,33–35,37–41,47,48,50–52,54,55,57,59,63,65,67–69] Most data were attained from neonates in the United States (142,934/311,359, 45.9%).[28,36,44] Eastern Mediterranean countries had the second highest representation, with 29.2% (91,133/311,359) of the included neonates from 6 studies.[31,39,41,53,68,70] Only 9.2% (28,773/311,359) of the included neonates were from African countries,[13,24,27,29,43,47,49–51,60,63,66] and 5.1% (15,758/311,359) evaluated neonates from Southeast Asia.[13,25,26,33–35,38,48,52,54,55,57,65,67,69]

In neonates with EOS of the 4347 significant pathogens isolated, 52.9% (2,301/4347) were Gram-negative and 46.8% (2,038/4,347) were Gram-positive bacteria. For neonates with LOS, of the 3894 significant pathogens reported 71% (2,765/3,894) were Gram-negative, and 29% (1,129/3,894) were Gram-positive (Table 1). Meta-analyses with random-effects weighting estimated a pooled prevalence of Gram-negative pathogens of 62.6% (95% CI: 52.1–72.3) for EOS, and 71.0% (95% CI: 63.5–78.1%) for LOS (Fig. 2).

Figure 2 and Figures S2–S16, Supplemental Digital Content 5, http://links.lww.com/INF/F655) reveal the results of the

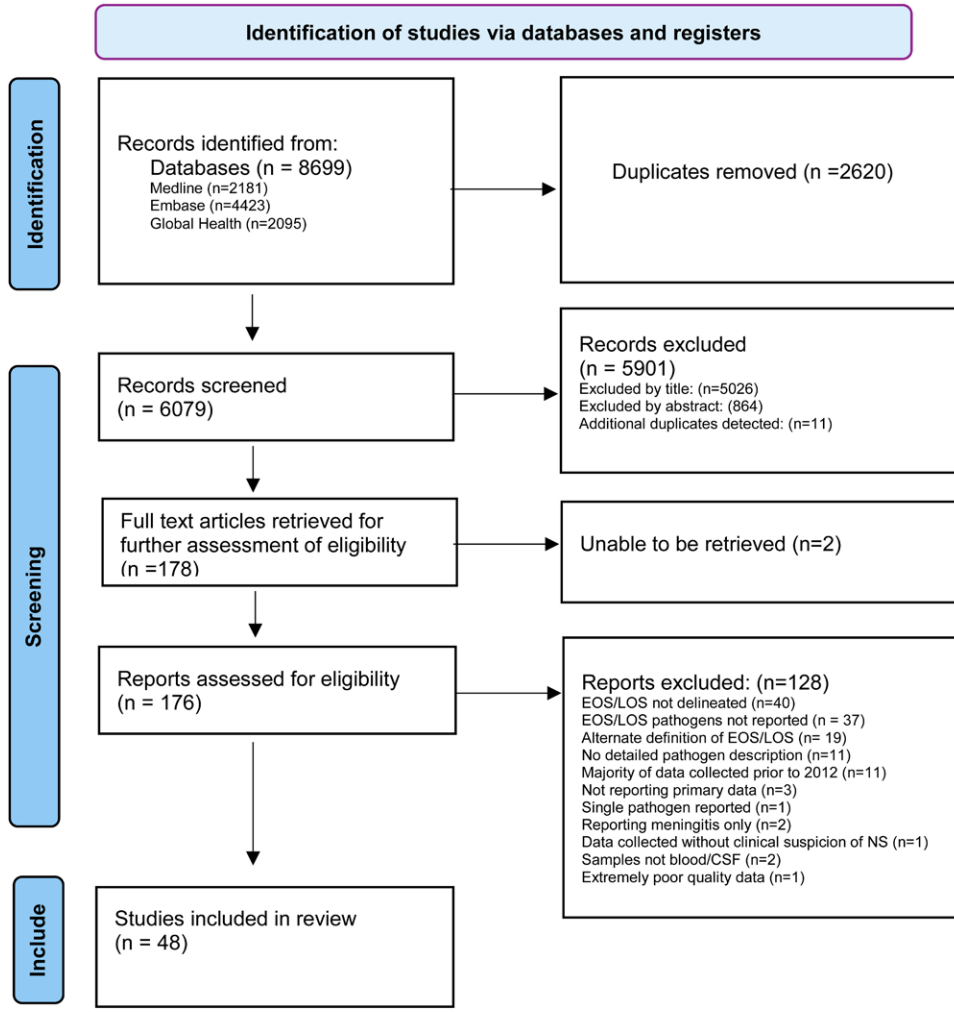

FIGURE 1. PRISMA diagram.

