Risk Factors of Early-Onset Neonatal Sepsis: A Systematic Review and Meta-Analysis

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Research

Keywords: Neonatal sepsis, risk factors, Meta-analysis

DOI: https://doi.org/10.21203/rs.3.rs-661481/v1

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Abstract

**Background:** Early-onset neonatal sepsis is a great managerial challenge worldwide. Studying the risk factors of early-onset neonatal sepsis is one of the most significant ways of reducing the incidence of sepsis and the associated health burden.

**Methods:** A literature search strategy was constructed, including PubMed, EMBASE, Web of Science, and the Cochrane Library. All publications until April 30, 2021 were retrieved; the key words were “neonatal sepsis” and “risk factors.” Moreover, the references of the retrieved articles were screened to identify related eligible studies. Data abstraction was performed in accordance with PRISMA guidelines. The Newcastle-Ottawa Scale and Agency for Healthcare Research and Quality scale scores were used to evaluate the quality of the included studies, and the fixed-effects model was used to combine the results. Risk factors related to the occurrence of early-onset neonatal sepsis, the pooled OR, and the 95% CI upper and lower limits were obtained to represent the correlation strength between risk factors and early-onset neonatal sepsis occurrence; the pooled $I^2$ value was used to determine the heterogeneity of the combined results.

**Results:** We included 21 articles with 44 non-repetitive risk factors, 91985 neonates, and 6627 cases of neonatal sepsis. We identified seven independent risk factors of early-onset neonatal sepsis, including at least three per vaginal examinations during labor and delivery, chorioamnionitis, premature rupture of membranes > 18 h, male fetus, gestational age < 37 weeks, neonatal resuscitation, and central venous catheterization, with the following ORs and 95% CIs: 7.18 (3.51-14.69), 6.56 (3.19-13.49), 2.74 (1.44-5.21), 3.03 (1.94-4.73), 4.08 (2.76-6.03), 2.6 (2.03-3.34), and 3.06 (1.64-5.73), respectively.

**Conclusions:** Our results suggest that frequent per vaginal examination during labor and delivery, chorioamnionitis, premature rupture of membranes (> 18 h), male fetus, gestational age (< 37 weeks), neonatal resuscitation, and central vascular catheterization are independent risk factors of early-onset neonatal sepsis. These findings support the use of empirical antibiotic therapy in neonates with these risk factors.

Introduction

Early-onset neonatal sepsis (EOS) is the main cause of neonatal mortality in the first week of life.$^{1-3}$ The mortality rate of EOS in some high-income countries can reach 16%; this may be higher in middle- and low-income countries, resulting in a huge health burden and medical care cost.$^4$ However, the initially asymptomatic nature of most cases of EOS and the non-specific clinical manifestations in some neonates render an early diagnosis of EOS difficult; moreover, the commonly used biomarkers for EOS diagnosis have low sensitivities and specificities.$^5$ In addition, despite improvements in knowledge regarding sepsis, effective intervention for patients with sepsis are largely limited. Therefore, an early prevention of neonatal sepsis is one of the most meaningful ways of reducing EOS-related complications and neonatal mortality.$^6,7$ To reduce the incidence of EOS, it is important to acquire more knowledge about the risk factors of EOS, monitor neonates closely, and administer antibiotics early to neonates with risk factors of EOS; this not only prevents the abuse of broad-spectrum antibiotics, but also controls the development of drug-resistant bacteria.$^8$

However, results of most previous studies that evaluated the risk factors of EOS are limited as most of these studies were single-center studies that performed partial screening for risk factors.$^9-29$ Hence, we aimed to
comprehensively explore the risk factors of EOS, using a systematic review wherein we extracted existing results of relevant risk factors. Further, we performed a meta-analysis of some of these factors to provide guidance for the prevention and management of EOS.

**Methods**

In accordance with the recommendations of the Quality of Reporting of Meta-analysis Statement, we developed a protocol before conducting this study. Our study was conducted following the Preferred Reporting Items for Systematic Reviews and Meta-analyses guidelines (Supplementary Material 1), and registration number is INPLASY202160049 (https://inplasy.com/), DOI number is 10.37766/inplasy2021.6.0049 (https://www.doi.org). All analyses were based on previously published articles; therefore, neither an ethics review nor consent of the patients’ families was required for this study.

**Search Strategy**

A literature search strategy was constructed, including PubMed, EMBASE, Web of Science, and the Cochrane Library. All publications until April 30, 2021, were retrieved; the key words used were “neonatal sepsis” and “risk factors.” Concurrently, the references of the retrieved articles were screened to identify related eligible studies.

