Neonatal sepsis or septicaemia is a clinical syndrome characterized by systemic signs of circulatory compromise (e.g., poor peripheral perfusion, pallor, hypotonia, poor responsiveness) caused by invasion of the bloodstream by bacteria in the first month of life. In the pre-antibiotic era neonatal sepsis was usually fatal. Case fatality rates in antibiotic-treated infants now range between 5% and 60% with the highest rates reported from the lowest-income countries [1]. The World Health Organization (WHO) estimates that 1 million deaths per year (10% of all under-five mortality) are due to neonatal sepsis and that 42% of these deaths occur in the first week of life [2]. There are wide disparities in neonatal care between high- and low-income countries. In high-income countries the major concern is the increasing numbers of extremely premature infants with high nosocomial infection rates due to multiresistant organisms in intensive care units. Health facility infections are also a major problem in low-income countries, but the more pressing issues are the high proportion of home deliveries in unclean environments predisposing to sepsis and ensuring that all neonates have access to effective interventions from health care providers in the first days of life [2]. Indeed, new strategies that can prevent, diagnose, and treat neonates with sepsis are needed in both low- and high-income settings.

Pathogenesis of Neonatal Infections

Distal risk factors for neonatal sepsis include poverty and poor environmental conditions. Proximate factors include prolonged rupture of membranes, preterm labour, maternal pyrexia, unhygienic intrapartum and postnatal care, low birth weight, and prelacteal feeding of contaminated foods and fluids [3–5].

The bacteria that cause neonatal sepsis are acquired shortly before, during, and after delivery (Figure 1). They can be obtained directly from mother’s blood, skin, or vaginal tract before or during delivery or from the environment during and after delivery. *Streptococcus agalactiae* (Group B streptococcus, GBS) is the most common cause of neonatal sepsis in many countries, though low rates are reported from many low-income countries, especially those in south Asia.[6–8]; gram-negative bacilli (*Escherichia coli*, *Klebsiella spp.*, *Pseudomonas spp.*, *Acinetobacter spp.*), and gram-positive cocci (*Staphylococcus aureus* and *Staphylococcus epidermidis*) are other important causes [6–8]. However, there are many difficulties in interpreting aetiological neonatal sepsis data, because many studies report selected populations of high-risk infants. Specimens from infants in the first 24 hours of life are also seriously under-represented, especially those from low birth-weight babies and babies born outside health facilities [6,9–11]. Intrapartum antibiotic prophylaxis against *S. agalactiae* has also led to a substantial change in the bacteria responsible for early onset neonatal sepsis; gram-negative bacilli and *Staphylococcus* spp. predominate in countries implementing these programs [12].

There are also many other important neonatal infectious disease pathogens that are not associated with the sepsis syndrome including: *Treponema pallidum*, rubella virus, herpes simplex virus, cytomegalovirus, toxoplasmosis, *Clostridium tetani*, HIV, hepatitis B virus, and *Bordetella pertussis* (Figure 1) [1,7,13]. These infectious pathogens cause serious morbidities in young infants and multifaceted disease syndromes including congenital anomalies, developmental disabilities, chronic liver disease, neonatal tetanus, and apnoea. They are also important causes of morbidity and mortality in older age groups. However, only pathogens that cause neonatal sepsis are discussed in this paper.

Neonatal Immunity

Neonates have a functionally immature immune system. They have extremely low immunoglobulin (Ig) levels except for IgG to specific maternal antigens transferred passively across the placenta during the last trimester of pregnancy [14,15]. T cell function is relatively unimpaired but complement activity is half that of healthy adults. Neonates have a low neutrophil storage pool, and their existing neutrophils have impaired capacity to migrate from the blood to sites of infection [16].

The basal expression of Toll-like receptors (TLRs, receptors that detect the presence of microbes) is similar in the neonate and adult [17]. However, innate immune responses of neonatal mononuclear cells are characterised by markedly reduced release of the proinflammatory Th1-polarizing cytokines tumour necrosis factor-alpha (TNF-α) and interferon-gamma (IFN-γ) with relative preservation of...
Five Key Papers on Preventing, Diagnosing, and Treating Neonatal Sepsis


- Stevens DY, Petri CR, Osborn JL, Spicar-Mihalic P, McKenzie KG, et al (2008) Enabling a microfluidic immunoassay for the developing world by integration of on-card dry reagent storage. Lab Chip 8: 2038–2045. One of a series of studies that has demonstrated that microfluidic microtechnologies can be used to create robust point-of-care diagnostic systems for low-income countries and that results can be fast, accurate, and reproducible.