meta-analyses comparing the relative prevalence estimates for pathogens causing EOS and LOS (Table, Supplemental Digital Content 6, http://links.lww.com/INF/F716). These revealed no significant difference in the likelihood of *Acinetobacter* spp. (P = 0.656), *Citrobacter* spp. (P = 0.913), *E. coli* (P = 0.582), *Serratia* spp. (P = 0.711), *Enterobacter* spp. (P = 0.172), *Proteus spp.* (P = 0.084), *Pseudomonas* spp. (P = 0.082), *Salmonella* spp. (P = 1.62), *Enterococcus* spp. (P = 0.376) and *Streptococcus pyogenes* (P = 0.272) to cause EOS or LOS.

However, there was a significant difference in the likelihood of some bacteria causing EOS versus LOS. In particular, *Streptococcus agalactiae* ($P \leq 0.001$) and *Listeria* spp. (P = 0.006) predominated in EOS, and *Klebsiella* spp. (P = 0.018) and *S. aureus* (P = 0.026) predominated in LOS. The Luis Furuya–Kanamori index classified the risk of bias as none (<|1|) or minor (|1| to |1.99|) for the majority of analyses, with the exception of *Listeria* spp., *S. pyogenes*, *S. pneumoniae* and *Acinetobacter* spp., where the risk was considered major (>|2|; Figures S17-S21, Supplemental Digital Content 6, http://links.lww.com/INF/F655).

Although the overall burden of Gram-negative pathogens in EOS was high, the predominant pathogen causing EOS was *S. agalactiae* (23.3%, 1,015/4347), followed by *E.coli* (18.0%, 783/4,347), *Klebsiella* spp. (14.8%, 644/4,347) and *S. aureus* (9.7%, 424/4,347) (Table 1, Figure S22, Supplemental Digital Content 6, http://links.lww.com/INF/F655). However, when the results were stratified for HICs versus LLMICs, the predominant pathogens causing EOS differed significantly, with *Klebsiella* spp. (31.7%, 95% CI: 24.1–39.7%) and *S. aureus* (17.5%, 95% CI: 8.5–28.4%) most prevalent in LLMICs, with *S. agalactiae* only responsible for a small proportion of EOS (13.8%, 95% CI: 3.4–28.7) in LLMICs (Fig. 3).

The predominant bacterial species causative of LOS overall were *Klebsiella* spp. (30.7%, 1,197/3,894), *E. coli* (16.5%, 643/3,894) and *S. aureus* (15.5%, 605/3,894) (Table 1 and Fig. 4). Only a small difference was found when stratifying between HIC and LLMIC in the meta-analysis for LOS, with *Klebsiella* spp. still the predominant bacteria causing LOS in LLMICs (30.6%, 95% CI: 24.9–36.7), followed by *S. aureus* (25.6%, 95% CI: 17.4–34.8%), and *E. coli* (9.7%, 95% CI: 7.4–12.1%) (Figure S21 Supplemental Digital Content 5, http://links.lww.com/INF/F655 and Supplemental Digital Content 7, http://links.lww.com/INF/F717).

DISCUSSION

We provide a comprehensive and systematic review of the current bacterial pathogens causing neonatal sepsis. Our results revealed Gram-negative bacteria are the most frequent cause

TABLE 1. Gram-negative and Gram-positive Pathogens Causative of Early-onset Sepsis and Late-Onset Sepsis