**Eligibility Criteria**

The selection of articles for this study was based on the following criteria: 1) EOS diagnosed based on clear laboratory and/or clinical standards; 2) the use of multivariate regression analysis to determine risk factors; and 3) the presence of complete literature data, including OR and 95% CI of risk factors. We excluded case reports, comments, abstracts, letters, and agreements.

**Data Extraction**

Data were evaluated and extracted by two researchers (L.G. and G.P.) using a data extraction table. In case of disagreement between the researchers, a third person (J.S.) was consulted, and all three researchers worked together to reach a consensus. The contents of the data extraction table included: 1) basic information (the first author of each study, year of publication, country or region of publication, and EOS-associated risk factors), 2) Study data (the study sample size, number of neonates with EOS, ORs of risk factors obtained using multivariate logistic regression analysis, and the upper and lower limits of 95% CIs). If necessary, the authors (D.W. and G.P.) obtained supplementary data by contacting the authors of the published studies.

**Risk Bias Evaluation of Included Studies**

Articles were selected by two independent reviewers to reduce publication bias and heterogeneity; the Newcastle-Ottawa Scale score was used to evaluate the quality of case-control and cohort studies, whereas the Agency for Healthcare Research and Quality scale score was used to evaluate the quality of cross-sectional studies.

**Statistical Analysis**

Based on the results of multivariate analysis of the current research data, the adjusted ORs and 95% CI upper limits (ul) and lower limits (ll) were obtained to represent the correlation strength between risk factors and EOS occurrence. Further, the results were converted to the corresponding logarithms: ln(OR), ln(ul), and ln(ll). The
sampling error (SE) of each risk factor was calculated as $SE = (\ln(ul) - \ln(ll)) / 3.92$, and the transformed $\ln(OR)$ and SE values were entered into the Review Manager software for meta-analysis. According to the detailed analysis, the $I^2$ value and total $p$ value of forest plots were obtained. $I^2 = 0$ indicated that the differences between studies were caused by sampling errors; $I^2$ values of 25%-50%, 50%-75%, and $\geq 75\%$ were considered as low, moderate, and high heterogeneities, respectively. In the absence and presence of heterogeneity, fixed- (Mantel Haenszel method) and random (Dersimonian Laird method)-effects models were used for analysis, respectively. Sensitivity analysis performed to summarize results with heterogeneity. Research results that had a significant influence on the change in $I^2$ value before and after removal were considered as the source of heterogeneity and re-analyzed after removal. Funnel plots were used to qualitatively examine publication bias (the more symmetrical the plots, the lower the risk of publication bias). The above-mentioned statistical methods were completed using RevMan software (5.3 Vision; Copenhagen: The Nordic Cochrane Center, The Cochrane Collaboration, 2014), and a $p$ value less than 0.05 was considered to be statistically significant. To ensure the accuracy and reliability of the results, two authors (Q.W. and L.G.) independently input the data into the software, and the obtained results were checked for consistency.

Results

Study Characteristics

After a systematic search and screening of existing research, 5450 articles were retrieved, 5429 articles were excluded based on the eligibility criteria, and 21 articles were included in the study (Fig. 1). All included studies were observational studies; ten, seven, and four were case-control, cohort, and cross-sectional studies, respectively. Nine of the included studies focused on EOS, and the remaining 12 studies did not distinguish between EOS and late-onset sepsis (LOS). Results of the quality of included studies are shown in the attached tables (Supplementary Material 2–4). A total of 91985 neonates were included, of which 6627 (7.2%) had neonatal sepsis. The study population mainly originated from Asia (48%), followed by North America, Europe, and Africa.

Three and one articles focused on nosocomial and community-acquired infections, respectively; no relevant information was provided in the remaining articles. Eight studies focused on neonates undergoing intensive care, and no relevant information was provided for the rest of the neonates. The number of premature neonates (gestational age [GA] < 37 weeks) included in the study was 39228, accounting for 42.6% of the total number of included neonates. Two, four, one, one and one studies focused on neonates with very low birth weight (< 1500 g), premature neonates, the risk factors of neonatal sepsis from the perspective of bacterial etiology, congenital diseases (diaphragmatic hernia) of neonates, and the risk factors of multiple drug resistance, respectively. Patients with neonatal sepsis included those with clinical suspicion and those with positive blood cultures. Of patients with neonatal sepsis, 1093 neonates had positive blood cultures, accounting for 18.9% of the corresponding neonatal sepsis. Among them, 54% for gram-negative bacteria, 43% for gram-positive bacteria, and 3% for fungi. The main bacterial groups were Escherichia coli (n = 215), coagulase-negative Staphylococci (CONS) (n = 160), Klebsiella pneumoniae (n = 158), Group B Streptococcus (n = 81), Staphylococcus aureus (n = 76), Enterobacter cloacae (n = 7), and other bacteria. The main fungal species was Candida spp. (n = 30). The most common drug-resistant strains of neonatal sepsis-
causing bacteria were *A. baumannii* and *K. pneumoniae* carbapenemase-producing bacteria. Most (96%-99%) *K. pneumoniae* bacteria were resistant to 94%-97% of third-generation cephalosporins. The main source of infection was a contaminated catheter, whereas the main infection sites were the lungs, urinary tract, and intestinal tract.

