

Challenges in the diagnosis and management of neonatal sepsis

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SUMMARY

Neonatal sepsis is the third leading cause of neonatal mortality and a major public health problem, especially in developing countries. Although recent medical advances have improved neonatal care, many challenges remain in the diagnosis and management of neonatal infections. The diagnosis of neonatal sepsis is complicated by the frequent presence of noninfectious conditions that resemble sepsis, especially in preterm infants, and by the absence of optimal diagnostic tests. Since neonatal sepsis is a high-risk disease, especially in preterm infants, clinicians are compelled to empirically administer antibiotics to infants with risk factors and/or signs of suspected sepsis. Unfortunately, both broad-spectrum antibiotics and prolonged treatment with empirical antibiotics are associated with adverse outcomes and increase antimicrobial resistance rates. Given the high incidence and mortality of sepsis in preterm infants and its long-term consequences on growth and development, efforts to reduce the rates of infection in this vulnerable population are one of the most important interventions in neonatal care. In this review, we discuss the most common questions and challenges in the diagnosis and management of neonatal sepsis, with a focus on developing countries.

In recent years, a significant decrease in childhood mortality has been achieved worldwide [1]. However, neonatal mortality has decreased at much lower rates, and currently represents 40% of all childhood mortality [1, 2]. Every year 2.6 million neonates die; three-fourths of these deaths occur in the first week of life, and almost all (99%) in low- and middle-income countries [1, 3]. Neonatal sepsis is the third leading cause of neonatal mortality, only behind prematurity and intrapartum-related complications (or birth asphyxia) [2]. It is responsible for 13% of all neonatal mortality, and 42% of deaths in the first week of life [2, 3]. Developing countries lack a surveillance system, and a high proportion of newborns in these

countries die at home before they are registered. Consequently, neonatal sepsis is likely underreported in these countries, suggesting that its impact on mortality may be even higher [4].

Newborns, especially preterms, are more susceptible to infections than children at any other age period [5]. Innate immunity is affected by impaired cytokine production, decreased expression of adhesion molecules in neutrophils and a reduced response to chemotactic factors [6]. Also, transplacental passage of antibodies starts during the second trimester and achieves its maximal speed during the third trimester. As a result, most preterm newborns have significantly reduced humoral responses [7]. Cytotoxic T-cell

activity is also impaired during the newborn period [5]. The multiple skin punctures and invasive procedures that preterm newborns commonly undergo increase even more the risk of infections in this population.

Advances in perinatal and neonatal intensive care have reduced the mortality rate of preterm infants, but improvements in survival have not been accompanied by proportional reductions in the incidence of disabilities in this population [8]. In developing countries, clinically diagnosed sepsis is present in 49–170 per 1000 live births, culture-proven sepsis in 16 per 1000 live births and neonatal meningitis in 0.8–6.1 per 1000 live births [4]. Infants with neonatal infections are more likely to have adverse neurodevelopmental outcomes at follow up, including cerebral palsy, lower mental and psychomotor development index scores, visual impairment and impaired growth [8, 9]. This increases the social and economic burden of this condition in already poor settings.

Despite the high burden of neonatal sepsis, high-quality evidence in diagnosis and treatment is lacking. The susceptibility of the population, lack of consensus in definitions and variability between regions hinder the development of clinical trials and global recommendations [10]. Physicians caring for infected neonates face multiple challenges in diagnostic and treatment decisions. The situation in developing countries is further complicated by a lack of reliable surveillance systems and high proportion of home births [4]. Some low- and middle-income countries, which are implementing tertiary care centers, are also experiencing the challenges of developed countries [11]. In this review we address the most frequent questions about the diagnosis and treatment of neonatal sepsis, with a focus on developing countries.

WHAT ARE THE MOST COMMON CAUSES OF NEONATAL SEPSIS IN DEVELOPED COUNTRIES?

