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ARTICLE

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ANTIBIOTIC RESISTANCE AND NEONATAL SEPSIS IN INDIA: A STORM IN THE CRADLE

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Neonatal sepsis is a major cause of neonatal death in India. Treatment of sepsis is often challenging due to limitation of antibiotic choices and emergence of drug-resistant infections, further narrowing treatment options in this vulnerable population. This review highlights the different factors contributing to sepsis and techniques for its diagnosis, importance of antibiotics, and mechanisms of antibiotic resistance. Along with it, government policies to curb neonatal mortality and updated treatment guidelines for saving newborn lives are also discussed here.

"There can be no keener revelation of a society's soul than the way in which it treats its children."

- Nelson Mandela

The birth of a child is one of the happiest moments for any family and the period just after birth is also the most critical. The first 28 days of life is known as the neonatal period and it is at this time when the newborn is at its most vulnerable[1]. Newborn deaths can be due to many reasons such as preterm birth complications, suffocation during birth, and infection[2]. When infection causing germs like bacteria or fungi reach the bloodstream of the newborn, it is known as neonatal sepsis[1]. A large proportion of newborns particularly in resource-poor settings die due to sepsis. To combat sepsis in neonates, antibiotics emerged as a potent weapon. However, here arises the next level of complexity. First, not all antibiotics can be administered to neonates and second, antibiotic resistance has limited the treatment options. Antibiotic/ antimicrobial resistance (AMR) has gained recognition as a major public health issue[3]. Bacteria are progressively becoming resistant to antibiotics leading to the emergence of antibiotic-resistant bacteria or 'Superbugs'[3]. Bugs have devised techniques to evade the different antibiotics such as enzymes to degrade them, pumps to pump out antibiotics or they even change themselves so that antibiotics cannot enter them[4]. This means that standard treatments no longer work; the risk of the spread of infection to others is increased; hospital stays are prolonged, economic burden increases; and the risk of death is greater. Uncontrolled use and the misuse of antibiotics in human and animal sector has also compounded the problem of AMR[5,6].

This review discusses the factors contributing to neonatal sepsis, the bacteria that cause neonatal sepsis, the importance of antibiotics and the mechanisms by which the bacteria can evade antibiotics. It also highlights, government policies for saving newborn lives, newer techniques for diagnosis of sepsis and updated treatment policies. The ultimate aim remains reduction in number of deaths due to neonatal sepsis, for which it is essential to curb the menace of AMR.

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Antibiotics, their Lost Glory and Usage in India

Antibiotics are among the most common medicines consumed in India[7]. They are used to treat different types of infection. Some common antibiotics are penicillin, azithromycin, ciprofloxacin, gentamicin and cefotaxime. Antibiotics work on a particular type of germ called bacteria. However, they are also frequently and incorrectly used in other types of infection where they have no role, for e.g. viral infections.

India is the largest consumer of antibiotics in the world[7]. In fact, India is one of the leading manufacturers as well, and well on its way to become the world's pharmaceutical hub. Antibiotics are easily available, and are often sold over the counter without the mandatory requirement of prescription. This is dangerous as the increased consumption of antibiotics can lead to increased resistance. Antibiotic resistance refers to the ability of microorganisms to withstand the effects of an antibiotic[8]. Bacteria are said to develop resistance when they are no longer inhibited or killed by a given antibiotic. Put simply, this means that the antibiotic may not work and will not cure the infection it is actually meant to. The danger is that there are very few new antibiotics being discovered or developed. Bacteria causing common infections already have high levels of resistance to the existing arsenal of available antibiotics. This can lead to a situation where infections become simply untreatable– a return to the pre-antibiotic era. AMR is a global challenge and the World Health Organization (WHO) has declared AMR as one of the top 10 global public health threats[3].

In 2019, globally 4.95 million people suffered from drug-resistant infections, of which 1.27 million died of AMR[3]. In India (2019), 2.97 million deaths were attributable to AMR, while 10.42 million deaths were associated with AMR. It is estimated that deaths due to AMR in India is higher compared to deaths due to other diseases such as cancer, respiratory infections, tuberculosis, enteric diseases, diabetes, maternal and neonatal[9]. According to One Health Trust (formerly known as Centre for Disease Dynamics, Economics & Policy-CDDEP), in 2015, drug resistance index (DRI), was found to be quite high in India (DRI: 71) compared to USA (DRI: 13), and Europe (DRI: 27-56)[10].

AMR is increasing due to two major factors- increased use of antibiotics in human and spread of antibiotic-resistant bacteria during travel across the globe. Use of antibiotics in animal sector, poor infection control measures in healthcare setting, lack of awareness about hygiene, sanitation and disposal of medical and pharmaceutical wastes leading to environmental contamination are other major contributors to increased AMR[11,12]. Absence of accurate diagnosis of infection, shortage of effective vaccines and improper uptake of vaccines due to lack of awareness accentuate the problem of AMR manifold

The WHO has classified antibiotics as AWaRe- ACCESS, WATCH and RESERVE, which is based on the impact on antibiotic resistance[13]. It means that doctors and patients need to be alert about three groups of antibiotics- those which can be readily used (ACCESS- e.g. amoxicillin, amoxicillin-clavulanic acid for ear infections), those which have to be used with care and monitored by health authorities for resistance (WATCH- e.g. Clarithromycin for sore throat; ciprofloxacin for kidney infections) and those which need to be used very sparingly, as a last resort for life threatening infections (RESERVE-e.g. carbapanem for sepsis). Antibiotics in ACCESS group are usually taken orally (60%) while fewer oral antibiotics are available in the WATCH (40%) and the RESERVE (10%) group[13] (Fig. 1).

Recent studies have provided insights into the pattern of antibiotic consumption in India[7,14]. In a nutshell, there is increase in the usage of oral antibiotics in India, with disproportionately high increase in liquid antibiotic consumption. This means that there is higher usage of antibiotics in children. There is also an increase in usage of injectable antibiotics, which means higher usage in hospitals.

The share of RESERVE group antibiotics increased while there was a small decline in the use of the Access Group. There was also an increase in consumption of new generation antibiotics compared to older ones.

India in 2015, was estimated to have a consumption of 4950 DDD (Defined daily dose) per 1000 population in hospital/retail sector[15]. In a survey of 20 tertiary care hospitals across India in 2021-22, antibiotic usage was observed in an average of 72% of hospital admissions, varying between 37% to 100 % at individual sites. There was more than 85% usage of injectable antibiotics. A cause of concern is that more than 50% of the prescribed antibiotics belonged to the WATCH category[16].

Consumption pattern of antibiotics in the private sector varied across the country[14]. For e.g., Delhi followed by Punjab were in the lead while Madhya Pradesh and Bihar were the lowest consumers. There was some good news with Kerala actively reducing the use of unapproved fixed combinations of antibiotics[14]. A silver lining is that national consumption in the private sector is falling since

2016 and Delhi and Tamil Nadu have reduced the use of antibiotics by nearly a third.

Newborn Lives and How India Plans to Take Care of Them

Every year nearly 26 million babies are born in India[17]. Of these, 1.2 million neonates die in the first 28 days or the neonatal period[18]. Neonatal mortality rate (NMR) in India was 20 deaths/ 1000 live births in 2020[19] which declined to 18 deaths/ 1000 live births in 2022[20] (Fig. 2). Despite ardent efforts, the vulnerable newborn is at high risk of being sick or dying in the neonatal period. The risk of death during this time is around 30-fold higher than in the later stages of infancy[1].