Based on the inclusion criteria, 44 non-repetitive risk factors were included in the study (Table 1). Our meta-analysis included ten risk factors that were present in at least two studies. These risk factors included: at least three per vaginal (PV) examinations during labor and delivery in two studies; chorioamnionitis, based on clinical and laboratory diagnosis, in five studies; premature rupture of membranes (PROM) (> 12 h, > 18 h, and > 24 h) in six studies; caesarean section in two studies; birth weight in five studies (< 1500 g and < 2500 g); GA in 11 studies (< 32 weeks and < 37 weeks; two studies did not specify the GA); the presence of central venous catheter (CVC) in three studies; neonatal resuscitation in three studies; sex in two studies; and APGAR score in four studies (1 and 5 min).
Table 1
Main Characteristics of the Included Studies

| Country | Source                  | Neonates (n) | Neonatal Sepsis (n) | Risk Factors                  | OR  | Low Limit | Up Limit | P   |
|---------|-------------------------|--------------|---------------------|-------------------------------|-----|-----------|----------|-----|
| Canada  | Beaulieu et al,9 2017   | 13449        | 83                  | Neutropenia                   | 8.80| 1.80      | 44.10    | **  |
|         |                         |              |                     | Chorioamnionitis              | 6.20| 1.60      | 24.90    | **  |
| Ethiopia| Alemu et al,10 2019     | 246          | 82                  | PROM (Yes)                    | 2.81| 1.02      | 7.79     | *   |
|         |                         |              |                     | Gestational age < 37 weeks    | 6.90| 2.76      | 17.28    | *** |
|         |                         |              |                     | APGAR at 5 minute < 7         | 17.67| 6.11      | 51.12    | *** |
|         |                         |              |                     | Cry immediately (No)          | 2.85| 1.09      | 7.47     | *   |
|         |                         |              |                     | Resuscitation (Yes)           | 2.85| 1.09      | 7.47     | *   |
| Mexico  | Pérez et al,11 2015     | 14207        | 67                  | Mother ≤ 15 years             | 3.50| 1.56      | 7.85     | *   |
|         |                         |              |                     | PROM > 18 hours               | 2.65| 1.18      | 5.92     | *   |
|         |                         |              |                     | Maternal fever                | 6.04| 1.54      | 23.60    | *   |
|         |                         |              |                     | Birth weight ≤ 2500 g         | 4.82| 2.38      | 9.75     | *   |
|         |                         |              |                     | Gestational age < 37 weeks    | 3.14| 1.58      | 6.22     | *   |
| Zambia  | Kabwe et al,12 2016     | 342          | 103                 | Nasal flaring                 | 0.54| 0.31      | 0.96     | *   |
|         |                         |              |                     | Pallor                        | 0.36| 0.14      | 0.94     | *   |
|         |                         |              |                     | Increasing parity             | 1.18| 1.01      | 1.37     | *   |
|         |                         |              |                     | Maternal HIV infection        | 0.46| 0.23      | 0.93     | *   |
| Belgium | Mahieu et al,13 2000    | 559          | 80                  | Lipid emulsion (Yes)          | 4.22| 1.59      | 12.49    | **  |
|         |                         |              |                     | Nonumbilical CVC (Yes)        | 2.49| 1.11      | 5.70     | *   |
|         |                         |              |                     | Total parenteral nutrition (Yes)| 2.87| 1.12      | 8.02     | *   |
| Country     | Source                        | Neonates (n) | Neonatal Sepsis (n) | Risk Factors                          | OR  | Low Limit | Up Limit | P   |
|------------|-------------------------------|--------------|---------------------|---------------------------------------|-----|-----------|----------|-----|
| Florida    | Yancey et al, 1996            | 833          | 15                  | Chorioamnionitis                      | 26.00 | 14.10     | 47.90    | *   |
|            |                               |              |                     | Preterm delivery                      | 4.00 | 2.30      | 7.00     | *   |
|            |                               |              |                     | PROM > 12 hours                       | 2.80 | 1.70      | 4.70     | *   |
|            |                               |              |                     | Endometritis                          | 3.00 | 1.20      | 7.50     | *   |
|            |                               |              |                     | Group B streptococcus                 | 1.90 | 1.10      | 3.10     | *   |
|            |                               |              |                     | Age                                   | 0.90 | 0.90      | 0.99     | *   |
| France     | Levy et al, 2017              | 82           | 28                  | Gestational age                       | 0.44 | 0.22      | 0.86     | *   |
|            |                               |              |                     | Birth weight                          | 1.00 | 1.00      | 1.01     | *   |
|            |                               |              |                     | Intra-thoracic liver                  | 8.32 | 1.44      | 48.10    | *   |
|            |                               |              |                     | Central vascular catheter             | 34.58 | 2.86      | 417.64   | **  |
| Ethiopia   | Tsehaynesh et al, 2017        | 251          | 251                 | Caesarean section                     | 5.19 | 2.36      | 11.37    | *** |
|            |                               |              |                     | Gestational age                       | 8.99 | 4.18      | 19.38    | *** |
|            |                               |              |                     | Birth weight                          | 12.37 | 4.14     | 37.04    | *** |
|            |                               |              |                     | APGAR score                           | 0.44 | 0.22      | 0.89     | *   |
| Uganda     | Kayom et al, 2018             | 338          | 35                  | Paternal financial support (NO)       | 3.87 | 1.40      | 10.68    | **  |
