

Early-Onset Neonatal Sepsis

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SUMMARY

Early-onset sepsis remains a common and serious problem for neonates, especially preterm infants. Group B streptococcus (GBS) is the most common etiologic agent, while *Escherichia coli* is the most common cause of mortality. Current efforts toward maternal intrapartum antimicrobial prophylaxis have significantly reduced the rates of GBS disease but have been associated with increased rates of Gram-negative infections, especially among very-low-birth-weight infants. The diagnosis of neonatal sepsis is based on a combination of clinical presentation; the use of non-specific markers, including C-reactive protein and procalcitonin (where available); blood cultures; and the use of molecular methods, including PCR. Cytokines, including interleukin 6 (IL-6), interleukin 8 (IL-8), gamma interferon (IFN- γ), and tumor necrosis factor alpha (TNF- α), and cell surface antigens, including soluble intercellular adhesion molecule (sICAM) and CD64, are also being increasingly examined for use as nonspecific screening measures for neonatal sepsis. Viruses, in particular enteroviruses, parechoviruses, and herpes simplex virus (HSV), should be considered in the differential diagnosis. Empirical treatment should be based on local patterns of antimicrobial resistance but typically consists of the use of ampicillin and gentamicin, or ampicillin and cefotaxime if meningitis is suspected, until the etiologic agent has been identified. Current research is focused primarily on development of vaccines against GBS.

INTRODUCTION

Neonatal sepsis is a systemic infection occurring in infants at ≤ 28 days of life and is an important cause of morbidity and mortality of newborns (1). Early-onset neonatal sepsis (EOS) has been variably defined based on the age at onset, with bacteremia or bacterial meningitis occurring at ≤ 72 h in infants hospitalized in the neonatal intensive care unit (NICU), versus < 7 days in term infants (2–4). In preterm infants, EOS is most consistently defined as occurring in the first 3 days of life and is caused by bacterial pathogens transmitted vertically from mother to infant before or during delivery (3). Late-onset sepsis (LOS) is sepsis occurring after 72 h in NICU infants and 7 days of life in term infants, has been variably defined as occurring up to the age of < 90 or 120 days, and may be caused by vertically or horizontally acquired pathogens (2, 3, 5–7). Early-onset neonatal infections of viral or

fungal etiology may also occur at < 7 days of life and must be distinguished from bacterial sepsis (8, 9).

In this article, we review the seminal literature and recent advances related to early-onset sepsis in term and preterm neonates in developed-country settings, predominantly North America.

EPIDEMIOLOGY AND PATHOPHYSIOLOGY

The incidence of culture-proven early-onset neonatal sepsis in the United States is estimated to be 0.77 to 1 per 1,000 live births (10–12). The incidence and mortality are higher when very-low-birth-weight (VLBW) infants are considered exclusively; for infants with a body weight of $< 1,000$ g, the incidences are estimated to be 26 per 1,000 and 8 per 1,000 live births in premature infants with a birth weight of between 1,000 and 1,500 g. Select populations of neonates are at much higher risk, including term black infants (0.89/1,000 live births) and nonblack preterm infants (2.27/1,000 live births), with black preterm infants having the highest rates of both infection (5.14/1,000 live births) and mortality (24.4% case fatality ratio) (Table 1) (12).

Organisms causing early-onset neonatal sepsis are typically colonizers of the maternal genitourinary tract, leading to contamination of the amniotic fluid, placenta, cervix, or vaginal canal. The pathogen may ascend when the amniotic membranes rupture or prior to the onset of labor, causing an intra-amniotic infection (13). Thus, the infant may acquire the pathogen either *in utero* or intrapartum. Risk factors for early-onset neonatal sepsis include both maternal and infant factors. Maternal risks, such as dietary intake of contaminated foods, can arise before labor and delivery, with *Listeria monocytogenes* contamination of refrigerated foods such as deli meats being the most important example. Procedures during pregnancy, such as cervical cerclage and amniocentesis, which disrupt the amniotic cavity, may also increase the rates of intra-amniotic infection and subsequent neonatal sepsis (14). During labor, maternal risk factors include prolonged rupture of membranes, fever, vaginal colonization with group B streptococcus (GBS), and GBS bacteriuria (15–20). A history of a previous infant with GBS infection is another identified maternal risk factor in subsequent pregnancies (21–23). In addition, adequacy of the maternal immune response is an important risk factor for neonatal sepsis. Maternal serum IgG antibodies against specific capsular polysaccharides of GBS have been shown to be protective against infection with the relevant GBS strain in their infants, and an increased risk for GBS EOS has been demonstrated in infants de-

TABLE 1 Invasive early-onset^a neonatal sepsis cases and deaths, Active Bacterial Core Surveillance Program, 2005 to 2008^b

Yr or pathogen	Total		Black preterm ^c		Black term ^d		Nonblack preterm ^c		Nonblack term ^d	
	No. of cases (no. of cases/1,000 live births)	No. of deaths (CFR [%]) ^e	No. of cases (no. of cases/1,000 live births)	No. of deaths (CFR [%])	No. of cases (no. of cases/1,000 live births)	No. of deaths (CFR [%])	No. of cases (no. of cases/1,000 live births)	No. of deaths (CFR [%])	No. of cases (no. of cases/1,000 live births)	No. of deaths (CFR [%])
Yr										
All	658 (0.77)	72 (10.9)	131 (5.14)	32 (24.4)	120 (0.89)	2 (1.7)	158 (2.27)	34 (21.5)	249 (0.040)	4 (1.6)
2005 ^f	122 (0.77)	10 (8.2)	23 (4.25)	5 (21.7)	30 (1.07)	1 (3.3)	26 (2.00)	4 (15.4)	43 (0.38)	0 (0)
2006	185 (0.79)	20 (10.8)	30 (4.45)	10 (33.3)	39 (1.11)	1 (2.6)	47 (2.41)	8 (17.0)	69 (0.40)	1 (1.4)
2007	174 (0.75)	16 (9.2)	40 (6.01)	7 (17.5)	25 (0.69)	0 (0)	39 (2.11)	8 (20.5)	70 (0.41)	1 (1.4)
2008	177 (0.76)	26 (14.7)	38 (5.71)	10 (26.3)	26 (0.72)	0 (0)	46 (2.48)	14 (30.4)	67 (0.39)	2 (3.0)
Pathogens										
GBS	249 (0.29)	17 (6.8)	40 (1.57)	9 (22.5)	75 (0.55)	0 (0)	38 (0.55)	8 (21.1)	96 (0.15)	0 (0)
<i>Escherichia coli</i>	159 (0.19)	39 (24.5)	46 (1.81)	17 (37.0)	16 (0.12)	1 (6.3)	66 (0.95)	19 (28.8)	31 (0.05)	2 (6.5)
Amp ^g <i>E. coli</i> ^h	81 (0.09)	16 (19.8)	31 (1.22)	9 (29.0)	3 (0.02)	0 (0)	31 (0.45)	6 (19.4)	16 (0.03)	1 (6.3)
Viridans group streptococci	118 (0.14)	3 (2.5)	16 (0.63)	2 (12.5)	16 (0.12)	0 (0)	18 (0.26)	0 (0)	68 (0.11)	1 (1.5)
<i>Staphylococcus aureus</i>	26 (0.03)	2 (7.7)	2 (0.08)	1 (50.0)	5 (0.04)	1 (20.0)	1 (0.01)	0 (0)	18 (0.03)	0 (0)
<i>Haemophilus influenzae</i>	26 (0.03)	4 (15.4)	10 (0.39)	1 (10.0)	0 (0)	0 (0)	12 (0.17)	3 (25.0)	4 (0.006)	0 (0)
Other ^h	80 (0.09)	7 (8.8)	17 (0.67)	2 (11.8)	8 (0.06)	0 (0)	23 (0.33)	4 (17.4)	32 (0.05)	1 (3.1)

^a Occurring in infants aged 0 to 2 days (0 to 72 h of life).^b Adapted from reference 12 with permission of the publisher.^c Preterm is classified as infants born at <37 weeks of gestation.^d Term infants are born at ≥37 weeks of gestation.^e CFR, case fatality ratio.^f Includes cases from Connecticut and selected counties in California and Georgia; statewide surveillance in Minnesota began in 2006.^g Amp^g, ampicillin resistant. Antimicrobial susceptibility data was available for 121 (76%) of 159 *E. coli* cases; those with missing susceptibility information were excluded from incidence and case fatality ratio calculations for ampicillin-resistant *E. coli*.^h Other (*n* = 80) includes *Enterococcus* (*n* = 14), *Listeria monocytogenes* (*n* = 9), *Streptococcus pneumoniae* (*n* = 8), *Citrobacter koseri* (*n* = 7), group D streptococcus (*n* = 7), *Klebsiella pneumoniae* (*n* = 6), group A streptococcus (*n* = 3), *Streptococcus bovis* (*n* = 3), *Streptococcus* not otherwise identified to the species level (*n* = 3), *Bacteroides fragilis* (*n* = 2), group G streptococcus (*n* = 2), *Peptostreptococcus* (*n* = 2), *Streptococcus* not group D (*n* = 2), and 1 each for the following pathogens: *Aerococcus viridans*, *Actinomyces*, *Clostridium septicum*, *Enterobacter aerogenes*, *Kingella denitrificans*, *Klebsiella ornithinolytica*, *Moraxella* species, *Pseudomonas aeruginosa*, *Pseudomonas oryzae*, *Pseudomonas stutzeri*, *Serratia marcescens*, and *Shigella* species.

livered to mothers with low titers (24). Chorioamnionitis, defined by maternal fever, leukocytosis (>15,000 white blood cells [WBCs]/mm³), maternal tachycardia, uterine tenderness, foul odor of amniotic fluid, and fetal tachycardia at delivery, is also a major risk factor for neonatal sepsis. Maternal factors associated with the development of chorioamnionitis include longer length of labor and membrane rupture, multiple digital vaginal examinations, placement of internal fetal or uterine monitoring devices, spontaneous onset of labor, and meconium-stained amniotic fluid (25). The risk of EOS increases to 1% when membranes are ruptured ≥18 h prior to delivery (26, 27), and the EOS risk of infants delivered to mothers with evidence of chorioamnionitis is estimated to be between 1 and 4% (27, 28). *In utero* inhalation or swallowing of infected amniotic fluid by the fetus may lead to intrapartum sepsis, which may partially explain the high sepsis incidence in infants delivered of mothers with chorioamnionitis; alternatively, colonization of the skin and mucus membranes by pathogens involved in chorioamnionitis may cause infection shortly after birth when these barriers lose their integrity (27).

Infant factors associated with early-onset sepsis in addition to the factors noted for the mother include prematurity/low birth weight, congenital anomalies, complicated or instrument-assisted delivery, and low APGAR scores (score of ≤6 at 5 min). Immaturity of the premature neonatal immune system, including low immunoglobulin levels related to decreased transplacental transfer of maternal IgG, also increases the risk of sepsis in preterm infants (29). Barrier function of the skin and mucus membranes is dimin-

ished in premature infants and is additionally compromised in ill premature infants by multiple invasive procedures, including intravenous (i.v.) access and intubation. Poor or late prenatal care, low socioeconomic status of the mother, poor maternal nutrition, maternal substance abuse, male sex, and African American mother (higher rate of GBS colonization) are additional ethnic and social factors associated with neonatal sepsis (11, 30, 31).

PATHOGENS

The organisms most frequently involved in early-onset neonatal sepsis of term and preterm infants together are GBS and *Escherichia coli*, which account for approximately 70% of infections combined. Additional pathogens to consider, which account for the remaining minority of cases, are other streptococci (most commonly viridans group streptococci but also *Streptococcus pneumoniae*) (32), *Staphylococcus aureus*, *Enterococcus* spp., Gram-negative enteric bacilli such as *Enterobacter* spp., *Haemophilus influenzae* (virtually all nontypeable *Haemophilus* spp. in the *H. influenzae* type b [Hib] vaccine era), and *Listeria monocytogenes* (12, 33, 34). When preterm and VLBW infants are considered separately, the burden of disease attributable to *E. coli* and other Gram-negative rods is increased, making Gram-negative sepsis the most common etiology of EOS in this population (3, 12). It is also important to note that while these bacterial pathogens are most likely to be confirmed by culture methods, there are many episodes of clinical neonatal sepsis that are managed empirically with antibiotics despite having no pathogen isolated.

Historically, GBS emerged as an important pathogen in the 1960s and replaced *S. aureus* as the most common cause of neonatal sepsis (35, 36). Current epidemiological trends are showing a decrease in the frequency of early-onset GBS disease related directly to prenatal screening and treatment with intrapartum antibiotics (IPA) (6, 37, 39). The use of intrapartum maternal prophylaxis for GBS has reduced the incidence of early-onset GBS disease by at least 80%; however, GBS remains one of the leading causes of EOS (11). In one large series of preterm infants, EOS was associated with higher rates of infections by Gram-negative organisms (667/1,219 episodes; 55%) than by Gram-positive organisms (459/1,219; 38%), fungal pathogens such as *Candida* sp. (58/1219; 5%), and other unclassified organisms (35/1,219; 2%) (40).