A UNICEF report clearly states that though enormous progress has been made in improving child survival, the neonate requires more attention. The rate of mortality in children up to five years (under-five mortality) has seen a steady decline[20], from 93 deaths/1000 live births in 1990 to 37 deaths/ 1000 live births in 2022[21]. Till 2022, NMR was high in Low- and Middle-Income countries (LMICs) such as different African nations, Pakistan, India, Bangladesh, etc[22]. Although, India has successfully reduced NMR, the rate of decline in NMR (64%) is less compared to infant mortality (70%) and under-five mortality (74%), which is also a global trend[23]. Of all the deaths that occur in under-five children, the majority (47%) die in the neonatal period[24]. It has thus been recognised that for reduction of under-five mortality, newborns should be prioritised.

Major contributors of neonatal mortality are prematurity and low birth weight, suffocation and trauma during birth, and neonatal pneumonia followed by infections (sepsis, diarrhoea, etc.), and congenital anomalies[17]. Preterm neonates get inadequate time in the uterus resulting in low transfer of immunoglobulins from mother. As a result, they are unable to produce mature immune cells to carry out complete immune response. Apart from these, several maternal conditions also play an important role in neonatal mortality. In India, hypertensive disorder like pre-eclampsia and eclampsia in the mother contribute to 33% of neonatal deaths followed by maternal or fetal vascular malperfusion (loss of blood supply to vital organs) (35%), intrauterine infections (19%) and preterm labour (14%)[25].

In line with the sustainable development goal (SDG) goal 3.2, India proposes to reduce neonatal mortality to 12/ 1000 live births by 2030[26]. Though India has been successful in reducing NMR, the results are not similar across the different states or districts of India. Only six of 28 states in India, (Kerala, Delhi, Tamil Nadu, Maharashtra, Jammu & Kashmir and Punjab) have been successful in attaining the SDG target for NMR[19]. NMR in urban areas is lower than in rural (12 deaths vs 23 deaths/ 1000 livebirths)[19]. It is estimated that India might fail to meet the SDG target as the NMR in certain states like Madhya Pradesh (33 deaths/ 1,000 livebirths), Bihar (24 deaths/ 1,000 livebirths), Rajasthan (25 deaths/ 1,000 livebirths), Uttar Pradesh (35 deaths/ 1,000 livebirths), etc. are still high[26].

India has progressed in minimizing neonatal death by implementation of certain action plans proposed by Ministry of Health and Family Welfare, India. India Newborn Action Plan (INAP) released on 2014 is one such plan which has provided insights and measures to ensure reduction in NMR[17]. INAP aims to attain a single digit NMR by 2030. It also focused in minimizing preventable deaths in neonates, improvement of quality of neonatal care, prioritization of preterm neonates, and provision of infrastructure for states/districts. The National Health Mission (NHM)[17] is also committed to reduce NMR (Fig. 2).

It has been realised that to overcome these problems and ensure a healthy life for neonates, care should be continuous and effective. This care includes complete physical examination, vaccination, adequate and exclusive breastfeeding, hygiene of the neonates, administration of prophylactic medications and evaluation of the need for resuscitation etc[1]. Antenatal care (ANC) or care for the mother during pregnancy is another way to improve neonatal health and curb neonatal mortality. WHO recommends at least four ANC visits during pregnancy. Scrutinizing maternal health, nutrition, infections, and immunization against tetanus have been found to significantly reduce neonatal morbidity and mortality[27]. However, as is the case with newborn care in India, ANC continues to be relatively inequitable, particularly between states and districts. Apart from maternal health, awareness relating to after birth care of neonates has also found to decrease neonatal mortality. UNICEF (2016-2022) reported that the lowest level of ANC is observed in sub-Saharan Africa and South Asia which probably culminated into poor neonatal health[28]. In order to ensure survival and allow a child thrive to their full potential, improvement of neonatal and maternal health is extremely important. A healthy mother is a prelude to a healthy newborn.

Understanding the Importance of Neonatal Sepsis and its Diagnosis-

When germs such as bacteria reach the bloodstream,

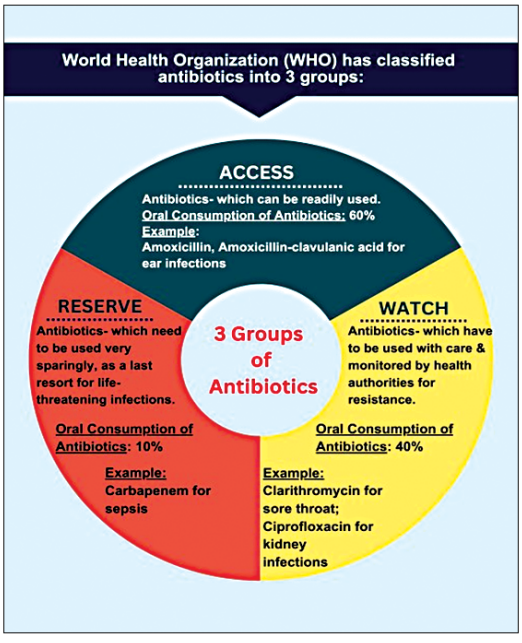

Fig. 1: WHO's AWaRe categorization of antibiotics.

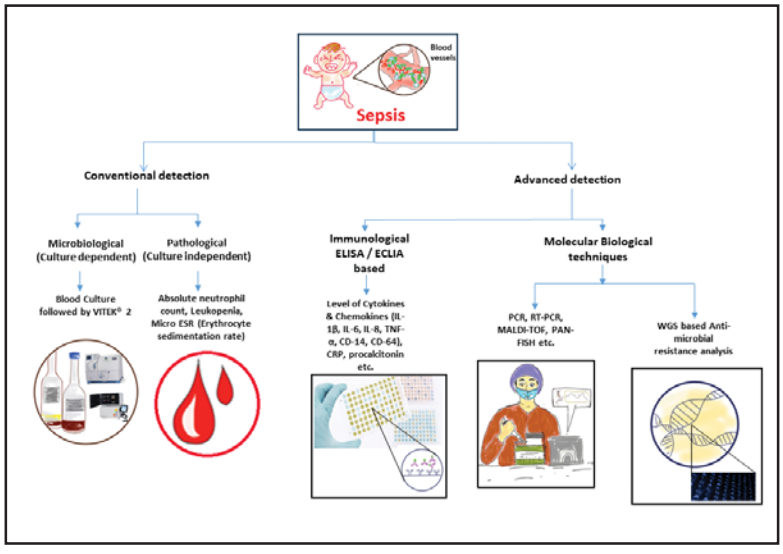

Fig. 3: Techniques and tools used for detection of neonatal sepsis.

