REVIEW ARTICLE

Prevalence of Gram-negative Bacteria in Maternal Cervical Secretions: A Systematic Review and Meta-analysis

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Abstract

Background: In neonates, early-onset sepsis (EOS) occurring within 72 hours after birth is an important cause of mortality worldwide. Emerging data show that EOS may occur more frequently in tropical and peri-equatorial regions with more gram-negative bacteria than in the Western countries. This systematic review aimed to estimate the prevalence of gram-negative bacteria in the maternal genital tract during the peripartum period.

Materials and methods: We explored the primary research studies that reported the presence of gram-negative bacteria in the maternal genital tract using the software STATA, version 17.1. Five databases, PubMed, Embase, Scopus, Web of Science, and ProQuest were searched until October 2022. Data were analyzed using random-effects meta-analyses to determine the prevalence of gram-negative bacteria in the maternal genital tract.

Results: Fifteen studies qualified for analysis by our predetermined inclusion criteria. The overall prevalence of gram-negative bacteria in cervical secretions was 23.20% (95% CI [confidence interval]: 11.77–37.08, I^2: 99.79%). *Escherichia coli* (15.3%) and *Acinetobacter* (0.36%) species reported the highest and lowest prevalent bacteria, respectively. The prevalence of other gram-negative species was *Klebsiella pneumoniae* (0.47%), *Pseudomonas* (2.81%), *Enterobacter* (3.33%), *Alcaligenes faecalis* (1.32%), *Proteus vulgaris* (10.0%), and *Providencia alcalifaciens* (10%). Most of the studies were from tropical countries, and there was a positive linear relationship between the studies.

Conclusion: Gram-negative colonization of the maternal cervical-vaginal tract may be more frequent than previously recognized in tropical/peri-equatorial regions of the world. Early identification of these bacterial pathogens may help in timely evaluation and treatment of these infants.

Keywords: Critical care devices, Early-onset sepsis, Gram-negative sepsis, Intestinal disorders, Newborn, Premature, Prolonged hospitalization.

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Key Points

- There are important differences in bacterial pathogens causing EOS in different parts of the world. In the West, gram-positive bacteria such as group B streptococci (GBS) are an important cause. However, in tropical and peri-equatorial regions, gram-negative pathogens are frequently identified in EOS whereas pathogens such as GBS are uncommon.
- In EOS, the pathogenic bacteria identified in maternal cervical–vaginal flora are believed to play an important role.
- We performed a systematic review and meta-analysis of the prevalence of gram-negative bacteria in maternal cervical secretions to (1) determine the relative frequency of these bacteria in these secretions and (2) determine whether there are geographical variations in the maternal genital flora, even if the evidence is limited, to determine the need for future studies.
- Fifteen studies qualified for analysis based on the inclusion criteria. The overall prevalence of gram-negative bacteria was 23.20% (95% CI: 11.77–37.08, I^2: 99.79%). We need focused studies to study the maternal genital flora in tropical and peri-equatorial regions.

Introduction

Neonatal sepsis affects up to 20% of newborn infants and is one of the leading causes of morbidity and mortality in these

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patients.[1–5] Neonatal mortality has improved over the last 3 decades, but this progress has been slower than older age groups.[5,6] Deaths in newborn infants contribute greater than 40% of all mortality in children under 5 years of age.[6,7] The Sustainable Development Goals target a reduction of neonatal mortality in all countries to less than 12 deaths per 1,000 live births by 2030.[8]

In EOS, neonates become bacteremia and develop some degree of systemic inflammation within 72 hours following birth.[9,10] These infants likely acquire the bacterial pathogens from the maternal cervical/vaginal secretions during the perinatal period.[9,11,12] And consistent with this possibility, the known predisposing factors associated with EOS include conditions with altered bacterial flora in the birth canal such as during maternal chorioamnionitis or cervical/vaginal colonization with bacteria such as GBS. In other cases, there could be abnormal exposure to various pathogens following procedures such as cervical cerclage and amniocentesis, which can disrupt the amniotic cavity, premature and/or prolonged rupture of membranes, and premature onset of labor.[10,12–19] The infants who get exposed to infectious agent(s) *in utero* or during delivery are at risk of developing sepsis because of their immature immunological responses, and also because they have not had any access to appropriate medical treatment for variable periods of time following the exposure to bacteria prior to delivery.[15,20] The most frequently identified pathogens include gram-positive bacteria such as GBS, *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Streptococcus viridans*, and *Enterococcus* spp., and gram-negative pathogens such as *E. coli*, *Klebsiella* spp., and *Haemophilus influenzae*.[9,21–24]