Neonatal sepsis is divided into early-onset (if symptoms start before 72 h of life) and late-onset (if symptoms start afterward). Various cutoff points have been used, from 48 h to 7 days, but most epidemiological studies use 72 h [12]. Early-onset

sepsis is caused by maternally transmitted pathogens. Chorioamnionitis, maternal intrapartum fever, prematurity, prolonged rupture of membranes and inadequate intrapartum antibiotic prophylaxis increase its risk [13]. Late-onset sepsis is caused by nosocomial infections and is more common in preterms and in newborns with prolonged hospitalizations, use of central lines, parenteral feeding and mechanical ventilation [14].

The incidence of early-onset neonatal sepsis in developed countries is 0.9–1.5 per 1000 live births [15, 16]. The most common cause of early-onset sepsis is Group B *Streptococcus* (GBS), isolated in half of episodes, followed by *Escherichia coli*, isolated in one-fourth of episodes [15, 16]. The remaining cases of early-onset sepsis are caused by *Staphylococcus aureus*, Coagulase-negative *Staphylococcus* (CoNS), *Listeria monocytogenes* and other gram-negative bacteria [15, 16] (Table 1). In very-low-birth-weight newborns (<1500 g), *E. coli* is more common than GBS [16].

Late-onset sepsis presents mainly in very-low-birth-weight infants. Its incidence in developed countries is 3–3.7 per 1000 live births [15]. The main pathogen is CoNS, responsible for half of episodes. Other important etiologic agents are *E. coli*, *Klebsiella* sp and *Candida* sp. Together they cause one-third of episodes. Less common causes of late-onset sepsis include *S. aureus*, *Enterococcus* sp and *Pseudomonas aeruginosa* [14, 15] (Table 1). Late-onset pathogens are more resistant to antibiotics than early-onset pathogens [17].

WHAT ARE THE MOST COMMON CAUSES OF NEONATAL SEPSIS IN DEVELOPING COUNTRIES?

In developing countries, most pathogens isolated in the hospital setting before 72 h of life are similar to those isolated afterward; it is likely that highly unclean delivery practices lead to infections with nosocomial agents very early in life [18]. In addition, most neonates are born at the household and might get infected with community acquired pathogens even after 72 h [11]. As a result, several authors have classified neonatal sepsis in developing countries as community- and hospital-acquired instead of early- and late-onset.

Table 1. Common pathogens of neonatal sepsis in developed countries^a

Early-onset		Late-onset	
Pathogen	Frequency (%)	Pathogen	Frequency (%)
Group B <i>Streptococcus</i>	43–58	Coagulase-negative <i>Staphylococcus</i>	39–54
<i>E. coli</i>	18–29	<i>E. coli</i>	5–13
Other gram-negative bacteria	7–8	<i>Klebsiella</i> sp.	4–9
<i>S. aureus</i>	2–7	<i>S. aureus</i>	6–18
Coagulase-negative <i>Staphylococcus</i>	1–5	<i>Candida albicans</i>	6–8
<i>L. monocytogenes</i>	0.5–6	<i>Enterococcus</i> sp.	6–8
		<i>P. aureginosa</i>	3–5
		Other <i>Candida</i> species	3–4

^aData from references [14–16].

Table 2. Common pathogens of neonatal sepsis in developing countries^a

Community-acquired		Hospital-acquired	
Pathogen	Frequency (%)	Pathogen	Frequency (%)
<i>Klebsiella</i> sp.	14–21	<i>Klebsiella</i> sp.	16–28
<i>S. aureus</i>	13–26	<i>S. aureus</i>	8–22
<i>E. coli</i>	8–18	<i>E. coli</i>	5–16
Group B <i>Streptococcus</i>	2–8	Coagulase-negative <i>Staphylococcus</i>	8–28
<i>S. pneumoniae</i>	2–5	<i>Pseudomonas</i> sp.	3–10
<i>Salmonella</i> sp.	1–5	<i>Enterobacter</i> sp.	4–12
		<i>Candida</i> sp.	0.3–3

^aData from references [18–20].