During Labour and Delivery

There is strong evidence that clean delivery practices and handwashing during delivery reduces rates of neonatal sepsis in both home and health facility settings [27–29]. Interventions to improve handwashing rates have been remarkably successful in research settings [30,31]. The reasons for lack of successful scale-up of handwashing interventions into policy, programs, and behaviour change are less clear [32]. New studies from Malawi and Nepal indicate that maternal antiseptic interventions such as vaginal chlorhexidine during labour may have a significant impact on rates of neonatal mortality and sepsis in developing countries [33]. However, other studies from high-income countries have demonstrated little effect on rates of HIV or neonatal infections [34].

Intrapartum antibiotic prophylaxis has been highly effective in reducing both early-onset neonatal bacterial and maternal sepsis in developed countries [35]. Chemoprophylaxis in the US has halved the incidence of early-onset neonatal bacterial sepsis caused by *S. agalactiae* from 1.7 per 1,000 live births in 1993 to 0.6 per 1,000 in 1998 [36]. Clear protocols are in place in high-income countries for the management of women with risk factors for neonatal sepsis [37]. Risk factors for early-onset neonatal bacterial sepsis in low-income settings are probably similar to resource-rich settings, but have not been evaluated in the context of high rates of maternal undernutrition, anaemia, HIV, and malaria.

After Delivery

There is also strong evidence that handwashing by health care providers after delivery can reduce neonatal sepsis and infection rates, especially in hospitals [27,28]. There is less evidence for the importance of rigorous handwashing and use of antiseptics in mothers of their own infants.
In high-income settings, studies have not shown an advantage of antibiotics or antiseptics over simply keeping the umbilical cord clean [2]. However, umbilical stump chlorhexidine cleansing has recently been shown to substantially reduce neonatal deaths in Nepal [38]. Other studies investigating the effects of chlorhexidine on prevention of omphalitis are currently underway in several countries [39].

There is emerging evidence that neonatal skin antisepsis preparations such as sunflower seed oil provides cheap, safe, and effective protection against nosocomial infections in hospitalized preterm neonates and infants in studies in south Asia. Application of chlorhexidine to neonatal skin has also been shown to be effective in reducing neonatal sepsis in studies from south Asia [39,40].

Breastmilk contains secretory IgA, lysozymes, white blood cells, and lactoferrin and has been shown to encourage the growth of healthy lactobacilli and reduce the growth of *E. coli* and other gram-negative pathogenic bacteria [15]. RCTs that focused on increasing early initiation and exclusive breastfeeding rates demonstrated significant reductions in diarrhoea and acute respiratory infections in neonates and older infants in India [42]. Other observational studies have demonstrated impact on infection specific mortality rates and all-cause mortality during the neonatal period [43–45].

Neonatal micronutrient supplementation trials have focused on vitamin A supplementation. Older studies have shown significant reductions in respiratory disease in low birth-weight infants after the administration of parenteral vitamin A [46]. More recently, trials of newborn vitamin A supplementation have shown encouraging reductions in neonatal mortality, and more trials are underway [47].

In high-income countries, clinical trials of immune stimulants such as granulocyte/monocyte colony stimulating factor (GM-CSF) to enhance the quantity and quality of neonatal neutrophils and monocytes appear promising but have not yet shown a significant clinical benefit [15]. The evaluation of recombinant APPs as adjunctive therapy for neonatal infection are still under evaluation. The impact of TLR agonists to improve defences against microorganisms are also being evaluated [15].

**Advances in Diagnosis**

Neonatal clinical sepsis syndrome identification is difficult as the clinical signs of

![Figure 1. Pathogenesis of congenital and neonatal infections.](doi:10.1371/journal.pmed.1000213.g001)
neonatal sepsis can be very similar to those of other life-threatening diseases such as necrotising enterocolitis, hyaline membrane disease, and perinatal asphyxia [48,49]. However, recent studies in middle- and low-income countries have provided seven danger signs which can be used to identify infants with very severe disease including neonatal sepsis (Table 1) [49]. These signs provide high sensitivity and moderate specificity for detecting serious illness in newborns in low-resource settings and have now been incorporated into the new neonatal WHO Integrated Management of Childhood Illness (IMCI) guidelines.