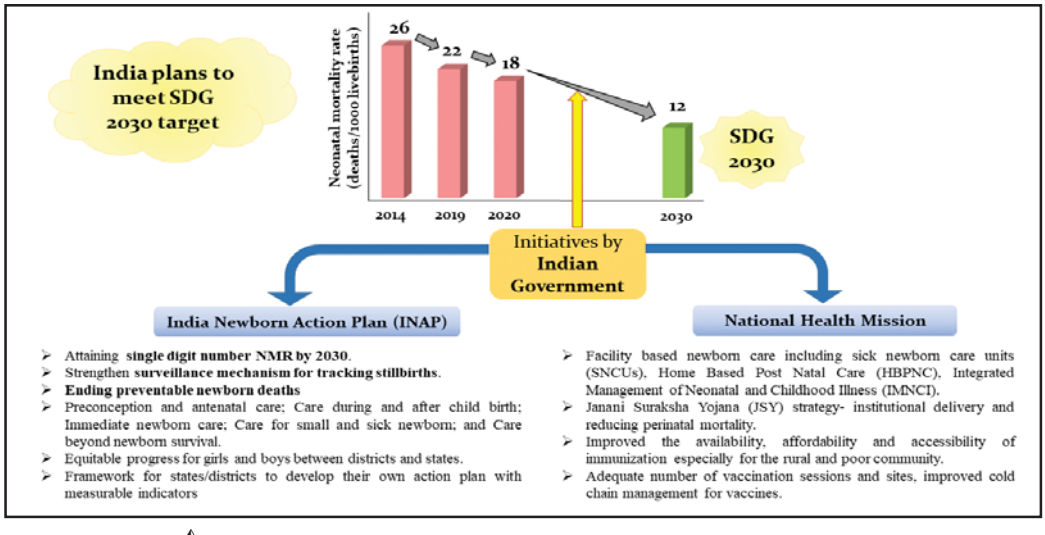

Fig. 2: Declining neonatal mortality rate in India and initiatives undertaken by Indian Government to meet SDG 2030 goal.

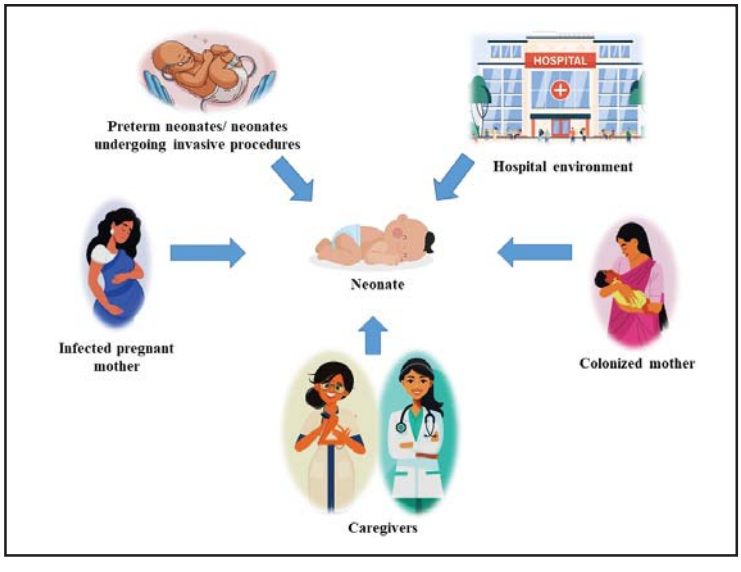

Fig. 4: Different routes for entry of germs causing neonatal sepsis.

they trigger an immune response in the entire body. This phenomenon in newborn is known as neonatal sepsis. Bacterial infection is the primary cause of neonatal sepsis but fungal, parasitic and viral infections can also lead to sepsis. Sepsis is the second major cause of neonatal mortality especially in LMICs[29]. More than a million neonates die every year due to sepsis[30]. Nearly 20 million of sepsis cases worldwide have been reported in children under 5 years of age[31]. A decline in the rate of neonatal sepsis from 111 to 19 per 1000 live births (1998 to 2019) was noted in India[32]. Despite this reduction, sepsis is responsible for 5.7% of neonatal deaths in India during 2015-2017[33].

Depending on the time of manifestation, neonatal sepsis can be divided into - i) Early-onset sepsis (EOS)- which occurs within the first 72 hours of life, and ii) Late onset sepsis (LOS)- sepsis which occurs after 72 hours of life. EOS is generally caused by the transmission of pathogen from mother's genito-urinary system to neonate at the time delivery. If a mother is suffering from urinary tract infections, pathogens can ascend to the uterus via the birth canal and infect the amniotic fluid. The neonate may also become infected by the vaginal pathogen, at the time of normal delivery. Several maternal factors such as infection of amniotic sac, or its rupture well before labor begins), and premature delivery before 37 weeks of gestation increases chance of sepsis in neonates[34].

LOS, on the other hand, is generally acquired by transmission from hospital or community environments via caregivers. Neonates admitted to intensive care units under many invasive procedures like ventilation, tube feeding, catheterization are also at risk of developing LOS[29]. Certain maternal and neonatal risk factors also predispose neonates to sepsis (Table 1).

Sepsis in neonates is difficult to diagnose as the signs of sepsis can be similar to other life threatening diseases and are non-specific. Sepsis is categorised as 'blood culture positive sepsis' or 'clinically suspected sepsis'. The growth of microorganisms in the blood in a laboratory culture is known as- 'blood culture positive sepsis'. Though blood culture remains the gold-standard for confirmation of sepsis, it is also fraught with difficulties because of the small amounts of blood that can be drawn from newborns as well as low bacterial load. When the blood culture does not yield organisms, but the baby shows clinical signs and symptoms such as fever, poor sucking, respiratory distress, lethargy, brady/tachycardia, metabolic acidosis, etc., it is categorised as 'clinically suspected sepsis'[36,37].

The treatment of sepsis in neonates is challenging. It demands quick diagnosis and immediate treatment. The first step to diagnosis often depends on the observation by the clinicians/ caregivers. This is followed by the laboratory detection of sepsis - i) conventional or ii) non-conventional methods[38] (Fig. 3). Conventional methods include both culture-based and culture-independent techniques. Culture-based techniques involve use of automated microbial detection system/ blood culture in incubators followed by identification of bacteria in an automated identification system/ or conventional identification tests. Culture-based method is considered the gold standard for diagnosis of sepsis and should be done in all cases of suspected sepsis. But the turnover time for this method is generally high (24-72 hrs). Culture-independent techniques involve assessment of certain haematological parameters such as absolute neutrophil count of <1800 per cmm, along with leukopenia (total leucocyte count <5,000 /cmm, immature neutrophil to total neutrophil, I/T ratio >0.2), micro ESR (>10 mm in 1st hour).

Table 1: Risk factors associated with neonatal sepsis[35].

Maternal Factors	Neonatal Factors
Fever within 2 weeks prior to delivery.	Perinatal asphyxia (Apgar score <4 at 1 minute or age) or difficult resuscitation.
Intrapartum antibiotic prophylaxis	Low birth weight, preterm and gender (male neonates are at increased risk of neonatal sepsis)
Foul smelling and/ or meconium stained amniotic fluid.	Poor hygiene, poor cord care.
Prolonged rupture of membranes >18 hours (for LOS) and >24 hours (for EOS).	Prolonged hospitalization, mechanical ventilation.
More than 3 vaginal examinations during labour.	Use of invasive procedures and devices (i.e., intravascular catheters and endotracheal Tubes).
Prolonged and difficult delivery with instrumentation.	Bottle-feeding and prelacteal feeds.