Gram-negative bacteria dominate in community-acquired sepsis, except in some parts of Africa [19]. The most common pathogens are *Klebsiella* sp, *E. coli* and *S. aureus* [19, 20]. GBS, the most common pathogen in developed countries, is responsible for only 2–8% of cases in developing countries [19, 20] (Table 2). It is possible that infants with GBS infection are underreported since this pathogen usually presents very early in life, and infected newborns might die before coming to medical attention [11].

Gram-negative bacteria, mainly *Klebsiella* sp and *E. coli*, and *S. aureus* are the most commonly isolated pathogens in hospital-acquired infections [18]. In contrast to high-income countries, CoNS is responsible for a lower proportion of hospital-acquired infections; overall, only 12% of hospital-acquired sepsis is caused by CoNS (Table 2). In Latin American and Southeast Asian countries that are implementing

sophisticated tertiary neonatal units, CoNS prevalence has risen to 28% [18]. This surge might be the result of increasing care to very-low-birth-weight newborns without assessing the dangers of common outbreak sources—similar to what happened in developed countries 50 years ago [18].

DIAGNOSIS

One of the major difficulties in the management of neonatal sepsis is getting an accurate diagnosis. Unlike older patients, newborns have very subtle presentations, and multiple conditions resemble neonatal sepsis [5]. Auxiliary tests have limited value and are difficult to interpret due to low sensitivity and changing normal ranges during the neonatal period [5]. Blood cultures also lack sensitivity due to specific characteristics of the neonatal population [5]. As a

result, a combination of findings is necessary to provide a correct diagnosis of neonatal sepsis. Deciding how to incorporate these tests is under great controversy.

What are the main clinical signs of neonatal sepsis?

Sepsis share a similar clinical presentation to other common conditions in the neonatal period. Auxiliary tests are paramount for its diagnosis, but access to laboratory tests in developing countries is limited [12]. The World Health Organization identified seven clinical signs—difficulty feeding, convulsions, movement only when stimulated, respiratory rate >60 per min, severe chest in drawing and axillary temperature >37.5 °C or <35.5 °C—that should prompt neonatal referral to a hospital [21]. Other authors have also included cyanosis and grunting [22]. Training community health workers to identify sick infants using these signs and referring them to the hospital significantly reduces neonatal mortality [23, 24].

What is the value of blood cultures in the diagnosis of neonatal sepsis?

Blood culture is the gold standard for the diagnosis of neonatal sepsis. However, its positivity rate is low and is affected by blood volume inoculated, prenatal antibiotic use, level of bacteremia and laboratory capabilities [5]. In developing countries, culture-negative sepsis is responsible for the majority of episodes [4]. Currently, the recommended minimal blood volume for cultures in newborns is 1 ml, but most samples taken are of less than 0.5 ml [25]. One classic study, focusing on *E. coli* infection, found that neonates have high-colony-count bacteremia [26]. However, a more recent study including other common neonatal-sepsis pathogens found that 68% of septic infants have low-level bacteremia (≤ 10 Colony-forming units (CFU)/ml) and 42% have counts ≤ 1 CFU/ml [27]. In low-colony-count bacteremia, as many as 60% of cultures will be falsely negative with 0.5 ml sample volumes [28]. Multiple blood cultures could help increase the yield of this test, but studies in the neonatal period have shown conflicting results [29, 30].

Important advances have been made in molecular diagnosis for the identification of pathogens, including polymerase chain reaction (PCR), real-time PCR,

pyrosequencing, use of microfluidic technology such as in the TaqMan Array Card, and other 'lab on a chip' devices [31]. A meta-analysis of 23 studies on molecular diagnosis of neonatal sepsis found that real-time PCR assays performed the best, with 96% sensitivity and 96% specificity [32]. Ribosomal RNA unique to bacteria are detected by 16s RNA. It has a high sensitivity, but has a high frequency of contamination, and it cannot determine bacterial antibiotic sensitivities [33]. These new assays require advanced molecular biology laboratories and special equipment, which are not available in many hospital settings.

What laboratory tests are useful in the evaluation of a newborn with signs of infection?