Identification of neonatal sepsis before delivery also remains challenging. A combination of maternal risk factors and clinical signs and symptoms is currently used [50]. However, peripartum proteomic analysis of the amniotic fluid is now offering the opportunity for early and accurate diagnosis of early-onset neonatal sepsis in the select population of women undergoing amniocentesis in high-risk pregnancies [51,52].

Confirmation of pathogenic organisms allows targeted antibiotic therapy. However, identification of pathogenic organisms in neonates with sepsis syndrome is fraught with difficulties. Bacterial load may be low due to mothers receiving antepartum or intrapartum antibiotics and because only small amounts of blood can often be taken from newborns [53]. Contamination rates may also be very high due to the technical difficulties of sterile venipuncture in small babies. There may also be misinterpretation of the role of coagulase-negative staphylococci (e.g., S. epidermidis), as these organisms are both normal skin flora and pathogenic organisms in preterms and infants with indwelling blood vessel catheters [54].

Automated blood culture systems have long been considered the gold standard for microbiological diagnosis. However, despite improvements in growth media and instrumentation, results of blood culture can be delayed by up to 48 hours [53,55]. The condition of a neonate with true sepsis can deteriorate quickly, thus the most common approach is to initiate empiric broad-spectrum antibiotic therapy in all young infants with suspected bacterial infection [49]. A negative blood culture after 48 hours may allow cessation of antibiotic therapy in a well infant. While appropriately cautious, this practice leads to antibiotic exposure in a large number of newborns for whom antibiotic treatment may be unnecessary since blood cultures are positive in only 5%–10% of suspected sepsis cases, even at highly resourced facilities [56].

Antigen detection techniques allow rapid detection and identification of microorganisms without culturing. The most commonly used commercially available test is the latex agglutination assay, which is based on specific agglutination by bacterial cell wall antigens of antibody-coated latex particles. However, these tests can only detect specific organisms such as S. agalactiae and are associated with high false positive and negative rates [57]. New urinary antigen tests for pneumococcus are more encouraging but are also associated with false positives from pneumococcal carriage [58].

The polymerase chain reaction (PCR) has been widely used in biomedical research laboratories for pathogen identification in neonatal sepsis and in some clinical hospital laboratories. The high sensitivity of PCR allows detection of bacterial DNA even when concentrations are low [57]. Conventional assays are being replaced by a newer “real-time” system, which is faster and associated with lower contamination rates because amplification and detection occur simultaneously in a closed system [59]. The real-time PCR is based on the measurement of a fluorescent signal generated during each amplification cycle. It produces quantitative results within 30 minutes and calculates bacterial load. Broad-range real-time PCR uses a single primer to detect the universal bacterial genome (16S RNA or 23S RNA) which is a conserved ribosomal genome sequence across all bacterial genera [60]. Broad-range real-time PCR can be used to distinguish bacterial septicemic disease from other causes of neonatal illness such as asphyxia or complications of prematurity. However, it has been used with varying success in the analysis of whole blood for neonatal sepsis; specificity is generally high but sensitivity can be as low as 40% [60,61]. In contrast, multiplex PCR involves the parallel amplification of different targets but is focused only on specific pathogens, and false negatives can occur if the aetiologic agent of interest is not included in the database [62]. Real-time PCR is now often used to screen for microbial load, followed by sequence-based targeting and identification of PCR amplicons (pyrosequencing) [62]. This process can detect very small copy numbers of specific nucleic acid sequences. There is also a new commercially available multiplex pyrosequencing PCR assay which can identify up to 40 different bacterial and fungal pathogens directly from whole blood [63]. Real-time PCR and pyrosequencing of the universal 23S rRNA gene has also recently been used successfully in neonatal blood culture samples [64]. Further tests on neonatal whole blood have been planned by a number of different research groups.