Early-onset Sepsis			**Late-onset Sepsis**		
Gram-negative	**n (%)**		**Gram-negative**	**n (%)**	
Escherichia spp.	783	(18.0)	*Klebsiella* spp.	1197	(30.7)
Klebsiella spp.	644	(14.8)	*Escherichia* spp.	643	(16.5)
Enterobacter spp.	147	(3.4)	*Acinetobacter* spp.	246	(6.3)
Acinetobacter spp.	132	(3.0)	*Enterobacter* spp.	211	(5.4)
Pseudomonas spp.	101	(2.3)	*Serratia* spp.	155	(4.0)
Serratia spp.	100	(2.3)	*Pseudomonas* spp.	118	(3.0)
Listeria spp.	96	(2.2)	*Citrobacter* spp.	26	(0.7)
Haemophilus spp.	48	(1.1)	*Proteus* spp.	13	(0.3)
Citrobacter spp.	28	(0.6)	*Salmonella* spp.	9	(0.2)
Proteus spp.	6	(0.1)	*Listeria* spp.	7	(0.2)
Salmonella spp.	6	(0.1)	*Neisseria* spp.	6	(0.2)
Neisseria spp.	3	(0.1)	Other Gram-negative	134	(3.4)
Other Gram-negative	207	(4.8)			
Total Gram-negative EOS	**N = 2301**	**53%**	**Total Gram-negative LOS**	**N = 2765**	**71%**
Gram-positive			Gram-positive		
Streptococcus agalactiae	1015	(23.3)	*Staphylococcus aureus*	605	(15.5)
Staphylococcus aureus	424	(9.8)	*Enterococcus* spp.	222	(5.7)
Enterococcus spp.	182	(4.2)	*Streptococcus agalactiae*	171	(4.4)
Streptococcus pyogenes	29	(0.7)	*Streptococcus pyogenes*	13	(0.3)
Streptococcus pneumoniae	23	(0.5)	*Streptococcus pneumoniae*	3	(0.1)
Other Gram-positive	365	(8.4)	Other Gram-positive	115	(3.0)
Total Gram-positive EOS	**N = 2038**	**47%**	**Total Gram-positive LOS**	**N = 1129**	**29%**

EOS indicates early-onset sepsis; LOS, late-onset neonatal sepsis.

of both EOS and LOS globally; of concern given the plasmid-mediated resistance these bacteria can easily transmit, contributing to the rising burden of AMR.[6] The significant rise in *Klebsiella* spp. as a dominant species in not only LOS but also EOS has also been noted in other recent epidemiological studies.[10,13,15,17,35,38,67]

Our review revealed *Klebsiella* spp. were the third most isolated bacteria causative of EOS globally, and the most common bacteria causative of neonatal sepsis in LLMICs. This is an important finding given the potential virulence and resistance profile commonly seen with this pathogen. Recent whole genome sequencing studies evaluating bacteria causing invasive neonatal infections identified an abundance of AMR genes and virulence factors in *Klebsiella* spp., exposing neonates to a high risk of mortality.[13]