Complete blood cell count is difficult to interpret in the neonatal period because it varies significantly with day of life and gestational age [5]. Low values of white blood cells, low values of absolute neutrophil counts and high immature/total ratio are associated with early-onset sepsis. In this type of sepsis, high values of white blood cells and absolute neutrophil counts are not informative [34]. High or low white blood cells counts, high absolute neutrophil counts, high immature/total ratio and low platelet counts are associated with late-onset sepsis [35]. Despite their association with infection, all of these findings have low sensitivities [34, 35].

A single value of C-reactive protein (CRP) has unacceptable low sensitivities, especially during the early stages of infection [36, 37]. Taking serial determinations 24–48 h after the onset of symptoms achieves a sensitivity of 74–89% and specificity of 74–95% [36, 37]. Different cutoff points have been used, ranging from 0.2–95 mg/l; the most commonly used cutoff is 10 mg/l [38]. Since CRP undergoes a physiological 3 day rise after birth and is lower in premature infants, using a single value for all newborns might be suboptimal. One recent study generated normal ranges based on gestational age and day of life [39]. CRP values are also affected by premature rupture of membranes, maternal fever, meconium aspiration, fetal distress and the etiology of the infection [37, 40].

Procalcitonin increases faster than CRP, making it a very appealing biomarker [5]. Its overall sensitivity is 81% and specificity is 79% [41]. In early-onset

BOX 1. CRITERIA FOR THE DIAGNOSIS OF NEONATAL SEPSIS (MODIFIED FROM REFERENCE [43])^a

Clinical variables

- Temperature instability
- Heart rate ≥ 180 beats/min or ≤ 100 beats/min
- Respiratory rate > 60 breaths/min plus grunting or desaturations
- Lethargy/altered mental status
- Glucose intolerance (plasma glucose > 10 mmol/l)
- Feed intolerance

Hemodynamic variables

- Blood pressure 2 SD below normal for age
- Systolic pressure < 50 mm Hg (newborn day 1)
- Systolic pressure < 65 mm Hg (infants ≤ 1 month)

Tissue perfusion variables

- Capillary refill > 3 s
- Plasma lactate > 3 mmol/l

Inflammatory variables

- Leukocytosis (WBC count $> 34\,000 \times 10^9/l$)
- Leukopenia (WBC count $< 5000 \times 10^9/l$)
- Immature neutrophils $> 10\%$
- Immature:Total neutrophil ratio > 0.2
- Thrombocytopenia $< 100\,000 \times 10^9/l$
- CRP > 10 mg/l or 2 SD above normal value
- Procalcitonin > 8.1 mg/dl or 2 SD above normal value
- IL-6 or IL-8 > 70 pg/ml
- 16S PCR positive

Interpretation

- Proven Sepsis: A positive blood culture or PCR in the presence of clinical signs and symptoms of infection. For CoNS two positive blood cultures or one positive blood culture plus a positive CRP.
- Probable Sepsis: Presence of signs and symptoms of infection and at least two abnormal laboratory results when blood culture is negative.
- Possible Sepsis: Presence of clinical signs and symptoms of infection plus raised CRP or IL-6/IL-8 level when blood culture is negative.

^aSD: standard deviation, WBC: white blood cell, CRP: C-reactive protein, IL: interleukin, PCR: polymerase chain reaction, CoNS: Coagulase-negative *Staphylococcus* or *Staphylococcus non-aureus*.

sepsis, its sensitivity is 70–77%, but values taken shortly after birth have a sensitivity of only 49% [41]. In late-onset sepsis, procalcitonin is more sensitive than CRP, achieving a sensitivity of 82–90% [41]. Most studies have used a cutoff between 0.3 and 2 ng/ml [38]. However, like CRP, procalcitonin is significantly affected by day of life and gestational

age, and these factors should be accounted to interpret its values [39].

Currently, there are new non-culture-based approaches that are being implemented to improve the diagnosis [5]. CD64 neutrophil marker has a high sensitivity and specificity. It has the additional advantage of requiring small amounts of

blood [42]. Multiple cytokines have been studied for the diagnosis of neonatal sepsis; interleukin 6 and 8 are the most widely studied [38]. They rise very rapidly after a bacterial infection but normalize before 24 h, limiting their clinical use [5]. Manose-binding lectin, important for the lectin pathway of the complement, is also being studied as a possible biomarker [5]. New alternatives under development include the use of genomics and proteomics for identification of host response biomarkers.