The biggest problem with real time PCR testing is that the specimen must be collected with a sterile venipuncture, which may be difficult in young neonates. Neonatal capillary heel prick specimens are easier to collect but highly contaminated by skin flora. There is also high potential for contamination of enrichment media, reagents, or the sample during collection and processing [61]. Other problems include low sensitivity due to competition from human DNA in whole blood, especially if white cell counts are high. Also, bacterial organisms require lysis before their DNA can be available for analysis, and gram-positive organisms are difficult to lyse because of their resilient cell wall [61]. Real-time PCR technologies are also expensive and currently can be used only by highly trained staff.

Important haematological tests include microscopic examination of the blood for white cells (total leucocyte count, differential, neutrophil count, and immature neutrophil to total neutrophil ratio). Advantages are that these specimens do not require sterility and a heel prick specimen can be used. However, many of these indices are falsely low in a septic neonate.

Biological biomarkers are human blood components that increase in response to infection. The most commonly used acute phase reactant is the C-reactive protein (CRP). However, the CRP takes 12–24 hours to increase to measurable levels; its half life is very long and it takes 3–7 days to normalize after eradication of the infective agent. Cytokines such as IL6, IL1, TNF-α, and procalcitonin have also been extensively studied [65,66]. Cytokines rise quickly after infection even in neonates, and are more sensitive to low concentra-

Table 1. Clinical symptoms and signs of severe neonatal illness including sepsis.

<table>
<thead>
<tr>
<th>Symptom</th>
<th>Description</th>
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<tbody>
<tr>
<td>History of difficulty feeding</td>
<td>Nonspecific but common in neonatal sepsis</td>
</tr>
<tr>
<td>History of convulsions</td>
<td>Nonspecific but common in neonatal sepsis</td>
</tr>
<tr>
<td>Movement only when stimulated</td>
<td>Common in neonatal sepsis</td>
</tr>
<tr>
<td>Respiratory rate</td>
<td>≥60 breaths per minute</td>
</tr>
<tr>
<td>Severe chest indrawing</td>
<td>Common in neonatal sepsis</td>
</tr>
<tr>
<td>Axillary temperature</td>
<td>≥37.5°C</td>
</tr>
<tr>
<td>Axillary temperature</td>
<td>&lt;35.5°C</td>
</tr>
</tbody>
</table>

From [49].

doi:10.1371/journal.pmed.1000213.t001
tions of pathogens than CRP [66]. However, cord and postnatal blood cytokine concentrations can be depressed in the presence of pregnancy-induced hypertension and can rise after induced vaginal or urgent cesarean delivery, delivery room intubation, muscular damage, and inflammation from other causes [57]. Simultaneous measurement of multiple biomarkers may improve both sensitivity and specificity [66,67]. However, biomarker assays are likely to be less acceptable to physicians who often place higher value on tests that confirm biological agents and allow targeting of antibiotic therapy [57].

Microtechnologies, especially microfluidics, have provided the greatest recent contribution to the diagnosis of neonatal sepsis. Microfluidics is the study of the behaviour, precise control, and manipulation of fluids geometrically constrained to submillimetre (nanolitre or picolitre) channels [68]. Microfluidic technology uses the unique properties of continuous flow micro-volume channels: viscosity, surface tension, energy dissipation, and fluidic resistance, and also includes micro pneumatic pump and valve systems. One specific application of microfluidics is bacterial DNA protein microarray hybridization [69]. In this test, DNA probes specific to selected targets are spotted on a glass or silicon slide in a known order. Target DNA fragments are labelled with a reporter molecule, combined into a single hybrid, and measured using fluorescent signals [62,68]. This technique has been used in the identification of the specific sepsis pathogen in bacterial meningitis, acute viral respiratory tract infections, and neonatal sepsis, and also in the detection of their antimicrobial resistance and virulence genes in research settings [63].

Microfluidic technology has also allowed sample preparation and a number of different assays to be combined in small, disposable, single-use diagnostic cartridges or cards that have been called a “lab on-a-chip” or LOC (Figure 2) [68]. Some LOCs have combined sample preparation, biomarkers, real-time PCR, and DNA microarrays to provide information about indices of inflammation, pathogen identification, and antimicrobial susceptibility patterns at the point of care [68,70]. LOCs have been reported to perform assays at sensitivity, specificity, and reproducibility levels similar to those of central laboratory analysers, but yet require little user input other than the insertion of the sample. Single drops of blood, faeces, and saliva have all been tested with encouraging results. LOCs are currently being evaluated for use in sepsis, endocarditis, HIV, tuberculosis, severe acute respiratory syndrome (SARS), and pneumonia [68]. However, they are not yet in clinical use nor licensed by regulatory authorities.