Emerging data show that the incidence of EOS in tropical and peri-equatorial regions is not only higher than the Western countries with relatively temperate climates, but the spectrum of the pathogenic bacteria may also be different. Sepsis due to gram-negative pathogens, and in some cases, Staphylococci, seems to be more frequent in the tropical and peri-equatorial regions.[25–29] In the West, gram-positive bacteria such as GBS, other Streptococci, and Staphylococci are seen more often than gram-negative bacteria.[26,27,30–32] Gram-negative EOS in tropical and peri-equatorial regions may result in higher mortality not only because of aggressive clinical disease but also because of limitations in health-care infrastructure.[28,33,34] *Escherichia coli* is the most frequently recorded cause of neonatal infections and is known to cause significant morbidity and mortality.[35] Vertical mother-to-infant transmission of *K. pneumoniae* can also cause neonatal sepsis;[36–38] in tropical and peri-equatorial countries, it may account for up to 20% of neonatal sepsis-related mortality.[39]

We currently have limited data to evaluate the possibility that maternal cervical secretions in the tropical and peri-equatorial regions contain more gram-negative pathogens. There is a need to evaluate the possibility that maternal cervical–vaginal mucosa in these regions is colonized differently with more gram-negative bacteria than in the West. To develop prevention strategies and set research priorities, a deeper understanding of the mechanisms by which infections are transmitted to the fetus/newborn infant is essential. In this study, we searched and evaluated existing relevant maternal and neonatal data. A systematic review was performed to assess the proportion of gram-negative bacteria in the cervical secretions of pregnant women.

Materials and Methods

Study Selection and Electronic Search

The preferred reporting items for systematic reviews and meta-analyses (PRISMA) criteria were followed to design this study (Flowchart 1).[40] We conducted a PRISMA-compliant literature search for relevant studies using phrases such as "cervical discharge," "gram-negative bacteria," and "Cervical mucus" in PubMed, Scopus, Web of Science, and EMBASE. The search criteria were broad in order to find all studies that included any health result, which would require a significant number of search phrases to be exhaustive. Appendix 1 details the search strategy.

Studies investigating the prevalence of gram-negative bacterial colonization in pregnant women were included. The reports published in languages other than English were excluded. Case reports and letters to the editor were not considered. Inclusion and exclusion criteria were defined as listed in Appendix 2. Mendeley Desktop was used to enter the search results.[41] DRP and SM evaluated the articles for the relevance of contents, title, and abstract, and the inclusion criteria were then applied to the full-text articles. If there was a disagreement among the reviewers, a discussion was held to reach an agreement; otherwise, help was sought from a third author.

Data Extraction and Management

We adopted a standardized form to evaluate each included study for the first author, year of publication, type of study, sample size, and prevalence rates of gram-negative bacterial infections.

Data Analysis

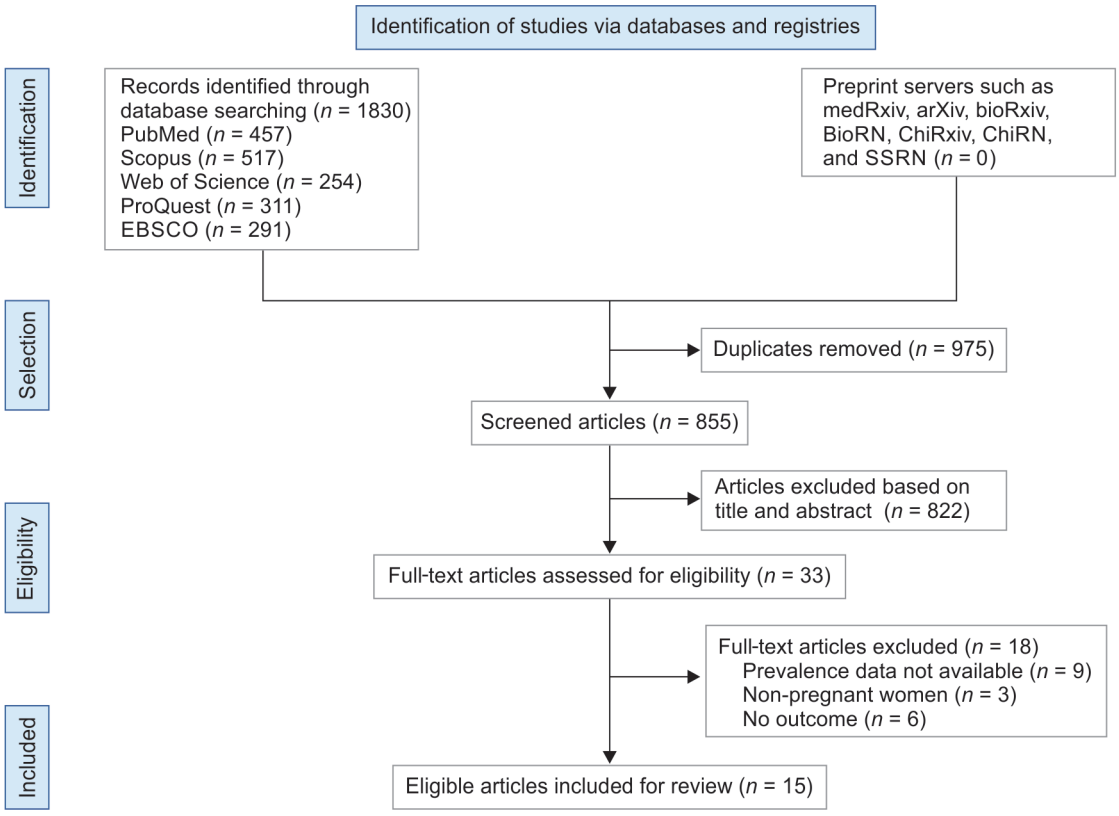
A random-effect meta-analysis[42] was used to group the prevalence of the presence of GNB in the cervical secretion of pregnant women, assuming heterogeneity between studies. To address the issues provided by the proportions near or on the boundaries of 0 and 1, we produced individual sample estimates with 95% CIs.[43] The percentage of variation resulting from heterogeneity was calculated using I^2, with values of more than 75% indicating significant heterogeneity.[44,45] The degree of funnel plot asymmetry was assessed using Egger's test when at least 10 estimates were available.[46] Funnel plots were made to visualize transformed proportions versus their standard errors. All analyses were carried out in STATA (version 17.0).