Viral infections, including herpes simplex virus (HSV), enteroviruses, and parechoviruses, are also implicated in early-onset neonatal sepsis and must be clinically differentiated from bacterial sepsis (42–50). There are other viruses associated with congenital infections, such as rubella virus, cytomegalovirus, lymphocytic choriomeningitis virus, and human immunodeficiency virus, for example. Additional seasonal viruses, including influenza virus, respiratory syncytial virus (RSV), adenoviruses, rhinoviruses, and rotaviruses, have been identified in hospitalized neonates, related primarily to horizontal transmission (41). However, these pathogens are not typically associated with an EOS presentation.

Fungal pathogens are rarely associated with early-onset neonatal sepsis, and *Candida* spp. are most likely, occurring primarily among VLBW infants (9). *Candida* infections may also present as congenital candidiasis that can occur in term or preterm infants, with symptoms occurring at birth or within the first 24 h of life (51, 52).

Group B Streptococcus

The U.S. incidence of EOS overall from 2005 to 2008 was 0.76 to 0.77 cases/1,000 live births (12). Recent population-based surveillance studies in the United States revealed GBS as the etiologic agent of EOS in 38 to 43% of all bacterial sepsis cases, with the incidence of neonatal GBS sepsis estimated to be 0.29 to 0.41/1,000 live births. The majority of these GBS EOS cases, 73%, were in term neonates (11, 12).

GBS (*Streptococcus agalactiae*) is a facultative Gram-positive diplococcus with virulence factors that include its polysaccharide capsule, capsular sialic acid residues, lipoteichoic acid, and deacylated glycerol teichoic acids (38, 53). In culture, GBS exhibits gray/white, flat, mucoid colonies of 3 to 4 mm in diameter on sheep blood agar with a narrow zone of beta-hemolysis. Ten type-specific polysaccharide capsular types have been described. Identification as Lancefield group B requires the use of group-specific antiserum targeting antigen and is most frequently performed in the clinical laboratory by using simple latex agglutination assays.

In pregnancy, GBS is harbored asymptomatically in mucous membrane sites, including the genital, rectal, and pharyngeal mucosa. Global colonization rates reveal significant regional variations in colonization prevalence. In the United States, rates of maternal colonization are estimated to be ~26% (54). Risk factors for maternal GBS colonization include African American race, maternal age of <20 years, low parity, and diabetes (55). Maternal GBS colonization results in infant colonization in approximately 50% of cases, and infants become colonized either intrapartum or through bacterial translocation despite intact membranes. An es-

timated 85% of EOS cases are now averted by intrapartum antibiotic prophylaxis, but the frequent use of antibiotics in the delivery setting may be driving higher proportions of neonatal sepsis attributable to ampicillin-resistant *E. coli* over time (56).

Escherichia coli

E. coli is the second leading cause of EOS in neonates, accounting for about 24% of all EOS episodes, with 81% of cases occurring in preterm infants (57). When VLBW infants are considered alone, *E. coli* is the most frequent cause of EOS, accounting for 33.4% of episodes in a large, multicenter study (3). This shift toward greater survival of VLBW infants may also be a factor accounting for the increasing proportion of EOS caused by *E. coli* observed in recent studies, and evidence is less clear as to whether the incidence is truly increasing (6, 9, 33). The recent population-based incidence in NICUs in the United States is reported to be 0.28/1,000 live births (11). The incidence of sepsis caused by Gram-negative organisms may be increasing in part due to the frequency of maternal antibiotic prophylaxis for GBS. Coliforms, including *E. coli*, are frequently colonizers of the maternal vaginal canal, and infants acquire them at or just before delivery. EOS secondary to *E. coli* often presents with bacteremia with or without meningitis at the time of delivery. Septic shock with clinical features associated with endotoxemia may be present.

While the antigenic structure of *E. coli* is diverse and complex, some virulence factors have been specifically identified as being important in neonatal sepsis. The K1 capsular antigen present in some strains is closely linked to neonatal meningitis and is the best-described virulence factor. It is a polysialic acid that impairs opsonophagocytic killing (58) and is immunochemically indistinct from the capsular antigen of serogroup B *Neisseria meningitidis*. Infants infected with K1 antigenic strains have increased morbidity and mortality compared to infants infected with other strains (59), and disease severity is related to the amount and persistence of K1 antigen in the cerebrospinal fluid. Other virulence factors linked to neonatal sepsis include complement resistance mediated by O-lipopolysaccharide and a group of surface proteins which aid in binding and invasion of brain endothelium (including OmpA, IbeA to IbeC, and CNF1) (60).

Listeria monocytogenes

Listeria monocytogenes is an intracellular pathogen that targets primarily the monocyte-macrophage cellular lineage. *Listeria* accounts for 5% of EOS in premature neonates; however, the overall incidence is low, at 2 to 13/100,000 live births, in the United States and Europe (61, 62). The organism is a Gram-positive bacillus which has the ability to survive environmentally in soil and is typically acquired in the diet through contamination of meats, poultry, dairy products, and fresh produce. Its ability to survive cold-temperature storage is an important biological advantage. *Listeria* infection during pregnancy can lead to fetal loss, early- or late-onset sepsis, and meningitis. Maternal findings can include chorioamnionitis with placental abscesses and preterm labor and delivery. Up to 70% of listerial infections of neonates are in infants delivered at <35 weeks of gestation (63).

The pathogenesis of human listeriosis is incompletely defined, but the gastrointestinal tract is the likely usual portal of entry as a result of food contamination. Gut translocation occurs quickly, and the organism is subsequently transported to the liver. Maternal listeriosis may be transmitted transplacentally to the fetus.

Fetal infection may also occur in relation to swallowing of contaminated amniotic fluid, based on the histopathological findings of the heaviest burden of infection in fetal lung and gut tissues. Premature infants are predisposed to invasive infection with *Listeria* due to the inherently diminished cell-mediated immune responses of premature infants, associated with decreased gamma interferon (IFN- γ) and interleukin-12 (IL-12) production; immature chemotaxis, phagocytosis, and killing macrophages; and decreased numbers and function of NK cells (64).

Neonatal listeriosis can present as EOS and may be associated with signs of maternal illness (50%). Maternal signs/symptoms may range from vague and mild malaise or myalgias to frank systemic illness with fever and chills and accompanying bacteremia. Most cases of EOS secondary to *Listeria* are clinically apparent in neonates at delivery; typical features include apnea, respiratory distress, cyanosis, and meconium-stained amniotic fluid (which can occur at any gestational age when *Listeria* infection is present). Pneumonia is also a common feature, and less frequently, a granulomatous skin rash, termed granulomatosis infantiseptica, is present, consisting of pinpoint pustules on an erythematous base. The rash is a feature consistent with severe infection, and biopsy reveals a leukocytic infiltrate with Gram-positive rods visible under a light microscope.

Gram-Negative Rods

Gram-negative rods, apart from *E. coli*, are less frequent causes of EOS but remain very important causes of LOS and are of increasing importance related to growing antimicrobial resistance concerns. Among the *Enterobacteriaceae*, *Enterobacter* spp., *Klebsiella* spp., and *Serratia* spp. are important causes of sepsis and possess polysaccharide capsules which contribute to their virulence by preventing opsonization, phagocytosis, and bacterial lysis. *Citrobacter* spp. and *Cronobacter sakasakii* account for <5% of bacterial sepsis cases in VLBW infants but are important due to their association with meningitis with brain abscesses and subsequent significant neurological sequelae (65).

Coagulase-Negative Staphylococci and *Staphylococcus aureus*

Both *S. aureus*, including community-associated methicillin-resistant *S. aureus*, and coagulase-negative staphylococci (CONS) are more frequent causes of late-onset, nosocomial sepsis in the neonatal period, especially in VLBW infants. However, there are reports of early-onset or maternal-fetal infections with *S. aureus*, including a case series from a single center describing seven premature infants with congenital *S. aureus* infection with both blood and amniotic fluid cultures being positive. Invasive procedures antenatally (amniocentesis or amnioinfusion) performed within a day of delivery were risk factors in 3 of these 7 cases (66). The species of CONS most commonly associated with neonatal sepsis in preterm infants is *Staphylococcus epidermidis*, which accounts for 60 to 93% of CONS bloodstream infections (67).

Fungal Infections

Early-onset fungal sepsis is an infrequent cause of neonatal sepsis, and risk factors include maternal fungal colonization and vaginal route of delivery. In the NICU setting, fungal infections, most commonly involving *Candida* spp., are more frequently associated with late-onset sepsis, with an incidence inversely proportional to the estimated gestational age (EGA) and birth weight.

The incidence of *Candida* species EOS in NICUs has been reported to be 1.4% (68).

Congenital candidiasis can occur uncommonly either as a skin infection presenting with pustules, small abscesses, and an erythematous maculopapular rash, which may desquamate, or as invasive disease, occurring more often in preterm neonates with immature skin barriers (70). It results from heavy exposure to maternal vaginal *Candida* colonization during delivery or from intrauterine infection. Predisposing risk factors include prolonged rupture of membranes and intrauterine foreign bodies, including intrauterine contraceptive devices and cervical cerclage (69, 70). Severe congenital candidiasis in a term infant should prompt diagnostic consideration of immunodeficiency. Placental histopathology in cases of congenital candidiasis reveals evidence of pseudohyphae, microabscesses, and granulomas (71).

Herpes Simplex Virus

Herpes simplex viruses can also cause sepsis in neonates, although presentation as EOS is uncommon. The estimated U.S. incidences of neonatal HSV have been reported to be between 12 and 60/100,000 live births in retrospective cohort and administrative claims database reviews and 30.8/100,000 live births in one prospective cohort study (72–76). Approximately 5% of neonatal HSV cases are acquired *in utero*, 85% are acquired peripartum, and 10% are acquired postnatally (77). HSV in neonates can present in one of three distinct forms (42): skin, eye, and mouth (SEM) involvement (45% of cases); meningoencephalitis (central nervous system [CNS]) (30% of cases); or disseminated (25% of cases) (77). Prenatally acquired infection is estimated to occur in 1/300,000 deliveries (43) and presents with cutaneous scarring or rash; hyperpigmentation or hypopigmentation; CNS abnormalities, including microcephaly, intracranial calcifications, and/or encephalomalacia; and ocular anomalies, including chorioretinitis, optic atrophy, or microphthalmia (42, 44, 45). Infants with SEM disease are diagnosed based on characteristic vesicular skin lesions that demonstrate HSV in viral culture, direct fluorescent antibody staining, and/or PCR. All infants being evaluated for neonatal HSV require lumbar puncture (LP) and cerebrospinal fluid (CSF) examination, with HSV-PCR of the CSF being the most reliable way to diagnose meningoencephalitis. Magnetic resonance imaging (MRI) is very sensitive in showing CNS abnormalities, with either temporal or multifocal areas of hyperintensity and hemorrhage being seen in the deep gray matters of more than one-half of patients, but it may be normal early in the course of the disease (78–80). Electroencephalogram (EEG) is also typically abnormal, with focal epileptiform discharges (50%), burst suppression (25%), focal electrographic seizures (25%), focal suppression (25%), and diffuse slowing (25%) (78). Disseminated infection is the disease form most likely to have an EOS presentation and characteristically involves multiple organ systems, with pneumonitis and hepatitis occurring most frequently. Importantly, neonates with disseminated HSV infection may not present with or develop skin vesicles, which could result in delayed diagnosis. Treatment of neonatal HSV infections with acyclovir intravenously is successful in reducing morbidity and mortality (46, 47, 77).

Enteroviruses and Parechoviruses

The enteroviruses and parechoviruses are RNA viruses representing 2 genera within the family *Picornaviridae*. They have under-

gone taxonomic reorganization since their original categorization in 1957 and are presently classified into 5 enterovirus groups: polioviruses and human enteroviruses (HEV-A, HEV-B, HEV-C, and HEV-D). Genomic and proteomic evaluations initially led to the determination that 2 viruses formerly classified as echoviruses 22 and 23 were sufficiently distinct as to be reassigned as human parechovirus 1 (HPeV1) and HPeV2 (48, 81), and currently, a total of 16 HPeVs have been described by molecular characterization (<http://www.picornaviridae.com/>).

Neonatal infection with enteroviruses is not rare and has been reported to occur in 12.8% of infants <29 days old in a single-center, prospective, U.S.-based study evaluating stool shedding during a typical enteroviral season (82). In that study, the majority (79%) of infants with fecal shedding of enteroviruses were asymptomatic. However, the high prevalence was associated with a high readmission rate (19%) for suspected sepsis among these neonates within the first month of life. Another prospective study of neonates (≤ 29 days of age) presenting with suspected serious systemic infections found that 3% of the episodes were enterovirus infections, which was equivalent to the 3% of infants in the series who were also diagnosed with a microbiologically confirmed cause of bacterial sepsis (83). Fewer data exist describing the epidemiology of the recently defined HPeVs. A single-center, U.S. retrospective cohort identified HPeV3 by PCR in 7% (58/780) of CSF specimens that were negative for bacterial pathogens in culture and by routine enteroviral PCR (which does not identify HPeVs), with infections occurring at a mean age of 6.6 ± 4.4 weeks (84). Additional cohort studies suggest that of neonates (<29 days of age) presenting with symptomatic enteroviral illnesses, about 20% will present with severe sepsis-like syndrome (85, 86), which is the disease form most likely requiring differentiation from bacterial EOS.

