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Michelle L. Harrison, Maria E. Villanueva-Uy, Erena Kasahara, Julie Jaynney Caparas, Nover Edward Duarte, Alkim Ozaygen, Patrick Cahill, Jill Hopkins, Tamalee Roberts, Yue Wu, Tom Snelling, H. Rogier van Doorn, Paul Turner & Phoebe C. M. Williams

Diagnostic challenges and gram-negative pathogen dominance in early- and late-onset neonatal infection in Manila, Philippines

Michelle L Harrison[1,2], A/Prof. Maria E. Villanueva-Uy[3,4] Dr. Erena Kasahara[3,4], Julie Jaynney Caparas[4], Nover Edward Duarte, [4] Dr. Alkim Ozaygen[1], Patrick Cahill[1], Jill Hopkins[5,6], Dr. Tamalee Roberts[5,7], Dr. Yue Wu[1,2], Prof. Tom Snelling[1,2], Prof. H Rogier van Doorn[5,8], Prof. Paul Turner[5,6], A/Prof. Phoebe CM Williams[1,2,9,]

[1]Sydney School of Public Health, Faculty of Medicine, The University of Sydney, Sydney, New South Wales, Australia
[2]Sydney Infectious Diseases Institute, Sydney, Australia
[3]Institute of Child Health and Human Development, National Institutes of Health, Manila, Philippines
[4]Philippine General Hospital, Manila, Philippines
[5]*Centre for Tropical Medicine and Global Health, Nuffield Department of Medicine, University of Oxford,* UK
[6]*Cambodia Oxford Medical Research Unit, Angkor Hospital for Children,* Cambodia
[7] *Lao- Oxford Mahosot Hospital Wellcome Trust Research Unit,* Laos
[8]*Oxford University Clinical Research Unit (OUCRU), Hanoi,* Vietnam
[9]*UNSW School of Women and Children's Health, UNSW, Sydney,* Australia

Corresponding author:
Michelle L Harrison: m.harrison@sydney.edu.au

ABSTRACT

Neonatal infections contribute to high mortality, yet data describing neonatal sepsis are predominantly from high-income countries. Epidemiology is likely to vary across settings, potentially affecting the way infections are managed. We aimed to describe the epidemiology of clinically-suspected infection in neonates (<28days of age) at the Philippine General Hospital in Manila, Philippines. We undertook prospective surveillance enrolling all neonates admitted to the Neonatal Intensive Care Unit (NICU) who had at least one episode of clinically-suspected infection between November 2023 and November 2024. We enrolled 799 neonates with 1,024 episodes of clinically-suspected infection. We found that only 1% of suspected early onset infections (<48 hours of life) returned a positive blood culture (6/757). 15% of neonates died (117/799), including 12% (93/754) of those with suspected a suspected infection that didn't return a positive culture and 53% (24/45) of those with confirmed culture-positive infections. We found a significant burden of culture-negative clinically-suspected infection and high mortality. There is a great need to improve case definitions and diagnostic capacity for neonatal infection and to reduce antibiotic overuse, in order to provide effective treatment and improve clinical outcomes for neonates.

Introduction:

Neonatal sepsis is associated with high mortality, posing a significant risk to attaining Sustainable Development Goal (SDG) 3·2 - to end preventable deaths of newborns and reduce neonatal mortality by the year 2030.[1] Mortality in children under five has improved in recent years. Improvements are largely due to targeted infection prevention and control (IPC) practices, and water, sanitation and hygiene (WASH) interventions, alongside improved vaccine availability. However, improvements in neonatal mortality have been much slower.[2] The immature immune systems of neonates predispose them to a high risk of infection,[3] and in many countries this is compounded by the global rise of antimicrobial resistance (AMR).[4,5] Long hospital stays and medical interventions secondary to prematurity, inadequate nurse-to-patient ratios, hospital

overcrowding, and incomplete integration of IPC interventions, all contribute to the risk of infection with hospital-acquired pathogens.[3]

In low-and middle-income countries (LMICs) in Southeast Asia, there is emerging evidence that gram-negative bacteria, which are typically associated with late-onset neonatal sepsis (clinical signs of infection ≥48 hours after birth)[6-8] are also a cause of early-onset neonatal sepsis (clinical signs of infection <48 hours after birth)[7,9-13] The differences in local pathogen prevalence across regions provoke a review of how neonatal infections are classified for empirical prescribing, particularly for neonates in low-resourced environments.[9,14] In Southeast Asia, there has been an increase in mortality in children under five attributable to AMR within gram-negative bacterial pathogens – particularly *Acinetobacter baumannii*, *Escherichia coli,* and *Klebsiella pneumoniae*.[2] However, published data describing the epidemiology of neonatal sepsis in these settings remain sparse.[9]

At the Philippine General Hospital (PGH) in Manila, Philippines, high rates of gram-negative sepsis and high AMR have been reported within the neonatal intensive care unit (NICU).[13] The high prevalence of AMR among gram-negative pathogens and the known impact on mortality in vulnerable populations intensifies the need to better understand the epidemiology of neonatal sepsis for effective treatment and prevention.[15] The World Health Organization's *"Pocketbook of Hospital Care for Children"* remains the recommended resource for the clinical management of children and neonates in hospitals globally.[16] These guidelines recommend ampicillin/benzylpenicillin and gentamicin for first-line therapy, and third-generation cephalosporins for second-line treatment.[16] However, like other international guidelines, these recommendations are primarily informed by data from high-income, resource-replete settings.

With the differing profile of neonatal sepsis between high- and low-income countries becoming increasingly evident, clinical sites are resorting to the use of divergent local empiric guidelines. At the PGH, recommended first-line therapy for early-onset sepsis (EOS) is amikacin and ampicillin, and for late-onset sepsis (LOS), guidelines recommend amikacin and ceftazidime. Meropenem is recommended as the second-line treatment for both EOS and LOS, creating a difficult balance between ensuring effective treatment and avoiding the overuse of broad-spectrum

antimicrobial agents. At PGH the decision to commence antibiotics is made by neonatology clinicians and consultants according to local guidelines for clinical care.

Widespread management of clinically-suspected infection using broad-spectrum empiric regimens calibrated to local susceptibility data creates the potential for further propagation of AMR. A greater understanding of disease epidemiology and causal pathways leading to both culture-negative and culture-positive sepsis in low-resourced settings is essential to inform new treatment strategies and to curb the rapidly growing rate of AMR in the region.

We aimed to describe clinically-suspected neonatal infection and to compare culture-negative and culture-positive, and early- and late-onset episodes. In doing so, we aim to better understand the factors that may predispose a neonate to these episodes, and to identify points at which targeted interventions may improve clinical outcomes. Systematic surveillance linking clinical and microbiological findings will help to address the paucity of data from Southeast Asia on the pathogens responsible for early- and late-onset neonatal sepsis, and can provide a basis for informed discussion of future antimicrobial prescribing strategies.