**Advances in Treatment**

As neonatal sepsis can be rapidly fatal if left untreated, highly effective antibiotic therapy must be used and delays in the provision of care must be minimised.

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*Figure 2. Example of “lab on a chip” point-of-care device.*

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Treatment must be effective against the causative pathogen, safe for the newborn, and feasible to deliver reliably in the hospital or community setting.

Parenteral (intravenous or intramuscular) regimens for neonatal sepsis currently recommended by national paediatric associations are a combination of penicillin/ampicillin and gentamicin, or third-generation cephalosporins (e.g., ceftriaxone or cefotaxime) for 10–14 days. These antibiotics are safe and retain efficacy when administered at extended intervals (e.g., twice daily or daily dosing) [56]. These regimens are very effective against Streptococcus spp., but Staphylococcus spp. can be highly resistant [71]. Gram-negative antimicrobial susceptibility to ampicillin and gentamicin can also be poor, especially for Klebsiella spp. [8,71]. Emerging E. coli resistance to ampicillin, gentamicin, and third-generation cephalosporins in hospital nurseries in both developed and developing countries is also causing increasing concern [8]. The potential for significant life-threatening toxicity among neonates associated with chloramphenicol makes it the least preferred empiric parenteral therapy [56].

Oral antibiotic therapy must be considered in settings where referral is not possible and there are no health care providers trained to give parenteral antibiotics [72]. The incremental benefit of injectable over oral antibiotics is not known, and oral antibiotic therapy is better than no antibiotic therapy at all. A series of trials are currently evaluating the impact of home and clinic-based short course (7 days) intramuscular and oral antibiotic therapy for neonatal sepsis in low-income countries [72]. Most data are available on the effect of oral cotrimoxazole in community-based treatment of serious neonatal bacterial infections from Nepal and India. However, there are concerns about high resistance rates, and side effects such as neonatal jaundice have been reported [71]. Oral amoxicillin is highly efficacious against Streptococcus spp. and some gram-negative bacilli and has an excellent safety record. However, it has no anti-Staphylococcus coverage and resistance is emerging in gram-negative bacilli such as E. coli. New, better-absorbed oral antibiotics are also being considered. The new second-generation cephalosporins (e.g., cefadroxil and cefuroxime) have an excellent safety profile, a spectrum of activity similar to cotrimoxazole, and may be more effective given the high resistance of neonatal pathogens to cotrimoxazole. Ciprofloxacin also is increasingly accepted as safe in neonates and warrants further investigation for treatment of infections in newborns. However, the current cost of these agents and potential for exacerbating antimicrobial resistance may limit widespread use in developing countries [72].

Poor maternal-neonatal health systems, low levels of care-seeking, and lack of access to sick newborns during the first day of life, when mortality risks are highest, are also important concerns.