Enteroviruses and HPeV are transmitted via fecal-oral and possible oral-oral (respiratory) routes in the community, and evidence supports transmission to neonates before, during, or after delivery. Neonatal infection may occur antenatally through maternal viremia and transplacental spread to the fetus; intrapartum by exposure to maternal blood, secretions, and/or stool; or postnatally from close contact with infected caregivers (85, 87–91). Health care-associated infection via contaminated hands of personnel and fomite transmission has been well documented (49, 50, 91, 92). Enteroviral and parechoviral EOS are more likely to be acquired vertically, as evidenced by cases of neonates with clinical illness presenting on the first day of life; cultures positive for enterovirus identified from amniotic fluid, umbilical cord blood, and neonatal organs; and detection of neutralizing IgM antibodies found in neonatal serum by the first day of life (89, 93–97). While the clinical presentation of enteroviruses and HPeVs during LOS is more frequently aseptic meningitis (95, 98, 99), EOS with these viruses typically presents as sepsis with fever, irritability, poor feeding, and sometimes rash, a presentation that is indistinct from that of bacterial sepsis (98). The pathogenesis of postnatally acquired neonatal infections begins with virus entry at the oral or respiratory tract, replication in the pharynx and lower alimentary tract, and direct extension to the reticuloendothelial tissues, with subsequent viremia. Once viremia occurs, this leads to dissemination of the virus throughout the tissues and infection of multiple organs, with CNS, myocardium, liver, pancreas, adrenal glands, lungs, skin, and mucous membranes potentially being involved (100).

CLINICAL PRESENTATION OF EOS

Clinical signs and symptoms of sepsis in newborns vary by gestational age and severity of infection. Rarely will infants present with fever unless they are born to a febrile mother and have fever immediately after delivery. It is much more common for a septic infant to be hypothermic upon presentation. This systemic sign is one of many nonspecific markers of sepsis. General symptoms include lethargy, hypothermia, and poor feeding, and nonspecific signs may include anuria and acidosis. As pneumonia is often the presenting infection, respiratory symptoms are common and may include apnea, tachypnea, grunting, nasal flaring, and intercostal retractions. Cardiac symptoms may include cyanosis, desaturation, bradycardia, poor perfusion, reduced capillary refill, and hypotension. It is important to realize that subtle changes in respiratory status of newborns, temperature instability, or feeding problems can be the first signs of a life-threatening infection.

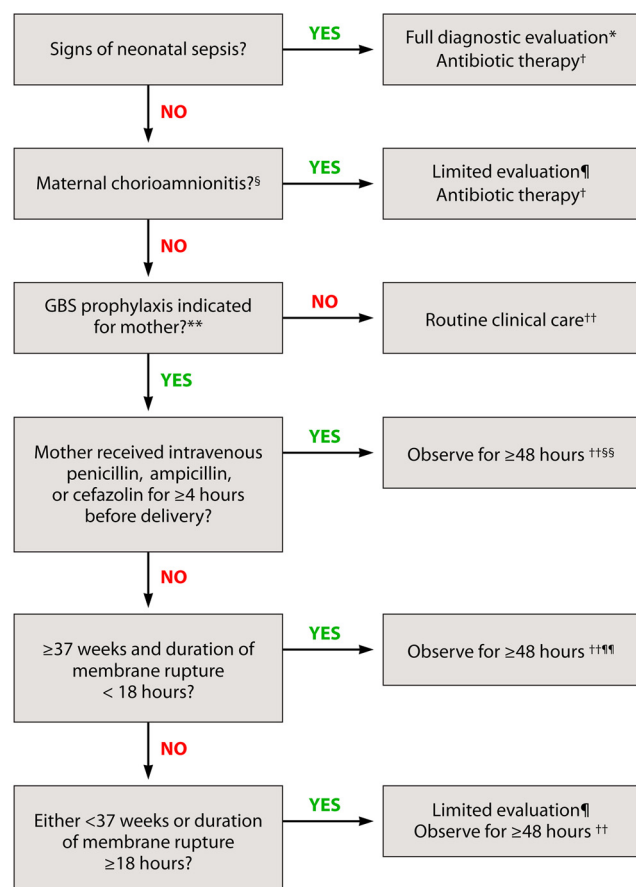
Preterm Infants

Preterm infants will often have apnea, bradycardia, and cyanosis (104/158; 65.8%) as the first sign of infection (101). Additionally, Lim et al. reported a high incidence of “poor activity,” presumably lethargy (77/158; 48.7%) and increased respiratory effort (68/158; 43.0%) (101). In general, symptoms are more severe with Gram-negative and fungal infections than with Gram-positive infections.

Term Infants

Signs of EOS in term infants typically present by the first 6 h, and the majority present usually within the first 24 h of life. Most infants will present with respiratory distress, which can masquerade as other diagnoses such as congenital heart disease, respiratory distress syndrome (RDS), pneumothorax, transitory tachypnea of newborns, congenital diaphragmatic hernia, and other congenital masses in the chest. Many of these can be detected or eliminated easily with chest radiographs and arterial blood gasses. Sepsis should be the initial differential diagnosis for each of these. In mildly symptomatic newborns, it is acceptable to monitor the newborn for 6 h before performing a complete blood count (CBC) and starting antibiotics. If the infant clinically improves, sepsis is very unlikely; if symptoms progress, blood culture and LP with CSF culture and studies should be obtained prior to initiation of antibiotics, and antibiotics should be started promptly.

Most cases (80% to 90%) of EOS will present in the first 24 to 48 h of life (27). When evaluating a newborn for suspected sepsis/meningitis, a thorough review of antenatal risk factors should be performed, as this may help guide therapy and is information that is necessary according to current Centers for Disease Control and Prevention (CDC) guidelines for GBS treatment (Fig. 1) (102). Such factors include documentation of maternal colonization status with GBS, gestational age of the infant, prolonged rupture of membranes, intra-amniotic infection, younger maternal age, black race, and previous delivery of an infant with invasive GBS diseases (15, 55, 103–107). Frequent evaluation of the newborn is critical in order to recognize the signs and symptoms of disease during the neonatal period, which can range from nonspecific to multiorgan failure. The presence of rash, seizures, meningoencephalitis, and hepatic or myocardial dysfunction should lead to suspicion of a viral infection, including HSV, enterovirus, and HPeV. Unfortunately, with the exception of myocarditis, which is most frequently associated with enteroviral infections, distinguishing between these viruses upon the presence of the other



* Full diagnostic evaluation includes a blood culture, a complete blood count (CBC) including white blood cell differential and platelet counts, chest radiograph (if respiratory abnormalities are present), and lumbar puncture (if patient is stable enough to tolerate procedure and sepsis is suspected).

† Antibiotic therapy should be directed toward the most common causes of neonatal sepsis, including intravenous ampicillin for GBS and coverage for other organisms (including *Escherichia coli* and other gram-negative pathogens) and should take into account local antibiotic resistance patterns.

§ Consultation with obstetric providers is important to determine the level of clinical suspicion for chorioamnionitis. Chorioamnionitis is diagnosed clinically and some of the signs are nonspecific.

¶ Limited evaluation includes blood culture (at birth) and CBC with differential and platelets (at birth and/or at 6–12 hours of life).

‡‡ If signs of sepsis develop, a full diagnostic evaluation should be conducted and antibiotic therapy initiated.

§§ If ≥37 weeks' gestation, observation may occur at home after 24 hours if other discharge criteria have been met, access to medical care is readily available, and a person who is able to comply fully with instructions for home observation will be present. If any of these conditions is not met, the infant should be observed in the hospital for at least 48 hours and until discharge criteria are achieved.

¶¶ Some experts recommend a CBC with differential and platelets at age 6–12 hours.

FIG 1 Centers for Disease Control and Prevention (CDC) algorithm for secondary prevention of early-onset group B streptococcus (GBS) disease among newborns. (Adapted from reference 102.)

clinical symptoms or signs alone is not possible (108). White matter injury can be frequently visualized with cranial ultrasonography or MRI in the form of increased echogenicity in the periventricular white matter of neonates with encephalitis due to HPeV, similar to lesions seen, albeit less frequently, with enteroviral encephalitis (98, 109). In contrast, neonatal HSV-2 encephalitis tends to be either diffuse, involving white and gray matters of the brain, or limited to the temporal lobes, brainstem, or cerebellum, and there is associated hemorrhage in more than one-half of patients on MRI or computed tomography (CT) scanning images (80). Parechoviral infections also seem to have a high preponderance of gastrointestinal illnesses associated with their presentation

(108, 110). Neonatal enteroviral illnesses tend to occur in the summer and fall seasons and are frequently (~60%) associated with recent maternal illness and the absence of other perinatal problems (81%) (86).

LABORATORY FINDINGS AND DIAGNOSTICS

The typical complete sepsis workup in a neonate consists of obtaining a complete white blood cell count with differential, a single blood culture, urine cultures, and a lumbar puncture for cell count and culture (28, 111). In addition, there may be a role for culture and Gram staining of tracheal aspirates in intubated neonates shortly after birth. Acute-phase reactants, such as C-reactive protein (CRP) and procalcitonin (PCT), along with hematologic scoring systems are increasingly being used to assist in the diagnosis of infants with suspected sepsis. The need for a chest radiograph is usually determined by the presence of respiratory symptoms. Patients suspected of having a viral etiology for their sepsis syndrome will typically need either immunologic response marker (e.g., changes in specific antibody levels) or specific viral studies to definitely determine the presence of virus. Special considerations in the diagnosis of viral sepsis are included below.

Blood Testing for Neonatal Sepsis

White blood count and differential. White blood cell (WBC) counts; differential, absolute neutrophil counts; and the ratio of immature to total neutrophils in the blood are widely used as screening tests for neonatal sepsis. Unfortunately, none of these tests have been particularly useful in identifying the majority of septic infants. Normal neutrophil values are age dependent, with a peak during the first 12 to 14 h of age (range, 7,800 cells/mm³ to 14,500 cells/mm³) (112). During 72 h to 240 h, the values range from 2,700 cells/mm³ (5th percentile) to 13,000 cells/mm³ (95th percentile) in full-term infants (113). Total white blood cells counts have a poor positive predictive value (PPV) for sepsis (114, 115). Neutropenia has greater specificity for neonatal sepsis, but the definition of neutropenia is dependent on gestational age, delivery method, and altitude (112, 116–119). Absolute immature neutrophil counts peak at 12 h of age, from a maximum value of 1,100 cells/mm³ to 1,500 cells/mm³ at 12 h (112). In contrast, a maximum normal ratio of immature to total white blood cells (I:T ratio) of 0.16 occurs at birth and reaches a nadir of 0.12 with increasing postnatal age. A single value of the I:T ratio (>0.3) has a very high negative predictive value (NPV) (99%) but a very poor positive predictive value (25%) for neonatal sepsis (116–118). In a study of 1,539 neonates, Murphy and Weiner found that a combination of 2 serial normal I:T ratios and a negative blood culture at 24 h in a neonate shortly after birth was accurate in ruling out neonatal sepsis (120). Typically, neonates with viral infections, including HSV, enteroviruses, and HPeV, have normal WBC counts or very mild leukopenia (108, 110).

Platelet counts. Platelet counts are not very sensitive or specific for the diagnosis of neonatal sepsis and not very helpful in monitoring the response to therapy (119, 121).

Blood cultures. All neonates suspected of having sepsis should have a blood sample sent for cultures. The volume of blood needed for cultures for neonates is substantially lower than that needed for adults because neonates tend to have a 1-log-higher concentration of bacteria in their bloodstream than adults. As a result, 0.5 ml was traditionally considered the standard volume of blood adequate to detect bacteremia in neonates. However, some

recent studies have shown that up to one-quarter of all neonates with sepsis have bacteremia involving low colony counts (≤ 4 CFU/ml), and two-thirds of those < 2 months old have colony counts of < 10 CFU/ml (122–124). A 0.5-ml volume of blood has been shown to be insufficient to detect most infants with these levels of bacteremia, while 1.0 ml doubles the likelihood of a positive yield (125). For these reasons, several experts now recommend that 1.0 ml of blood should be the minimum volume to be inserted into a single pediatric blood culture bottle (27). The blood is most frequently drawn from a peripheral vein, but samples obtained from an umbilical artery catheter shortly after insertion are also acceptable (126). Blood drawn from the umbilical vein has a much greater risk of being contaminated unless obtained during delivery from a carefully cleaned segment of a doubly clamped cord (127, 128).