Results:

NICU characteristics

The 70-bed NICU at PGH had an average occupancy of 97% (67/70) over the 12-month period. The highest occupancy reported was 119%, with 83 babies admitted into the 70-bed neonatal unit. Occupancy was at or over 100% for 25 weeks of the year.

Characteristics of enrolled neonates

Surveillance data were collected for 799 individual neonates who met the inclusion criteria during the study period (Figure 2). Figure 2 presents the consort diagram of enrolments through a relational database lens, categorising each section as ‘persons’, for factors specific to an individual; ‘episodes’, for factors specific to an episode of suspected infection, and ‘organisms’, for factors relating to microbiology. Characteristics of included neonates are outlined in Table 1. Of the 799 enrolled neonates, 45% (361/799) were female, median birthweight was 1930g (interquartile range (IQR) 1470-2485g), and median gestational age 34 weeks (IQR 31-36 weeks).

The median length of stay in the neonatal unit was 19 days (IQR 10-35 days), and the median age at the first episode of clinically-suspected bacterial infection was on the first day of life (Day 0, IQR 0-2 days). Most (74%, 597/799) were admitted to the NICU with suspected pulmonary disease. Of the total cohort, 95% (757/799) had a early-onset episode for their first episode of suspected infection (within the first 48 hours of life), and 42/799 (5%) had only late-onset suspected infection episodes (≥ 48 hours after birth). Six percent (45/799) experienced at least one episode caused by a positive culture with a significant pathogen.

Characteristics of episodes of suspected infection

There were 1,024 distinct episodes of clinically-suspected bacterial infection affecting 799 neonates, with an average of 1·3 suspected episodes per neonate.

Of the 1,024 episodes of suspected infection, 757 (74%) were early-onset (Table 2); of these, only 6 (1%) were culture-positive, and five were gram-negative (Figure 4). Of the 267 suspected late-onset episodes, 41 were culture-positive, and of these, 67% (27/41) were gram-negative. The proportions of early- and late-onset episodes in which the first blood culture was collected within 24 hours of admission/symptom onset were 93% (705/757) and 98% (262/267), respectively. The proportions of suspected early- and late-onset episodes in which antibiotics were administered before the first blood culture were 0·4% (3/758) and 13% (35/266), respectively.

The most common clinical sign reported was tachycardia (heart rate ≥ 160 bpm), affecting 73% of episodes (748/1,024), followed by 'abnormal temperature' (<35·5°C/>38·0°C axillary temperature), reported in 11% of episodes (110/1,024). Clinical severity scores were assigned to each episode (Table 2), and the proportion of culture-positive episodes by clinical severity score feature in Table 3.

Pathogens isolated and clinical management

Five percent of clinically-suspected episodes (47/ 1,024) were culture-positive, yielding 48 significant pathogens. Pathogen distribution across the study period is presented in Supplementary Figure 1. Overall, approximately two-thirds of isolated organisms were gram-negative bacteria (32/48). Of the culture-positive episodes, most were caused by gram-negative bacteria (32/47, 68%) with *Serratia marcescens* (n=11) and *Acinetobacter* spp. (n=10) the most commonly isolated gram-

negative pathogens (Figure 4). Three culture-positive episodes were polymicrobial; one had two significant pathogens (*Acinetobacter baumannii* and *Klebsiella pneumoniae*), and two had one significant pathogen together with CoNS, which were considered likely contaminants. CoNS was isolated and categorised as a probable contaminant from 12 other episodes.

Three of the 11 *S. marcescens* and five of the eight *A. baumannii* pathogens that were tested for antibiotic susceptibility were multidrug-resistant (non-susceptible to at least one agent in three classes of antibiotics[17]) (Supplementary Table 1.) Group B Streptococcus was cultured in only two episodes; both were identified after day seven of life and classified as late-onset episodes.

A total of 1990 empirical antibiotics were prescribed for the 1024 episodes of suspected infection. The most commonly prescribed empirical antibiotics were amikacin 40% (795/1990), ampicillin 30% (591/1990), ceftazidime 10% (191/1990), and meropenem 6% (122/1990). WHO-recommended gentamicin was prescribed for 7/1990 episodes.

Clinical outcomes

A mortality rate of 15% (117/799) was observed across all enrolled neonates (Supplementary Figure 2); 32 neonates (4%) were lost to follow-up and assumed to have survived. Mortality among neonates with at least one culture-positive episode and neonates with culture-negative episodes was 53% (24/45) and 12% (93/754), respectively (relative risk: 4 (95% CI 3-6), *(χ^2 $p < 0.0001$)*). The most common organisms identified among those who died were *S. marcescens* (n=10), *A. baumannii* (n=7), and *K. pneumoniae* (n=3); all of which were isolated during late-onset infections. Low birthweight (<2500g) and premature babies (<37 weeks gestational age) had a higher mortality rate ((16%, 98/601), and (17%, 104/615) respectively).

Discussion:

As overall mortality of children under five improves, only slow gains are being made in reducing neonatal mortality, and infection remains an important cause of death. Using the well-established ACORN2 methodology in a neonatal intensive care setting, we aimed to clarify the causal understanding of how factors mechanistically interact to drive episodes of suspected and confirmed infection and mortality in neonates admitted to the NICU at PGH. Among those prescribed an IV antibiotic for a presumed infection, we observed high mortality rates, high rates

of culture-negative infection, and a high proportion of culture-proven infections caused by gram-negative pathogens. With a large cohort that includes data from both culture-negative, clinically-suspected bacterial infections and culture-positive sepsis episodes, we provide much-needed insights from the populous region of Southeast Asia.

The mortality within this cohort was 15%; markedly higher than the mortality rate of 6.6% reported in neonates recruited with the same criteria within the ACORN2 multi-centre cohort across LMICs in Africa and Asia.[18] This is an important finding and further highlights the difficulties in the treatment of hospitalised neonates at PGH. While this comparison with other ACORN2 sites is possible as the methodology is the same, comparison of survival across other settings and surveillance systems is challenging due to variation in included case definitions, and the inclusion or exclusion of culture-negative cases of clinical sepsis can substantially alter the proportions for comparison. A large proportion of the total cohort at PGH were low birthweight (under 2,500g) and premature (<37 weeks' gestation), and a disproportionate number of these neonates died.