Abbreviation: Early onset sepsis (EOS), late onset sepsis (LOS)

Apart from haematological parameters, certain hormones such as procalcitonin (>1 ng/ml) and protein such as C-reactive protein (>1 mg/dl) are proposed as an important marker of sepsis[38] (Fig. 3). Sensitivity of these tests are around 99%, but these parameters can only suspect the occurrence of sepsis along with the clinical assessment of the signs and symptoms. As the condition of the neonate can deteriorate quickly after the onset of sepsis, a quick diagnosis can go a long way in saving lives and reducing the unnecessary use of antibiotics.

Some new methods or non-conventional methods for diagnosis of sepsis have come up. Some are immunological assays targeting cytokines & chemokines (IL-1β, IL-6, IL-8, TNF-α, CD-14, CD-64) (Fig. 3). Molecular biology techniques involving nucleic acid-based identification such as Fluorescence *in situ* hybridization using peptide nucleic acid probes (PNA-FISH), Matrix-assisted laser desorption/ ionization time-of-flight (MALDI-TOF), real time Polymerase Chain Reaction (RT-PCR), PCR coupled mass spectrophotometry[38,39] (Fig. 3). assess the presence of certain bacteria directly from patient blood. These estimations are highly sensitive and can detect pathogens even in culture negative samples. Bacteria isolated from blood are sometimes processed for whole genome sequencing (WGS)[38]. This can directly assess genes involved in AMR to be tracked. All these non-conventional techniques are used in research labs only, and are yet to be introduced in diagnostic labs for detection of sepsis.

It has been acknowledged that no marker alone can provide correct prediction of sepsis. Hence, multiple markers are considered together by the clinicians to decide upon the treatment of sepsis. In many medical facilities in LMICs, blood culture systems are scarce, and the capability to assess cytokines or perform PCRs is generally non-existent. Hence, clinicians and pathologists have to depend on haematological parameters and clinical signs to screen sepsis among neonates to initiate treatment procedures. Along with these parameters, assessment of risk factors for neonatal sepsis should also be taken into consideration as this will help in determining an ideal treatment regime for the neonates.

Recognizing the Varied Bugs that Cause Neonatal Sepsis-

There are many routes for the entry of microorganisms in the neonate (Fig. 4). As neonates have an under-developed immune system they cannot fight back against the entering germs and are thus vulnerable to sepsis.

Neonatal sepsis can occur due to bacteria (Gram-positive & Gram-negative bacteria) as well as fungus. Group B Streptococcus (GBS), *Staphylococcus aureus*, coagulase-negative Staphylococci (CoNS), *Listeria monocytogenes*, *Enterococcus* sp., etc. are the Gram-positive bacteria (GPB) causing neonatal sepsis. Among the Gram-negative bacteria (GNB), *Klebsiella pneumoniae, Escherichia coli, Acinetobacter baumannii, Haemophilus influenza, Enterobacter* sp., *Pseudomonas* sp., *Serratia* sp., etc. are the dominant pathogens that cause neonatal sepsis[40]. WHO has published a 'priority pathogen list' of ESKAPE organisms (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa*, and *Enterobacter* spp.) which are considered as 'critical' organisms due to their multidrug-resistant (MDR) nature and ability to cause serious infections. *K. pneumoniae* and *E. coli* are considered as the important MDR bacteria causing sepsis globally.

Depending on the time, location, onset of sepsis, duration of hospitalization, healthcare facilities, place of delivery (hospital or home deliveries) and lifestyle, the causative organism may vary. A difference in bacterial profile is often noticed in high-income countries (HICs) and LMICs. In HICs, neonatal sepsis is mainly mediated by GPB such as Group B Streptococcus (GBS), *Listeria* sp., *S. aureus*, *Enterococcus* sp., *Streptococcus* sp. followed by GNB (*E. coli*, *H. influenza*, *Enterobacter* spp.)[34,40] while in LMICs, it is mainly caused by GNB (*K. pneumoniae*, *E. coli*, *A. baumannii*, *Enterobacter* spp., etc.) followed by some GPB (*S. aureus*, *Streptococcus pyogenes*, etc.)[40,41]. Bacterial profile varies even in a single country such as in different zones of India, prevalence of certain bacteria is seen. *E. coli* were more prevalently found in isolates from Southern India[42], while Staphylococcus was common in the North India. Higher prevalence of Acinetobacter was noted in the Central and Northeast zones. Contrary to this, *Klebsiella* sp., *P. aeruginosa*, and Enterococcus were uniformly distributed across all the zones[43].

Bacterial profile often varies based on when the sepsis is occurring i.e. EOS or LOS. It has been noted that bacteria causing EOS are generally derived from mothers and responds well to antibiotic treatment indicating that the bacteria are susceptible to antibiotics. On the other hand, bacteria causing LOS are mostly acquired from community, hospital environment, caregivers and sometimes from colonized mothers. These bacteria have been found to be resistant to different antibiotics[44]. GBS and *E. coli* are the dominant EOS pathogens while CoNS are dominant during LOS of HICs[40]. In LMICs,

occurrence of *K. pneumoniae*, *S. aureus*, and *A. baumannii* in the EOS is well evident, while LOS generally showed prevalence of *K. pneumoniae* and CoNS[45]. However, several studies from LMICs reported incidences of EOS and LOS by similar causative agents[44,46]. Gram-negative bacteria are the prime causative agents of EOS and LOS in LMICs such as India[46].

The Ways by Which Bacteria Evade Antibiotics: Mechanisms of Antimicrobial Resistance-

The emergence of germs that are not killed by antibiotics has compounded the problem of effectively treating neonatal sepsis. Despite diverse modes of action, antibiotics are becoming futile in treating infections. With each generation, bacteria evolve to survive in antibiotic-loaded environment. In order to do so, bacteria develop certain machineries which help it to evade the damaging effect of antibiotics[4]. Some bacteria are resistant to multiple antibiotics and are commonly referred to as MDR bacteria[47].

Bacteria can develop resistance in many ways, be it chromosomal (changes in its own hereditary material) or acquired (genes acquired from other bacteria which can lead to antimicrobial resistance). To avoid the detrimental effect of antibiotics, bacteria use different strategies such as (i) drug target-site (binding site of drug) modifications to decrease the affinity for the drug, (ii) activation of multidrug efflux pumps to expel out the antibiotics, (iii) reduction of drug uptake by outer membrane impermeability (drug cannot enter the bacterial cell), and (iv) inactivation or alteration of drug by the production of different enzymes (Fig. 5). The first three mechanisms are generally due to chromosomal genes and the last mechanism is due to acquired genes from other bacteria[4].

i. Target-site modification- Antibiotics such as penicillin, cephalosporin, carbapenem, etc. target the bacterial cell wall and interrupts the cell wall formation (Fig. 5). Cationic antibiotics such as colistin bind with the surface molecules and destabilize bacterial membrane. Both mechanisms lead to bacterial cell death. However, bacteria can modify cell surface molecules leading to antibiotic resistance[48].