Quality Assessment

The risk of bias in observational research was evaluated by two authors (BKP and SM) using the Newcastle–Ottawa Scale.[47] Star ratings were assigned to individual quality components such as selection, comparability, and outcome. The collection included four items, and a star was awarded to each item. The comparison only used one item, which scored two stars. Three products, each with one star, were the result. Thus, a single study could have received up to nine stars. Each study was given a certain number of stars. The research was considered to be of good quality if it achieved six or more stars. Any disagreements or ambiguities were resolved by team members coming to an agreement. Appendix 3 depicts the quality of included studies in this review.

We have taken baseline data from three prospective cohort studies (Febriani et al.,[48] Ngonzi et al.,[49] and McDonald et al.) [50] and one randomized controlled trial (Husain et al.).[51] We treated it as a cross-sectional study while performing the quality assessment.

Flowchart 1: PRISMA flowchart for included studies in systematic review and meta-analysis of the prevalence of Gram-negative bacterial infection in maternal cervical secretion



Publication Bias

Egger's meta-regression test[52] and Funnel plots[52] were used to assess the small study effects. The study heterogeneity was reported using the I^2 measure of consistency.[53]

Results

Prevalence of Gram-negative Bacteria

Escherichia coli was the most extensively analyzed gram-negative bacterial species; it was examined in 15 studies.[48,50,54–60] *Klebsiella pneumoniae* was examined in 12.[48,50,54–56,58–62] *Pseudomonas* spp. in 7,[34,48,54,55,58,61] *Enterobacter* spp. in 4,[48,55,56,58] *Acinetobacter* spp. in 3,[48,54,55] and *P. vulgaris* in 4 studies.[48,50,54,55] *Alcaligenes faecalis* and *P. alcalifaciens* were reported in 1 study.[48] Figure 1 shows the overall pooled estimate of the prevalence of gram-negative bacteria in maternal cervical secretions through forest plots. The overall prevalence was found to be 23.20% (95% CI: 11.77–37.08).

Studies that qualified for further analysis in this project showed significant heterogeneity (Fig. 1). We used the DerSimonian and Laird random-effects model[44] to calculate the total pooled prevalence of gram-negative bacteria since it offers more conservative effect sizes. The publication year and study sample size were considered as potentially associated with the variation in prevalence. However, when we performed a univariate meta-regression analysis,[63] neither showed a statistically significant variation (Tables 1 and 2).

Publication Bias

A Funnel plot was used to test publication bias; upon initial inspection, it looked fairly asymmetrical (Fig. 2), indicating the presence of publication bias.[52] However, Egger's and Begg's tests[52] did not show any significant publication bias; the respective *p*-values were 0.0842 and 0.1836, respectively.

Relationship between Studies

We constructed a bubble graph to assess the relationship between studies.[64] A positive linear relationship was seen between studies with a larger sample size, which revealed a high prevalence of gram-negative bacteria (Fig. 3). In these depictions, the size of each bubble is determined by the effect estimated by the prevalence of individual studies. Larger bubbles signify a greater prevalence.

Subgroup Analysis

We performed subgroup analyses based on the type of gram-negative bacteria to evaluate potential sources of heterogeneity. The pooled prevalence of gram-negative bacteria was 23.20% (95% CI: 11.77–37.08, I^2: 99.79%). The highest and lowest prevalent gram-negative bacteria were identified as *E. coli* (15.34%, 95% CI: 6.89, 26.33) and *Acinetobacter* spp. (0.36%, 95% CI: 0, 1.48), respectively.