We found differences in risk factors between culture-positive and culture-negative cases, with mechanical ventilation and IV catheters more prevalent in culture-positive episodes than in clinically-suspected culture-negative infections, highlighting a potential avenue for improving IPC. Similarly, a larger proportion of neonates born extremely prematurely and at extremely low birthweight went on to have at least one episode of culture-positive sepsis. This concurs with previously reported risk factors for culture-positive sepsis in hospitalised neonates.[19]

The proportion of neonates delivered by caesarean section (C/S) in this cohort at PGH (63%) is far higher than the estimated rate of 16% for Southeast Asia, and the target caesarean of 10-15% set by the WHO.[20,21] While the Philippines consistently report C/S rates higher than the WHO-recommended rates,[22] our cohort rate is also 11% higher than the average caesarean rate of 52% for PGH in 2024.[23] It is possible that the high C/S proportion in our cohort is an over-ascertainment of neonates delivered by C/S, due to the high number of preterm babies delivered by emergency C/S who go on to acquire late-onset infections due to hospital pathogen exposure. These babies make up a large proportion of our cohort. High rates of C/S are a concern globally[20] and may be an important factor contributing to the acquisition of infection, potentially through the absence of contact with the vaginal microbiome.[24] Additionally, the combination of a high proportion of

babies who are low birth weight and premature delivered by C/S, who then go on to acquire a late-onset infection requiring antibiotics in addition to perinatal exposure to maternal antibiotics (one dose of cefazolin),[25] may have serious consequences for the development and maintenance of the infant microbiome, leading to longer-term health implications.[24] Further exploration of the impact of NICU admissions and the mode of delivery on the diversity of the microbiome and associated health outcomes is needed.

Concordant with the growing evidence from LMICs, we found gram-negative pathogens were responsible for a high proportion of both early- and late-onset culture-confirmed neonatal infections.[9,10,14,26] The high proportion of *S. marcescens* and *A. baumannii* neonatal infections in this setting is particularly concerning due to the antimicrobial-resistant properties these pathogens possess.[27,28] These pathogens may thrive in resource-constrained healthcare settings in tropical, humid climates, persisting in water sources despite infection control practices.[29] Their spread is increased by crowding, reuse of equipment, and the use of broad-spectrum antibiotics in intensive care units, which promotes selection pressure. The differences in pathogens responsible for infections between high- and LMICs[9] need to be considered by policy-makers to ensure treatment recommendations are appropriately targeted.

Within this cohort, a large number of suspected early-onset episodes were observed, a large proportion of which were culture-negative. There are a number of possible explanations, including falsely negative cultures due to suppression of growth by perinatal antibiotic exposure, but it must be considered that it may also be the case of cautious empiric 'over'-prescribing of antibiotics in the perinatal period, where clinical symptoms may be attributable to another diagnosis (such as transient tachypnoea of the newborn). It remains a limitation of this study that no antibiotic durations were recorded to determine if prescriptions were stopped early.

Diagnostic capabilities remain a significant concern globally, particularly in LMIC settings.[30] Blood cultures remain the gold standard despite clear limitations,[31] and the findings from this study highlight these challenges. Targeted treatment is subsequently hindered by low culture-positivity rates,[14] with only 10% culture-positivity for clinically suspected infections reported in some centres across the region.[13,32] Obtaining and processing blood cultures is difficult in neonates and is prone to inaccuracy, with risks of contamination, insufficient blood volume, and pre-treatment

with antibiotics, all of which are reported complicating factors.[33] In low-resource settings, limited access to microbiological testing contributes to a significantly higher rate of culture-negative sepsis cases compared with high-income settings.[33] Confounded by the absence of a consensus working definition for neonatal sepsis,[34] diagnostic limitations force clinicians to assume episodes are bacterial even in the absence of culture confirmation, and to use empirical regimens, local antibiograms, and local epidemiological knowledge to guide prescribing. Alternatives to conventional culture methods are limited, while multiplex-PCR remains costly and unfeasible for most LMICs.

At PGH, the low overall culture-positivity rate of 5% (47/1,024), which was lower still among clinically unstable neonates with suspected early-onset bacterial infection, must be considered when recommending empirical antibiotic therapy at this centre. The low positivity found at PGH may be driven, in part, by false negatives, which are a globally accepted limitation of the current diagnostic tools for treating neonatal sepsis. However, the implementation of robust culture methods, the standard practice of obtaining 1mL of blood for culture, and the low number of neonates receiving antibiotics prior to sampling, suggest low positivity may instead be driven by suspicion of sepsis in infants with a non-infectious cause of instability, a phenomenon previously reported in the literature.[35] The drivers of cautious antibiotic prescription are likely to be exacerbated by the non-specific features of neonatal sepsis and the fear of poor clinical outcomes if the neonate does indeed have a case of culture-negative sepsis.

A large proportion of episodes of clinically-suspected bacterial infection had only one recorded clinical sign of sepsis. Only six of the 1,024 episodes of suspected infection were assigned the highest clinical severity score classification of four, indicating four or more of the WHO severity signs of infection are present. The low severity scores likely point to a high index of suspicion or a highly cautious approach to antibiotic prescribing. Pre-emptive or prophylactic prescribing of antimicrobials creates challenges for stewardship, with antibiotic overuse known to drive further AMR.[35] Improved diagnostic methods for sepsis will be crucial for curtailing the use of antibiotics in LMIC settings; meanwhile, comprehensive, high-quality, systematically collected surveillance data will remain essential to identify causes of infection and adequately inform local empiric antibiotic guidelines.

Antimicrobial and diagnostic stewardship are essential in all neonatal care settings globally, with recent evidence suggesting that antimicrobial prescribing for neonatal sepsis within Southeast Asia is of particular concern.[36] In this cohort, empirical antibiotic prescribing is predominantly broad-spectrum; fewer than one-third received ampicillin as first-line therapy as recommended by WHO, and even fewer episodes were treated with gentamicin. This differs from that seen in high-income settings like in Australia, where benzylpenicillin and gentamicin were predominantly used in a multicentre study of neonates.[37] Further analysis of antimicrobial prescribing practices and antimicrobial stewardship, including a qualitative analysis of prescribing behaviours and decision making, is needed to better understand and minimise the impact of antimicrobial overprescribing. There is a distinct need for systems-level implementation studies to understand the barriers to practice change and to successfully implement antimicrobial stewardship programs.

The study indicated high adherence to two of the quality indicators of stewardship monitored in this surveillance network: blood cultures taken within 24 hours of NICU admission or symptom onset, and blood culture collection before antibiotic administration. Almost all blood cultures were obtained within the expected 24-hour timeframe, and in 90% of episodes, clinicians obtained cultures prior to antibiotic administration (Table 2). Of the 38 episodes in which antibiotics were administered prior to culture, 35 (92%) were late-onset. By comparison, the original multi-centre ACORN2 results also from LMICs found that only 64% of cultures were taken within the desired 24-hour window.[38]

Limitations to this study include those inherent to the observational reporting of a single centre study, limiting external validity and meaning the applicability of findings to other clinical sites should be carefully considered. As the ACORN2 protocol was designed as a pragmatic low-resource dependent surveillance protocol for use across all age groups, some factors specific to neonatal infection (respiratory rate, blood pressure and biomarkers, signs of organ dysfunction) and perinatal risk factors are not collected. Another specific limitation includes the lack of granularity of mortality data. In this cohort, the primary cause of death was not recorded, meaning we are unable to attribute the cause of death specifically to infection rather than co-morbidities like prematurity or low birthweight. Furthermore, data are constrained to neonates enrolled with a suspected infection (and not on all neonates) which introduces a selection bias that precludes assessment of the extent to which such episodes increased the risk of death. Given the high

mortality rate found here, we recommend that future studies collect more granular data on the relationship between infection and mortality, including timing and cause of death. Additional data on these factors, clinical signs, perinatal factors, and the timing of antibiotic prescription, would augment and further contextualise the findings and should be considered for future data collection.

