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Review

Transmission of Group B Streptococcus in late-onset neonatal disease: a narrative review of current evidence

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Francesca Miselli, Ilaria Frabboni, Marianna Di Martino, Isotta Zinani, Martina Buttera, Anna Insalaco, Francesca Stefanelli, Licia Lugli and Alberto Berardi

***Abstract*:** Group B streptococcus (GBS) late-onset disease (LOD, occurring from 7 through 89 days of life) is an important cause of sepsis and meningitis in infants. The pathogenesis and modes of transmission of LOD to neonates are yet to be elucidated. Established risk factors for the incidence of LOD include maternal GBS colonisation, young maternal age, preterm birth, HIV exposure and African ethnicity. The mucosal colonisation by GBS may be acquired perinatally or in the postpartum period from maternal or other sources. Growing evidence has demonstrated the predominant role of maternal sources in the transmission of LOD. Intrapartum antibiotic prophylaxis (IAP) to prevent early-onset disease reduces neonatal GBS colonisation during delivery; however, a significant proportion of IAP-exposed neonates born to GBS-carrier mothers acquire the pathogen at mucosal sites in the first weeks of life. GBS-infected breast milk, with or without presence of mastitis, is considered a potential vehicle for transmitting GBS. Furthermore, horizontal transmission is possible from nosocomial and other community sources. Although unfrequently reported, nosocomial transmission of GBS in the neonatal intensive care unit is probably less rare than is usually believed. GBS disease can sometime recur and is usually caused by the same GBS serotype that caused the primary infection. This review aims to discuss the dynamics of transmission of GBS in the neonatal LOD.

Keywords: group B streptococcus, late-onset disease, late-onset sepsis, *Streptococcus agalactiae*, transmission

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Correspondence to:
Alberto Berardi
Neonatal Intensive Care Unit, Policlinico University Hospital, 41124 Modena, Italy.
alberto.berardi@unimore.it

Francesca Miselli
Licia Lugli
Neonatal Intensive Care Unit, Azienda Ospedaliero-Universitaria Policlinico, Modena, Italy

Ilaria Frabboni
Marianna Di Martino
Isotta Zinani
Martina Buttera
Anna Insalaco
Francesca Stefanelli
Pediatric Post-Graduate School, University of Modena e Reggio Emilia, Modena, Italy

Introduction

Group B Streptococcus (GBS), also known as *Streptococcus agalactiae*, remains a leading pathogen responsible for neonatal sepsis, resulting in significant morbidity and mortality. Neonatal GBS disease may be divided into early-onset disease (EOD), occurring within the first week of life, and late-onset disease (LOD), occurring between 7 and 89 days of life. Incidence of LOD in infants older than 89 days (ultra-late-onset GBS disease) is rare and usually occurs in very preterm infants requiring prolonged hospitalisation or in infants with immunodeficiency syndromes.[1]

GBS bacteria are encapsulated gram-positive diplococci that cause beta-hemolysis. These organisms are classified into 10 serotypes based on their capsular polysaccharides: Ia, Ib and serotypes II–IX. Serotypes Ia, Ib, II, III, IV and V are the most prevalent, accounting for approximately 97% of neonatal invasive GBS infections. Different serotypes are associated with different types of GBS-associated invasive (iGBS) diseases. LOD is mainly caused by the predominant serotype III (56%), followed by the serotypes Ia (20%), V (8.3%), IV (6.2%) and Ib (6.1%). Collectively, these five serotypes are responsible for 99.7% of the LOD cases in the United States.[2]

Worldwide, serotypes III (61.5%), Ia (19.1%), V (6.7%), Ib (5.7%) and II (3.9%) are responsible for 96.9% invasive infections in infants.[3]

