

UNIVERSIDADE SÃO FRANCISCO
Programa de Pós-Graduação *Stricto Sensu* em Ciências da Saúde

PATRICIA KRAUSE ASKINIS

**PREVALÊNCIA E FATORES DE RISCO DE INFECÇÕES
BACTERIANAS EM RECÉM-NASCIDOS INTERNADOS NA
UNIDADE DE TERAPIA INTENSIVA NEONATAL**

Bragança Paulista
2026

PATRICIA KRAUSE ASKINIS – R.A. 202451582

**PREVALÊNCIA E FATORES DE RISCO DE INFECÇÕES
BACTERIANAS EM RECÉM-NASCIDOS INTERNADOS NA
UNIDADE DE TERAPIA INTENSIVA NEONATAL**

Dissertação apresentada ao Programa de Pós-Graduação *Stricto Sensu* em Ciências da Saúde da Universidade São Francisco, como requisito final para obtenção do Título de Mestre em Ciências da Saúde.

Área de Concentração: Ciências da Saúde

Orientador: Prof. Dr. Fernando Augusto Lima Marson

Bragança Paulista
2026

WS 420
A858p

Askinis, Patricia Krause

Prevalência e fatores de risco de infecções bacterianas em recém- nascidos internados na unidade de terapia intensiva neonatal / Patricia Krause Askinis. -- Bragança Paulista, 2026.

112 p.

Dissertação (Mestrado) – Programa de Pós-Graduação *Stricto Sensu* em Ciências da Saúde da Universidade São Francisco.

Orientação de: Fernando Augusto Lima Marson

1. Sepsis neonatal. 2. Unidade de terapia intensiva neonatal. 3. Resistência antimicrobiana. 4. Prematuridade. 5. Infecção hospitalar. I. Marson, Fernando Augusto Lima. II. Título.

FOLHA DE APROVAÇÃO



Educando
para a paz

ASKINIS, Patrícia Krause. "Prevalência e fatores de risco de infecções bacterianas em recém-nascidos internados na unidade de terapia intensiva neonatal". Dissertação defendida e aprovada no programa de Pós-Graduação *Stricto Sensu* em Ciências da Saúde da Universidade São Francisco em 24 de junho de 2026 pela Banca examinadora constituída pelos(as) professores(as):

Prof(a). Dr(a). Fernando Augusto de Lima Marson
Orientador(a) e Presidente
Universidade São Francisco

Prof(a). Dr(a). Mônica de Cássia Firmida
(por videoconferência)
Universidade do Estado do Rio de Janeiro

Prof(a). Dr(a). Thais Parisotto Ulmer
Universidade São Francisco

AGRADECIMENTOS

À Universidade São Francisco, pela oportunidade e pela concessão da bolsa de estudos, que possibilitou a concretização deste título e fortaleceu minha trajetória científica.

Ao Hospital Universitário São Francisco, pela autorização e pelo apoio na disponibilização dos prontuários, que tornaram viável a execução desta pesquisa.

Ao meu orientador, Prof. Dr. Fernando Marson, pela confiança, disponibilidade, incentivo e por todas as contribuições valiosas que tornaram este trabalho possível.

Aos estudantes de Iniciação Científica, pela colaboração nas etapas de coleta dos dados.

Ao Curso de Enfermagem da Universidade São Francisco, pela contribuição significativa para o meu desenvolvimento acadêmico e profissional.

Aos professores e colegas do Programa de Pós-Graduação em Ciências da Saúde, pelos ensinamentos, pela troca de.

Ao meu esposo, Sérgio, e aos meus filhos, Felipe e Miguel, pela paciência e compreensão ao longo deste percurso.

E, por fim, a todos que, direta ou indiretamente, contribuíram para a realização deste trabalho, deixo o meu sincero reconhecimento e gratidão.

RESUMO

As infecções bacterianas continuam sendo uma das principais causas de morbimortalidade neonatal, especialmente entre recém-nascidos prematuros e de baixo peso. A vulnerabilidade imunológica desses pacientes, somada à exposição a procedimentos invasivos e à crescente resistência antimicrobiana, representa um desafio constante nas unidades de terapia intensiva neonatal (UTIN). Este estudo teve como objetivo avaliar a incidência, os agentes etiológicos e os fatores associados à mortalidade por infecções bacterianas em recém-nascidos internados em uma UTIN de um hospital universitário entre 2017 e 2024. Trata-se de uma pesquisa retrospectiva, observacional e quantitativa, baseada na análise de 1.396 prontuários médicos. Foram avaliadas variáveis demográficas, obstétricas, clínicas e microbiológicas, incluindo o perfil de resistência antimicrobiana, o uso de antimicrobianos e os desfechos clínicos. As análises estatísticas foram realizadas no programa SPSS (versão 28.0), utilizando regressão logística bivariada e multivariável, com nível de significância de $p \leq 0,05$. Entre os 1.396 recém-nascidos incluídos, 1.062 (76,1%) tiveram pelo menos uma cultura coletada para análise microbiológica. A maioria dos casos foi classificada como sepse presumida de início precoce (61,1%), seguida por sepse presumida de início tardio (13,7%). Os casos confirmados laboratorialmente corresponderam a 9,8% do total, com predomínio de infecções bacterianas (8,3%) e menor frequência de infecções fúngicas (1,5%). Entre os microrganismos isolados, as bactérias Gram-positivas foram as mais prevalentes (5,8%), destacando-se *Staphylococcus coagulase negativa* e *Staphylococcus aureus*; entre as Gram-negativas, os principais agentes foram *Klebsiella spp.* e *Escherichia coli*; *Candida spp.* foi responsável por todos os isolamentos fúngicos. A taxa de mortalidade neonatal foi de 15,2%, associada significativamente à prematuridade extrema, ao peso extremamente baixos ao nascer, aos escores de Apgar reduzidos, à necessidade de ventilação mecânica e às infecções por bacilos Gram-negativos multirresistentes. A análise estatística apontou que os recém-nascidos que utilizaram gentamicina, cefepime, oxacilina e fluconazol estavam associados a maior mortalidade neonatal. Os resultados reforçam a necessidade de vigilância microbiológica contínua, uso racional de antibióticos e fortalecimento das práticas multiprofissionais de prevenção e controle de infecções. Conclui-se que a integração entre vigilância laboratorial, protocolos assistenciais e capacitação das equipes é essencial para reduzir a mortalidade neonatal e conter o avanço da resistência bacteriana nas UTIs neonatais.

Palavras-chave: Antibióticos. Infecção bacteriana. Mortalidade neonatal. Resistência antimicrobiana. Sepse neonatal.

ABSTRACT

Bacterial infections remain one of the leading causes of neonatal morbidity and mortality, especially among preterm and low-birth-weight infants. The immunological vulnerability of these patients, combined with exposure to invasive procedures and the growing problem of antimicrobial resistance, represents a constant challenge in neonatal intensive care units (NICUs). This study aimed to evaluate the incidence, etiological agents, and factors associated with mortality due to bacterial infections in newborns admitted to a NICU of a university hospital between 2017 and 2024. This was a retrospective, observational, and quantitative study based on the analysis of 1,396 medical records. Demographic, obstetric, clinical, and microbiological variables were assessed, including antimicrobial resistance profiles, antibiotic use, and clinical outcomes. Statistical analyses were performed using SPSS software (version 28.0), applying bivariate and multivariable logistic regression, with a significance level of $p \leq 0.05$. Among the 1,396 newborns included, 1,062 (76.1%) had at least one culture collected for microbiological analysis. Most cases were classified as presumed early-onset sepsis (61.1%), followed by presumed late-onset sepsis (13.7%). Laboratory-confirmed cases accounted for 9.8% of the total, with a predominance of bacterial infections (8.3%) and a lower frequency of fungal infections (1.5%). Among the isolated microorganisms, Gram-positive bacteria were the most prevalent (5.8%), mainly *coagulase-negative Staphylococcus* and *Staphylococcus aureus*; among Gram-negative bacteria, *Klebsiella spp.* and *Escherichia coli* were the main agents; *Candida spp.* accounted for all fungal isolates. The neonatal mortality rate was 15.2%, significantly associated with extreme prematurity, extremely low birth weight, low Apgar scores, need for mechanical ventilation, and infections caused by multidrug-resistant Gram-negative bacilli. Statistical analysis showed that newborns who received gentamicin, cefepime, oxacillin, and fluconazole had a higher risk of neonatal mortality. The results reinforce the need for continuous microbiological surveillance, rational antibiotic use, and strengthening of multiprofessional practices for infection prevention and control. It is concluded that the integration of laboratory surveillance, clinical care protocols, and team training is essential to reduce neonatal mortality and curb the spread of bacterial resistance in NICUs.

Keywords: *Antibiotics. Bacterial infection. Neonatal mortality. Antimicrobial resistance. Neonatal sepsis.*

LISTA DE SÍMBOLOS E ABREVIACÕES

- ABNT – Associação Brasileira de Normas Técnicas
- AINE – Anti-inflamatório não esteroideal
- ANVISA – Agência Nacional de Vigilância Sanitária
- AUC – *Area Under the Curve* (Área sob a curva)
- BPN – Baixo peso ao nascer
- CAPPesq – Comitê de Ética para Análise de Projetos de Pesquisa
- CAAE – Certificado de Apresentação para Apreciação Ética
- CDC – *Centers for Disease Control and Prevention* (Centro de Controle e Prevenção de Doenças)
- CFU – *Colony-Forming Unit* (Unidade formadora de colônia)
- CI 95% – *95% Confidence Interval* (Intervalo de confiança de 95%)
- COVID-19 – *Coronavirus Disease 2019*
- CPAP – *Continuous Positive Airway Pressure* (Pressão positiva contínua nas vias aéreas)
- CRM – Conselho Regional de Medicina
- CSF – *Cerebrospinal Fluid* (Líquor)
- DNA – *Deoxyribonucleic Acid* (Ácido desoxirribonucleico)
- E. coli* – *Escherichia coli*
- ESBL – *Extended-Spectrum Beta-Lactamase* (Beta-lactamase de espectro estendido)
- ETI – *Endotracheal Intubation* (Entubação endotraqueal)
- FHEMIG – Fundação Hospitalar do Estado de Minas Gerais
- GBS – *Group B Streptococcus* (Estreptococo do grupo B)
- GSA – *Global Sepsis Alliance* (Aliança Global contra a Sepsis)
- HMC – Hemocultura
- HUSF – Hospital Universitário São Francisco
- ICN – Índice de Condição Neonatal
- IL-6 – *Interleukin 6* (Interleucina 6)
- IOT – Intubação orotraqueal
- K. pneumoniae* – *Klebsiella pneumoniae*
- LBW – *Low Birth Weight* (Baixo peso ao nascer)
- MRSA – *Methicillin-Resistant Staphylococcus aureus* (Staphylococcus aureus resistente à metilina)
- NICU – *Neonatal Intensive Care Unit* (Unidade de Terapia Intensiva Neonatal)

OMS – Organização Mundial da Saúde

OR – *Odds Ratio* (Razão de chances)

PCR – Proteína C reativa

PN – Peso ao nascer

PNAISC – Política Nacional de Atenção Integral à Saúde da Criança

PPHN – *Persistent Pulmonary Hypertension of the Newborn* (Hipertensão pulmonar persistente do recém-nascido)

PPE – *Personal Protective Equipment* (Equipamento de proteção individual)

P. aeruginosa – *Pseudomonas aeruginosa*

RN – Recém-nascido

RNs – Recém-nascidos

SBP – Sociedade Brasileira de Pediatria

SDR – Síndrome do desconforto respiratório

S. aureus – *Staphylococcus aureus*

SCoN – *Staphylococcus coagulase negativa*

SPSS – *Statistical Package for the Social Sciences*

SUS – Sistema Único de Saúde

UTIN – Unidade de Terapia Intensiva Neonatal

VLBW – *Very Low Birth Weight* (Muito baixo peso ao nascer)

WHO – *World Health Organization* (Organização Mundial da Saúde)

SUMÁRIO

1. INTRODUÇÃO	10
1.1 Neonatologia, prematuridade e demanda por internação neonatal	10
1.2 Infecção bacteriana em neonatos	11
1.3 Uso empírico de antibióticos e resistência bacteriana em neonatos	13
2. OBJETIVOS.....	17
2.1. Objetivo geral	17
2.2. Objetivos Específicos	17
3. CAPÍTULO 1: ARTIGO 1 SUBMETIDO	18
4. CAPÍTULO 2: ARTIGO 2 SUBMETIDO	69
5. CONCLUSÃO	99
REFERÊNCIAS	100
ANEXO	103
Anexo 1 – Aprovação do CAAE.....	103
APÊNDICE	109
Apêndice A – Instrumento de Coleta de Dados	109

1. INTRODUÇÃO

1.1. Neonatologia, prematuridade e demanda por internação neonatal

A neonatologia ocupa um lugar fundamental na organização da atenção materno-infantil por concentrar o cuidado de uma população com alta vulnerabilidade clínica e grande risco de agravamento em curto espaço de tempo. O período neonatal, especialmente quando marcado por prematuridade e/ou baixo peso ao nascer, demanda vigilância contínua e intervenções precoces, muitas vezes em ambiente hospitalar, para garantir estabilidade térmica e hemodinâmica, suporte ventilatório quando indicado, adequação da oferta nutricional e manejo de intercorrências associadas à imaturidade fisiológica.

No Brasil mais de 31 milhões de nascimentos entre 2012 e 2022, com prematuridade em torno de 11,3% no período. Esses achados reforçam a prematuridade como um evento frequente e persistente, associado a maior risco de morbidade neonatal, mortalidade infantil e custos assistenciais, com impacto na capacidade de resposta dos serviços (Ministério da Saúde, 2024).

No plano global, a magnitude da prematuridade também segue elevada. Dados recentes indicam que, em 2020, aproximadamente 13,4 milhões de nascimentos ocorreram antes de 37 semanas de gestação, representando cerca de 9,9% dos nascidos vivos, com maior concentração em regiões historicamente marcadas por limitações estruturais na oferta de cuidado neonatal especializado (Ohuma et al., 2023). A Organização Mundial da Saúde também destaca que, em termos populacionais, cerca de 1 em cada 10 bebês nasce prematuro no mundo, com consequências relevantes para a sobrevivência e desfechos de curto e longo prazo (WHO, 2023).

Além da prematuridade, condições como baixo peso ao nascer e restrição do crescimento intrauterino representam importantes fatores de risco, associando-se a maior vulnerabilidade no início da vida, maior necessidade de suporte e maior probabilidade de internação em unidades neonatais. Evidências epidemiológicas indicam que essas condições também se associam a maior risco de complicações neonatais e desfechos desfavoráveis (Kale & Fonseca, 2023; Okwaraji et al., 2024). No Brasil, somam-se ainda desigualdades regionais na organização da rede, com diferenças de acesso e disponibilidade de serviços especializados, que podem influenciar tanto a necessidade quanto o desfecho das internações neonatais (Paho/OPAS, 2024).

É nesse contexto, de alta demanda por internação, necessidade frequente de procedimentos e permanência hospitalar prolongada, que os agravos infecciosos ganham especial relevância. A combinação entre imaturidade imunológica, fragilidade das barreiras

cutâneo-mucosas, exposição a terapias invasivas e tempo prolongado de internação cria um cenário favorável ao desenvolvimento de sepse neonatal, condição que permanece entre as principais causas de morbimortalidade nesse período e reforça a necessidade de análises específicas em serviços de alta complexidade.

1.2 Infecção Bacteriana em Neonatos

As infecções bacterianas no período neonatal constituem um importante problema de saúde pública, permanecendo como uma das principais causas de morbimortalidade no período neonatal, especialmente entre recém-nascidos prematuros e aqueles com baixo peso ao nascer. A imaturidade do sistema imunológico desses pacientes os torna particularmente suscetíveis à colonização e invasão por microrganismos patogênicos, com risco aumentado de evolução rápida para sepse e outras complicações graves (Miranda et al., 2024).

Um estudo multicêntrico do grupo BARNARDS (Burden of Antibiotic Resistance in Neonates from Developing Societies), realizado em sete países de baixa e média renda entre 2015 e 2018, acompanhou 30.557 recém-nascidos e identificou elevada incidência de sepse neonatal, com predominância de casos confirmados nos primeiros três dias de vida, caracterizando sepse neonatal precoce. O estudo também demonstrou mortalidade superior a 11% entre os casos de sepse, associada principalmente à dificuldade de diagnóstico precoce, à resistência antimicrobiana e às limitações no acesso aos cuidados de saúde, com predomínio de patógenos Gram-negativos multirresistentes (Milton et al., 2022).

No Brasil, embora os índices de mortalidade por sepse estejam alinhados com padrões globais, a elevada frequência de internações neonatais por infecções revela persistentes fragilidades na atenção perinatal, especialmente em regiões com menor cobertura de unidades de terapia intensiva neonatal e escassez de equipes especializadas (Almeida et al., 2022). A literatura destaca que fatores como infraestrutura deficiente, acesso limitado a exames laboratoriais rápidos e falhas nos protocolos de controle de infecção hospitalar contribuem para a manutenção desses indicadores (Almeida et al., 2022).

O diagnóstico precoce da sepse neonatal é desafiador, devido à inespecificidade dos sinais clínicos iniciais e à baixa acurácia dos biomarcadores nas primeiras horas de infecção, o que pode retardar a intervenção e comprometer os desfechos clínicos (Cohen et al., 2023).

Essas dificuldades diagnósticas, somadas à ausência de métodos rápidos e confiáveis, contribuem significativamente para a elevada mortalidade neonatal em escala global. Estima-se que aproximadamente 25% das mortes neonatais em países de baixa e média renda estejam

associadas à sepse, com taxas de mortalidade variando entre 10% e 29% nesses contextos, onde as barreiras de acesso à assistência qualificada são mais evidentes (Dramowski et al., 2025).

Embora o tratamento padrão da sepse neonatal envolve o uso de antibióticos empíricos de amplo espectro, conforme as recomendações da Organização Mundial da Saúde (OMS — *World Health Organization*, 2024) e de diretrizes nacionais, como os da Sociedade Brasileira de Pediatria (SBP, 2022), a crescente resistência bacteriana tem imposto desafios adicionais ao manejo clínico, exigindo revisão contínua dos esquemas terapêuticos, maior atenção à duração do tratamento e fortalecimento de estratégias de uso racional de antimicrobianos nos serviços neonatais.

Além da antibioticoterapia, o desfecho clínico do neonato com sepse depende da integração entre terapias de suporte e medidas preventivas, que atuam de forma complementar no controle da infecção, na redução de complicações e na recuperação clínica. Nesse sentido, diretrizes internacionais destacam a importância de abordagens que fortaleçam o cuidado integral ao recém-nascido, desde o período perinatal até a internação neonatal, como estratégia central para a redução da morbimortalidade associada às infecções (Rallis et al., 2023; OMS, 2024).

A equipe multiprofissional exerce papel fundamental na detecção precoce da sepse neonatal, na implementação de medidas preventivas e no monitoramento contínuo dos sinais clínicos e laboratoriais dos recém-nascidos. A atuação integrada dos diferentes profissionais é determinante para o reconhecimento de alterações sutis no estado clínico, favorecendo a comunicação ágil com os membros da equipe e a adoção de condutas imediatas. Além disso, a implementação coletiva de protocolos de prevenção de infecção e programas de *stewardship* antimicrobiano tem se mostrado essencial para a redução de eventos adversos e para a melhoria dos indicadores de segurança do paciente em unidades neonatais (Konlan et al., 2024; Abou El Fadl et al., 2023; Flannery et al., 2024).

A sepse neonatal é uma emergência médica que pode ter origem em infecções localizadas em diferentes órgãos, sendo seu manejo norteado por diretrizes clínicas reconhecidas, como as da Sociedade Brasileira de Pediatria (SBP, 2022), e pode ser causada por bactérias, vírus, fungos ou parasitas.

A confirmação diagnóstica é baseada principalmente em culturas positivas, especialmente hemoculturas e análise do líquido, e requer intervenção imediata para garantir a sobrevivência do recém-nascido. De acordo com Puopolo et al. (2018), essas intervenções incluem

a coleta adequada de amostras microbiológicas antes do início da antibioticoterapia, o início precoce de antimicrobianos empíricos de amplo espectro, o suporte hemodinâmico e respiratório conforme a gravidade clínica, além do monitoramento contínuo dos sinais vitais e da resposta ao tratamento.

No âmbito da prevenção, tanto a Organização Mundial da Saúde (OMS, 2024) quanto a *Global Sepsis Alliance* (GSA, 2023) ressaltam a importância da prevenção primária como estratégia central no enfrentamento das infecções neonatais.

Essas estratégias incluem a qualificação do cuidado pré-natal e intraparto, a identificação e o tratamento oportuno de infecções maternas, a adoção rigorosa de práticas de higiene das mãos, o uso seguro de dispositivos invasivos, a implementação de protocolos de prevenção de infecções relacionadas à assistência à saúde e a promoção do aleitamento materno. De forma integrada, essas medidas visam reduzir a exposição do recém-nascido a agentes infecciosos e minimizar o risco de sepse no período neonatal.

Diante da relevância clínica da sepse neonatal, da dificuldade diagnóstica nas fases iniciais e da gravidade dos desfechos associados, o uso precoce de antibióticos empíricos tornou-se uma prática amplamente incorporada na assistência neonatal. No entanto, a elevada frequência dessa conduta, associada à exposição prolongada e, muitas vezes, à ausência de confirmação microbiológica, tem levantado preocupações crescentes quanto ao impacto da antibioticoterapia sobre o perfil de resistência bacteriana e sobre os desfechos clínicos dos recém-nascidos. Nesse contexto, torna-se fundamental compreender os padrões de uso de antimicrobianos na neonatologia e suas implicações para a segurança do paciente e para a sustentabilidade das estratégias terapêuticas.

1.3. Uso Empírico de Antibióticos e Resistência Bacteriana em Neonatos

Na prática clínica neonatal, a antibioticoterapia empírica consolidou-se como uma estratégia no manejo inicial da sepse suspeita, sendo frequentemente iniciada antes da confirmação laboratorial. Essa conduta reflete a necessidade de intervenção rápida diante da potencial gravidade da infecção, em um contexto no qual os sinais clínicos iniciais são inespecíficos e os exames laboratoriais nem sempre oferecem respostas imediatas. No entanto, a ampliação e a duração do uso de antibióticos de amplo espectro têm sido progressivamente questionadas, sobretudo em razão do avanço da resistência bacteriana e de seus impactos sobre os desfechos neonatais.

Uma revisão recente aponta que cerca de metade dos patógenos envolvidos na sepse neonatal já apresenta resistência significativa a antibióticos considerados de primeira linha, como ampicilina, gentamicina e cefotaxima. A situação é ainda mais crítica quando se observam cepas multirresistentes de *Klebsiella pneumoniae* e *Escherichia coli*, produtoras de beta-lactamase de espectro estendido ESBL (*Extended-Spectrum Beta-Lactamase*) e resistentes a carbapenêmicos, um cenário cada vez mais comum em UTIs neonatais (Rallis et al., 2023).

De acordo com o Boletim de Segurança do Paciente e Qualidade em Serviços de Saúde da Agência Nacional de Vigilância Sanitária (ANVISA, 2024), os microrganismos mais frequentemente isolados em UTIs neonatais foram *Staphylococcus coagulase negativa* ($\approx 75\%$ de resistência), *Staphylococcus aureus* ($\approx 40\%$), *Klebsiella pneumoniae* ($\approx 25\%$), além de outros agentes como *Candida não-albicans*, *Escherichia coli*, *Acinetobacter* spp. e *Pseudomonas aeruginosa*.

Os impactos do uso excessivo de antimicrobianos em recém-nascidos vão além da resistência microbiana. A literatura tem apontado associações importantes entre a exposição prolongada a antibióticos e o aumento do risco para condições graves como enterocolite necrosante, displasia broncopulmonar, leucomalácia periventricular e retinopatia da prematuridade. Além disso, há repercussões indiretas, como interferência na amamentação e no estabelecimento do vínculo mãe-bebê (Rallis et al., 2023).

Embora a hemocultura permaneça como o padrão-ouro para o diagnóstico da sepse neonatal, sua sensibilidade é limitada, especialmente em neonatos já submetidos à antibioticoterapia prévia. Muitos recém-nascidos internados com suspeita clínica de infecção apresentaram culturas negativas, o que dificulta a confirmação etiológica. Nesses casos, a decisão terapêutica deve ser orientada por uma avaliação clínica criteriosa, aliada ao uso racional de biomarcadores, como proteína C reativa (PCR) e procalcitonina (Reyes, 2018).

Nessa perspectiva, é importante esclarecer que culturas positivas são aquelas que identificam o crescimento de microrganismos patogênicos em meio de cultura, confirmando a presença de infecção; enquanto culturas negativas não evidenciam crescimento bacteriano, podendo refletir a ausência de infecção ou limitações técnicas, como volume insuficiente de amostra, uso inadequado do frasco ou antibioticoterapia prévia. A sepse neonatal por cultura negativa representa até metade dos casos, sobretudo em prematuros e recém-nascidos de baixo peso, reforçando a importância do uso adequado dos frascos pediátricos e da coleta de volume mínimo de 0,5 a 1 mL de sangue para otimizar a sensibilidade diagnóstica (Vasilescu et al., 2025).

Frente a esse cenário, diversas iniciativas têm buscado racionalizar o uso de antibióticos na neonatologia. Um estudo realizado em um centro terciário na China demonstrou que a implementação de um programa de uso racional de antimicrobianos resultou em mudanças expressivas na prática clínica. Houve aumento na proporção de neonatos tratados por até três dias (de 2,1% para 38,2%) e redução significativa daqueles tratados por mais de sete dias (de 95,8% para 39,5%). Além disso, o uso total de antibióticos caiu de 27 para 10 dias, com melhoras nos desfechos clínicos observados (Chu et al., 2023).

De modo semelhante, revisões sistemáticas confirmam que estratégias multifatoriais, como os programas de *stewardship* antimicrobiano, têm sido eficazes na redução do uso desnecessário de antibióticos, sem comprometer a segurança clínica dos pacientes neonatais (Rajar et al., 2020). Tais intervenções incluem desde protocolos institucionais de início e suspensão da antibioticoterapia até capacitação da equipe multiprofissional e auditoria clínica.

Apesar dos avanços na redução no uso de antibióticos em unidades de terapia intensiva neonatal, ainda há grande variabilidade na prática entre diferentes unidades neonatais, o que evidencia oportunidades para otimizar a prescrição e racionalizar o tratamento (Boverman et al., 2022). Nesse contexto, a observação clínica rigorosa tem se mostrado uma abordagem segura e eficaz para recém-nascidos com sinais leves e transitórios, reduzindo não apenas o tempo de internação, mas também a exposição a exames invasivos e a antibióticos desnecessários (Montaner Ramón et al., 2023).

No campo preventivo, algumas medidas também têm se mostrado eficazes. Por exemplo, a administração profilática de antibióticos antes do clampeamento do cordão umbilical em cesarianas tem contribuído para reduzir infecções maternas, sem aumento da morbidade neonatal (Bollig et al., 2018).

Finalmente, dados epidemiológicos apontam que a sepse neonatal microbiologicamente confirmada ocorre em cerca de 0,29% dos nascidos vivos, com maior prevalência entre recém-nascidos de muito baixo peso internados em UTIs neonatais (Ortiz de Zárate et al., 2023). Esses dados reforçam a necessidade de equilíbrio entre precaução e racionalidade terapêutica, garantindo que o uso de antibióticos ocorra de forma criteriosa, segura e baseada em evidências.

Em estudo realizado na UTIN do Hospital Universitário Korle Bu, em Gana, verificou-se que as infecções da corrente sanguínea neonatal estavam associadas a um custo médio adicional de US\$ 1.359 por paciente, sendo 69% arcado pela instituição e 31% pelos

cuidadores, além de um prolongamento de cerca de 10 dias na hospitalização em comparação com neonatos sem infecção (Fenny et al., 2021).

Além das repercussões clínicas, as infecções neonatais representam um importante impacto econômico e social, tanto para o sistema de saúde quanto para as famílias. Estudos estimam que cada episódio de infecção em unidades neonatais pode aumentar em até 40% os custos de hospitalização, prolongando o tempo de internação e elevando a necessidade de suporte intensivo (Abreu et al., 2023). No contexto do Sistema Único de Saúde (SUS), esse cenário reforça a urgência de fortalecer políticas de vigilância epidemiológica, controle de infecção e uso racional de antibióticos nas unidades de terapia intensiva neonatal.

Apesar da reconhecida importância da sepse neonatal como causa de morbimortalidade, especialmente entre recém-nascidos prematuros e de baixo peso, observou-se que ainda são escassos os estudos nacionais que descrevem de forma sistemática a incidência, os fatores de risco e os agentes etiológicos envolvidos nas infecções bacterianas em UTINs. Essa lacuna limita a construção de estratégias preventivas e a implementação de protocolos baseados em evidências locais, particularmente diante do avanço da resistência antimicrobiana.

Nesse contexto, os achados do presente estudo contribuem para o aprimoramento da prática clínica, ao subsidiar estratégias mais efetivas de prevenção, diagnóstico e manejo das infecções bacterianas neonatais, além de auxiliar na racionalização do uso de antimicrobianos. Adicionalmente, os resultados podem colaborar para a redução do tempo de internação, dos custos hospitalares e para a qualificação da assistência prestada aos recém-nascidos em unidades de alta complexidade, fortalecendo a segurança do paciente e o cuidado neonatal baseado em evidências.

2. OBJETIVOS

2.1. Objetivo Geral

Avaliar a incidência, os padrões clínicos e microbiológicos, e os impactos das infecções bacterianas em recém-nascidos internados na unidade de terapia intensiva neonatal de um hospital universitário.

2.2. Objetivos Específicos

2.2.1. Identificar os principais agentes etiológicos e caracterizar os padrões de resistência antimicrobiana observados nas infecções bacterianas em recém-nascidos.

2.2.2. Descrever as características clínicas e perinatais dos neonatos acometidos, considerando idade gestacional, peso ao nascer, condições associadas e intervenções terapêuticas realizadas.

2.2.3. Analisar os desfechos clínicos relacionados às infecções bacterianas, incluindo mortalidade, tempo de internação, necessidade de ventilação mecânica e ocorrência de complicações.

3. CAPÍTULO 1: ARTIGO 1 SUBMETIDO

Title: Risk Factors, Prevalence of Bacterial Infections, and Mortality Among Neonates Admitted to a Neonatal Intensive Care Unit

Short Title: Bacterial Infections and Mortality Risk in Neonatal Intensive Care

Patricia Krause Askinis^{1,2,3,#}; Fernanda Ferreira Mariano^{1,2,3}; Gustavo Gomes Sacaizawa^{1,2,3}; Luiz Felipe Azevedo Marques^{1,2,3}; Ketlyn Oliveira Bredariol^{1,2,3}; Fernando Augusto Lima Marson^{1,2,3,*,#}

¹ Laboratory of Molecular Biology and Genetics, Postgraduate Program in Health Sciences, Postgraduate Program in Health Data Science, São Francisco University, Bragança Paulista, São Paulo, Brazil.

² Laboratory of Clinical and Molecular Microbiology, Postgraduate Program in Health Sciences, Postgraduate Program in Health Data Science, São Francisco University, Bragança Paulista, São Paulo, Brazil.

³ LunGuardian Research Group – Epidemiology of Respiratory and Infectious Diseases, Postgraduate Program in Health Sciences, Postgraduate Program in Health Data Science, São Francisco University, Bragança Paulista, São Paulo, Brazil.

*** Corresponding Author:** [FALM] Fernando Augusto Lima Marson, BSc, MSc, PhD.

Laboratory of Molecular Biology and Genetics, Laboratory of Clinical and Molecular Microbiology, LunGuardian Research Group – Epidemiology of Respiratory and Infectious Diseases, Postgraduate Program in Health Sciences, Postgraduate Program in Health Data Science, São Francisco University. Avenida São Francisco de Assis, 218. Jardim São José, Bragança Paulista 12916-900, São Paulo, Brasil. Phone Number: +55-19-999752911.

E-mail: fernandolimamarson@hotmail.com and fernando.marson@usf.edu.br.

[#] The authors contributed equally to this study.

Study authors' e-mails and ORCIDs:

PKA: patriciakrause14@gmail.com

ORCID: 0009-0000-8067-0677

FFM: fernandamariano011@gmail.com

ORCID: 0009-0003-5194-7050

GGS: gustavo.sacaizawa@mail.usf.edu.br

ORCID: 0009-0000-4310-844X

LFAM: luiz.azevedo@mail.usf.edu.br

ORCID: 0009-0008-5494-2171

KOB: Kbredariol@outlook.com

ORCID: 0009-0006-2066-8472

FALM: fernandolimamarson@hotmail.com and fernando.marson@usf.edu.br

ORCID: 0000-0003-4955-4234

Impact**What is the key message of your article?**

- Neonatal sepsis remains highly prevalent among preterm and low-birth-weight infants admitted to a tertiary NICU, with mortality strongly associated with extreme prematurity, invasive ventilation, and multidrug-resistant Gram-negative infections.

What does it add to the existing literature?

- This study provides longitudinal epidemiological and microbiological data from a Brazilian NICU, including antimicrobial resistance patterns and independent predictors of mortality in neonates hospitalized between 2017 and 2024.

What is the impact?

- The findings reinforce the importance of continuous microbiological surveillance, antimicrobial stewardship, and early risk stratification to improve neonatal outcomes and optimize empirical antimicrobial therapy in intensive care settings.

Abstract

Introduction: Bacterial infections remain major causes of neonatal morbidity and mortality, especially among preterm and low-birth-weight infants. This study evaluated the prevalence of bacterial infections, associated risk factors, predominant microorganisms, antimicrobial resistance patterns, and mortality among neonates admitted to a neonatal intensive care unit (NICU).

Methods: A retrospective observational study was conducted using medical records of newborns admitted to a university hospital NICU between 2017 and 2024. Demographic, obstetric, neonatal, microbiological, therapeutic, and clinical outcome data were collected. Antimicrobial resistance was assessed through antibiograms. Associations with mortality were analyzed using logistic regression ($\alpha = 0.05$).

Results: Among 1,396 neonates, 15.2% died during hospitalization. Most were preterm (65.0%) and low birth weight (68.5%). Sepsis was suspected or confirmed in 86.7% of cases, predominantly bacterial (68.5%; 9.5% confirmed). Gram-positive bacteria, mainly *Staphylococcus* spp., predominated, followed by *Klebsiella pneumoniae* and *Escherichia coli*. Resistance to fluoroquinolones and β -lactams was frequent, whereas carbapenem and glycopeptide resistance remained uncommon. Mortality was associated with extreme prematurity, extremely low birth weight, severe Apgar scores, invasive ventilation, and multidrug-resistant Gram-negative infections. Gentamicin, cefepime, oxacillin, and fluconazole use were independently associated with mortality.

Conclusions: Neonatal sepsis remains a major challenge in NICUs, reinforcing the need for microbiological surveillance, antimicrobial stewardship, and early risk stratification.

Keywords: Antimicrobial resistance; Gram-negative bacteria; Neonatal sepsis; Prematurity; Prognostic factors; *Staphylococcus* spp.

1. Introduction

Bacterial infections remain one of the leading causes of neonatal morbidity and mortality, particularly in preterm and low-birth-weight infants [1]. The immunological immaturity of these patients increases their susceptibility to pathogen colonization and the rapid development of sepsis [1], which has a high incidence in the first days of life and contributes significantly to mortality, even in neonatal hospitalization settings [2]. This high mortality is mainly associated with failures in early identification, antimicrobial resistance, and barriers to adequate healthcare access, with sepsis cases predominantly caused by multidrug-resistant Gram-negative pathogens [2].

In Brazil, the prevalence of preterm births remained stable between 2011 and 2021 [3]. In 2020, approximately 9.9% of births were preterm, with the highest concentration of cases occurring in Asia and Sub-Saharan Africa [4]. In the Brazilian context, the high frequency of neonatal hospitalizations due to sepsis highlights weaknesses in perinatal care, particularly in regions with limited neonatal intensive care unit (NICU) coverage and scarce resources [3]. Prematurity remains an important risk factor for neonatal mortality, being more strongly associated with death than intrauterine growth restriction [5,6]. In NICUs, the use of invasive devices and prolonged hospitalization further increase the risk of healthcare-associated infections. Additionally, disparities in healthcare infrastructure may contribute to differences in outcomes across settings.

Globally, prematurity remains a major public health challenge. In 2020, approximately 13.4 million infants were born before 37 weeks of gestation, representing 9.9% of all live births worldwide, with the highest burden occurring in sub-Saharan Africa and Southern Asia [4]. The World Health Organization also estimates that approximately one in every ten newborns is born preterm, highlighting the substantial impact of prematurity on neonatal survival and long-term health outcomes [5].

Neonatal sepsis is a medical emergency caused by bacteria, viruses, fungi, or parasites, and its management follows recognized clinical guidelines [7]. Diagnosis relies primarily on positive cultures, such as blood and cerebrospinal fluid cultures, requiring immediate intervention to ensure neonatal survival [8]. However, early diagnosis remains challenging due to nonspecific clinical signs and low-accuracy biomarkers, which delay interventions and increase mortality [9]. It is estimated that 25% of neonatal deaths in low- and middle-income countries are associated with sepsis, with mortality rates ranging from 10% to 29% [10].

Although the standard treatment for neonatal sepsis involves empirical broad-spectrum antibiotics, the rise of bacterial resistance poses an increasing challenge to clinical management [11]. Supportive care, such as assisted ventilation, continuous monitoring, and adequate nutrition, is essential for neonatal recovery [12], while prevention strategies include exclusive breastfeeding, maternal vaccination, and safe delivery practices [6,13]. Empirical antibiotic therapy is widely used in neonatology due to the nonspecific nature of initial clinical signs and the low accuracy of laboratory tests for confirming neonatal sepsis [14]. However, rising bacterial resistance compromises first-line antibiotics and increases the prevalence of multidrug-resistant *Klebsiella pneumoniae* and *Escherichia coli* strains [15]. Among the microorganisms most frequently isolated in NICUs are coagulase-negative *Staphylococcus*, *Staphylococcus aureus*, *K. pneumoniae*, *E. coli*, and *Pseudomonas aeruginosa*, with a predominance of Gram-positive cocci, particularly in late-onset infections [14]. Because antimicrobial susceptibility patterns vary substantially among institutions and over time, continuous local microbiological surveillance is essential to guide empirical treatment and antimicrobial stewardship strategies.

Excessive use of antimicrobials is associated with serious complications, such as necrotizing enterocolitis and bronchopulmonary dysplasia, and can also affect breastfeeding and mother–infant bonding [15]. Blood culture remains the gold standard for diagnosis, but negative cultures are common in preterm and low-birth-weight infants [16]. Stewardship programs and institutional protocols have reduced unnecessary antibiotic use without compromising clinical

safety [17,18], while careful observation allows for the safe management of neonates with mild signs [19,20]. Preventive measures, such as prophylactic antibiotics in cesarean deliveries, have also demonstrated effectiveness [21]. Despite the clinical relevance of neonatal bacterial infections, data from low- and middle-income countries remain limited, particularly regarding long-term epidemiological trends, causative microorganisms, antimicrobial resistance profiles, and factors associated with adverse outcomes in NICU settings. This gap is especially relevant in Latin America, where multicenter and longitudinal data remain scarce.

Confirmed neonatal sepsis occurs in approximately 0.29% of live births, with higher prevalence among very low-birth-weight neonates, impacting costs and length of hospital stay [22]. Neonatal infections are also associated with prolonged hospitalization, greater use of intensive care resources, and increased healthcare costs, reinforcing their burden on health systems [22].

Therefore, this study aimed to evaluate the prevalence of bacterial infections, characterize the microbiological and antimicrobial resistance profiles, and identify factors associated with adverse clinical outcomes and neonatal mortality in a university hospital NICU, thereby contributing to evidence-based management and rational antimicrobial use.

2. Methods

2.1. Study Design and Type

This was a retrospective observational study with a quantitative approach, conducted through the review of medical records of newborns admitted to the NICU of a university hospital. The data collection period included admissions that occurred between January 1, 2017, and December 31, 2024.

2.2. Study Population and Sampling Criteria

The study population comprised all newborns admitted to the NICU during the defined period. Initially, 1,630 patient records were considered. During the screening process, 234 records (14.4%) were excluded, of which 166 (10.2%) corresponded to patients older than 28 days at the time of admission, not meeting the definition of a newborn, and 68 (4.2%) were excluded due to incomplete or inaccessible information for analysis. The final sample consisted of 1,396 newborns, distributed across the different neonatal intensive care units as detailed in **Table 1**.

Data were obtained from both physical and electronic medical records using a standardized form designed to extract clinical, demographic, and perinatal variables. Clinical information was primarily extracted from physical medical records, whereas microbiological results were retrieved from electronic laboratory records. The data collection instrument was specifically developed for this study and pilot-tested before data extraction to ensure clarity and applicability.

The sample was divided into two groups: (i) newborns with laboratory-confirmed bacterial infection; and (ii) newborns without confirmed infection, who constituted the reference group. Laboratory-confirmed bacterial infection was defined as the isolation of a pathogenic bacterium from a normally sterile biological sample during hospitalization, interpreted in conjunction with compatible clinical findings. Cultures considered contaminants based on clinical and microbiological assessment were excluded from confirmed infection analyses.

Table 1. Demographic, obstetric, and neonatal characteristics and clinical outcomes of newborns admitted to the Neonatal Intensive Care Unit of a university hospital (2017–2024).

Markers	Category	N	(%)
Demographic data			
Type of healthcare coverage	Public	1,195	85.6%
	Insurance and private	201	14.4%
Coronavirus disease 2019 (COVID-19) pandemic	Pre-pandemic (2017–2019)	474	34.0%
	Pandemic (2020–2021)	327	23.4%
	Post-pandemic (2022–2024)	595	42.6%
Age in days at admission	First day of life	1,131	81.0%
	After the first day of life	265	19.0%
Sex	Male	787	56.4%
	Female	606	43.4%
	Ambiguous	3	0.2%
Gestational age	Post-term	23	1.6%
	Term	467	33.4%
	Moderate/late preterm	371	26.6%
	Very preterm	375	26.9%
	Extremely preterm	160	11.5%
Birth weight	Macrosomic	27	1.9%
	Appropriate	413	29.6%
	Low	487	34.9%
	Very low	301	21.6%
	Extremely low	168	12.0%
Apgar scores at 1 minute	Good	657	47.1%
	Moderate	596	42.7%
	Severe	143	10.2%
Apgar scores at 5 minutes	Good	1,149	82.3%
	Moderate	210	15.0%
	Severe	37	2.7%
Obstetric data			
Type of delivery	Cesarean	913	65.4%
	Vaginal (normal)	483	34.6%
Maternal antibiotic use before delivery	Yes	254	18.2%
	No	1,142	81.8%
Group B <i>Streptococcus</i> screening results	Positive	25	1.8%
	Negative	148	10.6%
	Unknown	1,223	87.6%
Amniotic membrane integrity	Intact	1,001	71.7%
	Ruptured	395	28.3%
Meconium in the amniotic fluid	Yes	159	11.4%
	No	1,237	88.6%
Neonatal data			
Recorded diagnostic hypotheses*	Respiratory disorders	1,246	89.3%
	Prematurity	1,000	71.6%
	Perinatal asphyxia	204	14.6%
	Hypoglycemia	148	10.6%
	Seizure episodes	86	6.2%
	Congenital heart defects	54	3.9%
	Jaundice	35	2.5%
	Atresias	11	0.8%
	Other associated clinical conditions	93	6.7%
Antimicrobials used	Yes	988	70.8%
	No	408	29.2%
Forms of respiratory support*	Supplemental oxygen	1,059	75.9%
	Continuous positive airway pressure	911	65.3%

Table 1. Demographic, obstetric, and neonatal characteristics and clinical outcomes of newborns admitted to the Neonatal Intensive Care Unit of a university hospital (2017–2024).