Systematic surveillance conducted in this study using the ACORN2 methodology, however, provides a platform to inform interventions that may interrupt the acquisition of hospital-acquired sepsis, better target the use of antibiotics, mitigate against the rise and impact of AMR, and improve clinical outcomes. The use of the descriptive DAG to explore mechanistic pathways to sepsis in the NICU provides a visual representation of the main challenges and opportunities for intervention, such as kangaroo mother care to improve immune system capacity and interrupt the causal pathway towards infection. The current manuscript describes the epidemiology of neonatal sepsis in our setting In future studies, we will use the DAG to design inferential analyses to assess the causal drivers of sepsis, estimate the expected reduction achievable by modifying these factors and seek to scale-up surveillance into settings beyond PGH.

Against a landscape of low culture positivity and predominance of gram-negative pathogens among culture-positive episodes of clinically-suspected bacterial infection, clinicians in low-resourced settings face a challenging task. A consensus case definition is needed for neonatal sepsis and that diagnostic capacity must be enhanced to provide better decision support for clinicians prescribing antimicrobial therapies for newborn care. Prevention, management and diagnosis of neonatal sepsis is increasingly difficult as AMR progresses, and mortality remains unacceptably high. A greater understanding of the epidemiology of neonatal infection through comprehensive, systematic surveillance that includes both clinical outcomes and microbiological data will help ensure that Southeast Asia is adequately represented globally and that diagnostics, treatment solutions and prevention strategies are appropriately designed.

Methods:

Ethical Considerations:

The Human Research Ethics Committee at the University of Sydney (2024/HE000366) and the Research Ethics Board at the University of the Philippines (UPMREB 2023-0315-01) provided

approval under a waiver of consent. The overarching ACORN2 protocol is approved by the Oxford Tropical Research Ethics Committee (OxTREC: Ref 524-21).

Study design:

This prospective observational surveillance study was conducted at the Philippine General Hospital in Manila, Philippines, from November 14th 2023, to November 13th 2024. Building on the previously well-documented ACORN2 project[38,39] participants were enrolled through daily surveillance of clinically-suspected infections in the neonatal intensive care unit (NICU).

Participants:

All patients admitted to the NICU were screened. Only those aged <28 days after correction for gestational age[40] were included in the analysis. We followed the ACORN2 protocol, yet the cohort enrolled differs from typical ACORN2 sites, as this surveillance ward admits only inborn infants, and therefore, no enrolled participants were born elsewhere or transferred in. There was no pre-determined sample size, rather a defined time-period within which all eligible participants were enrolled.

Admitted infants with a clinically suspected bacterial infection and prescribed intravenous (IV) antibiotics within 48 hours of admission were enrolled in active daily surveillance according to the published ACORN2 Protocol.[38] For the purposes of this cohort, an episode of clinically-suspected bacterial infection is defined as clinical instability and suspected bacterial infection in a neonate or young infant, for which clinicians decide to prescribe IV antibiotics.[38,41] In addition, weekly point-prevalence surveys (PPS) collected clinical data from infants prescribed an IV antibiotic for new-onset clinically-suspected bacterial infection, acquired ≥48 hours after admission to the NICU. The ACORN2 protocol distinguishes between community-acquired infections (CAI) and hospital-acquired infections (HAI) using the European Centre for Disease Prevention and Control definitions.[42] In this neonatal intensive care unit, all are inborn and 'admission' is synonymous with birth; we have therefore used early-onset (<48 hours) and late-onset (≥ 48 hours) episodes in place of CAI and HAI.

Definitions of early- and late-onset

Definitions regarding the timing of EOS vary throughout the literature,[43] with guidelines and studies reporting 48 hours,[6-8] 72 hours[6,9] and others as late as 7-days[44] as the cut-off. For this cohort, the definition of <48 hours was followed, in the context of increasing evidence that neonates may be colonised, and infected with, nosocomial pathogens very early in their postnatal period – with prior studies reporting high rates of bloodstream infections due to these pathogens (such as *A. baumannii*) as early as the day of birth – particularly in resource-constrained healthcare settings.[9,10,12] By contrast, global guidelines that use 72 hours as the cut-off for EOS/LOS are generally tailored to high-income country settings, such as the United States, where traditionally vertically-acquired bacteria (such as *Streptococcus agalactiae* or *Escherichia coli*) are capable of causing infection up to day three of life.

Therefore, in this resource-constrained healthcare setting, we have followed the EOS/LOS <48 hours cut-off to ensure we capture the distinction of vertical versus hospital-acquired pathogens closely. We have used EOI and LOI as the acronym to distinguish our cohort of clinically suspected early- and late-onset infections without meeting a case definition for sepsis in all cases.

Site Selection:

PGH is a tertiary referral Hospital in urban Manila, in the Philippines. The hospital has an 1,100 bed capacity with 70 beds in the neonatal intensive care unit (NICU). Despite an official 70-bed capacity, up to 90 babies may be admitted to the unit at one time. The NICU can provide incubator care, invasive and non-invasive ventilation, total parenteral nutrition (TPN) and ionotropic support.[13] In 2024 there were 1,009 NICU admissions following 3,776 live births at PGH.[23]

Data collection:

Data were collected according to the workflow outlined in Supplementary Figures 3 and 4. Data were captured and are reported according to the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) and STROBE for Newborns[14] (STROBE-NI) recommendations (Supplementary Table 2).[45,46]

Following enrolment, deidentified clinical data were collected from neonates' electronic medical record (EMR) and uploaded to an electronic REDCap database.[47] Clinical data were linked to microbiological data routinely recorded into the WHONET[48] database via the patient identification

number and the date of specimen collection. Clinical severity scores were calculated according to the ACORN2 protocol and case report form, taking into consideration general WHO severity signs for neonates, [39] scoring one point for each clinical sign from the following: inappropriate tachycardia, abnormal core temperature, altered mental state, reduced peripheral perfusion or prolonged capillary refill time, reduced level of activity, feeding difficulty or convulsions. The severity signs are rated from. Scores are attributed as 0 = no clinical signs, 1 = one clinical sign 2 = two clinical signs 3 = three clinical signs 4 = four or more clinical signs.