   Some antibiotics (fluoroquinolones like ciprofloxacin) can enter the bacteria and target proteins (enzymes) necessary for replication of bacterial DNA. They bind with DNA gyrase & topoisomerase IV (proteins necessary for DNA replication) and halt replication. To escape this activity of antibiotics, bacteria modify genes responsible for producing DNA gyrase and topoisomerase IV, respectively (*gyrA-gyrB* and *parC-parE*). As a result, now the antibiotic is unable to bind to these enzymes resulting in resistance[49].

ii. Activation of efflux pumps- Bacterial membrane contains different types of efflux pumps which can pump out antibiotics from bacteria (Fig. 5). Resistance-nodulation-division family efflux pump (RND) is the most prevalent ones[50] found in bacteria. Studies have shown that RND pump can pump out many antibiotics such as carbapenems, fluoroquinolones, colistin, etc. AcrAB-TolC pump is a prominent RND pump, composed of 3 protein units (AcrA, AcrB & TolC). Antibiotics entering the bacteria are pumped out through these pumps, so that the bacteria avoid the harmful effect of antibiotics.

iii. Outer membrane impermeability- Other than efflux pumps, bacterial membrane contains numerous gate-like protein called porins, which form channels to allow entry of antibiotics into the bacterial cell. Permeability of a bacterial membrane depends on the presence of these porins. By reducing the number of porins or by modifying them (by mutation), bacteria restrict the entry of antibiotics, resulting in resistance (Fig. 5). Research on porins have delineated certain porins such as OmpK35 and OmpK36 found in *K. pneumoniae* to be associated with carbapenem resistance[51].

iv. Drug inactivating enzymes- One of the most important mechanisms of resistance is the presence of enzymes that can degrade the respective antibiotics (Fig. 5). The above mechanisms of resistance are chiefly mediated by chromosomal genes but bacteria can acquire certain genes from other bacteria via plasmids and develop resistance towards certain group of antibiotics. These genes produce enzymes (β-lactamases) capable of hydrolysing β-lactam antibiotics (having a 4-carbon membered β-lactam ring at their active site) used for treating bacterial infections. They permanently degrade the antibiotics[52]. Till date different types of β-lactamases have been identified, such as extended spectrum β-lactamases (ESBLs) including $bla_{\text{CTX-M}}$ & AmpC-type cephalosporinases, carbapenemases

($bla_{NDM,KPC,IMI}$ etc), and oxacillinases ($bla_{OXA-1,23,24,48,54}$ etc.). β-lactamases are the major point of concern due to their association with plasmids indicating their tendency to spread within the bacterial community.

Bacteria use these resistance mechanisms in different combinations to evade the effect of antibiotics. Spread of resistance can be achieved by two ways- horizontal gene transfer (HGT) (transfer of DNA from one bacteria to other) and vertical gene transfer (VGT) (transfer of DNA from mother to daughter bacteria only)[53]. HGT or conjugation, is a biological process of exchanging genetic material within bacterial community. Different types of mobile genetic elements (MGEs) such as plasmids, transposons, integrons, etc. help in this genetic exchange[54]. Out of these MGEs, plasmid (genetic material other than chromosome) plays an important role in transmission of resistance. Plasmids bear many resistance genes which is readily transferred to other bacteria by HGT.

Antibiotics in Neonatal Treatment, Treatment Procedure and Empirical Therapy-

Treatment of neonatal sepsis is a major challenge due to several factors. This includes the clinical picture, which can often be misleading; the vast array of causative bacteria which are usually antibiotic resistant, and the delicate and vulnerable nature of the neonate itself. Antibiotics remain the primary treatment along with supportive therapy[2]. The choice of antibiotics largely depends on the particular bacteria which causes the infection, its response to different antibiotics, at what age the infection occurred, and the availability of laboratory results. Clinicians often start with empirical antibiotic therapy even before a confirmed blood culture report based on clinical symptoms. This therapy is often based on the diversity of pathogenic bacteria and their resistance profiles frequently identified in the given NICU or in the community settings.

For neonatal sepsis, the WHO recommends hospitalization and use of a combination of injections of gentamicin and benzylpenicillin or ampicillin for treatment, for at least 7–10 days. When referral to a hospital is not possible, injection of gentamicin and oral amoxicillin is recommended[13]. However, the success of the treatment depends on the causative bacteria actually responding to the treatment choices. With the emergence of antimicrobial resistance in bacteria causing neonatal sepsis, the recommended antibiotics often do not work at all, forcing the treating clinicians to opt for higher grade antibiotics in a bid to control the infection.

Nearly half of the pathogens causing neonatal sepsis are resistant to 1st and 2nd line antibiotics in neonates from LMICs such as India. They have been found to be highly resistant to the WHO recommended antibiotics (gentamicin)[55]. A multicentre study involving neonates from LMICs have shown 97%, 95%, 80%, and 60% resistance rates to ampicillin, amoxicillin, ceftriaxone, and gentamicin, respectively, in Gram-negative bacteria causing neonatal sepsis[55,56]. A study from India portrayed a similar finding with more than 56% GNB to be resistant to three or more classes of broad-spectrum antibiotics[57]. In GNB, resistance towards gentamicin has increased considerably compared to the past when dosing recommendations were developed. Studies have proposed to discontinue the use of ampicillin to treat neonatal sepsis in LMICs[56,58]. Hence, it is clear that re-evaluation of the current WHO-recommended regimen for neonatal sepsis is needed, along with an alternative first-line empiric regimen.

One way to circumvent this problem is the use of combination therapy. The use of novel antibiotics like avibactam with ceftazidime; vaborbactam with meropenem or the use of plazomicin have been effective against highly resistant strains of bacteria like *K. pneumonia*e. The drawback is that these antibiotics belong to the RESERVE group for which detailed clinical data are not available as yet[59].

The use of antimicrobials in newborns is fraught with several risks[60]. These are due to the side effects which are exaggerated in this age group because of the immaturity of the vital organs. So, the antibiotics stay longer in the body and are not broken down or removed as they would be in older children or adults. The dose has to be carefully adjusted and often doctors need to give lower doses to avoid toxicity., for e g with gentamicin and amikacin, which cause hearing loss. The flip side is that this dose may actually not be effective and may not kill the bacteria as it is supposed to.

In 2022, the Indian Academy of Pediatrics came out with Standard Treatment Guidelines for neonatal sepsis[61] (Fig. 6). It is important to note that the guidelines emphasize the need to obtain relevant local data regarding newborn infection. This is because of local variation in the pattern of bacteria causing neonatal sepsis as well as the different patterns of antibiotic resistance. Therefore, it is not possible to recommend a single antibiotic policy for use in all newborn units across the country. There are no one size fits all; as shown by studies from different parts of India.

The first line of antibiotics is the same as the WHO recommendation. The second line antibiotics recommended

are piperacillin-tazobactam (which is a variety of penicillin combined with a molecule which prevents the enzymatic destruction of the antibiotic itself) and amikacin (a higher-grade antibiotic of the same family as gentamicin). Cephalosporins are to be given along with amikacin, only if it is confirmed in the laboratory that there is meningitis (the coverings of the brain itself are infected). If this does not work, it is recommended to change the cephalosporin to a reserve antibiotic- meropenem.

Future Steps to Curb AMR in Neonates- The Way Forward

Antimicrobial resistance is with us to stay. Serious efforts are needed to combat this important threat to our healthcare system. All sectors of the community need to be involved in this. Some of the key measures which can be adapted are highlighted in Fig. 7.

1. Educational awareness programs involving the media, health professional groups and consumer groups with adequate communication and co-ordination are essential to ensure success. This is also highlighted as Strategic Priority 1 in India's National Action Plan on AMR.