### Table 2. Effective current measures and new approaches to prevent, diagnose, and treat neonatal sepsis.

<table>
<thead>
<tr>
<th>Category</th>
<th>Measure</th>
<th>Item</th>
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<tbody>
<tr>
<td>Prevention</td>
<td>Current measures</td>
<td>Improved maternal health and nutrition</td>
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<tr>
<td></td>
<td></td>
<td>Clean delivery practices and handwashing</td>
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<tr>
<td></td>
<td></td>
<td>Risk-based intrapartum antibiotic prophylaxis</td>
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<tr>
<td></td>
<td></td>
<td>Hand washing from health care providers</td>
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<tr>
<td></td>
<td></td>
<td>Promotion of early initiation of exclusive breastfeeding</td>
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<tr>
<td></td>
<td>New approaches</td>
<td>Maternal S. agalactiae and S. pneumoniae immunisation</td>
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<td></td>
<td></td>
<td>Maternal vaginal chlorhexidine other antisepsis preparations</td>
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<tr>
<td></td>
<td></td>
<td>Neonatal protective and antisepsis skin preparations</td>
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<tr>
<td></td>
<td></td>
<td>Neonatal vitamin A supplementation</td>
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<td></td>
<td></td>
<td>Recombinant active antimicrobial proteins</td>
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<td></td>
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<td>Toll like receptor agonists</td>
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<tr>
<td>Diagnosis</td>
<td>Current measures</td>
<td>Blood culture</td>
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<td></td>
<td></td>
<td>Antigen detection</td>
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<td></td>
<td></td>
<td>Blood neutrophil count and differential</td>
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<td></td>
<td></td>
<td>C-reactive protein</td>
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<td></td>
<td>New approaches</td>
<td>Proteomic amniotic fluid analysis</td>
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<tr>
<td></td>
<td></td>
<td>Improved clinical syndrome identification</td>
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<td></td>
<td></td>
<td>Real-time polymerase chain reaction</td>
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<td></td>
<td></td>
<td>Interleukin inflammatory indices</td>
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<td></td>
<td></td>
<td>Microfluidic microtechnologies</td>
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<tr>
<td></td>
<td></td>
<td>“Lab on a chip” point of care devices</td>
</tr>
<tr>
<td>Treatment</td>
<td>Current measures</td>
<td>Parenteral antibiotics (penicillin/amoxycillin and gentamicin or third generation cephalosporins) for 10–14 d</td>
</tr>
<tr>
<td></td>
<td>New approaches</td>
<td>Shorter courses of antibiotic therapy</td>
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<tr>
<td></td>
<td></td>
<td>Better-absorbed oral antibiotics especially second generation cephalosporins and ciprofloxacin</td>
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<tr>
<td></td>
<td></td>
<td>Programs to increase the access of neonates in remote areas to health care providers in the first days of life</td>
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doi:10.1371/journal.pmed.1000213.t002
Recent studies have shown that community health workers can deliver antibiotic treatment to neonates with very severe infections at home safely and acceptably when hospitalization is not feasible [74]. Trials are currently evaluating the effectiveness, quality of care, and coverage of these community health worker programmes in Asia and Africa [73]. Barriers to large-scale implementation include high cost, poor staff training and retention, and difficulties with referral (e.g., lack of ambulances and poor institutional links).

Summary and Next Steps

Newborn sepsis is a major cause of child mortality across the world. Industrialized countries have made remarkable progress in reducing newborn sepsis and sepsis-related mortality by providing access to hygienic skilled delivery for all women, risk-based intrapartum antibiotic prophylaxis, and high-quality intensive care for newborns that need it. Although resource constraints preclude whole-scale adoption of these strategies in developing countries, there are a number of low-cost proven interventions and promising approaches that have the potential to significantly reduce the burden of neonatal sepsis worldwide (Table 2).

However, practicability of implementing these new advances must be considered. Skilled attendance at delivery is increasing in low- and middle-income countries. Thus, intrapartum approaches such as risk-based antibiotic prophylaxis and improved hand washing during delivery are likely to be both cost-effective and feasible in these countries. More challenges face the implementation of diagnostic technologies. It may take many years for technologies such as the “lab-on-a-chip” to be sufficiently robust and affordable for scale-up to low-income countries. Home-based antibiotic treatment of neonatal sepsis also faces major obstacles to large-scale implementation. Concerns such as “one law for the rich and another for the poor” have already been raised. A careful assessment of the risks and benefits of new technologies and interventions is clearly needed. In low-income settings there are also difficulties with care-seeking for neonatal illnesses, and home visiting programs are needed to identify sick newborns early in life. Neonatal sepsis is also one of the most rapidly fulminating clinical diseases, and many practitioners, including experienced neonatologists, administer parenteral antibiotics rather than wait for the results of any diagnostic tests. These practitioners rightly consider that the individual patient’s health is more important than the potential risks of emerging antibiotic resistance.

Thus, front-line health workers and families must be partners in all research and evaluation planning. Detailed assessment of end-user attitudes and preferences using formative and qualitative research methods must be included in the development of programs to reduce morbidity and mortality from neonatal sepsis. Finally, advocacy for equitable resource allocation across and within countries must be a priority and modelling techniques to assess public health impact of neonatal sepsis interventions must be developed and used more widely.

Author Contributions

ICMJE criteria for authorship read and met: KE AZ. Agree with the manuscript’s results and conclusions: KE AZ. Designed the experiments/study: KE AZ. Analyzed the data: KE AZ. Collected data/did experiments for the study: KE AZ. Wrote the first draft of the paper: KE. Contributed to the writing of the paper: KE AZ.

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