At PGH, blood cultures are routinely collected before commencing antibiotics and repeated for every infection episode. Blood cultures were collected aseptically during the first IV insertion. One millilitre of blood is collected if possible, with a minimum of 0.5mL submitted for culture. Blood culture bottles are incubated for five days with continuous monitoring using the automated BACTEC FX System (Becton Dickinson) for culture identification. If no growth is detected in the first 48 hours, a preliminary 'negative growth' result is released, and if no growth is detected after five days, a result of 'no growth after five days of incubation' is reported. The VITEK 2 (BioMerieux, Inc. Durham, USA) was used for antimicrobial susceptibility testing with interpretation under guidelines of the Clinical and Laboratory Standards Institute (CLSI Edition: 35, 2025).[49]

Statistical Analysis:

The reporting and analysis plan were informed by a directed acyclic graph (DAG) which was based on the domain knowledge of the contributing authors (Figure 1 and Supplementary Table 3). The DAG aims to depict how bacterial infection is acquired in newborns and how it may be managed, diagnosed, and documented in the study data.

Clinical characteristics were described using R (version 4.2.1; R Foundation for Statistical Computing, Austria) within the RStudio environment (version 12.0; RStudio, USA).[50] Means or medians were reported alongside standard deviations or interquartile ranges as appropriate. Pathogen prevalence was calculated as the proportion of the total pathogens across all recorded episodes of clinically-suspected bacterial infection. Chi-square or Fisher's exact tests were used to compare mortality among those with culture-positive versus culture-negative clinically-suspected bacterial infection. Only bacterial species considered to be non-contaminants were included as

culture-positive episodes; common contaminants were reported separately and are detailed in Supplementary Table 4. Coagulase-negative *Staphylococcus* spp. were considered non-contaminants if isolated from a neonate with a long line in place (eg. peripherally inserted central catheter, central venous line), or with an umbilical line, or a neonate who was intubated and ventilated and had clinically-suspected infection in the absence of isolation of another significant pathogen. In cases where a common contaminant was detected together with a known pathogen, it was excluded from analysis (Supplementary Figure 5).

Data Availability:

Due to the institutional requirements and the need for hospital data sharing agreements for the sharing of human clinical data, source data is not publicly available, but access can be facilitated on request from the corresponding author, ML Harrison (m.harrison@sydney.edu.au) or from the NeoSEAP consortium via the website contact page: https://neoseap.com/contact-us/.

Code Availability:

Source R-code will also be made available alongside source data on request from the corresponding Author ML Harrison (m.harrison@sydney.edu.au) or from the NeoSEAP consortium via the website contact page: https://neoseap.com/contact-us/.

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Author Contributions: PT and HyD designed the ACORN2 protocol. JH and TR facilitated database access, training, and data extraction set up and support. MEVU, the site PI at PGH, supported local staff. EK and JC collected data and maintained data quality and monitoring. ND served as the local laboratory lead and supported the extraction of laboratory data. PC provided statistical support. AO and YW handled data processing, data engineering, and central data quality monitoring. PCMW and TS provided conceptual and analytic oversight, including data interpretation and DAG development. MH analysed the data, drafted the manuscript, and provided project oversight and quality monitoring. All authors contributed to manuscript preparation and review.

Declaration of Competing Interests: The authors have no competing interests to declare.

Table 1. Characteristics of enrolled participants

Variable	Overall	Only culture-negative clinically suspected infection	Any culture-positive infection[+]	1st suspected infection episode EOI[^]	Mortality[*]
	N (%)	n (%)	n (%)	n (%)	n (%)
	799 (100)	754 (94)	45 (6)	757 (95)	117(15)
Sex					
Female	**361 (45)**	339 (45)	22 (49)	339 (45)	57 (49)
Male	**436 (55)**	413 (55)	23 (51)	416 (55)	59 (50)
Undetermined	**2 (0·3)**	2 (0·3)	0 (0)	2 (0·3)	1 (1)
Birthweight (g) (med (IQR))	**1930 (1470-2485)**	1962 (1510-2498)	1440 (903-1830)	1915 (1460-2458)	1000 (790-2035)
Extremely Low birth weight (<1000g)	**81 (10)**	65 (9)	16 (36)	79 (10)	59 (50)
Very Low birth weight (1000-1500g)	**128 (16)**	118 (16)	10 (22)	125 (17)	19 (16)
Low birth weight <2500g)	**392 (49)**	381 (51)	11 (24)	372 (49)	20 (17)
Normal birth weight (2500-4499g)	**193 (24)**	185 (25)	8 (18)	177 (23)	19 (16)
High birth weight (≥4500g)	**3 (0·4)**	3 (0·4)	0 (0)	2 (0·3)	0 (0)
Missing	**2 (0·3)**	2 (0·3)	0 (0)	2 (0·3)	0 (0)
Gestational Age (weeks) (med (IQR))	**34 (31-36)**	34 (32-36)	31 (28-35)	34 (31-36)	29 (27-34)
Extremely preterm (<28 weeks' gestation)	**51 (6)**	43 (6)	8 (18)	49 (7)	39 (33)
Very preterm (28-32 weeks' gestation)	**163 (20)**	144 (19)	19 (42)	160 (21)	35 (30)
Mod-late preterm (32-37 weeks' gestation)	**401 (50)**	392 (52)	9 (20)	387 (51)	30 (26)
Term (>37 weeks' gestation)	**182 (23)**	173 (23)	9 (20)	159 (21)	13 (11)
Missing	**2 (0·2)**	2 (0·3)	0 (0)	2 (0·3)	0 (0)
Mode of delivery					
Elective Caesarean section	**109(14)**	102 (14)	7 (16)	99 (13)	12(10)
Emergency Caesarean section	**396 (50)**	369 (49)	27 (60)	379 (50)	65 (56)
Spontaneous Vaginal Delivery	**283 (35)**	272 (36)	11 (24)	269 (36)	40 (34)
Assisted Vaginal Delivery	**8 (1)**	8 (1)	0 (0)	8 (1)	0 (0)
Missing	**3 (0·4)**	3 (0·4)	0 (0)	2 (0·3)	0 (0)
Person-level suspected-infection Characteristics					
Age at first infection onset (days)(med (IQR))	**0 (0-2)**	0 (0-2)	0 (0-1)	0 (0-1)	0 (0-1)
Duration of hospital stay (days) med (IQR)	**19 (10-35)**	17 (10-33)	44 (33-71)	19 (10-35)	23 (3-44)
Admission Diagnosis					
Cardiovascular condition	**14 (2)**	13 (2)	1 (2)	14 (2)	3 (3)
Pulmonary disease	**597 (74)**	563 (75)	34 (76)	588 (79)	92 (79)
Infectious disease	**134 (17)**	127 (17)	7 (16)	106 (14)	10 (9)
Neurological disease	**20 (3)**	19 (3)	1 (2)	18 (2)	6 (5)
Gastrointestinal disease	**2 (0·2)**	2 (0·3)	0 (0)	1 (0·1)	0 (0)
Undetermined	**32 (4)**	30 (4)	2 (4)	30 (4)	6 (5)

^EOI: Early-onset clinically suspected infection onset <48 hours after birth
*32 neonates were lost to follow up (32/799, 4%), calculation of mortality percentage has been made based on the assumption that all those lost to follow up are alive.