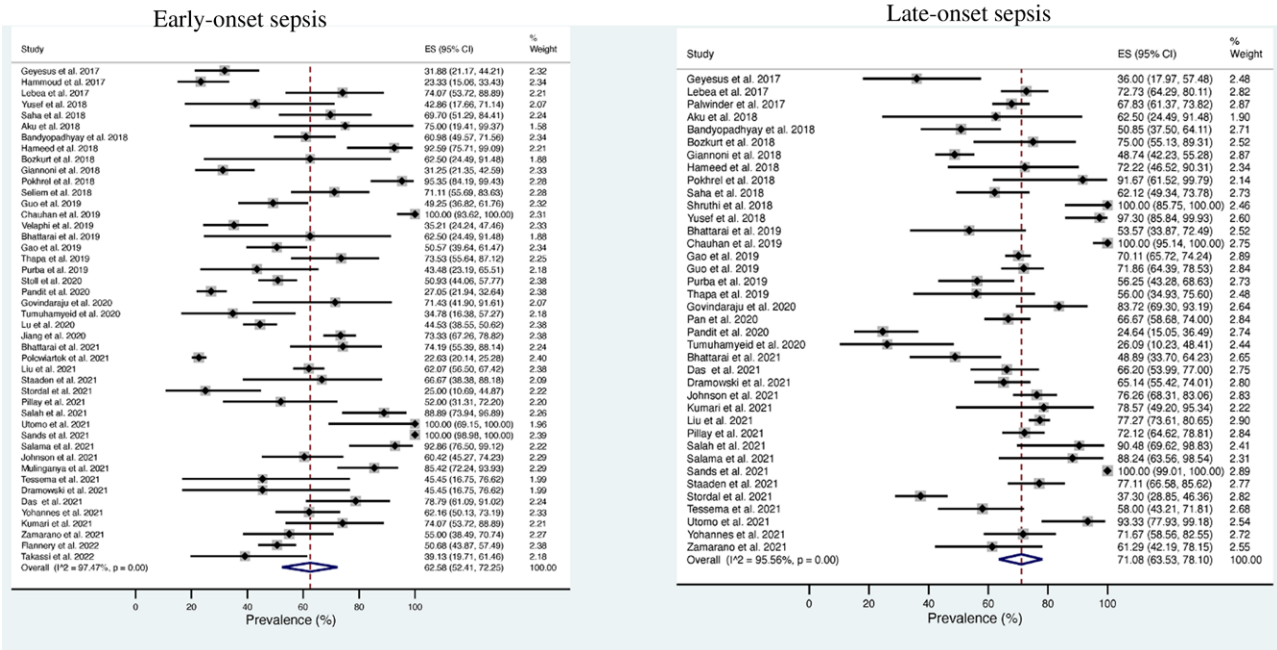

FIGURE 2. Meta-analysis of the proportion (%) of Gram-negative bacteria causative of early-onset (A) and late-onset (B) neonatal sepsis

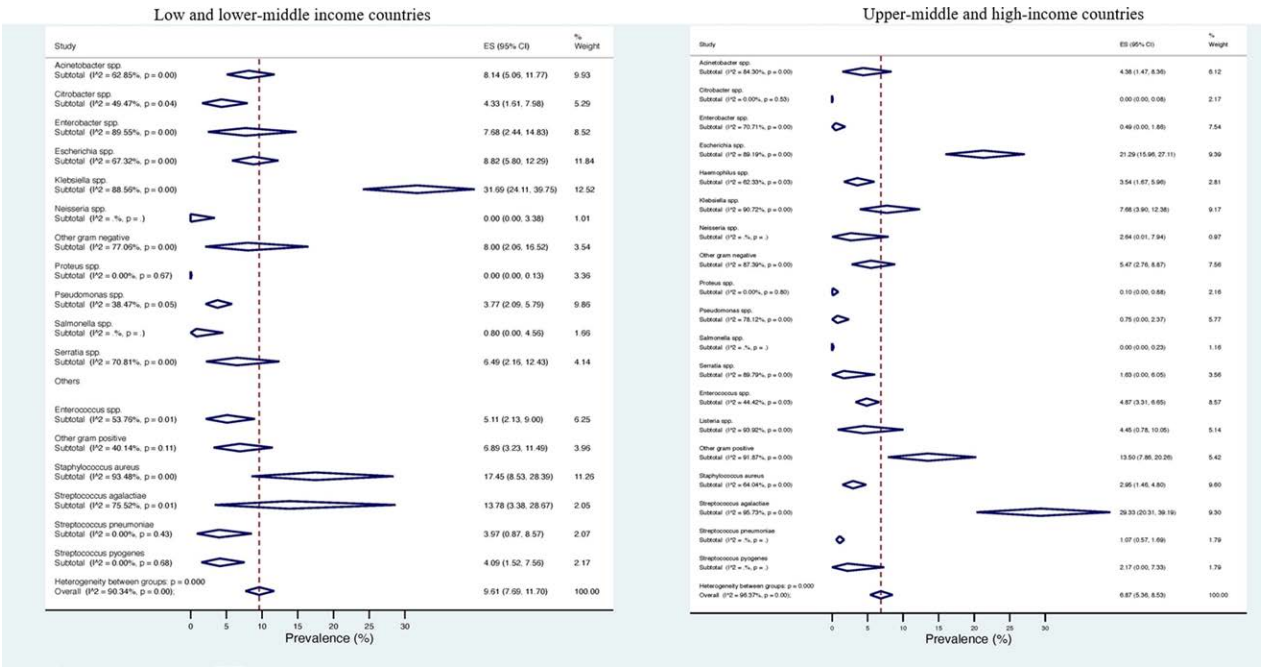

FIGURE 3. Meta-analysis of species prevalence causative of early-onset neonatal sepsis (%) in low- and lower-middle-income versus upper- and high-income countries.