How to interpret the results of multiple tests?

One of the major setbacks for the management, surveillance and research in neonatal sepsis is the lack of globally accepted case definitions [10, 43]. In adults, sepsis is defined by the presence of a systemic inflammatory response plus an infectious focus. This definition cannot be applied to newborns due to nonspecific clinical signs, common pathologies that resemble sepsis and the low positivity rate of cultures. Also, auxiliary tests do not have enough sensitivity and specificity to be used on their own [43]. Specific criteria for neonatal sepsis, using clinical and laboratory information, have been published [10, 43]. These criteria classify episodes according to the certainty of the diagnosis into culture-proven, probable and possible sepsis [43] (Box 1). Unfortunately, none of these classifications have been widely adopted.

Recently, we proposed an algorithm adapted from Haque's definitions to be used as a diagnostic surveillance tool in developing countries [44]. Epidemiological surveillance systems are necessary to develop interventions for the management of neonatal sepsis and to evaluate the results of such interventions. Long-term data from different regions or institutions can be analyzed to identify those with greater deficiencies and prioritize resources. To achieve this, neonatal sepsis definitions must be consistent and reproducible between institutions. Simplicity is also important for these case definitions in order to minimize the work-load to already busy and understaffed units. Currently, most developing countries do not have such definitions. As a result, neonatal sepsis diagnosis varies widely between institutions [44].

How to differentiate CoNS infection from contamination?

CoNS is the most common cause of late-onset sepsis, but it is also part of the skin flora and a common contaminant. Differentiating true infection from contamination is very challenging [45]. The National Institute of Child Health and Human Development (NICHD) Neonatal Research Network published specific criteria to define CoNS sepsis. Two positive blood cultures or one positive blood culture plus a positive CRP are required to diagnose culture-proven sepsis. If these conditions are not met, but the patient received more than 5 days of anti-staphylococcal therapy, the episode is considered probable sepsis [14]. Other criteria have also been published; most of them agree that two positive cultures are necessary to diagnose a CoNS infection [46].

Are lumbar punctures necessary when evaluating a newborn for sepsis?

The use of lumbar puncture for the evaluation of neonatal sepsis is controversial and varies significantly between centers [47]. The incidence of neonatal meningitis is 0.27–0.44 per 1000 live births and increases to 6.5–14 per 1000 in very-low-birth-weight newborns [48]. Meningitis is more common in infants evaluated for late-onset sepsis than for early-onset sepsis [48]. One-third of cases—and two-thirds of candida meningitis—have negative blood cultures [49, 50]. Despite reported adverse effects, Stoll et al. found that lumbar puncture was not associated with increased mortality, while meningitis significantly increased it [49]. In asymptomatic newborns evaluated for early-onset sepsis only due to maternal risk factors, the incidence of meningitis is nil; in these infants a lumbar puncture can be postponed [51]. In every symptomatic newborn evaluated for sepsis, a lumbar puncture must be performed, regardless of the time of presentation. All neonates with bacteremia, especially with gram-negative rods, should have a lumbar puncture done.

MANAGEMENT

The management of neonatal sepsis is highly heterogeneous [52]. Clinical trials evaluating the treatment of neonatal sepsis are scarce and failed to find an optimal antibiotic regimen [10]. The lack of an

accepted definition of sepsis in neonates is one of the main obstacles for the performance of these trials. Including only culture-proven sepsis would result in the exclusion of culture-negative sepsis that still require antibiotic therapy. Finding an adequate endpoint also obstructs the implementation and interpretation of trials [10]. In the absence of clinical trials, knowledge of the most common pathogens and their antibiotic resistance patterns should guide the management of neonatal sepsis [53].