+ Any culture-positive infection: This column includes infants who experienced at least one episode of culture-positive infection

Table 2. Characteristics of clinically-suspected episodes of suspected infection

	Overall	[#]Culture-positive episodes	[#]Culture-negative episodes	[^]Suspected EOI <48h	[*] Suspected LOI ≥48h
	N(%)	n(%)	n(%)	n(%)	n(%)
Episodes of suspected infection	**1,024 (100)**	**47 (5)**	**977 (95)**	**757 (74)**	**267 (26)**
Blood culture taken within 24hours of birth suspected EOI, or symptom onset for suspected LOI					
Yes	967 (94)	45 (96)	922 (94)	705 (93)	262 (98)
No	53 (5)	2 (4)	51 (5)	48 (6)	5 (2)
Unknown	4 (0·4)	0 (0)	4 (0·4)	4 (1)	0 (0)
≥1 dose of systemic antibiotic administered prior to blood culture collection					
Yes	38 (4)	4 (9)	34 (3)	3 (0·4)	35 (13)
No	921 (90)	41 (87)	880 (90)	696 (92)	225 (84)
Unknown	65 (6)	2 (4)	63 (6)	58 (8)	7 (3)
Medical devices insitu – HAI only					
Peripheral IV catheter	-	14 (30)	39 (4)	-	53 (20)
Intubation / Mechanical ventilation	-	28 (60)	100 (10)	-	128 (48)
Urinary catheter	-	4 (9)	8 (0·8)	-	12 (5)
Central IV catheter	-	2 (4)	11 (1)	-	13 (5)
Episode clinical signs recorded					
Temperature abnormality (<35.5°C/ >38.0°C axillary)	110 (11)	3 (6)	107 (11)	94 (12)	16 (6)
Tachycardia (≥160bpm)	748 (73)	37 (79)	711 (73)	570 (75)	178 (67)
Reduced peripheral perfusion	16 (2)	3(6)	13 (1)	9 (1)	7 (3)
Feeding difficulties	19 (2)	0 (0)	19 (2)	13 (2)	6 (2)
Reduced movement	36 (4)	3 (6)	33 (3)	23 (3)	13 (5)
Episode clinical severity score[^^]					
Clinical severity score 0	250 (24)	10 (21)	240 (25)	165 (22)	85 (32)
Clinical severity score 1	634 (62)	31 (66)	603 (62)	480 (63)	154 (58)
Clinical severity score 2	117 (11)	4 (9)	113 (12)	100 (13)	17 (6)
Clinical severity score 3	17 (2)	1 (2)	16 (2)	8 (1)	9(3)
Clinical severity score 4	6 (1)	1 (2)	5 (0·5)	4 (0·5)	2 (1)
28-day mortality by episode	219 (21)	28 (60)	191 (20)	110 (15)	109 (41)

[#]Culture-positive: infection where a blood culture has been taken and has returned a significant pathogen in accordance with supplementary information table 2.
[^]EOI: Early-onset Infection: suspected infection onset <48 hours after birth; [*]LOI: Late-onset infection, suspected infection onset >48hr after birth; IV: Intravenous, [^^]Clinical severity score: a rating of severity based on the presence of clinical signs: 0= No clinical signs, 1= 1 clinical sign, 2=2 clinical signs, 3= 3 clinical signs, 4 = ≥4 clinical signs

Table 3. Culture-positivity for clinical severity scores

	Clinical Severity score

	0 n (%)	1 n (%)	2 n (%)	3 n (%)	4 n (%)
Suspected culture-negative episode	240 (96)	603 (95)	113 (97)	16 (94)	5 (83)
Confirmed Culture-positive episode	10 (4)	31 (5)	4 (3)	1 (6)	1(17)

Clinical severity score: a rating of severity based on the presence of clinical signs: 0= No clinical signs, 1= 1 clinical sign, 2=2 clinical signs, 3= 3 clinical signs, 4 = ≥4 clinical sign

Figure Legends:

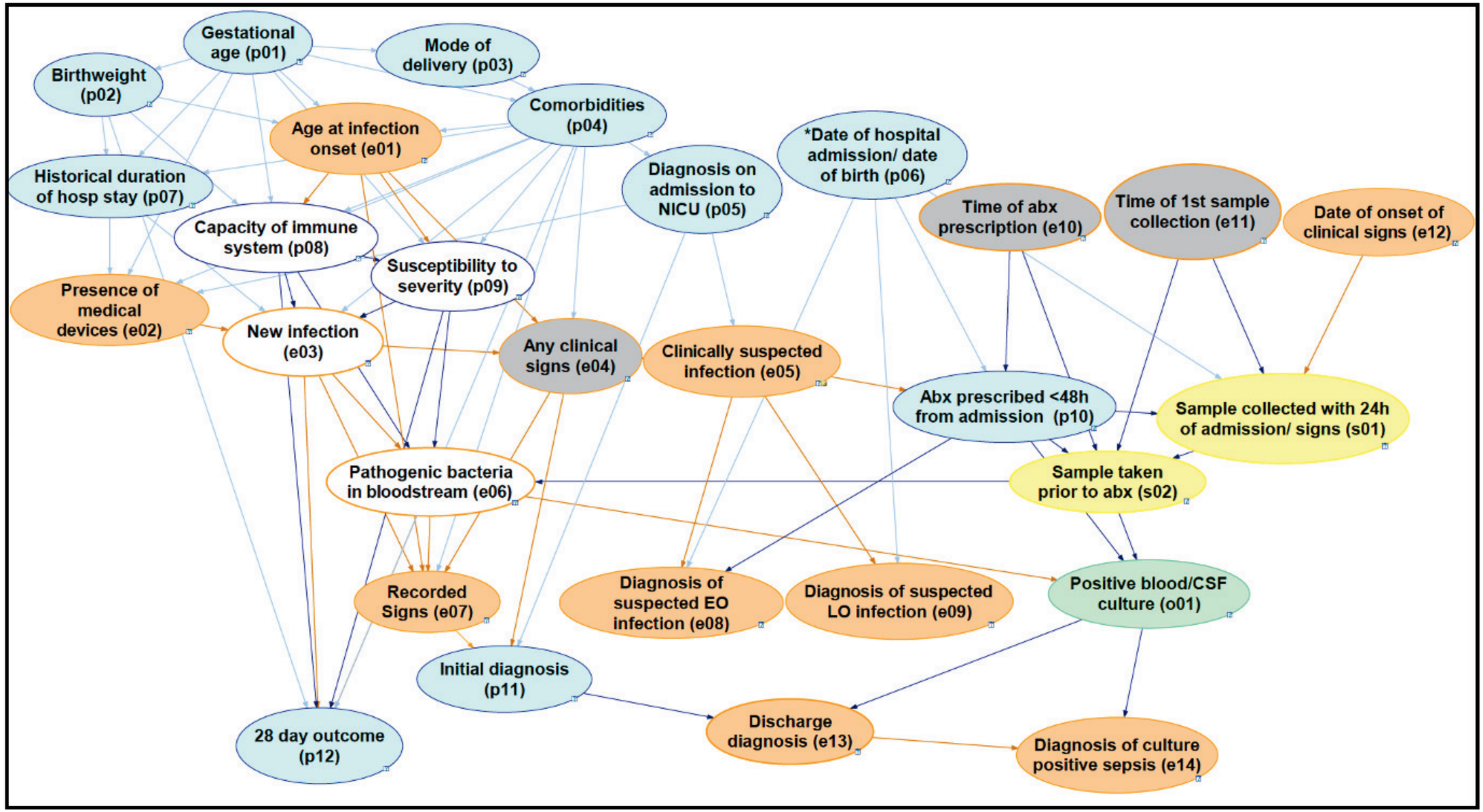
Figure 1. Directed-acyclic graph (DAG) outlining the mechanistic pathway to acquisition of infection and 28day clinical outcomes for neonates in the NICU. Directional arrows show the relationship between causal factors leading to the diagnosis of culture-positive sepsis, or the diagnosis of suspected early-onset infection (EOI) or suspected late-onset infection (LOI). Upstream factors influence factors further down in the directional flow. In line with the relational-database analytic method, Light-blue nodes are 'Person-level' factors belonging to an individual person, Orange nodes relate to 'Episode-level' factors influencing each distinct episode of infection, green nodes relate to Organism-level factors, and yellow nodes relate to the 'Specimen-level' factors. White nodes are latent concepts that have influence but are not measurable, and grey nodes are factors for which we don't have available data. To improve readability, all arcs from upstream person-level background factors are light blue, all arcs from episode-level variables are orange, and all other arcs are dark blue. Supplementary Table 2 consists of detailed definitions for each variable/node and how they are mechanistically affected by the parent nodes.