Markers	Category	N	(%)
Invasive procedures*	Mechanical ventilation	523	37.5%
	Umbilical catheters	1,095	78.4%
	Peripherally inserted central catheters	480	34.4%
	Surgical procedures	50	3.6%
	Thoracic drainage	16	1.1%
	Phlebotomy	13	0.9%
	Tracheostomy	9	0.6%
Complications occurring during hospitalization	Bronchopulmonary dysplasia	75	5.4%
	Pulmonary hypertension	61	4.4%
	Septic shock	51	3.7%
	Necrotizing enterocolitis	42	3.0%
	Meningitis	11	0.8%
Outcome			
Length of hospital stay	Up to seven days	402	28.8%
	Eight to twenty-one days	544	39.0%
	More than twenty-one days	450	32.2%
Endpoint	Death	212	15.2%
	Hospital discharge	1,184	84.8%

The data are presented as absolute (N) and relative (%) frequencies. *: The total may exceed N = 1,396, as a single newborn could have received more than one respiratory support device, undergone multiple invasive procedures, or presented with more than one complication or diagnosis during hospitalization.

2.3. Variables and Categories of Analysis

The variables analyzed were organized into specific categories, as described below:

(i) *Demographic data*: included the unit of admission, type of healthcare coverage, length of hospital stay, age in days at admission, sex, gestational age, birth weight, and Apgar scores at 1 and 5 minutes.

Admission units were classified according to the hospital's organizational structure. Type of healthcare coverage was categorized as public (*Sistema Único de Saúde* – SUS), insurance, or private. The length of hospital stay was defined according to the coronavirus disease 2019 (COVID-19) pandemic context in three phases: pre-pandemic (2017–2019), pandemic (2020–2021), and post-pandemic (2022–2024).

Age in days at admission was classified as newborns admitted on the first day of life or after the first day. Biological sex was recorded as male, female, or ambiguous.

Gestational age was classified as [23]: extremely preterm (< 28 completed weeks); very preterm (28 to < 32 weeks); moderate/late preterm (32 to < 37 weeks); term (37 to 41 weeks completed), and post-term ($\geq$ 42 weeks). Birth weight was categorized as [24]: appropriate (2,500–3,999 g); low (< 2,500 g); very low (< 1,500 g); extremely low (< 1,000 g), and macrosomic ($\geq$ 4,000 g).

Apgar scores at 1 and 5 minutes were categorized according to neonatal vitality as [25]: good (8–10 points); moderate (4–7 points), and severe (1–3 points).

(ii) *Obstetric data*: included the type of delivery, maternal antibiotic use before delivery, Group B *Streptococcus* screening results, amniotic membrane integrity, and the presence of meconium in the amniotic fluid.

The type of delivery was categorized as cesarean or vaginal (normal), according to obstetric medical records. Maternal antibiotic administration in the pre-delivery period was classified as yes or no, considering the use of prophylactic or therapeutic antimicrobial agents before birth, based on the maternal history documented during obstetric admission.

Group B *Streptococcus* (*Streptococcus agalactiae*) screening was recorded according to the maternal laboratory result and classified as positive, negative, or unknown (if no information was available in the medical record).

Amniotic membrane integrity at the time of delivery was determined based on clinical description and categorized as intact or ruptured. Rupture was considered either prior to or during delivery, regardless of the time elapsed until birth.

The presence of meconium in the amniotic fluid was assessed according to the obstetric record and classified as present (yes) or absent (no). This variable was included because of its association with fetal vitality and the risk of neonatal meconium aspiration.

(iii) *Neonatal data*: included recorded diagnostic hypotheses, microbiological results, antimicrobials used, forms of respiratory support, invasive procedures, and complications occurring during hospitalization.

Neonatal diagnostic hypotheses encompassed respiratory disorders, prematurity, perinatal asphyxia, hypoglycemia, seizure episodes, congenital heart defects, jaundice, atresias, and other associated clinical conditions. Variables related to management and clinical course during hospitalization were also analyzed. Antibiotic use was assessed based on medical prescriptions recorded in medical records, considering therapy initiated during the hospitalization period. Among the most frequently used antimicrobials were ampicillin, gentamicin, amikacin, cefepime, vancomycin, meropenem, piperacillin-tazobactam, metronidazole, oxacillin, and other antibiotics according to clinical indication.

Forms of respiratory support were classified as supplemental oxygen (nasal cannula, mask, halo, or tent), continuous positive airway pressure (CPAP), and mechanical ventilation, according to the type and level of support required. Invasive procedures included the use of umbilical catheters, peripherally inserted central catheters (PICC), thoracic drainage, phlebotomy, tracheostomy, and surgical procedures performed during hospitalization.

Neonatal complications considered included bronchopulmonary dysplasia, pulmonary hypertension, septic shock, necrotizing enterocolitis, and meningitis, according to clinical records and patient progression. Specific sepsis classifications, as well as the identification of isolated microorganisms and antimicrobial susceptibility profiles, are presented in the following section.

(iv) *Classification and management of neonatal sepsis*: Neonatal sepsis was classified according to the timing of clinical and laboratory signs, following World Health Organization guidelines and international recommendations [26]. Early-onset sepsis was defined as occurring within the first three days of life (≤ 72 hours after birth), and late-onset sepsis as occurring after this period (> 72 hours).

Early-onset sepsis is frequently associated with vertical transmission of microorganisms from mother to newborn during labor or at birth. Transmission can occur via transplacental, ascending (from vaginal colonization), or intrapartum (contact with contaminated maternal secretions) routes. Key maternal risk factors for early neonatal sepsis include premature or prolonged rupture of membranes (> 18 hours); chorioamnionitis or intra-amniotic infection; maternal colonization with Group B *Streptococcus* (*S. agalactiae*); intrapartum fever; preterm birth; acute fetal distress or meconium-stained amniotic fluid; and inadequate or absent intrapartum antibiotic prophylaxis in mothers colonized with Group B *S. agalactiae*.

The following diagnostic categories were defined:

Early-onset sepsis with positive culture: isolation of a pathogenic microorganism from blood, cerebrospinal fluid, or another sterile site, associated with compatible clinical signs.

Presumed early-onset sepsis: presence of suggestive clinical signs (e.g., temperature instability, apnea, respiratory distress, lethargy, irritability, abdominal distension, feeding intolerance, tachycardia, bradycardia, or hypotension) in a newborn exposed to maternal risk factors, associated with laboratory abnormalities and requiring antimicrobial treatment

according to institutional protocols, but without laboratory confirmation. In these cases, immediate empirical treatment with ampicillin plus gentamicin was initiated according to institutional protocol and international recommendations. This approach aims to cover the main vertically transmitted pathogens and reduce the risk of progression to severe sepsis.

Late-onset sepsis with positive culture: microbiological isolation after 72 hours of life, generally related to hospital exposure or the use of invasive devices.

Presumed late-onset sepsis: presence of compatible clinical signs after the third day of life without laboratory confirmation, but receiving broad-spectrum empirical treatment, including vancomycin combined with cefepime or piperacillin-tazobactam, according to institutional protocol.

Confirmed fungal sepsis: isolation of yeasts, mainly *Candida* spp., from a sterile culture site, with initiation of systemic antifungal therapy.

Presumed fungal sepsis: clinical suspicion of fungal infection (e.g., persistent thrombocytopenia, failure of bacterial antibiotic therapy, feeding intolerance, or hemodynamic instability) without laboratory confirmation, but with empirical antifungal treatment, usually fluconazole.

In all cases of suspected clinical sepsis, cultures were collected prior to the initiation of antibiotic therapy, according to institutional protocol, to avoid interference with microbiological results. The following types of biological materials were processed according to clinical indication and suspected infection site: blood (blood culture), urine (urine culture), cerebrospinal fluid (microbiological analysis), central venous catheter tip, and skin swab. Both the number of newborns and the total number of collected cultures were included in the analysis, considering that some patients had samples collected at different times during the hospitalization period. The isolated bacteria were classified according to Gram staining as Gram-positive or Gram-negative, allowing for a comparative evaluation of infection patterns and microbiological distribution among the analyzed groups.

All clinical samples were processed and analyzed at the accredited outsourced laboratory Unilab Medicina Diagnóstica, recognized by the Brazilian Society of Clinical Pathology and certified by the Clinical Laboratory Accreditation Program. For bloodstream infection identification, an automated blood culture system was used, including Renylab bottles and the Automated Blood Culture System – BT Series, which continuously monitors the bottles and emits audible alerts when positivity is detected.

For the isolation and cultivation of microorganisms, non-selective culture media were used, including Blood Agar, Blood/Chocolate Agar, and CLED (Cystine–Lactose–Electrolyte-Deficient agar), as well as TSB (Tryptic Soy Broth) and BHI (Brain Heart Infusion) for enrichment and maintenance of quality control strains. Selective media were also employed: MacConkey for Gram-negative bacilli, Sabouraud for fungi, and chromogenic media for detection of multidrug-resistant microorganisms (MDR), including KPC (*K. pneumoniae* carbapenemase), ESBL (Extended-Spectrum Beta-Lactamase), VRE (Vancomycin-Resistant Enterococcus), and MRSA (Methicillin-Resistant *S. aureus*).

Microorganism identification was performed using commercial ready-to-use tests (such as pyr and oxidase) and in-house biochemical assays, including lysine, arginine, and urease, in addition to commercial kits such as Rugai. Final microorganism determination was based on the Brazilian Ministry of Health (Detection and Identification of Medically Important Bacteria guidelines) and laboratory bench expertise. All positive cultures were evaluated by colony macroscopic and microscopic examination, including Gram staining, for preliminary confirmation prior to final identification. In addition, Gram staining was performed according to standardized microbiology protocols. Bacteria isolated from the cultures were subjected to sequential application of crystal violet, iodine solution, alcohol (or acetone), and safranin. Based on dye retention, the microorganisms were classified as Gram-positive or Gram-negative.

Antimicrobial susceptibility testing was conducted using the disk diffusion method, and resistance mechanism determination followed BrCAST/EUCAST (Brazilian Committee on Antimicrobial Susceptibility Testing and European

Committee on Antimicrobial Susceptibility Testing) standards, which are currently mandatory for all laboratories in Brazil. All techniques, interpretations, and quality controls comply with official documents available at <https://brcast.org.br/>, updated annually, usually in January or February.

Resistance profiles were assessed for the following agents: β -lactams and β -lactamase inhibitors (ampicillin, amoxicillin + clavulanic acid, ampicillin/sulbactam, oxacillin, penicillin G, cephalothin, cefotaxime, ceftriaxone, cefoxitin, cefepime, ceftazidime, aztreonam); aminoglycosides (gentamicin, amikacin); carbapenems (imipenem, meropenem); glycopeptides (vancomycin); fluoroquinolones (ciprofloxacin, levofloxacin); macrolides and lincosamides (erythromycin, azithromycin, clindamycin); other agents (tetracycline, metronidazole, trimethoprim/sulfamethoxazole); and antifungals (fluconazole).

Antimicrobial therapy was administered according to the institutional protocol, with initial empirical therapy adjusted based on culture and susceptibility results.

Regarding clinical outcomes, length of stay was categorized into three groups: up to seven days, eight to twenty-one days, and more than twenty-one days. The final clinical outcome was classified as hospital discharge or death, according to medical records.

2.4. Statistical Analysis

Data were initially recorded in Microsoft Excel spreadsheets (.xls) and subsequently analyzed using the Statistical Package for the Social Sciences (IBM SPSS Statistics for Macintosh, version 28.0). Categorical variables were described using absolute and relative frequencies. The association between independent variables and the clinical outcome (death) was assessed using binary logistic regression, with results presented as odds ratios (OR) and corresponding 95% confidence intervals (95% CI). In addition, the comparison of outcomes according to the NICU of admission was performed using the chi-square test.

Variables with $p \leq 0.05$ in the bivariate analysis (sepsis and antibiotic/antifungal use) were included in an exploratory multivariable logistic regression model using the Backward Stepwise method, with the clinical outcome (hospital discharge versus death) as the dependent variable. An additional exploratory multivariable logistic regression model was performed to assess the association between antibiotic/antifungal use and the diagnosis of sepsis. Model results were presented as regression coefficients, standard errors, Wald tests, ORs, and corresponding 95% CIs. Multicollinearity was assessed using tolerance values (< 0.1) and the variance inflation factor (> 10). For all analyses, the significance level was set at $\alpha = 0.05$.

Graphs were created using GraphPad Prism software, version 10.2.3 (GraphPad Software, San Diego, CA, USA).

2.5. Ethical Considerations

The study was approved by the institution's Research Ethics Committee [Certificate of Presentation for Ethical Appraisal (CAAE – *Certificado de Apresentação para Avaliação Ética*) number: 80862224.4.0000.5514], **Anexo 1**. As this was a retrospective analysis of secondary data without direct patient contact, informed consent was waived. All data were anonymized, ensuring participant confidentiality.

3. Results

3.1. Characteristics of Newborns Admitted to the Neonatal Intensive Care Unit

Supplementary Table 1 shows the distribution of newborns across the NICUs of the university hospital. Among the 1,396 newborns, 219 (15.7%) were admitted to NICU 01, 157 (11.2%) to NICU 02, 137 (9.8%) to NICU 03, 141

(10.1%) to NICU 04, 155 (11.1%) to NICU 05, 152 (10.9%) to NICU 06, 115 (8.2%) to NICU 07, and 320 (22.9%) to the Extra NICU.

Table 1 summarizes the demographic, obstetric, and neonatal characteristics, as well as clinical outcomes, of newborns admitted to the NICU between 2017 and 2024. The majority of infants were covered by the public healthcare system (85.6%), and hospitalizations were distributed across the pre-pandemic (34.0%), pandemic (23.4%), and post-pandemic periods (42.6%). Most newborns were admitted on the first day of life (81.0%), with a higher proportion of males (56.4%) compared to females (43.4%), and a very small percentage identified as ambiguous sex (0.2%).

Regarding gestational age, 33.4% were full-term, 11.5% extremely preterm, 26.9% very preterm, 26.6% moderate/late preterm, and 1.6% post-term. Birth weight was distributed as follows: extremely low (12.0%), very low (21.6%), low (34.9%), appropriate (29.6%), and macrosomic (1.9%). Apgar scores at 1 minute were good in 47.1% of cases, moderate in 42.7%, and severe in 10.2%, while at 5 minutes, 82.3% were good, 15.0% moderate, and 2.7% severe.

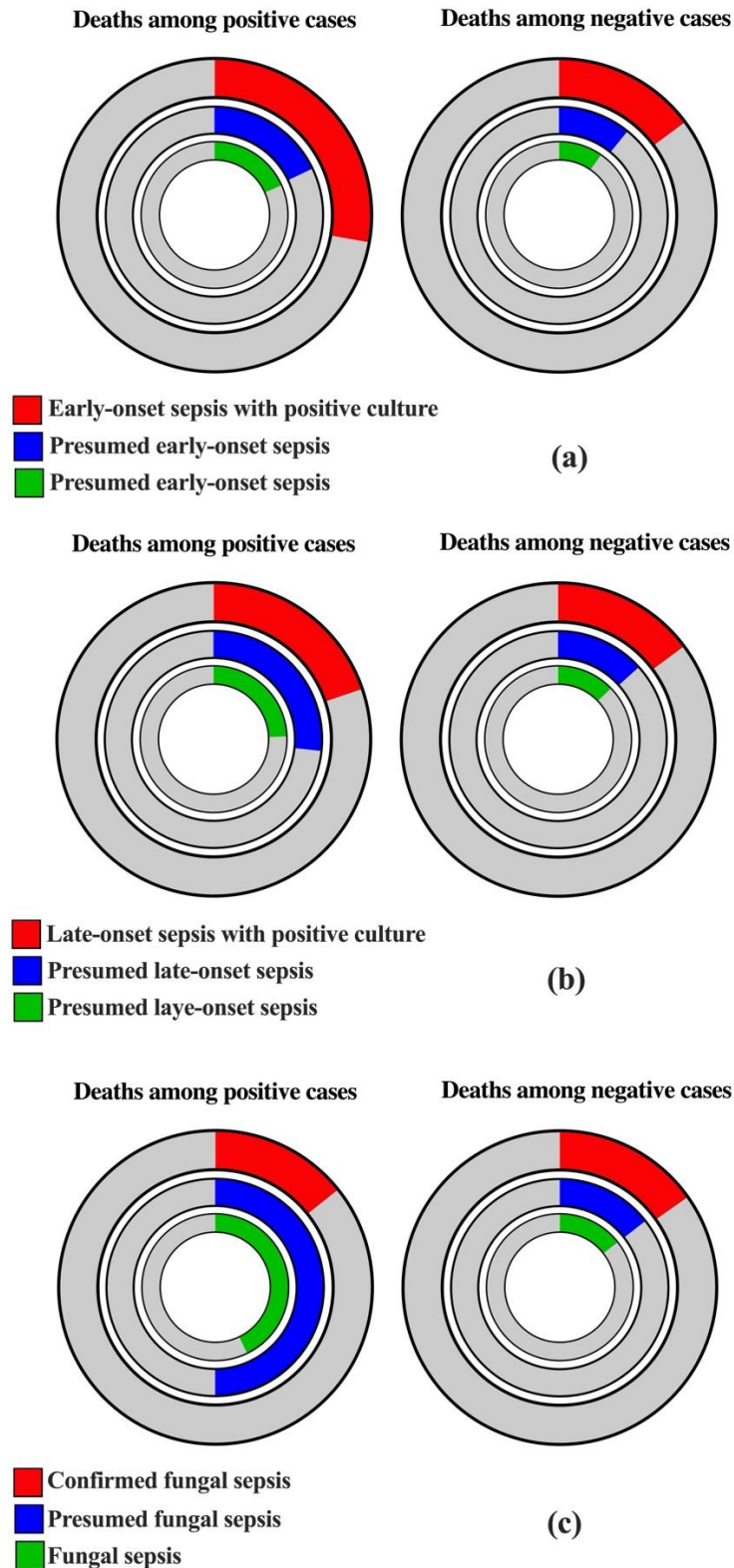
Obstetric data indicated a predominance of cesarean deliveries (65.4%), with 18.2% of mothers receiving antibiotics before delivery. Group B *Streptococcus* screening was positive in 1.8%, negative in 10.6%, and unknown in 87.6% of cases. Membrane integrity was intact in 71.7% of births, and meconium was present in the amniotic fluid in 11.4% of deliveries.

Neonatal diagnostic hypotheses most frequently recorded included respiratory disorders (89.3%) and prematurity (71.6%). Respiratory support was provided as supplemental oxygen in 75.9% of newborns, CPAP in 65.3%, and mechanical ventilation in 37.5%. Invasive procedures included umbilical catheters (78.4%), PICC (34.4%), surgical procedures (3.6%), thoracic drainage (1.1%), phlebotomy (0.9%), and tracheostomy (0.6%).

Complications during hospitalization were observed in a minority of patients, including bronchopulmonary dysplasia (5.4%), pulmonary hypertension (4.4%), septic shock (3.7%), necrotizing enterocolitis (3.0%), and meningitis (0.8%).

The length of hospital stay was distributed as follows: up to seven days (28.8%), eight to twenty-one days (39.0%), and more than twenty-one days (32.2%). The overall clinical outcome indicated a hospital discharge rate of 84.8% and a mortality rate of 15.2% (**Figure 1**).

Figure 1. Distribution of neonatal deaths according to sepsis status and infection type.



(a) Early-onset sepsis: proportion of deaths among neonates with culture-confirmed infection (red), presumed infection (blue), and combined confirmed and presumed cases (green). (b) Late-onset sepsis: proportion of deaths among neonates with culture-confirmed infection (red), presumed infection (blue), and combined confirmed and presumed cases (green). (c) Fungal sepsis: proportion of deaths among neonates with confirmed fungal infection (red), presumed fungal infection (blue), and combined confirmed and presumed cases (green). The concentric ring charts illustrate the relative mortality burden within each diagnostic category, highlighting differences between confirmed and presumed infections across the different types of neonatal sepsis.

3.2. Distribution of Neonatal Sepsis Cases, Isolated Microorganisms, Antimicrobial Resistance Profile, and Antimicrobial Therapy

Among the 1,396 newborns included in the study, 1,062 (76.1%) had at least one culture collected for microbiological analysis (**Table 2**). Most cases were classified as presumed early-onset sepsis (61.1% of all newborns; 80.3% among those with cultures), followed by presumed late-onset sepsis (13.7%; 18.0%), confirmed bacterial sepsis – either early (2.6%; 3.4%) or late (6.9%; 9.0%) – and a smaller number of confirmed (0.5%; 0.6%) or presumed (2.0%; 2.7%) fungal sepsis cases (**Table 2; Figure 2a**).

At least two or more culture collections were performed in 58.8% of all newborns (77.3% among those with cultures). The most frequently collected specimens were blood samples (101.1% relative to the total population; 132.9% among those with cultures), followed by skin swabs (15.9%; 20.9%), urine samples (10.4%; 13.6%), cerebrospinal fluid (CSF) (4.4%; 5.7%), and central venous catheter tips (3.1%; 4.1%) (**Table 2**).

Microorganism isolation was achieved in 9.8% of all newborns and 12.9% of those with cultures. Gram-positive bacteria were the most frequent (5.8%; 7.6%), followed by Gram-negative bacteria (3.5%; 4.6%) and fungi (0.5%; 0.6%) (**Figures 2b, c, d, and e**). Among Gram-positive isolates, coagulase-negative *Staphylococcus* (3.6%; 4.7%) and *S. aureus* (1.9%; 2.4%) predominated (**Table 2**). Among Gram-negative organisms, *Klebsiella* spp. (1.3%; 1.7%) and *E. coli* (1.2%; 1.6%) were most common (**Table 2**). *Candida* spp. accounted for all fungal isolates (**Table 2**).

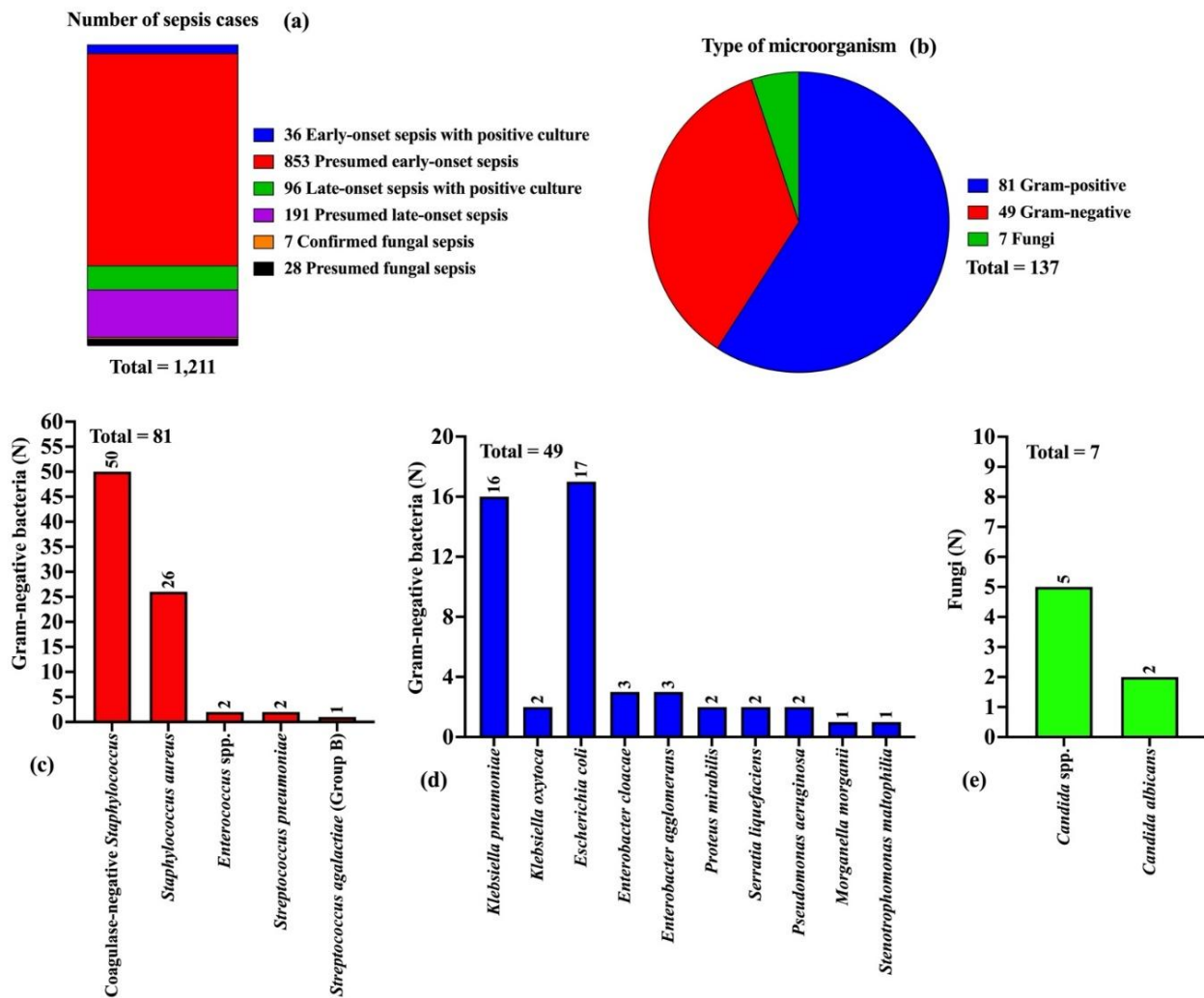
Supplementary Table 2 presents the antimicrobial resistance profile identified among neonates with positive blood cultures. Of the 1,396 newborns included in the study, 1,062 (76.1%) had cultures performed, and resistance analysis was restricted to those with confirmed microbial growth. Among culture-positive isolates, resistance to fluoroquinolones was the most frequently observed, particularly ciprofloxacin and levofloxacin, followed by trimethoprim/sulfamethoxazole. Regarding other antimicrobial classes, resistance to gentamicin and oxacillin – the latter indicating *methicillin-resistant Staphylococcus aureus* – was also common. Macrolide resistance, including erythromycin and azithromycin, was less frequent. Among β -lactams, resistance was detected to ampicillin, amoxicillin/clavulanic acid, ampicillin/sulbactam, and cephalosporins such as cefepime, ceftriaxone, and ceftazidime, though at relatively low rates. Resistance to broad-spectrum or last-line agents such as piperacillin/tazobactam, imipenem, and vancomycin was rare, suggesting a limited presence of multidrug-resistant strains. Overall, the findings indicate that first-line and intermediate-spectrum antimicrobials accounted for most resistance patterns observed among culture-confirmed neonatal sepsis cases, while resistance to carbapenems and glycopeptides remained uncommon.

Antimicrobial therapy was administered to 988 newborns (70.8%), while 408 (29.2%) did not receive any antimicrobial treatment. Empirical antimicrobial therapy most commonly included ampicillin (64.3%; 84.5%) and gentamicin (64.0%; 84.1%), followed by vancomycin (16.3%; 21.4%), cefepime (12.3%; 16.2%), and oxacillin (6.1%; 8.0%) (**Table 2; Figure 3**). Broader-spectrum or targeted regimens, including piperacillin/tazobactam, amikacin, meropenem, and metronidazole, were used less frequently, while fluconazole was administered in 2.2% of all newborns (3.0% among those with cultures) (**Table 2; Figure 3**).

Table 2. Distribution of neonatal sepsis cases by diagnostic hypothesis, collected specimens, isolated microorganisms, antimicrobial resistance profile, and treatment administered

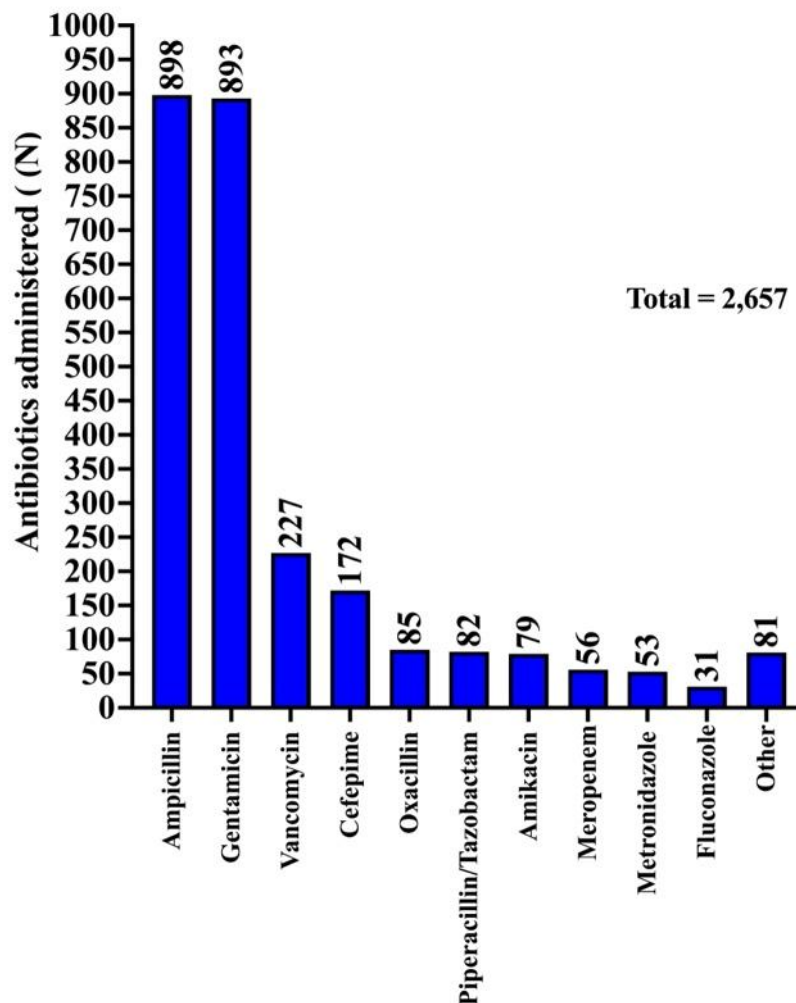
Markers	Category	Percentage relative to the total study population (n = 1,396)	Percentage relative to population with cultures (n = 1,062)
Sepsis cases by diagnostic hypothesis	Early-onset sepsis with positive culture	36 (2.6%)	36 (3.4%)
	Presumed early-onset sepsis	853 (61.1%)	853 (80.3%)
	Late-onset sepsis with positive culture	96 (6.9%)	96 (9.0%)
	Presumed late-onset sepsis	191 (13.7%)	191 (18.0%)
	Confirmed fungal sepsis	7 (0.5%)	7 (0.6%)
	Presumed fungal sepsis	28 (2.0%)	28 (2.7%)
≥ 2 culture collections	Yes	821 (58.8%)	821 (77.3%)
	No	241 (17.3%)	241 (22.7%)
Specimen type	Blood	1,411 (101.1%)	1,411 (132.9%)
	Skin swab	222 (15.9%)	222 (20.9%)
	Urine	145 (10.4%)	145 (13.6%)
	Cerebrospinal fluid	61 (4.4%)	61 (5.7%)
	Central venous catheter tip	44 (3.1%)	44 (4.1%)
Isolated bacteria/fungi	Isolated	137 (9.8%)	137 (12.9%)
	Not isolated	1,259 (90.2%)	1,259 (118.5%)
Type of microorganism	Gram-positive	81 (5.8%)	81 (7.6%)
	Gram-negative	49 (3.5%)	49 (4.6%)
	Fungi	7 (0.5%)	7 (0.6%)
Gram-positive bacteria	Coagulase-negative <i>Staphylococcus</i>	50 (3.6%)	50 (4.7%)
	<i>Staphylococcus aureus</i>	26 (1.9%)	26 (2.4%)
	<i>Enterococcus</i> spp.	2 (0.1%)	2 (0.2%)
	<i>Streptococcus pneumoniae</i>	2 (0.1%)	2 (0.2%)
	<i>Streptococcus agalactiae</i> (Group B)	1 (0.01%)	1 (0.1%)
Gram-negative bacteria	<i>Klebsiella pneumoniae</i>	16 (1.2%)	16 (1.5%)
	<i>Klebsiella oxytoca</i>	2 (0.1%)	2 (0.2%)
	<i>Escherichia coli</i>	17 (1.2%)	17 (1.6%)
	<i>Enterobacter cloacae</i>	3 (0.2%)	3 (0.3%)
	<i>Enterobacter agglomerans</i>	3 (0.2%)	3 (0.3%)
	<i>Proteus mirabilis</i>	2 (0.1%)	2 (0.2%)
	<i>Serratia liquefaciens</i>	2 (0.1%)	2 (0.2%)
	<i>Pseudomonas aeruginosa</i>	2 (0.1%)	2 (0.2%)
	<i>Morganella morganii</i>	1 (0.01%)	1 (0.1%)
	<i>Stenotrophomonas maltophilia</i>	1 (0.01%)	1 (0.1%)
Fungi	<i>Candida</i> spp.	5 (0.4%)	5 (0.5%)
	<i>Candida albicans</i>	2 (0.1%)	2 (0.2%)
Antibiotics administered	Ampicillin	898 (64.3%)	898 (84.5%)
	Gentamicin	893 (64.0%)	893 (84.1%)
	Vancomycin	227 (16.3%)	227 (21.4%)
	Cefepime	172 (12.3%)	172 (16.2%)
	Oxacillin	85 (6.1%)	85 (8.0%)
	Piperacillin/Tazobactam	82 (5.9%)	82 (7.7%)
	Amikacin	79 (5.6%)	79 (7.4%)
	Meropenem	56 (4.0%)	56 (5.3%)
	Metronidazole	53 (3.8%)	53 (5.0%)
	Fluconazole	31 (2.2%)	31 (3.0%)
	Other	81 (5.8%)	81 (7.6%)

Data are presented as absolute (N) and relative (%) frequencies. The total may exceed N = 1,396 or 1,062, as a single newborn could have had more than one specimen type collected.

Figure 2. Characterization of neonatal sepsis cases and distribution of isolated microorganisms.

a) Distribution of 1,211 neonatal sepsis cases according to clinical classification and culture confirmation. Presumed early-onset sepsis accounted for the majority (853 cases), followed by presumed late-onset sepsis (191 cases). Confirmed cases represented smaller proportions (36 early-onset and 96 late-onset), while fungal sepsis (confirmed and presumed) totaled 35 episodes. (b) Classification of 137 microorganisms isolated from positive cultures, showing predominance of Gram-positive bacteria (81; 59.1%), followed by Gram-negative bacteria (49; 35.8%) and fungi (7; 5.1%). (c) Distribution of Gram-positive isolates, highlighting *Staphylococcus* spp. (coagulase-negative, 50; *S. aureus*, 26) as the most frequent pathogens, followed by *Enterococcus* spp., *Streptococcus pneumoniae*, and *S. agalactiae* (Group B). (d) Distribution of Gram-negative isolates, mainly *Escherichia coli* (17) and *Klebsiella pneumoniae* (16), with additional species such as *Enterobacter cloacae*, *Proteus mirabilis*, *Serratia liquefaciens*, and *Pseudomonas aeruginosa*. (e) Distribution of fungal isolates, predominantly *Candida* spp. (5) and *Candida albicans* (2). These findings demonstrate the predominance of Gram-positive cocci in neonatal sepsis, particularly in late-onset cases, and the emergence of multidrug-resistant Gram-negative bacilli as relevant pathogens in neonatal intensive care units. The data are presented as absolute (N) frequencies.

Figure 3. Frequency of antimicrobial and antifungals agents administered to neonates with suspected or confirmed sepsis.



Bar chart showing the total of 2,657 antimicrobial administrations during the study period (2017–2024). The most frequently used agents were ampicillin (898; 33.8%) and gentamicin (893; 33.6%), followed by vancomycin (227; 8.5%) and cefepime (172; 6.5%). Broad-spectrum antibiotics such as oxacillin, piperacillin/tazobactam, amikacin, meropenem, and metronidazole were used less frequently (< 4% each). The antifungal fluconazole was administered in 31 cases (1.2%). These data reflect the empirical use of ampicillin and gentamicin as first-line therapy for neonatal sepsis, in accordance with institutional protocols and international guidelines, with subsequent adjustments based on microbiological results and resistance profiles.

The data are presented as absolute (N) and relative (%) frequencies.

3.3. Antimicrobial Susceptibility Patterns of Isolated Microorganisms

A total of 137 microorganisms were isolated from the infectious episodes identified among newborns admitted to the NICU (**Supplementary Table 3**). In general, Gram-positive bacteria showed higher sensitivity to β -lactam antibiotics and glycopeptides. *Staphylococcus coagulase-negative* isolates frequently exhibited resistance to macrolides (erythromycin, clindamycin) and fluoroquinolones (ciprofloxacin, levofloxacin), while maintaining susceptibility to vancomycin. *Staphylococcus aureus* isolates demonstrated similar behavior, with low resistance rates to oxacillin, cephalosporins, and aminoglycosides, and preserved sensitivity to glycopeptides.

Among Gram-negative organisms, *K. pneumoniae* and *E. coli* isolates displayed variable resistance profiles. Resistance to third- and fourth-generation cephalosporins (such as ceftazidime, ceftriaxone, and cefepime) and to β -lactam/ β -lactamase inhibitor combinations (ampicillin/sulbactam and piperacillin/tazobactam) was frequently observed. In contrast, susceptibility to carbapenems, particularly imipenem, remained high. *Enterobacter* species showed similar

resistance behavior, especially toward penicillins and early-generation cephalosporins. *P. aeruginosa* and *Stenotrophomonas maltophilia* isolates were rare but presented resistance to multiple antimicrobial classes, reinforcing their potential clinical relevance in nosocomial infections.

When evaluating clinical outcomes, most infections resulted in patient discharge after clinical improvement and completion of targeted antimicrobial therapy. However, infections caused by multidrug-resistant Gram-negative bacteria, especially *K. pneumoniae* and *E. coli*, were more frequently associated with higher mortality.

These findings emphasize the predominance of Gram-positive organisms in neonatal intensive care infections, the emerging threat of multidrug-resistant Gram-negative pathogens, and the importance of ongoing microbiological surveillance to guide empirical therapy and infection control strategies in the NICU setting.

3.4. Factors Associated with the Clinical Outcomes of Newborns with Neonatal Sepsis

Supplementary Table 1 shows the distribution of NICU in a university hospital between 2017 and 2024. There were no statistically significant differences in outcomes (death or hospital discharge) among the NICUs ($p = 0.108$).

Table 3 and **Figure 4** present the clinical, microbiological, and therapeutic characteristics of the newborns according to clinical outcome (hospital discharge or death). Among the 1,396 neonates included in the analysis, 212 (15.2%) died during hospitalization, while 1,184 (84.8%) were discharged alive.

Mortality varied substantially across demographic and perinatal characteristics. Newborns admitted on the first day of life had a mortality rate of 16.8%, corresponding to an OR of 2.23 (95% CI = 1.40 to 3.55) compared with those admitted after the first day (8.3%). Gestational age showed a strong and graded association with death: mortality reached 52.2% among post-term infants (OR = 13.89, 95% CI = 5.71 to 33.81) and 51.2% among extremely preterm infants (OR = 13.39, 95% CI = 8.39 to 21.35), compared with 7.3% among term newborns. Similarly, very preterm infants had an OR of 2.57 (95% CI = 1.65 to 4.00) for death, while moderate/late preterm infants showed a modest, nonsignificant difference (5.7%; OR = 0.76, 95% CI = 0.44 to 1.34).

Birth weight demonstrated a clear dose–response relationship with mortality. The death rate increased from 7.5% among newborns with appropriate weight to 13.6% in very low birth weight infants (OR = 1.94, 95% CI = 1.19 to 3.18) and peaked at 60.7% among extremely low birth weight infants (OR = 19.04, 95% CI = 11.79 to 30.76).

The severity of neonatal depression at birth also correlated strongly with mortality. Infants with moderate Apgar scores at 1 minute had a mortality of 19.0% (OR = 3.43, 95% CI = 2.36 to 4.98), and those with severe scores reached 39.9% (OR = 9.71, 95% CI = 6.14 to 15.34), compared with 6.4% among newborns with good scores. At 5 minutes, mortality increased progressively from 11.6% (good) to 29.0% (moderate; OR = 3.13, 95% CI = 2.21 to 4.43) and 48.6% (severe; OR = 7.24, 95% CI = 3.71 to 14.14).

Regarding obstetric variables, vaginal delivery was associated with a mortality of 19.3%, higher than 13.0% for cesarean births, yielding an OR of 1.59 (95% CI = 1.18 to 2.14). Likewise, maternal antibiotic use prior to delivery was associated with increased neonatal mortality (19.3% vs. 14.3%; OR = 1.44, 95% CI = 1.01 to 2.04).

In neonatal diagnostic hypotheses, deaths were more frequent among newborns with respiratory disorders (16.5%; OR = 4.02, 95% CI = 1.86 to 8.72), prematurity (17.7%; OR = 2.22, 95% CI = 1.51 to 3.25), and congenital heart defects (38.9%; OR = 3.83, 95% CI = 2.17 to 6.77). Other diagnoses such as hypoglycemia, perinatal asphyxia, and seizures did not demonstrate strong associations.

The need for respiratory support and invasive procedures markedly influenced outcomes. Mortality was 7.9% among those receiving supplemental oxygen (OR = 0.14, 95% CI = 0.10 to 0.19), 9.4% with continuous positive airway pressure (OR = 0.30, 95% CI = 0.22 to 0.40), and 38.8% among neonates requiring mechanical ventilation, corresponding to an extremely high OR of 60.90 (95% CI = 30.86 to 120.19). The use of umbilical catheters was also associated with

higher mortality (17.5%; OR = 2.99, 95% CI = 1.85 to 4.83), as were surgical procedures (38.0%; OR = 3.66, 95% CI = 2.03 to 6.61), phlebotomy (46.2%; OR = 4.90, 95% CI = 1.63 to 14.72), and tracheostomy (55.6%; OR = 7.13, 95% CI = 1.90 to 26.76).