   A recent example is artwork at Khan Market, New Delhi, to raise public awareness about this silent pandemic. The mural was put up by students who learnt about the key points of this pressing problem from experts at an AMR awareness workshop (times of india.com) (Fig. 8).

2. In the arena of public health, measures such as WASH (Water, sanitation and hygiene) practices and vaccinations are commonly used to prevent and control infections. The World Bank has highlighted the usefulness of these practices to tackle AMR, and introduced the terms 'AMR-sensitive' and 'AMR-specific'[62]. These describe interventions that can indirectly or directly contribute to reducing AMR, respectively. For e.g., WASH is identified as essential to support AMR strategies with the potential to indirect, and indirectly combats AMR. This gives the added benefit of reducing the number of diarrhea cases. Investment in these interventions would therefore be considered as 'AMR-Smart'.

   An example of vaccination for tackling AMR is the use of the typhoid vaccine which actually contributes to reduction in AMR of the bacteria which causes typhoid (*Salmonella typhi*). The most important aetiology for neonatal sepsis in our country is the bacteria named *K. pneumoniae*. While there is currently no vaccine available for preventing this infection in newborns, a Vaccine Value Profile (VVP) has been developed by WHO in consultation with experts based on available information[63]. This profile actually considers the vaccination of pregnant women to protect their babies from birth through to at least three months of age from infection with *K. pneumoniae*.

3. Antimicrobial stewardship is a key initiative for reducing the inappropriate use of antimicrobials. This approach works by the education and support of health care professionals and encourages the use of appropriate guidelines for antibiotics[64]. The successful application of this intervention helps to decrease the use of, and duration of treatment with antibiotics. It also encourages the use of the right antibiotic for any infection. Sometimes, it can serve as a tap on the shoulder to remind a doctor, "Do you think you should use this antibiotic? Do you want to re check how long you want to give this antibiotic?". This initiative helps to improve overall outcomes for patients and reduce the side effects they experience.

4. Despite the urgent need for new antibiotics, the antibacterial development pipeline is drying up, and the number of new antibiotics reaching the market is very low. In case of neonates, there is the added danger of serious side effects. According to a report published by WHO, while several new antibiotics are presently being developed, none of them are projected to be effective against the most severe forms of antibiotic-resistant bacteria. Alternate and novel strategies need to be considered for mitigation of AMR[65] (Fig. 9).

   One such method is the use of immune antibiotics[66], which use the host immune system for better control and treatment of infection. Other approaches include improving the performance of antibiotics by blocking key metabolic pathways of the target bacteria. This is being considered as a viable alternative to the "one antibiotic one target model". Phytochemicals, which are derived from plants, have been used for many years to treat various ailments. They have shown good anti-cancer and anti-inflammatory activity. Some derivatives like polyphenolic compounds have good effect against an important bacterium causing neonatal infections- *E. coli*[67].

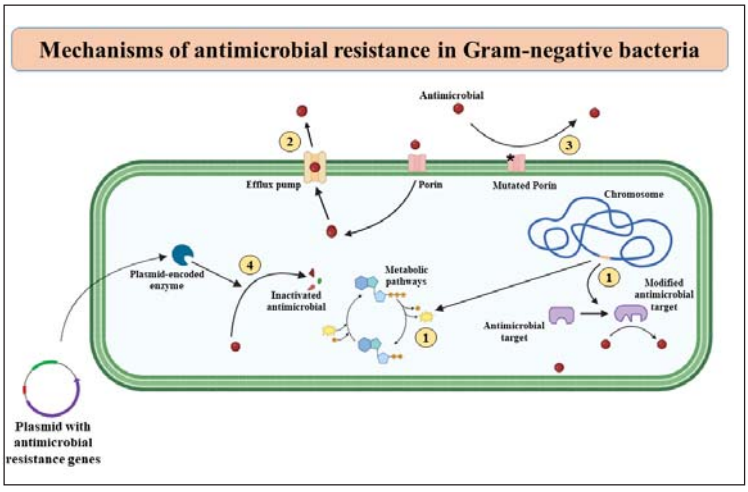

Fig. 5: Schematic diagram highlighting the antimicrobial resistance mechanisms (AMR) in bacteria- 1. Drug target-site modification, 2. Activation of efflux pumps, 3. Outer membrane impermeability, 4. Drug inactivating enzymes. (*Image developed using Biorender.com*).

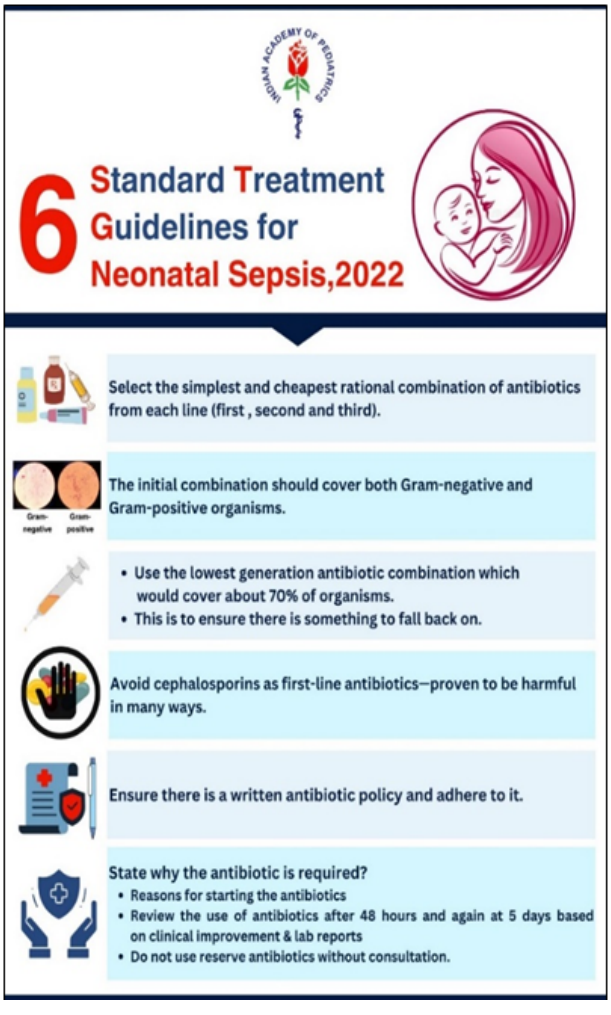

Fig. 6: Standard treatment guidelines for neonatal sepsis, 2022, recommended by Indian Academy of Pediatrics.

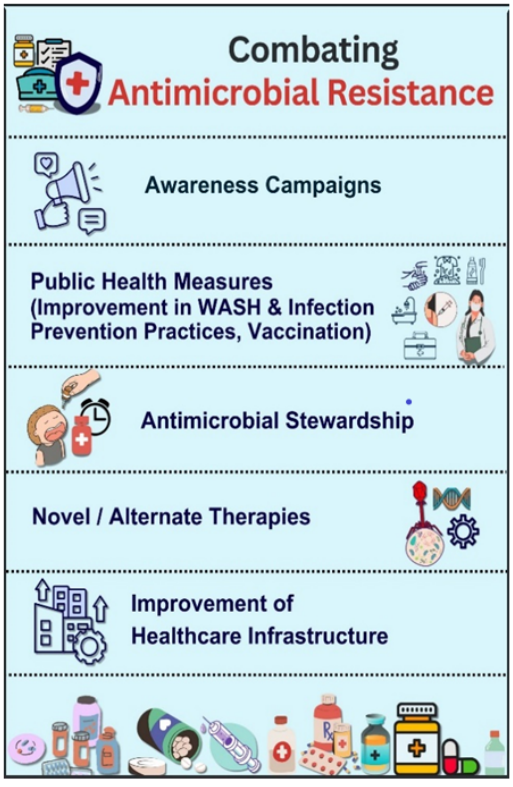

Fig. 7: Key measures for combating antimicrobial resistance.