GBS is a common coloniser of gastrointestinal and genitourinary tract in adults,[4] and asymptomatic vaginorectal (VR) colonisation occurs in 15–35% of pregnant women globally.[5] The gastrointestinal tract – namely, the rectal site – has been suggested as the principal reservoir for GBS.[6] The urinary tract is a relevant site of infection, and GBS bacteriuria is a marker of heavy maternal colonisation. In patients with a weakened immune system, GBS colonisation can progress to an invasive stage (iGBS), resulting in a wide spectrum of diseases. In the elderly, immunocompromised individuals and infants, GBS causes severe infections, such as sepsis, pneumonia and meningitis.[6] The worldwide mean incidence of iGBS in infants aged 0–89 days has been estimated at 0.53 cases per 1000 live births.[7] Before prevention was introduced, the rate of incidence of EOD was approximately fourfold higher than that of LOD; however, the administration of antibiotics during delivery (intrapartum antibiotic prophylaxis, IAP) substantially reduced the rate of incidence of EOD,[1,2] with no apparent impact on the incidence of LOD.[8] Thus, in the clinical settings where the application of IAP is widespread, LOD has become the most common manifestation of iGBS during infancy.[1] Current incidence of EOD and LOD in the United States is 0.23 and 0.31 cases per 1000 live births, respectively.[2] The worldwide burden of iGBS infection during infancy remains substantial, however. In 2015, iGBS affected approximately 320,000 neonates, and 90,000 of them died;[8] mortality rates were highest in low-income countries. According to recent estimates, GBS colonisation occurred in approximately 20 million pregnant women in 2020, and 393,000 iGBS cases occurred in infants (231,000 EOD and 162,000 LOD). The outcome of these infections was severe, with an estimated 37,100 children developing moderate-to-severe neurodevelopmental impairment after the recovery from iGBS. The infections also led to the death of 91,000 children, with a disproportionate burden on Sub-Saharan Africa.[9] Globally, LOD is estimated to have a case fatality rate of 7%, ranging from 4% in developed countries to 12% in Africa.[3] It should be noted that LOD case fatality rate in preterm infants born before 37 weeks' gestation is roughly twice that in the term infants (7.8% *versus* 3.4%) and almost threefold higher when meningitis is present (9.7 *versus* 3.5%).[2]

This review aims to elucidate the dynamics of GBS pathogenesis and its modes of transmission to newborns and improve the currently limited preventative measures because of the poor understanding of these aspects.

Pathogenesis

EOD and LOD differ regarding the timing and mode of exposure, clinical presentation, mortality and morbidity. EOD is vertically acquired and modes of transmission are well known. GBS is carried at VR site by an asymptomatically colonised mother and is transmitted to the neonate causing the EOD.[10] Most neonates, however, do not develop the disease and remain colonised at mucocutaneous surfaces. Without any intervention (such as IAP), the neonatal colonisation occurs in approximately 50% of neonates delivered from a colonised mother.[10,11] The inoculum (i.e. the number of organisms) in maternal genital tract is the main factor associated with the likelihood of GBS transmission to the newborn. Heavily colonised mothers are more likely to have GBS-colonised or infected neonates, and heavily colonised neonates are more likely to develop both EOD or LOD.[6]

In EOD, exposure to GBS may occur hours or a few days before birth, or during passage through the birth canal. GBS is aspirated into the lungs before birth, with exposure of the respiratory epithelial surface and subsequent invasion of pulmonary vessels. During delivery, aspiration and swallowing of vaginal secretions result in bacterial adherence to respiratory and gastrointestinal mucous membranes with subsequent bacterial invasion of the bloodstream. In both cases, infants typically present with pneumonia or sepsis at birth or soon thereafter.[6,10] The mechanisms underlying GBS transmission in LOD have not been fully elucidated yet, however. GBS may be acquired at mucosal surfaces at the time of delivery or in the postpartum period, from maternal or other sources. By overcoming the epithelial barriers and innate cellular immunity, GBS leads to LOD. Prior studies have shown that intestinal colonisation at 12 weeks of age is found approximately in 40% of infants who were colonised at birth.[12] Furthermore, studies have shown that intestinal colonisation is an important ensuing factor for the incidence of LOD: in a prospective study, among 21 infants with LOD, 10 were colonised at birth (oropharyngeal and rectal swab)

with the serotype subsequently causing disease.[13] In an animal model, oral administration of GBS precedes the development of systemic disease.[14] The pathogenesis of LOD involves GBS adhesion to mucosal surfaces, followed by invasion of the epithelium and, subsequently, of the bloodstream. The kinetics of gastrointestinal colonisation by GBS and the role of GBS-specific immunoglobulin G (IgG) production were recently reported in a study employing exposed offspring and juvenile mice. The progression to iGBS after perinatal and postnatal exposure to GBS occurred in 21% and 27% of cases, respectively; of note, experimental animals developed iGBS after sustained gastrointestinal colonisation.[15] It is, however, unclear why GBS, a mucocutaneous coloniser present in approximately 10% of infants in the initial weeks of life, causes LOD only in less than 0.1% of individuals.[16] Factors at the mucosal immunity and local microbiome levels that mediate persistent intestinal colonisation or promote the progression to iGBS remain unknown.