Acute-phase reactants. CRP and procalcitonin are the two most commonly studied acute-phase reactants in neonatal sepsis. CRP levels rise within 6 to 8 h of infection and peak at 24 h (129, 130). Inflammation triggers the release of IL-6, which stimulates an increase in CRP concentrations. Depending on the study, individual CRP values of 0.2 to 95 mg/liter (mean, 1.7 mg/liter; median, 10 mg/liter) have a sensitivity range of 41 to 96% and a specificity range of 72 to 100% for neonatal sepsis (131). A value of 10 mg/liter is the most commonly used cutoff in most published studies. Viral infections are not usually associated with an elevated CRP level, and if the CRP level is elevated, it is usually < 5 mg/liter (132, 133). CRP has its best predictive value if measured within 24 to 48 h of onset of infection. An increasing CRP level is a better predictor than individual values. Two normal CRP determinations (8 to 24 h after birth and 24 h later) have been shown to have a negative predictive value of 99.7% and a negative likelihood ratio of 0.15 for proven neonatal sepsis (133). Thus, repeatedly normal CRP values are strong evidence against bacterial sepsis and can enable antibiotics to be safely discontinued.

Procalcitonin is a propeptide of calcitonin produced mainly by monocytes and hepatocytes that is significantly elevated during infections in neonates, children, and adults (134). The half-life is about 24 h in peripheral blood. The normal level for neonates > 72 h of age is usually < 0.1 ng/ml (135). While procalcitonin has been used primarily in research settings, it is increasingly being used as a guide in managing infections in real time by clinical laboratories (http://www.nebraskamed.com/app_files/pdf/careers/education-programs/asp/procalcitonin-guidance.pdf) and generally takes about 90 min to 2 h to process (136). In general, procalcitonin is more sensitive for earlier detection of sepsis than is CRP. The procalcitonin level is more likely to be elevated during bacterial infections than during viral ones (131) and declines rapidly with appropriate therapy. However, a physiologic increase in the procalcitonin concentration occurs within the first 24 h of birth, and elevated levels in serum can occur under noninfectious conditions (e.g., infants with respiratory distress syndrome, hemodynamic instability, and diabetic mothers). Procalcitonin is also useful for detecting neonatal nosocomial sepsis (137). The probability of nosocomial sepsis is doubled with a PCT of > 0.5 ng/ml for VLBW infants ($< 1,501$ g).

Other biomarkers. Cytokines, including interleukin 6 (IL-6), interleukin 8 (IL-8), gamma interferon (IFN- γ), and tumor necrosis factor alpha (TNF- α), and cell surface antigens, including soluble intercellular adhesion molecule (sICAM) and CD64, have

also all been studied as measures for neonatal sepsis, but none are currently in routine clinical use.

They all generally have very similar sensitivities and specificities. Using a cutoff of 30 pg/ml, IL-6 has a mean sensitivity of 78% (median, 80%) and a mean specificity of 79% (median, 78%) for detection of neonatal sepsis (138). IL-6 has a characteristic early appearance during sepsis, and based on its short half-life, it may be appropriate for monitoring the appropriateness of therapy. Indeed, IL-6 levels peak up to 48 h prior to the onset of clinical sepsis (138). Age-specific levels should be taken into consideration in determining the significance of levels of IL-6 measured. The kinetics of IL-6 during the first 48 h of life in healthy infants are different in preterm infants compared with kinetics in term neonates, suggesting a gestational age-dependent effect on IL-6 values over the first 48 h of life (139).

IL-8 has a slightly lower mean sensitivity of 73% (median, 80%) using a cutoff value of 70 pg/ml and a mean specificity of 81% (median, 82%) (131). TNF- α has a mean sensitivity of 79% (median, 80%) and a median specificity of 93% using a median cutoff value of 7.5 pg/ml. Similarly, a median cutoff value of sICAM of 275 pg/ml yields a mean sensitivity of 79% (median, 80%) and a somewhat lower mean specificity of 76% (median, 76%). CD64 has been measured by using different units, and on average, studies yielded a mean sensitivity of 82% (median, 92%) and a mean specificity of 83% (median, 88%) (131). Based on a systematic review of the global literature, Meem et al. have classified biomarkers for detection neonatal sepsis into three categories: early phase (IL-6, IL-8, CD64, sICAM, TNF- α , and IFN- γ), mid-phase (PCT), and late phase (CRP). IFN- γ levels are particularly responsive early in detection of viral infections (131).

The sequential appearance and disappearance of these biomarkers during sepsis may enable them to be packaged into a multiplex kit to detect neonatal infections irrespective of the stage. A study by Celik et al. (140) showed that the combination of IL-6 and CRP was superior to the use of each one individually for the early detection of neonatal sepsis. Similarly, Abdollahi et al. (141) showed that the combination of IL-6, CRP, and PCT was highly predictive for the diagnosis of early-onset sepsis. While the costs of each of these biomarkers are significant in relation to the cost of antibiotics, their potential utility in reducing hospitalization makes their use more attractive.

Molecular testing. Molecular methods for detection of neonatal sepsis in blood include PCR- and DNA microarray-based methods. Most of these tests hold the promise of rapid detection directly from blood without prior culture combined with high sensitivity and specificity in relation to cultures. Of these tests, PCR-based methods have been the most studied for neonates (142).

PCR techniques are increasingly being used for the diagnosis of neonatal sepsis in research and some clinical laboratories. They have a high sensitivity in relation to culture when positive organisms identified are considered the gold standard as a result of detection of bacterial DNA, and pathogens can be detected at much lower concentrations (143). Furthermore, there is promise of faster diagnosis (as quickly as 30 min) and quicker time to begin appropriate targeted therapy with the use of real-time PCR that utilizes the detection of fluorescent signals generated during each amplification cycle and is able to give some measure of bacterial load. The challenge with this approach is that individual PCR methods fail to detect most causes of neonatal infections. As a result, several investigators and laboratories have described mul-

tiplex PCR in which the DNAs of several potential neonatal bacterial and fungal pathogens are amplified in parallel (144, 145). This approach holds much promise but is currently still limited primarily to research laboratories due to the relatively high cost and the detection limit of the organisms targeted in the kit. Furthermore, false-negative results may still occur if the etiologic organism is not included in the kit. In addition, the use of sterile venipuncture to collect the specimen may prove tricky for some neonates. Specimens collected by heel prick are subject to easy contamination by skin organisms. Finally, individual kits fail to detect the presence of antimicrobial resistance, unless such markers are built into the PCR assay.

Increasingly, the use of broad-range-based PCR amplification methods to detect conserved 16S rRNA or 23S rRNA has been reported to distinguish neonatal sepsis from other conditions that may mimic it, including respiratory distress syndrome. These tests rely on the fact that while all bacteria have these 16S or 23S rRNA genes, different bacterial species possess different numbers of copies, tied to their rate of growth. Following amplification, the amplicons are identified by various methods, including hybridization with specific probes, capillary sequencing analysis, or pyrosequencing.

Real-time 16S rRNA gene PCR using the highly conserved RW01 and DG74 primers followed by pyrosequencing of the 380-bp amplicon generated was compared to blood culture in a large study involving 1,233 neonates. Compared to culture, 16S rRNA gene PCR yielded high specificity (97.5%) and negative predictive values (99.2%) but low sensitivity (up to 60%) (146, 147). Contamination during the use of heel prick may lead to lower specificity and should be avoided.

Reier-Nilsen et al., comparing a broad-range 16S rRNA gene PCR with conventional blood cultures in 48 neonates with suspected sepsis, showed similar results for PCR, with 66.7% sensitivity and 87.5% specificity but with positive and negative predictive values of 95.4% and 75%, respectively (148). However, whereas only one patient with a positive blood culture had a negative PCR result, six patients with positive PCR results had negative blood cultures. Five of these six patients were diagnosed with clinical sepsis, suggesting that the blood cultures were falsely negative. Receipt of maternal intrapartum antibiotic prophylaxis, inadequate volume of blood drawn, or low-grade bacteremia below the level of culture detection may all account for these “false-negative” culture results.

Commercial kits are now available for a multiplex pyrosequencing PCR technique that identifies up to 40 bacterial and fungal pathogens directly from whole blood in several studies summarized by Andrade et al. (149, 150). These tests are not yet readily available or in routine use and are not yet FDA approved. Other laboratories are employing a combination of real-time PCR and 23S rRNA pyrosequencing (149, 150). The use of any form of real-time PCR for neonatal sepsis diagnosis is complicated by the need to collect specimens using sterile techniques via venipuncture. There is a greater risk of contamination with capillary heel prick specimens. Furthermore, competition with human DNA in the blood may lead to a lower sensitivity.

The region between the 16S and 23S sequences, known as the internal transcribed spacer (ITS), is also being studied as a region useful for identifying microbes. Because the 16S-23S ITS contains more variable regions and polymorphic sites than the 16S sequences, there appears to be better discrimination of distinct bacterial species, which can be achieved by amplifying and sequenc-

ing this region (145, 151), but published clinical studies in neonates are lacking.

DNA microarrays, in which DNA probes specific to selected microbial targets are spotted onto glass or silicon slides in a known order, are also being studied for diagnosis of neonatal and other types of sepsis. Fragments of the target DNA are labeled with a reporter molecule and then hybridized to the array to form duplexes. Detection of the duplexes formed is achieved with specific probes by measurement of fluorescent signals on advanced platforms (152–154). In a study by Shang et al., blood samples from 172 neonates with suspected clinical sepsis were evaluated by using PCR targeting the 16S rRNA gene followed by DNA microarray hybridization (153). Compared to blood cultures, the microarray approach was considered to be 100% sensitive and 97.9% specific. Microarrays have the potential advantage of the added ability to detect antimicrobial resistance and/or virulence genes in addition to identification of the specific sepsis pathogen and may shorten the time to diagnosis.

Urine Testing

Neonates with suspected sepsis in the first few days of life (<72 h) do not need urine obtained for chemical and microscopic analysis because most infections of the urinary tract in this population are secondary to hematogenous seeding of the kidney by bacteremia (155–157). However, subsequent workups for sepsis should include careful consideration of a urinalysis and urine culture, especially in symptomatic neonates. Only specimens obtained by suprapubic aspiration or urethral catheterization are appropriate for urine cultures due to the risk of bacterial contamination. Catheter-obtained urine cultures have a sensitivity of ~95% and a specificity of ~99% compared to suprapubic tap specimens when >1,000 CFU/ml of bacteria of a single colony are identified (158–160). In contrast, bag urine specimens have a sensitivity of 100% but low specificity (14% to 84%) (161). Urine analysis may be helpful in providing adjunctive information to support or rule out the diagnosis of urinary tract infection (UTI). Both reagent “dipstick” tests and microscopic examination of the urine specimen for leukocytes and bacteria are routinely used. The dipstick reagent tests related to possible UTI are leukocyte esterase (LE), blood, nitrite, and protein tests. The LE sensitivity ranges from 67% to 94% depending on the preexisting likelihood of UTI, and the specificity ranges from 63% to 92% (162, 163). The nitrite test has a high specificity (90% to 100%) but a low sensitivity (16% to 82%). It is thus a good test to suggest the presence of a UTI but not to rule one out. The dipstick blood and protein tests have very poor sensitivity (25% to 64%) and specificity (60% to 89%) for UTI and have minimal roles in diagnosing UTI. Detection of the presence of leukocytes under a microscope has a sensitivity of 32 to 100% and a specificity of 45 to 98%, while the bacteria seen usually correlate with a variable sensitivity of 16 to 99% and a specificity of 45% to 98%. Having positive results by more than one test usually raises the sensitivity substantially, but the specificity margins remain wide.

Cerebrospinal Fluid Testing

While lumbar puncture (LP) is an important means of obtaining cerebrospinal fluid (CSF) to rule out the presence of meningitis in infants with suspected sepsis, its routine use in neonates is controversial (27). The risk of concomitant meningitis in high-risk neonates

who appear healthy or those whose clinical signs appear to be due to noninfectious conditions such as RDS is very low (111, 164).

Up to 23% of neonates with bacteremia will also have concomitant meningitis (165). For this reason, there should be a very low threshold for obtaining CSF through LP in neonates who have a strong clinical picture suggestive of neonatal sepsis or who end up with a positive blood culture and who have not previously had an LP. Furthermore, up to 38% of those with meningitis will have a negative blood culture; hence, lumbar puncture should be a component of every neonatal sepsis evaluation and not just performed if cultures return positive (27, 166). It should be noted that an LP done in the setting of previous receipt of antibiotics by the neonate could lead to falsely negative CSF cultures. Lumbar puncture would also aid in ruling out neonatal herpesvirus infection or enteroviral or parechovirus meningitis or meningoencephalitis. Conditions that may lead to a delay or cancellation of lumbar puncture include severely ill infants with either cardiovascular or respiratory distress, tense or bulging anterior fontanelle (for which a CT scan or MRI may be indicated to rule out significantly raised intracranial pressure prior to LP), the presence of severe thrombocytopenia, or infection around the lumbosacral region (167–170).