|            |                               |              |                     | PROM ≥ 18 hours                       | 12.60 | 4.74      | 33.64    | *** |
| Jordan     | Yusef et al, 2016             | 68           | 19                  | Prematurity (< 37 weeks)              | 0.07 | 0.01      | 0.67     | *   |
| Ethiopia   | Agnche et al, 2019            | 338          | 158                 | Per vaginal examination > 3 times     | 6.06 | 2.45      | 14.99    | **  |
|            |                               |              |                     | Male                                  | 3.73 | 1.76      | 7.89     | **  |
|            |                               |              |                     | Resuscitation (Yes)                   | 6.11 | 1.71      | 21.84    | *   |
| Country | Source | Neonates (n) | Neonatal Sepsis (n) | Risk Factors | OR    | Low Limit | Up Limit | P   |
|---------|--------|--------------|---------------------|--------------|-------|-----------|----------|-----|
|         |        |              |                     | Training of Health workers (NO) | 2.14  | 1.04      | 4.44     | *   |
|         |        |              |                     | Maternal age (30 – 34 years) | 0.19  | 0.05      | 0.81     | *   |
|         |        |              |                     | History of UTI (Yes) | 6.26  | 1.16      | 33.62    | *   |
|         |        |              |                     | Place of delivery | 3.05  | 1.19      | 7.79     | *   |
| Korea   | Heo et al,2015 | 26345 | 242 | Central vascular catheter | 4.10  | 1.56      | 10.80    | **  |
|         |        |              |                     | Hospital stay | 1.01  | 1.00      | 1.02     | *   |
| Ghana   | Adatara et al,21 | 900   | 103 | Parity 1 | 0.16  | 0.07      | 0.39     | *   |
|         |        |              |                     | Mode of delivery Instrumental | 0.35  | 0.04      | 3.45     | *   |
|         |        |              |                     | Caesarean section | 0.14  | 0.09      | 0.24     | *   |
|         |        |              |                     | Vacuum | 0.23  | 0.05      | 0.32     | *   |
|         |        |              |                     | Bleeding disorder (Yes) | 0.07  | 0.02      | 0.25     | *** |
|         |        |              |                     | PROM (Yes) | 0.34  | 0.09      | 1.40     | *** |
| Spain   | Cernada et al,22 | 147   | 10 | Chorioamnionitis | 44.03 | 3.62      | 535.42   | **  |
|         |        |              |                     | APGAR at 1 min | 0.41  | 0.25      | 0.68     | *** |
| Israel  | Klinger et al,23 | 15839 | 383 | Antenatal care (NO) | 1.94  | 1.32      | 2.86     | *   |
|         |        |              |                     | Resuscitation | 2.49  | 1.91      | 3.24     | *   |
| India   | Dutta et al,24 | 728   | 85 | Birth weight < 1500 g | 2.80  | 1.54      | 5.09     | *** |
|         |        |              |                     | Gestational age < 30 weeks | 1.99  | 1.12      | 3.53     | *   |
|         |        |              |                     | Per vaginal examinations ≥ 3 times | 9.52  | 2.97      | 30.53    | *** |
|         |        |              |                     | Intrapartum antibiotics not Received | 2.07  | 1.05      | 4.11     | *   |
| Country   | Source                     | Neonates (n) | Neonatal Sepsis (n) | Risk Factors                                      | OR  | Low Limit | Up Limit | P    |
|-----------|----------------------------|--------------|---------------------|---------------------------------------------------|-----|-----------|----------|------|
| USA       | Palatnik et al,²⁵ 2019     | 779          | 40                  | Male                                              | 2.70| 1.56      | 4.70     | ***  |
|           |                            |              |                     | Clinical chorioamnionitis                         | 8.84| 1.81      | 43.17    |      |
|           | Maternal obesity           | 1.05         | 0.48                | 2.30                                              |     |           |          | *    |
|           | Antibiotics administration in labor | 0.32 | 0.11                | 0.89                                              |     |           |          | *    |
|           | Magnesium sulfate in labor | 0.57         | 0.20                | 1.60                                              |     |           |          | *    |
|           | Fever in labor ≥ 38.0°C    | 3.38         | 1.07                | 10.69                                             |     |           |          | *    |
|           | Presence of meconium-stained amniotic fluid | 3.63 | 1.04                | 12.65                                             |     |           |          | *    |
|           | Birth weight < 1500 g      | 0.03         | 0.01                | 111.90                                            |     |           |          | *    |
| Germany   | Martius et al,²⁶ 1998      | 343          | 143                 | Gestational age                                   | 0.90| 0.91      | 0.96     | *    |
|           | Funisitis and/or chorioamnionitis | 4.10 | 1.36                | 12.12                                             |     |           |          | *    |
|           | PROM                       | 2.90         | 1.00                | 8.56                                              |     |           |          | *    |
|           | APGAR at 1 min             | 0.70         | 0.53                | 0.96                                              |     |           |          | *    |
| Israel    | Salem et al,²⁷ 2006        | 786          | 50                  | Tocolytics                                        | 4.80| 1.10      | 1.60     | *    |
|           | Gestational age            | 0.09         | 0.02                | 0.01                                              |     |           |          | *    |
| Germany   | Köstlin-Gille et al,²⁸ 2020| 14926        | 4527                | Gestational age                                   | 0.87| 0.81      | 0.93     | ***  |
|           | Multiple pregnancies (%)   | 0.66         | 0.45                | 0.96                                              |     |           |          | *    |
|           | intra-amniotic infection   | 3.39         | 2.32                | 4.94                                              |     |           |          | ***  |
|           | Spontaneous               | 2.02         | 1.06                | 3.85                                              |     |           |          | *    |
### Maternal Risk Factors of EOS