Figure 2. Consort Diagram of Enrolled Neonates and their associated outcomes within the main analytic concepts. The diagram presents the enrolments and findings according to a relational database concept. The 'Persons' concept relates to factors inherent to each individual enrolled, the 'Episodes' concept represents factors related to each individual episode of suspected infection, and the 'Organism' concept represents factors related to the isolated organism. Data were analysed using these main analytic concepts. The 28-day clinical outcome relates to the clinical outcome of each episode of suspected episode.

Figure 3. Culture-positivity for episodes of clinically-suspected infection in Neonates at the Philippine General Hospital, Manila.

Figure 4. Pathogens responsible for Early- and Late-onset infections in this cohort. EOI: Early-onset Infection <48h from birth, LOI: Late-onset Infection ⩾48h from birth. Culture-positive episodes of infection, n=47, with causative pathogens, n=48.

Systematic surveillance of all clinically suspected infections in a Neonatal Intensive Care Unit revealed a high burden of clinically-suspected but culture-negative infection and high mortality supporting an urgent need for improved definitions, diagnostics and reduced antibiotic overuse.
Peer review information: *Nature Communications* thanks the anonymous reviewers for their contribution to the peer review of this work. A peer review file is available.


Gestational age (p01)
Mode of delivery (p03)
Birthweight (p02)
Comorbidities (p04)
Age at infection onset (e01)
*Date of hospital admission/ date of birth (p06)
Historical duration of hosp stay (p07)
Diagnosis on admission to NICU (p05)
Time of abx prescription (e10)
Time of 1st sample collection (e11)
Date of onset of clinical signs (e12)
Capacity of immune system (p08)
Susceptibility to severity (p09)
Presence of medical devices (e02)
New infection (e03)
Any clinical signs (e04)
Clinically suspected infection (e05)
Abx prescribed <48h from admission (p10)
Sample collected with 24h of admission/ signs (s01)
Pathogenic bacteria in bloodstream (e06)
Sample taken prior to abx (s02)
Recorded Signs (e07)
Diagnosis of suspected EO infection (e08)
Diagnosis of suspected LO infection (e09)
Positive blood/CSF culture (o01)
Initial diagnosis (p11)
28 day outcome (p12)
Discharge diagnosis (e13)
Diagnosis of culture positive sepsis (e14)

Persons concept

Data Analysis and Monitoring Concepts

Episodes concept

Organism concept

Total Enrolled N= 799
14/11/2023 - 13/11/2024

Total episodes of infection: n= 1024

Early-onset infection
n= 758
(Admitted to the NICU and prescribed IV antibiotic with a presumed infection within 48 hours of admission)

Late-onset Hospital-acquired infection
n= 266
(Infant with onset of a new infection episode and prescribed IV antibiotics >48 hours after admission to the NICU)

Culture-positive infection n=6

Culture-negative infection n=752

Culture-negative infection n=225

Culture-positive infection n=41
(n=42 significant pathogens)