CSF PARAMETERS IN SUSPECTED NEONATAL MENINGITIS

In most studies, the normal white blood cell count in healthy, uninfected preterm or term neonates is <10 cells/mm³ (171–176), with $>95\%$ having counts of <20 cells/mm³. However, the levels are age dependent (177), with the highest values during the first week. Neonates with proven bacterial meningitis born after 34 weeks of gestation typically have a higher median white cell count of over 400 cells/mm³, while those born prior to 34 weeks of gestation have a much lower elevation of their median cell count of ~ 110 cells/mm³ (178). Higher cell counts are usually associated with Gram-negative versus Gram-positive meningitis (179). While the red blood cell (RBC) level considered to represent a “traumatic” tap in CSF for patients with suspected meningitis has ranged from >500 cells/mm³ to $>1,000$ cells/mm³ (180, 181), there has been no proven diagnostic benefit in adjusting the CSF WBC count for the number of red blood cells (182). Furthermore, the number of immature white cells, such as bands in the CSF, is not predictive of meningitis. While bacterial meningitis is most commonly associated with CSF pleocytosis with a polymorphonuclear predominance, viral meningitis is more commonly lymphocytic in nature (90). However, polymorphonuclear predominance has been reported in up to one-third of all neonates with enteroviral meningitis (85, 183). A delay in analysis of collected CSF specimens by 2 to 4 h is associated with drops in measured white cell counts of between 23% and 39% (184). Normal protein levels in uninfected term infants are usually less than 100 mg/dl, while preterm infants have higher levels (reported upper limits of 150 to 290 mg/dl) that decline with increased gestational age (171–174, 176, 185–189). These levels are usually, but not always, elevated above normal levels in neonates with meningitis. The normal CSF glucose level in neonates is between 70 and 80% of the serum level. This level usually drops significantly with bacterial meningitis but, as in the case with protein levels, may sometimes remain normal, even in infants with significant bacterial colony counts (166, 178).

Unlike the enteroviruses, where most cases have a CSF pleocytosis, in parechovirus aseptic meningitis, the CSF cell count and protein concentration are normal in most cases (99).

Diagnosis of Sepsis Due to Viruses

The most common viruses to consider in the differential diagnosis of neonatal sepsis are human enteroviruses, HPeVs, and neonatal HSV. Human enteroviruses and HPeVs are frequent causes of clinical neonatal sepsis syndrome. Due to their replication in the gastrointestinal tract, stool samples are the traditional sources of isolation for these viruses (190–193). Viral culture of stool specimens, nasal and throat swabs, and cerebrospinal fluid and bronchoalveolar lavage specimens are considered the gold standard for diagnosis, but they typically take several days to weeks to yield positive results and are labor-intensive. As a result, investigators have more recently developed real-time PCR-based techniques to identify these viruses in stool, blood, and other body fluids, including cerebrospinal fluid. Different investigators have demonstrated the importance of these viruses in neonatal clinical sepsis and that the yield of the virus was very similar in blood specimens compared to stool specimens in neonates with clinical sepsis (190–193).

Neonatal herpes simplex virus can present in up to 25% of cases as disseminated disease, with signs and symptoms that are indistinguishable from those of neonatal sepsis syndrome (194, 195). Fever, vesicular rash, and abnormal CSF findings, especially with seizures, are important diagnostic clues. Presentation is typically between the first and second weeks of life but can occur sooner or later. In these patients, the bacterial cultures will be negative, and typically, there is evidence of significant hepatic dysfunction. While the presence of a rash (especially vesicular) should always lead to the consideration of neonatal herpes in differential diagnoses, a significant minority will not have a rash, and the systemic signs may precede skin lesions (196). HSV easily grows in cell culture media and leads to cytopathic effects 1 to 3 days following inoculation. Following the cytopathic effect, HSV infection can be confirmed by a variety of methods, including fluorescent antibody staining, enzyme immunoassay (EIA), or monolayer culture with typing (197). For suspected neonatal HSV, swab specimens should be obtained from the mouth, mucous membranes (nasopharynx, conjunctivae, and anal introitus), and skin, especially if vesicular lesions are present, and transported in viral transport media for cultures. Some experts note that the same swab may be used for all the specimens, with the anus sampled last, and transported in a single viral transport container (198). It may be prudent, though, to avoid using the same swab applied to an obviously infected site to swab another site, to avoid the risk of secondary inoculation. PCR to detect viral DNA is increasingly being used for the diagnosis of neonatal HSV, particularly when there is CNS involvement, for which it is the diagnostic method of choice (sensitivity of 75% to 100% and specificity of 71% to 100%) (199–201), and should always be used in suspected cases of neonatal HSV. It is also useful for diagnosing HSV from whole-blood specimens and fluid from vesicular skin lesions (202, 203). It is best to wait at least 12 to 24 h after birth to obtain surface samples in neonates without obvious skin disease to ensure that there is no maternal contamination during the intrapartum period. Direct fluorescent antibody and EIA staining of scrapings from vesicles can also be used for rapid diagnosis and typing of HSV but are slightly less sensitive than culture (198). Serology is not of significant clinical value for the diagnosis of neonatal HSV due to the possibility of transplacentally acquired maternal IgG, which reflects previous maternal infection (201). The IgM response is poor in neonatal HSV, and cross-reactivity with other

IgM assays is common. Furthermore, negative serology may occur in the setting of first-episode maternal primary infection if the infection is early on.

Previously, HPeV1 and HPeV2 could be identified only by neutralization assays following virus isolation in cell culture, using standardized antiserum pools (204). However, this method has been hampered by the poor growth of parechoviruses in culture, the fact that most laboratories do not routinely use the Vero cell line that provides the most optimal growth, and the lack of broad availability of antigenic typing reagents for HPeV3 to -6. Diagnosis of HPeVs is now routinely possible with the use of reverse transcriptase PCR (RT-PCR) on blood, CSF, stool, or nasopharynx (98, 205–208). Enteroviral and HPeV RT-PCR methods that can detect the majority of members of both genera target the conserved 5′ untranslated region (UTR) or nontranslated region of the enteroviral and HPeV genomes, respectively (209, 210). Investigators are also reporting newer assays that can simultaneously detect enteroviral and HPeV RNAs in clinical samples by using one- or two-step real-time RT-PCR. While several commercial kits are now available for detection of enteroviral and HPeV infections (211, 212), there are no FDA-approved assays yet for HPeV detection.

Wolthers et al. (99) reported that addition of a HPeV-specific PCR to CSF tests conducted on 761 children <5 years of age (46% of whom were neonates) led to a 31% increase in detection of a viral cause of neonatal sepsis or central nervous system symptoms, suggesting that they are underdiagnosed. Of these 761 children, 108 had enteroviral infections, while 31 had HPeV infections. While that study identified HPeV as the second most common cause of viral sepsis and meningitis in young children, those authors did not specify if this was the case when only neonates were considered. Nonetheless, that study adds to the impetus for rapid identification of HPeV by PCR in order to shorten the duration of both antibiotic use and hospital stay for neonates (99).

TREATMENT

Antimicrobials used to treat sepsis in neonates usually include beta-lactams such as ampicillin, oxacillin, and cefotaxime; extended-spectrum beta-lactams such as piperacillin-tazobactam; and the carbapenem meropenem. These are bactericidal agents that inhibit the synthesis of the peptidoglycan layer of the bacterial cell wall (213). Additional antimicrobial classes of agents used for treatment of neonatal sepsis include the glycopeptide vancomycin and aminoglycosides. Through a time-dependent killing process, vancomycin is bactericidal for Gram-positive organisms by inhibiting key peptide subunits from being incorporated into the peptidoglycan bacterial cell wall layer, thus inhibiting cell wall synthesis (214). Aminoglycosides bind to the bacterial 30S ribosomal subunit, thus inhibiting protein synthesis. They exert a concentration-dependent killing effect that could be bacteriostatic or bactericidal. Both glycopeptide and aminoglycoside drugs require therapeutic-drug monitoring to achieve the correct dose and limit toxicity, mainly ototoxicity and nephrotoxicity (213, 214).

As noted previously, neonatal sepsis due to HSV types 1 and 2 is treated with acyclovir, a nucleoside analogue that acts as an inhibitor of herpesvirus DNA polymerase (201). Management of neonatal fungal infections includes the use of polyenes such as amphotericin B and liposomal amphotericin B, azoles such as fluconazole, and the echinocandins, including caspofungin and micafungin. The polyenes bind to the fungal cell membrane compo-

nent ergosterol, thus forming a transmembrane channel that causes leakage of key ions, leading to fungal cell death. Fluconazole inhibits the fungal cytochrome P450 enzyme, thus preventing the conversion of lanosterol to ergosterol, which is essential to the fungal cell membrane; it is primarily a fungistatic agent. Echinocandins act on the fungal cell wall by noncompetitive inhibition of the enzyme $\beta(1,3)$ -D-glucan synthase and can be both fungistatic and fungicidal (215).

CDC/AAP Guidelines on GBS Management

Given that GBS continues to be a leading cause of early-onset neonatal sepsis and meningitis in the United States, when assessing a child for full/limited evaluation and for treatment and observation, the CDC has developed an algorithm to help guide practitioners who are evaluating neonates with sepsis (Fig. 1) (102).

Empirical Antibiotic Therapy

The appropriate empirical antibiotic selection during early-onset neonatal sepsis is based on the likely etiologic pathogens based on epidemiologic surveillance. Once blood and, in most clinical scenarios, CSF and/or urine samples are promptly obtained, the combination of ampicillin and gentamicin is still the most appropriate coverage for the most common organisms, GBS and *E. coli*, which still predominate as etiologic pathogens in this age group (11). There has, however, been an increased prevalence of community extended-spectrum beta-lactamase (ESBL) producers as etiologic agents of neonatal sepsis (216, 217). ESBLs, found mostly in nosocomial *E. coli* and *Klebsiella pneumoniae* infections, are enzymes that confer resistance to beta-lactam antibiotics, including penicillins, cephalosporins, and the monobactam aztreonam (218, 219). In addition, most ESBL producers demonstrate resistance to aminoglycosides as well (219). An increasing prevalence in community-acquired ESBLs has also been observed across the globe, including Europe, Asia, and South America, and there are reports of neonatal sepsis secondary to community-acquired ESBL producers in India (216, 219–222). A recent report showed an increase in the prevalence of ESBL producers in the United States, with 36% of all *E. coli* infections in that study being caused by community-acquired ESBL producers (223). There is, however, a paucity of neonatal community-acquired ESBL epidemiologic data in the United States in the setting of EOS. For now, based on available reports, most neonatal infections secondary to *E. coli* in the United States are community acquired and remain gentamicin susceptible (27, 56). The ongoing emergence of ESBL-producing organisms in the community calls for vigilance in monitoring local patterns of susceptibility to gentamicin, as this may eventually render this aminoglycoside less useful in the setting of empirical therapy for EOS. Ampicillin and gentamicin have demonstrated a synergistic effect in laboratory and animal models of *L. monocytogenes* (224). If there is concern of meningitis and while awaiting final cultures and susceptibilities, cefotaxime may be added as an agent empirically. The highly protein-bound agent ceftriaxone is not recommended for neonates with concerns of meningitis due to the risk of acute bilirubin encephalopathy from displacement of free bilirubin by the drug (225). It has also been rarely associated with biliary pseudolithiasis, nephrolithiasis, and pulmonary impairment due to precipitation with calcium ions in neonates with both elevated and normal serum calcium levels (226–230).

Alternative Empirical Therapy

An alternative initial empirical treatment that has been proposed is a combination of ampicillin and cefotaxime (27). However, there is evidence that in early neonatal sepsis, this combination leads to more resistant Gram-negative organisms being isolated in neonatal intensive care units (NICUs), and there may be an increase in serious complications such as necrotizing enterocolitis (NEC) and death (27). Moreover, some studies have noted an increase in the prevalence of invasive candidiasis in NICUs where cefotaxime is used extensively as an initial empirical antimicrobial in early neonatal sepsis (27). Therefore, unless there are epidemiological concerns or concerns based on susceptibility of the organism isolated in cultures, the recommended empirical treatment for neonatal sepsis remains ampicillin and gentamicin.

Antibiotic Resistance in an Era of Intrapartum Prophylaxis

While maternal GBS intrapartum prophylaxis has decreased early-onset GBS sepsis, the use of intrapartum antibiotics has affected the prevalence of other neonatal pathogens such as Gram-negative organisms, particularly *E. coli* (11). Although prophylaxis has overall been beneficial, concerns have been raised regarding increasing Gram-negative resistance to ampicillin and gentamicin. In a 12-year review of neonatal infections before standardized intrapartum prophylaxis, the use of ampicillin and gentamicin for neonatal sepsis in an area with antimicrobial stewardship failed to show an increase in resistance of Gram-negative organisms to ampicillin and gentamicin (231). Although some studies have shown an increase in *E. coli* resistance in cases of early neonatal sepsis where mothers received intrapartum prophylaxis, other reviews have shown that in the community in general, there is an increase in ampicillin and gentamicin resistance in Gram-negative organisms without a history of intrapartum antibiotics, prompting the theory that more than just intrapartum prophylaxis may be at play (56). Thus, currently in the United States, although there is increased ampicillin and gentamicin *E. coli* resistance, this combination of antibiotics is still appropriate for empirical coverage of early-onset neonatal sepsis (56).