Antibiotics are among the most used medications in the neonatal intensive care units (NICU) [54]. Almost all neonates in an NICU receive antibiotics during their hospitalization, but only 5% have a positive blood culture [55]. Most of the antibiotic courses are given empirically before 72 h of life, and 60% of these courses are prolonged for more than 48–72 h despite negative blood culture and a stable clinical condition [55]. Patel et al. found that 35% of neonates receive at least one inappropriate course of antibiotics during their NICU stay [56].

What are the consequences of excessive antibiotic use?

Inappropriate antibiotic use is associated to the development and spread of resistant pathogens in the NICUs [57]. One study compared amoxicillin plus cefotaxime vs. penicillin plus tobramycin for suspected early-onset sepsis. The authors found that amoxicillin plus cefotaxime increased by 18-fold the risk of colonization with resistant pathogens [58]. A study of hospital-acquired infections, comparing cefotaxime vs. tobramycin, found that newborns who received cefotaxime in the previous 30 days were 33 times more likely to develop an extended-spectrum beta-lactamase infection [59].

Antibiotics are also associated with adverse outcomes [57]. The use of third-generation cephalosporins is associated with increased risk of candida-invasive disease [odds ratio (OR): 2.157] and death (OR: 1.5) [60, 61]. Prolonged antibiotic therapy increases the risk of late-onset sepsis (OR: 2.45), necrotizing enterocolitis (OR: 1.10) and death (OR 1.12) [62, 63]. Adverse effects of antibiotics transcend the neonatal period; some studies found an association between neonatal antibiotics and wheezing during childhood [64].

What is the best empiric therapy for neonatal sepsis?

Neonates with risk factors for early-onset sepsis or compatible clinical condition should receive prompt empiric antibiotic therapy [53]. Poupolo et al. developed a risk stratification tool to select neonates that need empiric therapy [13]. GBS and *E. coli* account for most episodes of early-onset sepsis in developed countries [16]. Since the reported antibiotic resistance to the combination of ampicillin plus aminoglycosides in the past 10 years has remained at less than 10%, this should be the initial therapy for suspected early-onset sepsis [16, 17]. This regimen has the additional advantage of having synergistic activity against GBS and *Listeria monocytogenes* [53].

Every neonate with signs of late-onset sepsis should receive empiric antibiotic therapy [53]. In developed countries, almost three-fourths of CoNS isolated are resistant to methicillin. Also, one-fourth of gram-negative pathogens are resistant to third-generation cephalosporins but only 10% are resistant to aminoglycosides [15, 17]. Considering the high resistance to methicillin, some experts recommend using vancomycin plus an aminoglycoside as empiric therapy for late-onset sepsis [53]. However, CoNS infections are rarely fulminant and starting therapy with an anti-staphylococcal penicillin plus an aminoglycoside is a safe option. Vancomycin should be reserved for confirmed cases of methicillin-resistant pathogens [14, 65]. Newborn with risk factors for candida sepsis—central vascular access, endotracheal intubation, thrombocytopenia, exposure to broad-spectrum cephalosporins or carbapenems and extreme prematurity—should receive fungal empiric therapy [66].

When to stop antibiotics in newborns with negative blood cultures?

Antibiotics can be safely stopped at 48–72 h in neonates with negative blood cultures who are clinically stable [53]. Around 90% of positive blood cultures grow by 48 h, and 97% by 72 h. Most cultures that turn positive after 72 h are contaminants [67]. Stopping antibiotics after the blood culture is reported negative at 48 h in clinically stable patients does not increase treatment failure [68]. Continuing antibiotics for more than 7 days vs. stopping them after 3 days in extremely-low-birth-weight neonates

(<1000 g) with negative blood cultures increased the hospitalization length but had no effect on survival [69].

CRP has emerged as a valuable tool to guide and reduce the duration of antibiotic therapy [53]. Single and serial values taken after 24 h of onset of symptoms have a high negative predictive value (98–100%) [70]. However, a recent study found that CRP has a negative predictive value of only 86% at 48 h [71]. Previous studies have excluded high-risk infants like those with central lines, mechanical ventilation or birth asphyxia. The value of CRP to guide antimicrobial therapy might be limited to a selected population [53]. Immature/total neutrophil ratio and procalcitonin have also been tested to guide therapy with encouraging results, but larger trials need to be performed before they can be used for this indication [72, 73].