The prevalence of *K. pneumoniae* was estimated to be 2.65% (95% CI: 0.63, 5.86), 0.47% for *Pseudomonas* spp. (95% CI: 0.00, 1.54), 2.81% for *Enterobacter* spp. (95% CI: 0.59, 6.40), 3.33% for *A. faecalis* (95% CI: 1.14, 9.35), 1.32% for *P. vulgaris* (95% CI: 0.29, 2.94), and 10% (95% CI: 5.35, 17.92) of *Providencia alcalifaciens* (Table 3).

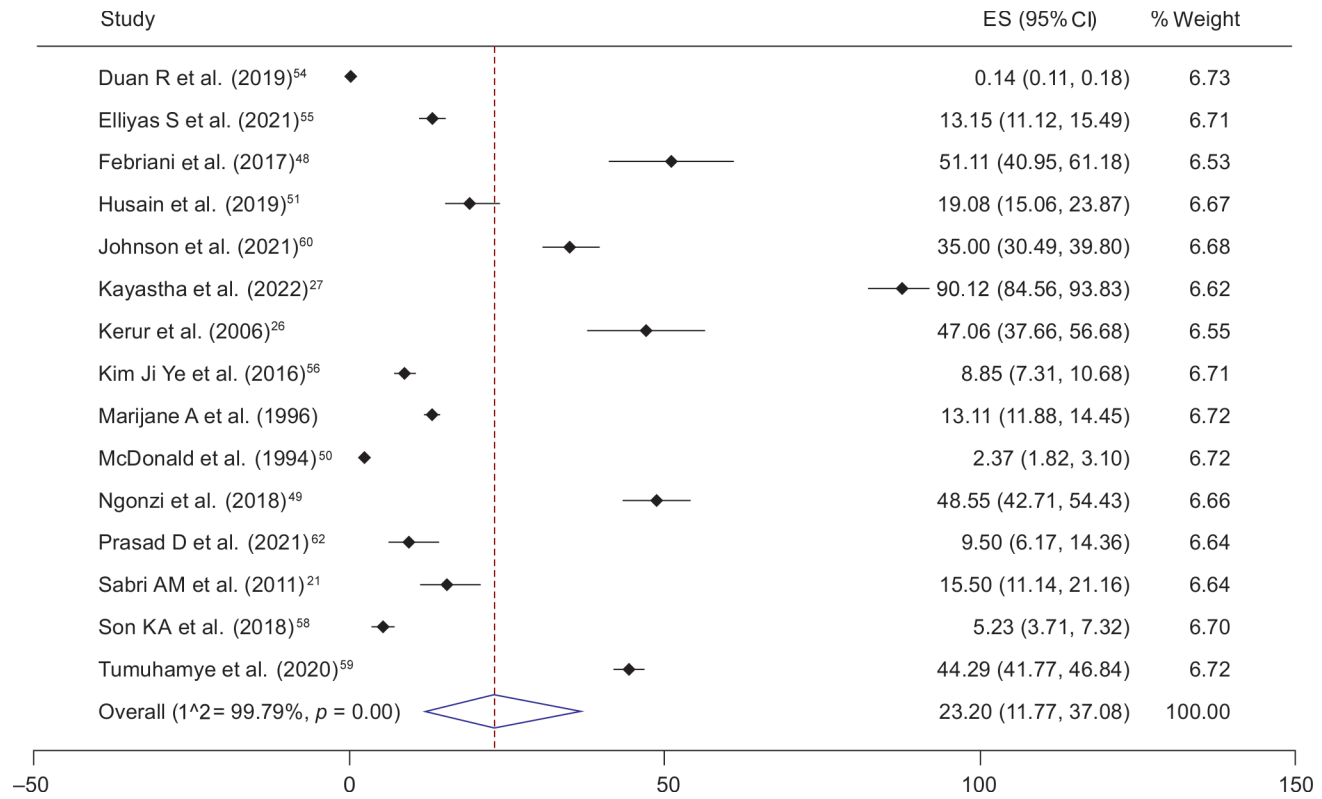


Fig. 1: Forest plot of the pooled magnitude of the prevalence of Gram-negative bacterial infection in maternal cervical secretion

Discussion

This meta-analysis reported a pooled prevalence of gram-negative bacteria of 23.2% in maternal secretions. Most studies reported were from tropical countries. A global systematic review of multidrug-resistant gram-negative bacteria and their transmission to newborn infants identified a pooled proportion of 19% neonates who acquired these infections through mother-to-child transmission.[24]

In vertically transmitted mother-to-infant infections, an intrauterine pathogen can enter the amniotic sac, replicate, and can cause fetal/neonatal sepsis.[65] Transmission can occur during early pregnancy, even if the embryonic membranes remain intact, and infection may persist for a few weeks. Hematogenous infections of the fetus can also arise through placental infection. Furthermore, the transmission of gram-negative bacteria can also occur during delivery when the fetus comes into contact with contaminated vaginal secretions.[66] It is a potentially serious problem because these bacteria are often resistant to antibiotics, causing severe infections that are difficult to treat. In this meta-analysis, we report a pooled prevalence of gram-negative bacteria of 5% among pregnant women. However, the problem might be larger; a global systematic review of multidrug-resistant gram-negative bacteria and their transmission rate to newborns identified a pooled proportion of 19% of the neonates who acquired infection after mother-to-child transmission.[34]