Pathogen-Directed Therapy

Group B streptococcus. In an era of intrapartum prophylaxis and widespread antimicrobial usage in the NICU, GBS remains essentially susceptible to penicillin. In 2008, researchers from Japan described GBS isolates with reduced susceptibility to penicillin and then subsequently reported a cluster of multidrug-resistant, penicillin-resistant GBS isolates (232). The mechanism of resistance is thought to be due to mutations in amino acids of the penicillin binding protein (232). In a study of 99 GBS isolates with elevated MICs of one or more beta-lactam antibiotics, 22 isolates seemed to carry mutations that conferred MICs of beta-lactams that were well above baseline levels. These isolates, however, were still considered to be penicillin, ampicillin, and cefotaxime sensitive (233). If treatment with ampicillin and gentamicin is started empirically, gentamicin may be discontinued once cultures confirm GBS infection, and treatment may be completed with ampicillin alone. Uncomplicated bacteremia is treated for 10 days, while uncomplicated GBS meningitis is usually treated for 14 days; however, some experts recommend a minimum of 21 days, especially for severely ill neonates (234). With GBS meningitis, some experts advocate for performing a repeat LP at 24 to 48 h into treatment to document clearance, as it may have therapeutic

(duration of treatment) and prognostic value (234). In cases of prolonged bacteremia or a clinically complicated course, some experts advocate for a longer course of therapy. Septic arthritis and osteomyelitis may require 3 to 4 weeks of treatment, and endocarditis and ventriculitis may need at least 4 weeks or more (234). While therapy may be completed with penicillin G alone, continuing ampicillin and gentamicin therapy for synergy until documentation of clearance of bacteremia and CNS infection prior to narrowing to penicillin G may be prudent. In this setting, consultation with a pediatric infectious disease specialist may help guide clinicians on optimal management (235).

***Escherichia coli* and other Gram-negative bacilli.** Uncomplicated bacteremia due to ampicillin-susceptible *E. coli* should be treated for 14 days from the first negative culture, while meningitis should be treated for a minimum of 21 days (231). In the United States, while there is increased ampicillin and gentamicin resistance in *E. coli* isolates, this combination of antibiotics is still appropriate for empirical coverage of early-onset neonatal sepsis (56). *E. coli* resistance to ampicillin is mediated primarily by β -lactamases. In these cases, cefotaxime may be used. Gentamicin is usually continued until final susceptibilities are obtained. In the case of bacteremia with susceptible strains, monotherapy with ampicillin or cefotaxime is appropriate. In cases of meningitis, the aminoglycoside may be continued until CSF is sterile (120) or for the first 7 to 14 days of a 21-day meningitis treatment (236). Complications of meningitis such as ventriculitis, subdural effusions, or brain abscess warrant a longer treatment duration (234). With the increase in the prevalence of community-acquired ESBL-producing *E. coli* infections, penicillins, cephalosporins, and aminoglycosides would become less useful empirical therapeutic options. In these ESBL-producing *E. coli* infections, meropenem has been used successfully in neonates (216, 217, 222, 237–239). For other Gram-negative organisms, the treatment duration is similar to that for *E. coli*, but the greater incidence of some complications of meningeal infections, such as brain abscess associated with *Citrobacter*, *Enterobacter*, and *Serratia* spp., may necessitate a longer treatment duration (240–242).

Listeria monocytogenes. The combination of ampicillin and gentamicin is the optimal therapy for *Listeria monocytogenes* (243). Cephalosporins are inactive against *Listeria*, and many treatment failures with vancomycin have been reported (243). Uncomplicated bacteremia should be treated for 10 to 14 days (243). If the infection is mild, it can be completed with ampicillin alone once the patient has improved. For invasive infections associated with meningitis, most experts recommend 14 to 21 days of treatment (243).

***Staphylococcus aureus* and coagulase-negative staphylococci.** When Gram-positive organisms other than GBS are suspected based on the clinical pattern, vancomycin should be started empirically until susceptibility is known. If the organism identified is methicillin-susceptible *S. aureus* (MSSA), treatment should be narrowed to nafcillin or oxacillin due to their better bactericidal activity (244). As a result of the enhanced MSSA bactericidal activity, some experts recommend the use of vancomycin and nafcillin combination therapy until susceptibility results become available (244). Coagulase-negative staphylococcal and methicillin-resistant *S. aureus* (MRSA) infections usually require treatment with vancomycin. Some studies have shown that linezolid, an oxazolidinone that inhibits protein synthesis, can be an effective and well-tolerated alternative to vancomycin in the treatment

of resistant Gram-positive infections in neonates (245), including reports of cases of successful use with CNS infections (246–248).

Candida spp. Amphotericin B deoxycholate (1 mg/kg of body weight/dose every 24 h [q24h] i.v.) is the empirical treatment of choice for neonatal candidiasis and is usually well tolerated in neonates compared to older children (249, 250). Liposomal amphotericin B (5 mg/kg/dose q24h i.v.) can also be used, especially if a fungal urinary infection has been excluded, as it covers the urinary tract poorly (249, 250), and amphotericin B deoxycholate should be used in cases of urinary tract infections (251). If the organism isolated is *Candida albicans*, fluconazole (6 to 12 mg/kg/dose q24h i.v./orally [p.o.]) is an effective alternative treatment; however, if the neonate had been receiving fluconazole prophylaxis, a different class of antifungal would be more appropriate due to potential resistance (27). Echinocandins such as caspofungin (25 mg/m² or approximately 2 mg/kg i.v. per dose q24h) have been used effectively in neonatal fungal infections, especially for tissue, bone, and hepatosplenic infections; however, if there are concerns of CNS infection, amphotericin or fluconazole achieves better CNS penetration (249, 250, 252). Of note, standard deoxycholate amphotericin B and fluconazole remain the preferred treatments of choice over liposomal amphotericin formulations for invasive candidiasis. A recent multicenter retrospective review demonstrated increased mortality when the liposomal formulation of amphotericin was compared to these two agents (253).

Herpes simplex virus. In neonates with HSV infection, parenteral acyclovir at a dose of 60 mg/kg/day intravenously in 3 divided doses is the treatment of choice regardless of clinical manifestations and findings (201). The treatment duration is 14 days in skin eye mucous membrane disease, and a minimum of 21 days should be used in cases of CNS disease or disseminated disease (201). In cases of CNS disease, a repeat HSV PCR should be done on the CSF, and treatment may need to be prolonged if the result is still positive at 21 days (201). Oral acyclovir suppressive therapy at 300 mg/m²/dose, administered 3 times daily for 6 months following treatment of neonatal HSV disease, improves neurodevelopmental outcomes for infants with CNS disease (254).

Therapeutic-Drug Monitoring

Aminoglycosides exert a concentration-dependent killing effect and have nephrotoxicity and ototoxicity, for which therapeutic-drug monitoring is essential (255). Neonates should have peak and trough values obtained at around the third dose, and if there are concerns about renal damage, levels may be determined earlier to help tailor dosing frequency based on renal function. Vancomycin has toxicity similar to that of aminoglycosides but exerts a time-dependent killing. Therefore, therapeutic monitoring should be done with the third dose to ensure that trough levels are adequate (10 to 15 µg/ml for bacteremia and 15 to 20 µg/ml for CNS and bone infections and endocarditis) (214).

Alternative/Adjunctive Therapy

In a 9-country randomized controlled clinical trial, 3,493 infants with suspected or proven sepsis from 113 hospitals were randomized to receive antibiotics with placebo versus antibiotics with immunoglobulin. Intravenous immunoglobulin (IVIG) therapy in this setting did not demonstrate any improvement of patient outcome versus antimicrobial therapy alone (256). Recombinant granulocyte colony-stimulating factor (rG-CSF) administered to neonates with neutropenia and sepsis showed no differences in

severity of illness, morbidity, or mortality compared to placebo (257). So far, no adjunctive therapy to antibiotics has been proven beneficial in the management of neonatal sepsis (258).

Duration of Treatment/Response to Therapy

Positive cultures. In general, in most cases of neonatal sepsis, infants respond to treatment clinically in the first 24 to 48 h of effective treatment (27). Within 72 h, the white blood cell (WBC) count usually trends toward normal, the I:T ratio improves, and the level of C-reactive protein (CRP) also tends to normalize during that time (259). At 72 h, repeat blood, CSF, and urine cultures are usually negative when sampling is indicated (260). The treatment duration for culture-proven sepsis varies from at least 10 to 14 days based on the organism, and when there is meningitis, the duration may be 21 days or more (27). Pathogen-specific duration of therapy is addressed above. Antimicrobial courses may be prolonged if there are any complications such as brain abscesses, osteomyelitis, or endocarditis. In such cases, a pediatric infectious disease consultation is strongly recommended to help guide management.

Negative cultures. In many cases, an etiologic agent may not be identified in cultures, yet the neonate has a concerning clinical picture. Cultures may remain negative in the setting of maternal antimicrobial treatment prior to delivery. In these cases, continuation of empirical antimicrobial therapy with ongoing monitoring of the infant is warranted. Often, an empirical 10-day course of antimicrobial therapy is completed (27). Serial WBC and CRP measurements may help evaluate clinical severity and/or response to empirical treatment (261). In general, both the WBC count and CRP levels should decline with treatment. They may, however, have limited therapeutic implications, as they may not be reliable in critically ill patients or may not fall within normal ranges in an otherwise improved child with negative cultures (261). The choice of antimicrobial regimen should be based initially on epidemiologic empirical coverage and on clinical response. When cultures that are drawn prior to initiation of antimicrobial therapy remain negative and the neonate is otherwise well appearing, discontinuation of antimicrobial therapy at 48 h is recommended, as studies have shown that prolonged empirical therapy may increase adverse outcomes (27). Strong suspicion should be given to the possibility of viral infections, such as HSV, in the setting of a severely ill neonate with negative cultures. In cases of neonatal sepsis where no cultures were obtained or where blood but no CSF was obtained prior to empirical antibiotic treatment being started and where the clinical picture is concerning, the duration of therapy should be individualized, and a pediatric infectious disease consultation is recommended.

Follow-up testing. Repeat blood cultures should be obtained, usually within 24 h of presumed effective therapy, to document clearance, as persistent positive cultures could mean failure of antimicrobial therapy or evidence of intravascular site infection, and antibiotic coverage and duration may need to be adjusted (260). Follow-up testing may include monitoring the trends in the WBC counts, CRP levels, and I:T ratios to assess the response to therapy (260). Circumstances in which obtaining repeat CSF samples is warranted include neonatal HSV CNS infection, where HSV PCR of CSF should be repeated at the end of therapy, with therapy being prolonged if the specimen remains positive (198). HSV in the CNS is frequently detected in the first 24 h of illness by PCR. However, in the case of an initially negative HSV PCR of the

CSF and persistent clinical or imaging findings consistent with HSV infection, a repeat CSF sample should be sent, since there have been cases of negative results early in the course of the illness (262, 263). Some experts advocate performing a repeat LP at 24 to 48 h into treatment for GBS meningitis to document clearance for therapeutic and prognostic value (234). In cases of *E. coli* meningitis, some experts recommend repeating a LP to document CSF sterility and make therapeutic adjustments, such as discontinuing aminoglycoside treatment (236). If there is persistent fever, increasing elevations of peripheral WBC counts and CRP levels, and abnormal I:T ratios in a neonate with CNS infection, repeat CSF cell count and culture should be done, and head imaging should be obtained to rule out abscesses (236), especially with certain pathogens, such as *Citrobacter*, *Enterobacter*, and *Serratia* spp. (240–242). These organisms may ultimately be ESBL producers, even when cephalosporin MICs are in the susceptible range (264). In this instance, broadening of coverage to a carbapenem may be indicated (264). Repeated positive cultures must also lead to a careful search for additional foci of infections, such as osteomyelitis or endocarditis, which will lead to longer therapy (260).

MORTALITY

Mortality rates have been stable during the last decade, a time period during which screening-based GBS maternal intrapartum prophylaxis became widespread (265). GBS remains the leading cause of EOS, while *E. coli* is responsible for the majority of deaths among all patient populations (Table 1) (12). Taking into account the differences in incidence rates by gestational age and race, decreasing health care disparities for both general care and pregnancy outcomes in the black population may be critical components in decreasing rates of early-onset sepsis.

Preterm Mortality

Mortality risk from early-onset sepsis increases with increasing degree of prematurity and associated morbidities. Very-low-birth-weight (VLBW) infants are at the greatest risk of infection because of compromised immunity. Neither the innate or adaptive immune systems are functioning at optimal levels in the perinatal period, significantly increasing the preterm infant's risk of developing invasive disease (266). Infants with early-onset sepsis may be septic due to their prematurity, or they may be born premature secondary to their infection and/or maternal intra-amniotic infection. VLBW infants are more likely to have sepsis from Gram-negative organisms and *E. coli* rather than GBS or other Gram-positive organisms (101). In VLBW infants, up to 20% of mortality is related to sepsis. Even with adjustment for gestational age, sex, and comorbidities, the relative risk of death is 3 times greater for VLBW infants with sepsis, and surviving VLBW infants are at risk for prolonged hospital stays with long-term morbidity, including bronchopulmonary dysplasia (BPD) and neurodevelopmental impairment (9, 267–269).

Preterm infants with EOS were shown in one study to have a much higher mortality rate than preterm infants with LOS, 40% versus 5% ($P < 0.01$), with *E. coli* causing the highest mortality rates (101). Infants dying of EOS caused by Gram-negative organisms had the highest rates of death in the first 72 h (270). Other series examining all causes of EOS in the preterm population have reported mortality rates of 26 to 37% (9, 271). The risk of mortality from EOS in premature infants may not occur in the first week of life, and diagnosis of EOS is associated with higher all-cause

mortality in the first 120 days (266). These figures highlight the large and disproportionate burden of mortality, both early and late, in the preterm infant population and the very serious nature of early-onset infection for this population.