Sources and mode of GBS transmission

Transmission from person to person plays a primary role in GBS dissemination. Data regarding GBS transmission have been largely obtained from mother–infant pairs, whereas the contribution of faecal–oral GBS transmission by other family members (father and siblings) to the neonates has not been clearly elucidated. It is reported that sexual partners usually share the same strain of GBS, whereas casual contact because of cohabitation is not a likely cause of GBS transmission. Here, we report GBS transmission *via* maternal (intra- or postpartum) or nonmaternal (nosocomial or community-acquired) sources. Recurrence and concurrence of disease in cases of multiple births are analysed separately. In these cases, it is difficult to assess whether the source of GBS is maternal or nonmaternal and often remains undetermined.

Maternal sources

Maternal sources are one of the predominant modes of GBS transmission to neonates. A case–control study carried out in the United States reported high rates of VR colonisation during prenatal screening in mothers of infants with LOD (46 of 122 mothers, i.e. 38%). The risk of LOD increased by a factor of 4.15 [95% confidence interval (CI) = 1.27–13.60] in infants whose mothers were positive for GBS.[17] In a subsequent population-based case–control study involving 77 cases of LOD (2003–2011), maternal prenatal GBS positivity remained associated with an increased risk of LOD, as shown by multivariate analyses, after the adjustment of other risk factors (RFs), including preterm birth, multiple births, young maternal age, maternal ethnicity and prenatal smoking [odds ratio (OR) = 1.90; 95% CI = 1.09–3.31; $p = 0.02$].[18] Multivariate analysis performed in a case–control study involving 46 infants from South Africa confirmed that LOD was associated with maternal GBS colonisation (OR = 2.44; 95% CI = 0.88–6.79; $p = 0.088$) and GBS bacteriuria (OR = 3.49; 95% CI = 1.17–10.40; $p = 0.025$), which serves as a marker of significant maternal colonisation.[19] These studies, however, have investigated the maternal origin of LOD based only on prenatal VR screening: as maternal colonisation may be falsely negative[1] and intermittent,[20] prenatal screening may not accurately detect all mothers carrying GBS during the postpartum period.[21,22] Thus, the proportion of GBS carriers among mothers of infants with LOD is probably underestimated by prenatal VR screening. Indeed, a recent Italian cohort study investigated 98 neonates by culturing the GBS isolated from their mothers at the time of LOD diagnosis. The information regarding maternal prenatal screening was made available for all the cases of LOD, allowing for a 'full assessment of maternal carriage'. GBS was isolated from the VR site (at the time of screening and LOD diagnosis), urine or breast milk samples in up to 67% of the mothers.[23] Furthermore, GBS-colonised mothers had often GBS bacteriuria at the time of LOD diagnosis. Investigators concluded that the mother, often heavily colonised, was the primary source of GBS in most LOD cases.

Despite preventing perinatal transmission of GBS, IAP cannot eradicate GBS from the mother's reservoir,[18] which consequently remains a potential source of GBS transmission to the newborn during the postpartum period.[21,22,24] In fact, even after the IAP administration, up to 77% of women (GBS-positive during the antenatal screening) remain culture-positive carriers at the time of discharge from the hospital.[22] Among neonates born to GBS-positive women, IAP-exposed neonates present with lower rates of colonisation at birth (<5%) than the IAP-unexposed

neonates (~40%); however, GBS can be transmitted from the mother at home when it is no longer suppressed by IAP. About a quarter of these newborns are colonised after their discharge from hospital (26.3% *versus* 57.7%).[22,25] The predominant routes of GBS transmission and severity of LOD may still be influenced by IAP administration, however. A study involving 100 LOD cases from Italy reported that IAP-unexposed newborns were younger at LOD presentation (median = 24 days) than newborns exposed to IAP (median = 44 days, $p < 0.01$); the duration of IAP did not affect these results.[21] In addition, IAP exposure was significantly associated with a lower severity of the LOD. The authors speculated that IAP could delay the onset of LOD by changing the routes of GBS transmission from vertical to horizontal, leading to a less heavy neonatal colonisation and reducing its severity.