Klebsiella pneumoniae is known to harbor numerous plasmids and can rapidly acquire resistance to multiple antibiotics. These species can survive in the environment and within the human gut, and are known to be one of the commonest reasons for sepsis outbreaks in hospitals in tropical and peri-equatorial regions.[67] *Klebsiella pneumoniae* has been identified as a cause of fetal death as early as 18 weeks of gestation due to acute suppurative placentitis.[68] Cervical–vaginal colonization with *K. pneumoniae* can result in severe infections that invade the uterine cavity, cause chorioamnionitis, preterm delivery, and fetal complications including sepsis, respiratory distress, and patent ductus arteriosus.[69,70]

Escherichia coli is the second most extensively studied species of gram-negative bacteria. A few studies have reported that higher rates of *E. coli* colonization might reflect increased vaginal exams during labor or increased vaginal contamination by anorectal bacteria resulting from prolonged or obstructed labor.[56–58,61] Interrupted labor may be seen in 13–49% mothers. It is known to cause serious illness in infants and lead to various adverse outcomes, including neonatal sepsis, meningitis, and death. *Escherichia coli* infections are a significant cause of infant morbidity and mortality worldwide, and early diagnosis and treatment of *E. coli* infections is essential to improve outcomes.

Pseudomonas aeruginosa seems to cause EOS less frequently, even in infants with identifiable risk factors.[71] However, the mortality rate from *P. aeruginosa* infections in neonates can be as high as 56%. These bacterial species are known to account for approximately 2% of the commensal vaginal flora and, therefore, could have been undetected in vaginal discharge.[72] The pooled prevalence of multidrug-resistant *Pseudomonads* can be high, which can be a cause for concern.[59]

Acinetobacter spp. are the second most common nonfermenting gram-negative pathogens isolated from clinical samples after the Pseudomonads. It is listed by the American Society of Infectious Diseases as one of the six most dangerous microorganisms.[73] The most well-recognized species is *Acinetobacter baumannii*. *Acinetobacter lwoffii*, *Acinetobacter haemolyticus*, and *Acinetobacter*

Table 1: Baseline characteristics of the studies included for meta-analysis

Author (year)	*Country*	*Study design*	*Study population*	*Gram-negative bacteria isolated (n)*
Duan et al. (2019)[54]	China	Cross-sectional	49,496	*Escherichia coli* (*n* = 38) *Klebsiella pneumoniae* (*n* = 4) *Acinetobacter* species (*n* = 17) *Pseudomonas* species (*n* = 10)
Elliyas et al. (2021)[55]	India	Cross-sectional	920	*Escherichia coli* (*n* = 82) *Klebsiella pneumoniae* (*n* = 18) *Enterobacter* spp. (*n* = 15) *Pseudomonas aeruginosa* (*n* = 3) *Acinetobacter* species (*n* = 3)
Febriani et al. (2017)[48]	Indonesia	Prospective cohort	90	*Escherichia coli* (*n* = 3) *Klebsiella pneumoniae* (*n* = 3) *Enterobacter* (*n* = 21) *Proteus vulgaris* (*n* = 3) *Providencia alcalifaciens* (*n* = 9) *Pseudomonas aeruginosa* (*n* = 1) *Alcaligenes faecalis* (*n* = 3) *Acinetobacter* species (*n* = 3)
Husain et al. (2020)[51]	UK	Randomized controlled trial	304	*Escherichia coli* (*n* = 58)
Johnson et al. (2021)[60]	Uganda	Cross-sectional	400	*Klebsiella pneumoniae* (*n* = 52) *Escherichia coli* (*n* = 40) *Pseudomonas aeruginosa* (*n* = 7) *Proteus vulgaris* (*n* = 7)
Kayastha et al. (2022)[27]	Nepal	Cross-sectional	162	*Escherichia coli* (*n* = 146)
Kerur et al. (2006)[26]	India	Cross-sectional	102	*Escherichia coli* (*n* = 39) *Klebsiella pneumoniae* (*n* = 5) *Enterobacter* (*n* = 2) *Pseudomonas aeruginosa* (*n* = 1) *Acinetobacter* species (*n* = 1)
Kim et al. (2016)[56]	Korea	Cross-sectional	1096	*Escherichia coli* (*n* = 63) *Klebsiella pneumoniae* (*n* = 23) *Enterobacter* (*n* = 11)
Krohn et al. (1996)[57]	US	Cross-sectional	2646	*Escherichia coli* (*n* = 347)
McDonald et al. (1994)[50]	Australia	Cohort	2190	*Escherichia coli* (*n* = 52) *Klebsiella pneumoniae* (*n* = 6) *Proteus vulgaris* (*n* = 16)
Ngonzi et al. (2018)[49]	Uganda	Prospective cohort	276	*Escherichia coli* (*n* = 134)
Prasad et al. (2021)[62]	India	Cross-sectional	200	*Escherichia coli* (*n* = 15) *Klebsiella pneumoniae* (*n* = 4)
Sabri et al. (2011)[21]	Iraq	Cross-sectional	200	*Escherichia coli* (*n* = 8) *Klebsiella pneumoniae* (*n* = 4) *Pseudomonas aeruginosa* (*n* = 3)
Son et al. (2018)[58]	Korea	Cross-sectional	593	*Escherichia coli (n = 23)* *Klebsiella pneumoniae (n = 5)* *Pseudomonas aeruginosa (n = 1)* *Enterobacter (n = 1)*
Tumuhamye et al. (2020)[59]	Uganda	Cross-sectional	1472	*Escherichia coli (n = 508)* *Klebsiella pneumoniae (n = 144)*