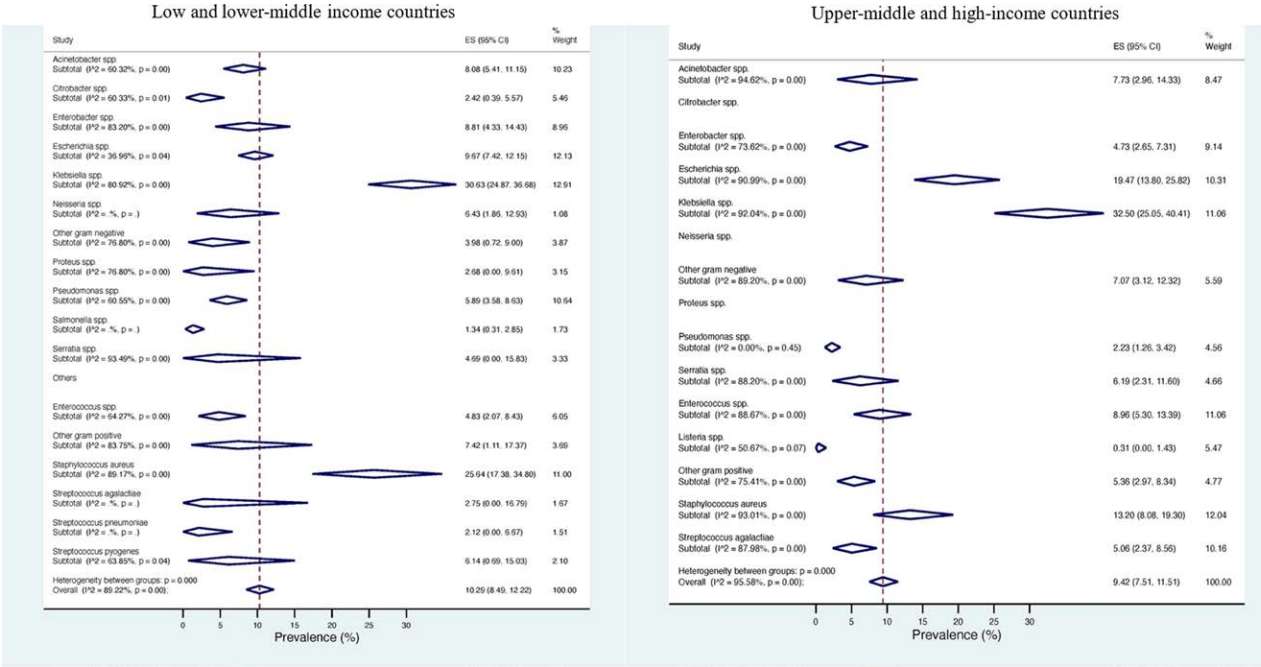

FIGURE 4. Proportion of EOS and LOS caused by these pathogens.*NB: pathogen proportions <1% have been omitted from graph, see Table 1 for full description of causative pathogens.

While our review found *S. agalactiae* to be the highest isolated single pathogen in EOS overall, this was likely impacted by the overrepresentation of data from high-income settings. When using a meta-analysis to stratify data from LLMICs independently, the most prevalent bacteria was *Klebsiella* spp. in both early- and late-onset sepsis. In these resource-constrained settings, *S. agalactiae* is only the third most prevalent bacteria causing EOS. Overcrowding, poor sanitation and restricted infection, prevention and control resource availability may predispose infants in LLMIC to early colonization with Gram-negative bacteria, which may explain these findings.

Our data from HIC support the historical assumptions that EOS is predominantly caused by *S. agalactiae,* followed by *E. coli*. The

TABLE 2. Suggested Contemporary Classification of Neonatal Sepsis, Likely Causative Pathogens and Potential Efficacious Empiric Regimens