Four of the ten risk factors included in the meta-analysis were maternal risk factors, including at least three PV examinations, chorioamnionitis, PROM (>18 h), and cesarean section. The heterogeneity of the four risk factors was tested. Studies with obvious heterogeneity were excluded from sensitivity analysis. After excluding the sources of heterogeneity, the $I^2$ values of at least three PV examinations, chorioamnionitis, PROM (>18 h) were 0 ($p = 0.55$), 3% ($p = 0.38$), and 0 ($p = 0.90$), respectively; hence, no significant heterogeneity was observed (Fig. 2A-C). The pooled results showed that the ORs and 95% CIs of at least three PV examinations, chorioamnionitis, and PROM (>18 h) were 7.18 (3.51–14.69; $p < 0.001$), 6.56 (3.19–13.49; $p < 0.001$), and 2.74 (1.44–5.21; $p = 0.002$), respectively (Fig. 2A-C). In two studies with a strong heterogeneity of cesarean section after the combination ($I^2 = 98%$; $p < 0.001$), it was not possible to determine whether cesarean section was an independent risk factor for neonatal sepsis by meta-analysis.

### Neonatal Risk Factors

There were six neonatal risk factors, including male sex, GA (<37 weeks), birth weight (<1500 g), neonatal resuscitation, CVC, and low APGAR score. After heterogeneity testing and the exclusion of heterogeneity sources, the $I^2$ values of male sex, GA (<37 weeks), birth weight (<1500 g), neonatal resuscitation, and CVC were all 0, with $p$ values of 0.50, 0.40, 1.00, 0.39, and 0.44, respectively. There was no heterogeneity among the risk factors. The pooled ORs and 95% CIs of male sex, GA (<37 weeks), neonatal resuscitation, and CVC were 3.03 (1.94–4.73; $p < 0.001$), 4.08 (2.76–6.03; $p < 0.001$), 2.6 (2.03–3.34; $p < 0.001$), and 3.06 (1.64–5.73; $p = 0.004$), respectively (Fig. 3D-G). However, in two studies on low Apgar score at 1 min, the combined $I^2$ value was 69%, with moderate heterogeneity; moreover, in two other studies on low Apgar score at 5 min, the combined $I^2$ value was 97%, with strong heterogeneity. Heterogeneity testing was performed on studies with neonates who weighed <1500 g and <2500 g at birth, and the results showed that there was strong heterogeneity, which was not eliminated by sensitivity testing; therefore, the meta-analysis could not be used to merge data.

In this meta-analysis, seven independent risk factors of EOS can were identified, including at least three PV examinations during labor and delivery, chorioamnionitis, PROM (>18 h), male sex, GA (<37 weeks), resuscitation, and CVC.