johnsonii have also been noted in clinical samples.[74,75] Saha et al.[74] reported a similar prevalence of 2.9% of *Acinetobacter* spp. Patients in NICUs are immunocompromised because of prematurity or high severity of illness, which places them at high risk of these infections, particularly with *A. baumannii*.

Gram-negative bacteria can be an important cause of neonatal sepsis in certain geographical and climatic conditions. There is also a high incidence of resistance to empirical first- and second-line antibiotics recommended by the World Health Organization. Further research is needed to determine whether antimicrobial stewardship programs would be adequate to reduce the incidence of these neonatal infections. Additionally, a global commitment is necessary to address the management of gram-negative bacterial infects in pregnant women to reduce vertically transmitted infections to newborn infants.[76]

Recommendations

Given the high prevalence of gram-negative bacteria and the possibility of vertical transmission to the offspring, the risks of fetal/neonatal infections need to be prioritized globally. Many

Table 2: Factors pertaining to the heterogeneity of Gram-negative bacterial prevalence in the current meta-analysis (univariate meta-regression model)

Variables	*Co-efficient*	*p-value*
Publication year	0.0080988	0.256
Sample size	−6.19e-06	0.214

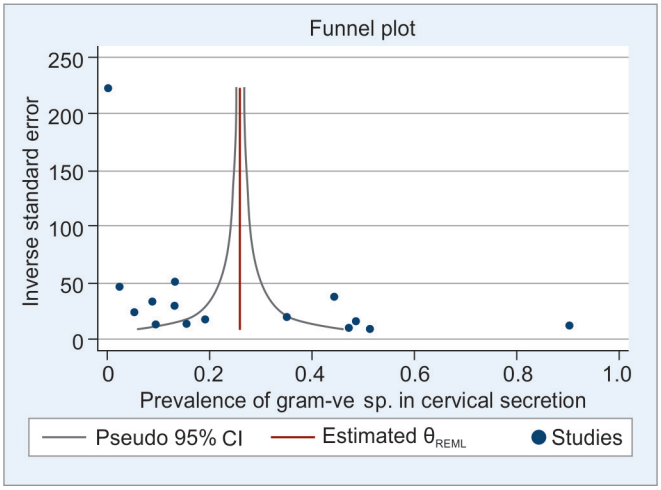


Fig. 2: Funnel plot to assess publication bias of total studies included in the analysis ($n = 15$)

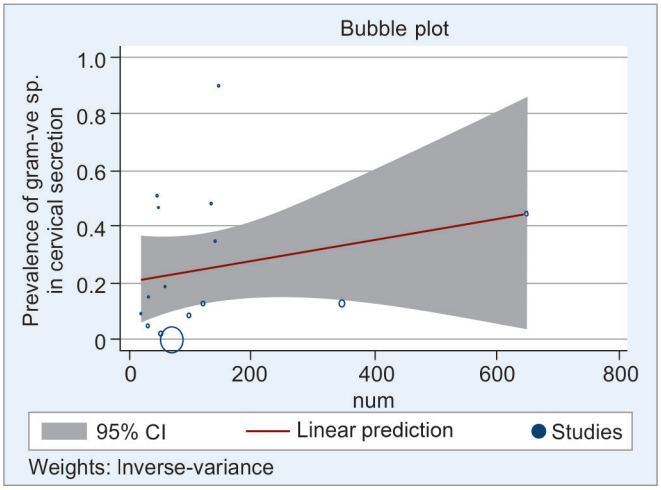


Fig. 3: Bubble plot with 95% CI of pooled prevalence of Gram-negative bacterial infection in maternal cervical secretion

studies have limited these discussions as a problem of low- and middle-income level countries, but the issues might extend beyond economics and may reflect the impact of environmental and climatic conditions. Early detection and appropriate management are essential to efficiently monitor and manage infections among pregnant women and the health of their offspring.

