ANTIMICROBIAL-RESISTANT GRAM-NEGATIVE INFECTIONS IN NEONATES: BURDEN OF DISEASE AND CHALLENGES IN TREATMENT

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ABSTRACT

Purpose of review. This review summarises the main challenges of antimicrobial resistance (AMR) in the neonatal population with a special focus on multidrug-resistant (MDR) Gram-negative (GN) pathogens.

Recent findings. MDR-GN bacteria are a great concern in the neonatal population, with a worldwide rise in the reported incidence and with very limited therapeutic options. Extended-Spectrum β-Lactamase (ESBL) and Carbapenem-Resistant *Enterobacteriaceae* (CRE) have been reported as responsible for NICU outbreaks. Hospital data from low/middle-income countries show high proportions of isolates from neonates resistant to the WHO first-line and second-line recommended treatments. The spread of CRE has resulted in old antibiotics, such as colistin and fosfomycin, to be considered as alternative treatment options, despite the paucity of available data on safety and appropriate dosage.

Summary. Improved global neonatal AMR surveillance programmes including both epidemiology and clinical outcomes are critical for defining the burden and designing interventions. The optimal empiric treatment for neonatal sepsis in settings of high rates of AMR is currently unknown. Both strategic trials of older antibiotics and regulatory trials of new antibiotics are required to improve clinical outcomes in MDR-GN neonatal sepsis.

Keywords: Sepsis; Infant, Newborn; Drug resistance, bacterial; Gram-negative bacteria

INTRODUCTION

Maternal and child deaths have halved worldwide over the past two decades [1]. However, neonatal mortality has remained unacceptably high, with an estimated 2.9 million deaths in newborns within the first 28 days of life every year. Among them, nearly a quarter is directly due to infectious causes (23%), with neonatal sepsis accounting for 15% [1]. Severe bacterial infections (SBI) in neonates account for about 3% of all disability-adjusted life years (DALY) [2]. Infection-attributable deaths, together with survivors of severe sepsis in the neonatal period, contribute largely to the global burden of disease.

Appropriate empirical treatment of SBI is crucially important in reducing mortality. However, it requires knowledge of the relative proportions of causative organisms at a local level as well as their resistance patterns collected using standardised methods. Multidrug-resistant (MDR) pathogens are now a challenge in both high-income (HIC) and low/middle-income countries (LMIC) [3]. Neonates admitted to Neonatal Intensive Care Units (NICU), especially premature infants, have been identified to be at high-risk for the selection and transmission of MDR pathogens. Recently, the first estimate of neonatal deaths attributable to resistant sepsis has been published, with antimicrobial resistance (AMR) potentially responsible for around 30% of all global neonatal sepsis deaths [4**].

This review summarises the main challenges of AMR in the neonatal population with a special focus on MDR Gram-negative (GN) pathogens.

GLOBAL EPIDEMIOLOGY AND CLINICAL IMPACT OF AMR IN NEONATES

Historically, the main resistant pathogens encountered in the hospital setting were Gram-positive bacteria. Among them, methicillin-resistant *Staphylococcus aureus* (MRSA) was a critically important pathogen in the NICU population, being associated with both endemic and epidemic infections [5].

Enterococci spp. are less-frequent, however, ampicillin-resistant and, more recently, vancomycin-resistant enterococci have been described in NICUs [6*].

Currently, it is MDR Gram-negative bacteria that are the greatest concern in the neonatal population, with a worldwide rise in the reported incidence and with very limited therapeutic options [7]. AMR among the *Enterobacteriaceae* represents a major problem in both healthcare-associated and community-acquired neonatal infections.

Extended-Spectrum β-Lactamase (ESBL)–Producing *Enterobacteriaceae*

The ESBLs are the result of a single nucleotide polymorphism in the *blaSHV* gene producing a family of enzymes able to hydrolyse the β-lactam ring [8]. There are many phenotypic and genetic differences among β-lactamases, with a common result that β-lactam antibiotics, such as penicillins, cephalosporins, and monobactams, are inactivated, leading to ineffective therapy. ESBLs are inhibited by β-lactam inhibitors (clavulanic acid, tazobactam, and sulbactam) [9]. β-lactamase genes are mostly carried on mobile genetic elements, frequently harbouring other resistance genes conferring resistance to other antibiotic classes [10]. The most common ESBLs are members of the SHV, TEM, and CTX-M (Ambler Class A) families (Table 1) [9].