Historical Classification	Likely Pathogens	Timing of Acquisition	Empiric Antibiotic Regimens to Target Likely Pathogens(s)
Early-onset sepsis	*Streptococcus agalactiae; Escherichia coli*	Within 72 hours of birth	Aminopenicillin(s) and gentamicin; third-generation cephalosporins
Late-onset sepsis	*E. coli, S. agalactiae, Streptococcus aureus,* coagulase-negative staphylococci	After 72 hours of birth	Aminopenicillin(s) and gentamicin; third-generation cephalosporins; flucloxacillin if *S. aureus* infection suspected; vancomycin (where available)
Proposed Contemporaneous Classification	**Likely Pathogens**	**Timing of Acquisition**	**Antibiotic Regimens Likely to be Efficacious**
Vertically-acquired	*S. agalactiae* (GBS), *E. coli., Klebsiella* spp., *Enterobacter* spp., *S. aureus*	Within 72 hours after birth life	While in many contexts aminopenicillin(s) and gentamicin, or third-generation cephalosporins, remain efficacious, there is increasing evidence that in many high-burden AMR settings, antibiotics with activity against extended-spectrum beta lactamases, metallo-beta lactamases and carbapenem-resistant *Acinetobacter* spp. may be necessary.
Horizontally-acquired; nosocomial setting	*Klebsiella* spp., *E. coli, Acinetobacter* spp. *S. aureus,* coagulase-negative staphylococci	These pathogens may be acquired during the delivery process, very early in the perinatal period or over the course of a prolonged hospitalisation following a premature delivery.	
Horizontally-acquired; community setting	*S. agalactiae, E coli, S. aureus*	Within the first month of life	While community-acquired pathogens tend to be less likely to be multidrug resistant, in some settings, there is a high community prevalence of ESBL-producing Gram-negative bacteria, which should be considered when selecting empiric antibiotic regimens.

clear contrast in causative bacteria isolated from resource-constrained settings compared to well-resourced healthcare settings, particularly in EOS, warrants further research to consider how LLMICs might reduce the current burden of EOS caused by Gram-negative bacteria—pathogens against which currently-recommended empiric antibiotic regimens are unlikely to be effective.[15,18]

Klebsiella spp., *E.coli* and *S. aureus* were the pathogens primarily responsible for LOS in our review, supporting the evolving international literature highlighting the overall burden of Gram-negative bacteria in neonatal sepsis.[10] In fact, less than one-third of all bacteria isolated in neonates with LOS were Gram-positive within our comprehensive review.

In the context of increasing facility-based births globally,[73] alongside prolonged hospital stays following the successful resuscitation of very premature infants, early colonization of infants with Gram-negative bacteria may be one factor driving the altered etiology our study reveals. The historical consideration that EOS is caused by bacteria that are vertically-acquired, while LOS is horizontally-acquired, is increasingly becoming less clear—as neonates may be admitted to hospital units with a heavy environmental prevalence of multidrug-resistant Gram-negative bacteria. Selection pressure due to a high burden of MDR infections in resource-constrained hospital facilities propagates AMR, and if these MDR bacteria are colonizing neonates quickly in the perinatal period, it is essential to ensure empirical treatment guidelines address the local contemporary causes of neonatal sepsis to avoid unnecessary neonatal morbidity and mortality.

In keeping with evolving themes emerging from single epidemiological studies,[10,74,75] and in support of recently published literature,[14] the results of our review query the traditional definitions and assumptions behind EOS and LOS (and the bacteria typically associated with these defined clinical syndromes, which guide current empirical antibiotic regimen recommendations). Our results confirm that it is clear that EOS and LOS assumptions have poor utility in LLMICs. In these settings, community-acquired versus hospital-acquired definitions[76] may be more useful in predicting the bacteria (and resistance profiles) empiric antibiotic regimens need to target, yet data pertaining to community-acquired infections—particularly in LLMICs—are sparse in the published literature. New definitions, with less emphasis on the timing of symptom onset, that instead consider the most likely source of infection, may enable a more accurate selection of efficacious empirical antibiotic regimens to ensure infants receive appropriate antibiotic therapy-Magiorakos, 2012 #20 targeting the most likely bacteria causative of their infection (Table 2). Furthermore, classification defining infections by community acquired infection and hospital acquired infection will harmonize neonatal sepsis definitions with the pediatric and adult definitions currently in use.

Our research has a number of limitations. The heterogeneity of study designs in included studies, and the poor quality of many published observational studies, reduces confidence in the estimate of the effect observed. Many studies meeting the inclusion criteria provide insufficient microbiological methodological detail and limited denominator data. Additionally, the published and available data includes almost exclusively inborn neonates, with the majority of studies undertaken in hospital settings and only 1 study recruiting neonates from the community.[38] This likely biases the results toward an overrepresentation of hospital-acquired pathogens. To enhance the quality of published literature in this space, future observational studies should follow strengthening the reporting of observational studies in epidemiology for newborn infection guidelines which have been developed to ensure the quality of observational research in the neonatal population.[77] Ensuring studies comply with these guidelines will enable high-quality published data that can be readily compared across sites and regions.