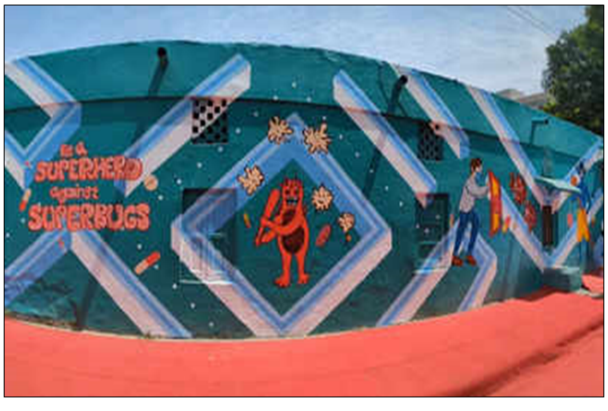

Fig. 8: Mural art (at Khan Market, New Delhi) for raising awareness towards AMR.

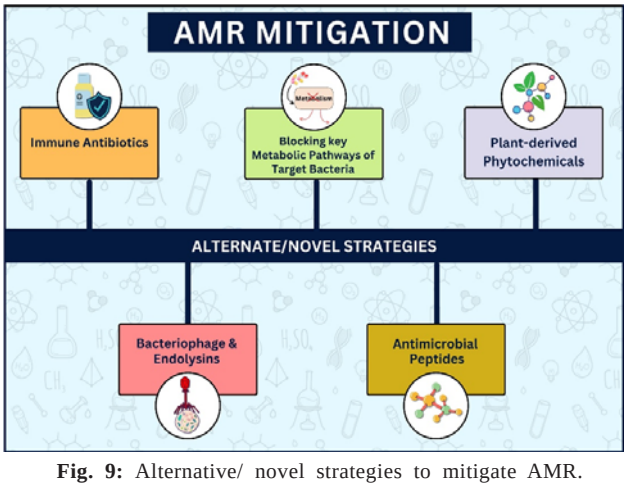

Fig. 9: Alternative/ novel strategies to mitigate AMR.

Viruses which infect bacteria are called bacteriophages and they have been considered for treatment of bacteria like *E. coli* which cause sepsis. While they have their own advantage that development of resistance does not occur, they have an intrinsic disadvantage that only oral usage is possible. This may not work in neonatal sepsis. An alternate approach proposed is the use of endolysins which are enzymes that bacteriophages produce to break down their host bacteria[68].

Another novel strategy is the use of antimicrobial peptides which are small protein molecules produced by the host to resist bacterial or other infections[66,67].

5. Optimal health care, encompassing the presence and availability of health infrastructure including health care facilities, pharmacies, trained manpower, diagnostics, and availability of prescribed antibiotics, is essential to curb the AMR pandemic. Such an improvement, focusing on antenatal immunisation and obstetric care has been shown to have an impact on the development of AMR. The highest reduction in resistance, on implementation of such measures, was noted for ceftriaxone[68]. Interventions to improve health infrastructure emphasizes the need to involve key players in the system, from patients to healthcare staff and policy makers. This approach provides opportunities to improve health outcomes of infectious diseases, and at the same time provides an opportunity to tackle the AMR crisis.

In conclusion, newborns represent the future of every nation, and safeguarding their health is essential for a nation's well-being. To secure a healthy future, urgent action is required to tackle the growing threat of antimicrobial resistance (AMR) in neonatal sepsis. Early and accurate diagnosis is crucial to preventing life-threatening complications, and it also reduces the unnecessary use of antibiotics. AMR is a complex issue involving humans, animals, and the environment, with constantly evolving patterns, which calls for a multifaceted solution. The careful, timely, and appropriate use of antibiotics is key to addressing this urgent health crisis. Striking the right balance in antibiotic use will help manage this significant challenge. Additionally, prioritizing the development of alternative therapies is essential to ensuring effective treatments while limiting the spread of AMR. Every child deserves to be born into a safer and healthier world.

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References

1. B.P. Perez, M.D. Mendez. Routine Newborn Care. [Updated 2023 Jul 24]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2024
2. Pocket Book of Hospital Care for Children: Guidelines for the Management of Common Childhood Illnesses. 2nd edition. Geneva: World Health Organization; 2013. Problems of the neonate and young infant. Available from: https://www.ncbi.nlm.nih.gov/books/NBK154443/
3. https://www.who.int/health-topics/antimicrobial-resistance (Last accessed on 15.08.2024).
4. S. Roy, S. Naha, A. Rao, S. Basu. *Prog Mol Biol Transl Sci.* **178**, 123-174 (2021).
5. E. Castro-Sánchez, L.S. Moore, F. Husson, A.H. Holmes, *BMC Infect Dis.* **16**, 465 (2016).
6. P. Collignon, J.J. Beggs, *Antibiotics (Basel).* **8**, 86 (2019).
7. A. Mehta, P. Brhlikova, P. McGettigan, A.M. Pollock, P. Roderick, H.H. Farooqui, *Bull World. Health. Organ.* **100**, 610-619 (2022).
8. https://www.who.int/news-room/fact-sheets/detail/antimicrobial-resistance (Last accessed on 24.09.2024).
9. https://www.healthdata.org/sites/default/files/files/Projects/GRAM/India_0.pdf (Last accessed on 16.08.2024).
10. https://resistancemap.onehealthtrust.org/DRI.php (Last accessed on 21.08.2024).
11. E. Castro-Sánchez, L.S. Moore, F. Husson, A.H. Holmes, *BMC Infect Dis.* **16**, 465 (2016).
12. P. Collignon, J.J. Beggs, *Antibiotics (Basel).* **8**, 86 (2019).
13. https://www.who.int/publications/i/item/WHO-MHP-HPS-EML-2023.04 (Last accessed on 20.07.2024).
14. S.K. Fazaludeen, S. Ganesh, S. Selvaraj, V.J. Wirtz, S. Galea, P.C. Rockers, *JAC. Antimicrob. Resist.* **4**, dlac112 (2022).
15. https://resistancemap.onehealthtrust.org/AntibioticUse.php (Last accessed on 25.09.2024).
16. Report of the First Multicentric Point Prevalence Survey of antibiotic use at 20 NAC NET sites, India 2021-22. National Program for AMR Containment, NCDC, Directorate General of Health Services. New Delhi.
17. https://nhm.gov.in/index1.php?lang=1&level=2&sublinkid=819&lid=219#:~:text=In%20India%2C%20an%20estimated%2026,total%20population%20in%20the%20Country. (Last accessed on 25.07.2024).
18. https://www.who.int/news-room/fact-sheets/detail/newborn-mortality#:~:text=The%20first%20month%20of%20life,decreased%20by%2044%25%20since%202000. (Last accessed on 06.08.2024)
19. https://pib.gov.in/PressReleaseIframePage.aspx?PRID=1861710 (Last accessed on 24.07.2024).
20. https://www.unicef.org/topics/child-survival (Last accessed 22.07.2024)
21. https://data.worldbank.org/indicator/SH.DYN.MORT (Last accessed on 22.07.2024)