Severe complications during hospitalization were also strongly predictive of death. Mortality was 42.6% in neonates with pulmonary hypertension (OR = 4.59, 95% CI = 2.70 to 7.80), 43.1% in those with septic shock (OR = 4.61, 95% CI = 2.60 to 8.20), and 28.6% in cases of necrotizing enterocolitis (OR = 2.31, 95% CI = 1.16 to 4.58). Conversely, bronchopulmonary dysplasia appeared to be protective (6.7%; OR = 0.38, 95% CI = 0.15 to 0.96), reflecting the predominance of survivors in prolonged ventilatory support groups.

Length of hospital stay also showed a strong association with survival. Newborns hospitalized for up to seven days had a mortality of 31.8%, compared with 10.5% for stays of 8–21 days (OR = 0.25, 95% CI = 0.18 to 0.35) and 6.0% for stays longer than 21 days (OR = 0.14, 95% CI = 0.09 to 0.21).

Sepsis-related variables were among the most significant predictors of death (**Figures 3a, b, and c**). Infants with early-onset sepsis with positive culture had a mortality of 27.8% (OR = 2.21, 95% CI = 1.05 to 4.64), while those with presumed early-onset sepsis reached 17.9% (OR = 1.79, 95% CI = 1.30 to 2.47). Late-onset sepsis was also associated with increased mortality (24.4%; OR = 2.20, 95% CI = 1.59 to 3.03), particularly when presumed (26.7%; OR = 2.36, 95% CI = 1.65 to 3.39). Sepsis caused by bacteria presented a mortality of 18.3% (OR = 2.44, 95% CI = 1.68 to 3.55), whereas fungal sepsis, although less frequent, had a proportionally higher fatality (42.9%; OR = 4.43, 95% CI = 2.23 to 8.80).

Among the therapeutic variables, the use of broad-spectrum antibiotics and antifungal agents was strongly associated with fatal outcomes. The mortality rate was 18.2% for ampicillin (OR = 2.03, 95% CI = 1.45 to 2.86), 18.3% for gentamicin (OR = 2.07, 95% CI = 1.47 to 2.91), 25.6% for vancomycin (OR = 2.26, 95% CI = 1.61 to 3.19), 29.1% for cefepime (OR = 2.69, 95% CI = 1.86 to 3.88), 28.6% for meropenem (OR = 2.34, 95% CI = 1.28 to 4.25), and 34.0% for metronidazole (OR = 3.05, 95% CI = 1.69 to 5.49). The highest mortality was observed among neonates who received fluconazole (48.4%; OR = 5.56, 95% CI = 2.70 to 11.42), suggesting that antifungal therapy often reflected severe or refractory infections.

Further analyses detailing clinical and therapeutic variables among neonates with confirmed or presumed sepsis are available in **Supplementary Table 4**, which provides complementary data to those summarized in **Table 3** and reinforces the associations between infection type, antimicrobial therapy, and mortality.

Table 3. Clinical, microbiological, and therapeutic characteristics of newborns according to clinical outcome (hospital discharge or death).

Markers	Category	Death [N (%)]	Hospital discharge [N (%)]	Total	P	OR (95% CI)
Demographic data						
Type of healthcare coverage	Public	182 (15.2%)	1013 (84.8%)	1,195	0.911	1.024 (0.674 to 1.557)
	Insurance and private	30 (14.9%)	171 (85.1%)	201		Reference
Coronavirus disease 2019 (COVID-19) pandemic	Pre-pandemic (2017–2019)	68 (14.3%)	406 (85.7%)	474	0.160	Reference
	Pandemic (2020–2021)	59 (18.0%)	268 (82.0%)	327		1.314 (0.898 to 1.925)
	Post-pandemic (2022–2024)	85 (14.3%)	510 (85.7%)	595		0.995 (0.705 to 1.404)
Age in days at admission	First day of life	190 (16.8%)	941 (83.2%)	1,131	0.001	2.230 (1.403 to 3.545)
	After the first day of life	22 (8.3%)	243 (91.7%)	265		Reference
Sex	Female	99 (16.3%)	507 (83.7%)	606	0.249	1.189 (0.886 to 1.596)
	Male	111 (14.1%)	676 (85.9%)	787		Reference
	Ambiguous	2 (66.7%)	1 (33.3%)	3		Not used
Gestational age	Post-term	12 (52.2%)	11 (47.8%)	23	< 0.001	13.893 (5.708 to 33.814)
	Term	34 (7.3%)	433 (92.7%)	467		Reference
	Moderate/late preterm	21 (5.7%)	350 (94.3%)	371	0.348	0.764 (0.436 to 1.340)
	Very preterm	63 (16.8%)	312 (83.2%)	375	< 0.001	2.572 (1.653 to 4.000)
	Extremely preterm	82 (51.2%)	78 (48.8%)	160	< 0.001	13.388 (8.394 to 21.354)
Birth weight	Macrosomic	1 (3.7%)	26 (96.3%)	27	0.471	0.474 (0.062 to 3.611)
	Appropriate	31 (7.5%)	382 (92.5%)	413		Reference
	Low	37 (7.6%)	450 (92.4%)	487	0.959	1.013 (0.617 to 1.664)
	Very low	41 (13.6%)	260 (86.4%)	301	0.008	1.943 (1.188 to 3.179)
	Extremely low	102 (60.7%)	66 (39.3%)	168	< 0.001	19.044 (11.791 to 30.759)
Apgar scores at 1 minute	Good	42 (6.4%)	615 (93.6%)	657		Reference
	Moderate	113 (19.0%)	483 (81.0%)	596	< 0.001	3.426 (2.358 to 4.978)
	Severe	57 (39.9%)	86 (60.1%)	143	< 0.001	9.705 (6.139 to 15.343)
Apgar scores at 5 minutes	Good	133 (11.6%)	1,016 (88.4%)	1,149		Reference
	Moderate	61 (29.0%)	149 (71.0%)	210	< 0.001	3.127 (2.207 to 4.431)
	Severe	18 (48.6%)	19 (51.4%)	37	< 0.001	7.237 (3.705 to 14.136)
Obstetric data						
Type of delivery	Cesarean	119 (13.0%)	794 (87.0%)	913	0.002	Reference
	Vaginal (normal)	93 (19.3%)	390 (80.7%)	483		1.591 (1.182 to 2.142)
Maternal antibiotic use before delivery	Yes	49 (19.3%)	205 (80.7%)	254	0.045	1.436 (1.009 to 2.043)
	No	163 (14.3%)	979 (85.7%)	1,142		Reference
Group B <i>Streptococcus</i> screening results	Positive	3 (12.0%)	22 (88.0%)	25	0.907	0.926 (0.253 to 3.393)
	Negative	19 (12.8%)	129 (87.2%)	148		Reference
	Unknown	190 (15.5%)	1,033 (84.5%)	1,223		Not used
Amniotic membrane integrity	Intact	152 (15.2%)	849 (84.8%)	1,001	0.998	Reference
	Ruptured	60 (15.2%)	335 (84.8%)	395		1.000 (0.723 to 1.384)

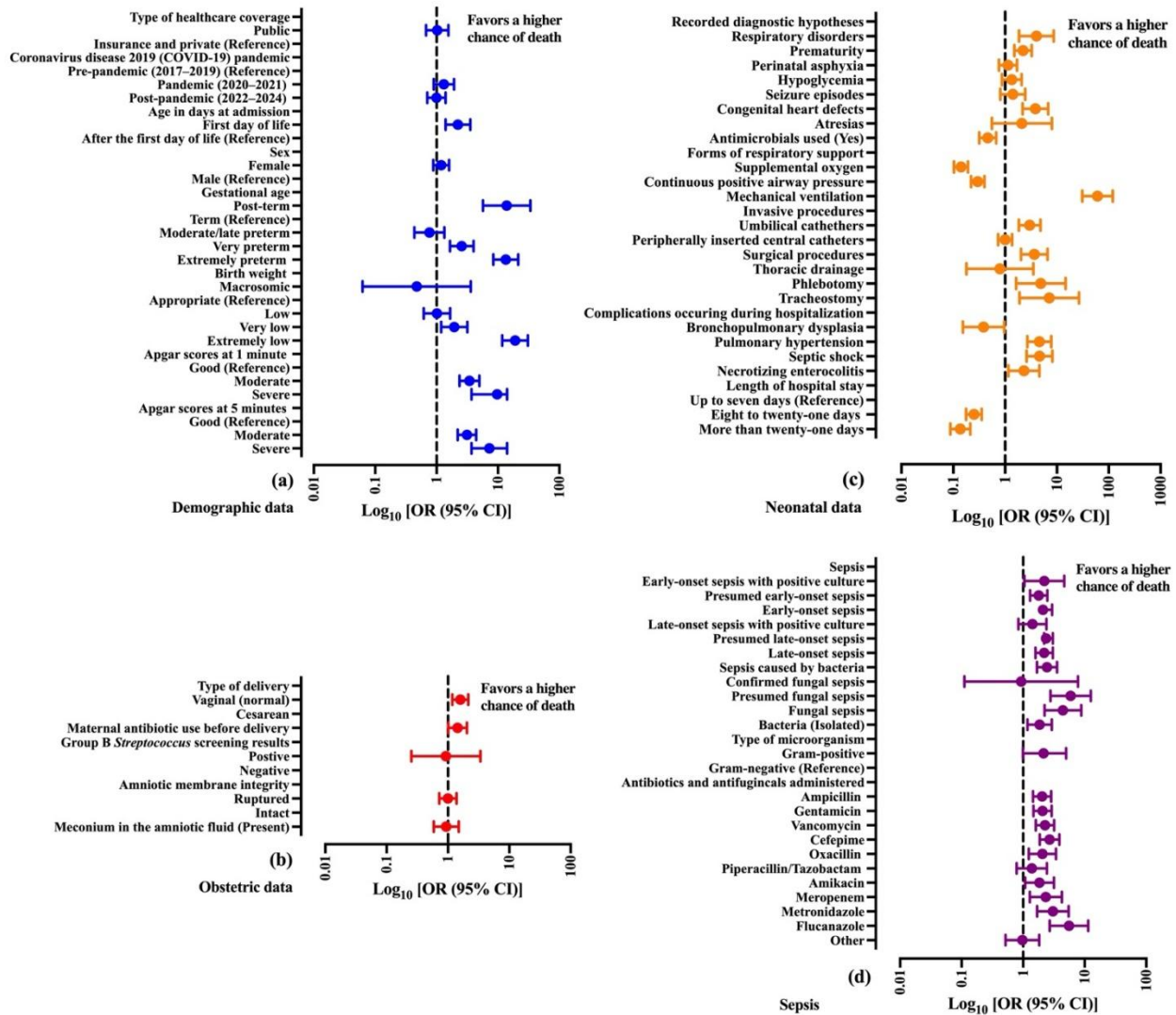
Table 3. Clinical, microbiological, and therapeutic characteristics of newborns according to clinical outcome (hospital discharge or death).

Markers	Category	Death [N (%)]	Hospital discharge [N (%)]	Total	P	OR (95% CI)
Meconium in the amniotic fluid	Yes	23 (14.5%)	136 (85.5%)	159	0.788	0.938 (0.587 to 1.498)
	No	189 (15.3%)	1,048 (84.7%)	1,237		Reference
Neonatal data						
Recorded diagnostic hypotheses	Respiratory disorders	205 (16.5%)	1,041 (83.5%)	1,246	< 0.001	4.023 (1.856 to 8.718)
	Prematurity	177 (17.7%)	823 (82.3%)	1,000	< 0.001	2.218 (1.512 to 3.254)
	Perinatal asphyxia	34 (16.7%)	170 (83.3%)	204	0.524	1.139 (0.763 to 1.702)
	Hypoglycemia	28 (18.9%)	120 (81.1%)	148	0.182	1.349 (0.869 to 2.095)
	Seizure episodes	17 (1.8%)	69 (80.2%)	86	0.224	1.409 (0.811 to 2.447)
	Congenital heart defects	21 (38.9%)	33 (61.1%)	54	< 0.001	3.835 (2.173 to 6.768)
	Jaundice	0 (0.0%)	35 (100.0%)	35	NA	NA
	Atresias	3 (27.3%)	8 (72.7%)	11	0.273	2.110 (0.555 to 8.018)
Antimicrobials used	Yes	175 (17.7%)	813 (82.3%)	988	< 0.001	0.463 (0.318 to 0.674)
	No	37 (9.1%)	371 (90.9%)	408		Reference
Forms of respiratory support	Supplemental oxygen	84 (7.9%)	975 (92.1%)	1,059	< 0.001	0.141 (0.103 to 0.192)
	Continuous positive airway pressure	86 (9.4%)	825 (90.6%)	911	< 0.001	0.297 (0.220 to 0.401)
	Mechanical ventilation	203 (38.8%)	320 (61.2%)	523	< 0.001	60.900 (30.857 to 120.192)
Invasive procedures	Umbilical catheters	192 (17.5%)	903 (82.5%)	1,095	< 0.001	2.987 (1.849 to 4.826)
	Peripherally inserted central catheters	73 (15.2%)	407 (84.8%)	480	0.987	1.003 (0.737 to 1.364)
	Surgical procedures	19 (38.0%)	31 (62.0%)	50	< 0.001	3.662 (2.028 to 6.612)
	Thoracic drainage	2 (12.5%)	14 (87.5%)	16	0.764	0.796 (0.180 to 3.528)
	Phlebotomy	6 (46.2%)	7 (53.8%)	13	0.005	4.897 (1.629 to 14.719)
	Tracheostomy	5 (55.6%)	4 (44.4%)	9	0.004	7.126 (1.898 to 26.756)
Complications occurring during hospitalization	Bronchopulmonary dysplasia	5 (6.7%)	70 (93.3%)	75	0.042	0.384 (0.153 to 0.964)
	Pulmonary hypertension	26 (42.6%)	35 (57.4%)	61	< 0.001	4.589 (2.700 to 7.801)
	Septic shock	22 (43.1%)	29 (56.9%)	51	< 0.001	4.612 (2.595 to 8.195)
	Necrotizing enterocolitis	12 (28.6%)	30 (71.4%)	42	0.017	2.308 (1.162 to 4.583)
	Meningitis	0 (0.0%)	11 (100.0%)	11	NA	NA
Length of hospital stay	Up to seven days	128 (31.8%)	274 (68.2%)	402		Reference
	Eight to twenty-one days	57 (10.5%)	487 (89.5%)	544	< 0.001	0.251 (0.177 to 0.354)
	More than twenty-one days	27 (6.0%)	423 (94.0%)	450	< 0.001	0.137 (0.088 to 0.213)
Sepsis						
Early-onset sepsis with positive culture	Yes	10 (27.8%)	26 (72.2%)	36	0.037	2.205 (1.047 to 4.642)
	No	202 (14.9%)	1,158 (85.1%)	1,360		Reference
Presumed early-onset sepsis	Yes	153 (17.9%)	700 (82.1%)	853	< 0.001	1.793 (1.299 to 2.474)
	No	59 (10.9%)	484 (89.1%)	543		Reference
Early-onset sepsis	Yes	163 (18.3%)	726 (81.7%)	889	< 0.001	2.099 (1.494 to 2.949)
	No	49 (9.7%)	458 (90.3%)	507		Reference

Table 3. Clinical, microbiological, and therapeutic characteristics of newborns according to clinical outcome (hospital discharge or death).

Markers	Category	Death [N (%)]	Hospital discharge [N (%)]	Total	P	OR (95% CI)
Late-onset sepsis with positive culture	Yes	19 (19.8%)	77 (80.2%)	96	0.195	1.415 (0.837 to 2.392)
	No	193 (14.8%)	1,107 (85.2%)	1,300		Reference
Presumed late-onset sepsis	Yes	51 (26.7%)	140 (73.3%)	191	< 0.001	2.362 (1.646 to 3.389)
	No	161 (13.4%)	1,044 (86.6%)	1,205		Reference
Late-onset sepsis	Yes	70 (24.4%)	217 (75.6%)	287	< 0.001	2.197 (1.592 to 3.031)
	No	142 (12.8%)	967 (87.2%)	1,109		Reference
Sepsis caused by bacteria	Yes	175 (18.3%)	781 (81.7%)	956	< 0.001	2.441 (1.678 to 3.549)
	No	37 (8.4%)	403 (91.6%)	440		Reference
Confirmed fungal sepsis	Yes	1 (14.3%)	6 (85.7%)	7	0.947	0.930 (0.111 to 7.768)
	No	211 (15.2%)	1,178 (84.8%)	1,389		Reference
Presumed fungal sepsis	Yes	14 (50.0%)	14 (50.0%)	28	< 0.001	5.909 (2.775 to 12.584)
	No	198 (14.5%)	1,170 (85.5%)	1,368		Reference
Fungal sepsis	Yes	15 (42.9%)	20 (57.1%)	35	< 0.001	4.431 (2.231 to 8.802)
	No	197 (14.5%)	1,164 (85.5%)	1,361		Reference
Bacteria	Isolated	30 (22.7%)	100 (77.3%)	130	0.008	1.854 (1.177 to 2.919)
	Not isolated	127 (15.6%)	809 (84.4%)	936		Reference
Type of microorganism	Gram-positive	15 (18.5%)	66 (81.5%)	81	0.076	2.147 (0.922 to 4.998)
	Gram-negative	15 (30.6%)	34 (69.4%)	49		Reference
Antibiotics and antifungals administered	Ampicillin	163 (18.2%)	735 (81.8%)	898	< 0.001	2.032 (1.446 to 2.856)
	Gentamicin	163 (18.3%)	730 (81.7%)	893	< 0.001	2.069 (1.472 to 2.907)
	Vancomycin	58 (25.6%)	169 (74.4%)	227	< 0.001	2.262 (1.605 to 3.188)
	Cefepime	50 (29.1%)	122 (70.9%)	172	< 0.001	2.687 (1.859 to 3.883)
	Oxacillin	22 (25.9%)	63 (74.1%)	85	0.005	2.060 (1.238 to 3.428)
	Piperacillin/Tazobactam	16 (19.5%)	66 (80.5%)	82	0.262	1.383 (0.784 to 2.438)
	Amikacin	19 (24.1%)	60 (75.9%)	79	0.026	1.844 (1.077 to 3.159)
	Meropenem	16 (28.6%)	40 (71.4%)	56	0.006	2.335 (1.282 to 4.251)
	Metronidazole	18 (34.0%)	35 (66.0%)	53	< 0.001	3.046 (1.691 to 5.487)
	Fluconazole	15 (48.4%)	16 (51.6%)	31	< 0.001	5.558 (2.704 to 11.424)
	Other	12 (14.8%)	69 (85.2%)	81	0.970	0.970 (0.516 to 1.823)

Data are presented as absolute (N) and relative (%) frequencies. NA (not applicable).

Figure 4. Demographic, obstetric, and neonatal factors associated with neonatal mortality.

Panel of four forest plots representing odds ratios (OR) and 95% confidence intervals (CI) for variables associated with neonatal death. (a) Demographic data: mortality was significantly associated with prematurity, birth weight, and severe Apgar scores. (b) Obstetric data: absence of maternal antibiotic use before delivery showed a tendency toward increased mortality risk. (c) Neonatal data: mechanical ventilation, use of antimicrobials, umbilical catheterization, and complications such as septic shock and bronchopulmonary dysplasia were strongly associated with death. (d) Microbiological and therapeutic data: sepsis caused by Gram-negative bacteria and exposure, for example, to gentamicin, cefepime, oxacillin, and fluconazole were significantly associated with higher mortality. These results indicate that severe clinical conditions and specific antimicrobial exposures are key determinants of adverse outcomes among neonates admitted to intensive care. The association between independent variables and the clinical outcome (death) was assessed using binary logistic regression. For all analyses, the significance level was set at $\alpha = 0.05$.

3.5. Multivariable Models Identifying Antibiotic Classes and Sepsis Subtypes Associated with Neonatal Mortality

Table 4 and **Figure 5** present the final multivariable logistic regression model assessing the relationship between antimicrobial use, sepsis subtype, and mortality among neonates in the intensive care unit. The model demonstrated excellent discriminative ability (AUC = 0.976; SE = 0.005; 95% CI = 0.966 to 0.987), indicating high predictive accuracy.

Across 12 backward stepwise iterations, five antimicrobial agents remained consistently associated with mortality in the final model. Gentamicin use showed a significant and independent association with death (OR = 1.90; 95% CI = 1.34 to 2.69), suggesting that treatment with this aminoglycoside was more frequent among neonates with

severe clinical conditions. Similarly, cefepime demonstrated a significant association (OR = 1.91; 95% CI = 1.28 to 2.85), as did fluconazole, with a twofold increase in the odds of death (OR = 2.50; 95% CI = 1.13 to 5.56).

Although oxacillin (OR = 1.74; 95% CI = 1.01 to 2.98) reached the significance threshold, its wide confidence interval suggests variability in outcomes depending on infection etiology. Metronidazole, while not statistically significant, showed a trend toward higher mortality (OR = 1.78; 95% CI = 0.93 to 3.40). The clinical diagnosis of early-onset sepsis (confirmed or presumed) did not retain significance in the final model (OR = 1.64; 95% CI = 0.74 to 3.61), suggesting that mortality was more closely linked to infection severity and therapeutic exposure than to the timing of infection onset.

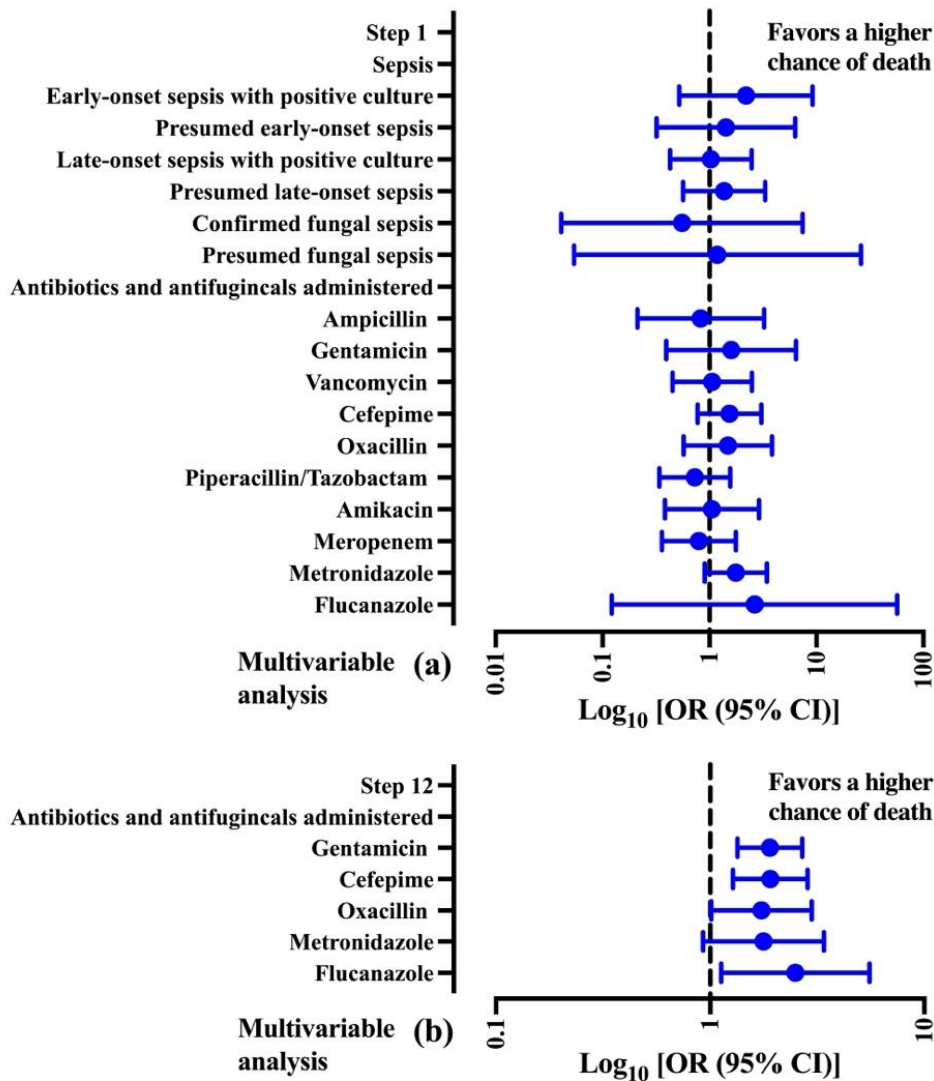
Table 4. Multivariable analysis to identify the main antibiotics and antifungals and types of sepsis associated with mortality among Neonatal Intensive Care Unit patients of a university hospital.

Steps	Markers	B	SE	Wald	df	P	OR (95% CI)
Step 1	Ampicillin	-0.188	0.697	0.073	1	0.788	0.829 (0.212 to 3.247)
	Gentamicin	0.468	0.713	0.431	1	0.511	1.597 (0.395 to 6.459)
	Amikacin	0.054	0.517	0.011	1	0.918	1.055 (0.383 to 2.909)
	Cefepime	0.432	0.349	1.528	1	0.216	1.540 (0.776 to 3.056)
	Vancomycin	0.060	0.436	0.019	1	0.891	1.061 (0.452 to 2.493)
	Meropenem	-0.228	0.406	0.314	1	0.575	0.796 (0.359 to 1.765)
	Piperacillin/Tazobactam	-0.315	0.390	0.651	1	0.420	0.730 (0.340 to 1.568)
	Metronidazole	0.570	0.342	2.785	1	0.095	1.769 (0.905 to 3.457)
	Oxacillin	0.397	0.486	0.667	1	0.414	1.488 (0.574 to 3.858)
	Fluconazole	0.972	1.568	0.385	1	0.535	2.644 (0.122 to 57.114)
	Early-onset sepsis with positive culture	0.785	0.732	1.152	1	0.283	2.193 (0.523 to 9.199)
	Presumed early-onset sepsis	0.354	0.762	0.216	1	0.642	1.424 (0.320 to 6.341)
	Late-onset sepsis with positive culture	0.030	0.448	0.005	1	0.946	1.031 (0.428 to 2.482)
	Presumed late-onset sepsis	0.315	0.450	0.491	1	0.484	1.371 (0.567 to 3.312)
	Confirmed fungal sepsis	-0.588	1.325	0.197	1	0.657	0.555 (0.041 to 7.449)
	Presumed fungal sepsis	0.168	1.578	0.011	1	0.915	1.183 (0.054 to 26.073)
	Constant	-2.379	0.159	222.996	1	< 0.001	0.093
Step 2	Ampicillin	-0.190	0.696	0.075	1	0.785	0.827 (0.212 to 3.233)
	Gentamicin	0.471	0.711	0.439	1	0.508	1.602 (0.397 to 6.458)
	Amikacin	0.065	0.491	0.017	1	0.895	1.067 (0.408 to 2.790)
	Cefepime	0.439	0.334	1.726	1	0.189	1.551 (0.806 to 2.985)
	Vancomycin	0.074	0.382	0.037	1	0.847	1.077 (0.509 to 2.277)
	Meropenem	-0.228	0.406	0.315	1	0.574	0.796 (0.359 to 1.765)
	Piperacillin/Tazobactam	-0.310	0.384	0.652	1	0.419	0.734 (0.346 to 1.556)
	Metronidazole	0.569	0.341	2.780	1	0.095	1.767 (0.905 to 3.451)
	Oxacillin	0.401	0.483	0.690	1	0.406	1.493 (0.580 to 3.846)
	Fluconazole	0.976	1.567	0.388	1	0.533	2.653 (0.123 to 57.208)
	Early-onset sepsis with positive culture	0.782	0.730	1.148	1	0.284	2.187 (0.523 to 9.145)
	Presumed early-onset sepsis	0.354	0.762	0.216	1	0.642	1.424 (0.320 to 6.336)
	Presumed late-onset sepsis	0.293	0.309	0.898	1	0.343	1.341 (0.731 to 2.458)
	Confirmed fungal sepsis	-0.568	1.291	0.193	1	0.660	0.567 (0.045 to 7.113)
	Presumed fungal sepsis	0.165	1.577	0.011	1	0.917	1.179 (0.054 to 25.946)
	Constant	-2.378	0.159	223.583	1	< 0.001	0.093
Step 3	Ampicillin	-0.191	0.695	0.075	1	0.784	0.826 (0.212 to 3.229)
	Gentamicin	0.474	0.711	0.444	1	0.505	1.606 (0.399 to 6.465)
	Amikacin	0.070	0.488	0.021	1	0.886	1.073 (0.413 to 2.790)
	Cefepime	0.440	0.334	1.742	1	0.187	1.553 (0.808 to 2.988)
	Vancomycin	0.071	0.382	0.035	1	0.852	1.074 (0.508 to 2.269)
	Meropenem	-0.232	0.405	0.329	1	0.566	0.793 (0.359 to 1.752)
	Piperacillin/Tazobactam	-0.307	0.383	0.644	1	0.422	0.736 (0.347 to 1.557)
	Metronidazole	0.566	0.340	2.769	1	0.096	1.762 (0.904 to 3.433)
	Oxacillin	0.394	0.478	0.679	1	0.410	1.483 (0.581 to 3.786)
	Fluconazole	1.132	0.464	5.964	1	0.015	3.102 (1.250 to 7.696)
	Early-onset sepsis with positive culture	0.783	0.730	1.150	1	0.283	2.187 (0.523 to 9.145)
	Presumed early-onset sepsis	0.352	0.761	0.214	1	0.643	1.422 (0.320 to 6.324)
	Presumed late-onset sepsis	0.296	0.308	0.924	1	0.336	1.345 (0.735 to 2.459)

	Confirmed fungal sepsis	-0.632	1.153	0.300	1	0.584	0.532 (0.056 to 5.092)
	Constant	-2.378	0.159	223.625	1	< 0.001	0.093
Step 4	Ampicillin	-0.182	0.696	0.069	1	0.793	0.833 (0.213 to 3.257)
	Gentamicin	0.460	0.707	0.423	1	0.516	1.584 (0.396 to 6.332)
	Cefepime	0.437	0.333	1.721	1	0.190	1.548 (0.806 to 2.974)
	Vancomycin	0.072	0.382	0.036	1	0.850	1.075 (0.508 to 2.273)
	Meropenem	-0.236	0.404	0.343	1	0.558	0.789 (0.358 to 1.742)
	Piperacillin/Tazobactam	-0.309	0.383	0.653	1	0.419	0.734 (0.347 to 1.554)
	Metronidazole	0.572	0.338	2.867	1	0.090	1.772 (0.914 to 3.437)
	Oxacillin	0.446	0.309	2.091	1	0.148	1.563 (0.853 to 2.862)
	Fluconazole	1.132	0.464	5.950	1	0.015	3.101 (1.249 to 7.697)
	Early-onset sepsis with positive culture	0.780	0.732	1.135	1	0.287	2.182 (0.519 to 9.168)
	Presumed early-onset sepsis	0.357	0.763	0.219	1	0.640	1.429 (0.320 to 6.379)
	Presumed late-onset sepsis	0.300	0.307	0.958	1	0.328	1.350 (0.740 to 2.465)
	Confirmed fungal sepsis	-0.657	1.142	0.331	1	0.565	0.518 (0.055 to 4.865)
	Constant	-2.377	0.159	224.455	1	< 0.001	0.093
Step 5	Ampicillin	-0.183	0.696	0.069	1	0.792	0.832 (0.213 to 3.260)
	Gentamicin	0.462	0.708	0.425	1	0.514	1.587 (0.396 to 6.356)
	Cefepime	0.476	0.260	3.355	1	0.067	1.610 (0.967 to 2.680)
	Meropenem	-0.218	0.393	0.309	1	0.578	0.804 (0.373 to 1.735)
	Piperacillin/Tazobactam	-0.277	0.343	0.654	1	0.419	0.758 (0.387 to 1.483)
	Metronidazole	0.574	0.338	2.885	1	0.089	1.775 (0.915 to 3.444)
	Oxacillin	0.435	0.303	2.056	1	0.152	1.545 (0.852 to 2.801)
	Fluconazole	1.132	0.464	5.949	1	0.015	3.101 (1.249 to 7.700)
	Early-onset sepsis with positive culture	0.784	0.734	1.142	1	0.285	2.190 (0.520 to 9.224)
	Presumed early-onset sepsis	0.359	0.765	0.220	1	0.639	1.431 (0.320 to 6.404)
	Presumed late-onset sepsis	0.323	0.283	1.306	1	0.253	1.382 (0.794 to 2.405)
	Confirmed fungal sepsis	-0.641	1.138	0.317	1	0.573	0.527 (0.057 to 4.900)
	Constant	-2.377	0.159	224.332	1	< 0.001	0.093
Step 6	Gentamicin	0.387	0.664	0.340	1	0.560	1.473 (0.401 to 5.407)
	Cefepime	0.474	0.260	3.333	1	0.068	1.607 (0.966 to 2.673)
	Meropenem	-0.222	0.392	0.320	1	0.571	0.801 (0.371 to 1.728)
	Piperacillin/Tazobactam	-0.280	0.342	0.670	1	0.413	0.756 (0.386 to 1.478)
	Metronidazole	0.566	0.336	2.833	1	0.092	1.762 (0.911 to 3.406)
	Oxacillin	0.436	0.304	2.062	1	0.151	1.546 (0.853 to 2.803)
	Fluconazole	1.141	0.463	6.080	1	0.014	3.130 (1.264 to 7.754)
	Early-onset sepsis with positive culture	0.701	0.675	1.079	1	0.299	2.015 (0.537 to 7.563)
	Presumed early-onset sepsis	0.255	0.667	0.146	1	0.703	1.290 (0.349 to 4.767)
	Presumed late-onset sepsis	0.322	0.283	1.300	1	0.254	1.380 (0.793 to 2.403)
	Confirmed fungal sepsis	-0.640	1.138	0.317	1	0.574	0.527 (0.057 to 4.905)
	Constant	-2.381	0.158	226.647	1	< 0.001	0.093
Step 7	Gentamicin	0.631	0.179	12.485	1	< 0.001	1.880 (1.325 to 2.668)
	Cefepime	0.479	0.259	3.412	1	0.065	1.615 (0.971 to 2.684)
	Meropenem	-0.220	0.392	0.316	1	0.574	0.802 (0.372 to 1.730)
	Piperacillin/Tazobactam	-0.282	0.342	0.678	1	0.410	0.755 (0.386 to 1.475)
	Metronidazole	0.565	0.337	2.822	1	0.093	1.760 (0.910 to 3.404)
	Oxacillin	0.436	0.303	2.064	1	0.151	1.546 (0.853 to 2.802)
	Fluconazole	1.132	0.461	6.022	1	0.014	3.102 (1.256 to 7.663)
	Early-onset sepsis with positive culture	0.492	0.402	1.498	1	0.221	1.635 (0.744 to 3.594)
	Presumed late-onset sepsis	0.324	0.283	1.311	1	0.252	1.382 (0.794 to 2.405)
	Confirmed fungal sepsis	-0.634	1.138	0.311	1	0.577	0.530 (0.057 to 4.929)
	Constant	-2.376	0.157	227.808	1	< 0.001	0.093
Step 8	Gentamicin	0.627	0.178	12.364	1	< 0.001	1.873 (1.320 to 2.657)
	Cefepime	0.462	0.257	3.220	1	0.073	1.587 (0.958 to 2.628)
	Piperacillin/Tazobactam	-0.293	0.341	0.738	1	0.390	0.746 (0.382 to 1.456)
	Metronidazole	0.562	0.336	2.788	1	0.095	1.753 (0.907 to 3.389)
	Oxacillin	0.418	0.303	1.910	1	0.167	1.519 (0.840 to 2.749)
	Fluconazole	1.027	0.421	5.960	1	0.015	2.794 (1.225 to 6.374)
	Early-onset sepsis with positive culture	0.487	0.403	1.462	1	0.227	1.627 (0.739 to 3.582)
	Presumed late-onset sepsis	0.317	0.282	1.264	1	0.261	1.374 (0.790 to 2.389)
	Confirmed fungal sepsis	-0.676	1.141	0.351	1	0.553	0.509 (0.054 to 4.761)
	Constant	-2.374	0.157	227.881	1	< 0.001	0.093

Step 9	Gentamicin	0.627	0.178	12.346	1	< 0.001	1.872 (1.320 to 2.656)
	Cefepime	0.463	0.257	3.248	1	0.072	1.589 (0.960 to 2.630)
	Piperacillin/Tazobactam	-0.288	0.341	0.714	1	0.398	0.750 (0.385 to 1.462)
	Metronidazole	0.547	0.335	2.668	1	0.102	1.728 (0.896 to 3.331)
	Oxacillin	0.395	0.301	1.729	1	0.188	1.485 (0.824 to 2.676)
	Fluconazole	0.989	0.414	5.694	1	0.017	2.688 (1.193 to 6.055)
	Early-onset sepsis with positive culture	0.494	0.402	1.511	1	0.219	1.639 (0.745 to 3.605)
	Presumed late-onset sepsis	0.332	0.281	1.389	1	0.239	1.393 (0.803 to 2.419)
	Constant	-2.377	0.157	228.391	1	< 0.001	0.093
Step 10	Gentamicin	0.622	0.178	12.158	1	< 0.001	1.862 (1.313 to 2.641)
	Cefepime	0.507	0.248	4.182	1	0.041	1.660 (1.021 to 2.697)
	Metronidazole	0.544	0.334	2.651	1	0.103	1.724 (0.895 to 3.320)
	Oxacillin	0.419	0.296	1.999	1	0.157	1.520 (0.851 to 2.717)
	Fluconazole	0.931	0.408	5.201	1	0.023	2.537 (1.140 to 5.645)
	Early-onset sepsis with positive culture	0.494	0.403	1.507	1	0.220	1.639 (0.745 to 3.608)
	Presumed late-onset sepsis	0.236	0.253	0.870	1	0.351	1.266 (0.771 to 2.078)
	Constant	-2.382	0.157	229.527	1	< 0.001	0.092
Step 11	Gentamicin	0.633	0.178	12.665	1	< 0.001	1.883 (1.329 to 2.668)
	Cefepime	0.634	0.205	9.529	1	0.002	1.885 (1.260 to 2.820)
	Metronidazole	0.553	0.333	2.751	1	0.097	1.738 (0.904 to 3.339)
	Oxacillin	0.514	0.278	3.410	1	0.065	1.672 (0.969 to 2.884)
	Fluconazole	0.945	0.408	5.366	1	0.021	2.573 (1.157 to 5.724)
	Early-onset sepsis with positive culture	0.474	0.402	1.395	1	0.237	1.607 (0.731 to 3.531)
	Constant	-2.377	0.157	228.870	1	< 0.001	0.093
Step 12	Gentamicin	0.643	0.178	13.090	1	< 0.001	1.901 (1.342 to 2.693)
	Cefepime	0.647	0.205	10.001	1	0.002	1.910 (1.279 to 2.853)
	Metronidazole	0.575	0.332	3.003	1	0.083	1.776 (0.928 to 3.402)
	Oxacillin	0.552	0.275	4.033	1	0.045	1.738 (1.013 to 2.979)
	Fluconazole	0.918	0.407	5.091	1	0.024	2.504 (1.128 to 5.559)
	Constant	-2.374	0.157	228.354	1	< 0.001	0.093

The multivariable logistic regression model was performed using the backward stepwise method, with the clinical outcome (hospital discharge versus death) as the dependent variable. Model results were presented as regression coefficients, standard errors (SE), Wald statistics, odds ratios (ORs), and corresponding 95% confidence intervals (CIs). The model demonstrated excellent discriminative ability, with an area under the receiver operating characteristic (ROC) curve (AUC) of 0.976 (SE = 0.005; 95% CI = 0.966 to 0.987; $p < 0.001$). For all analyses, the significance level was set at $\alpha = 0.05$.

Figure 5. Multivariable analysis of factors associated with neonatal mortality.

a) Initial multivariable model: includes sepsis categories (confirmed and presumed) and antimicrobial use. Gentamicin, cefepime, oxacillin, and fluconazole remained independently associated with increased mortality. (b) Final model (Step 12): after adjustment for clinical and microbiological variables, gentamicin, cefepime, oxacillin, metronidazole, and fluconazole persisted as independent predictors of neonatal death. These findings emphasize the importance of continuous microbiological surveillance and antimicrobial stewardship, particularly regarding aminoglycosides and fourth-generation cephalosporins, to minimize adverse outcomes and preventable deaths in neonatal sepsis. Panel of four forest plots representing odds ratios (OR) and 95% confidence intervals (CI) for variables associated with neonatal death. Variables with $p \leq 0.05$ in the bivariate analysis (sepsis and antibiotic/antifungal use) were included in an exploratory multivariable logistic regression model using the Backward Stepwise method, with the clinical outcome (hospital discharge versus death) as the dependent variable. Multicollinearity was assessed using tolerance values (< 0.1) and the variance inflation factor (> 10). For all analyses, the significance level was set at $\alpha = 0.05$.

In summary, the multivariable analysis identified gentamicin, cefepime, oxacillin, and fluconazole as the strongest independent predictors of mortality among neonates with sepsis. **Supplementary Tables 5 to 7** expand these results by analyzing antibiotic–sepsis associations separately by sepsis subtype and infection onset.