Case reports have recognised infected breast milk, with or without occurrence of mastitis, as a possible vehicle for transmitting GBS.[21] In some cases of LOD, no source of GBS other than breast milk was identified.[26,27] The relationship between infected breast milk and occurrence of LOD is yet to be elucidated, however.[22] A recent study in Australia investigating 92 cases of LOD and 368 controls found that breastfeeding was not associated with an increased risk of LOD (OR = 1.2; 95% CI = 0.7–2.3). In addition, although approximately 0.8–3.5% of the mothers carry GBS in their breast milk,[28,29] the incidence of LOD is considerably lower (<0.5 cases per 1000 live births), indicating that a majority of the breastfed infants are unaffected by GBS. Even though some cases of LOD could be acquired through infected breast milk, the benefits of breast milk surpass this limitation and protect the newborn from numerous invasive diseases.[30] Notably, breast milk is considered to be the main source of nonpathogenic bacteria in the gastrointestinal tract of the infant. Intestinal bacteria are one of the most important stimuli for the development of mucosa-associated lymphoid tissue in the intestine of the neonate. Breast milk consumption leads to a critical modulation of the host immune system.[31] Furthermore, as the levels of natural secretory immunoglobulin IgA and IgM are low in neonates, colostrum and breast milk containing natural antibodies confer protection from invasive pathogens.[30] Moreover, breast milk contains high concentrations of nonspecific protective molecules, such as lactoferrin and human milk oligosaccharides (HMOs), which show antimicrobial and antibiofilm activities against GBS.[30–32] Interestingly, not all women produce the same concentrations of HMOs[31] and, in animal models, postnatal exposure to maternal GBS antibodies present in milk results in improved neonatal survival after the GBS infection, with the rate of survival being directly related to maternal GBS antibody titres present in the milk.[33,34] Undoubtedly, the delivery procedure, treatment and storage methods of breast milk are potential routes of GBS contamination. GBS-positive milk is associated with heavy neonatal colonisation.[22] Preterm birth and high bacterial inoculum are considered the RFs for developing an infection after ingesting GBS-contaminated breast milk.[35]

GBS transmission through breast milk does not necessarily require the presence of mastitis[28] but could be associated with alternative factors, such as milk stasis and bacterial load.[30] Two main mechanisms underlying GBS infection have been proposed. During passage through the mother's birth canal, GBS colonises the oropharyngeal mucosa of the newborn and then infects the mammary ducts of the mother during breastfeeding, which can lead to bacterial overgrowth in breast milk and reinfection of the newborn during breastfeeding. Alternatively, recent studies have suggested that the bacteria from maternal digestive tract may also colonise the breast and, through the milk, may reach the infant's intestinal mucosa.[36] It is, however, unclear whether LOD is related to a recent GBS infection from breast milk or is a result of gut translocation from an already GBS-colonised infant.[30] Interestingly, in their prospective cohort study, Carl *et al.*[37] documented enteric colonisation of pathogens, including GBS, that subsequently caused LOD; however, GBS gut colonisation was not evident from birth but occurred closer to the days on which the sepsis occurred.[36] Thus, the isolation of GBS from the stool may predict an ensuing bloodstream infection. Therefore, to guide early diagnosis and prompt treatment, colonised infants may undergo an increased vigilance for the earliest signs of infection.

Non maternal sources

GBS colonisation of maternal VR sites does not always occur in the LOD cases, indicating that some cases of LOD are horizontally acquired postpartum from alternative sources, such as caregivers or health-care workers. Studies performed

prior to the introduction of IAP have been inconclusive in determining the predominant sources (maternal, nosocomial or community) of postnatal GBS transmission.[10,11,38–42] Although most studies have recognised that the transmission occurs predominantly during the delivery process,[11,38–43] horizontal transmission from community and hospital sources is an important, albeit less frequent, mode for GBS transmission to neonates. Nosocomial transmission of GBS in the nurseries, through the hands of health-care workers, has been frequently reported a few decades ago, when mothers and their infants remained hospitalised for several days after delivery.[44–46] Most neonates currently remain in hospital only for a few days, and usually 'room in' with their own mothers, however.[21,22,47] Therefore, this mode of transmission is probably less common. Furthermore, in the recent past, nosocomial transmission of GBS within neonatal intensive care units (NICUs) has rarely been reported,[47,48] but it is probably not exceptional. A 2-year surveillance of LOD cases in an NICU in the United Kingdom identified 4 clusters with 12 LOD cases, of which 11 were associated with at least one or the other LOD isolate, identified by serotyping and genome sequencing.[45] Notably, GBS hospital clusters can be challenging to identify as long intervals between consecutive cases (up to 50 days) may occur. The hospital stay of affected infants may not overlap, so identifying the potential contamination source may be difficult.[45] Even a single nosocomial LOD case warrants prompt investigation and improved efforts for prevention because it may not necessarily indicate a sporadic occurrence.[49] A recent systematic review[44] investigated GBS transmission in hospital clusters of iGBS and identified the following possible contributing factors: unsatisfactory practices for the prevention of infection (8 out of 17 studies), excessive proximity of the cots (7 out of 17 studies), inadequate disinfection of equipment and surfaces, such as shared breast milk equipment and formula preparation facilities (6 out of 17 studies), crowding and high patient-to-nurse ratio (5 out of 17 studies). Finally, the role of persistent GBS carriage in the NICU personnel seeding clusters is a highly sensitive topic that requires further investigation. Efforts to prevent LOD should focus on developing practices for prevention and control of infections, including catheter care and hand hygiene.