There is a need to develop clear guidelines for early identification of pregnant women with symptoms of bacterial colonization. Further studies are needed to examine the infection patterns in various parts of the globe and deepen our understanding of the pathogenesis. Antimicrobial stewardship programs are obviously important, but we also need large-scale studies to establish the effectiveness of preventive strategies to intervene in the vertical mother-to-child transmission of these bacteria.

Limitations

In our analysis, one clear limitation is the small number of available studies. A larger number of studies are available from the West, which can be a source of bias. Environmental factors might also be an important variable and it might not be prudent to extrapolate conclusions from one region of the world to another without due thought. Socioeconomic status, awareness, demographic characteristics, and even genetic susceptibility may all need to be factored in. Finally, this review incorporates only observational research.

Conclusion

Understanding the causes of neonatal and maternal bacterial infections is very important on a global scale. Improved Identification of the infectious agents seen in pre- and intrapartum periods is important, which requires the easier access to newer microbiological technology. The access to newer technology could potentially facilitate earlier diagnosis and institution of appropriate treatment.

Authors' Contributions

All authors contributed equally to this work.

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Table 3: Subgroup analysis based on the type of Gram-negative bacteria present in maternal cervical secretion

Organisms	*No. of studies*	*Prevalence with 95% CI*	*I^2 (%)*	*p-value*
Escherichia coli	15	15.34% (6.89, 26.33)	98.64	<0.001
Klebsiella pneumoniae	11	2.65 (0.63, 5.86)	56.80	0.02
Proteus vulgaris	3	1.32 (0.29, 2.94)	0.03	0.96
Providencia alcalifaciens	1	10 (5.35, 17.92)	Not applicable	
Pseudomonas sp.	7	0.47 (0.00, 1.54)	0.00	1.00
Acinetobacter sp.	4	0.36 (0.29, 2.94)	1.00	0.99
Alcaligenes faecalis	1	3.33 (1.14, 9.35)	Not applicable	
Enterobacter	5	2.81 (0.59, 6.40)	0.04	0.35

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Appendix 1

The adjusted search terms as per searched electronic databases

Database	*No.*	*Search Query*	*Results*
PubMed	#1	"Gram-negative bacteria" OR "Gram-Negative Bacteria"[Mesh] OR Klebsiella OR "Klebsiella pneumoniae"[Mesh] OR Haemophilus OR "Haemophilus influenzae"[Mesh] OR Shigella OR "Shigella"[Mesh] OR Salmonella OR "Salmonella"[Mesh] OR *Acinetobacter* OR "*Acinetobacter* baumannii"[Mesh] OR "*Acinetobacter* calcoaceticus"[Mesh] OR Citrobacter OR "Citrobacter"[Mesh] OR Neisseria OR "Neisseria meningitidis"[Mesh] OR *Enterobacter* OR "*Enterobacter*"[Mesh] OR "*Enterobacter* aerogenes"[Mesh] OR "*Enterobacter* cloacae"[Mesh] OR Brucella OR "Brucella"[Mesh] OR Pasteurella OR "Pasteurella"[Mesh] OR "Mycoplasma"[Mesh] OR Mycoplasma OR Bacteroides OR "Bacteroides"[Mesh]	943,806
	#2	Pregnan* OR "Pregnancy"[Mesh] OR Trimeste* OR "Pregnancy Trimesters"[Mesh] OR Antenatal OR "Prenatal Care"[Mesh] OR obstetri* OR maternal	1,572,639
	#3	"Cervical Mucus" OR "Cervix mucus" OR "Endocervical secretion" OR "Cervical discharge" OR " Cervical secretions" OR "Cervix Mucus"[Mesh]	4,776
	#4	#1 AND #2 AND #3	457
Scopus			
	#1	(TITLE-ABS-KEY (Gram-Negative Bacteria) OR TITLE-ABS-KEY (Klebsiella) OR TITLE-ABS-KEY (Shigella) OR TITLE-ABS-KEY (Salmonella) OR TITLE-ABS-KEY (*Acinetobacter*) OR TITLE-ABS-KEY (Citrobacter) OR TITLE-ABS-KEY (Neisseria) OR TITLE-ABS-KEY (*Enterobacter*) OR TITLE-ABS-KEY (Brucella) OR TITLE-ABS-KEY (Pasteurella)	
	#2	(TITLE-ABS-KEY (Pregnan*) OR TITLE-ABS-KEY (Trimeste*) OR TITLE-ABS-KEY (maternal) OR TITLE-ABS-KEY (Pregnancy) OR TITLE-ABS-KEY (Antenatal))	
	#3	(TITLE-ABS-KEY (Cervical Mucus) OR TITLE-ABS-KEY (Cervix mucus) OR TITLE-ABS-KEY (Endocervical secretion) OR TITLE-ABS-KEY ("Cervical discharge") OR TITLE-ABS-KEY ("Cervical secretions"))	
	#4	#1 AND #2 AND #3	517
Web of Science			
	#1	Gram-Negative Bacteria (All Fields) or Klebsiella (All Fields) or Shigella (All Fields) or Salmonella (All Fields) or *Acinetobacter* (All Fields) or Citrobacter (All Fields) or Neisseria (All Fields) or *Enterobacter* (All Fields) or Brucella (All Fields) or Pasteurella (All Fields)	
	#2	Pregnancy (All Fields) or Pregnan* (All Fields) or Trimeste* (All Fields) or Maternal (All Fields) or Antenatal (All Fields) or Postnatal (All Fields)	
	#3	Cervical Mucus (All Fields) or Cervi* (All Fields) or Cervix mucus (All Fields) or Cervical discharge (All Fields) or Cervical secretions (All Fields)	
		#1 AND #2 AND #3	254
ProQuest			
	#1	(Gram-Negative Bacteria OR Klebsiella OR Shigella OR Salmonella OR *Acinetobacter* OR Citrobacter OR Neisseria OR *Enterobacter* OR Brucella OR Pasteurella) AND (Pregnancy OR Maternal OR Antenatal OR Postnatal OR Pregnan* OR Trimeste*) AND (Cervical Mucus OR Cervix mucus OR Cervical discharge OR Cervical secretions)	311
EBSCO Host-Academic Search Complete			
	#1	TX Gram-Negative Bacteria OR TX Klebsiella OR TX Shigella OR TX Salmonella OR TX *Acinetobacter* OR TX Citrobacter OR TX Neisseria OR TX *Enterobacter* OR TX Brucella OR TX Pasteurella	
	#2	TX pregnan* or TX gestat* or TX gravid* or TX maternal or TX mother* or TX puerper* or TX antenat*	
	#3	TX Cervical Mucus OR TX Cervix mucus OR TX Cervical discharge OR TX Cervical secretions	
	#4	#1 AND #2 AND #3	291