Recently, many studies have been conducted globally on the prevalence of both community- and hospital-acquired ESBL-infections in neonates. ESBL-producing *Enterobacteriaceae* have been responsible for an increasing number of NICU outbreaks [11-15*].

Data from HIC showed a consistent rise in the rate of ESBL-positive isolates among neonatal units. A two-centre case–control study of children aged 0-17 years conducted in the US showed that 11/30 ESBL-producing isolates were in babies on NICU [10]. European data from the ARPEC study reported rates of resistance to 3rd-generation cephalosporins between 6.6 and 39.5% among *Enterobacteriaceae* in children less than 1 year [16]. A study conducted in Taiwan on 393 cases of GN

bacteraemia in NICU patients showed that the most frequent mechanism of resistance was ESBL-production that accounted for 67.1% of MDR strains [17]. MDR-GN bacteraemia was associated with a poorer outcome, with significantly higher rates of complications and overall case fatality. Another case-control analysis conducted in Taiwan between 2001 and 2012 reported that 14.2% of all neonatal GN late-onset sepsis were due to ESBL-producing strains (62.3% of *Klebsiella* spp, 20.8% of *Echerichia coli*, and 16.9% of *Enterobacter* spp). The SHV-type ESBLs were the most prevalent among these patients (67% of ESBLs), with most strains being genetically unrelated. In this study, neonates infected with ESBL-GN strains had higher rates of infectious complications (p = 0.008) and adverse outcomes (p=0.049) compared to non-ESBLs [18].

Although ESBL-producing *Enterobacteriaceae* are of global concern, they represent an even greater burden in LMIC. Asian studies showed a global rise in rates of ESBLs among hospitalised neonates. Data from a recent Indian study showed that 87% of *E. coli* isolated from septic neonates were ESBL-producers. CTX-M-15 was the most commonly identified ESBL (81%), reflecting its global dissemination [14]. The rates of β-lactamase production reported in 103 neonates in a referral hospital in Iran was 95.5% among *Klebsiella pneumoniae* and 78.6% among *E. coli* isolates. Infections caused by ESBL-producing pathogens were associated with longer hospital stay [13].

The same trend was highlighted in studies from South America, where an overall rate of 73.3% of ESBL-producers was identified in Peru among neonates infected with *K. pneumoniae*, with CTX-M-15 and CTX-M-2 the most commonly detected ESBLs [19]. Another study in Venezuela described the first nosocomial outbreak of ESBL-producing *Enterobacter ludwigiian* in neonates [12].

ESBL outbreaks in NICUs have been associated with suboptimal hygiene standards, with understaffing reported as a major risk factor [15*]. In non-outbreak settings, variables independently associated with ESBL infections in neonates included prematurity, low birth weight, prolonged hospitalization, invasive devices, and previous antibiotic use [8].

Carbapenem-Resistant *Enterobacteriaceae* (CRE)

The use of carbapenems has increased worldwide during the last decade due to the spread of ESBL-producing pathogens. Carbapenemases genes have been recently identified on mobile genetic elements, therefore favouring both person-to-person and species-to-species spread. Dynamics of spread and associated-resistance phenotypes vary according to different resistance determinants [20**]. Molecular classification of carbapenemases includes Ambler class A, B, and D, distinguished by the site of the hydrolytic mechanism (Table 1) [21].

Various studies have been published recently on the geographical expansion of CRE worldwide. After the global dissemination of *K. pneumoniae* carbapenemase (KPC)-producers, OXA-48 and New Delhi metallo-β-lactamase (NDM)-positive strains are now increasingly being reported [22]. However, limited data have been published on the epidemiology of CRE infections in the neonatal population, and the available data is largely limited to case reports or small case series. Nosocomial outbreaks have been reported in NICUs, and the geographic distribution is broadly comparable with data reported in adults [23]. It is unclear at present whether ESBL and CRE prevalence on neonatal units broadly reflects national resistance patterns or neonatal unit specific resistance profiles.