Undetermined source: recurrence and multiple births

Although uncommon (0.5–3% of the cases), the infection can recur after an adequate antibiotic treatment of a first episode of iGBS.[34,50–54] Recurrence is usually caused by the same GBS serotype responsible for the primary infection. The low rate of GBS recurrence suggests that LOD is usually a singular accident rather than a result of an immune deficiency. Several case reports and series have been elaborated in the literature, but the pathogenesis of recurrent infections remains poorly understood. Early studies suggest that an undrained focus on infection, inadequate course of antibiotics, impaired mucosal surface integrity, immunodeficiency and enhanced bacterial virulence are the causative factors of recurrence.[55] Currently, the most acceptable hypotheses relate the recurrence to the persistence of GBS colonisation in the mucosal sites and inappropriate antimicrobial therapy.[34,54] Repeated translocation from the natural ecological habitat to the bloodstream must be discriminated from the persistence of infectious foci due to insufficient antibiotic treatment.[34] Cases of GBS infection recurrence were analysed in a recent retrospective study by Freudenhammer *et al.*,[34] in which recurrence was associated with a shorter antibiotic course (<10 days: OR = 4.2; 95% CI = 1.3–18.0). The relationship between a shorter antibiotic course and an increased recurrence risk may highlight a subgroup of insufficiently treated cases. This finding is in contrast with a previous retrospective cohort study showing that as compared with long-duration courses (10 days), short-duration intravenous antibiotic courses (⩽8 days) for treating uncomplicated LOD in infants were not associated with a higher recurrence rate and treatment failure.[56] The authors concluded that the duration of antibiotic treatment in LOD should be determined by the clinical presentation (10 days of intravenous antibiotic administration for GBS bacteraemia and 14 days for meningitis) and should not be extended beyond what is usually recommended to prevent the risk of recurrence.[1] In fact, prolonged antibiotic courses in the perinatal period are known to have undisputed costs, including increased susceptibility to sepsis in preterm neonates.[57] Preterm birth increases the risk of LOD recurrence, as it is associated with various alterations in the host resistance (transient hypogammaglobulinemia, complement deficiency

and neutropenia) and with disruption of the gut microbiota. In the previously cited study by Freudenhammer *et al.*,[34] 57% of the recurrent GBS cases were preterm, and recurrence was associated with a very low birth weight (birth weight under 1500 g: OR = 9.7; 95% CI = 2.8–33.3). Mastitis was reported in 32% of the mothers, and 81% of breast milk samples tested positive during culture screening or molecular testing. Among the 11 breast milk samples tested, the GBS serotypes present were identical to those found in the infants.

The incidence of LOD in case of multiple births provides intriguing insights into the mechanisms underlying GBS transmission and pathogenesis. In some cases, the simultaneous onset of LOD in siblings within 48 h, occurring many weeks after birth, is highly suggestive of an acute infection in infants from an external source, predominantly from the mother. Alternatively, the infectious GBS clone may be acutely transferred from one sibling to the other through the mother's breast. In other cases, a longer interval of up to 18 days in the LOD onset between twin siblings suggests fluctuations in the individual host immunity rather than an acute infection.[34] If a twin develops LOD, prophylactic antibiotic treatment of the other multiple(s) does not seem to be justified, given the highly variable interval in the onset of LOD between the siblings; however, parental education is crucial.

Risk Factors

In some high-income countries, LOD has become the most common presentation of neonatal GBS disease; hence, investigation of the associated RFs has become fundamental.[49] Established RFs for LOD include preterm birth, maternal GBS colonisation, young maternal age, HIV exposure and African ethnicity. Breastfeeding and twin delivery have been suggested as RFs in case reports and series, but their role has not been confirmed by evidence-based data.[49]