*Contaminants n=12

Gram-negative bacteria
n= 5,83%

Gram-positive bacteria
n= 1, 17%

Gram-negative bacteria
n=27,67%

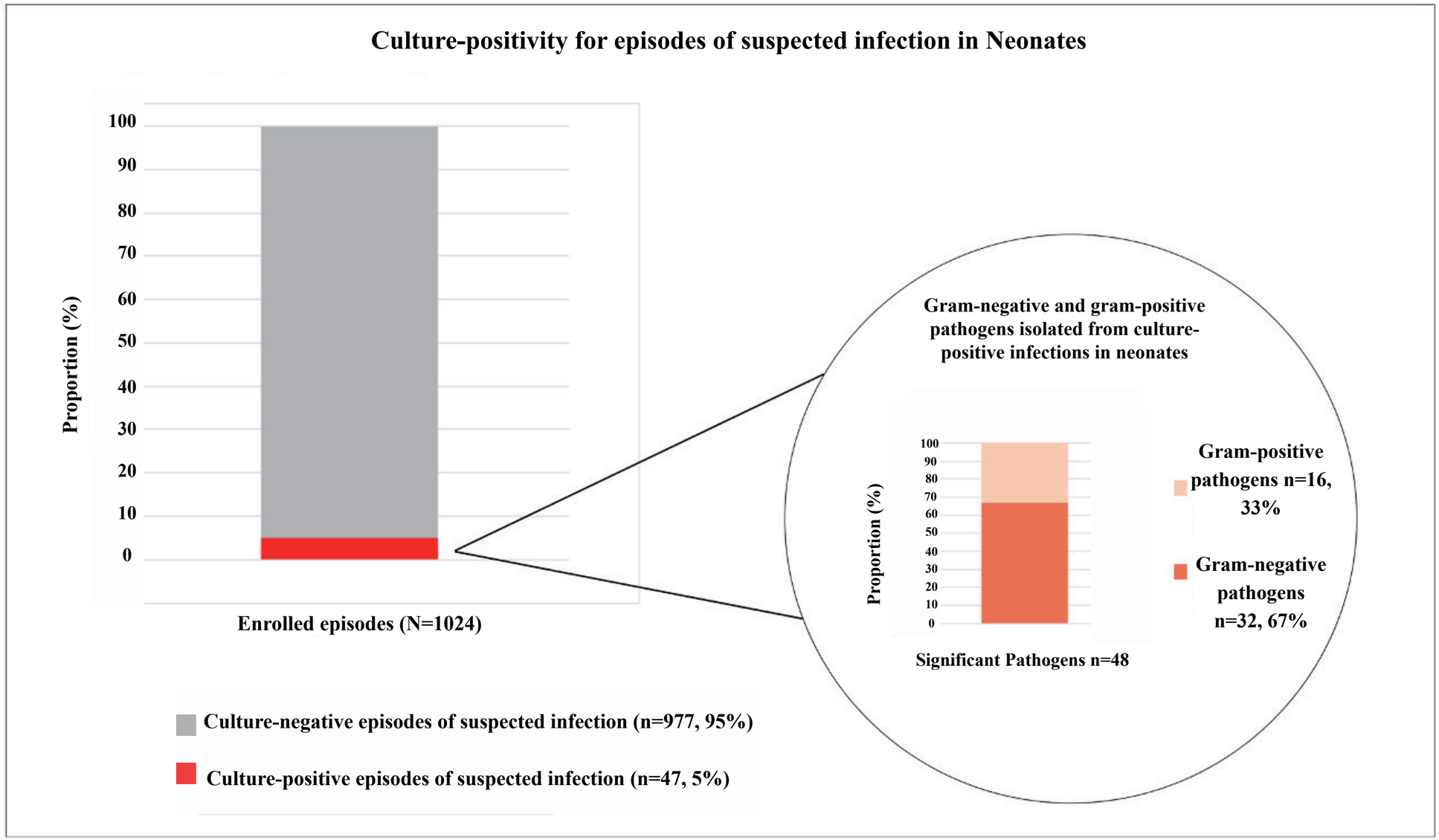
Gram-positive bacteria
n=15, 35%

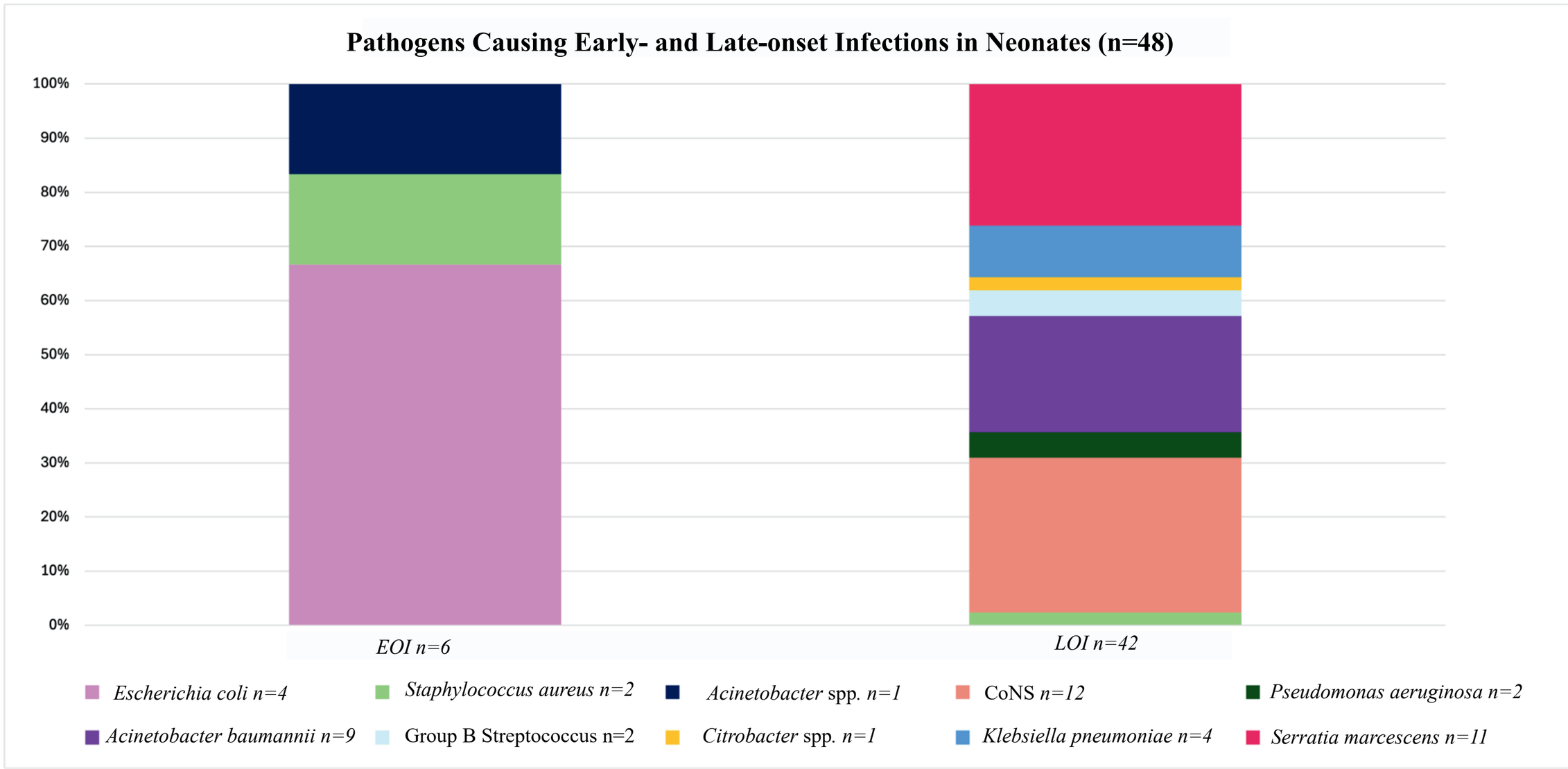
28 day episode outcome Deceased n=2

28 day episode outcome Deceased n=109

28 day episode outcome Deceased n=82

28 day episode outcome Deceased n=26


Culture-positivity for episodes of suspected infection in Neonates
Proportion (%)
100
90
80
70
60
50
40
30
20
10
0
Enrolled episodes (N=1024)
Culture-negative episodes of suspected infection (n=977, 95%)
Culture-positive episodes of suspected infection (n=47, 5%)
Gram-negative and gram-positive pathogens isolated from culture-positive infections in neonates
Proportion (%)
100
90
80
70
60
50
40
30
20
10
0
Significant Pathogens n=48
Gram-positive pathogens n=16, 33%
Gram-negative pathogens n=32, 67%


Pathogens Causing Early- and Late-onset Infections in Neonates (n=48)
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
EOI n=6
LOI n=42
Escherichia coli n=4
Staphylococcus aureus n=2
Acinetobacter spp. n=1
CoNS n=12
Pseudomonas aeruginosa n=2
Acinetobacter baumannii n=9
Group B Streptococcus n=2
Citrobacter spp. n=1
Klebsiella pneumoniae n=4
Serratia marcescens n=11