22. https://childmortality.org/wp-content/uploads/2024/03/UNIGME-2023-Child-Mortality-Report.pdf (Last accessed on 23.09.2024).
23. India State-Level Disease Burden Initiative Child Mortality Collaborators (2020), *Lancet*. **395**, 1640–1658 (2020).
24. https://www.unicef.org/reports/global-annual-results-2021-goal-area-1 (Last accessed on 06.08.2024).
25. S. M. Dhaded, S. Saleem, S.S. Goudar, S. S. Tikmani, K. Hwang, G. Guruprasad, *et al*., *The Lancet. Global health.* **10**, e1575–e1581 (2022).
26. V. Datta, S. Ghosh, & L. D. Aquino, *BMJ open quality*. **11**, e001971 (2022).
27. A.T. Wondemagegn, A. Alebel, C. Tesema, W. Abe, *Public Health Rev.* **39**, 33 (2018).
28. F. G. Gebeyehu, B. M. Geremew, A. K. Belew, & M. A. Zemene. *PLOS Glob Public Health.* **2**, e0001180 (2022).
29. M. Singh, M. Alsaleem, C.P. Gray. Neonatal Sepsis. [Updated 2021 Oct 10]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2021 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK531478/
30. I. Abubakar, T. Tillmann, A. Banerjee, *Lancet*. **385**, 117–171 (2015).
31. https://www.who.int/health-topics/sepsis#tab=tab_1 (Last accessed on 06.08.2024).
32. A. Bang, M. Deshmukh, S. Baitule, J. Duby, *Pediatr Infect Dis J*. **40**, 1029-1033 (2021).
33. https://www.data.gov.in/resource/stateut-wise-neonatal-mortality-rate-nmr-srs-during-2018 (Last accessed on 10.08.2024).
34. K.A. Simonsen, A.L. Anderson-Berry, S.F. Delair, H.D. Davies, *ClinMicrobiol Rev*. **27**, 21-47 (2014).
35. B.C. Araújo, H. Guimarães, *J Pediatr Neonat Individual Med.* **9**, e090206 (2020).
36. M.J. Sankar, R. Agarwal, A.K. Deorari, V.K. Paul, *Indian J Pediatr*. **5**, 261-6 (2008).
37. K.N. Haque, *J Med Sci*. **3**:11-27 (2010).
38. I. H. Celik, M. Hanna, F. E. Canpolat, P. Mohan, *Pediatric research*. **91**, 337–350 (2022).
39. P.Y.I. Tam, C. Bendel, *Pediatr Res*. **82**, 574–583 (2017).
40. S. Vergnano, M. Sharland, P. Kazembe, C. Mwansambo, P.T. Heath, *Arch Dis Child Fetal Neonatal Ed*. **90**, F220-4 (2005).
41. M.A. Rafi, M.M.Z. Miah, M.A. Wadood, M.G. Hossain, *PLoS One*. **15**, e0242275 (2020).
42. B.P. Zakariya, V. Bhat, B.N. Harish, T. Arun Babu, N.M. Joseph, *Indian J Pediatr*. **78**, 413-7 (2011).
43. J. Kaur, Singh H, Sethi T., *Lancet Reg Health Southeast Asia*. **26**, 100412 (2024).
44. S. Basu, Eur J Clin Microbiol Infect Dis. 34, 215–222 (2015).
45. B. Sathyamurthi, K.V. Leela, R. Narayanababu, S. Padmanaban, S. Sreedevi, Sujatha, *et al*., *Int J Sci Study*. **4**, 57-60 (2016).
46. R. Viswanathan, A. K. Singh, S. Basu, S. Chatterjee, S. Sardar, D. Isaacs, *Archives of disease in childhood.* **97**, F182–F187 (2012).
47. R.I. Aminov, *Front Microbiol*. **1**, 134 (2010).
48. S.S. Tang, A. Apisarnthanarak, L.Y. Hsu, *Adv Drug Deliv Rev.* **78**:3–13 (2014).
49. D.C. Hooper. *Lancet Infect Dis*. **2**, 530–538 (2002).
50. Sun J. Sun, Z. Deng, A. Yan. *Biochem Biophys Res Commun*. **453**, 254–267 (2014).
51. Y. F. Liu, J. J. Yan, H. Y. Lei, C. H. Teng, M. C. Wang, C. C. Tseng, *et al*., *Infection and immunity*, **80**, 1815–1822 (2012).
52. H.C. Neu, *Science*. **257**:1064–1073 (1992).
53. G.D. Wright, *Nat Rev Microbiol*. **5**, 175–186 (2007).
54. L.S. Frost, R. Leplae, A.O. Summers, A. Toussaint, *Nat Rev Microbiol*. **3**, 722–732 (2005).
55. K. Sands, M.J. Carvalho, E. Portal, K. Thomson, C. Dyer, C. Akpulu, *et al*., *Nat Microbiol*, **6**, 512–523 (2021).
56. K. M. Thomson, C. Dyer, F. Liu, K. Sands, E. Portal, M.J. Carvalho, *et al*., *Lancet Infect Dis*, **21**, 1677–1688 (2021).
57. Investigators of the Delhi Neonatal Infection Study (DeNIS) collaboration, *Lancet Glob Health*, **4**, e752–e760 (2016).
58. A. Fuchs, J. Bielicki, S. Mathur, M. Sharland, J.N. Van Den Anker, *Paediatr Int Child Health*., **38**, S3–S15 (2018).
59. S. Mukherjee, S. Mitra, S. Dutta, S. Basu. *Front Med (Lausanne).* **8**:634349 (2021).
60. N. Fleiss, K. Schwabenbauer, T.M. Randis, R Polin, *Arch Dis Child Fetal Neonatal Ed*, **108**, F10–F14 (2023).
61. https://iapindia.org/pdf/STG-Neonatal-Sepsis-005.pdf (Last accessed on 15.09.2024).
62. Addressing the Grand Pandemic of Antimicrobial Resistance. World Bank Group (2024).
63. Z. Dangor, N. Benson, J.A. Berkley, J. Bielicki, M.W. Bijsma, J. Broad, et al., *Vaccine*. **42**, S125-S141 (2024).
64. E. Mendelsohn, N. Ross, C. Zambrana-Torrelio, T.P. Van Boeckel, L. Ramanan, P. Daszak. *Proc Biol Sci*. **290**, 20231085 (2023).
65. G. Boscarino, R. Romano, C. Iotti, F. Tegoni, S. Perrone, S. Esposito, *Antibiotics*, **13**, 250 (2024).
66. B. Singha, V. Singh, V. Soni, *Front. Drug Discov*. **4**, 1385460 (2024).
67. J. Murugaiyan, P.A. Kumar, G.S. Rao, K. Iskandar, S. Hawser, J.P. Hays, *et al*., *Antibiotics*, **11**, 200 (2022).
68. K. Loosli, A. Davis, A. Muwonge, T. Lembo, *PLoS Negl Trop Dis*. **15**, e0009529 (2021).