- Preterm birth: it is one of the main RFs for the incidence of LOD, and each week of decreasing gestation results in an increase in the risk of LOD by a factor of 1.34.[17] Approximately, 42% of all the LOD cases afflict preterm infants born before 37 weeks' gestation.[2] A recent study confirmed that the annual LOD incidence rates (2006–2015) were more than sixfold higher among preterm infants than in those at term.[2] The increased susceptibility of preterm infants to LOD can be attributed to the immaturity of their immune system, as the transplacental transfer of maternal antibodies peaks after 32–33 weeks of gestation.[58] Prematurity is also characterised by disturbances in gut microbiota development (as a result of repeated exposure to antibiotics),[59] prolonged hospital stay, formula feeding, and reduced contact with maternal microbiome.[34,44,60]
- Maternal GBS colonisation: a maternal culture positive for GBS at the time of delivery or LOD occurrence is significantly associated with the risk of LOD.[18,22] Notably, the risk of a neonate acquiring carriage at mucosal membranes is directly correlated with the intensity of maternal GBS colonisation (inoculum size), being higher in neonates born to heavily colonised women than in those born to women with low colony counts of GBS in the vaginal cultures at the time of delivery.[61,62] The role of maternal colonisation is more prominent in full-term infants exposed to more frequent and closer contact with their mothers. They may be infected from any of the numerous reservoirs of GBS in the colonised women, with the main ones being contaminated stool, urine, vagina, body surface and upper respiratory tract.[28]
- HIV: infants diagnosed with HIV are more susceptible to LOD. In addition, infants exposed to HIV but not subsequently diagnosed with HIV also show a fivefold greater incidence of LOD than HIV-unexposed infants.[63] This can be attributed to maternal GBS colonisation. In fact, mothers living with HIV might have a higher rate of GBS colonisation, and they could be colonised by a higher number of pathogens or by more virulent organisms, compared with mothers not living with HIV.[64] Of clinical relevance, an inverse correlation between maternal CD4 cell count at the time of delivery and mortality in infants exposed to HIV has been reported.[65]
- Young maternal age: infants born to teenage mothers (<20 years of age) present a higher risk of LOD and are unaffected by the implementation of universal GBS screening and IAP administration.[18] Higher

maternal GBS carriage, altered immunity or higher incidence of coinfections is possible mechanisms linking young mothers to a higher LOD incidence in their infants.[66]

- African ethnicity: the annual LOD incidence rates are nearly threefold higher among Black infants compared with that in White infants.[2] A recent study found that GBS-colonised women were more likely than GBS-negative women to be Black or African American (45.7% *versus* 31.6%, $p < 0.001$).[67] In addition to the higher carriage of GBS in African women, a higher LOD risk in Black infants is associated with lack of access to healthcare, socioeconomic disparities and poor health outcomes.[68]
- Siblings: in cases of multiple births, a sibling with iGBS is a major RF for LOD, and the risk in the other sibling has been estimated to be tenfold higher as compared with that predicted based on maternal VR colonisation.[34]

Prevention

Considering the mode of transmission and the RFs known to promote GBS infections, there are some strategies, discussed below, that could help to reduce their occurrence.

First, an upcoming prevention strategy could be the vaccination of pregnant women in the second or third trimester. That timing seems to be optimal both to reduce the risk for teratogenicity and to ensure that a sufficient maternal antibody production occurs prior to delivery.[69,70] Thus, not only vaccination would confer to the newborns passive immunity through the transplacental transfer of IgG antibodies, protecting them by GBS-related infections up to 3 months of life, but it could be effective in preventing maternal VR colonisation as well.[69,71,72] As a result, the vaccination strategy could reduce both the occurrence of LOD in term infants and of the GBS-associated stillbirths and preterm births. As the transplacental transfer of antibodies mainly occurs after 34 week's gestation, however, the vaccination strategy may be less effective in protecting very preterm neonates from GBS invasive infections.[49] Finally, vaccination could also help in preventing the development of GBS resistance to antibiotics, by representing an alternative strategy to IAP. Capsular polysaccharide vaccines, including trivalent (targeting serotypes Ia, Ib and III) and hexavalent (targeting serotypes Ia, Ib, II, III, IV and V) protein-polysaccharide conjugate vaccines have reached phase II clinical trials.[73] In addition, vaccines targeting antigenic surface proteins and pili subunits, that could overcome the limited serotype coverage, are in study as an alternative.[74–82]

Moreover, as a healthy gut microbiota is known to play a key role in maintaining a balanced immune response and in developing the intestinal barrier in the perinatal period, elements that could help its development, such as breastfeeding and probiotic supplementation, must be encouraged.[83] Studies showed that exclusive human milk feeding and probiotic supplementation with combinations of *Lactobacillus* and *Bifidobacteria* could reduce the risk of late-onset sepsis in preterm infants.[84–86] Considering that an abnormal intestinal flora is a recognised RF for nosocomial pathogen transmission, including GBS, and disruption in early microbiota may predispose to LOD, efforts should be also made to minimise unnecessary neonatal exposure to antibiotics.[17] Furthermore, in order to prevent nosocomial infections, practices like catheter care and hand hygiene must be developed.[87]