How long to treat a newborn with culture-proven sepsis?

One trial comparing 10 vs. 14 days of therapy found that there was no difference in treatment failure if the neonate was asymptomatic and with normal CRP at the seventh day, but the 10-day group had significantly shorter hospitalizations [74]. Another trial testing 7 vs. 14 days of therapy, in asymptomatic newborns at day 7, found a nonsignificant trend toward greater treatment failure in the short course arm [75]. Both of these trials had small sample sizes and were performed in neonates with a gestational age >32 weeks and birth weight >1500 g. The length of optimal duration might also depend on the pathogen. In *S. aureus* infection, a short course of antibiotic (7 vs. 14 days) is significantly associated with higher treatment failures [75]. Conversely, treatments of only 3 days have been effective in CoNS sepsis [76]. Newborns with culture-proven sepsis must receive a full antibiotic course for 10–14 days. In selected cases (>32 weeks gestational age, >1500 g birth-weight and not *S. aureus* infection) a course of 7–10 days might be sufficient [53].

How to treat neonatal meningitis?

The management of neonatal meningitis is based on expert recommendations; no clinical trials have evaluated the choice and duration of antibiotic therapy

[53]. In a neonate with early-onset meningitis (<72 h), ampicillin plus cefotaxime or ampicillin plus an aminoglycoside is recommended [77]. In the case of late-onset meningitis, vancomycin plus a third-generation cephalosporin must be used [53]. The recommended duration of therapy is 14 days for gram-positive meningitis, 21 days for gram-negative meningitis and >21 days for *L. monocitogenes* meningitis [77]. All neonates with meningitis should have central nervous system imaging (ultrasound or computed tomography) to rule out complications; some pathogens have a higher likelihood of being associated with brain abscess (i.e., *Serratia*, *Citrobacter*, *Enterobacter*). Newborns with complicated meningitis required prolonged antibiotic therapy [53, 77].

A trial testing the adjunctive use of dexamethasone in 52 neonates showed no difference in mortality, neurological deficits or hearing impairment at 2 years of age [78]. A more recent trial found that dexamethasone decreased mortality and hearing impairment, but this trial has several limitations [79]. Considering the lack of high-quality evidence and the poor understanding of the effect of steroids on the developing brain, adjunctive dexamethasone is not recommended in neonatal meningitis [77].

Are there special considerations of neonatal sepsis treatment in developing countries?

In developing countries, antibiotic resistance of community-acquired infections has increased significantly in the past 20 years [80]. *Klebsiella* sp. resistance to gentamicin is 60–72%, to amikacin is 43% and to third-generation cephalosporins is 57–66%. *Escherichia coli* resistance to gentamicin is 13–48%, to amikacin is 15% and to third-generation cephalosporins is 19–64%. In the case of *S. aureus*, 4% are resistant to methicillin [20, 80]. Despite these levels of resistance, current recommendations state that a newborn with suspicion of sepsis should be hospitalized and treated with ampicillin plus gentamicin. However, physicians must keep in mind the local resistance patterns when deciding empiric therapy [80].

Resistance of hospital-acquired infections is also very high in developing countries [18]. Around 30–90% of *Klebsiella* sp isolates in hospital settings

are resistant to commonly used antibiotics against gram-negative bacteria, and resistance rates are alarmingly high in Southeast Asia. *Escherichia coli* resistance rates are slightly lower but still very high. Overall resistance of *S. aureus* to methicillin is 38% in developing countries but rises to 56% in South Asia [18]. High resistance levels force physicians to use broad-spectrum antibiotics, like carbapenems and vancomycin, as first-line regimens. In these low-resource communities, many families can not afford the cost of these medications. If they are obtained, health-care workers might try to prolong their use by using the leftovers on other patients, leading to contamination and outbreaks of resistant bacteria [18].

How to treat infected newborns who cannot be hospitalized?