Term Mortality

While mortality rates are significantly lower in the term infant population than in preterm infants, EOS still plays an important role in neonatal mortality. Term infants are at higher risk of infection if they have comorbidities such as impaired immune function, meconium aspiration, galactosemia, and underlying cardiac or pulmonary abnormalities (57).

Black term infants are a select population noted to be at higher risk of EOS than nonblack infants. Black women have higher rates of colonization rates by GBS, black infants have a higher incidence of EOS, and these infants have a similar case fatality ratio (1.6 to 1.7%) compared to nonblack infants, placing a disproportionate burden of mortality on this population (12). This health disparity, similar to others, has not been associated with factors such as poor prenatal care, socioeconomic status, or maternal age and remains an area for continued research and improvement.

In the term infant population, the organism contributing most to mortality is *E. coli*, despite fewer total infections than GBS. In the term population, there were no deaths from GBS in a study from 2005 to 2008 capturing over 850,000 live births (12).

Viral Mortality

The case fatality rate for disseminated HSV has ranged from 85% prior to the era of antiviral treatment to 57% initially with treatment by vidarabine (272, 273) and subsequently to 29% in the current era of high-dose acyclovir (60 mg/kg/day for 21 days) (46). Lethargy, severe hepatitis, and delayed initiation of treatment have also been associated with greater mortality (175). The mortality rate of CNS HSV infections in neonates is 4% (77).

For enteroviruses, a wide range of mortality, ranging from 0% (majority) to 42%, has been reported, depending on the gestational age of the neonate (prematurity is a risk factor), the serotype of enterovirus involved, the onset of symptoms within the first week of life, and the absence of specific maternal antibodies to the infecting neonatal serotype (274). Coxsackievirus B4 has the highest observed mortality rate, at 40%, while coxsackievirus B5 seems to have a very low associated mortality rate (87). Echovirus 11 has also been associated with a high case fatality rate. The presence of symptoms and signs associated with EOS in general is associated with worse outcomes (8, 275). For example, higher WBC counts (WBC count of $>15,000$ cells/mm³), lower hemoglobin levels (<10.7 g/dl), and earlier onset (<7 days) have been associated with hepatic necrosis with coagulopathy, which has a high mortality rate of 24% to 31% (8, 275). Myocarditis and hepatitis are two complications with poor prognoses (8, 85, 183, 276). Mortality and long-term sequelae in patients with enteroviral infections are most often related to myocarditis (108). Mortality from parechovirus in the neonatal period is not well described.

MORBIDITY

Preterm

In preterm infants, there can be significant acute physiologic decompensation associated with EOS. Hypotension requiring pressor support; respiratory distress or suppression requiring intuba-

tion or noninvasive ventilation; and hyper- and hypoglycemia, thrombocytopenia, and disseminated intravascular coagulation (DIC) requiring medical management and blood product transfusion are all frequently associated with sepsis. Treatment of EOS with broad-spectrum antibiotics can also predispose to candidal infections, including invasive sepsis and meningitis, and localized disease such as diaper dermatitis and oral thrush. There is no definitive evidence that preterm infants are at subsequent risk for development of LOS after EOS. Lin et al. reported no association between EOS and the development of LOS (odds ratio [OR], 0.92 [95% confidence interval {CI}, 0.74, 1.16]) or NEC (odds ratio, 0.89 [95% CI, 0.70, 1.12]) (266). However, Leviton et al. reported that infants <28 weeks of gestational age or younger have an increased risk of LOS (odds ratio, 2.2 [95% CI, 1.4, 3.3]) with a history of EOS (277).

Long-Term Morbidities in Preterm Infants

Perinatal infection and bronchopulmonary dysplasia. EOS and exposure to intrauterine inflammation from chorioamnionitis are associated with an increased risk of development of bronchopulmonary dysplasia (BPD) in preterm infants. BPD, a chronic lung disease of prematurity, is diagnosed by oxygen requirement at a corrected gestational age (CGA) of 36 weeks in infants born weighing <1,500 g and is associated with poor long-term developmental outcomes, prolonged initial hospitalization, increased readmission during the first year of life, increased wheezing and asthma later in life, and increased cost of care during the first year of life (278).

Several proinflammatory cytokines and macrophages have been isolated from the amniotic fluid of affected pregnancies and from the endotracheal secretions of infected infants after delivery. This exposure is associated with moderate protection against respiratory distress syndrome (RDS) in the immediate postpartum period. Later in hospitalization, there are significantly higher rates of BPD in infants who are exposed to early inflammation secondary to EOS. Watterberg et al. reported that RDS rates for preterm infants exposed to chorioamnionitis were 33%, compared to 82% RDS rates in infants without exposure (279). That same group noted that chorioamnionitis-exposed infants were diagnosed with BPD 63% of the time, compared to their unexposed counterparts, who had BPD rates of 21%. Multiple inflammatory markers were associated with the clinical pattern of lower rates of RDS and higher rates of BPD, including tracheal IL-1 β , LTB₄, thromboxane B₂, and prostaglandin E₂ (279). This exposure to inflammatory mediators during early stages of development provides favorable conditions for apoptosis leading to abnormal or decreased alveolarization, as demonstrated by Kramer et al. in a fetal lamb model (280). Another study evaluating fetal lamb surfactant protein B maturation after fetal endotoxin injection showed a direct correlation with maturation and increasing levels of the proinflammatory cytokines endotoxin and IL-1 (281). In a review of these interactions, Adams-Chapman (282) implicated the potential of cytokine-mediated injury to progress from pulmonary inflammation to BPD. This result is not universal and is likely dependent on bacterial factors and individual host responses as well as the timing of the injury during development.

Perinatal infection and brain injury. Multiple studies have associated perinatal infection and inflammation with brain injury, including periventricular leukomalacia (PVL), neurodevelopmental delays, and cerebral palsy. These associations are very often confounded by multiple factors, including the degree of prematurity and other comorbidities; however, after regression analysis,

strong associations between clinical and histological chorioamnionitis, funisitis, and EOS persist. Polin eloquently delineated the multiple pathways that may lead to neuronal brain injury from early exposure to inflammatory mediators or different strains of bacteria (283). Problematic is the lack of a consistent inflammatory response or injury pattern to similar exposures in the preterm population, indicating that host factors, including immune system function, gestational age, and timing of the exposure, among others, lead to variable outcomes across populations. EOS has been reported to double the risk of multiple negative outcomes in work performed by Klinger et al. Incidences of BPD, PVL, intraventricular hemorrhage (IVH), retinopathy of prematurity (ROP), and death were all increased in infants with EOS (282, 284). In one of the longest-term follow-up studies of VLBW infants looking at cognitive function across a series of domains, septicemia (both EOS and LOS) could not be associated with decreased function in any domain at the age of 16 years (269). Although that study was underpowered to comment on EOS separately, this information provides one longitudinal perspective.

While evaluating the association between EOS and brain injury, another group noted significant associations between levels of interleukin-6, interleukin-8, and tumor necrosis factor alpha measured at the time when VLBW infants were evaluated for EOS. Among the study infants, those with proven EOS and NEC who had elevated levels of these cytokines were at high risk for white matter injury (285). This information helps to further define the mechanisms of EOS-induced long-term morbidity in this fragile patient population (Fig. 2) (286).

Term Morbidities

Term infants who are affected by GBS infection have a high complication rate with life-altering significance. Up to 50% of these infants will suffer serious neurologic sequelae, including seizures, blindness, deafness or significant hearing loss, and cognitive delays in speech and language (287). Other very rare complications develop from sepsis-associated endocarditis and thrombosis and can include valvular damage, pulmonary embolism, and secondary infectious thromboembolism.

Viral Morbidity

Treatment with acyclovir has also improved morbidity in survivors of disseminated neonatal HSV infection. While only 50% of neonates had normal development at 12 months of age in the pretreatment era, with current high-dose acyclovir treatment, 83% of survivors of disseminated neonatal disease have normal development at 12 months of age. CNS disease survivors have not had such dramatic improvement in morbidity, with 33% having normal development at 12 months in the pretreatment era, largely unchanged from the 31% developing normally in the treatment era. Morbidity for SEM survivors has improved from 38% with developmental abnormalities at 12 months without therapy to none in the current high-dose acyclovir era (272).

Most neonates with HPeV infection have an unremarkable recovery. However, HPeVs have been associated with CNS infections in neonates and subsequent neurodevelopmental delays (108). Upon evaluation with cranial ultrasound or MRI, white matter abnormalities can often be identified. Poor neurologic outcomes have been associated with these lesions, including flaccid paralysis (288). Neonatal enteroviral sepsis is also generally asso-

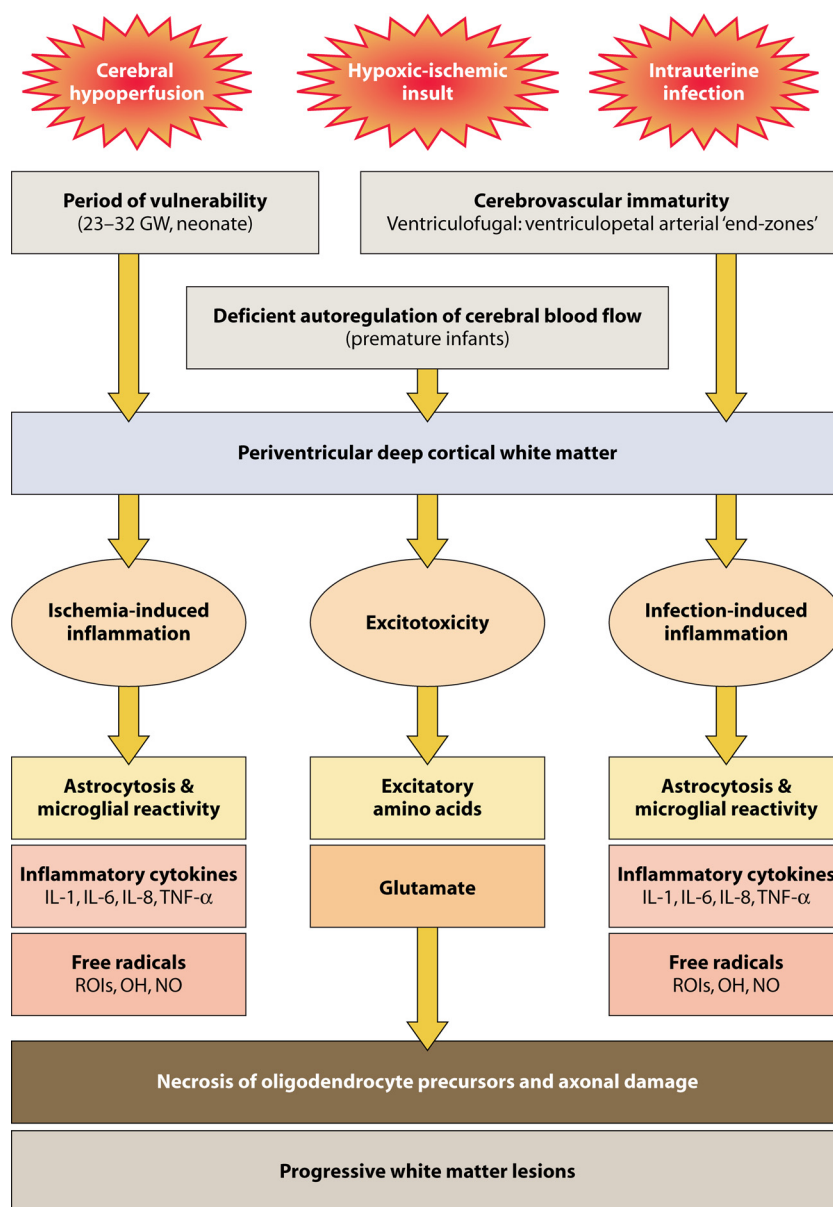


FIG 2 Schematic representation of events associated with the formation of deep cortical white matter lesions in periventricular leukomalacia. GW, gestational weeks. (Adapted from reference 286 with permission of the publisher.)

ciated with a benign recovery. Fever typically lasts for 3 to 5 days, while other symptoms resolve within 4 to 7 days (85, 90, 183).

PREVENTION

GBS prenatal screening and subsequent screening-based intrapartum antibiotics (IPA) have been adopted in the United States based on recommendations from the CDC and American College of Obstetrics and Gynecology (ACOG) (108). This method has replaced risk-based IPA which lowered GBS rates to only 0.66/1,000 live births and has been considerably more effective at decreasing the burden of neonatal invasive GBS disease in the United States (289). Most series reported rates of 0.35 to 0.41/1,000 live births for early-onset GBS disease, a decrease from 1.8/1,000 live births at the end of the last century (11). Despite compliance rates of 85% for prenatal screening and 85% for delivery of IPA, rates of

early-onset neonatal GBS disease have remained stable since 2003 (290). Additional measures are necessary to eliminate transmission to neonates during delivery. Currently, resources are focused on two areas, a rapid testing method effective for guidance of IPAs when the mother presents in labor and a maternal vaccine aimed at the most prevalent serotypes of GBS.