Supplementary Table 5 (Early-onset sepsis): The model revealed that ampicillin (OR = 171.2; 95% CI = 50.5 to 580.8) and gentamicin (OR = 377.4; 95% CI = 110.3 to 1,291.4) were the most strongly associated antibiotics with early-

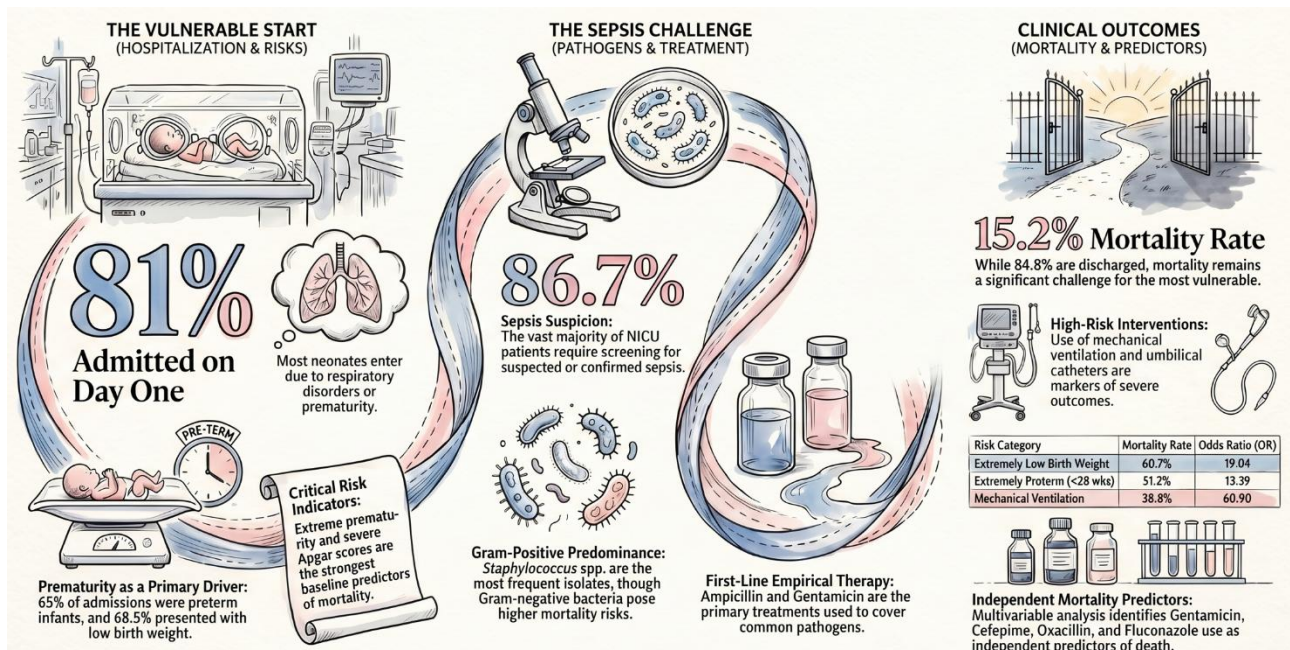
onset sepsis. Both variables remained robust across model iterations (AUC = 0.996; 95% CI = 0.992 to 0.999), indicating a high predictive capacity for early infection identification.

Supplementary Table 6 (Late-onset sepsis): For late-onset infections, the model demonstrated strong associations with vancomycin (OR = 82.9; 95% CI = 32.9 to 209.1), amikacin (OR = 69.8; 95% CI = 18.6 to 261.7), cefepime (OR = 18.2; 95% CI = 6.9 to 47.8), and oxacillin (OR = 16.3; 95% CI = 4.4 to 59.9). Fluconazole also showed a significant association (OR = 13.7; 95% CI = 1.1 to 165.1). The model's discriminative ability remained excellent (AUC = 0.970; 95% CI = 0.956 to 0.984).

Supplementary Table 7 (Combined early and late sepsis): When both sepsis types were analyzed together, the antibiotics most predictive of diagnosis were vancomycin (OR = 2,721.6; 95% CI = 421.6 to 17,568.6), oxacillin (OR = 266.9; 95% CI = 38.2 to 1,866.8), gentamicin (OR = 271.1; 95% CI = 71.8 to 1,023.1), and ampicillin (OR = 132.8; 95% CI = 36.7 to 481.4), with an AUC = 0.997 (95% CI = 0.994 to 1.000), reinforcing the model's high discriminatory power.

Figure 6 summarizes the main hospitalization characteristics, sepsis-related findings, therapeutic approaches, and mortality predictors observed among neonates admitted to the NICU.

Figure 6. Overview of hospitalization characteristics, sepsis burden, and clinical outcomes among neonates admitted to the neonatal intensive care unit (NICU).



The figure summarizes the clinical profile of hospitalized neonates, highlighting the high frequency of admission on the first day of life, the predominance of prematurity and respiratory disorders, the burden of suspected or confirmed sepsis, the predominance of Gram-positive pathogens, and the empirical antimicrobial therapies most frequently used. Additionally, the figure illustrates mortality rates and the main predictors associated with adverse outcomes, including extremely low birth weight, extreme prematurity, and the need for mechanical ventilation. Independent predictors of mortality identified in multivariable analysis are also presented.

Abbreviations: NICU, neonatal intensive care unit; OR, odds ratio; wks, weeks; spp., species.

4. Discussion

The present study analyzed a retrospective cohort of 1,396 newborns admitted to a tertiary-level NICU between 2017 and 2024. Most neonates were admitted during the post-pandemic period and through the public healthcare system. A predominance of male infants was observed. Regarding delivery characteristics, more than half of the births occurred via cesarean section, with a low rate of prophylactic antibiotic administration.

Concerning neonatal characteristics, preterm infants predominated, mainly within the moderate-to-late preterm range. Most newborns had low birth weight and presented respiratory complications. Additionally, a high demand for ventilatory support and antibiotic therapy during hospitalization was identified. Among sepsis cases, early-onset presumed sepsis was the most frequent, followed by late-onset presumed sepsis. Gram-positive bacteria were the most commonly identified pathogens, particularly coagulase-negative *Staphylococcus*. Ampicillin and gentamicin were the most frequently used antibiotics for empirical treatment. Finally, the majority of patients were discharged from the hospital.

These findings are consistent with those described in a 40-year systematic review and meta-analysis conducted across 14 countries, which demonstrated that neonates with lower gestational age and birth weight are more vulnerable to severe complications such as sepsis, prolonged hospitalization, and mortality [27].

4.1. Clinical-epidemiological Profile and Perinatal Factors Associated with Neonatal Vulnerability

Prematurity is defined as birth occurring before 37 completed weeks of gestation and may be spontaneous or medically induced. In addition to being a global public health problem, it represents the leading cause of neonatal morbidity and mortality [28]. According to the World Health Organization (WHO), it is estimated that 1 in every 10 babies worldwide is born preterm, and no significant reduction in these rates has been observed over the past decade [29]. In Brazil, approximately 11.2% of all births are preterm, the majority being male (52.8%), of mixed race (54.2%), with adequate birth weight (>2,500 g), and Apgar scores above 7 at both the first (82.6%) and fifth minutes (93.1%) [30].

In this study, the combined prematurity rate was 65%, consisting of 11.5% extremely preterm (<28 weeks), 26.9% very preterm (28 to <32 weeks), and 26.6% moderate-to-late preterm (32 to <37 weeks). This rate is substantially higher than both the global average of approximately 10% [29] and the national estimate of 11.2% [30]. This discrepancy can be attributed to the specific care profile of the study institution, which serves as a tertiary referral center for high-risk pregnancies and newborns, thereby concentrating more severe cases and a higher likelihood of preterm births. Furthermore, as this is a single-center study based on a hospital sample restricted to neonates admitted to a NICU, a natural overrepresentation of preterm births occurs compared to broader population-based studies.

In this context, studies conducted in high-complexity neonatal units in Brazil have also demonstrated elevated prematurity rates. Barreto et al. (2024), analyzing a cohort of all live births admitted to a tertiary hospital (n = 9,778), reported a 23% preterm birth rate – nearly twice the national average. However, when analyzing only neonates admitted to the NICU (n = 480; 4.9%), this proportion increased to 76.9%, including 41 (11.1%) extremely preterm, 63 (17.1%) very preterm, 92 (24.9%) moderately preterm, and 176 (46.9%) late preterm infants [31]. These findings align with those of the present study, in which the majority of preterm cases were concentrated in the moderate and late preterm groups.

Similarly, low-birth-weight – defined as a birth weight below 2.5 kg regardless of gestational age – is also a major public health concern and a strong predictor of neonatal health and survival [32]. According to global estimates, low birth weight remains alarming: 1 in 7 newborns (or 14.7%) are affected [33]. In the Americas, and particularly in Brazil, the percentage of low birth weight increased from 8.1% in 2002 to 8.7% in 2019 [34].

The overall prevalence of low birth weight in this study was 68.5%, distributed as 12.0% extremely low birth weight, 21.6% very low birth weight, and 34.9% low birth weight. Once again, these values are substantially higher than global averages, even considering regional differences. For instance, South Asia and Sub-Saharan Africa account for approximately 55.5% of all low birth weight cases worldwide, whereas countries in North America, Australia, New Zealand, Central Asia, and Europe have much lower mean rates, averaging 7.2% [32].

International data corroborate the Brazilian scenario. In the United States, the rate of low-birth-weight infants reaches 9.31% [35]. Similarly, Tessema et al. (2021) found an average low birth weight rate of 9.76% across 35 Sub-

Saharan African countries, and Shimes et al. (2022) reported a 13.8% rate in a Guatemalan hospital – higher than that country's national average of 10.9% [36,37].

Although numerous studies have investigated risk factors for low-birth-weight in newborns [38–41], few have aimed to estimate its prevalence among NICU populations. For instance, a study in an Indian tertiary hospital NICU found a 64.63% low birth weight rate among 4,275 admissions [42]. Conversely, Berhane et al. (2019) reported a much lower prevalence of 14.6% in a cohort of 1,486 NICU admissions in Ethiopia [43]. To date, no population-based Brazilian study has estimated the prevalence of low-birth-weight among NICU-admitted neonates.

Both conditions discussed in this section – prematurity and low birth weight – are strongly associated with systemic and infectious complications in newborns. Premature and low-birth-weight infants are more susceptible to respiratory distress syndrome, necrotizing enterocolitis, sepsis, hypotension, and acute kidney injury [28]. Fortunately, mortality and sequelae associated with these conditions have declined over recent decades, owing to advances in perinatal and neonatal care. Between 1990 and 2019, there was a marked decrease in deaths attributable to these two conditions, with global mortality declining from 3.2 million (95% UI: 3.0–3.4) to 1.8 million (95% UI: 1.5–2.1) [44]. Conversely, due to improved survival, individuals born under these conditions are at higher risk of experiencing long-term complications of prematurity and low-birth-weight, including neurological impairment, cerebral palsy, behavioral disorders, short stature, and chronic kidney disease [28,44].

4.2. Neonatal Sepsis: Prevalence, Microbiological Profile, and Antimicrobial Resistance

Neonatal sepsis is a clinical syndrome resulting from the presence of pathogenic microorganisms in normally sterile body fluids during the first month of life [45]. It is a potentially fatal condition and a leading cause of neonatal morbidity and mortality [46]. Although global cases have declined over recent decades [47], the true incidence of neonatal sepsis remains uncertain, as many countries lack adequate surveillance systems. Nonetheless, available data suggest an incidence of approximately 2,824 cases per 100,000 live births [27]. In Brazil, between 2008 and 2018, 47,554 newborns were hospitalized with sepsis, corresponding to 148.04 cases per 100,000 live births [48].

In this study, the overall prevalence of bacterial sepsis was 68.48%, and fungal sepsis accounted for 2.51%, with a low number of culture-positive cases. Most cases were classified as presumed early-onset sepsis and presumed late-onset sepsis. Other NICU-based studies have also reported high sepsis rates. In Uganda, for example, 41.2% of neonates developed sepsis, whereas a study from Nepal reported a lower prevalence of 36.41% [49,50]. In line with the present findings, the majority of cases in these studies were early-onset, followed by late-onset sepsis [50,51].

The high prevalence of sepsis, particularly in presumed cases, is likely associated with the clinical and epidemiological characteristics of newborns admitted to tertiary NICUs. A systematic review and meta-analysis by Traoré et al. (2024) identified several major risk factors for neonatal sepsis, including Apgar score <7, meconium-stained amniotic fluid, prolonged rupture of membranes (>12 h), male sex, intrapartum fever, and a history of maternal urinary tract infection [52]. Consistently, Seyoum et al. (2023) reported that delayed initiation of breastfeeding was also a significant risk factor for neonatal sepsis [53].

Regarding maternal factors, a study from Uganda identified primigravidity, antepartum hemorrhage, multiple vaginal examinations after membrane rupture, a long decision-to-delivery interval, and inter-facility referral as the most significant predictors of early-onset neonatal sepsis [54]. Both maternal and neonatal factors highlight how prenatal conditions and pregnancy complexity directly influence the likelihood of developing sepsis – particularly early-onset sepsis, which occurs within 72 hours of life and is typically caused by vertical transmission of maternal infections during delivery [55]. The main pathogen associated with early-onset neonatal sepsis is *Group B Streptococcus*, which colonizes the female genitourinary and gastrointestinal tracts. In this study, 87.6% of *Group B Streptococcus* results were unknown,

suggesting possible underreporting and underscoring the pathogen's importance in vertical transmission leading to early-onset neonatal sepsis.

The microbiological profile in this cohort revealed a predominance of Gram-positive bacteria, mainly *Coagulase-negative Staphylococcus* and *S. aureus*. Gram-negative bacteria such as *Klebsiella* spp. and *E. coli*, as well as *Candida* species, were also identified. A meta-analysis of 52 studies by Zelellw et al. (2021) found similar results, identifying *Klebsiella* spp. (26.36%), *S. aureus* (23.22%), *Coagulase-negative Staphylococcus* (23.22%), and *E. coli* (15.30%) as the main pathogens [56]. Likewise, pooled data from 29 additional studies reported comparable microbial distributions, indicating that the etiological pattern of neonatal sepsis remains consistent across different geographic regions [57].

The association between *S. aureus* or *Coagulase-negative Staphylococcus* infections and intravascular catheters is well documented. These microorganisms have a high capacity for adhesion and biofilm formation on prosthetic surfaces (umbilical, central, and peripherally inserted central catheters), facilitating colonization and bloodstream invasion, which may result in bacteremia and sepsis [58]. Studies on catheter-related infections consistently demonstrate that neonates with intravascular devices are at greater risk of bloodstream infection, with *Coagulase-negative Staphylococcus* being the dominant pathogen in many settings [58,59].

In our cohort of 1,396 hospitalized newborns, the predominant empirical antimicrobial therapy consisted of ampicillin combined with gentamicin, administered in 64% of patients. This institutional practice follows a protocol designed to cover both vertically transmitted and hospital-acquired pathogens [60]. The resistance profile observed in this study showed low documented resistance rates to first-line agents suggesting that, in our center, the ampicillin–gentamicin combination still provides broad empirical antimicrobial coverage.

Internationally, large multicenter cohorts in low- and middle-income countries have reported much lower efficacy of this regimen, with observational studies showing non-susceptibility rates to ampicillin among Gram-negative organisms reaching several tens of percent, and overall empirical coverage of the ampicillin–gentamicin combination as low as 30–40% in some series [61,62].

Regarding the global resistance pattern observed in our cohort, the highest resistance was found against fluoroquinolones, while resistance to carbapenems remained very low, indicating preserved efficacy of second-line options for confirmed multidrug-resistant infections. This pattern of low resistance to aminoglycosides and broad-spectrum β -lactams contrasts with several national and regional reports documenting outbreaks of ESBL-producing *Enterobacterales* and high resistance rates to cephalosporins and aminoglycosides in Brazilian NICUs, suggesting marked geographic heterogeneity within the country [63].

4.3. Factors Associated with Neonatal Mortality

The global infant mortality rate reached a historic low, declining to 4.9 million deaths in 2022, while the under-five mortality rate has decreased by 51% since 2000 [64]. However, reducing neonatal mortality remains a major challenge, as the first month of life represents the most critical period for child survival [65]. Prematurity and its related complications are the leading direct causes of neonatal deaths worldwide, accounting for approximately 35% of all neonatal deaths [66]. Given that prematurity and extremely low birth weight are closely interrelated [67], low birth weight has been strongly associated with an increased risk of neonatal death, especially among preterm infants [28,68].

This occurs because premature infants experience long-term adverse consequences related to immaturity, affecting nutritional status, growth, neurodevelopment, and cardiac function [28,69], in addition to increased susceptibility to infection due to developmental immaturity of the immune system [70]. In a study conducted in Lusaka, Zambia, including 2,340 neonates admitted to the NICU, 69% were preterm and 51% had low birth weight, with a

mortality rate of 40% [71]. Similarly, a study carried out in Rio de Janeiro, Brazil, found a higher risk of neonatal death among preterm infants small for gestational age, with a mortality rate of 78.1 per 1,000 live births [72]. Both studies align with the findings of Paixão et al. (2021), who reported a 15.9-fold higher mortality risk among preterm infants and a 25.8-fold higher risk among those with low birth weight [73].

The Apgar score assesses, at 1 and 5 minutes after birth, variables such as color, heart rate, reflexes, muscle tone, and respiration to estimate the newborn's hemodynamic condition. Although it is a rapid method to evaluate a newborn immediately after birth and in response to resuscitation, it has limited prognostic value since it provides subjective information about the baby's physiology at a specific time point [74,75]. Low Apgar scores, which may reflect biological immaturity or perinatal asphyxia [76], have shown a positive correlation with neonatal mortality, especially among those with severely depressed scores at 5 minutes.

Since the Apgar score does not identify the cause of distress and only reflects the newborn's condition after delivery, low scores are more common among premature infants [77]. However, studies have shown that even term, non-malformed infants born with low Apgar scores are at higher risk of adverse long-term outcomes [78,79]. In a Swedish cohort of 113,300 newborns, infants born at 22–24 weeks with Apgar scores of 0 or 1 had nearly five times higher neonatal mortality compared to those with scores of 9 or 10, while among term infants, the relative risk exceeded 300-fold [77]. Similarly, Ehrhardt et al. (2025), although not stratifying by gestational age, found an approximately 20% difference in mortality between infants with 5-minute Apgar <7 and those ≥ 7 [79]. Other studies have also shown that reduced Apgar scores are significantly associated with higher neonatal mortality [80,81].

Advances in neonatal intensive care, particularly the implementation of respiratory support, have had a major impact on reducing neonatal morbidity and mortality [82]. However, the association between mechanical ventilation and higher lethality observed in this study has also been documented in other research. This correlation exists because the need for mechanical ventilation usually reflects severe clinical conditions and may lead to ventilator-induced lung injury [83,84]. Furthermore, mechanical ventilation itself may contribute to adverse outcomes through the increased incidence of ventilator-associated respiratory infections, suggesting a significant impact on mortality [85].

Studies indicate that neonates undergoing mechanical ventilation have significantly higher mortality rates, particularly with prolonged use [86,87]. In the study by Iqbal et al. (2015), mortality among mechanically ventilated neonates was substantially higher than that of the overall NICU population, with respiratory distress syndrome, sepsis, and asphyxia being the main conditions leading to intubation [82].

Complications such as septic shock, pulmonary hypertension, and necrotizing enterocolitis (are not only highly prevalent among neonatal morbidities but are also associated with high mortality rates. Septic shock, the most severe form of sepsis, is characterized by circulatory, cellular, or metabolic dysfunction. In neonates, due to organ immaturity, this condition is linked to elevated mortality [88]. A study conducted in India involving 3,271 neonatal admissions found that shock episodes occurred in a significant proportion of patients, reaching a fatality rate of 62%, reinforcing septic shock as a marker of severity and a predictor of mortality [89].

Neonatal pulmonary hypertension, characterized by failure of postnatal circulatory adaptation, has a reported mortality rate of 7.6%, reaching 32% in cases associated with congenital respiratory anomalies [90,91]. Although several risk factors influence prognosis, pulmonary hypertension alone can markedly increase neonatal mortality [92]. Regarding necrotizing enterocolitis, some studies have identified this complication as one of the leading short-term morbidities contributing to neonatal death, with mortality rates reaching up to 23.5% [93,94].

When evaluating risk factors associated with neonatal mortality, a significant proportion of deaths could potentially be prevented through early identification of risk factors and optimized clinical intervention. Maternal, gestational, and contextual factors – such as inadequate prenatal care, low maternal education, and other social

determinants – contribute to preventable infant mortality [95,96]. Furthermore, prematurity remains one of the leading causes of neonatal death, being complex to prevent and influenced by multiple interrelated factors [96].

Intervention strategies should therefore focus primarily on preventing prematurity through adequate maternal care during pregnancy, emphasizing the quality of prenatal monitoring, organization of neonatal services, and the preparedness of healthcare teams [95,96].

4.4. Practical implications, limitations, and perspectives

Continuous microbiological surveillance and early diagnosis of neonatal sepsis are essential pillars for reducing morbidity and mortality in NICUs. Rapid identification of etiologic agents and their resistance profiles allows for timely therapeutic adjustments and the rational use of antimicrobials, preventing both infection progression and the emergence of multidrug-resistant strains [97,98].

In this context, strategies based on antibiotic stewardship protocols, monitoring, and prescription audits have demonstrated a positive impact on reducing adverse events and hospital length of stay. These measures also contribute to optimizing institutional resources and strengthening antimicrobial stewardship practices in neonatal units [99,100].

The role of nurses is fundamental in the early detection of clinical deterioration, proper collection of microbiological samples, and assurance of adherence to infection prevention and control measures. The integrated work of the multidisciplinary team reinforces patient safety through standardized care, compliance with aseptic techniques, and the promotion of an active surveillance culture within NICUs [101,102].

Ongoing education of healthcare teams, combined with systematic monitoring of epidemiological indicators – such as catheter- and ventilator-associated sepsis rates, adherence to hand hygiene, and rational antimicrobial use – constitutes a key tool for continuous quality improvement and the reduction of healthcare-associated infections [98,103].

Strict adherence to institutional protocols for hand hygiene, surface disinfection, and appropriate catheter management remains one of the most effective measures to prevent cross-transmission of microorganisms in neonatal units. This practice is particularly relevant in light of the higher mortality associated with infections caused by Gram-negative bacilli, such as *Klebsiella* spp. and *E. coli*, as observed in this study [104,105].

Laboratory monitoring of bacterial resistance – through continuous and standardized surveillance of isolated microorganisms and their antimicrobial susceptibility profiles – should be periodically updated to allow evidence-based adjustments tailored to local microbiological realities. This practice is crucial to promptly identify changes in resistance patterns and to guide more accurate therapeutic protocols, being widely recommended in NICUs and contributing significantly to infection control and prevention strategies [98,106].

Among the main limitations of this study is its retrospective design, which relies on data extracted from medical records and therefore depends on the accuracy and completeness of documentation. This design also limits the ability to control for confounding factors, such as clinical heterogeneity among patients.

Conducting the study in a single center may limit the generalizability of the findings to other institutions, as structural and care-related differences can influence outcomes. Nevertheless, the large number of cases and the extended observation period represent important strengths of this sample.

Future research should prioritize multicenter, prospective designs capable of evaluating preventive interventions, the impact of antimicrobial stewardship programs, and the outcomes of continuing education initiatives focused on the early recognition and management of neonatal sepsis [107,108]. Furthermore, the use of digital surveillance technologies that integrate clinical and laboratory data in real time – combined with predictive analytics and artificial intelligence models – represents a promising strategy to identify infection risks early and support faster, more accurate clinical decision-making [109,110].

5. Conclusion

Neonatal sepsis remains a major public health challenge, particularly among preterm and low-birth-weight infants. In this cohort, presumed early-onset sepsis predominated, reflecting the persistent diagnostic challenges associated with neonatal infections. Prematurity, low birth weight, invasive respiratory support, and Gram-negative bacterial infections were associated with worse clinical outcomes and higher mortality. Although first-line empirical antimicrobial therapy remained appropriate according to the observed resistance profile, continuous microbiological surveillance and antimicrobial stewardship remain essential to optimize treatment and prevent antimicrobial resistance. Overall, these findings contribute to a better understanding of the epidemiological, microbiological, and clinical characteristics of neonatal sepsis and support strategies aimed at improving diagnosis, infection prevention, and neonatal outcomes.

6. References

1. Miranda S, Harahap A, Husada D, Faramarisa FN. Risk factors of multidrug-resistant organisms neonatal sepsis in Surabaya tertiary referral hospital: a single-center study. *BMC Pediatr*. **2024**; 24(1):153.
2. Milton R, Gillespie D, Dyer C, et al. Neonatal sepsis and mortality in low-income and middle-income countries from a facility-based birth cohort: an international multisite prospective observational study. *Lancet Glob Health*. **2022**; 10(5):e661–e672.
3. Almeida NRC de, Pontes GF, Jacob FL, et al. Analysis of trends in sepsis mortality in Brazil and by regions from 2010 to 2019. *Rev Saude Publica*. **2022**; 56:25.
4. Ohuma EO, Moller A-B, Bradley E, et al. National, regional, and global estimates of preterm birth in 2020, with trends from 2010: a systematic analysis. *Lancet*. **2023**; 402(10409):1261–1271.
5. Kale PL, Fonseca SC. Intrauterine growth restriction, prematurity, and low birth weight: risk phenotypes of neonatal death, Rio de Janeiro State, Brazil. *Cad Saude Publica*. **2023**; 39(6):e00231022.
6. Procianoy RS, Silveira RC. The challenges of neonatal sepsis management. *J Pediatr (Rio J)*. **2020**; 96 Suppl 1(Suppl 1):80–86.
7. Santos RP, Tristram D. A Practical Guide to the Diagnosis, Treatment, and Prevention of Neonatal Infections. *Pediatr Clin North Am*. **2015**; 62(2):491–508.
8. Puopolo KM, Benitz WE, Zaoutis TE, COMMITTEE ON FETUS AND NEWBORN, COMMITTEE ON INFECTIOUS DISEASES. Management of Neonates Born at ≤ 34 6/7 Weeks' Gestation With Suspected or Proven Early-Onset Bacterial Sepsis. *Pediatrics*. **2018**; 142(6):e20182896.
9. Cohen R, Romain O, Tauzin M, Gras-Leguen C, Raymond J, Butin M. Neonatal bacterial infections: Diagnosis, bacterial epidemiology and antibiotic treatment. *Infect Dis Now*. **2023**; 53(8S):104793.
10. Dramowski A, Bolton L, Fitzgerald F, Bekker A, NeoNET AFRICA Partnership. Neonatal Sepsis in Low- and Middle-income Countries: Where Are We Now? *Pediatr Infect Dis J*. **2025**; 44(6):e207–e210.
11. Soni P, Matoria R, Nagalli MM. Antibiotic strategies for neonatal sepsis: navigating efficacy and emerging resistance patterns. *Eur J Pediatr*. **2025**; 184(7):439.
12. Rallis D, Giapros V, Serbis A, Kosmeri C, Baltogianni M. Fighting Antimicrobial Resistance in Neonatal Intensive Care Units: Rational Use of Antibiotics in Neonatal Sepsis. *Antibiotics (Basel)*. **2023**; 12(3):508.
13. Sepsis [Internet]. [cited 2025 Oct 22]. Available from: <https://www.who.int/news-room/fact-sheets/detail/sepsis>
14. Mariani M, Parodi A, Minghetti D, et al. Early and Late Onset Neonatal Sepsis: Epidemiology and Effectiveness of Empirical Antibacterial Therapy in a III Level Neonatal Intensive Care Unit. *Antibiotics (Basel)*. **2022**; 11(2):284.
15. Rallis D, Giapros V, Serbis A, Kosmeri C, Baltogianni M. Fighting Antimicrobial Resistance in Neonatal Intensive Care Units: Rational Use of Antibiotics in Neonatal Sepsis. *Antibiotics (Basel)*. **2023**; 12(3):508.
16. Vasilescu DI, Dan AM, Stefan LA, Vasilescu SL, Dima V, Cirstoiu MM. Assessment of Culture-Negative Neonatal Early-Onset Sepsis: Risk Factors and Utility of Currently Used Serum Biomarkers. *Children (Basel)*. **2025**; 12(3):355.
17. Chu M, Lin J, Wang M, et al. Restrictive Use of Empirical Antibiotics Is Associated with Improved Short Term Outcomes in Very Low Birth Weight Infants: A Single Center, Retrospective Cohort Study from China. *Antibiotics (Basel)*. **2023**; 12(4):741.

18. Rajar P, Saugstad OD, Berild D, et al. Antibiotic Stewardship in Premature Infants: A Systematic Review. *Neonatology*. **2020**; 117(6):673–686.
19. Boverman G, Perez C, Vij S, et al. Neonatal ICU antibiotic use trends within an integrated delivery network. *Antimicrob Resist Infect Control*. **2022**; 11(1):21.
20. Montaner Ramón A, Castilla Fernández Y, Frick MA, et al. How to assess early-onset neonatal sepsis? Comparison of three detection strategies. *An Pediatr (Engl Ed)*. **2023**; 98(2):92–98.
21. Bollig C, Nothacker M, Lehane C, et al. Prophylactic antibiotics before cord clamping in cesarean delivery: a systematic review. *Acta Obstet Gynecol Scand*. **2018**; 97(5):521–535.
22. Ortiz de Zárate M, Sáenz C, Cimbaro Canella R, et al. Prevalence of microbiologically confirmed neonatal sepsis at a maternity center in the City of Buenos Aires. *Arch Argent Pediatr*. **2023**; 121(3):e202202779.
23. Making every baby count [Internet]. [cited 2025 Oct 22]. Available from: <https://www.who.int/publications/i/item/9789241511223>
24. Preterm birth [Internet]. [cited 2025 Oct 22]. Available from: <https://www.who.int/news-room/fact-sheets/detail/preterm-birth>
25. AMERICAN ACADEMY OF PEDIATRICS COMMITTEE ON FETUS AND NEWBORN, AMERICAN COLLEGE OF OBSTETRICIANS AND GYNECOLOGISTS COMMITTEE ON OBSTETRIC PRACTICE. The Apgar Score. *Pediatrics*. **2015**; 136(4):819–822.
26. Odabasi IO, Bulbul A. Neonatal Sepsis. *Sisli Etfal Hastan Tip Bul*. **2020**; 54(2):142–158.
27. Fleischmann C, Reichert F, Cassini A, et al. Global incidence and mortality of neonatal sepsis: a systematic review and meta-analysis. *Arch Dis Child*. **2021**; 106(8):745–752.
28. Jańczewska I, Wierzbą J, Jańczewska A, Szczurek-Gierczak M, Domżalska-Popadiuk I. Prematurity and Low Birth Weight and Their Impact on Childhood Growth Patterns and the Risk of Long-Term Cardiovascular Sequelae. *Children (Basel)*. **2023**; 10(10):1599.
29. 1 in 10 babies worldwide are born early, with major impacts on health and survival [Internet]. [cited 2025 Nov 7]. Available from: <https://www.who.int/news/item/06-10-2023-1-in-10-babies-worldwide-are-born-early--with-major-impacts-on-health-and-survival>
30. Boletim Epidemiológico - Volume 55 - nº 13 — Ministério da Saúde [Internet]. [cited 2025 Nov 7]. Available from: <https://www.gov.br/saude/pt-br/centrais-de-conteudo/publicacoes/boletins/epidemiologicos/edicoes/2024/boletim-epidemiologico-volume-55-no-13.pdf/view>
31. Barreto MGP, Manso MC, Barreto RP, Barreto RP, Vasconcelos LMT de, Silva C. Frequency and Risk Factors Associated with Prematurity: A Cohort Study in a Neonatal Intensive Care Unit. *J Clin Med*. **2024**; 13(15):4437.
32. Okwaraji YB, Krasevec J, Bradley E, et al. National, regional, and global estimates of low birthweight in 2020, with trends from 2000: a systematic analysis. *Lancet*. **2024**; 403(10431):1071–1080.
33. Joint low birthweight estimates [Internet]. [cited 2025 Nov 7]. Available from: <https://www.who.int/teams/nutrition-and-food-safety/monitoring-nutritional-status-and-food-safety-and-events/joint-low-birthweight-estimates>
34. Paho/Ops. Perfil de país - Brasil [Internet]. Health in the Americas. 2024 [cited 2025 Nov 7]. Available from: <https://hia.paho.org/en/perfis-de-pais/brasil>
35. Zheng S, Cao Y, Strasser S, Wei H. Prevalence and risk factors of low birth weight in the United States: An analysis of 2016–2021 data. *Am J Hum Biol*. **2024**; 36(4):e24016.
36. Tessema ZT, Tamirat KS, Teshale AB, Tesema GA. Prevalence of low birth weight and its associated factor at birth in Sub-Saharan Africa: A generalized linear mixed model. *PLoS One*. **2021**; 16(3):e0248417.
37. Himes ES, Rivera C, Nacht AS, et al. Prevalence and Predictors of Low Birth Weight in a Rural Guatemalan Community. *Obstet Gynecol Res*. **2022**; 5:1–9.
38. Saldanha J, Moniz C, Machado MC. [Very Low Birth Weight Infants in a Portuguese Intensive Care Unit and the Vermont Oxford Network: 15 Years of Registry Data]. *Acta Med Port*. **2019**; 32(11):686–692.
39. Chee YY, Wong MS, Wong RM, Wong KY. Neonatal outcomes of preterm or very-low-birth-weight infants over a decade from Queen Mary Hospital, Hong Kong: comparison with the Vermont Oxford Network. *Hong Kong Med J*. **2017**; 23(4):381–386.
40. Wondie WT, Zeleke KA, Wubneh CA. Incidence and predictors of mortality among low birth weight neonates in the first week of life admitted to the neonatal intensive care unit in Northwestern Ethiopia comprehensive specialized hospitals, 2022. Multi-center institution-based retrospective follow-up study. *BMC Pediatr*. **2023**; 23(1):489.

41. Mengstie LA, Gebeyehu MT, Zeneb Teklehaimanot W, et al. Incidence and predictors of mortality among low birth weight neonates admitted to neonatal intensive care unit in selected public hospitals, North Shoa zone, Amhara Region, Ethiopia: multi-center retrospective follow-up study. *Front Pediatr.* **2025**; 13:1524966.
42. Chauhan AV, Gosai D, Patel U. Morbidity profile and outcome of low-birth-weight neonates in NICU of a tertiary care hospital. *International Journal of Contemporary Pediatrics.* **2023**; 10(1):63–67.
43. Berhane M, Workineh N, Girma T, et al. Prevalence of Low Birth Weight and Prematurity and Associated Factors in Neonates in Ethiopia: Results from a Hospital-based Observational Study. *Ethiop J Health Sci.* **2019**; 29(6):677–688.
44. Liu T, Xu Y, Gong Y, Zheng J, Chen Z. The global burden of disease attributable to preterm birth and low birth weight in 204 countries and territories from 1990 to 2019: An analysis of the Global Burden of Disease Study. *J Glob Health.* **2024**; 14:04109.
45. Shane AL, Sánchez PJ, Stoll BJ. Neonatal sepsis. *Lancet.* **2017**; 390(10104):1770–1780.
46. Miranda S, Harahap A, Husada D, Famarisa FN. Risk factors of multidrug-resistant organisms neonatal sepsis in Surabaya tertiary referral hospital: a single-center study. *BMC Pediatr.* **2024**; 24:153.
47. Mu C, Liu F, Tian T, et al. Analysis of the global burden and key risk factors of neonatal sepsis and other neonatal infections in 204 countries and territories, 1990–2021. *Front Med (Lausanne).* **2025**; 12:1536948.
48. Abreu MFCW de, Ogata JFM, Fonseca MCM, Sansone D, Guinsburg R. The financial impact of neonatal sepsis on the Brazilian Unified Health System. *Clinics (Sao Paulo).* **2023**; 78:100277.
49. Katugume B, Muzungu J, Okello N, Kigongo E, Namutebi DA. Prevalence of neonatal sepsis and associated factors among neonates admitted in the neonatal intensive care unit at Lira Regional Referral Hospital, Northern Uganda. *PLoS One.* **2025**; 20(1):e0315794.
50. Devkota K, Kanodia P, Joshi B. Sepsis among Neonates Admitted to a Neonatal Intensive Care Unit in a Tertiary Care Centre. *JNMA J Nepal Med Assoc.* **2024**; 62(270):76–78.
51. Traoré FB, Camara BS, Diallo EM, et al. Prevalence and factors associated with neonatal sepsis in Mali: a cross-sectional study. *BMJ Open.* **2025**; 15(1):e082066.
52. Traoré FB, Sidibé CS, Diallo EHM, et al. Prevalence and factors associated with maternal and neonatal sepsis in sub-Saharan Africa: a systematic review and meta-analysis. *Front Public Health.* **2024**; 12:1272193.
53. Seyoum K, Sahiledengle B, Kene C, et al. Determinants of neonatal sepsis among neonates admitted to neonatal intensive care units in Ethiopian hospitals: A systematic review and meta-analysis. *Heliyon.* **2023**; 9(9):e20336.
54. Maisaba JM, Migisha R, Owaraganise A, et al. Maternal factors associated with early-onset neonatal sepsis among caesarean-delivered babies at Mbarara Regional Referral Hospital, Uganda: a case-control study. *BMC Pregnancy Childbirth.* **2024**; 24:707.
55. Raturi A, Chandran S. Neonatal Sepsis: Aetiology, Pathophysiology, Diagnostic Advances and Management Strategies. *Clin Med Insights Pediatr.* **2024**; 18:11795565241281337.
56. Zelellw DA, Dessie G, Worku Mengesha E, Balew Shiferaw M, Mela Merhaba M, Emishaw S. A Systemic Review and Meta-analysis of the Leading Pathogens Causing Neonatal Sepsis in Developing Countries. *Biomed Res Int.* **2021**; 2021:6626983.
57. Yu Y-Q, He X-R, Wan L-J, Yang Y-H, Chen P-Y. Etiology, antimicrobial resistance, and risk factors of neonatal sepsis in China: a systematic review and meta-analysis from data of 30 years. *J Matern Fetal Neonatal Med.* **2022**; 35(25):7541–7550.
58. Cho HJ, Cho H-K. Central line-associated bloodstream infections in neonates. *Korean J Pediatr.* **2019**; 62(3):79–84.
59. Mobley RE, Bizzarro MJ. Central line-associated bloodstream infections in the NICU: Successes and controversies in the quest for zero. *Semin Perinatol.* **2017**; 41(3):166–174.
60. 19/05/2025 17h23 P em. Anvisa lança Diretriz Nacional para Gerenciamento de Antimicrobianos em Serviços de Neonatologia [Internet]. Agência Nacional de Vigilância Sanitária - Anvisa. [cited 2025 Nov 7]. Available from: <https://www.gov.br/anvisa/pt-br/assuntos/noticias-anvisa/2025/anvisa-lanca-diretriz-nacional-para-gerenciamento-de-antimicrobianos-em-servicos-de-neonatologia>
61. Russell NJ, Stöhr W, Plakkal N, et al. Patterns of antibiotic use, pathogens, and prediction of mortality in hospitalized neonates and young infants with sepsis: A global neonatal sepsis observational cohort study (NeoOBS). *PLoS Med.* **2023**; 20(6):e1004179.
62. Sands K, Carvalho MJ, Portal E, et al. Characterization of antimicrobial-resistant Gram-negative bacteria that cause neonatal sepsis in seven low- and middle-income countries. *Nat Microbiol.* **2021**; 6(4):512–523.

63. Ferreira IC da S, Menezes R de P, Lopes MSM, Araújo LB de, Ferreira DM de LM, Röder DVD de B. Multidrug-resistant *Klebsiella pneumoniae* in critically ill neonates: evidence from a Brazilian cohort. *Aust Crit Care*. **2025**; 38(6):101444.
64. Relatório sobre Mortalidade Infantil 2023 | As Nações Unidas no Brasil [Internet]. [cited 2025 Nov 7]. Available from: <https://brasil.un.org/pt-br/263674-relat%C3%B3rio-sobre-mortalidade-infantil-2023>
65. Levels and trends in child mortality: report 2021 [Internet]. [cited 2025 Nov 7]. Available from: <https://www.who.int/publications/m/item/levels-and-trends-in-child-mortality-report-2021>
66. Birhanu D, Gebremichael B, Tesfaye T, et al. Survival status and predictors of mortality among preterm neonates admitted to neonatal intensive care unit of Addis Ababa public hospitals, Ethiopia, 2021. A prospective cohort study. *BMC Pediatr*. **2022**; 22(1):153.
67. Bernardino FBS, Gonçalves TM, Pereira TID, Xavier JS, Freitas BHBM de, Gaíva MAM. Trends in neonatal mortality in Brazil from 2007 to 2017. *Cien Saude Colet*. **2022**; 27(2):567–578.
68. Nwanze LD, Siuliman A, Ibrahim N. Factors associated with infant mortality in Nigeria: A scoping review. *PLoS One*. **2023**; 18(11):e0294434.
69. Chawla D, Agarwal R. Preterm births and deaths: from counting to classification. *Lancet Glob Health*. **2022**; 10(11):e1537–e1538.
70. Fleiss N, Tarun S, Polin RA. Infection prevention for extremely low birth weight infants in the NICU. *Semin Fetal Neonatal Med*. **2022**; 27(3):101345.
71. Tembo D, Abobo FDN, Kaonga P, Jacobs C, Bessing B. Risk factors associated with neonatal mortality among neonates admitted to neonatal intensive care unit of the University Teaching Hospital in Lusaka. *Sci Rep*. **2024**; 14:5231.
72. Kale PL, Fonseca SC. Intrauterine growth restriction, prematurity, and low birth weight: risk phenotypes of neonatal death, Rio de Janeiro State, Brazil. *Cad Saude Publica*. 39(6):e00231022.
73. Paixao ES, Blencowe H, Falcao IR, et al. Risk of mortality for small newborns in Brazil, 2011–2018: A national birth cohort study of 17.6 million records from routine register-based linked data. *Lancet Reg Health Am*. **2021**; 3:100045.
74. Simon LV, Shah M, Bragg BN. APGAR Score. StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2025 [cited 2025 Nov 7]. Available from: <http://www.ncbi.nlm.nih.gov/books/NBK470569/>
75. Rozycki HJ, Yitayew M. The Apgar score in clinical research: for what, how and by whom it is used. *J Perinat Med*. **2023**; 51(4):580–585.
76. Mu Y, Li M, Zhu J, et al. Apgar score and neonatal mortality in China: an observational study from a national surveillance system. *BMC Pregnancy Childbirth*. **2021**; 21:47.
77. Cnattingius S, Johansson S, Razaz N. Apgar Score and Risk of Neonatal Death among Preterm Infants. *N Engl J Med*. **2020**; 383(1):49–57.
78. Razaz N, Cnattingius S, Joseph K. Association between Apgar scores of 7 to 9 and neonatal mortality and morbidity: population based cohort study of term infants in Sweden. *BMJ*. **2019**; 365:l1656.
79. Ehrhardt H, Behboodi S, Maier RF, et al. Five-Minute Apgar Scores and Its Prognostic Value for Mortality and Severe Morbidity in Very Preterm Infants: A Multinational Cohort Study. *BJOG*. **2025**; .
80. Chen H-Y, Blackwell SC, Chauhan SP. Association between apgar score at 5 minutes and adverse outcomes among Low-Risk pregnancies. *J Matern Fetal Neonatal Med*. **2022**; 35(7):1344–1351.
81. Blundell PDM, Chakraborty M. Relationship between Apgar Scores and Morbidity and Mortality Outcomes in Preterm Infants: A Single-Centre Cohort Study. *Neonatology*. **2020**; 117(6):742–749.
82. Iqbal Q, Younus MM, Ahmed A, et al. Neonatal mechanical ventilation: Indications and outcome. *Indian J Crit Care Med*. **2015**; 19(9):523–527.
83. Kaltsogianni O, Dassios T, Greenough A. Neonatal respiratory support strategies—short and long-term respiratory outcomes. *Front Pediatr*. **2023**; 11:1212074.
84. Chakkarapani AA, Adappa R, Mohammad Ali SK, et al. “Current concepts of mechanical ventilation in neonates” – Part 1: Basics. *Int J Pediatr Adolesc Med*. **2020**; 7(1):13–18.
85. Mello Freitas FT de, Viegas APB, Romero GAS. Neonatal healthcare-associated infections in Brazil: systematic review and meta-analysis. *Arch Public Health*. **2021**; 79:89.
86. Jo HS, Lim MN, Cho S-I. Required biological time for lung maturation and duration of invasive ventilation: a Korean cohort study of very low birth weight infants. *Front Pediatr*. **2023**; 11:1184832.
87. Sauthier M, Sauthier N, Bergeron Gallant K, et al. Long-Term Mechanical Ventilation in Neonates: A 10-Year Overview and Predictive Model. *Front Pediatr*. **2021**; 9:689190.