Some mothers refuse to hospitalize their children, or hospitals might be unavailable in developing countries [81]. In these cases, community management of neonatal sepsis, including antibiotic therapy at home, reduces mortality significantly [23, 24]. Community management includes several interventions, but one study estimated that home antibiotics alone reduce case fatalities by 35% [82]. Simplified antibiotic regimens are being developed to make home-management feasible [81, 83]. Intramuscular gentamicin, procaine penicillin and ceftriaxone offer wide coverage and can easily be administered once a day [83]. Oral antibiotics like cotrimoxazole, cefuroxime and amoxicillin are also potential options in the community setting [81]. Home treatment with intramuscular procaine penicillin plus intramuscular gentamicin, intramuscular ceftriaxone alone and oral cotrimoxazole plus intramuscular gentamicin significantly reduced neonatal mortality in rural communities. However, cotrimoxazole plus gentamicin seems to be less effective than the other two regimens [84]. Currently, ongoing trials are testing new simplified regimens for home-based treatment.

What are the most effective strategies to prevent neonatal sepsis?

Multiple preventive interventions have been designed to decrease sepsis rates in neonates. Hand-washing and clean practices during delivery and afterward reduce neonatal sepsis significantly [85].

Interventions to increase hand washing rates have been successful; however, several hospitals in developing areas lack the basic facilities to implement them. Using chlorhexidine in vaginal washes during labor, to cleanse the umbilical cord stump, or as neonatal skin antiseptic has also reduced the incidence of neonatal sepsis in developing countries [86].

Breast feeding is another effective strategy in term and preterm infants that improves cognitive and behavior skills, and decreases rates of infection [87, 88]. The protective effects of human milk are due primarily to the multiple anti-infective, anti-inflammatory and immunoregulatory factors transmitted through milk. Lactoferrin is one of these factors [89]. Oral supplementation with bovine lactoferrin significantly reduced the incidence of late-onset sepsis in an Italian trial and in a second trial in Turkey [90, 91]. In a pilot study in Peru, our group found a nonsignificant reduction of sepsis in the lactoferrin group; however, the sample size was small. (Accepted for publication) Bovine lactoferrin has the additional advantage of being very cheap. Multiple trials are ongoing to test the value of lactoferrin in prevention of neonatal sepsis using different doses and populations. This information will help to define lactoferrin's role in clinical settings [89].

Chemoprophylaxis has also been used to prevent neonatal sepsis. GBS screening and intrapartum antibiotic prophylaxis have significantly reduced early-onset neonatal sepsis in developed countries. In the USA, clear protocols for generalized testing and treatment of GBS colonization in pregnant women have been established for many years-[92]. Also, fungal prophylaxis with fluconazole has demonstrated efficacy in reducing invasive *Candida* infections in extremely-low-birth-weight neonates (<1000 g) [93]. However, a recent trial did not find a reduction in the composite outcome of invasive candidiasis and death, raising questions on the universal use of prophylactic fluconazole [94].

CONCLUSION

Neonatal sepsis is a major public health problem especially in developing countries. The susceptibility of this naïve population, lack of consensus in the definitions and pathogen variability between different

regions hinder the development of clinical trials and practice guidelines. Physicians taking care of these patients face multiple questions when making diagnosis and treatment decisions. Most of them feel pressured to treat every newborn with suspicion of sepsis aggressively. As a result, many newborns receive prolonged antibiotic therapies without considering the adverse effects of such regimens. The management of neonatal sepsis in developing countries is aggravated by increased levels of antibiotic resistance, shortages of medical personnel and high numbers of home-births. Multiple studies, some of them still ongoing, have addressed these difficulties. Additionally, some developing countries have started to implement tertiary care units and are now facing the challenges of developed countries as well. Given the high incidence and high morbidity and mortality of sepsis in preterm infants, efforts to reduce the rates of infection in this vulnerable population are one of the most important interventions in neonatal care. Among these preventive interventions, early and exclusive breastfeeding is one of the most important interventions to reduce neonatal sepsis and overall mortality.

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