### Publication Bias
Although we constructed funnel plots of publication bias testing for different risk factors, the final number of studies combined was small due to the elimination of heterogeneous studies, and therefore there may be a certain level of publication bias (Supplementary Material 5).

Discussion

This systematic review and meta-analysis identified seven independent risk factors for EOS, three and four of which were maternal and neonatal risk factors, respectively. Based on the significance of the combined after-effect, the most independent risk factor for EOS was at least three PV examinations. From our analysis, the incidence of EOS in neonates with this risk factor was approximately 7.18 times that of other neonates from our analysis. At least three PV examinations during labor and delivery were performed in 212 mothers, who were from cities in developing countries. This may be due to a low level of medical care, the non-practice of strict aseptic procedures, and the direct introduction of vaginal microbes into the uterus through the cervical canal during PV examination or under aseptic conditions. Additionally, repeated PV examination can easily cause an imbalance of the vaginal microecology and eventually lead to a significant increase in the risk of neonatal infection. Moreover, frequent PV examination may also lead to the occurrence of acute or subacute chorioamnionitis (7.4% in our study population). Chorioamnionitis is mainly caused by microorganisms invading the amniotic fluid, and the most common causative bacterium is \textit{Ureaplasma urealyticum}. Amniotic fluid infection may cause vasospasm in the umbilical cord and placenta, and reduce fetal perfusion; further, fetal inhalation or ingestion of bacteria in the amniotic fluid may cause congenital pneumonia or systemic infection, and increase perinatal asphyxia, which increases the incidence of EOS and related mortality. In addition, chorioamnionitis can be caused by PROM, which is directly related to the duration of rupture. From our findings, when PROM lasts for more than 18 h, microorganisms tend to enter the amniotic cavity through the cervix, thereby causing chorioamnionitis, fetal distress, and asphyxia. This leads to intrauterine infection and induces a 2.74-fold increase in EOS risk. PROM may also lead to premature delivery. In neonates with lower GA, innate and adaptive immunity may be more immature, and hence, the risk of infection is 4.08 times that in full-term infants. Premature neonates may have an impaired immune function due to a lack of maternal IgG placental transfer, which occurs in late pregnancy. Moreover, the colonization of \textit{Ureaplasma} in the respiratory tract of premature neonates with maternal chorioamnionitis after PROM can lead to bronchopulmonary dysplasia, which increases the risk of neonatal respiratory tract infection and perinatal asphyxia. It is necessary to resuscitate neonates with respiratory failure; however, neonatal peripheral airway stenosis, rich respiratory secretions, atelectasis, and effective resuscitation treatment may lead to neonatal vulnerable mucosa secondary injury and become the entry point of microorganisms. In addition, during resuscitation, catheters inserted into the trachea or blood vessels may not be sterile, and these equipments may introduce microorganisms into the lungs of neonates whose immune system is not well developed. This exposes the neonate to bacteria from the mother or exogenous pathogens during resuscitation and increases the risk of infection. Currently, few studies have focused on the bacterial colonization of an inserted intravenous catheter by added catheter culture to a scoring system for predicting neonatal sepsis occurrence, which significantly improves the discrimination of the latter. However, from the current research, bacterial culture of an implanted catheter in asymptomatic neonates has a certain predictive value for the occurrence of hospital-acquired neonatal sepsis. Furthermore, male sex was an independent risk factor of EOS. From a genetic perspective, innate immune cells carry a gene pool of alleles. In females, the difference of innate immune cells in
X-linked parental alleles with different regulatory and activation abilities can represent a more effective immune system, which can better adapt to the dynamic changes during the inflammatory response. Moreover, different sexes have different levels of sex hormones, especially estrogen. Animal studies have shown that exogenous estrogen injection can improve the immunosuppressive state of male animals, and a high serum concentration of estrogen can eliminate the toxic effects of active free radicals, and reduce inflammatory damage. Therefore, male sex is a risk factor of EOS. In addition to genetic effects, sex hormone levels are an important cause of differences in EOS occurrence in males and females.

Although birth weight, APGAR score, and cesarean section were also included in our meta-analysis, these variables showed strong heterogeneity after combination, which could not guarantee the reliability of the combined results. Further clinical studies are needed to confirm the role of these variables in EOS occurrence. In particular, many studies reported birth weight as an important risk factor for EOS. Five of the included studies (except the study by Levy et al., which did not explicitly present neonates with low birth weight), mainly divided the birth weights into two groups (<1500 g and <2500 g); nevertheless, the combined results had strong heterogeneity. Although it is impossible to determine the influence of low birth weight in EOS occurrence by meta-analysis, this risk factor cannot be ignored, and further clinical research is needed.