88. Spaggiari V, Passini E, Crestani S, et al. Neonatal septic shock, a focus on first line interventions. *Acta Biomed.* **2022**; 93(3):e2022141.
89. Saini SS, Shrivastav AK, Kumar J, et al. Predictors of Mortality in Neonatal Shock: A Retrospective Cohort Study. *Shock.* **2022**; 57(2):199–204.
90. Steurer MA, Jelliffe-Pawlowski LL, Baer RJ, Partridge JC, Rogers EE, Keller RL. Persistent Pulmonary Hypertension of the Newborn in Late Preterm and Term Infants in California. *Pediatrics.* **2017**; 139(1):e20161165.
91. Huang Y, Yang T, Liang X, et al. Global, regional and national trends in the burden of persistent pulmonary hypertension of the newborn and essentials of its management from 1993 to 2023: a scoping review. *Front Pediatr.* **2025**; 13:1502385.
92. Steurer MA, Baer RJ, Oltman S, et al. Morbidity of Persistent Pulmonary Hypertension of the Newborn in the First Year of Life. *J Pediatr.* **2019**; 213:58–65.e4.
93. Lima RG, Vieira VC, Medeiros DS de. Determinants of preterm infants' deaths at the Neonatal Intensive Care Units in the Northeast Countryside in Brazil. *Rev Bras Saude Mater Infant. Instituto de Medicina Integral Prof. Fernando Figueira*; **2020**; 20:535–544.
94. Jones IH, Hall NJ. Contemporary Outcomes for Infants with Necrotizing Enterocolitis-A Systematic Review. *J Pediatr.* **2020**; 220:86–92.e3.
95. Kreutz IM, Santos IS. Contextual, maternal, and infant factors in preventable infant deaths: a statewide ecological and cross-sectional study in Rio Grande do SUL, Brazil. *BMC Public Health.* **2023**; 23(1):87.
96. Sousa MRM, Parada CMG de L, Nunes HR de C. Factors associated with preventable infant mortality in 2020: a Brazilian population-based study. *Rev Bras Enferm.* 77(4):e20230072.
97. Klingenberg C, Kornelisse RF, Buonocore G, Maier RF, Stocker M. Culture-Negative Early-Onset Neonatal Sepsis — At the Crossroad Between Efficient Sepsis Care and Antimicrobial Stewardship. *Front Pediatr.* **2018**; 6:285.
98. De Rose DU, Ronchetti MP, Martini L, et al. Diagnosis and Management of Neonatal Bacterial Sepsis: Current Challenges and Future Perspectives. *Trop Med Infect Dis.* **2024**; 9(9):199.
99. Mascarenhas D, Ho MSP, Ting J, Shah PS. Antimicrobial Stewardship Programs in Neonates: A Meta-Analysis. *Pediatrics.* **2024**; 153(6):e2023065091.
100. Dramowski A, Prusakov P, Goff DA, et al. Prospective antimicrobial stewardship interventions by multidisciplinary teams to reduce neonatal antibiotic use in South Africa: The Neonatal Antimicrobial Stewardship (NeoAMS) study. *Int J Infect Dis.* **2024**; 146:107158.
101. Konlan KD, Nukpezah RN, Doat AR. Nurses' experiences in neonatal sepsis prevention and management: a qualitative cross-sectional study at selected hospitals in a metropolitan area. *BMC Health Serv Res.* **2024**; 24(1):1298.
102. Abou El Fadl DK, Aly YAF, Darweesh EAG, Sabri NA, Ahmed MA. Assessment of neonatal intensive care unit nurses' compliance with standard precautions of infection control and identification of enabling factors. *Futur J Pharm Sci.* **2023**; 9(1):6.
103. Dramowski A, Aucamp M, Beales E, et al. Healthcare-Associated Infection Prevention Interventions for Neonates in Resource-Limited Settings. *Front Pediatr.* **2022**; 10:919403.
104. Flannery DD, Chiotos K, Gerber JS, Puopolo KM. Neonatal multidrug-resistant gram-negative infection: epidemiology, mechanisms of resistance, and management. *Pediatr Res.* **2022**; 91(2):380–391.
105. Artelt T, Kaase M, Bley I, et al. Transmission Risk on a Neonatal Intensive Care Unit: *Escherichia coli* versus *Klebsiella pneumoniae*. *Can J Infect Dis Med Microbiol.* **2018**; 2018:1525072.
106. Graziano G, Notarbartolo V, Priano W, et al. Surveillance of Multidrug-Resistant Pathogens in Neonatal Intensive Care Units of Palermo, Italy, during SARS-CoV-2 Pandemic. *Antibiotics (Basel).* **2023**; 12(9):1457.
107. Maalouf FI, Saad T, Zakhour R, Yunis K. Successful establishment and five-year sustainability of a neonatal-specific antimicrobial stewardship program in a low middle-income country. *Front Pharmacol.* **2022**; 13:1076392.
108. Abdelaal NH, Aziz NHRA, Abdelaziz AM, Khalil SBH, Abdel-Latif MMM. Clinical impact of an antibiotic stewardship program in a neonatal intensive care unit at a tertiary care hospital: a prospective quasi-experimental clinical study. *J Pharm Health Care Sci.* **2025**; 11(1):81.
109. Duci M, Verlato G, Moschino L, Uccheddu F, Fascetti-Leon F. Advances in Artificial Intelligence and Machine Learning for Precision Medicine in Necrotizing Enterocolitis and Neonatal Sepsis: A State-of-the-Art Review. *Children (Basel).* **2025**; 12(4):498.

110. Sullivan BA, Kausch SL, Fairchild KD. Artificial and human intelligence for early identification of neonatal sepsis. *Pediatr Res.* **2023**; 93(2):350–356.

Declarations

Funding: [LFAM] received a scientific initiation grant from the São Paulo Research Foundation (*Fundação de Amparo à Pesquisa do Estado de São Paulo – FAPESP*), grant number: 2024/20061-9. This work was supported by a Productivity Scholarship (FALM) from the CNPq, grant number: 305906/2024-0.

Conflicts of interest: The authors declare no conflicts of interest related to the development of this study.

Availability of data and material: The materials used in this study are available from the corresponding author upon reasonable request.

Ethical approval: The study was conducted in accordance with the principles of the Declaration of Helsinki and was approved by the Institutional Ethics Committee (Ethical approval number: 80862224.4.0000.5514 Study approval number: 6.994.663).

Authors' contributions: [PKA, FALM] were responsible for data collection and tabulation. [PKA, FFM, GGS, KOB, LFAM, FALM] contributed to the interpretation of the study findings. [PKA, FFM, GGS, KOB, LFAM, FALM] were involved in drafting and critically revising the manuscript prior to submission. All authors [PKA, FFM, GGS, KOB, LFAM, FALM] approved the final version of the manuscript and consented to its submission to the scientific journal.

Acknowledgments: The Article Processing Charge for this publication was covered by the Brazilian Coordination for the Improvement of Higher Education Personnel (*Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – CAPES*), ROR number: 00x0ma614. For open access purposes, the authors have assigned the Creative Commons CC BY license to any accepted version of the article.

Supplementary Material

Title: Risk Factors, Prevalence of Bacterial Infections, and Mortality Among Neonates Admitted to a Neonatal Intensive Care Unit

Short Title: Bacterial Infections and Mortality Risk in Neonatal Intensive Care

Patricia Krause Askinis^{1,2,3}; Fernanda Ferreira Mariano^{1,2,3}; Gustavo Gomes Sacaizawa^{1,2,3}; Luiz Felipe Azevedo Marques^{1,2,3}; Ketlyn Oliveira Bredariol^{1,2,3}; Fernando Augusto Lima Marson^{1,2,3,*}

¹ Laboratory of Molecular Biology and Genetics, Postgraduate Program in Health Sciences, Postgraduate Program in Health Data Science, São Francisco University, Bragança Paulista, São Paulo, Brazil.

² Laboratory of Clinical and Molecular Microbiology, Postgraduate Program in Health Sciences, Postgraduate Program in Health Data Science, São Francisco University, Bragança Paulista, São Paulo, Brazil.

³ LunGuardian Research Group – Epidemiology of Respiratory and Infectious Diseases, Postgraduate Program in Health Sciences, Postgraduate Program in Health Data Science, São Francisco University, Bragança Paulista, São Paulo, Brazil.

*** Corresponding Author:** [FALM] Fernando Augusto Lima Marson, BSc, MSc, PhD.

Laboratory of Molecular Biology and Genetics, Laboratory of Clinical and Molecular Microbiology, LunGuardian Research Group – Epidemiology of Respiratory and Infectious Diseases, Postgraduate Program in Health Sciences, Postgraduate Program in Health Data Science, São Francisco University. Avenida São Francisco de Assis, 218. Jardim São José, Bragança Paulista 12916-900, São Paulo, Brasil. Phone Number: +55-19-999752911.

E-mail: fernandolimamarson@hotmail.com and fernando.marson@usf.edu.br.

Study authors' e-mails and ORCIDs:

PKA: patriciakrause14@gmail.com

ORCID: 0009-0000-8067-0677

FFM: fernandamariano011@gmail.com

ORCID: 0009-0003-5194-7050

GGS: gustavo.sacaizawa@mail.usf.edu.br

ORCID: 0009-0000-4310-844X

LFAM: luiz.azevedo@mail.usf.edu.br

ORCID: 0009-0008-5494-2171

KOB: Kbredariol@outlook.com

ORCID: 0009-0006-2066-8472

FALM: fernandolimamarson@hotmail.com and fernando.marson@usf.edu.br

ORCID: 0000-0003-4955-4234

Supplementary Table 1. Distribution of newborns by neonatal intensive care unit (NICU) in a university hospital (2017–2024).

NICU unit	Death [N (%)]	Hospital discharge [N (%)]	N	(%)
NICU 01	39 (17.8%)	180 (82.2%)	219	15.7%
NICU 02	34 (21.7%)	123 (78.3%)	157	11.2%
NICU 03	21 (15.3%)	116 (84.7%)	137	9.8%
NICU 04	19 (13.5%)	122 (86.5%)	141	10.1%
NICU 05	23 (14.8%)	132 (85.2%)	155	11.1%
NICU 06	15 (9.9%)	137 (90.1%)	152	10.9%
NICU 07	20 (17.4%)	95 (82.6%)	115	8.2%

The data are presented as absolute (N) and relative (%) frequencies. There was no statistically significant difference in the outcome between the groups analyzed ($p = 0.108$).

Supplementary Table 2. Distribution of neonatal sepsis cases by antimicrobial resistance profile.

Antimicrobial resistance profile	Percentage relative to the total study population (n = 1,396)	Percentage relative to population with cultures (n = 1,062)
Ciprofloxacin	46 (3.3%)	46 (4.3%)
Levofloxacin	46 (3.3%)	46 (4.3%)
Trimethoprim/Sulfamethoxazol	38 (2.7%)	38 (3.6%)
Gentamicin	35 (2.5%)	35 (3.3%)
Oxacillin	35 (2.5%)	35 (3.3%)
Erythromycin	33 (2.4%)	33 (3.1%)
Ampicillin	30 (2.1%)	30 (2.8%)
Clindamycin	30 (2.1%)	30 (2.8%)
Amoxicillin/Clavulanic acid	16 (1.1%)	16 (1.5%)
Ampicillin/Sulbactam	14 (1.0%)	14 (1.3%)
Cefoxitin	8 (0.6%)	8 (0.7%)
Penicillin	8 (0.6%)	8 (0.7%)
Aztreonam	6 (0.4%)	6 (0.6%)
Cefepime	6 (0.4%)	6 (0.6%)
Ceftazidime	6 (0.4%)	6 (0.6%)
Ceftriaxone	6 (0.4%)	6 (0.6%)
Piperacillin/Tazobactam	5 (0.3%)	5 (0.5%)
Tetracycline	5 (0.3%)	5 (0.5%)
Vancomycin	3 (0.2%)	3 (0.3%)
Azithromycin	2 (0.2%)	2 (0.2%)
Cefalotin	2 (0.2%)	2 (0.2%)
Imipenem	1 (0.02%)	1 (0.1%)

The data are presented as absolute (N) and relative (%) frequencies.

Supplementary Table 3. Antimicrobial susceptibility profiles of microorganisms isolated from the infection episodes in Neonatal Intensive Care Unit patients of a university hospital.

[illegible]

Supplementary Table 3. Antimicrobial susceptibility profiles of microorganisms isolated from the infection episodes in Neonatal Intensive Care Unit patients of a university hospital.																								
P	Micro	AC+AMX	AMP	AMP/SUL	AZI	AZT	CEFAL	CEFEP	CFOX	CEFT	CFT	CIP	CLI	ERI	GEN	IMI	LEVO	OXA	PEN	PIP/TAZ	TET	TMP/SMX	VAN	Outcome
37	CoNS	S	S	S	S	S	S	S	S	S	S	R	R	R	R	S	R	R	S	S	S	R	S	DIS
38	EC	S	R	S	S	S	S	S	S	S	S	R	S	S	S	S	R	S	S	S	S	S	S	DTH
39	SA	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	DTH
40	CSP	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	DIS
41	KO	S	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	DTH
42	CA	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	DIS
43	CoNS	S	S	S	S	S	S	S	S	S	S	R	R	R	R	S	R	R	S	S	S	R	S	DIS
44	KP	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	DIS
45	SA	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	DIS
46	CoNS	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	DIS
47	SA	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	DTH
48	KP	R	R	R	S	S	S	S	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	DIS
49	KP	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	DIS
50	CoNS	S	S	S	S	S	S	S	S	S	S	R	R	R	S	S	R	S	S	S	S	S	S	DIS
51	CoNS	S	S	S	S	S	S	S	S	S	S	R	R	R	R	S	R	R	S	S	S	R	S	DIS
52	EC	R	R	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	DIS
53	SA	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R	R	S	S	S	S	S	DIS
54	CoNS	S	S	S	S	S	S	S	S	S	S	R	S	S	S	S	R	S	S	S	S	S	S	DIS
55	CoNS	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	DIS
56	SA	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	DIS
57	CoNS	S	S	S	S	S	S	S	S	S	S	R	R	R	R	S	R	R	S	S	R	R	S	DIS
58	KP	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	DIS
59	EAGG	R	R	R	S	S	S	S	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	DIS
60	KP	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	DIS
61	EAGG	R	R	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	DIS
62	EC	S	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	DTH
63	EC	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	DIS
64	SA	S	S	S	S	S	S	S	S	S	S	R	R	R	R	S	R	R	R	S	S	R	S	DTH
65	CoNS	S	S	S	S	S	S	S	S	S	S	R	R	R	R	S	R	R	S	S	S	S	S	DIS
66	MM	R	R	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	DTH
67a	EC	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	DIS
67b	KP	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	DIS
68	CoNS	S	S	S	S	S	S	S	S	S	S	R	S	S	S	S	R	S	S	S	S	S	S	DIS
69	CoNS	S	S	S	S	S	S	S	S	S	S	R	S	S	S	S	R	S	S	S	S	S	S	DIS
70	CoNS	S	S	S	S	S	S	S	S	S	S	R	S	S	S	S	R	S	S	S	S	S	S	DIS
71	ECLO	R	R	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	DIS
72	CoNS	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S	DIS

Supplementary Table 3. Antimicrobial susceptibility profiles of microorganisms isolated from the infection episodes in Neonatal Intensive Care Unit patients of a university hospital.																								
P	Micro	AC+AMX	AMP	AMP/SUL	AZI	AZT	CEFAL	CEFEP	CFOX	CEFT	CFT	CIP	CLI	ERI	GEN	IMI	LEVO	OXA	PEN	PIP/TAZ	TET	TMP/SMX	VAN	Outcome
107	CSP	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	DTH
108	EC	S	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R	S	DTH
109	SA	S	S	S	S	S	S	S	S	S	S	R	R	R	R	S	R	R	R	S	S	R	S	DIS
110	SPN	S	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	DIS
111	CoNS	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R	DIS
112	CoNS	S	S	S	S	S	S	S	S	S	S	S	R	R	R	S	S	S	S	S	S	S	S	DIS
113	CoNS	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S	S	S	S	DIS
114	CoNS	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S	S	S	S	DIS
115	SA	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S	S	S	DIS
116	CoNS	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S	S	S	S	S	S	S	S	DTH
117	CoNS	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	DTH
118	CoNS	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	DTH
119	CoNS	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	DTH
120a	SA	S	S	S	S	S	S	S	S	S	S	R	R	R	R	S	R	R	R	S	S	R	S	DIS
120b	SA	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	DIS
120c	SA	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	DIS
121	CoNS	S	S	S	S	S	S	S	S	S	R	R	R	R	R	S	R	R	S	S	S	R	S	DTH
122a	CoNS	S	S	S	S	R	S	R	S	R	S	R	S	R	R	R	R	R	S	R	S	R	S	DTH
122b	PA	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	DTH
123a	CoNS	S	R	R	S	R	S	R	S	R	R	R	R	R	S	S	R	S	S	R	S	S	S	DTH
123b	KP	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	DTH
124	EC	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	DIS
125	CoNS	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S	S	R	S	S	S	S	S	DTH
126	KP	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	DIS
127	ECLO	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	DIS

Antibiotics: AC+AMX (Clavulanic Acid + Amoxicillin), AMP (Ampicillin), AMP/SUL (Ampicillin/Sulbactam), AZI (Azithromycin), AZT (Aztreonam), CEFAL (Cephalothin), CEFEP (Cefepime), CFOX (Cefoxitin), CEFT (Ceftazidime), CFT (Ceftriaxone), CIP (Ciprofloxacin), CLI (Clindamycin), ERI (Erythromycin), GEN (Gentamicin), IMI (Imipenem), LEVO (Levofloxacin), OXA (Oxacillin), PEN (Penicillin), PIP/TAZ (Piperacillin sodium + tazobactam), TET (Tetracycline), TMP/SMX (Trimethoprim/Sulfamethoxazole), VAN (Vancomycin), R (Resistant), S (Susceptible), - (not informed).

The microorganisms were abbreviated as follows: SA (*Staphylococcus aureus*), CoNS (*Staphylococcus coagulase-negative*), SPN (*Streptococcus pneumoniae*), GBS (*Streptococcus agalactiae* Group B), ENT (*Enterococcus spp.*), EC (*Escherichia coli*), KP (*Klebsiella pneumoniae*), KO (*Klebsiella oxytoca*), ECLO (*Enterobacter cloacae*), EAGG (*Enterobacter agglomerans*), PM (*Proteus mirabilis*), SLQ (*Serratia liquefaciens*), PA (*Pseudomonas aeruginosa*), SMAL (*Stenotrophomonas maltophilia*), MM (*Morganella morganii*), CA (*Candida albicans*), and CSP (*Candida spp.*).

Table fields: P (Participant), OUT (Outcome), DIS (Discharge), DTH (Death).

Supplementary Table 4. Clinical and therapeutic characteristics of newborns with neonatal sepsis according to clinical outcome (hospital discharge or death).

Markers	Category	Groups	Death [N (%)]	Hospital discharge [N (%)]	Total
Recorded diagnostic hypotheses	Respiratory disorders	Yes	205 (16.5%)	1,041 (83.5%)	1,246
		No	7 (4.7%)	143 (95.3%)	150
	Prematurity	Yes	177 (17.7%)	823 (82.3%)	1,000
		No	35 (8.8%)	361 (91.2%)	396
	Perinatal asphyxia	Yes	34 (16.7%)	170 (83.3%)	204
		No	178 (14.9%)	1,014 (85.1%)	1,192
	Hypoglycemia	Yes	28 (18.9%)	120 (81.1%)	148
		No	184 (14.7%)	1,064 (85.3%)	1,248
	Seizure episodes	Yes	17 (1.8%)	69 (80.2%)	86
		No	195 (14.9%)	1,115 (85.1%)	1,310
	Congenital heart defects	Yes	21 (38.9%)	33 (61.1%)	54
		No	191 (14.2%)	1,151 (85.8%)	1,342
	Jaundice	Yes	0 (0.0%)	35 (100.0%)	35
		No	212 (15.6%)	1,149 (84.4%)	1,361
Forms of respiratory support	Atresias	Yes	3 (27.3%)	8 (72.7%)	11
		No	209 (15.1%)	1,176 (84.9%)	1,385
	Supplemental oxygen	Yes	84 (7.9%)	975 (92.1%)	1,059
		No	128 (38.0%)	209 (62.0%)	337
	Continuous positive airway pressure	Yes	86 (9.4%)	825 (90.6%)	911
		No	126 (26.0%)	359 (74.0%)	485
	Mechanical ventilation	Yes	203 (38.8%)	320 (61.2%)	523
		No	9 (1.0%)	864 (99.0%)	873
Invasive procedures	Umbilical catheters	Yes	192 (17.5%)	903 (82.5%)	1,095
		No	20 (6.6%)	281 (93.4%)	301
	Peripherally inserted central catheters	Yes	73 (15.2%)	407 (84.8%)	480
		No	139 (15.2%)	777 (84.8%)	916
	Surgical procedures	Yes	19 (38.0%)	31 (62.0%)	50
		No	193 (14.3%)	1,153 (85.7%)	1,346
	Thoracic drainage	Yes	2 (12.5%)	14 (87.5%)	16
		No	210 (15.2%)	1,170 (84.8%)	1,380
	Phlebotomy	Yes	6 (46.2%)	7 (53.8%)	13
		No	206 (14.9%)	1,177 (85.1%)	1,383
	Tracheostomy	Yes	5 (55.6%)	4 (44.4%)	9
		No	207 (14.9%)	1,180 (85.1%)	1,387
Complications occurring during hospitalization	Bronchopulmonary dysplasia	Yes	5 (6.7%)	70 (93.3%)	75
		No	207 (15.7%)	1,114 (84.3%)	1,321
	Pulmonary hypertension	Yes	26 (42.6%)	35 (57.4%)	61
		No	186 (13.9%)	1,149 (86.1%)	1,335
	Septic shock	Yes	22 (43.1%)	29 (56.9%)	51
		No	190 (14.1%)	1,155 (85.9%)	1,345
	Necrotizing enterocolitis	Yes	12 (28.6%)	30 (71.4%)	42
		No	200 (14.8%)	1,154 (85.2%)	1,354
	Meningitis	Yes	0 (0.0%)	11 (100%)	11
		No	212 (15.3%)	1,173 (84.7%)	1,385
Antibiotics and antifungals administered	Ampicillin	Yes	163 (18.2%)	735 (81.8%)	898
		No	49 (9.8%)	449 (90.2%)	498
	Gentamicin	Yes	163 (18.3%)	730 (81.7%)	893
		No	49 (9.7%)	454 (90.3%)	503
	Vancomycin	Yes	58 (25.6%)	169 (74.4%)	227
		No	154 (13.2%)	1,015 (86.8%)	1,169
	Cefepime	Yes	50 (29.1%)	122 (70.9%)	172
		No	162 (13.2%)	1,062 (86.8%)	1,224
	Oxacillin	Yes	22 (25.9%)	63 (74.1%)	85
		No	190 (14.5%)	1,121 (85.5%)	1,311
	Piperacillin/Tazobactam	Yes	16 (19.5%)	66 (80.5%)	82
		No			

Supplementary Table 4. Clinical and therapeutic characteristics of newborns with neonatal sepsis according to clinical outcome (hospital discharge or death).

Markers	Category	Groups	Death [N (%)]	Hospital discharge [N (%)]	Total
Amikacin		No	16 (14.9%)	1,118 (85.1%)	1,314
		Yes	19 (24.1%)	60 (75.9%)	79
Meropenem		No	193 (14.7%)	1,124 (85.3%)	1,317
		Yes	16 (28.6%)	40 (71.4%)	56
Metronidazole		No	196 (14.6%)	1,144 (85.4%)	1,340
		Yes	18 (34.0%)	35 (66.0%)	53
Fluconazole		No	194 (14.4%)	1,149 (85.6%)	1,343
		Yes	15 (48.4%)	16 (51.6%)	31
Other		No	197 (14.4%)	1,168 (85.6%)	1,365
		Yes	12 (14.8%)	69 (85.2%)	81
		No	200 (15.2%)	1,115 (84.8%)	1,396

The data are presented as absolute (N) and relative (%) frequencies.

Supplementary Table 5. Multivariable analysis to identify the main antibiotics associated with early-onset sepsis (early-onset sepsis with positive culture and presumed early-onset sepsis) among Neonatal Intensive Care Unit patients of a university hospital.

Step	Marker	B	SE	Wald	df	P	OR (95% CI)
Step 1	Ampicillin	5.142	0.629	66.798	1	< 0.001	171.089 (49.851 to 587.176)
	Gentamicin	5.952	0.655	82.662	1	< 0.001	384.684 (106.617 to 1,387.979)
	Amikacin	0.100	1.385	0.005	1	0.943	1.105 (0.073 to 16.674)
	Cefepime	0.004	1.185	< 0.001	1	0.997	1.004 (0.098 to 10.255)
	Vancomycin	0.906	1.324	0.469	1	0.494	2.475 (0.185 to 33.146)
	Meropenem	0.770	1.791	0.185	1	0.667	2.159 (0.065 to 72.248)
	Piperacillin/Tazobactam	-1.838	1.351	1.849	1	0.174	0.159 (0.011 to 2.250)
	Metronidazole	-0.813	1.321	0.379	1	0.538	0.444 (0.033 to 5.904)
	Oxacillin	0.993	1.557	0.407	1	0.524	2.699 (0.128 to 57.088)
	Fluconazole	-0.694	1.523	0.208	1	0.649	0.500 (0.025 to 9.878)
	Constant	-5.979	0.686	75.962	1	< 0.001	0.003
Step 2	Ampicillin	5.142	0.624	67.952	1	< 0.001	171.141 (50.390 to 581.257)
	Gentamicin	5.952	0.648	84.368	1	< 0.001	384.550 (107.986 to 1,369.428)
	Amikacin	0.099	1.385	0.005	1	0.943	1.105 (0.073 to 16.667)
	Vancomycin	0.910	0.885	1.058	1	0.304	2.484 (0.439 to 14.069)
	Meropenem	0.771	1.780	0.187	1	0.665	2.161 (0.066 to 70.786)
	Piperacillin/Tazobactam	-1.840	1.147	2.575	1	0.109	0.159 (0.017 to 1.503)
	Metronidazole	-0.812	1.319	0.380	1	0.538	0.444 (0.033 to 5.881)
	Oxacillin	0.993	1.557	0.407	1	0.524	2.699 (0.128 to 57.077)
	Fluconazole	-0.693	1.507	0.212	1	0.646	0.500 (0.026 to 9.582)
	Constant	-5.979	0.684	76.373	1	< 0.001	0.003
Step 3	Ampicillin	5.145	0.622	68.318	1	< 0.001	171.511 (50.639 to 580.905)
	Gentamicin	5.941	0.629	89.298	1	< 0.001	380.332 (110.921 to 1,304.103)
	Vancomycin	0.920	0.875	1.104	1	0.293	2.508 (0.451 to 13.950)
	Meropenem	0.770	1.787	0.186	1	0.666	2.161 (0.065 to 71.750)
	Piperacillin/Tazobactam	-1.845	1.145	2.595	1	0.107	0.158 (0.017 to 1.491)
	Metronidazole	-0.770	1.192	0.417	1	0.518	0.463 (0.045 to 4.789)
	Oxacillin	1.074	1.067	1.014	1	0.314	2.928 (0.362 to 23.704)
	Fluconazole	-0.712	1.488	0.229	1	0.632	0.491 (0.027 to 9.071)
	Constant	-5.971	0.674	78.550	1	< 0.001	0.003
Step 4	Ampicillin	5.143	0.623	68.103	1	< 0.001	171.225 (50.477 to 580.816)
	Gentamicin	5.933	0.628	89.361	1	< 0.001	377.392 (110.289 to 1,291.379)
	Vancomycin	0.971	0.874	1.235	1	0.267	2.641 (0.476 to 14.645)
	Piperacillin/Tazobactam	-1.765	1.140	2.399	1	0.121	0.171 (0.018 to 1.598)
	Metronidazole	-0.634	1.166	0.295	1	0.587	0.530 (0.054 to 5.218)
	Oxacillin	1.045	1.066	0.961	1	0.327	2.845 (0.352 to 22.993)
	Fluconazole	-0.556	1.433	0.150	1	0.698	0.574 (0.035 to 9.519)
	Constant	-5.975	0.675	78.450	1	< 0.001	0.003
Step 5	Ampicillin	5.175	0.617	70.367	1	< 0.001	176.867 (52.782 to 592.663)
	Gentamicin	5.896	0.619	90.643	1	< 0.001	363.422 (107.973 to 1,223.232)
	Vancomycin	0.942	.870	1.171	1	0.279	2.564 (0.466 to 14.117)
	Piperacillin/Tazobactam	-1.798	1.142	2.477	1	0.115	0.166 (0.018 to 1.554)
	Metronidazole	-0.741	1.150	0.416	1	0.519	0.476 (0.050 to 4.535)
	Oxacillin	1.057	1.068	0.979	1	0.323	2.878 (0.354 to 23.364)
	Constant	-5.979	0.676	78.181	1	< 0.001	0.003
Step 6	Ampicillin	5.122	0.610	70.394	1	< 0.001	167.676 (50.679 to 554.772)
	Gentamicin	5.956	0.617	93.300	1	< 0.001	386.149 (115.312 to 1,293.116)
	Vancomycin	0.968	0.873	1.230	1	.267	2.633 (0.476 to 14.582)
	Piperacillin/Tazobactam	-1.874	1.133	2.736	1	0.098	0.153 (0.017 to 1.414)
	Oxacillin	1.096	1.062	1.066	1	0.302	2.994 (0.374 to 23.988)
	Constant	-6.029	0.681	78.417	1	< 0.001	0.002
Step 7	Ampicillin	5.068	0.604	70.388	1	< 0.001	158.827 (48.613 to 518.909)
	Gentamicin	5.875	0.600	95.927	1	< 0.001	355.901 (109.843 to 1,153.152)
	Vancomycin	1.074	0.862	1.553	1	0.213	2.927 (0.541 to 15.850)

Supplementary Table 5. Multivariable analysis to identify the main antibiotics associated with early-onset sepsis (early-onset sepsis with positive culture and presumed early-onset sepsis) among Neonatal Intensive Care Unit patients of a university hospital.

Step	Marker	B	SE	Wald	df	P	OR (95% CI)
	Piperacillin/Tazobactam	-1.783	1.117	2.546	1	0.111	0.168 (0.019 to 1.502)
	Constant	-5.886	0.649	82.288	1	< 0.001	0.003
Step 8	Ampicillin	5.159	0.594	75.525	1	< 0.001	173.978 (54.350 to 556.918)
	Gentamicin	5.727	0.567	102.000	1	< 0.001	307.021 (101.040 to 932.911)
	Piperacillin/Tazobactam	-0.912	0.863	1.118	1	0.290	0.402 (0.074 to 2.180)
	Constant	-5.690	0.599	90.383	1	< 0.001	0.003
Step 9	Ampicillin	5.094	0.587	75.291	1	< 0.001	163.042 (51.592 to 515.245)
	Gentamicin	5.737	0.564	103.626	1	< 0.001	310.147 (102.766 to 936.024)
	Constant	-5.752	0.603	90.904	1	< 0.001	0.003

The multivariable logistic regression model was performed using the backward stepwise method, with the diagnosis of sepsis as the dependent variable. Model results were presented as regression coefficients, standard errors (SE), Wald statistics, odds ratios (ORs), and corresponding 95% confidence intervals (CIs). The model demonstrated excellent discriminative ability, with an area under the receiver operating characteristic (ROC) curve (AUC) of 0.996 (SE = 0.002; 95% CI = 0.992 to 0.999; $p < 0.001$). For all analyses, the significance level was set at $\alpha = 0.05$.

Supplementary Table 6. Multivariable analysis to identify the main antibiotics associated with late-onset sepsis (late-onset sepsis with positive culture and presumed late-onset sepsis) among Neonatal Intensive Care Unit patients of a university hospital.

Step	Marker	B	SE	Wald	df	P	OR (95% CI)
Step 1	Ampicillin	-0.888	0.895	0.984	1	0.321	0.412 (0.071 to 2.379)
	Gentamicin	1.770	0.894	3.917	1	0.048	5.873 (1.017 to 33.903)
	Amikacin	4.500	0.741	36.885	1	< 0.001	90.051 (21.074 to 384.799)
	Cefepime	2.905	0.503	33.403	1	< 0.001	18.259 (6.818 to 48.896)
	Vancomycin	4.593	0.539	72.587	1	< 0.001	98.796 (34.344 to 284.201)
	Meropenem	-0.338	0.729	0.214	1	0.643	0.713 (0.171 to 2.980)
	Piperacillin/Tazobactam	3.379	0.952	12.601	1	< 0.001	29.342 (4.542 to 189.562)
	Metronidazole	-0.431	0.697	0.382	1	0.536	0.650 (0.166 to 2.549)
	Oxacillin	2.678	0.690	15.064	1	< 0.001	14.549 (3.764 to 56.241)
	Fluconazole	2.652	1.274	4.335	1	0.037	14.183 (1.168 to 172.188)
	Constant	-4.765	0.407	136.847	1	< 0.001	0.009
Step 2	Ampicillin	0.895	0.889	1.013	1	0.314	0.409 (0.072 to 2.334)
	Gentamicin	1.771	0.887	3.985	1	0.046	5.878 (1.033 to 33.454)
	Amikacin	4.524	0.740	37.398	1	< 0.001	92.213 (21.631 to 393.095)
	Cefepime	2.931	0.497	34.790	1	< 0.001	18.738 (7.076 to 49.617)
	Vancomycin	4.489	0.485	85.717	1	< 0.001	89.041 (34.425 to 230.310)
	Piperacillin/Tazobactam	3.429	0.944	13.197	1	< 0.001	30.853 (4.850 to 196.252)
	Metronidazole	-0.436	0.692	0.398	1	0.528	0.646 (0.167 to 2.509)
	Oxacillin	2.647	0.689	14.774	1	< 0.001	14.111 (3.659 to 54.421)
	Fluconazole	2.533	1.264	4.014	1	0.045	12.595 (1.057 to 150.122)
	Constant	-4.765	0.407	137.085	1	< 0.001	0.009
Step 3	Ampicillin	-0.974	0.857	1.292	1	0.256	0.377 (0.070 to 2.026)
	Gentamicin	1.832	0.858	4.563	1	0.033	6.248 (1.163 to 33.560)
	Amikacin	4.399	0.706	38.796	1	< 0.001	81.362 (20.383 to 324.767)
	Cefepime	2.946	0.497	35.134	1	< 0.001	19.036 (7.186 to 50.429)
	Vancomycin	4.427	0.472	87.797	1	< 0.001	83.672 (33.146 to 211.221)
	Piperacillin/Tazobactam	3.493	0.935	13.968	1	< 0.001	32.889 (5.266 to 205.411)
	Oxacillin	2.696	0.681	15.663	1	< 0.001	14.825 (3.900 to 56.349)
	Fluconazole	2.351	1.241	3.590	1	0.058	10.499 (0.922 to 119.525)
	Constant	-4.765	0.406	137.472	1	< 0.001	0.009
Step 4	Ampicillin	0.967	0.412	5.515	1	0.019	2.630 (1.173 to 5.895)
	Amikacin	4.245	0.675	39.606	1	< 0.001	69.762 (18.597 to 261.690)
	Cefepime	2.901	0.492	34.726	1	< 0.001	18.200 (6.934 to 47.771)
	Vancomycin	4.418	0.472	87.564	1	< 0.001	82.903 (32.864 to 209.130)
	Piperacillin/Tazobactam	3.405	0.938	13.170	1	< 0.001	30.113 (4.787 to 189.413)
	Oxacillin	2.791	0.664	17.660	1	< 0.001	16.303 (4.435 to 59.933)
	Fluconazole	2.615	1.271	4.235	1	0.040	13.674 (1.133 to 165.071)
	Constant	-4.842	0.408	141.125	1	< 0.001	0.008

The multivariable logistic regression model was performed using the backward stepwise method, with the diagnosis of sepsis as the dependent variable. Model results were presented as regression coefficients, standard errors (SE), Wald statistics, odds ratios (ORs), and corresponding 95% confidence intervals (CIs). The model demonstrated excellent discriminative ability, with an area under the receiver operating characteristic (ROC) curve (AUC) of 0.970 (SE = 0.007; 95% CI = 0.956 to 0.984; $p < 0.001$). For all analyses, the significance level was set at $\alpha = 0.05$.

Supplementary Table 7. Multivariable analysis to identify the main antibiotics associated with early-onset sepsis and late-onset among Neonatal Intensive Care Unit patients of a university hospital.

Steps	Markers	B	SE	Wald	df	P	OR (95% CI)
Step 1	Ampicillin	4.872	0.679	51.549	1	< 0.001	130.550 (34.530 to 493.571)
	Gentamicin	5.774	0.700	68.124	1	< 0.001	321.964 (81.713 to 1,268.591)
	Amikacin	3.381	1.164	8.435	1	0.004	29.397 (3.002 to 287.852)
	Cefepime	0.846	1.092	0.600	1	0.438	2.330 (0.274 to 19.804)
	Vancomycin	7.203	1.249	33.256	1	< 0.001	1,344.076 (116.190 to 15,548.147)
	Meropenem	20.116	3464.711	< 0.001	1	0.995	544,594,886.189 (< 0.001 to .)
	Piperacillin/Tazobactam	2.918	2.678	1.187	1	0.276	18.499 (0.097 to 3,521.832)
	Metronidazole	-1.592	0.971	2.687	1	0.101	0.203 (0.030 to 1.365)
	Oxacillin	4.640	1.124	17.033	1	< 0.001	103.513 (11.431 to 937.375)
	Fluconazole	-1.428	1.696	.709	1	0.400	0.240 (0.009 to 6.658)
	Constant	-5.424	0.615	77.680	1	< 0.001	0.004
Step 2	Ampicillin	4.929	0.671	53.951	1	< 0.001	138.260 (37.109 to 515.131)
	Gentamicin	5.673	0.679	69.796	1	< 0.001	290.869 (76.861 to 1,100.752)
	Amikacin	3.066	1.083	8.020	1	0.005	21.453 (2.570 to 179.049)
	Vancomycin	7.763	1.072	52.398	1	< 0.001	2,351.513 (287.416 to 19,239.068)
	Meropenem	19.514	3528.433	< 0.001	1	0.996	298,428,506.567 (< 0.001 to .)
	Piperacillin/Tazobactam	2.863	2.671	1.149	1	0.284	17.515 (0.093 to 3291.286)
	Metronidazole	-1.462	.973	2.258	1	0.133	0.232 (0.034 to 1.561)
	Oxacillin	4.973	1.031	23.250	1	< 0.001	144.407 (19.132 to 1,089.967)
	Fluconazole	-1.299	1.660	0.612	1	0.434	0.273 (0.011 to 7.063)
	Constant	-5.369	0.600	80.100	1	< 0.001	0.005
Step 3	Ampicillin	5.004	0.663	57.022	1	< 0.001	148.936 (40.643 to 545.773)
	Gentamicin	5.606	0.674	69.259	1	< 0.001	272.176 (72.680 to 1,019.252)
	Amikacin	3.030	1.083	7.824	1	0.005	20.697 (2.477 to 172.968)
	Vancomycin	7.667	1.059	52.408	1	< 0.001	2,136.067 (268.018 to 17,024.152)
	Meropenem	18.305	3,714.140	< 0.001	1	0.996	89,115,164.113 (< 0.001 to .)
	Piperacillin/Tazobactam	2.922	2.646	1.219	1	0.270	18.570 (0.104 to 3,321.572)
	Metronidazole	-1.400	0.970	2.082	1	0.149	0.247 (0.037 to 1.652)
	Oxacillin	5.026	1.034	23.616	1	< 0.001	152.391 (20.070 to 1,157.126)
	Constant	-5.406	0.605	79.840	1	< 0.001	0.004
Step 4	Ampicillin	4.890	0.660	54.962	1	< 0.001	132.962 (36.498 to 484.378)
	Gentamicin	5.640	0.683	68.280	1	< 0.001	281.492 (73.871 to 1,072.652)
	Amikacin	2.593	1.074	5.829	1	0.016	13.368 (1.629 to 109.700)
	Vancomycin	7.260	0.972	55.839	1	< 0.001	1,421.847 (211.784 to 9,545.790)
	Meropenem	17.344	4,125.166	< 0.001	1	0.997	34,083,505.283 (< 0.001 to .)
	Piperacillin/Tazobactam	2.899	2.687	1.164	1	0.281	18.157 (0.094 to 3,515.916)
	Oxacillin	5.235	1.060	24.400	1	< 0.001	187.761 (23.522 to 1,498.774)
	Constant	-5.417	0.611	78.563	1	< 0.001	0.004
Step 5	Ampicillin	4.876	0.659	54.795	1	< 0.001	131.167 (36.064 to 477.060)
	Gentamicin	5.625	0.682	68.105	1	< 0.001	277.341 (72.913 to 1,054.930)
	Amikacin	2.616	1.076	5.908	1	0.015	13.685 (1.660 to 112.833)
	Vancomycin	7.702	0.959	64.552	1	< 0.001	2212.305 (337.974 to 14,481.260)
	Meropenem	17.211	4,102.625	< 0.001	1	0.997	29,839,005.289 (< 0.001 to .)
	Oxacillin	5.224	1.060	24.286	1	< 0.001	185.759 (23.257 to 1,483.684)
	Constant	-5.393	0.609	78.348	1	< 0.001	0.005
Step 6	Ampicillin	4.889	0.657	55.384	1	< 0.001	132.837 (36.654 to 481.417)
	Gentamicin	5.603	0.678	68.366	1	< 0.001	271.112 (71.843 to 1,023.081)
	Amikacin	2.327	1.017	5.234	1	0.022	10.247 (1.396 to 75.226)
	Vancomycin	7.909	0.951	69.092	1	< 0.001	2,721.598 (421.611 to 17,568.568)
	Oxacillin	5.587	0.992	31.688	1	< 0.001	266.876 (38.153 to 1,866.791)
	Constant	-5.377	0.606	78.740	1	< 0.001	0.005

The multivariable logistic regression model was performed using the backward stepwise method, with the diagnosis of sepsis as the dependent variable. Model results were presented as regression coefficients, standard errors (SE), Wald statistics, odds ratios (ORs), and corresponding 95% confidence intervals (CIs). The model demonstrated excellent discriminative ability, with an area under the receiver operating characteristic (ROC) curve (AUC) of 0.997 (SE = 0.002; 95% CI = 0.994 to 1.000; $p < 0.001$). For all analyses, the significance level was set at $\alpha = 0.05$.