Geographic bias in the available published literature also limits the generalizability of our findings, with a stark paucity of published data on the causative pathogens responsible for neonatal sepsis across Southeast Asia, South America, the Pacific and Africa. The lack of epidemiological data in the regions is concerning, given their high birth rates and infant mortality rates.[78] In 2017, South Asia and sub-Saharan Africa accounted for 79% of all

neonatal deaths.[79] Yet this review reveals the paucity of representative epidemiological data available to inform policy and guideline change from these regions with the highest burden of infant mortality, where the need for robust evidence is greatest.[16]

Moreover, data from the United States of America accounted for 46% of the published data in this review, which is likely to significantly bias the overall prevalence of Gram-positive organisms more commonly seen in high-resourced healthcare settings. We rectified this by stratifying and analyzing our data by country income status; and despite the high representation of North American sites in the overall dataset, our review still reveals an almost equal proportion of Gram-negative and Gram-positive bacteria are responsible for EOS globally. The concerningly high proportions of *E. coli* and *Klebsiella* spp. causative of EOS reinforces the changing pathogen distribution for this clinical syndrome globally, and the urgent need to ensure antibiotic regimens to treat EOS provide coverage against these bacteria.[10]

Our meta-analysis supports the hypothesis that the causative bacteria responsible for EOS and LOS in neonates are similar. This was particularly evident across Gram-negative organisms and stands contrary to prior assumptions regarding the traditional causes of EOS and LOS. The findings support the evolving literature revealing the changing etiology of neonatal sepsis and the importance of considering new definitions to guide efficacious empiric antibiotic regimens against neonatal sepsis.[10] Our findings reflect those revealed by the multicenter NeoOBS study, which highlighted the presence of common pathogens in both EOS and LOS and the low prevalence of *S. agalactiae* (3.4%) isolated within their global cohort.[15] Additionally, our findings concur with the Delhi Neonatal Infection Study study,[10] and the Neonatal Sepsis in Southeast Asia and the Pacific study observational study across Southeast Asia,[17] which reveal a low prevalence of *S. agalactiae* causing neonatal sepsis after the first day of life in low-resource healthcare settings.[10,15] It remains unclear what is causing this low prevalence, with difficulty isolating this pathogen in low-resourced settings likely to impact the findings.[14] Further granular data representative of all regions to provide insight into the timing of acquisition of particular pathogens are needed with the exploration of the causal mechanisms of ultra-early horizontal transmission occurring within the neonatal intensive care unit. These data can ultimately ensure a more robust categorization of neonatal sepsis based on the likely pathogens and the means of transmission, to ensure empiric therapies are optimized to avoid unnecessary neonatal mortality.

Our results raise the importance of empirical antibiotic treatment recommendations that need to be region- and setting-specific to reflect the local causative pathogens and known resistance patterns responsible for neonatal sepsis more closely. The challenge remains; however, that to derive locally-recommended regimens from sufficient observational data, prospective and systematic surveillance is required.

The findings from this review, while limited in their direct application, support the evolving literature suggesting a higher proportion of Gram-negative pathogens are causing both EOS and LOS and an equitable distribution of bacteria are responsible for infections across the early- and late-neonatal period, suggests new definitions to define the acquisition of invasive infections to the empirical antibiotic guidelines are needed. The predominance of Gram-negative bacteria identified in our review as the leading cause of early-onset sepsis in LLMICs further highlights the need to update the current empirical antibiotic recommendations in neonatal sepsis to ensure they reflect the contemporaneous etiology and epidemiology of neonatal sepsis.

Most importantly, in the context of the rising prevalence of Gram-negative bacteria, and their potential for acquisition of resistant mechanisms, it is vital that neonates are prioritized in drug development programs and clinical trials to enable access to efficacious agents to treat MDR infections.[4] To attain sustainable development goals, the pressing burden of neonatal sepsis requires urgent attention to ensure infant and child health outcomes can be optimized, and the unacceptable burden of mortality due to neonatal sepsis can be curtailed.

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