Appendix 2

Inclusion and exclusion criteria

	Inclusion	*Exclusion*
Participants	• Female • Any trimester of pregnancy • Any age-group	• Nonpregnant women
Disease	• Gram-negative bacteria	• Bacteria causing sexually transmitted infections • Gram-positive bacteria
Outcome	• Proportion of women colonized with Gram-negative bacteria	
Study	Prevalence studies, cross-sectional studies, cohort studies, case–control studies, and surveys English Language Published and unpublished data	Qualitative, policy, opinion, case series, and letter to the editor (if not providing data on desired outcome)

Quality assessment of studies examining the prevalence of Gram-negative bacterial infection in maternal cervical secretion ($N = 15$)

	Newcastle–Ottawa quality assessment scale for cross-sectional studies								
	Selection				*Comparability*	*Outcome*			
Study	*Representativeness of the sample*	*Sample size*	*Nonrespondents*	*Ascertainment of the exposure (risk factor)*	*The subjects in different outcome groups are comparable based on the study design or analysis. Confounding factors are controlled. Maximum: ☆☆*	*Assessment of outcome Maximum: ☆☆*	*Statistical test*	*Score*	*Evidence quality*
Duan et al. (2019)	☆	☆	☆	☆☆	☆	☆	☆	*8*	Low risk of bias
Elliyas et al. (2021)	☆	☆	☆	☆	☆☆	☆☆	☆	*9*	Very low risk of bias
Febriani et al. (2017)	☆	☆	☆	☆	☆	☆☆	☆	*8*	Low risk of bias
Hussain et al. (2019)	☆	☆	☆	☆	☆☆	☆☆	☆	*9*	Very low risk of bias
Johnson et al. (2021)	☆	☆	☆	☆	☆☆	☆☆	☆	*9*	Very low risk of bias
Kayastha et al. (2022)	☆			☆	☆	☆☆	☆	*6*	Low risk of bias
Kerur et al. (2006)	☆	☆	☆	☆	☆	☆☆		*7*	Low risk of bias
Kim et al. (2016)	☆	☆	☆	☆☆	☆☆	☆☆	☆	*10*	Very low risk of bias
Marijane et al. (1996)	☆	☆	☆	☆	☆	☆☆		*7*	Low risk of bias
McDonald et al. (1994)	☆	☆	☆	☆	☆	☆☆		*7*	Low risk of bias
Ngonzi et al. (2018)	☆	☆	☆	☆	☆☆	☆☆	☆	*9*	Very low risk of bias
Prasad et al. (2021)	☆	☆	☆	☆	☆	☆☆	☆	*8*	Low risk of bias
Sabri et al. (2011)	☆	☆	☆	☆	☆	☆☆		*7*	Low risk of bias
Son et al. (2018)	☆	☆	☆	☆☆	☆	☆☆	☆	*9*	Very low risk of bias
Tumuhamye et al., (2020)	☆	☆	☆	☆	☆	☆☆	☆	*8*	Low risk of bias