4. CAPÍTULO 2: ARTIGO 2 SUBMETIDO

Title: Clinical Marker Profiles Comparing Confirmed and Presumed Neonatal Sepsis in Early- and Late-Onset Sepsis within a Neonatal Intensive Care Unit Cohort

Short Title: Clinical Markers in Early- and Late-Onset Neonatal Sepsis

Patricia Krause Askinis ^{1,2,3,#}, Fernando Augusto Lima Marson ^{1,2,3,*,#}

¹ Laboratory of Molecular Biology and Genetics, Postgraduate Program in Health Sciences, Postgraduate Program in Health Data Science, São Francisco University, Bragança Paulista, São Paulo, Brazil.

² Laboratory of Clinical and Molecular Microbiology, Postgraduate Program in Health Sciences, Postgraduate Program in Health Data Science, São Francisco University, Bragança Paulista, São Paulo, Brazil.

³ LunGuardian Research Group — Epidemiology of Respiratory and Infectious Diseases, Postgraduate Program in Health Sciences, Postgraduate Program in Health Data Science, São Francisco University, Bragança Paulista, São Paulo, Brazil.

*** Corresponding Author:** [FALM] Fernando Augusto Lima Marson, BSc, MSc, PhD.

Laboratory of Molecular Biology and Genetics, Laboratory of Clinical and Molecular Microbiology, LunGuardian Research Group — Epidemiology of Respiratory and Infectious Diseases, Postgraduate Program in Health Sciences, Postgraduate Program in Health Data Science, São Francisco University. Avenida São Francisco de Assis, 218, Jardim São José, Bragança Paulista 12916-900, São Paulo, Brazil. Phone number: +55 19 99975-2911.

E-mail: fernandolimamarson@hotmail.com and fernando.marson@usf.edu.br.

[#] The authors contributed equally to this study.

Declarations

Funding: This work was supported by a Productivity Scholarship (FALM) from the CNPq, grant number: 305906/2024-0.

Conflicts of interest: The authors declare no conflicts of interest related to the development of this study.

Availability of data and material: The materials used in this study are available from the corresponding author upon reasonable request.

Ethical approval: The study was conducted in accordance with the principles of the Declaration of Helsinki and was approved by the Institutional Ethics Committee (Ethical approval number: 80862224.4.0000.5514; Study approval number: 6.994.663).

Authors' contributions: [PKA and FALM] were responsible for data collection and tabulation. [PKA and FALM] contributed to the interpretation of the study findings. [PKA, FALM] were involved in drafting and critically revising the manuscript prior to submission. All authors [PKA and FALM] approved the final version of the manuscript and consented to its submission to the scientific journal.

Acknowledgments: The Article Processing Charge for this publication was covered by the Brazilian Coordination for the Improvement of Higher Education Personnel (*Coordenação de Aperfeiçoamento de Pessoal de Nível*

Superior — CAPES), ROR number: 00x0ma614. For open access purposes, the authors have assigned the Creative Commons CC BY license to any accepted version of the article.

Study authors' e-mails and ORCIDs:

PKA: patriciakrause14@gmail.com

ORCID: 0009-0000-8067-0677

FALM: fernandolimamarson@hotmail.com and fernando.marson@usf.edu.br

ORCID: 0000-0003-4955-4234

Abstract

Introduction: Neonatal sepsis remains a major cause of morbidity and mortality in neonatal intensive care units, particularly among preterm infants. Distinguishing confirmed from presumed sepsis and early- from late-onset sepsis remains challenging because of nonspecific clinical manifestations and limited microbiological sensitivity. Therefore, this study aimed to evaluate the profile of clinical markers according to neonatal sepsis classification in a neonatal intensive care unit cohort.

Methods: This retrospective observational study analyzed secondary clinical and microbiological data from newborns admitted to the NICU of a tertiary university hospital between 2017 and 2024. A total of 956 newborns with clinical suspicion of neonatal sepsis and at least one microbiological culture were included. Sepsis was classified as early- or late-onset and as confirmed or presumed according to microbiological findings and clinical criteria. Clinical, demographic, microbiological, and outcome-related variables were collected from medical records. The study was approved by the institutional Research Ethics Committee.

Results: A total of 956 newborns with clinical suspicion of neonatal sepsis were included in the study. Presumed sepsis predominated in both early-onset neonatal sepsis and late-onset neonatal sepsis. Prematurity was highly prevalent across all groups and demonstrated significant associations with confirmed early-onset neonatal sepsis ($p = 0.019$) and confirmed late-onset neonatal sepsis ($p = 0.014$). Confirmed late-onset neonatal sepsis also demonstrated significant temporal variation across pre-pandemic, pandemic, and post-pandemic periods ($p = 0.003$). Maternal antibiotic exposure before delivery was significantly more frequent among confirmed late-onset neonatal sepsis cases ($p = 0.013$). Respiratory distress, apnea, and perinatal depression were observed in more than 95% of cases, while prolonged hospitalization and elevated mortality rates were identified in both early-onset neonatal sepsis and late-onset neonatal sepsis groups. Coagulase-negative *Staphylococci* represented the most frequently isolated microorganisms in confirmed sepsis, followed by *Staphylococcus aureus*, *Escherichia coli*, and *Klebsiella pneumoniae*. Empirical antimicrobial therapy was nearly universal, particularly involving ampicillin and gentamicin.

Conclusions: Neonatal sepsis in critically ill newborns was strongly associated with prematurity, prolonged hospitalization, intensive care interventions, and substantial mortality. Although presumed sepsis predominated, microbiologically confirmed cases demonstrated important differences in gestational age distribution, temporal patterns, microbiological profile, and antimicrobial utilization. The predominance of coagulase-negative *Staphylococci* and the extensive use of empirical antimicrobial therapy reinforce the diagnostic and therapeutic challenges associated with neonatal sepsis in NICU settings. These findings contribute to improved understanding of real-world neonatal sepsis profiles and may support more individualized diagnostic and antimicrobial stewardship strategies.

Keywords: Antimicrobial Stewardship. Blood Culture. Healthcare-Associated Infection. Neonatal Intensive Care Unit. Nosocomial Infection. Premature Infant.

1. Introduction

Neonatal sepsis remains one of the leading causes of morbidity and mortality among newborns admitted to neonatal intensive care units (NICUs), particularly among preterm infants and those with low birth weight ^{1,2}. The immaturity of the neonatal immune system, combined with the frequent need for intensive support and exposure to invasive procedures, substantially increases susceptibility to infection and contributes to the rapid progression of infectious conditions in this population ^{3,4}.

From a clinical perspective, neonatal sepsis is characterized by marked heterogeneity in presentation, ranging from subtle and nonspecific manifestations to severe conditions associated with hemodynamic instability, respiratory failure, and multiple organ dysfunction ⁵. This variability makes early recognition particularly challenging, especially during the first hours of life, when clinical signs may be minimal and laboratory tests may not yet fully reflect the systemic inflammatory response ⁵.

The classification of neonatal sepsis into early-onset and late-onset forms, as well as into confirmed and presumed sepsis, is widely adopted in clinical practice and international guidelines because it reflects important differences in infection acquisition pathways, etiological profiles, prognosis, and therapeutic management ^{1,6,7}. Nevertheless, despite these classification systems, distinguishing true infection from nonspecific inflammatory responses remains a persistent challenge in neonatal intensive care settings ^{8–10}.

Although blood culture remains the gold standard for etiological confirmation of neonatal sepsis, its diagnostic sensitivity is limited in neonates, particularly among infants previously exposed to empirical antimicrobial therapy or when insufficient blood volumes are collected for microbiological analysis ^{8,11}. Consequently, a substantial proportion of cases are classified as presumed sepsis, often leading to prolonged antimicrobial exposure even in the absence of microbiological confirmation ¹⁰.

In this context, the assessment of clinical markers associated with different neonatal sepsis classifications plays a critical role in supporting diagnostic reasoning and therapeutic decision-making ^{5,12,13}. However, despite the extensive literature on neonatal sepsis, relatively few studies have comprehensively evaluated clinical markers according to sepsis classification, particularly using real-world data derived from NICUs and heterogeneous populations of critically ill newborns ^{12,13}.

Furthermore, a considerable proportion of international studies has focused predominantly on isolated laboratory biomarkers or highly selected patient populations, which may limit the applicability of their findings to routine clinical practice ^{9,10,14}. Evaluating clinical markers alongside routinely available microbiological findings may provide a more pragmatic and clinically applicable approach, especially in healthcare settings with heterogeneous diagnostic and therapeutic resources ^{5,11,12}.

Therefore, the present study aimed to evaluate the profile of clinical markers according to neonatal sepsis classification in a cohort of newborns admitted to a neonatal intensive care unit, comparing confirmed and presumed sepsis in both early-onset and late-onset sepsis, while also analyzing microbiological findings obtained during hospitalization. By exploring these associations, this study seeks to contribute to the improvement of early clinical recognition of neonatal sepsis and to support therapeutic decision-making in high-complexity neonatal care settings.

2. Methods

2.1. Study Design and Setting

This retrospective observational study with a quantitative approach was conducted through secondary analysis of clinical and microbiological data obtained from newborns admitted to the NICU of a tertiary university hospital specialized in high-risk pregnancies and neonatal care. The study period extended from January 1, 2017, to December 31, 2024. The primary objective was to evaluate the profile of clinical and microbiological markers according to neonatal sepsis classification, comparing confirmed and presumed sepsis in both early- and late-onset neonatal sepsis.

2.2. Study Population and Sampling Criteria

The study population consisted of newborns admitted to the NICU of the university hospital between January 1, 2017, and December 31, 2024.

After approval by the institutional Research Ethics Committee, a list of hospitalized patients containing identification and admission-related information was obtained from the institutional statistics department. Based on this list, a standardized electronic database was developed to organize the study sample and systematize data collection procedures.

Clinical, demographic, obstetric, and healthcare-related information was retrospectively extracted from physical medical records. Microbiological data, particularly culture results and antimicrobial resistance profiles, were complemented through consultation of electronic laboratory medical records.

Initially, 1,630 medical records of newborns admitted to the NICU during the study period were identified. A total of 166 patients older than 28 days at admission and 68 records with insufficient clinical or microbiological information for neonatal sepsis classification were excluded. Additionally, 440 medical records were excluded because the newborns did not present clinical suspicion or diagnosis of neonatal sepsis. These data were not included in the present analyses because they were outside the scope and objectives of the study. The final study population consisted of 956 newborns.

For analytical purposes, newborns presenting clinical suspicion of sepsis and at least one microbiological culture collected during hospitalization were included in the sepsis-related analyses, totaling 956 newborns, regardless of microbiological confirmation.

2.3. Variables and Classification Criteria

The analyzed variables were organized into a standardized electronic spreadsheet developed according to institutional data availability and the study-specific data collection instrument. The following variables were included:

(i) Clinical and Perinatal Characteristics: Gestational age, birth weight, sex, Apgar scores at the 1st and 5th minutes of life, and major associated clinical diagnoses were collected.

(ii) Microbiological Markers: Microbiological variables included type of biological sample collected, culture result (positive or negative), isolated microorganism, Gram staining classification (Gram-positive or Gram-negative), and antimicrobial resistance profile described in laboratory reports.

(iii) Neonatal Sepsis Classification: Neonatal sepsis was classified according to the timing of symptom onset as early-onset sepsis (≤ 72 hours of life) or late-onset sepsis (> 72 hours of life). In addition, cases were categorized as confirmed sepsis when microbiological isolation was identified in culture, or presumed sepsis in the absence of microbiological confirmation but in the presence of suggestive clinical manifestations requiring empirical antimicrobial therapy according to institutional protocols. Some newborns experienced more than one sepsis episode during hospitalization and could therefore be simultaneously classified as having early- and late-onset sepsis for analytical purposes.

(iv) Clinical Outcomes: Final clinical outcomes were categorized as hospital discharge or in-hospital death.

2.4. Statistical Analysis

Statistical analyses were performed using IBM SPSS Statistics software, version 28.0. Categorical variables were described using absolute and relative frequencies. Comparisons of clinical and microbiological markers between neonatal sepsis groups were performed using Pearson's chi-square test or Fisher's exact test, when appropriate.

The association between neonatal sepsis classifications and the clinical outcome of death was evaluated using binary logistic regression analysis, with estimation of odds ratios (ORs) and corresponding 95% confidence intervals (95% CIs). Statistical significance was defined as a two-tailed p-value ≤ 0.05 .

2.5. Ethical Considerations

The study was approved by the institutional Research Ethics Committee under CAAE number 80862224.4.0000.5514 (**Anexo 1**). Because this was a retrospective study based exclusively on secondary data analysis without direct participant contact, the requirement for written informed consent was waived. All data were anonymized prior to analysis and were used exclusively for scientific purposes.

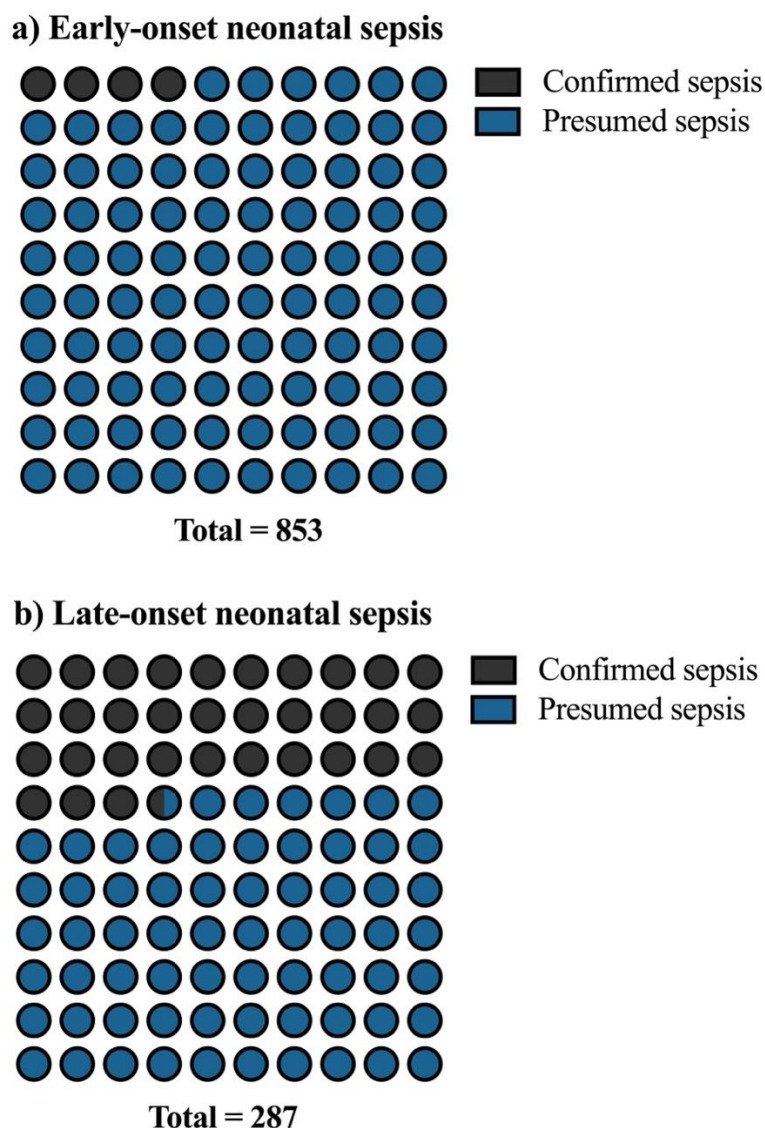
3. Results

3.1. Study population and general characteristics

A total of 1,630 medical records of newborns admitted to the NICU between January 2017 and December 2024 were initially screened. After exclusion of patients older than 28 days at admission, records with insufficient clinical or microbiological information, and newborns without clinical suspicion of neonatal sepsis, the final study population consisted of 956 newborns evaluated for neonatal sepsis. Among these, cases were categorized according to timing of onset as early-onset neonatal sepsis or late-onset neonatal sepsis, and further stratified into confirmed and presumed sepsis based on microbiological findings.

Presumed sepsis represented the majority of cases in both early-onset neonatal sepsis and late-onset neonatal sepsis groups. Early-onset presumed sepsis accounted for 853 cases, whereas only 36 newborns fulfilled criteria for confirmed early-onset neonatal sepsis (**Figure 1**). Similarly, 191 newborns were classified as presumed late-onset neonatal sepsis and 96 as confirmed late-onset neonatal sepsis, highlighting the predominance of clinically suspected sepsis episodes without microbiological confirmation in routine NICU practice (**Figure 1**).

Figure 1. Distribution of early- and late-onset neonatal sepsis according to microbiological confirmation status in newborns admitted to the neonatal intensive care unit (NICU).



This figure illustrates the distribution of neonatal sepsis cases stratified according to timing of onset and microbiological confirmation status among newborns admitted to the NICU between January 2017 and December 2024. Sepsis episodes were categorized as early-onset neonatal sepsis (≤ 72 hours of life) or late-onset neonatal sepsis (> 72 hours of life), and further classified as confirmed sepsis when microbiological isolation was identified in culture or presumed sepsis when clinical manifestations compatible with sepsis required empirical antimicrobial therapy despite negative cultures. The figure demonstrates the marked predominance of presumed sepsis in both early-onset and late-onset neonatal sepsis groups, highlighting the substantial frequency of clinically suspected but culture-negative sepsis episodes in neonatal intensive care practice. Absolute frequencies and corresponding percentages are presented for each subgroup.

3.2. Perinatal and demographic characteristics

The distribution of demographic and perinatal characteristics according to neonatal sepsis classification is summarized in **Table 1**. Among newborns with early-onset neonatal sepsis, confirmed sepsis was significantly associated with prematurity patterns when compared with presumed sepsis ($p = 0.019$). Moderate-to-late prematurity was proportionally more frequent among confirmed early-onset neonatal sepsis cases (44.4%),

whereas term newborns were more commonly observed in presumed early-onset neonatal sepsis (26.6%). Similarly, among late-onset neonatal sepsis cases, confirmed sepsis demonstrated a significantly higher proportion of extremely preterm newborns (38.5%) compared with presumed sepsis (20.4%) ($p = 0.014$).

Low birth weight, very low birth weight, and extremely low birth weight predominated across all neonatal sepsis groups, reinforcing the strong association between neonatal immaturity and susceptibility to infectious complications requiring NICU admission. No statistically significant differences were identified regarding sex distribution, healthcare coverage, postnatal age classification, or Apgar scores between confirmed and presumed sepsis groups in either early-onset neonatal sepsis or late-onset neonatal sepsis analyses.

Despite the absence of statistical significance for most variables, a predominance of male sex was consistently observed across all groups. In addition, moderate impairment at the 1st-minute Apgar score represented the most frequent category in both early-onset neonatal sepsis and late-onset neonatal sepsis populations, whereas most newborns demonstrated recovery to mild impairment ranges at the 5th minute of life.

Table 1. Perinatal and demographic characteristics according to early- and late-onset neonatal sepsis classification.

Early-onset neonatal sepsis					
Marker	Groups	Confirmed sepsis [n (%)]	Presumed sepsis [n (%)]	Total	p-value
Type of healthcare coverage	Public healthcare system	33 (91.7%)	747 (87.6%)	780 (87.7%)	0.609
	Private healthcare/insurance	3 (8.3%)	106 (12.4%)	109 (12.3%)	
Postnatal age classification	Day 1 of life	28 (77.8%)	706 (82.8%)	734 (82.6%)	0.440
	Postnatal days >1	8 (22.2%)	147 (17.2%)	155 (17.4%)	
Sex	Female	14 (38.9%)	353 (41.4%)	367 (41.3%)	0.761
	Male	22 (61.1%)	499 (58.6%)	521 (58.7%)	
Gestational age	Term	5 (13.9%)	227 (26.6%)	232 (26.1%)	0.019
	Post-term	0 (0.0%)	23 (2.7%)	23 (2.6%)	
	Moderate-late preterm	16 (44.4%)	166 (19.5%)	182 (20.5%)	
	Very preterm	10 (27.8%)	296 (34.7%)	306 (34.4%)	
	Extremely preterm	5 (13.9%)	141 (16.5%)	146 (16.4%)	
Birth weight	Appropriate birth weight	5 (13.9%)	219 (25.7%)	224 (25.2%)	0.244
	Macrosomia	—	10 (1.2%)	10 (1.1%)	
	Low birth weight	18 (50.0%)	271 (31.8%)	289 (32.5%)	
	Very low birth weight	8 (22.2%)	210 (24.6%)	218 (24.5%)	
	Extremely low birth weight	5 (13.9%)	143 (16.8%)	148 (16.6%)	
	Mild impairment (7–10)	14 (38.9%)	368 (43.1%)	382 (43.0%)	
Moderate impairment (4–6)	18 (50.0%)	392 (46.0%)	410 (46.1%)		
Severe impairment (0–3)	4 (11.1%)	93 (10.9%)	97 (10.9%)		
Apgar scores at the 5th minutes of life	Mild impairment (7–10)	32 (88.9%)	693 (81.2%)	725 (81.6%)	0.084
	Moderate impairment (4–6)	2 (5.6%)	141 (16.5%)	143 (16.1%)	
	Severe impairment (0–3)	2 (5.6%)	19 (2.2%)	21 (2.4%)	
Late-onset neonatal sepsis					
Type of healthcare coverage	Public healthcare system	76 (79.2%)	165 (86.4%)	241 (84.0%)	0.116
	Private healthcare/insurance	20 (20.8%)	26 (13.6%)	46 (16.0%)	
Postnatal age classification	Day 1 of life	84 (87.5%)	165 (86.4%)	249 (86.8%)	0.793
	Postnatal days >1	12 (12.5%)	26 (13.6%)	38 (13.2%)	
Sex	Female	45 (47.4%)	83 (43.5%)	128 (44.8%)	0.531
	Male	50 (52.6%)	108 (56.5%)	158 (55.2%)	
Gestational age	Term	14 (14.6%)	37 (19.4%)	51 (17.8%)	0.014
	Post-term	1 (1.0%)	10 (5.2%)	11 (3.8%)	
	Moderate-late preterm	9 (9.4%)	25 (13.1%)	34 (11.8%)	
	Very preterm	35 (36.5%)	80 (41.9%)	115 (40.1%)	
	Extremely preterm	37 (38.5%)	39 (20.4%)	76 (26.5%)	
Birth weight	Appropriate birth weight	18 (18.8%)	29 (15.2%)	47 (16.4%)	0.466
	Macrosomia	—	3 (1.6%)	3 (1.0%)	

	Low birth weight	16 (16.7%)	41 (21.5%)	57 (19.9%)	
	Very low birth weight	29 (30.2%)	65 (34.0%)	94 (32.8%)	
	Extremely low birth weight	33 (34.4%)	53 (27.7%)	86 (30.0%)	
Apgar scores at the 1st minute of life	Mild impairment (7–10)	42 (43.8%)	74 (38.7%)	116 (40.4%)	0.415
	Moderate impairment (4–6)	40 (41.7%)	95 (49.7%)	135 (47.0%)	
	Severe impairment (0–3)	14 (14.6%)	22 (11.5%)	36 (12.5%)	
Apgar scores at the 5th minute of life	Mild impairment (7–10)	77 (80.2%)	152 (79.6%)	229 (79.8%)	0.701
	Moderate impairment (4–6)	17 (17.7%)	31 (16.2%)	48 (16.7%)	
	Severe impairment (0–3)	2 (2.1%)	8 (4.2%)	10 (3.5%)	

This table presents the distribution of demographic and perinatal characteristics among newborns diagnosed with early-onset neonatal sepsis and late-onset neonatal sepsis, stratified according to microbiological confirmation status (confirmed sepsis versus presumed sepsis). Variables include healthcare coverage type, postnatal age at evaluation, sex, gestational age classification, birth weight category, and Apgar scores at the 1st and 5th minutes of life. Gestational age was categorized as term, post-term, moderate-to-late preterm, very preterm, and extremely preterm according to standard neonatal definitions. Birth weight was classified as appropriate birth weight, macrosomia, low birth weight, very low birth weight, and extremely low birth weight. Apgar scores were grouped into mild (7–10), moderate (4–6), and severe impairment (0–3). Data are expressed as absolute frequency and percentage [n (%)]. Comparisons between confirmed and presumed sepsis groups were performed using Pearson's chi-square test or Fisher's exact test, as appropriate. Statistically significant p-values (≤ 0.05) indicate differences in marker distribution according to neonatal sepsis classification.

3.3. Temporal distribution of neonatal sepsis

The temporal distribution of neonatal sepsis according to pre-pandemic, pandemic, and post-pandemic periods is presented in **Table 2** and **Figure 2**. Early-onset neonatal sepsis did not demonstrate statistically significant temporal variation throughout the study periods ($p = 0.254$). However, confirmed late-onset neonatal sepsis showed a significant difference according to study period ($p = 0.003$).

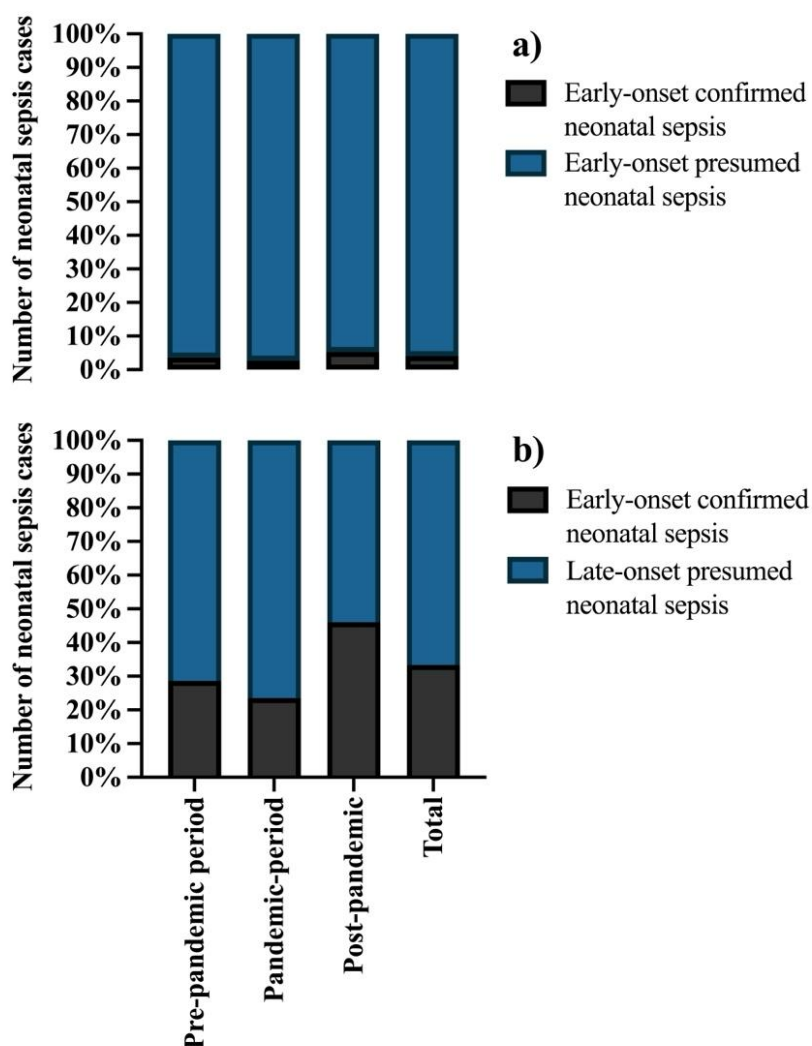
Among confirmed late-onset neonatal sepsis cases, the highest proportion was observed during the post-pandemic period (46.0%), followed by the pre-pandemic period (28.6%) and the pandemic period (23.5%). In contrast, presumed late-onset neonatal sepsis demonstrated a progressive reduction from the pre-pandemic to post-pandemic periods. These findings suggest potential temporal modifications in microbiological confirmation patterns, healthcare-associated infection dynamics, or institutional diagnostic practices during and after the COVID-19 pandemic period.

Table 2. Temporal distribution of early- and late-onset neonatal sepsis according to pre-pandemic, pandemic, and post-pandemic periods.

Sepsis classification	Pre-pandemic period [n (%)]	Pandemic period [n (%)]	Post-pandemic period [n (%)]	Total [n]	p-value
Early-onset confirmed neonatal sepsis	11 (3.6%)	6 (2.7%)	19 (5.3%)	36 (4.0%)	0.254
Early-onset presumed neonatal sepsis	294 (96.4%)	220 (97.3%)	339 (94.7%)	853 (96.0%)	
Late-onset confirmed neonatal sepsis	34 (28.6%)	16 (23.5%)	46 (46.0%)	96 (33.4%)	0.003
Late-onset presumed neonatal sepsis	85 (71.4%)	52 (76.5%)	54 (54.0%)	191 (66.6%)	

This table describes the temporal distribution of neonatal sepsis cases according to study period and sepsis classification. Neonatal sepsis episodes were categorized into early-onset confirmed sepsis, early-onset presumed sepsis, late-onset confirmed sepsis, and late-onset presumed sepsis. The study period was divided into pre-pandemic, pandemic, and post-pandemic phases according to the institutional epidemiological timeline associated with the coronavirus disease 2019 (COVID-19) pandemic. Data are presented as absolute frequency and percentage [n (%)]. Statistical comparisons were performed to evaluate potential differences in the distribution of neonatal sepsis classifications across the three study periods using Pearson's chi-square test. A statistically significant association was observed for late-onset confirmed neonatal sepsis, suggesting temporal variation in microbiologically confirmed healthcare-associated infections during the evaluated periods.

Figure 2. Temporal distribution of early- and late-onset neonatal sepsis according to pre-pandemic, pandemic, and post-pandemic periods.



This figure presents the temporal distribution of neonatal sepsis classifications during the pre-pandemic, pandemic, and post-pandemic periods associated with the coronavirus disease 2019 (COVID-19) pandemic. Neonatal sepsis cases were stratified into early-onset confirmed sepsis, early-onset presumed sepsis, late-onset confirmed sepsis, and late-onset presumed sepsis. The figure highlights the relative stability of early-onset neonatal sepsis across the evaluated periods, whereas confirmed late-onset neonatal sepsis demonstrated significant temporal variation, with a higher proportion observed during the post-pandemic period. In contrast, presumed late-onset neonatal sepsis demonstrated a progressive reduction throughout the evaluated timeline. These findings may reflect changes in infection control practices, microbiological surveillance, healthcare-associated infection dynamics, antimicrobial use, and institutional diagnostic strategies during and after the COVID-19 pandemic. Data are presented as percentages within each temporal period.

3.4. Maternal and obstetric characteristics

Maternal and obstetric variables are summarized in **Table 3**. In the early-onset neonatal sepsis analysis, no obstetric variable reached statistical significance, although maternal antibiotic exposure before delivery

demonstrated a trend toward higher frequency among confirmed sepsis cases (36.1% versus 22.6%; $p = 0.060$). Vaginal delivery was also proportionally more common among confirmed early-onset neonatal sepsis episodes.

In contrast, among late-onset neonatal sepsis cases, maternal antibiotic exposure before delivery was significantly more frequent in confirmed sepsis compared with presumed sepsis (28.1% versus 15.7%; $p = 0.013$). This finding may reflect greater maternal infectious burden, increased neonatal vulnerability, or more complex perinatal conditions associated with microbiologically confirmed late-onset infections.

Cesarean delivery predominated among late-onset neonatal sepsis cases regardless of microbiological confirmation status. No statistically significant associations were observed for maternal Group B *Streptococcus* screening status, rupture of membranes, or meconium-stained amniotic fluid in either early-onset neonatal sepsis or late-onset neonatal sepsis analyses.

Table 3. Maternal and obstetric characteristics according to early- and late-onset neonatal sepsis classification.

Early-onset neonatal sepsis					
Marker	Groups	Confirmed sepsis [n (%)]	Presumed sepsis [n (%)]	Total	p-value
Mode of delivery	Vaginal delivery	20 (55.6%)	346 (40.6%)	366 (41.2%)	0.073
	Cesarean delivery	16 (44.4%)	507 (59.4%)	523 (58.8%)	
Maternal antibiotic exposure before delivery	Yes	13 (36.1%)	193 (22.6%)	206 (23.2%)	0.060
	No	23 (63.9%)	660 (77.4%)	683 (76.8%)	
Maternal group B <i>Streptococcus</i> screening	Positive	—	16 (16.7%)	16 (16.0%)	0.492
	Negative	4 (100.0%)	80 (83.3%)	84 (84.0%)	
Prematurity	Yes	31 (86.1%)	648 (76.0%)	679 (76.4%)	0.160
	No	5 (13.9%)	205 (24.0%)	210 (23.6%)	
Amniotic membrane status at delivery	Intact membranes	20 (55.6%)	583 (68.3%)	603 (67.8%)	0.108
	Rupture of membranes	16 (44.4%)	270 (31.7%)	286 (32.2%)	
Meconium-stained amniotic fluid at delivery	Positive	6 (16.7%)	107 (12.5%)	113 (12.7%)	0.467
	Negative	30 (83.3%)	746 (87.5%)	776 (87.3%)	
Late-onset neonatal sepsis					
Mode of delivery	Vaginal delivery	37 (38.5%)	68 (35.6%)	105 (36.6%)	0.626
	Cesarean delivery	59 (61.5%)	123 (64.4%)	182 (63.4%)	
Maternal antibiotic exposure before delivery	Yes	27 (28.1%)	30 (15.7%)	57 (19.9%)	0.013
	No	69 (71.9%)	161 (84.3%)	230 (80.1%)	
Maternal group B <i>Streptococcus</i> screening	Positive	3 (33.3%)	2 (8.3%)	5 (15.2%)	0.111
	Negative	6 (66.7%)	22 (91.7%)	28 (84.8%)	
Amniotic membrane status at delivery	Intact membranes	81 (84.4%)	149 (78.0%)	230 (80.1%)	0.202
	Rupture of membranes	15 (15.6%)	42 (22.0%)	57 (19.9%)	
Meconium-stained amniotic fluid at delivery	Positive	12 (12.5%)	18 (9.4%)	30 (10.5%)	0.422
	Negative	84 (87.5%)	173 (90.6%)	257 (89.5%)	

This table summarizes maternal and obstetric characteristics associated with neonatal sepsis classifications among newborns admitted to the neonatal intensive care unit. Variables analyzed include mode of delivery, maternal antibiotic exposure before delivery, maternal Group B *Streptococcus* screening status, prematurity, amniotic membrane status at delivery, and presence of meconium-stained amniotic fluid. Sepsis cases were stratified into confirmed and presumed sepsis within both early-onset and late-onset neonatal sepsis groups. Maternal antibiotic exposure refers to any systemic antimicrobial therapy administered before delivery. Amniotic membrane status was classified as intact membranes or rupture of membranes at delivery. Data are presented as absolute frequencies and percentages [n (%)]. Comparisons between groups were performed using Pearson's chi-square test or Fisher's exact test, depending on expected cell frequencies. Significant p-values indicate differences in obstetric risk factor distribution according to neonatal sepsis classification.

3.5. Clinical characteristics, intensive care interventions, and neonatal complications

The clinical profile of neonatal sepsis is presented in **Table 4**. Respiratory distress, apnea, or perinatal depression represented the most prevalent clinical manifestations across all sepsis classifications, affecting more

than 95% of newborns in both early-onset neonatal sepsis and late-onset neonatal sepsis groups. Prematurity also remained highly prevalent, particularly among confirmed late-onset neonatal sepsis cases (85.4%).

The need for advanced respiratory support and invasive procedures was substantial across all groups. Mechanical ventilation was required in approximately half of early-onset neonatal sepsis cases and more than two-thirds of late-onset neonatal sepsis cases. Continuous positive airway pressure and supplemental oxygen therapy were also frequently required, reinforcing the high clinical complexity of this NICU population.

Umbilical catheterization and peripherally inserted central catheter use were highly prevalent among both confirmed and presumed sepsis groups, especially in late-onset neonatal sepsis, reflecting the extensive need for invasive vascular access during prolonged hospitalization and intensive care support.

Among neonatal complications, bronchopulmonary dysplasia, septic shock, pulmonary hypertension, necrotizing enterocolitis, and meningitis were identified in both confirmed and presumed sepsis groups, although without statistically significant differences. Septic shock occurred in approximately 5% of early-onset neonatal sepsis cases and 8% of late-onset neonatal sepsis cases, whereas necrotizing enterocolitis affected 4.3% and 10.5% of early-onset neonatal sepsis and late-onset neonatal sepsis populations, respectively.

Antibiotic exposure was nearly universal. In early-onset neonatal sepsis, all presumed sepsis cases received antimicrobial therapy, whereas 91.7% of confirmed cases received antibiotics ($p < 0.001$). These findings emphasize the substantial dependence on empirical antimicrobial therapy in neonatal sepsis management, particularly in clinically suspected cases without microbiological confirmation.

Table 4. Clinical characteristics, interventions, and neonatal complications according to early- and late-onset neonatal sepsis classification.

Early-onset neonatal sepsis					
Marker	Groups	Confirmed sepsis [n (%)]	Presumed sepsis [n (%)]	Total	p-value
Respiratory distress, apnea, or perinatal depression	Yes	35 (97.2%)	813 (95.3%)	848 (95.4%)	1.000
	No	1 (2.8%)	40 (4.7%)	41 (4.6%)	
Prematurity	Yes	31 (86.1%)	648 (76.0%)	679 (76.4%)	0.160
	No	5 (13.9%)	205 (24.0%)	210 (23.6%)	
Perinatal asphyxia or anoxia	Yes	4 (11.1%)	141 (16.5%)	145 (16.3%)	0.494
	No	32 (88.9%)	712 (83.5%)	744 (83.7%)	
Neonatal hypoglycemia	Yes	5 (13.9%)	96 (11.3%)	101 (11.4%)	0.626
	No	31 (86.1%)	757 (88.7%)	788 (88.6%)	
Neonatal seizures	Yes	3 (8.3%)	57 (6.7%)	60 (6.7%)	0.729
	No	33 (91.7%)	796 (93.3%)	829 (93.3%)	
Congenital heart disease	Yes	1 (2.8%)	27 (3.2%)	28 (3.1%)	1.000
	No	35 (97.2%)	826 (96.8%)	861 (96.9%)	
Neonatal jaundice	Yes	0 (0.0%)	4 (0.5%)	4 (0.4%)	1.000
	No	36 (100.0%)	849 (99.5%)	885 (99.6%)	
Esophageal, intestinal, or duodenal atresia	Yes	0 (0.0%)	9 (1.1%)	9 (1.0%)	1.000
	No	36 (100.0%)	844 (98.9%)	880 (99.0%)	
Antibiotic use	Yes	33 (91.7%)	853 (100%)	886 (99.7%)	<0.001
	No	3 (8.3%)	—	3 (0.3%)	
Mechanical ventilation	Yes	18 (50.0%)	411 (48.2%)	429 (48.3%)	0.831
	No	18 (50.0%)	442 (51.8%)	460 (51.7%)	
Continuous positive airway pressure	Yes	26 (72.2%)	635 (74.4%)	661 (74.4%)	0.765
	No	10 (27.8%)	218 (25.6%)	228 (25.6%)	
Supplemental oxygen use	Yes	30 (83.3%)	675 (79.1%)	705 (79.3%)	0.542
	No	6 (16.7%)	178 (20.9%)	184 (20.7%)	
Umbilical catheterization	Yes	33 (91.7%)	749 (87.8%)	782 (88.0%)	0.610
	No	3 (8.3%)	104 (12.2%)	107 (12.0%)	
	Yes	20 (55.6%)	384 (45.0%)	404 (45.4%)	

Table 4. Clinical characteristics, interventions, and neonatal complications according to early- and late-onset neonatal sepsis classification.

Early-onset neonatal sepsis					
Marker	Groups	Confirmed sepsis [n (%)]	Presumed sepsis [n (%)]	Total	p-value
Peripherally inserted central catheter use	No	16 (44.4%)	469 (55.0%)	485 (54.6%)	
Surgical procedures	Yes	1 (2.8%)	45 (5.3%)	46 (5.2%)	1.000
	No	35 (97.2%)	808 (94.7%)	843 (94.8%)	
Phlebotomy	Yes	—	12 (1.4%)	12 (1.3%)	1.000
	No	36 (100.0%)	841 (98.6%)	877 (98.7%)	
Chest tube drainage	Yes	—	15 (1.8%)	15 (1.7%)	1.000
	No	36 (100.0%)	838 (98.2%)	874 (98.3%)	
Tracheostomy	Yes	—	6 (0.7%)	6 (0.7%)	1.000
	No	36 (100.0%)	847 (99.3%)	883 (99.3%)	
Bronchopulmonary dysplasia	Yes	3 (8.3%)	66 (7.7%)	69 (7.8%)	0.754
	No	33 (91.7%)	787 (92.3%)	820 (92.2%)	
Pulmonary hypertension	Yes	1 (2.8%)	52 (6.1%)	53 (6.0%)	0.718
	No	35 (97.2%)	801 (93.9%)	836 (94.0%)	
Septic shock	Yes	3 (8.3%)	41 (4.8%)	44 (4.9%)	0.416
	No	33 (91.7%)	812 (95.2%)	845 (95.1%)	
Necrotizing enterocolitis	Yes	3 (8.3%)	35 (4.1%)	38 (4.3%)	0.196
	No	33 (91.7%)	818 (95.9%)	851 (95.7%)	
Meningitis	Yes	—	10 (1.2%)	10 (1.1%)	1.000
	No	36 (100.0%)	843 (98.8%)	879 (98.9%)	
Late-onset neonatal sepsis					
Respiratory distress, apnea, or perinatal depression	Yes	92 (95.8%)	187 (97.9%)	279 (97.2%)	0.448
	No	4 (4.2%)	4 (2.1%)	8 (2.8%)	
Prematurity	Yes	82 (85.4%)	159 (83.2%)	241 (84.0%)	0.636
	No	14 (14.6%)	32 (16.8%)	46 (16.0%)	
Perinatal asphyxia or anoxia	Yes	17 (17.7%)	29 (15.2%)	46 (16.0%)	0.582
	No	79 (82.3%)	162 (84.8%)	241 (84.0%)	
Neonatal hypoglycemia	Yes	14 (14.6%)	23 (12.0%)	37 (12.9%)	0.544
	No	82 (85.4%)	168 (88.0%)	250 (87.1%)	
Neonatal seizures	Yes	8 (8.3%)	24 (12.6%)	32 (11.1%)	0.282
	No	88 (91.7%)	167 (87.4%)	255 (88.9%)	
Congenital heart disease	Yes	3 (3.1%)	7 (3.7%)	10 (3.5%)	1.000
	No	93 (96.9%)	184 (96.3%)	277 (96.5%)	
Neonatal jaundice	Yes	0 (0.0%)	1 (0.5%)	1 (0.3%)	1.000
	No	96 (100.0%)	190 (99.5%)	286 (99.7%)	
Esophageal, intestinal, or duodenal atresia	Yes	1 (1.0%)	3 (1.6%)	4 (1.4%)	1.000
	No	95 (99.0%)	188 (98.4%)	283 (98.6%)	
Antibiotic use	Yes	94 (97.9%)	191 (100.0%)	285 (99.3%)	0.111
	No	2 (2.1%)	—	2 (0.7%)	
Mechanical ventilation	Yes	63 (65.6%)	139 (72.8%)	202 (70.4%)	0.211
	No	33 (34.4%)	52 (27.2%)	85 (29.6%)	
Continuous positive airway pressure	Yes	78 (81.2%)	155 (81.2%)	233 (81.2%)	0.984
	No	18 (18.8%)	36 (18.8%)	54 (18.8%)	
Supplemental oxygen use	Yes	73 (76.0%)	131 (68.6%)	204 (71.1%)	0.189
	No	23 (24.0%)	60 (31.4%)	83 (28.9%)	
Umbilical catheterization	Yes	86 (89.6%)	173 (90.6%)	259 (90.2%)	0.789
	No	10 (10.4%)	18 (9.4%)	28 (9.8%)	
Peripherally inserted central catheter use	Yes	78 (81.3%)	150 (78.5%)	228 (79.4%)	0.591
	No	18 (18.8%)	41 (21.5%)	59 (20.6%)	
Surgical procedures	Yes	9 (9.4%)	12 (6.3%)	21 (7.3%)	0.343
	No	87 (90.6%)	179 (93.7%)	266 (92.7%)	
Phlebotomy	Yes	2 (2.1%)	3 (1.6%)	5 (1.7%)	1.000
	No	94 (97.9%)	188 (98.4%)	282 (98.3%)	
Chest tube drainage	Yes	2 (2.1%)	6 (3.1%)	8 (2.8%)	0.723

Table 4. Clinical characteristics, interventions, and neonatal complications according to early- and late-onset neonatal sepsis classification.