The prenatal screening test has been shown to have poor positive predictive value (PPV) compared to GBS screening done during labor, with the PPV of prenatal screening ranging from 44 to 63% and with a NPV of 91.7%. The PPV increased for those women whose cultures were taken within 1 week of delivery (291). Previously reported large differences in maternal colonization between prenatal screening and onset of labor likely account for the continued disease burden currently seen in the newborn population. Several different methods of rapid screening during labor are

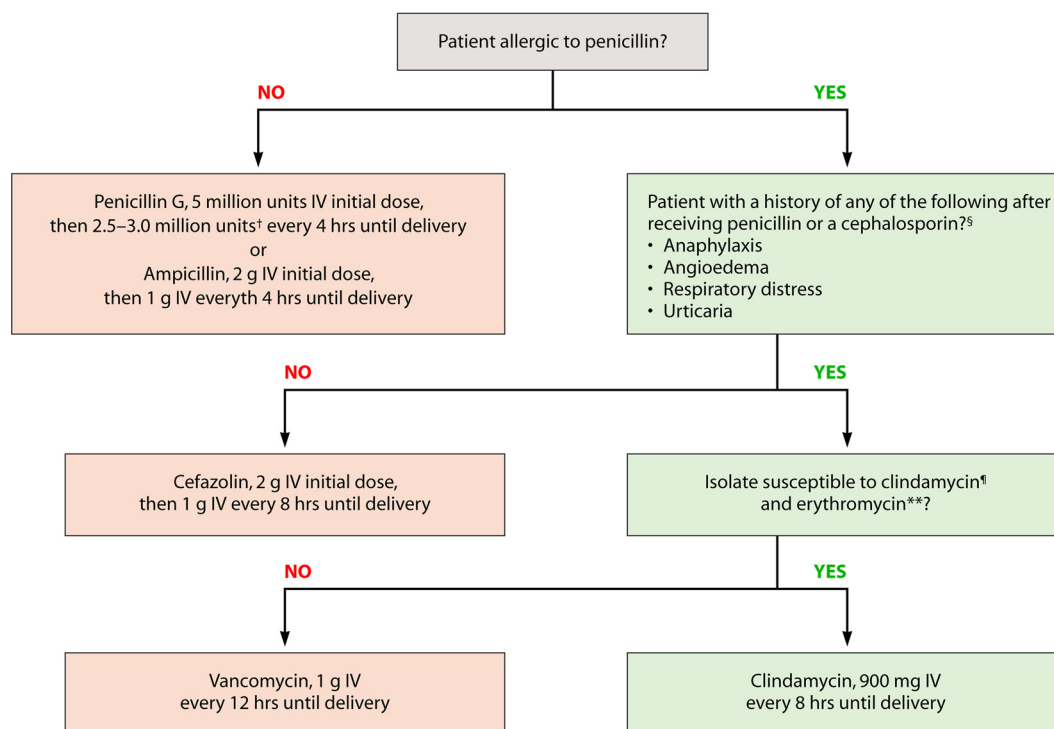


FIG 3 CDC-recommended regimens for intrapartum antibiotic prophylaxis for prevention of early-onset GBS disease. IV, intravenously. †, broader-spectrum agents, including an agent active against GBS, might be necessary for treatment of chorioamnionitis. ‡, doses ranging from 2.5 to 3.0 million units are acceptable for the doses administered every 4 hours following the initial dose. The choice of dose within that range should be guided by which formulations of penicillin G are readily available to reduce the need for pharmacies to specially prepare doses. §, penicillin-allergic patients with a history of anaphylaxis, angioedema, respiratory distress, or urticaria following administration of penicillin or a cephalosporin are considered to be at high risk for anaphylaxis and should not receive penicillin, ampicillin, or cefazolin for GBS intrapartum prophylaxis. For penicillin-allergic patients who do not have a history of those reactions, cefazolin is the preferred agent because pharmacologic data suggest it achieves effective intra-amniotic concentrations. Vancomycin and clindamycin should be reserved for penicillin-allergic women at high risk for anaphylaxis. ¶, if laboratory facilities are adequate, clindamycin and erythromycin susceptibility testing should be performed on prenatal GBS isolates from penicillin-allergic women at high risk for anaphylaxis. If no susceptibility testing is performed or the results are not available at the time of labor, vancomycin is the preferred agent for GBS intrapartum prophylaxis for penicillin-allergic women at high risk for anaphylaxis. **, resistance to erythromycin is often but not always associated with clindamycin resistance. If an isolate is resistant to erythromycin, it might have inducible resistance to clindamycin, even if it appears susceptible to clindamycin. If a GBS isolate is susceptible to clindamycin and resistant to erythromycin and testing for inducible clindamycin resistance has been performed and is negative (no inducible resistance), then clindamycin can be used for GBS intrapartum prophylaxis instead of vancomycin. (Adapted from reference 102.)

being evaluated for efficacy. The majority of work is focused on the development of GBS DNA-based RT-PCR (292–294). The use of this technology is infrequent at this time due to limitations of time in the clinical setting, cost, and availability (102).

Timing of Screening

The timing of prenatal GBS screening may have the largest effect on its PPV. In a series of prenatal and intrapartum cultures obtained by Lin et al., the PPVs were 29% if timing of prenatal culture was unknown and 61% for those with known timing ($P = 0.001$) (295). Current CDC recommendations are for universal prenatal culture at an estimated gestational age (EGA) of between 35 and 37 weeks (102). Women who do not seek prenatal care; women who deliver very shortly after their GBS screen is performed, before results are available; and women who deliver prematurely are not able to take full advantage of the current screening recommendations and should all be treated with intrapartum antibiotics under the current CDC protocol.

Intrapartum Prophylaxis

Chemoprophylaxis with penicillin is currently the recommended therapy for mothers with prenatal GBS-positive cultures or for mothers with unknown GBS status (Fig. 3) (102).

Treatment recommendations for women with significant penicillin allergy currently include vancomycin; however, clinical trends show vancomycin to be underutilized, with an increasing use of clindamycin instead, despite sensitivity patterns demonstrating increasing levels of resistance to this therapy or a lack of sensitivity results (296–298). In another study, only 65.5% of indicated sensitivity testing was performed, and only 26.5% of patients prescribed clindamycin had sensitivity testing completed (299). The exact recommended algorithm for different scenarios of penicillin allergy is detailed in Fig. 3.

Since the initial recommendations for universal screening and treatment for GBS in pregnant women were made in 1996, there have been serious concerns about development of antibiotic resistance and increasing neonatal Gram-negative disease (300). Ecker et al. showed increasing numbers of *Candida* infections in newborn infants ($P = 0.006$), increasing numbers of Gram-negative and *Candida* infections in VLBW infants ($P = 0.009$), and increased resistance to ampicillin in *Escherichia coli* infections ($P = 0.006$) since IPA initiation. Regression analysis also showed increasing resistance to both ampicillin and penicillin, temporally associated with IPA (OR, 2.05) (301). Evaluating reports from the NICHD Neonatal Research Network (NRN) from before and af-

ter IPA adoption, the primary cause of disease in the preterm infant population has changed, from a predominance of GBS in the early 1990s to a majority of Gram-negative organisms in the most recent reports from 2012 (34, 57). Concern has been raised about reports of increasing ampicillin resistance in *E. coli* strains from this high-risk population in recent years (267, 302). In a recent study evaluating positive blood culture rates before and after IPA adoption in a cohort of 716,000 neonates, EOS GBS rates decreased from 3.5 to 2.6/1,000 admissions, EOS *E. coli* rates remained unchanged at 1.4/1,000 admissions, and LOS GBS (0.9 to 1.1/1,000 admissions) and *E. coli* (2.2 to 2.5/1,000 admissions) rates increased in the two evaluated time periods (6). This method of evaluating rates varies from the traditional method of calculating rates/1,000 live births. Looking forward, a GBS vaccine may help to avoid these concerns and cost-effectively eliminate neonatal GBS sepsis.

Update on Current Status of Vaccine Development

Five GBS capsular polysaccharide types, types III, Ia, V, Ib, and II, account for 95% of neonatal disease (316). Neonatal susceptibility to invasive GBS disease is due to low neonatal concentrations of GBS capsular polysaccharide-specific serum antibodies (303, 304). With 10 to 26% of women screening positive for GBS during pregnancy, a vaccine aimed at pregnant women may afford the most effective reduction in disease burden (295). While a multivalent vaccine will cover a large percentage of invasive GBS disease, the vaccine will likely not eradicate all GBS carriage, and all women may not respond to vaccination, leaving a continued but reduced role for IPA. Benefits of vaccination over IPA include lowered risks of development of resistant GBS strains, stopping the surge of EOS *E. coli* and other Gram-negative infections that have increased since the introduction of IPA, and the ability to reach a broad group of women during pregnancy. With recent public backlash against current pediatric vaccine recommendations and many mothers refusing immunization for their newborns, there is cause for concern about universal adoption of a maternal vaccine program (305). As Johri et al. noted, “conducting a double blind, placebo-controlled study of a vaccine in the era of IPA is unlikely, and efficacy will likely need to be based on serological immune response, a less than optimal primary outcome for evaluation of a therapy for a potentially fatal illness” (306). On the other hand, several vaccines have now been demonstrated to be safe and recommended by the Centers for Disease Control and Prevention to be administered during pregnancy either routinely (inactivated influenza virus vaccine and the tetanus, diphtheria, and acellular pertussis vaccine [Tdap] from 27 to 36 weeks) or if indicated (hepatitis A virus vaccine, hepatitis B virus vaccine, either the polysaccharide or conjugate meningococcal vaccines, and the polysaccharide pneumococcal vaccine) (see http://www.cdc.gov/vaccines/pubs/downloads/f_preg_chart.pdf and <http://www.cdc.gov/vaccines/vpd-vac/pertussis/tdap-pregnancy-hcp.htm#tdap>).

Initial research on a GBS vaccine began in the 1970s, with human trials in the 1980s showing variable immunogenicity with a capsular polysaccharide-based vaccine. The vaccine was well tolerated in the intended population of pregnant women, and for those women who did mount an immune response (307), their infants were shown to have active GBS antibodies at the age of 2 to 3 months, potentially decreasing the burden of late-onset GBS disease as well (304, 308).

More recent vaccine development has focused on conjugate vaccines aimed at various combinations of GBS types Ia, Ib, II, and

III. Continued work is necessary to define optimal doses and administration schedules. In Europe, the DEVANI (Design a Vaccine against Neonatal Infections) project has been funded by the European Union after statistical modeling identified a maternal immunization program as being the most effective at decreasing GBS disease burden (309). This model and others have estimated that such a program would potentially decrease preterm births by as much as 1 to 4% and decrease stillbirths by 5 to 10% (4, 309, 310). Globally, serotypes of GBS differ dramatically from those in the United States and Europe, making a serotype-specific vaccine designed for U.S. populations less likely to have a global impact.

One possible solution would be to base a GBS vaccine on antigens, in which case the vaccine would not be dependent on a specific GBS serotype. New technologies are being employed to develop and test such a vaccine but have not yet produced a vaccine that targets all nine GBS serotypes. Using one novel method, reverse vaccinology, researchers are using genomic information acquired from GBS serotypes to determine which universal antigens might be acceptable candidates for a vaccine (307).

Currently, there is increased research activity by a vaccine manufacturer in a phase 3 efficacy trial in pregnant women testing a GBS vaccine containing GBS types Ia, Ib, and III conjugated to CRM₁₉₇ (4). Glycoconjugate vaccines have shown 40 to 60% peak antibody concentrations and good *in vitro* activity at 18 to 24 months postvaccination (311–314). With continued research and development in this area, vaccine development should lead to reductions of both morbidity and mortality in the neonatal population.

Prenatal Care To Prevent Infection and Premature Delivery

Aside from vaccination and improving health disparities, a third intervention that would decrease the incidence of fetal loss, stillbirth, premature delivery, and neonatal sepsis is adequate prenatal care for all pregnant women. Women and infants with GBS sepsis and appropriate IPA have improved outcomes compared to women who are not screened or who are screened at times other than an EGA of 35 to 37 weeks. Decreasing the burden of disease can also be accomplished by routine prenatal care, including screening for GBS bacteriuria, and appropriate treatment of preterm labor. Importantly, not only must women be given screening tests as part of prenatal care, appropriate therapy also must be administered to achieve optimal maternal and newborn outcomes. In one survey of prenatal practices across the country from 2003 to 2004, 80% of women and infants failed to receive the indicated treatment after routine prenatal screening was performed (315).

Prevention of Viral Infection

Prevention of neonatal HSV has been successful with the implementation of cesarean sections for women with active genital lesions who have first-episode disease, and viral suppression during pregnancy is also helpful. As most neonatal HSV is due to primary HSV disease in the mother and is often not prenatally diagnosed, prevention can be difficult (77). For women diagnosed with enteroviral infection, postponement of delivery by 5 to 7 days can improve maternal antibody transmission to the fetus and potentially decrease the burden of disease (87). The most effective way of prevention of neonatal infection with HPeV is not known at this time.

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