Finally, there is a lack of consensus in the literature on the prevention and management of LOD associated with contaminated breast milk. First, it is debatable whether screening breast milk for contamination is necessary in cases of mastitis. Most authors do not recommend this screening, as the level of GBS present in breast milk is low, and the presence of the pathogen in breast milk has an unknown predictive value for LOD.[36] When LOD occurs, screening the breast milk for GBS is not routinely recommended but could be considered in cases such as mastitis, preterm birth, recurrent LOD and concurrent disease in case of multiple births.[34,36,87,88] When breast milk is found GBS-contaminated, temporarily ceasing breastfeeding or pasteurising the milk is frequently recommended. According to some authors,[89] however, it is safe to continue nursing in the cases of isolated milk stasis, in which bacterial counts and leukocyte levels are low. It could be safer to temporarily discard breast milk when bacterial counts and leukocyte levels are high. Finally, in the cases of GBS-positive milk culture, maternal antibiotic therapy with amoxicillin (for 7–10 days) may be considered, even though it is

not always successful. Rifampicin (continued for 7 days) should be considered after failure of amoxicillin courses, due to increased risk of resistance.[36]

Therapy and management

Antibiotics

For GBS, pan-susceptibility to first-line antibiotic treatment (penicillin) is still described, although few reports warned of reduced susceptibility to beta-lactams (including penicillin) in some countries. In recent years, rising levels of GBS resistance to other commonly used second-line antibiotics (erythromycin, clindamycin, gentamicin and fluoroquinolones) have been noted in a multitude of studies. Even more worrying, two reports documented resistance to vancomycin.[90] Second- and third-line antibiotics should only be used for treatment if a penicillin or cephalosporin is not appropriate, and susceptibility of the organism has been determined.[91]

Despite its high burden, high-quality evidence assessing the beneficial and harmful effects of different antibiotic regimens for late-onset sepsis is scarce. The Cochrane Review published in 2021 concluded that evidence from randomised controlled trials (RCTs) in favour of any particular antibiotic regimen for the treatment of suspected late-onset sepsis was insufficient.[92]

For empiric therapy of late-onset sepsis in infants from 8 to 28 days of life who are not critically ill and do not have evidence of meningitis, an antibiotic treatment based on the use of ampicillin plus gentamicin or cefotaxime (or cefepime if cefotaxime is not available) is recommended.[1,5] Gentamicin-based regimens, however, should be preferred to cefotaxime-based treatments, considering the lower levels of susceptibility to cefotaxime and the need to limit exerting selective pressure for resistance and invasive candidiasis.[93] If meningitis is suspected, ampicillin plus cefotaxime should be used; gentamicin should not be used if meningitis is suspected.[5] For infants from 29 to 90 days of age, the use of ceftriaxone is recommended. If there is evidence of meningitis or critical illness, vancomycin should be added to expand empiric coverage, including β-lactamases-resistant *Streptococcus pneumoniae*. For a preterm infant hospitalised beyond 72 h, empiric treatment for sepsis should cover health-care-associated pathogens, as well as those responsible for neonatal sepsis, including GBS.[1,5] In such cases, vancomycin plus an aminoglycoside is recommended.[94]

When GBS is identified as the pathogen responsible for sepsis, penicillin G or ampicillin is indicated. Recommended doses of penicillin G for infants older than 7 days are 50,000 U/kg every 8 h in bacteraemia and 125,000 U/kg every 6 h in meningitis. Recommended doses of ampicillin in bacteraemia for newborns older than 7 days are 75 mg/kg every 12 h (gestational age $\leq$ 34 weeks) and 50 mg/kg every 8 h (gestational age $>$ 34 weeks). In meningitis, recommended dosing is higher: for penicillin G 125,000 U/kg every 6 h and for ampicillin 75 mg/kg every 6 h.[1,5]

Regarding the treatment of GBS meningitis, the doses of ampicillin are not differentiated on the basis of gestational age. Pharmacokinetic studies, however, showed that ampicillin half-life appeared nearly two times longer in infants $\leq$ 34 weeks' gestation compared with those $>$ 34 weeks' gestation. Thus, as high ampicillin concentration have been associated with seizures, the high ampicillin dose for GBS meningitis should be used with caution in preterm infants and doses should be deescalated to sepsis-dosing as soon as meningitis is ruled out.[95]