Early-onset neonatal sepsis					
Marker	Groups	Confirmed sepsis [n (%)]	Presumed sepsis [n (%)]	Total	p-value
Tracheostomy	No	94 (97.9%)	185 (96.9%)	279 (97.2%)	0.690
	Yes	3 (3.1%)	4 (2.1%)	7 (2.4%)	
Bronchopulmonary dysplasia	No	93 (96.9%)	187 (97.9%)	280 (97.6%)	0.752
	Yes	17 (17.7%)	31 (16.2%)	48 (16.7%)	
Pulmonary hypertension	No	79 (82.3%)	160 (83.8%)	239 (83.3%)	0.226
	Yes	4 (4.2%)	16 (8.4%)	20 (7.0%)	
Septic shock	No	92 (95.8%)	175 (91.6%)	267 (93.0%)	0.990
	Yes	8 (8.3%)	16 (8.4%)	24 (8.4%)	
Necrotizing enterocolitis	No	88 (91.7%)	175 (91.6%)	263 (91.6%)	0.672
	Yes	9 (9.4%)	21 (11.0%)	30 (10.5%)	
Meningitis	No	87 (90.6%)	170 (89.0%)	257 (89.5%)	0.488
	Yes	4 (4.2%)	5 (2.6%)	9 (3.1%)	
	No	92 (95.8%)	186 (97.4%)	278 (96.9%)	

This table presents the clinical characteristics, neonatal complications, invasive procedures, respiratory support requirements, and therapeutic interventions observed among newborns with early-onset and late-onset neonatal sepsis, stratified by confirmed and presumed sepsis status. Clinical variables include respiratory distress, apnea, perinatal depression, prematurity, perinatal asphyxia or anoxia, hypoglycemia, seizures, congenital heart disease, jaundice, gastrointestinal atresias, bronchopulmonary dysplasia, pulmonary hypertension, septic shock, necrotizing enterocolitis, and meningitis. Therapeutic and intensive care variables include antibiotic use, mechanical ventilation, continuous positive airway pressure, supplemental oxygen therapy, umbilical catheterization, peripherally inserted central catheter use, surgical procedures, phlebotomy, chest tube drainage, and tracheostomy. Data are expressed as absolute frequencies and percentages [n (%)]. Statistical comparisons between confirmed and presumed sepsis groups were performed using Pearson's chi-square test or Fisher's exact test, as appropriate. This analysis aimed to characterize the severity profile and healthcare complexity associated with different neonatal sepsis classifications.

3.6. Clinical outcomes

Clinical outcomes and hospitalization duration are summarized in **Table 5**. Prolonged hospitalization was highly prevalent in both neonatal sepsis groups, particularly among late-onset neonatal sepsis cases. More than 69% of newborns with late-onset neonatal sepsis remained hospitalized for more than 21 days, whereas approximately 41% of early-onset neonatal sepsis cases presented similarly prolonged hospitalization periods (**Figure 3**).

Mortality rates were elevated across all groups. Among early-onset neonatal sepsis cases, mortality reached 27.8% in confirmed sepsis and 17.9% in presumed sepsis. In late-onset neonatal sepsis, mortality was observed in 19.8% of confirmed cases and 26.7% of presumed cases (**Figure 4**). Although these differences did not achieve statistical significance, the findings reinforce the substantial burden of neonatal sepsis on NICU mortality.

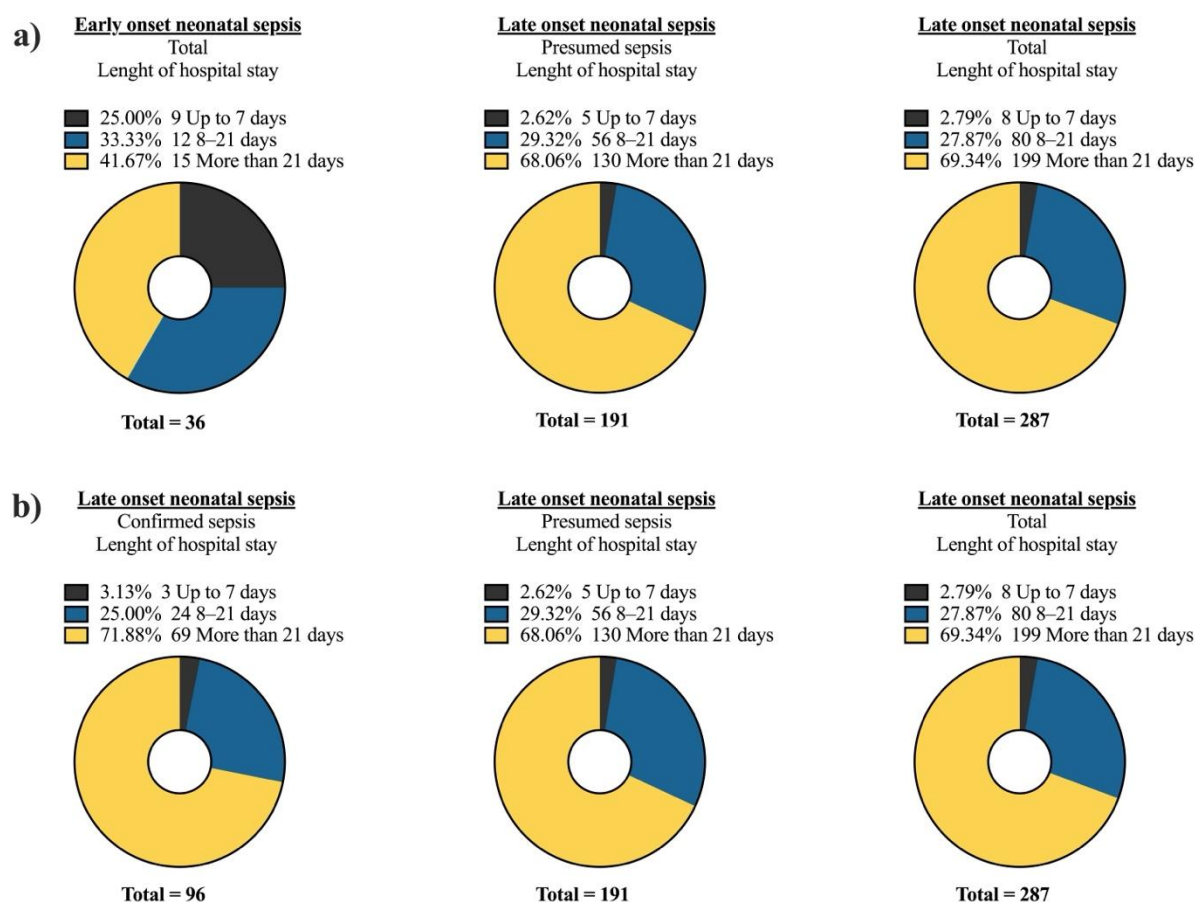
Hospital discharge remained the predominant outcome in all groups; however, the persistently elevated mortality rates highlight the severe clinical impact associated with neonatal infectious syndromes in critically ill newborns.

Table 5. Length of hospital stay and clinical outcomes according to neonatal sepsis classification.

Marker	Groups	Confirmed sepsis [n (%)]	Presumed sepsis [n (%)]	Total	p-value
Early-onset neonatal sepsis					
Length of hospital stay	Up to 7 days	9 (25.0%)	171 (20.0%)	180 (20.2%)	0.713
	8–21 days	12 (33.3%)	330 (38.7%)	342 (38.5%)	
	More than 8–21 days	15 (41.7%)	352 (41.3%)	367 (41.3%)	
Clinical outcome	Hospital discharge	26 (72.2%)	700 (82.1%)	726 (81.7%)	0.183
	Death	10 (27.8%)	153 (17.9%)	163 (18.3%)	
Late-onset neonatal sepsis					
Length of hospital stay	Up to 7 days	3 (3.1%)	5 (2.6%)	8 (2.8%)	0.716
	8–21 days	24 (25.0%)	56 (29.3%)	80 (27.9%)	
	More than 8–21 days	69 (71.9%)	130 (68.1%)	199 (69.3%)	
Clinical outcome	Hospital discharge	77 (80.2%)	140 (73.3%)	217 (75.6%)	0.198
	Death	19 (19.8%)	51 (26.7%)	70 (24.4%)	

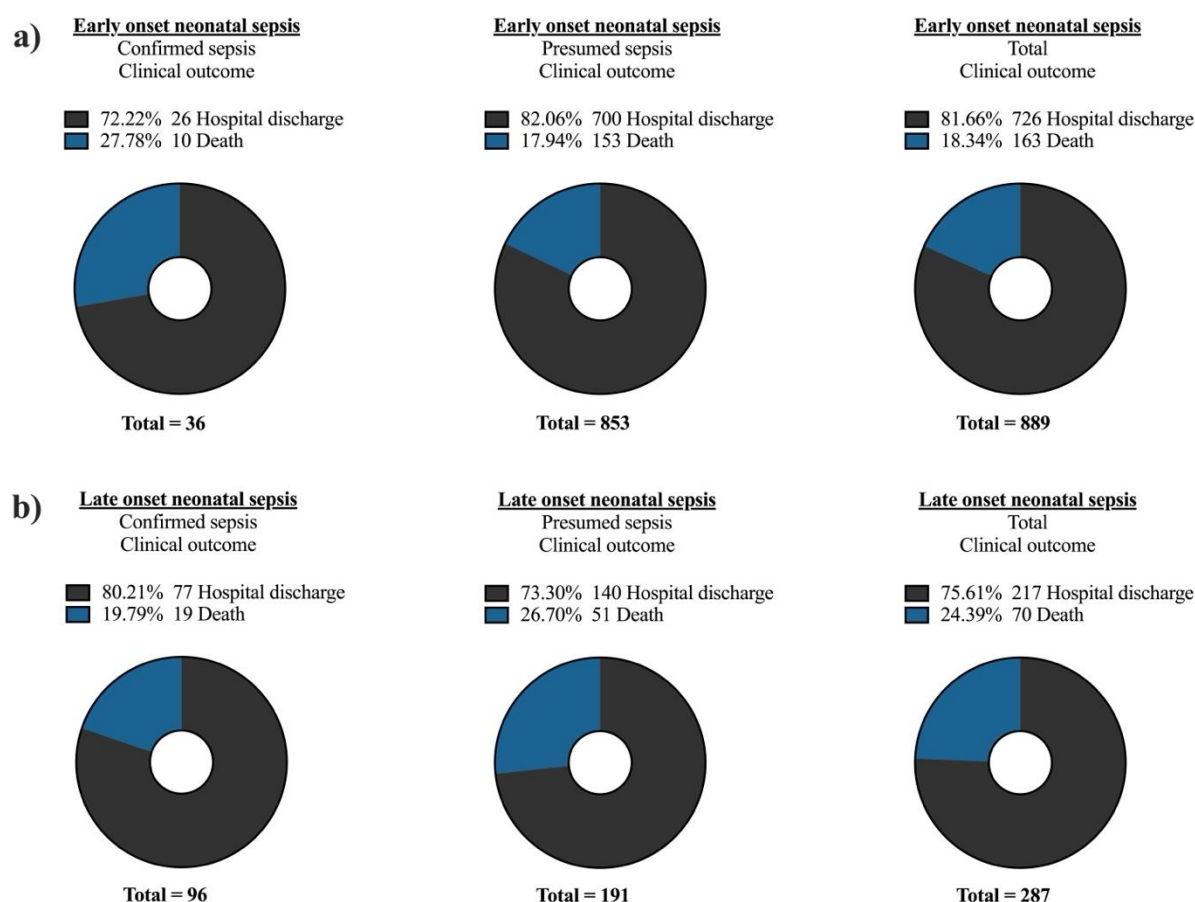
This table describes hospitalization duration and clinical outcomes among newborns with early-onset and late-onset neonatal sepsis according to microbiological confirmation status. Length of hospital stay was categorized as up to 7 days, 8–21 days, and more than 21 days. Clinical outcomes were classified as hospital discharge or in-hospital death. Neonatal sepsis cases were stratified into confirmed and presumed sepsis groups for both early-onset and late-onset sepsis analyses. Data are presented as absolute frequencies and percentages [n (%)]. Statistical comparisons were conducted using Pearson's chi-square test or Fisher's exact test to evaluate potential differences in hospitalization duration and mortality according to sepsis classification. These analyses were performed to explore the association between microbiological confirmation and adverse neonatal outcomes.

Figure 3. Length of hospital stay according to neonatal sepsis classification and microbiological confirmation status.



This figure illustrates the distribution of hospitalization duration among newborns with early-onset and late-onset neonatal sepsis according to microbiological confirmation status. Length of hospital stay was categorized as up to 7 days, 8–21 days, and more than 21 days. Both confirmed and presumed late-onset neonatal sepsis groups demonstrated a predominance of prolonged hospitalization exceeding 21 days, reflecting the greater clinical complexity, increased exposure to invasive procedures, and prolonged intensive care requirements associated with healthcare-associated infections in critically ill newborns. Early-onset neonatal sepsis cases also demonstrated substantial rates of prolonged hospitalization, although at lower frequencies compared with late-onset sepsis. The figure emphasizes the significant healthcare burden associated with neonatal sepsis and its impact on neonatal intensive care unit occupancy and resource utilization. Data are expressed as percentages within each neonatal sepsis subgroup.

Figure 4. Clinical outcomes according to neonatal sepsis classification and microbiological confirmation status.



This figure presents the clinical outcomes of newborns diagnosed with neonatal sepsis according to timing of onset and microbiological confirmation status. Outcomes were categorized as hospital discharge or in-hospital death. Elevated mortality rates were observed across all neonatal sepsis groups, particularly among confirmed early-onset neonatal sepsis and presumed late-onset neonatal sepsis cases. Although hospital discharge remained the predominant outcome in all groups, the persistently high mortality frequencies reinforce the severe clinical burden associated with neonatal infectious syndromes in critically ill newborns admitted to the neonatal intensive care unit. The figure also highlights the substantial morbidity associated with prolonged hospitalization and intensive care support in this population. Data are presented as percentages within each neonatal sepsis classification group.

3.7. Microbiological profile of confirmed neonatal sepsis

The microbiological profile of confirmed neonatal sepsis is presented in **Table 6**. In early-onset neonatal sepsis, coagulase-negative *Staphylococci* represented the most frequently isolated pathogens ($n = 14$), followed by *Escherichia coli* ($n = 8$), *Staphylococcus aureus* ($n = 5$), and *Klebsiella pneumoniae* ($n = 3$).

In late-onset neonatal sepsis, the microbiological spectrum was broader and demonstrated predominance of healthcare-associated pathogens. Coagulase-negative *Staphylococci* were again the most frequent isolates ($n = 34$), followed by *Staphylococcus aureus* ($n = 17$), *Klebsiella pneumoniae* ($n = 10$), and *Escherichia coli* ($n = 8$). Additional Gram-negative organisms, including *Enterobacter cloacae*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Morganella morganii*, and *Stenotrophomonas maltophilia*, were identified exclusively or predominantly in late-onset neonatal sepsis.

Polymicrobial infections were uncommon but identified in both early-onset neonatal sepsis and late-onset neonatal sepsis groups. Overall, the microbiological profile demonstrated predominance of Gram-positive organisms, particularly coagulase-negative *Staphylococci*, while Gram-negative bacteria remained clinically relevant pathogens, especially among severe and late-onset infections.

Table 6. Microbiological profile of confirmed early- and late-onset neonatal sepsis cases.

Early-onset neonatal sepsis	
Microbiological profile	Confirmed sepsis [n]
<i>Enterobacter agglomerans</i>	2
<i>Escherichia coli</i>	8
<i>Klebsiella pneumoniae</i>	3
<i>Staphylococcus aureus</i>	5
<i>Staphylococcus aureus</i> + <i>Escherichia coli</i>	1
Coagulase-negative <i>Staphylococci</i>	14
<i>Streptococcus pneumoniae</i>	1
Missing data	2
Late-onset neonatal sepsis	
	Confirmed sepsis [n]
Gram-positive cocci	1
<i>Enterobacter agglomerans</i>	1
<i>Enterobacter cloacae</i>	3
<i>Enterococcus</i> spp.	2
<i>Escherichia coli</i>	8
<i>Klebsiella oxytoca</i>	2
<i>Klebsiella pneumoniae</i>	10
<i>Morganella morganii</i>	1
Partial Gram-positive cocci	1
<i>Proteus mirabilis</i>	2
<i>Pseudomonas aeruginosa</i>	1
<i>Serratia liquefaciens</i>	2
<i>Staphylococcus aureus</i>	17
<i>Staphylococcus aureus</i> + <i>Escherichia coli</i>	1
<i>Staphylococcus aureus</i> + coagulase-negative <i>Staphylococci</i>	1
Coagulase-negative <i>Staphylococci</i>	34
<i>Stenotrophomonas maltophilia</i>	1
Group B <i>Streptococcus agalactiae</i>	1
<i>Streptococcus pneumoniae</i>	1
Missing data	6

This table presents the microbiological profile of culture-confirmed neonatal sepsis episodes identified during hospitalization in the neonatal intensive care unit. Isolated microorganisms are described separately for early-onset neonatal sepsis and late-onset neonatal sepsis. The identified pathogens include Gram-positive and Gram-negative bacteria, as well as polymicrobial infections. Coagulase-negative *Staphylococci*, *Staphylococcus aureus*, *Escherichia coli*, and *Klebsiella pneumoniae* were among the most frequently isolated microorganisms. Data are expressed as absolute frequencies [n]. Cases categorized as “missing data” correspond to records with incomplete microbiological identification despite classification as confirmed sepsis. This microbiological characterization provides insight into the etiological spectrum associated with neonatal sepsis in a tertiary neonatal intensive care setting.

3.8. Anti-Infective therapy and microbiological investigation

The anti-infective therapies administered according to neonatal sepsis classification are summarized in **Table 7** and **Supplementary Tables 1–2**. Ampicillin and gentamicin represented the cornerstone empirical regimens in both early-onset neonatal sepsis and late-onset neonatal sepsis populations, being administered to more than 75% of newborns in all groups.

In early-onset neonatal sepsis, cefepime use was significantly more frequent among confirmed sepsis cases (27.8% versus 15.1%; $p = 0.041$), while oxacillin use was also significantly higher in confirmed sepsis (22.2% versus 4.8%; $p < 0.001$). In late-onset neonatal sepsis, vancomycin use was significantly more frequent among presumed sepsis cases (82.2% versus 61.5%; $p < 0.001$), whereas cefepime was more frequently prescribed in presumed late-onset neonatal sepsis as well (59.7% versus 46.9%; $p = 0.039$).

Supplementary microbiological data demonstrated that blood cultures represented the most frequently collected biological specimens in both early-onset neonatal sepsis and late-onset neonatal sepsis evaluations, followed by urine cultures and catheter-related samples. Additional antimicrobial regimens included antifungal agents, glycopeptides, cephalosporins, macrolides, aminoglycosides, and combination therapies, reflecting the heterogeneity and complexity of neonatal infectious management in critically ill newborns.

A graphical abstract summarizing the main differences between early- and late-onset neonatal sepsis, including microbiological confirmation, prematurity profile, respiratory support requirements, hospitalization duration, mortality, predominant pathogens, and empirical antibiotic use, is presented in **Supplementary Figure 1**.

Table 7. Anti-infective therapy according to early- and late-onset neonatal sepsis classification.**Early-onset neonatal sepsis**

Anti-infective therapy	Group	Confirmed sepsis [n (%)]	Presumed sepsis [n (%)]	Total	p-value
Ampicillin	Yes	29 (80.6%)	853 (100.0%)	882 (99.2%)	<0.001
	No	7 (19.4%)	—	7 (0.8%)	
Gentamicin	Yes	29 (80.6%)	853 (100.0%)	882 (99.2%)	<0.001
	No	7 (19.4%)	—	7 (0.8%)	
Amikacin	Yes	4 (11.1%)	40 (4.7%)	44 (4.9%)	0.097
	No	32 (88.9%)	813 (95.3%)	845 (95.1%)	
Cefepime	Yes	10 (27.8%)	129 (15.1%)	139 (15.6%)	0.041
	No	26 (72.2%)	724 (84.9%)	750 (84.4%)	
Vancomycin	Yes	11 (30.6%)	176 (20.6%)	187 (21.0%)	0.152
	No	25 (69.4%)	677 (79.4%)	702 (79.0%)	
Meropenem	Yes	3 (8.3%)	44 (5.2%)	47 (5.3%)	0.431
	No	33 (91.7%)	809 (94.8%)	842 (94.7%)	
Piperacillin/tazobactam	Yes	2 (5.6%)	63 (7.4%)	65 (7.3%)	1.000
	No	34 (94.4%)	790 (92.6%)	824 (92.7%)	
Metronidazole	Yes	4 (11.1%)	38 (4.5%)	42 (4.7%)	0.084
	No	32 (88.9%)	815 (95.5%)	847 (95.3%)	
Oxacillin	Yes	8 (22.2%)	41 (4.8%)	49 (5.5%)	<0.001
	No	28 (77.8%)	812 (95.2%)	840 (94.5%)	
Fluconazole	Yes	1 (2.8%)	26 (3.0%)	27 (3.0%)	1.000
	No	35 (97.2%)	827 (97.0%)	862 (97.0%)	
Other medications	Yes	2 (5.6%)	49 (5.7%)	51 (5.7%)	1.000
	No	34 (94.4%)	804 (94.3%)	838 (94.3%)	

Late-onset neonatal sepsis

Anti-infective therapy	Group	Confirmed sepsis [n (%)]	Presumed sepsis [n (%)]	Total	p-value
Ampicillin	Yes	73 (76.0%)	152 (79.6%)	225 (78.4%)	0.492
	No	23 (24.0%)	39 (20.4%)	62 (21.6%)	
Gentamicin	Yes	73 (76.0%)	147 (77.0%)	220 (76.7%)	0.862
	No	23 (24.0%)	44 (23.0%)	67 (23.3%)	
Amikacin	Yes	23 (24.0%)	50 (26.2%)	73 (25.4%)	0.684
	No	73 (76.0%)	141 (73.8%)	214 (74.6%)	
Cefepime	Yes	45 (46.9%)	114 (59.7%)	159 (55.4%)	0.039
	No	51 (53.1%)	77 (40.3%)	128 (44.6%)	
Vancomycin	Yes	59 (61.5%)	157 (82.2%)	216 (75.3%)	<0.001
	No	37 (38.5%)	34 (17.8%)	71 (24.7%)	
Meropenem	Yes	18 (18.8%)	33 (17.3%)	51 (17.8%)	0.758
	No	78 (81.2%)	158 (82.7%)	236 (82.2%)	
Piperacillin/tazobactam	Yes	20 (20.8%)	60 (31.4%)	80 (27.9%)	0.059
	No	76 (79.2%)	131 (68.6%)	207 (72.1%)	
Metronidazole	Yes	10 (10.4%)	20 (10.5%)	30 (10.5%)	0.989
	No	86 (89.6%)	171 (89.5%)	257 (89.5%)	
Oxacillin	Yes	22 (22.9%)	55 (28.8%)	77 (26.8%)	0.289
	No	74 (77.1%)	136 (71.2%)	210 (73.2%)	
Fluconazole	Yes	10 (10.4%)	19 (9.9%)	29 (10.1%)	0.901
	No	86 (89.6%)	172 (90.1%)	258 (89.9%)	
Other medications	Yes	16 (16.7%)	21 (11.0%)	37 (12.9%)	0.176
	No	80 (83.3%)	170 (89.0%)	250 (87.1%)	

This table describes the antimicrobial and anti-infective therapies administered to newborns with early-onset and late-onset neonatal sepsis according to microbiological confirmation status. Evaluated medications included ampicillin, gentamicin, amikacin, cefepime, vancomycin, meropenem, piperacillin/tazobactam, metronidazole, oxacillin, fluconazole, and other anti-infective agents. Sepsis episodes were categorized as confirmed or presumed sepsis within both early-onset and late-onset neonatal sepsis groups. Data are presented as absolute frequencies and percentages [n (%)]. Comparisons between groups were performed using Pearson's chi-square test or Fisher's exact test, as appropriate. Significant differences may reflect variations in empirical antimicrobial protocols, infection severity, microbiological confirmation, or suspected pathogen profile during neonatal intensive care management.

4. Discussion

The present study provides a comprehensive evaluation of neonatal sepsis classifications in a tertiary NICU over an eight-year period, including demographic, obstetric, clinical, microbiological, therapeutic, and outcome-related variables. Presumed neonatal sepsis represented most cases in both early-onset and late-onset sepsis, reinforcing the persistent diagnostic challenges associated with neonatal infections in critically ill newborns. This predominance likely reflects the nonspecific clinical manifestations of neonatal sepsis, the limited sensitivity of blood cultures, and the frequent need for empirical antimicrobial therapy in NICU practice. These findings are consistent with previous studies reporting the high frequency of culture-negative sepsis in neonatal intensive care settings ^{8,9,11,12}.

Prematurity and low birth weight were strongly associated with both confirmed and presumed sepsis, particularly among confirmed late-onset infections, emphasizing the role of immunological immaturity, prolonged hospitalization, invasive procedures, and healthcare exposure in neonatal infection susceptibility. Previous studies have consistently demonstrated that extremely preterm and very-low-birth-weight newborns represent the neonatal population most vulnerable to severe infectious complications and healthcare-associated infections ^{1,4,15,16}.

The microbiological profile demonstrated predominance of coagulase-negative *Staphylococci* and *Staphylococcus aureus*, especially in late-onset sepsis, while Gram-negative organisms such as *Escherichia coli* and *Klebsiella pneumoniae* also remained clinically relevant pathogens. Empirical antimicrobial therapy was widely used across all neonatal sepsis groups, with ampicillin and gentamicin representing the most common regimens, whereas broader-spectrum agents such as cefepime and vancomycin were more frequently used in late-onset sepsis. In addition, neonatal sepsis was associated with prolonged hospitalization, high rates of respiratory support, extensive use of invasive devices, and elevated mortality rates, highlighting the substantial clinical burden of neonatal infections in critically ill newborns. Similar microbiological and therapeutic patterns have been reported in recent international neonatal sepsis cohorts, particularly among critically ill NICU populations ^{3,7,17,18}.

The predominance of presumed cases observed in this cohort is consistent with findings reported in neonatal units across different regions of the world, where empirical treatment is frequently initiated in the presence of subtle or nonspecific clinical manifestations, even in the absence of microbiological confirmation. Neonatal sepsis remains a major diagnostic challenge, particularly among critically ill newborns, due to the limited blood volume available for collection, the low sensitivity of blood cultures, and prior exposure to antimicrobial agents ^{8–11}. This scenario contributes to substantial antimicrobial exposure during neonatal hospitalization and highlights the ongoing challenge of balancing early sepsis treatment with antimicrobial stewardship strategies ^{12,19,20}.

Prematurity and low birth weight represented central characteristics of the studied population and were strongly associated with confirmed neonatal sepsis, particularly in cases of late-onset sepsis. Similar findings have been consistently described in both national and international neonatal cohorts ^{1,4,16,21}. Preterm newborns present marked immunological immaturity, prolonged neonatal intensive care unit hospitalization, and frequent need for invasive procedures, all of which significantly increase susceptibility to healthcare-associated infections ¹⁵. In addition, extremely preterm and very-low-birth-weight newborns often require prolonged respiratory and vascular support, thereby increasing exposure to nosocomial pathogens ^{22–24}.

In addition to prematurity, the high frequency of respiratory distress and the extensive need for ventilatory support observed in this study also reflect the clinical severity of the evaluated neonatal population. Mechanical ventilation, continuous positive airway pressure, and oxygen therapy were required in a large proportion of cases, especially during episodes of late-onset sepsis. Previous studies have demonstrated that prolonged respiratory support is associated with increased infectious risk, particularly among preterm infants undergoing prolonged intensive care management ^{25–27}.

The microbiological profile identified in this cohort demonstrated a predominance of Gram-positive microorganisms, particularly coagulase-negative *Staphylococcus* and *Staphylococcus aureus*, especially in late-onset neonatal sepsis. This pattern has been widely described in neonatal units and is commonly associated with catheter-related infections, prolonged hospitalization, and frequent manipulation of invasive devices ^{7,24,28,29}. Coagulase-negative *Staphylococcus* species remain among the pathogens most frequently associated with healthcare-associated infections in neonatology due to their ability to form biofilms and persist on vascular devices.

Despite the predominance of Gram-positive bacteria, clinically relevant Gram-negative microorganisms were also identified, including *Klebsiella pneumoniae*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Enterobacter* species. These pathogens deserve particular attention because of their association with antimicrobial resistance, septic shock, and increased neonatal mortality ^{17,18,30,31}. Recent international reports have demonstrated a growing burden of neonatal infections caused by multidrug-resistant Gram-negative bacteria, particularly in low- and middle-income countries ^{3,13}.

The antimicrobial therapy profile observed in this study was consistent with current recommendations for neonatal sepsis management, with predominance of empirical ampicillin and gentamicin use ^{5,6}. However, broad-spectrum antimicrobials, such as cefepime and vancomycin, were more frequently used in late-onset and microbiologically confirmed cases, suggesting therapeutic escalation in response to suspected healthcare-associated infections and more severe clinical presentations. These findings reinforce the importance of continuous microbiological surveillance and implementation of antimicrobial stewardship programs in neonatal units ^{12,20,32}.

The temporal differences observed in confirmed cases of late-onset neonatal sepsis across the pre-pandemic, pandemic, and post-pandemic periods may reflect changes in infection control practices, antimicrobial use, healthcare workflows, and the dynamics of healthcare-associated infections during the COVID-19 pandemic. Similar epidemiological changes in neonatal infections have been described in neonatal units during the pandemic period ³³.

Finally, neonatal sepsis remained associated with prolonged hospitalization, high mortality rates, and frequent need for intensive support in this cohort. Late-onset sepsis, in particular, was associated with longer hospital stays and greater exposure to invasive devices. These findings reinforce the persistent impact of neonatal sepsis on morbidity and mortality in neonatal intensive care units, especially among highly vulnerable neonatal populations ^{2,4,34–39}.

4.1. Limitations

This study has some limitations. Its retrospective single-center design may limit causal inference and the generalizability of the findings. In addition, neonatal sepsis classification partially relied on clinical suspicion and blood culture results, whose sensitivity is limited in neonates because of low blood volumes and prior antimicrobial

exposure, potentially leading to misclassification of presumed cases. Finally, inflammatory biomarkers, molecular diagnostic methods, antimicrobial resistance phenotypes, and long-term neonatal outcomes were not evaluated. Despite these limitations, the large cohort and extended study period provide a comprehensive overview of neonatal bacterial infections and sepsis in a tertiary NICU. Despite these limitations, the large cohort, extended study period, and detailed clinical and microbiological characterization strengthen the robustness and clinical relevance of the findings.

5. Conclusion

Neonatal sepsis in critically ill newborns was associated with prematurity, low birth weight, prolonged hospitalization, intensive care interventions, and increased mortality. Although presumed sepsis predominated, microbiologically confirmed and late-onset sepsis were associated with greater clinical severity, longer hospitalization, and broader antimicrobial use. These findings reinforce the importance of continuous microbiological surveillance, accurate diagnostic strategies, and antimicrobial stewardship to optimize neonatal sepsis management. Overall, this study provides a comprehensive characterization of neonatal sepsis and contributes to improving evidence-based clinical decision-making.

6. References

1. Shane AL, Sánchez PJ, Stoll BJ. Neonatal sepsis. *Lancet* 2017;390(10104):1770–1780.
2. Milton R, Gillespie D, Dyer C, Taiyari K, Carvalho MJ, Thomson K, Sands K, Portal EAR, Hood K, Ferreira A, et al. Neonatal sepsis and mortality in low-income and middle-income countries from a facility-based birth cohort: an international multisite prospective observational study. *Lancet Glob Health* 2022;10(5):e661–e672.
3. Dramowski A, Bolton L, Fitzgerald F, Bekker A, NeoNET AFRICA Partnership. Neonatal Sepsis in Low- and Middle-income Countries: Where Are We Now? *Pediatr Infect Dis J* 2025;44(6):e207–e210.
4. Fleischmann C, Reichert F, Cassini A, Horner R, Harder T, Markwart R, Tröndle M, Savova Y, Kissoon N, Schlattmann P, et al. Global incidence and mortality of neonatal sepsis: a systematic review and meta-analysis. *Arch Dis Child* 2021;106(8):745–752.
5. Cohen R, Romain O, Tauzin M, Gras-Leguen C, Raymond J, Butin M. Neonatal bacterial infections: Diagnosis, bacterial epidemiology and antibiotic treatment. *Infect Dis Now* 2023;53(8S):104793.
6. Puopolo KM, Benitz WE, Zaoutis TE, COMMITTEE ON FETUS AND NEWBORN, COMMITTEE ON INFECTIOUS DISEASES. Management of Neonates Born at $\leq 34\frac{6}{7}$ Weeks' Gestation With Suspected or Proven Early-Onset Bacterial Sepsis. *Pediatrics* 2018;142(6):e20182896.
7. Mariani M, Parodi A, Minghetti D, Ramenghi LA, Palmero C, Ugolotti E, Medici C, Saffioti C, Castagnola E. Early and Late Onset Neonatal Sepsis: Epidemiology and Effectiveness of Empirical Antibacterial Therapy in a III Level Neonatal Intensive Care Unit. *Antibiotics (Basel)* 2022;11(2):284.
8. Klingenberg C, Kornelisse RF, Buonocore G, Maier RF, Stocker M. Culture-Negative Early-Onset Neonatal Sepsis — At the Crossroad Between Efficient Sepsis Care and Antimicrobial Stewardship. *Front Pediatr* 2018;6:285.
9. Montaner Ramón A, Castilla Fernández Y, Frick MA, Camba Longueira F, Céspedes Domínguez MC, Ribes Bautista C, Castillo Salinas F. How to assess early-onset neonatal sepsis? Comparison of three detection strategies. *An Pediatr (Engl Ed)* 2023;98(2):92–98.

10. Vasilescu DI, Dan AM, Stefan LA, Vasilescu SL, Dima V, Cîrstoiu MM. Assessment of Culture-Negative Neonatal Early-Onset Sepsis: Risk Factors and Utility of Currently Used Serum Biomarkers. *Children (Basel)* 2025;12(3):355.
11. Procianoy RS, Silveira RC. The challenges of neonatal sepsis management. *J Pediatr (Rio J)* 2020;96 Suppl 1(Suppl 1):80–86.
12. Rallis D, Giapros V, Serbis A, Kosmeri C, Baltogianni M. Fighting Antimicrobial Resistance in Neonatal Intensive Care Units: Rational Use of Antibiotics in Neonatal Sepsis. *Antibiotics (Basel)* 2023;12(3):508.
13. Flannery DD, Chiotos K, Gerber JS, Puopolo KM. Neonatal multidrug-resistant gram-negative infection: epidemiology, mechanisms of resistance, and management. *Pediatr Res* 2022;91(2):380–391.
14. Duci M, Verlato G, Moschino L, Uccheddu F, Fascetti-Leon F. Advances in Artificial Intelligence and Machine Learning for Precision Medicine in Necrotizing Enterocolitis and Neonatal Sepsis: A State-of-the-Art Review. *Children (Basel)* 2025;12(4):498.
15. Fleiss N, Tarun S, Polin RA. Infection prevention for extremely low birth weight infants in the NICU. *Semin Fetal Neonatal Med* 2022;27(3):101345.
16. Ohuma EO, Moller A-B, Bradley E, Chakwera S, Hussain-Alkhateeb L, Lewin A, Okwaraji YB, Mahanani WR, Johansson EW, Lavin T, et al. National, regional, and global estimates of preterm birth in 2020, with trends from 2010: a systematic analysis. *Lancet* 2023;402(10409):1261–1271.
17. Russell NJ, Stöhr W, Plakkal N, Cook A, Berkley JA, Adhisivam B, Agarwal R, Ahmed NU, Balasegaram M, Ballot D, et al. Patterns of antibiotic use, pathogens, and prediction of mortality in hospitalized neonates and young infants with sepsis: A global neonatal sepsis observational cohort study (NeoOBS). *PLoS Med* 2023;20(6):e1004179.
18. Sands K, Carvalho MJ, Portal E, Thomson K, Dyer C, Akpulu C, Andrews R, Ferreira A, Gillespie D, Hender T, et al. Characterization of antimicrobial-resistant Gram-negative bacteria that cause neonatal sepsis in seven low- and middle-income countries. *Nat Microbiol* 2021;6(4):512–523.
19. Rajar P, Saugstad OD, Berild D, Dutta A, Greisen G, Lausten-Thomsen U, Mande SS, Nangia S, Petersen FC, Dahle UR, et al. Antibiotic Stewardship in Premature Infants: A Systematic Review. *Neonatology* 2020;117(6):673–686.
20. Mascarenhas D, Ho MSP, Ting J, Shah PS. Antimicrobial Stewardship Programs in Neonates: A Meta-Analysis. *Pediatrics* 2024;153(6):e2023065091.
21. Barreto MGP, Manso MC, Barreto RP, Barreto RP, Vasconcelos LMT de, Silva C. Frequency and Risk Factors Associated with Prematurity: A Cohort Study in a Neonatal Intensive Care Unit. *J Clin Med* 2024;13(15):4437.
22. Saldanha J, Moniz C, Machado MC. [Very Low Birth Weight Infants in a Portuguese Intensive Care Unit and the Vermont Oxford Network: 15 Years of Registry Data]. *Acta Med Port* 2019;32(11):686–692.
23. de Mello Freitas FT, Viegas APB, Romero GAS. Neonatal healthcare-associated infections in Brazil: systematic review and meta-analysis. *Arch Public Health* 2021;79:89.
24. Cho HJ, Cho H-K. Central line-associated bloodstream infections in neonates. *Korean J Pediatr* 2019;62(3):79–84.
25. Iqbal Q, Younus MM, Ahmed A, Ahmad I, Iqbal J, Charoo BA, Ali SW. Neonatal mechanical ventilation: Indications and outcome. *Indian J Crit Care Med* 2015;19(9):523–527.

26. Kaltsogianni O, Dassios T, Greenough A. Neonatal respiratory support strategies—short and long-term respiratory outcomes. *Front Pediatr* 2023;11:1212074.
27. Jo HS, Lim MN, Cho S-I. Required biological time for lung maturation and duration of invasive ventilation: a Korean cohort study of very low birth weight infants. *Front Pediatr* 2023;11:1184832.
28. Zelellw DA, Dessie G, Worku Mengesha E, Balew Shiferaw M, Mela Merhaba M, Emishaw S. A Systemic Review and Meta-analysis of the Leading Pathogens Causing Neonatal Sepsis in Developing Countries. *Biomed Res Int* 2021;2021:6626983.
29. Yu Y-Q, He X-R, Wan L-J, Yang Y-H, Chen P-Y. Etiology, antimicrobial resistance, and risk factors of neonatal sepsis in China: a systematic review and meta-analysis from data of 30 years. *J Matern Fetal Neonatal Med* 2022;35(25):7541–7550.
30. Miranda S, Harahap A, Husada D, Faramarisa FN. Risk factors of multidrug-resistant organisms neonatal sepsis in Surabaya tertiary referral hospital: a single-center study. *BMC Pediatr* 2024;24:153.
31. Ferreira IC da S, Menezes R de P, Lopes MSM, Araújo LB de, Ferreira DM de LM, Röder DVD de B. Multidrug-resistant *Klebsiella pneumoniae* in critically ill neonates: evidence from a Brazilian cohort. *Aust Crit Care* 2025;38(6):101444.
32. Maalouf FI, Saad T, Zakhour R, Yunis K. Successful establishment and five-year sustainability of a neonatal-specific antimicrobial stewardship program in a low middle-income country. *Front Pharmacol* 2022;13:1076392.
33. Graziano G, Notarbartolo V, Priano W, Maida CM, Insinga V, Rinaudo G, Russo A, Palermo R, Vitale F, Giuffrè M. Surveillance of Multidrug-Resistant Pathogens in Neonatal Intensive Care Units of Palermo, Italy, during SARS-CoV-2 Pandemic. *Antibiotics (Basel)* 2023;12(9):1457.
34. Almeida NRC de, Pontes GF, Jacob FL, Deprá JVS, Porto JPP, Lima FR de, Albuquerque MRT de. Analysis of trends in sepsis mortality in Brazil and by regions from 2010 to 2019. *Rev Saude Publica* 2022;56:25.
35. Mu C, Liu F, Tian T, Feng M, Dang X, Xie L, Liu J, Li X. Analysis of the global burden and key risk factors of neonatal sepsis and other neonatal infections in 204 countries and territories, 1990-2021. *Front Med (Lausanne)* 2025;12:1536948.
36. Bernardino FBS, Gonçalves TM, Pereira TID, Xavier JS, Freitas BHB de, Gaíva MAM. Trends in neonatal mortality in Brazil from 2007 to 2017. *Cien Saude Colet* 2022;27(2):567–578.
37. Tembo D, Abobo FDN, Kaonga P, Jacobs C, Bessing B. Risk factors associated with neonatal mortality among neonates admitted to neonatal intensive care unit of the University Teaching Hospital in Lusaka. *Sci Rep* 2024;14:5231.
38. Paixao ES, Blencowe H, Falcao IR, Ohuma EO, Rocha A dos S, Alves FJO, Costa M da CN, Suárez-Idueta L, Ortelan N, Smeeth L, et al. Risk of mortality for small newborns in Brazil, 2011-2018: A national birth cohort study of 17.6 million records from routine register-based linked data. *Lancet Reg Health Am* 2021;3:100045.
39. Sousa MRM, Parada CMG de L, Nunes HR de C. Factors associated with preventable infant mortality in 2020: a Brazilian population-based study. *Rev Bras Enferm* 77(4):e20230072.