Apart from the abovementioned risk factors, many risk factors were not included in the meta-analysis due to the limitations of the current research results. However, these factors are very important, such as the presence of meconium-stained amniotic fluid, early maternal age, and the absence of crying immediately after birth. Anna et al. suggested that the OR of the presence of meconium-stained amniotic fluid as a risk factor was 3.63 (95% CI: 1.04–12.65). Compared to neonates without fecal infection, those with meconium-stained amniotic fluid have a higher risk of infection, probably because the meconium-stained amniotic fluid enters into fetal lungs, leading to dyspnea, hypoxemia, pneumonia, and finally EOS. In addition, maternal age ≤ 15 years is a risk factor for EOS, which is related to differences in the microbial flora of the vagina. Lactobacilli in the vaginal microbiota of normal adult women can prevent infection by reducing vaginal pH and producing antimicrobial substances, such as hydrogen peroxide. However, the dominant bacteria in the vaginal microbiota of young women under age 15 were different; most of them had *E. coli*, which alters the acidic environment of the vagina that is important against infections, weakens the ability of the vagina to inhibit pathogenic bacterial action, and increases the probability of neonatal infection. A case-control study conducted by Alemu et al. found that neonates who did not cry at birth were 2.85 times more likely to develop sepsis than those who cried at birth. Crying at birth may be the manifestation of a series of changes in lung function when the neonate takes the first breath, and it is the most critical part in the process of physiological adjustment of the neonate to survive outside the uterus after the umbilical cord is cut off. Initial breathing is the result of reflexes triggered by changes in pressure, temperature, noise, light, and other sensations associated with the birth process. Neonates may not be able to cry due to respiratory interference. Failure to breathe and/or cry would require immediate resuscitation, increase the chances of clinically invasive procedures, and increase the risk of infection.

From the perspective of causative microorganisms, a few patients in this study had multiple positive blood culture results; thus, the actual proportion of patients with positive blood culture results should be lower. Among them, 160 patients had CONS, which can produce false-positive blood culture results since it is a skin-colonizing bacterium. The number of infections caused by CONS in the included studies may have been overestimated. In addition, CONS infection usually shows low virulence. Therefore, in the blood culture diagnosis of neonatal
sepsis, some scholars treat CONS as a bacterial contamination of samples, not as an infectious cause of neonatal sepsis. Group B Streptococcus, a vagina-colonizing bacterium, has always been considered to constitute the main bacterial group causing EOS. Nonetheless, we found that *E. coli* accounted for the largest proportion in each flora, followed by CONS and *Klebsiella pneumoniae*; Group B Streptococcus ranked fourth. This probably indicates that, due to the frequent long-term use of antibiotics against gram-positive bacteria and the use of medical procedures such as prenatal PV examination, neonatal resuscitation, and central venous catheterization, the causative pathogen of EOS has gradually changed from gram-positive to gram-negative bacteria. Shane et al. and Stoll et al. reported that *E. coli* was the most common cause of EOS, which was akin to our findings. In addition, we found that fungi caused approximately 3% of EOS, all of which were caused by Candida. However, because three articles on fungal infection in this study did not distinguish between EOS and LOS, the fungal infection rate is probably underestimated in the current study. Compared with the LOS study, fungal infections seem to occur more frequently in patients with LOS. During hospitalization, due to the application of broad-spectrum antibiotics, normal body floras are out of balance. Many antibiotic-sensitive bacteria are killed, and therefore fungi can reproduce and grow. Thus, fungal infections can easily occur in patients with LOS. Compared to infections caused by gram-positive and gram-negative bacteria, sepsis caused by fungal infections are often more severe and result in higher mortality.

**Strengths and limitations**

Our study had the following strengths. First, we evaluated the risk factors of EOS, which provides an effective prevention and treatment strategy to reduce the serious damage caused by EOS in the clinic. Seven independent risk factors of EOS were identified through quantitative meta-analysis. Second, we also systematically summarized the causative bacteria of EOS, and found that *E. coli*, not Group B Streptococcus, was the main cause of EOS.

We acknowledge that this study has some shortcomings. First, in the two studies on CVC, the analysis of neonatal sepsis did not distinguish between EOS and LOS. Therefore, although there was no heterogeneity in the results of our combination, it cannot be ascertained whether CVC is a risk factor for EOS or LOS. Second, to ensure the accuracy of our results, we eliminated most of the studies with heterogeneity through sensitivity analysis, resulting in a small number of studies, which can weaken the results of the current meta-analysis for each risk factor. Furthermore, the inclusion of the neonatal sepsis population had a certain degree of skewness, as two, four, and one studies were based on very low birth weight neonates (<1500 g), premature neonates (<37 weeks), and neonates with sepsis and congenital disease (diaphragmatic hernia), respectively; thus, our results may have publication bias.