The majority of reports on the dissemination of CRE among the neonatal population in the last years has been published from South-East Asia and Western Pacific Regions. An outbreak of NDM-1-producing *K. pneumoniae* was recently reported in the neonatal ward of a teaching hospital in mainland China [24]. The isolates were shown to be clonally related and harboured a sequence type 17. The first *blaNDM-1*-positive *K. pneumoniae* in China was isolated in 2012. Since then, a number of reports have been published showing a continuous spread of *blaNDM-1*. In other areas of NDM endemicity, such as India, NDM-1 has been the predominant carbapenemase among neonates. In a 5-year study on neonatal sepsis in a tertiary care NICU in Kolkata, India, NDM-1 was identified in 14% of the 105 isolates, and was the only carbapenemase detected [25]. The same trend in the same hospital was highlighted in another 7-year study on carbapenem-resistant *Acinetobacter baumannii*

causing neonatal sepsis. Again, *blaNDM−1* was the major contributor to carbapenem-resistance [26]. Another *K. pneumoniae* sepsis outbreak described in a NICU in central India reported that all the isolates were positive for *blaNDM-1* with co-association of *blaCTX-M-15* [27]. The first study using whole-genome sequencing to characterise an extended outbreak caused by carbapenem-resistant *K. pneumoniae* in a neonatal unit in Nepal revealed that the case clusters were all caused by a single NDM *K. pneumoniae* with a conserved set of four plasmids, one carrying *blaNDM-1* gene [28]. In this study, the mortality rate among infected neonates was 64% (16/25).

Relatively few data has been published on the epidemiology of neonatal CRE in the African region, mainly from the North African countries. A large study conducted on 1,287 newborns in Morocco showed that 34 out of 157 isolated *Enterobacteriaceae* were carbapenemase-producing, with OXA-48 the predominant type [29]. However, no statistically significant difference in mortality was shown between neonates infected with CRE and non carbapenem-resistant strains.

Data from neonatal units in Europe and the US generally reflect the distribution of CRE in adults. A recent study published in Italy on a national cohort of 69 children infected or colonised with CRE confirmed the endemic role of the KPC carbapenemase in Southern Europe and the Mediterranean region, with 61.4% of CRE being KPC-producing and 13.6% harbouring the OXA-48 type [30].

Plasmid-mediated Colistin-Resistant Enterobacteriaceae

With the global increase in the prevalence of CRE, polymyxins have become a last-resort treatment option, with an increase in the human consumption of colistin and an inevitable risk of emerging resistance. Recently, the emergence of plasmid-mediated *mcr-1* gene conferring colistin-resistance has become a great challenge to public health [31]. This gene has been identified firstly in animals and humans in China, but is now being reported in several other countries [32]. New agents effective

against MDR-GN pathogens are lacking, and the impact of mobile colistin resistance genes on global health cannot be underestimated.

The first report published in 2016 on the detection of *mcr-1* gene in *K. pneumoniae* and *E. coli* isolated from an infant with diarrhoea in China raised huge concerns [33**]. Due to the very limited therapeutic options, the emergence of *mcr-1*-positive isolates in neonates represents an enormous challenge. Furthermore, the simultaneous isolation of this gene in two different pathogens from the same patient is likely to suggest horizontal transmission.

CHALLENGES IN AVAILABLE TREATMENTS

Hospital data from LMIC reported in a recent systematic review shows that nearly half of pathogens causing SBI in neonates are resistant to the WHO first-line recommended regimen of ampicillin (or penicillin) and gentamicin, and to the second-line treatment with 3rd-generation cephalosporins [4**, 34-36]. These findings could potentially have significant implications for global antibiotic recommendations in neonatal settings [37-38*-39]. The current *WHO's Pocketbook of Hospital Care for Children* provides the same empiric guidance for probable SBI in both early-onset and late-onset sepsis in both the community and hospital setting. However, the risks of MDR-GN sepsis may vary considerably between early-onset in the rural population and late-onset nosocomial infections in large neonatal units.