Supplementary Material

Title: Clinical Marker Profiles Comparing Confirmed and Presumed Neonatal Sepsis in Early- and Late-Onset Sepsis within a Neonatal Intensive Care Unit Cohort

Short Title: Clinical Markers in Early- and Late-Onset Neonatal Sepsis

Patricia Krause Askinis^{1,2,3,#}; Fernando Augusto Lima Marson^{1,2,3,*,#}

¹ Laboratory of Molecular Biology and Genetics, Postgraduate Program in Health Sciences, Postgraduate Program in Health Data Science, São Francisco University, Bragança Paulista, São Paulo, Brazil.

² Laboratory of Clinical and Molecular Microbiology, Postgraduate Program in Health Sciences, Postgraduate Program in Health Data Science, São Francisco University, Bragança Paulista, São Paulo, Brazil.

³ LunGuardian Research Group — Epidemiology of Respiratory and Infectious Diseases, Postgraduate Program in Health Sciences, Postgraduate Program in Health Data Science, São Francisco University, Bragança Paulista, São Paulo, Brazil.

*** Corresponding Author:** [FALM] Fernando Augusto Lima Marson, BSc, MSc, PhD.

Laboratory of Molecular Biology and Genetics, Laboratory of Clinical and Molecular Microbiology, LunGuardian Research Group — Epidemiology of Respiratory and Infectious Diseases, Postgraduate Program in Health Sciences, Postgraduate Program in Health Data Science, São Francisco University. Avenida São Francisco de Assis, 218, Jardim São José, Bragança Paulista 12916-900, São Paulo, Brazil. Phone number: +55 19 99975-2911.

E-mail: fernandolimamarson@hotmail.com and fernando.marson@usf.edu.br.

[#] The authors contributed equally to this study.

Declarations

Funding: This work was supported by a Productivity Scholarship (FALM) from the CNPq, grant number: 305906/2024-0.

Conflicts of interest: The authors declare no conflicts of interest related to the development of this study.

Availability of data and material: The materials used in this study are available from the corresponding author upon reasonable request.

Ethical approval: The study was conducted in accordance with the principles of the Declaration of Helsinki and was approved by the Institutional Ethics Committee (Ethical approval number: 80862224.4.0000.5514; Study approval number: 6.994.663).

Authors' contributions: [PKA and FALM] were responsible for data collection and tabulation. [PKA and FALM] contributed to the interpretation of the study findings. [PKA, FALM] were involved in drafting and critically revising the manuscript prior to submission. All authors [PKA and FALM] approved the final version of the manuscript and consented to its submission to the scientific journal.

Acknowledgments: The Article Processing Charge for this publication was covered by the Brazilian Coordination for the Improvement of Higher Education Personnel (*Coordenação de Aperfeiçoamento de Pessoal de Nível*

Superior — CAPES), ROR number: 00x0ma614. For open access purposes, the authors have assigned the Creative Commons CC BY license to any accepted version of the article.

Study authors' e-mails and ORCIDs:

PKA: patriciakrause14@gmail.com

ORCID: 0009-0000-8067-0677

FALM: fernandolimamarson@hotmail.com and fernando.marson@usf.edu.br

ORCID: 0000-0003-4955-4234

Supplementary Table 1. Distribution of biological samples collected for microbiological investigation according to neonatal sepsis classification.

Early-onset neonatal sepsis	Confirmed sepsis	Presumed sepsis	Total
	[n]	[n]	
Cerebrospinal fluid	2	41	43
Skin	12	81	93
Blood	58	1,052	1,110
Urine	7	102	109
Catheter top	3	35	38
Inguinal tip	2	53	55
Secretation	0	3	3
Nasal swab	0	2	2

Late-onset neonatal sepsis	Confirmed sepsis	Presumed sepsis	Total
	[n (%)]	[n (%)]	
Cerebrospinal fluid	12	36	48
Skin	11	11	22
Blood	196	372	568
Urine	33	71	104
Catheter top	19	18	37
Inguinal tip	14	13	27
Secretation	2	0	2
Nasal swab	2	0	2

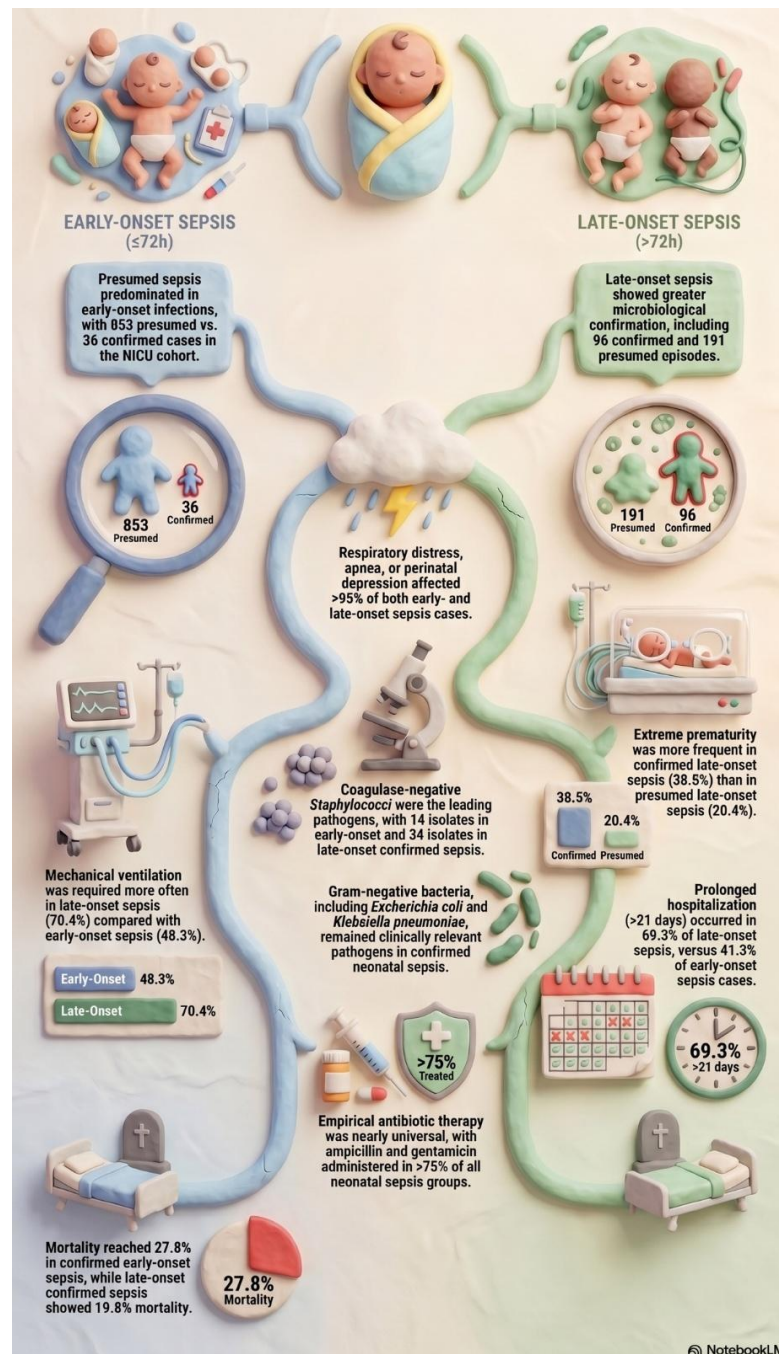
This supplementary table presents the distribution of biological samples collected for microbiological investigation among newborns with early-onset and late-onset neonatal sepsis. Sample types included cerebrospinal fluid, skin cultures, blood cultures, urine cultures, catheter tip cultures, inguinal swabs, secretion cultures, and nasal swabs. Data are stratified according to confirmed and presumed neonatal sepsis classifications and expressed as absolute frequencies [n]. Because multiple biological samples could be collected from the same newborn during hospitalization, the total number of samples may exceed the total number of patients included in the study. This table provides an overview of institutional microbiological diagnostic practices and specimen utilization patterns in neonatal sepsis evaluation.

Supplementary Table 2. Additional antimicrobial agents used in the management of early- and late-onset neonatal sepsis.

Other antimicrobial agents	Early-onset neonatal sepsis			Late-onset neonatal sepsis		
	Confirmed sepsis [n]	Presumed sepsis [n]	Total [n]	Confirmed sepsis [n]	Presumed sepsis [n]	Total [n]
Amoxicillin/clavulanate	—	3	3	—	2	3
Amphotericin B	1	3	4	—	4	4
Azithromycin	—	1	1	—	—	—
Cephalexin	—	1	1	—	—	—
Cephalothin	—	2	2	2	—	2
Cephalothin + cephalexin	—	1	1	1	—	1
Cefazolin	—	1	1	—	1	1
Cefotaxime	1	3	4	1	4	5
Ceftriaxone	—	4	4	1	—	1
Cefuroxime	—	3	3	1	1	2
Cefuroxime + teicoplanin	—	1	1	—	1	1
Clarithromycin	—	3	3	1	2	3
Clarithromycin + trimethoprim/sulfamethoxazole	—	1	1	1	—	1
Clindamycin	—	3	3	1	2	3
Clindamycin + ceftriaxone	—	1	1	—	—	—
Erythromycin	—	2	2	1	1	2
Benzathine penicillin	—	4	4	1	—	1
Penicillin G	—	5	5	2	—	2
Penicillin G + penicillin G procaine	—	1	1	—	—	—
Penicillin G + cefotaxime	—	1	1	—	—	—
Polymyxin + amphotericin B + trimethoprim/sulfamethoxazole	—	1	1	1	—	1
Teicoplanin	—	1	1	1	1	2
Tobramycin	—	3	3	1	2	3

This supplementary table summarizes less frequently prescribed antimicrobial and anti-infective agents administered during the management of neonatal sepsis in the neonatal intensive care unit. Therapies are presented separately for early-onset and late-onset neonatal sepsis and stratified according to confirmed and presumed sepsis classifications. Medications included beta-lactams, macrolides, glycopeptides, antifungal agents, aminoglycosides, and combination antimicrobial regimens. Data are presented as absolute frequencies [n]. These therapies likely reflect individualized clinical decision-making based on microbiological findings, suspected antimicrobial resistance patterns, disease severity, or specific infectious complications identified during hospitalization.

Supplementary Figure 1. Key findings comparing early- and late-onset neonatal sepsis in a NICU cohort (2017–2024). **Graph Abstract**



A total of 956 newborns with suspected neonatal sepsis were evaluated over an 8-year period. Presumed sepsis predominated in early-onset infections, whereas late-onset sepsis demonstrated a higher rate of microbiological confirmation. Late-onset sepsis was associated with greater extreme prematurity, increased need for invasive respiratory support, and prolonged hospitalization. Respiratory distress, apnea, or perinatal depression affected more than 95% of cases. Coagulase-negative *Staphylococci* were the leading pathogens in confirmed infections, followed by clinically relevant Gram-negative bacteria, including *Escherichia coli* and *Klebsiella pneumoniae*. Empirical antibiotic therapy, mainly ampicillin and gentamicin, was administered in more than 75% of neonatal sepsis cases. Mortality remained high in both groups, particularly among confirmed early-onset sepsis cases. This graphical abstract was developed with the assistance of artificial intelligence using NotebookKLM (Google) for visual design support. Scientific interpretation, data selection, and final content validation were performed by the authors.

5. CONCLUSÃO

Este estudo caracterizou a incidência e o perfil clínico, epidemiológico e microbiológico das infecções bacterianas em recém-nascidos internados em uma unidade de terapia intensiva neonatal. Observou-se elevada ocorrência de sepse neonatal, com predomínio de casos presumidos e de início precoce, em uma população composta majoritariamente por prematuros e recém-nascidos de baixo peso ao nascer. Os principais agentes etiológicos identificados foram estafilococos coagulase-negativos (CoNS) e *Staphylococcus aureus*, seguidos por bactérias Gram-negativas, com destaque para *Klebsiella pneumoniae* e *Escherichia coli*. De modo geral, observou-se baixa resistência aos antimicrobianos de primeira linha, embora a presença de microrganismos multirresistentes reforce a necessidade de vigilância microbiológica contínua. As infecções bacterianas estiveram associadas à maior necessidade de suporte intensivo, ventilação mecânica, tempo prolongado de internação, complicações clínicas e maior mortalidade, especialmente entre recém-nascidos extremamente prematuros, com extremo baixo peso ao nascer e baixos escores de Apgar. Em conjunto, os achados ampliam o conhecimento sobre o perfil das infecções bacterianas em unidades neonatais de alta complexidade e reforçam a importância da prevenção de infecções, da vigilância microbiológica e do uso racional de antimicrobianos para a melhoria da assistência neonatal.

REFERÊNCIAS

- ABOU EL FADL, D. K.; ALY, Y. A. F.; DARWEESH, E. A. G.; SABRI, N. A.; AHMED, M. A. Assessment of neonatal intensive care unit nurses' compliance with standard precautions of infection control and identification of enabling factors. *Future Journal of Pharmaceutical Sciences*, v. 9, n. 1, p. 6, 2023.
- ALMEIDA, N. R. C.; PONTES, G. F.; JACOB, F. L.; DEPRÁ, J. V. S.; PORTO, J. P. P.; LIMA, F. R.; ALBUQUERQUE, M. R. T. C. Analysis of trends in sepsis mortality in Brazil and by regions from 2010 to 2019. *Revista de Saúde Pública*, v. 56, p. 25, 2022.
- AGÊNCIA NACIONAL DE VIGILÂNCIA SANITÁRIA (ANVISA). Boletim Segurança do Paciente e Qualidade em Serviços de Saúde nº 32 – Avaliação dos Indicadores Nacionais de Infecções Relacionadas à Assistência à Saúde (IRAS) e Resistência aos Antimicrobianos (RAM): anos 2012 a 2024. Brasília: ANVISA, 2024. Disponível em: <https://www.gov.br/anvisa/>. Acesso em: 9 jun. 2025.
- BOLLIG, C.; NOTHACKER, M.; LEHANE, C.; MOTSCHALL, E.; LANG, B.; MEERPOHL, J. J.; SCHMUCKER, C. M. Prophylactic antibiotics before cord clamping in cesarean delivery: a systematic review. *Acta Obstetrica et Gynecologica Scandinavica*, v. 97, n. 5, p. 521–535, 2018.
- BOVERMAN, G.; PEREZ, C.; VIJ, S.; TGAVALEKOS, K.; RAVINDRANATH, S.; ANTONESCU, C.; CHAMBERS-HAWK, B. Neonatal ICU antibiotic use trends within an integrated delivery network. *Antimicrobial Resistance & Infection Control*, v. 11, n. 1, p. 21, 2022.
- CHU, M.; LIN, J.; WANG, M.; LIAO, Z.; CAO, C.; HU, M.; DING, Y.; LIU, Y.; YUE, S. Restrictive use of empirical antibiotics is associated with improved short-term outcomes in very low birth weight infants: a single-center retrospective cohort study from China. *Antibiotics (Basel)*, v. 12, n. 4, p. 741, 2023.
- COHEN, R.; ROMAIN, O.; TAUZIN, M.; GRAS-LEGUEN, C.; RAYMOND, J.; BUTIN, M. Neonatal bacterial infections: diagnosis, bacterial epidemiology and antibiotic treatment. *Infectious Diseases Now*, v. 53, n. 8S, p. 104793, 2023.
- DE ABREU, M. F. C. W.; OGATA, J. F. M.; FONSECA, M. C. M.; SANSONE, D.; GUINSBURG, R. The financial impact of neonatal sepsis on the Brazilian Unified Health System. *Clinics (São Paulo)*, v. 78, p. 100277, 2023.
- DRAMOWSKI, A.; BOLTON, L.; FITZGERALD, F.; BEKKER, A.; NEONET AFRICA PARTNERSHIP. Neonatal sepsis in low- and middle-income countries: where are we now?. *Pediatric Infectious Disease Journal*, v. 44, n. 6, p. e207–e210, 2025.
- KONLAN, K. D.; NUKPEZAH, R. N.; DOAT, A. R. Nurses' experiences in neonatal sepsis prevention and management: a qualitative cross-sectional study at selected hospitals in a metropolitan area. *BMC Health Services Research*, v. 24, n. 1, p. 1298, 2024.
- FLANNERY, D. D.; COGGINS, S. A.; MEDORO, A. K. Antibiotic stewardship in the neonatal intensive care unit. *Journal of Intensive Care Medicine*, v. 40, n. 8, p. 862–875, 2024.
- FENNY, A. P.; ENEMARK, U.; ASANTE, F. A.; HANSEN, K. S. Cost and length of stay associated with neonatal bloodstream infections in a teaching hospital in Ghana. *PLoS ONE*, v. 16, n. 1, p. e0244860, 2021.

FLEISCHMANN, C.; REICHERT, F.; CASSINI, A.; HORNER, R.; HARDER, T.; MARKWART, R.; TRÖNDLE, M.; SAVOVA, Y.; KISSOON, N.; SCHLATTMANN, P.; REINHART, K.; ALLEGGRANZI, B.; ECKMANN, T. Global incidence and mortality of neonatal sepsis: a systematic review and meta-analysis. *Archives of Disease in Childhood*, v. 106, n. 8, p. 745–752, 2021.

GLOBAL SEPSIS ALLIANCE. Why sepsis is a global health priority. Global Sepsis Alliance, 2023. Disponível em: <https://www.global-sepsis-alliance.org/>. Acesso em: 17 jun. 2025.

MILTON, R.; GILLESPIE, D.; DYER, C.; TAIYARI, K.; CARVALHO, M. J.; THOMSON, K.; SANDS, K.; PORTAL, E. A. R.; HOOD, K.; FERREIRA, A.; et al. Neonatal sepsis and mortality in low-income and middle-income countries from a facility-based birth cohort: an international multisite prospective observational study. *The Lancet Global Health*, v. 10, n. 5, p. e661–e672, 2022.

MINISTÉRIO DA SAÚDE (BR). Secretaria de Vigilância em Saúde e Ambiente. Perfil epidemiológico dos nascimentos prematuros no Brasil, 2012 a 2022. *Boletim Epidemiológico*, v. 55, n. 13, 2024.

MIRANDA, S.; HARAHAP, A.; HUSADA, D.; FARAMARISA, F. N. Risk factors of multidrug-resistant organisms neonatal sepsis in Surabaya tertiary referral hospital: a single-center study. *BMC Pediatrics*, v. 24, n. 1, p. 153, 2024.

MONTANER RAMÓN, A.; CASTILLA FERNÁNDEZ, Y.; FRICK, M. A.; CAMBA LONGUEIRA, F.; CÉSPEDES DOMÍNGUEZ, M. C.; RIBES BAUTISTA, C.; CASTILLO SALINAS, F. How to assess early-onset neonatal sepsis? Comparison of three detection strategies. *Anales de Pediatría (English Edition)*, v. 98, n. 2, p. 92–98, 2023.

OHUMA, E. O.; et al. National, regional, and global estimates of preterm birth in 2020, with trends from 2010: a systematic analysis. *The Lancet*, v. 402, n. 10403, p. 1261–1271, 2023.

ORTIZ DE ZÁRATE, M.; SÁENZ, C.; CIMBARO CANELLA, R.; DÍAZ, M.; MUCCI, J.; DINERSTEIN, A.; SOLANA, C. Prevalence of microbiologically confirmed neonatal sepsis at a maternity center in the City of Buenos Aires. *Archivos Argentinos de Pediatría*, v. 121, n. 3, p. e202202779, 2023.

PUOPOLO, K. M.; BENITZ, W. E.; ZAOUTIS, T. E. Management of neonates born at $\leq 34\frac{6}{7}$ weeks' gestation with suspected or proven early-onset bacterial sepsis. *Pediatrics*, v. 142, n. 6, p. e20182896, 2018.

RAJAR, P.; SAUGSTAD, O. D.; BERILD, D.; DUTTA, A.; GREISEN, G.; LAUSTEN-THOMSEN, U.; MANDE, S. S.; NANGIA, S.; PETERSEN, F. C.; DAHLE, U. R.; HAALAND, K. Antibiotic stewardship in premature infants: a systematic review. *Neonatology*, v. 117, n. 6, p. 673–686, 2020.

RALLIS, D.; GIAPROS, V.; SERBIS, A.; KOSMERI, C.; BALTOGIANNI, M. Fighting antimicrobial resistance in neonatal intensive care units: rational use of antibiotics in neonatal sepsis. *Antibiotics (Basel)*, v. 12, n. 3, p. 508, 2023.

REYES, A. Hemocultivos negativos en sepsis neonatal en UCIN. *Revista Chilena de Infectología*, v. 35, n. 2, p. 216–217, 2018.

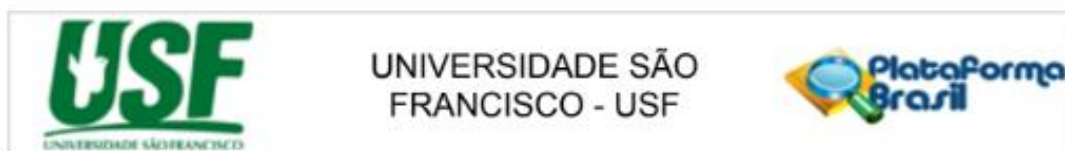
SOCIEDADE BRASILEIRA DE PEDIATRIA. Seps neonatal precoce e abordagem do RN de risco: recomendações para a prática clínica. Departamento Científico de Neonatologia da SBP, Rio de Janeiro, 2022.

VASILESCU, D. I.; DAN, A. M.; STEFAN, L. A.; VASILESCU, S. L.; DIMA, V.; CÎRSTOIU, M. M. Assessment of culture-negative neonatal early-onset sepsis: risk factors and utility of currently used serum biomarkers. *Children (Basel)*, v. 12, n. 3, p. 355, 2025.

WORLD HEALTH ORGANIZATION. Sepsis. World Health Organization, 2024. Disponível em: <https://www.who.int/news-room/fact-sheets/detail/sepsis>. Acesso em: 17 jun. 2025.

ANEXO

Anexo 1. Aprovação do CAAE



PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: Prevalência e fatores de risco associados a infecções de caráter bacteriano nos recém-nascidos que foram hospitalizados na Unidade de Terapia Intensiva Neonatal de um Hospital Universitário, antes, durante e após a pandemia da coronavirus disease (COVID)-19

Pesquisador: Fernando Augusto de Lima Marson

Área Temática:

Versão: 2

CAAE: 80862224.4.0000.5514

Instituição Proponente: Universidade São Francisco-SP

Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 6.994.663

Apresentação do Projeto:

Este estudo será do tipo observacional e retrospectivo, abrangendo o período de 01 de janeiro de 2015 a 31 de maio de 2024. A pesquisa será conduzida na Unidade de Terapia Intensiva Neonatal de um Hospital Universitário. A população do estudo será composta por todos os recém-nascidos admitidos na Unidade de Terapia Intensiva do Hospital Universitário durante o período especificado. A amostra irá incluir todos os recém-nascidos que desenvolveram infecções bacterianas confirmadas por exames laboratoriais, conforme registrado nos prontuários médicos, bem como, os pacientes hospitalizados na unidade e que não apresentaram evolução clínica com a presença de infecção bacteriana. A presença de infecção pelo vírus SARS-CoV-2 será computada perante a presença de positividade no exame molecular realizado pela técnica real-time polymerase chain reaction. Os dados serão coletados a partir dos prontuários médicos eletrônicos e físicos dos recém-nascidos admitidos na Unidade de Terapia Intensiva Neonatal. As informações a serem extraídas incluem: dados demográficos (idade gestacional, peso ao nascer, sexo); diagnósticos clínicos; resultados de exames microbiológicos; tratamentos administrados (antibióticos, suporte respiratório e dentre outros); desfechos clínicos (tempo de internação, complicações, mortalidade) e períodos temporais específicos (pré-pandêmico: 01/01/2015 a 31/12/2019; pandêmico: 01/01/2020 a 31/12/2021;

Endereço: Av. São Francisco de Assis, 218, sala 35, prédio central

Bairro: Cidade Universitária

CEP: 12.916-900

UF: SP

Município: BRAGANÇA PAULISTA

Telefone: (11)2454-8302

E-mail: comiteetica@usf.edu.br



UNIVERSIDADE SÃO
FRANCISCO - USF



Continuação do Parecer: 6.994.663

pós-pandêmico: 01/01/2022 a 31/05/2024).

Objetivo da Pesquisa:

Objetivo Primário: Avaliar a incidência, padrões e impacto das infecções bacterianas em recém-nascidos hospitalizados na Unidade de Terapia Intensiva Neonatal de um Hospital Universitário, investigando as variações temporais e os efeitos associados à pandemia da COVID-19.

Objetivo Secundário: 1. Determinar a incidência de infecções bacterianas em recém-nascidos admitidos na Unidade de Terapia Intensiva Neonatal do Hospital Universitário durante o período de 01/01/2015 a 31/05/2024. 2. Identificar os principais agentes etiológicos das infecções bacterianas e padrão de resistência aos antibióticos em recém-nascidos na Unidade de Terapia Intensiva Neonatal, por meio das análises de prontuários. 3. Analisar as características clínicas dos recém-nascidos afetados por infecções bacterianas na Unidade de Terapia Intensiva Neonatal do Hospital Universitário em cada período temporal da pandemia, incluindo idade gestacional, peso ao nascer, presença de comorbidades e necessidade de intervenções médicas. 4. Avaliar os desfechos clínicos dos recém-nascidos com infecções bacterianas na Unidade de Terapia Intensiva Neonatal do Hospital Universitário durante os diferentes períodos temporais, incluindo taxa de mortalidade, tempo de internação, necessidade de ventilação mecânica e complicações associadas. 5. Investigar os fatores associados às variações temporais na incidência e padrões de infecções bacterianas, incluindo o padrão de resistência aos antibióticos, em recém-nascidos na Unidade de Terapia Intensiva Neonatal do Hospital Universitário, incluindo medidas de controle de infecções, mudanças nos protocolos de atendimento e impacto da pandemia da COVID-19. 6. Propor recomendações para aprimorar as estratégias de prevenção, diagnóstico e tratamento das infecções bacterianas em recém-nascidos na Unidade de Terapia Intensiva Neonatal do Hospital Universitário, com base nos resultados da pesquisa e nas lições aprendidas durante a pandemia da COVID-19.

Avaliação dos Riscos e Benefícios:

Riscos: O estudo será submetido ao Comitê de Ética em Pesquisa do Hospital Universitário para aprovação. Serão garantidos o anonimato e a confidencialidade dos dados dos pacientes. Como se trata de um estudo retrospectivo, o risco para os participantes é mínimo, e será solicitada a dispensa do Termo de Consentimento Livre e Esclarecido. Dentro o risco, o principal fator a ser considerado é a possibilidade de quebra de anonimato com a identificação do participante da Pesquisa. Dentre os principais benefícios destaca-se a possibilidade de conhecer o perfil epidemiológico dos pacientes unidade de terapia intensiva neonatal e a presença de infecções

Endereço: Av. São Francisco de Assis, 218, sala 35, prédio central
Bairro: Cidade Universitária **CEP:** 12.916-900
UF: SP **Município:** BRAGANÇA PAULISTA
Telefone: (11)2454-8302

E-mail: comiteetica@usf.edu.br



UNIVERSIDADE SÃO
FRANCISCO - USF



Continuação do Parecer: 6.994.663

bacterianas. Neste contexto, será possível elencar possíveis meios de controle de infecção com o intuito de prover melhores cuidados e, possivelmente, desfechos clínicos para pacientes a serem hospitalizados na unidade referida.

Benefícios: O estudo será submetido ao Comitê de Ética em Pesquisa do Hospital Universitário para aprovação. Serão garantidos o anonimato e a confidencialidade dos dados dos pacientes. Como se trata de um estudo retrospectivo, o risco para os participantes é mínimo, e será solicitada a dispensa do Termo de Consentimento Livre e Esclarecido. Dentro o risco, o principal fator a ser considerado é a possibilidade de quebra de anonimato com a identificação do participante da Pesquisa. Dentre os principais benefícios destaca-se a possibilidade de conhecer o perfil epidemiológico dos pacientes unidade de terapia intensiva neonatal e a presença de infecções bacterianas. Neste contexto, será possível elencar possíveis meios de controle de infecção com o intuito de prover melhores cuidados e, possivelmente, desfechos clínicos para pacientes a serem hospitalizados na unidade referida.

Comentários e Considerações sobre a Pesquisa:

A metodologia de análise de dados compreenderá: a) aquisição de dados: os dados serão obtidos por meio de um arquivo .xls digitado pelos pesquisadores a partir das fichas de coleta. O arquivo será visualizado no software Statistical Package for the Social Sciences (SPSS) (IBM SPSS Statistics for Macintosh, versão 27.0.). b) análise de dados ausentes/perdidos (missing data): Serão excluídas as características dos pacientes que apresentaram mais de 40% de dados faltantes. Os dados perdidos serão imputados usando o XLSTAT Statistical Software para Excel. Para os dados qualitativos (categóricos), serão estimados os dados perdidos utilizando o algoritmo NIPALS (Nonlinear Iterative Partial Least Squares); também, para os dados quantitativos (numéricos), serão estimados os dados perdidos usando o algoritmo de imputação múltipla da cadeia MCMC (Markov Chain Monte Carlo). O XLSTAT Statistical Software gera um novo conjunto de dados em formato de leitura em excel (.xls) e será usado para realizar as análises estatísticas no software SPSS e análise inferencial. c) Análise descritiva: Serão realizadas as análises descritivas utilizando o número de indivíduos (N) e o percentual (%) para os dados categóricos. Também serão apresentadas a média $\pm$ desvio padrão [ou média (mediana) e intervalo de confiança de 95% (IC95%)] para os dados numéricos. Para as análises estatísticas inferenciais, quando possível, também serão apresentadas a razão de chances (OR, do inglês Odds Ratio) e o IC95%. f) Análise bivariada: Será utilizada a análise estatística bivariada utilizando os softwares SPSS e o OpenEpi (OpenEpi: Open-Source

Endereço: Av. São Francisco de Assis, 218, sala 35, prédio central

Bairro: Cidade Universitária

CEP: 12.916-900

UF: SP

Município: BRAGANCA PAULISTA

Telefone: (11)2454-8302

E-mail: comiteetica@usf.edu.br



UNIVERSIDADE SÃO
FRANCISCO - USF



Continuação do Parecer: 6.994.663

Epidemiologic Statistics for Public Health, Versão. www.OpenEpi.com, 2013/04/06). Será utilizado o teste de Qui-quadrado (e o teste Exato de Fisher) para avaliar a frequência de óbitos de acordo com os dados epidemiológicos entre os indivíduos com e sem infecção bacteriana. Serão calculados o OR e o IC95% para estimar a associação de cada marcador com a presença de óbito. Será calculado o OR usando o software OpenEpi para tabelas 2 x 2, incluindo o valor para cada característica do paciente. Ademais, o risco de óbito será comparado com marcadores numéricos pelo uso do Teste T ou de Mann-Whitney, a depender da distribuição dos dados. A normalidade dos dados numéricos será avaliada pelas seguintes técnicas: (i) análise de medidas descritivas para tendência central; (ii) método gráfico (gráfico Q-Q normal, gráfico Q-Q sem tendência e boxplot; e (iii) método por teste estatístico (testes de normalidade); Kolmogorov-Smirnov e Shapiro-Wilk. Os resultados serão resumidos em tabelas e números. g) Análise multivariada: Será utilizada a análise multivariada utilizando o Modelo de Regressão Logística Binária com o método Stepwise Backward e serão incluídos no modelo de regressão os marcadores com $P \leq 0,05$ na análise bivariada. A variável resposta será o desfecho (recuperação, alta hospitalar ou óbito). As características de pacientes serão incluídas na análise multivariada e não serão utilizadas as características dos pacientes com $P > 0,05$. No Modelo de Regressão Logística serão apresentados os seguintes dados (i) coeficiente B [incluindo o EP (erro padrão)], que para a constante será chamado de interceptação; (ii) o teste Qui-quadrado de Wald e sua significância; (iii) graus de liberdade para o teste Qui-quadrado de Wald; e (iv) $\text{Exp}(B)$ que representa a exponenciação do coeficiente B (OR), incluindo seu IC95%. Antes de realizar a análise estatística, serão testados os marcadores para multicolinearidade considerando como pontos de cortes 10 para fator de inflação de variância. h) Os gráficos (figuras) serão construídos usando o GraphPad Prism versão 8.0.0 para Mac, GraphPad Software, San Diego, Califórnia, EUA (<http://www.graphpad.com>).

Considerações sobre os Termos de apresentação obrigatória:

O pesquisador principal encaminha na presente documentação a retificação do termo de confidencialidade ("TermoConfidencialidade.pdf") atendendo ao último parecer deste Comitê que emitiu pendência no projeto "Prevalência e fatores de risco associados a infecções de caráter bacteriano nos recém-nascidos que foram hospitalizados na Unidade de Terapia Intensiva Neonatal de um Hospital Universitário, antes, durante e após a pandemia da coronavirus disease (COVID)-19".

Recomendações:

Vide "Conclusões ou Pendências e Lista de Inadequações".

Endereço: Av. São Francisco de Assis, 218, sala 35, prédio central

Bairro: Cidade Universitária

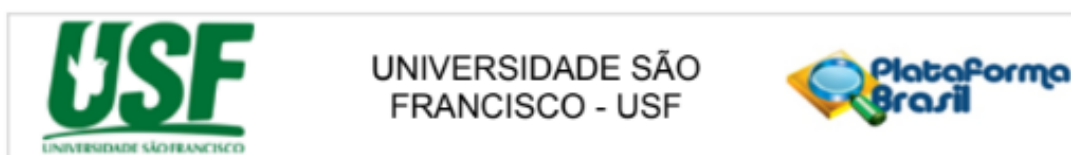
CEP: 12.916-900

UF: SP

Município: BRAGANÇA PAULISTA

Telefone: (11)2454-8302

E-mail: comiteetica@usf.edu.br



Continuação do Parecer: 6.994.663

Conclusões ou Pendências e Lista de Inadequações:

Não foram observados óbices éticos.

Considerações Finais a critério do CEP:

APÓS DISCUSSÃO EM REUNIÃO DO DIA 08/08/2024, O COLEGIADO DELIBEROU PELA APROVAÇÃO DO PROJETO DE PESQUISAS. SENDO OBRIGATÓRIO O ENVIO DE RELATÓRIOS PARCIAIS DE SEIS EM SEIS MESES E APÓS A CONCLUSÃO DO PROJETO É OBRIGATÓRIO O ENVIO DO RELATÓRIO FINAL PARA ENCERRAMENTO DO PROJETO.

Este parecer foi elaborado baseado nos documentos abaixo relacionados:

Tipo Documento	Arquivo	Postagem	Autor	Situação
Informações Básicas do Projeto	PB_INFORMAÇÕES_BÁSICAS_DO_PROJETO_2363303.pdf	03/07/2024 15:44:50		Aceito
Outros	RespostaPendencia.pdf	03/07/2024 15:40:56	Fernando Augusto de Lima Marson	Aceito
Cronograma	Cronograma.pdf	03/07/2024 15:39:48	Fernando Augusto de Lima Marson	Aceito
Outros	TermoConfidencialidade.pdf	03/07/2024 15:39:09	Fernando Augusto de Lima Marson	Aceito
Projeto Detalhado / Brochura Investigador	ProjetoV2.docx	03/07/2024 15:38:13	Fernando Augusto de Lima Marson	Aceito
Folha de Rosto	FolhaRostoAssinado.pdf	21/06/2024 13:46:12	Fernando Augusto de Lima Marson	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	TermoDeDispensaPatricia.docx	21/06/2024 13:46:00	Fernando Augusto de Lima Marson	Aceito
Outros	TermoCompromisso.pdf	21/06/2024 13:45:23	Fernando Augusto de Lima Marson	Aceito
Outros	AutorizacaoPesquisadorResponsavel.pdf	21/06/2024 13:44:58	Fernando Augusto de Lima Marson	Aceito
Outros	AutorizacaoCoordenadorSetorColeta.pdf	21/06/2024 13:44:33	Fernando Augusto de Lima Marson	Aceito
Outros	AutorizacaoCoordenadoraPosGraduacao.pdf	21/06/2024 13:44:00	Fernando Augusto de Lima Marson	Aceito
Declaração de concordância	DiretorHospitalFiliponeAssinado.pdf	21/06/2024 13:42:45	Fernando Augusto de Lima Marson	Aceito

Situação do Parecer:

Endereço: Av. São Francisco de Assis, 218, sala 35, prédio central

Bairro: Cidade Universitária

CEP: 12.916-900

UF: SP

Município: BRAGANCA PAULISTA

Telefone: (11)2454-8302

E-mail: comiteetica@usf.edu.br



UNIVERSIDADE SÃO
FRANCISCO - USF



Continuação do Parecer: 6.994.663

Aprovado

Necessita Apreciação da CONEP:

Não

BRAGANCA PAULISTA, 09 de Agosto de 2024

Assinado por:

Miguel Simão Haddad Filho
(Coordenador(a))

Endereço: Av. São Francisco de Assis, 218, sala 35, prédio central

Bairro: Cidade Universitária

CEP: 12.916-900

UF: SP

Município: BRAGANCA PAULISTA

Telefone: (11)2454-8302

E-mail: comiteetica@usf.edu.br

APÊNDICE

Apêndice A – Instrumento de Coleta de Dados

1. Identificação do Paciente

- Número do prontuário: _____
- Data de nascimento: ____ / ____ / ____
- Data de admissão: ____ / ____ / ____
- Data de alta / óbito / transferência: ____ / ____ / ____

2. Dados Demográficos

- Idade gestacional (semanas completas): _____
- Peso ao nascer (g): _____
- Sexo: ☐ Masculino ☐ Feminino ☐ Indeterminado
- Apgar:
 - 1º minuto: _____
 - 5º minuto: _____

3. Dados do Parto

- Tipo de parto: ☐ Cesárea ☐ Vaginal
- Bolsa amniótica: ☐ Íntegra ☐ Rota
- Presença de mecônio: ☐ Sim ☐ Não
- Streptococcus do grupo B: ☐ Positivo ☐ Negativo ☐ Desconhecido
- Uso materno de antibiótico antes do parto: ☐ Sim ☐ Não

4. Diagnósticos Clínicos Neonatais

Diagnósticos principais (marcar todos que se aplicam):

- ☐ Prematuridade
- ☐ Desconforto respiratório
- ☐ Asfixia perinatal
- ☐ Hipoglicemia
- ☐ Cardiopatia congênita
- ☐ Malformação congênita
- ☐ Convulsões
- ☐ Icterícia
- ☐ Atresias
- ☐ Outros (especificar): _____

5. Resultados de Exames Microbiológicos

Data da coleta: ____ / ____ / ____

Tipo de cultura:

- ☐ Hemocultura
- ☐ Urocultura
- ☐ Líquido cefalorraquidiano (LCR)
- ☐ Ponta de cateter
- ☐ *Swab* de pele
- ☐ Outros: _____

Agente etiológico identificado:

- ☐ *Escherichia coli*
- ☐ *Staphylococcus aureus*
- ☐ *Klebsiella pneumoniae*
- ☐ *Acinetobacter spp.*
- ☐ *Enterococcus spp.*
- ☐ *Streptococcus agalactiae* (grupo B)
- ☐ *Pseudomonas aeruginosa*
- ☐ *Enterobacter spp.*
- ☐ *Candida spp.*
- ☐ *Streptococcus pneumoniae*
- ☐ *Listeria monocytogenes*
- ☐ *Neisseria meningitidis*
- ☐ *Cryptococcus neoformans*
- ☐ *Staphylococcus saprophyticus*
- ☐ *Proteus mirabilis*
- ☐ *Haemophilus influenzae*
- ☐ *Salmonella spp.*
- ☐ *Shigella spp.*
- ☐ *Campylobacter jejuni*
- ☐ Outros (especificar): _____

Antibiograma – Perfil de resistência:

- ☐ Sensível
- ☐ Multirresistente (MDR)
- ☐ ESBL
- ☐ KPC
- ☐ MRSA
- ☐ VRE

☐ Outro: _____

6. Classificação da Seps Neonatal

· Tempo de início:

☐ Precoce (≤ 72 horas de vida)

☐ Tardia (> 72 horas de vida)

· Tipo:

☐ Confirmada (cultura positiva)

☐ Presumida (cultura negativa)

7. Tratamentos Administrados

Antibióticos utilizados (marcar todos):

☐ Ampicilina

☐ Amicacina

☐ Amoxicilina

☐ Amoxicilina + clavulanato

☐ Cefotaxima

☐ Ceftriaxona

☐ Clindamicina

☐ Gentamicina

☐ Imipenem

☐ Penicilina G

☐ Meropenem

☐ Piperacilina-tazobactam

☐ Vancomicina

☐ Outros (especificar): _____

Antifúngicos:

☐ Fluconazol

☐ Anfotericina B

☐ Outros: _____

Suporte respiratório:

☐ Não

☐ Sim → ☐ Ventilação mecânica ☐ CPAP ☐ Oxigênio suplementar

Procedimentos invasivos:

☐ Cateter umbilical

☐ PICC

- ☐ Dreno de tórax
- ☐ Cateter venoso central (CDL)
- ☐ Flebotomia
- ☐ Traqueostomia
- ☐ Procedimento cirúrgico

8. Desfechos Clínicos

- Tempo de internação (dias): _____
- Complicações (especificar): _____

Desfecho final:

- ☐ Alta hospitalar ☐ Óbito ☐ Transferência