**Conclusion**

Our results suggest that frequent per vaginal examination during labor and delivery, chorioamnionitis, premature rupture of membranes (>18 h), male fetus, gestational age (<37 weeks), neonatal resuscitation, and central vascular catheterization are independent risk factors of early-onset neonatal sepsis. These findings support the use of empirical antibiotic therapy in neonates with these risk factors.

**Abbreviations**
Declarations

Acknowledgements

Not applicable.

Author contributions

PGX, GLB, and SJH contributed to the data curation; ZL and DJ contributed to the project administration; WDL and HSY contributed to patient data resourcing and software development, and WQ and ZL contributed to writing the original draft. All authors read and approved the final manuscript.

Funding

This work was supported by the National Natural Science Foundation of China (81772403); Chongqing Science and Technology Talents, Chongqing Special Project for Academicians (cstc2020yszx-jcyjX0004); Project of Trauma, the Burns and Combined Injury State Key Laboratory (SKLYQ201901 and SKLKF201802); Project of the Science and Technology Fund of Guizhou Provincial Department of Health (gzwjkj2020-1-106); Training plan of the innovation ability of military medical frontier research (2019CXJSB014).

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Ethical approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Figures
Figure 1. Study Selection Flowchart

Figure 1

Study Selection Flowchart
**Figure 2.** Forest plot showing a Fixed-effects meta-analysis of neonates with and without sepsis

| Study or Subgroup | log(Odds Ratio) | SE | Weight | Odds Ratio | Odds Ratio |
|-------------------|----------------|----|--------|------------|------------|
|                   |                |    |        | IV. Fixed. 95% CI | IV. Fixed. 95% CI |
| A                 |                |    |        |             |             |
| Sourabh 2009      | 2.2532898      | 0.59459732 | 37.7% | 9.52 [2.97, 30.53] |             |
| Zelaïem 2019      | 1.8017098      | 0.46206512 | 62.3% | 6.06 [2.45, 14.99] |             |
| **Total (95% CI)**| **100.0%**     | **7.18 [3.51, 14.69]** |       |             |             |
| Heterogeneity: Chi² = 0.36, df = 1 (P = 0.55); I² = 0% |             |       |             |             |
| Test for overall effect: Z = 5.40 (P < 0.00001) |             |       |             |             |

| Study or Subgroup | log(Odds Ratio) | SE | Weight | Odds Ratio | Odds Ratio |
|-------------------|----------------|----|--------|------------|------------|
|                   |                |    |        | IV. Fixed. 95% CI | IV. Fixed. 95% CI |
| B                 |                |    |        |             |             |
| Beaulieu 2017     | 1.82454929     | 0.70022045 | 27.6% | 6.20 [1.57, 24.48] |             |
| Joachim 1998      | 1.41098697     | 0.55900313 | 43.4% | 4.10 [1.37, 12.24] |             |
| Marie 2011        | 3.78487122     | 1.27463711 | 8.3%  | 44.03 [3.62, 535.45] |             |
| Sourabh 2009      | 2.1795131      | 0.80899068 | 20.7% | 8.84 [1.81, 43.17] |             |
| **Total (95% CI)**| **100.0%**     | **6.56 [3.19, 13.49]** |       |             |             |
| Heterogeneity: Chi² = 3.08, df = 3 (P = 0.38); I² = 3% |             |       |             |             |
| Test for overall effect: Z = 5.12 (P < 0.00001) |             |       |             |             |

| Study or Subgroup | log(Odds Ratio) | SE | Weight | Odds Ratio | Odds Ratio |
|-------------------|----------------|----|--------|------------|------------|
|                   |                |    |        | IV. Fixed. 95% CI | IV. Fixed. 95% CI |
| C                 |                |    |        |             |             |
| Joachim 1998      | 1.06471074     | 0.54671127 | 36.2% | 2.90 [0.99, 8.47] |             |
| René 2015         | 0.7745964      | 0.41143419 | 63.8% | 2.65 [1.18, 5.64] |             |
| **Total (95% CI)**| **100.0%**     | **2.74 [1.44, 5.21]** |       |             |             |
| Heterogeneity: Chi² = 0.02, df = 1 (P = 0.90); I² = 0% |             |       |             |             |
| Test for overall effect: Z = 3.06 (P = 0.002) |             |       |             |             |

**Figure 2**

Forest plot showing a Fixed-effects meta-analysis of neonates with and without sepsis
Figure 3. Forest plot showing a Fixed-effects meta-analysis of neonates with and without sepsis

Forest plot showing a Fixed-effects meta-analysis of neonates with and without sepsis

Supplementary Files

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