The duration of antibiotic treatment varies according to the site and the severity of the infection. Ten days course of parenteral treatment is recommended for infants with bacteraemia without a defined focus or with an isolated urinary tract infection without bacteraemia. For infants with uncomplicated meningitis, 14 days of intravenous treatment is recommended, with longer courses of treatment provided for infants with prolonged or complicated courses. Septic arthritis or osteomyelitis requires treatment from 3 to 4 weeks. Endocarditis or ventriculitis require treatment for at least 4 weeks.[1,5]

Supportive care

In addition to antibiotic treatment, general supportive care should be provided to the infants, including respiratory support, maintenance of adequate tissue perfusion with intravenous fluids and inotropic drugs, blood product administration, temperature and glucose control and phototherapy.

For neonatal meningitis, some experts recommend that a second lumbar puncture should be performed approximately from 24 to 48 h after the beginning of antibiotic therapy. This should allow the health-care providers to improve the management of the infection as well as define the prognosis. If cerebrospinal fluid sterility is not achieved or if increasing protein concentration is noted, a complication (such as cerebral infarcts, cerebritis, ventriculitis, subdural empyema and ventricular obstruction) is more likely. Additional lumbar punctures and intracranial imaging are recommended if neurologic abnormalities persist or focal neurological deficits occur. In fact, late-onset GBS meningitis can be also complicated by cerebrovascular disease, including arterial ischemic stroke and cerebral sinus venous thrombosis. When cerebrovascular complications occur, anticoagulant therapy may be considered for secondary prevention.[96] A failed hearing screening test or abnormal neurologic examination at discharge mandates careful follow-up.[1,5]

Immunoglobulin

Routine administration of intravenous immunoglobulin (IVIG) in infants with suspected or proven neonatal infection is not recommended, because there are evidences that showed no impact on mortality or major disability at 2 years of age.[97] Nevertheless, promising results concerning the use of specific antibodies targeting GBS to treat and prevent LOD are available. In animal studies, the use of GBS-specific hyperimmune IVIG demonstrated an increased survival even in case of severe infections.[98] Clearly, the effectiveness of the use of GBS-specific hyperimmune IVIG in LOD should be still determined in neonates and infants.

Conclusion

In conclusion, GBS remains a leading cause of serious bacterial infections in infants younger than 3 months. LOD is an important cause of bacterial meningitis in the initial months of life. IAP has significantly reduced the incidence of EOD, but the widespread use of antibiotics is a major concern because of the increasing antibiotic resistance to pathogens. Furthermore, IAP has not shown notable effects on the incidence of LOD, as GBS may be acquired postpartum from maternal or nonmaternal sources. Maternal GBS carriage at the VR site is common when assessed during LOD diagnosis. Nosocomial transmission of GBS, however, may also occur, and premature neonates, who remain hospitalised for long periods, are at higher risk. Furthermore, growing evidence shows that the progression of GBS from mucosal surface colonisation to the development of an invasive disease often results from gut flora changes, and antibiotics in early stages of life may affect the nascent gut microbiome with short- and long-term risks. The role of GBS transmission among adults and community transmission of GBS to newborns from nonmaternal sources should be studied in detail in the future.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Author contributions

Francesca Miselli: Conceptualisation; Formal analysis; Investigation; Methodology; Supervision; Writing – original draft; Writing – review & editing.

Ilaria Frabboni: Conceptualisation; Data curation; Methodology; Resources; Writing – original draft; Writing – review & editing.

Marianna Di Martino: Conceptualisation; Data curation; Methodology; Resources; Writing – original draft.

Isotta Zinani: Conceptualisation; Data curation; Resources; Writing – original draft.

Martina Buttera: Conceptualisation; Data curation; Investigation; Resources; Writing – original draft.

Anna Insalaco: Conceptualisation; Investigation; Resources; Writing – original draft.

Francesca Stefanelli: Conceptualisation; Resources; Writing – original draft.

Licia Lugli: Conceptualisation; Data curation; Methodology; Resources; Supervision; Writing – review & editing.

Alberto Berardi: Conceptualisation; Data curation; Formal analysis; Investigation; Methodology;

Project administration; Resources; Supervision; Validation; Writing – review & editing.

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ORCID iDs
Francesca Miselli https://orcid.org/0000-0001-5991-8949

Alberto Berardi https://orcid.org/0000-0002-3534-7499

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