Babies are particularly at risk for adverse outcomes from sepsis due to MDR-*Enterobacteriaceae*, with few available effective antimicrobials approved for neonatal use and the pipeline for novel antibiotics in advanced development limited (Table 2).

The role of β-lactam–β-lactamase inhibitors (BL/BLIs) for the treatment of ESBL infections is still controversial since many organisms have been shown to produce multiple ESBLs simultaneously, therefore reducing the effectiveness of the inhibitor. The available literature is limited to

observational studies where BL/BLIs have been compared with carbapenems in neonates with ESBL-producing *Enterobacteriaceae*, showing no difference in clinical outcomes [42]. Carbapenems have become the treatment of choice for neonatal ESBL-infections, and should be used as monotherapy since no evidence of benefit from addition of an aminoglycoside has been demonstrated [40].

The optimal treatment for CRE infections in adults has not been defined yet due to the lack of large randomised studies evaluating different therapeutic approaches. Small case series seem to support the use of combination therapy to significantly reduce mortality [22].

The emergence and spread of CRE among NICU patients has led to old antibiotics, such as colistin and fosfomycin, to be reconsidered, even if the increasing prevalence of colistin-resistant CRE is of high concern [32, 33**, 43].

Colistin is increasingly used in the neonatal population. However, few studies have been published about its use in neonatal sepsis, and none of them focussed specifically on infections caused by CRE. Colistin is associated with renal toxicity, with significant rates of acute kidney injury reported in adults [20**]. In a retrospective study on 12 neonates infected with MDR-GN bacteria, the intravenous treatment with colistin seemed to be safe and efficacious, including for preterm babies [44]. Another retrospective study on 21 neonates treated with colistin for MDR *Acinetobacter* sepsis showed a surviving rate of 91%, with no renal impairment being documented [45]. A similar report on preterm neonates with nosocomial sepsis caused by *A. baumannii* showed a recovery rate of 81% in babies receiving IV colistin, with acute kidney injury reported in 19% [46]. A recent PK study on 7 critically ill neonates with GN infections highlighted that a single IV dose of colistin at the currently recommended dosage resulted in suboptimal plasma concentrations [47].

There is little experience of using fosfomycin in neonates, and it should be considered as a final-resort option for extensively-drug resistant GN [48]. This drug has a broad-spectrum of bactericidal activity and is active against the majority of CRE isolates, with the advantage that it achieves excellent concentrations in urine, plasma, bronchoalveolar and cerebrospinal fluid, with low rates of

toxicity [40]. Resistance to fosfomycin can develop rapidly if used as monotherapy, but if combined with other agents the chance of developing resistance is limited [42].

Tigecycline is active against many difficult-to-treat MDR-GN. However, due to the possible effects on bone growth, the use in neonates could only be justified when other effective antibiotics are not available [40, 42, 48].

At the moment, 37 new antibiotics are listed on the Pew Charitable Trusts Antibiotic Pipeline, with few of them, such as aztreonam-avibactam, carbavance (vaborbactam+meropenem) and plazomicin, specifically targeting infections caused by CRE [49]. However, there is only one study currently ongoing on the safety and pharmacokinetics of carbavance in children that includes neonates [50].

CONCLUSIONS AND FUTURE PERSPECTIVES

Standardised global surveillance programmes collecting neonatal AMR data including risk factors and clinical outcomes are critical to allow benchmarking and design interventions. Studies published so far reported conflicting data about the relationship between currently-defined MDR isolates and neonatal mortality. This should be addressed properly through prospective studies focussing on clinical impact of AMR in neonatal sepsis.

It should be noted that although the increasing emphasis on in-hospital births in LMIC may led to improve neonatal outcomes in terms of birth complications and stillbirths, this may be at the expense of increased exposure of babies to highly-resistant hospital flora, and to the subsequent spread of AMR.

The impact of different risk factors at both baby- and hospital-level is still debated. Due to the paucity and high heterogeneity of available studies, firm conclusions on the correlation between GNB colonisation and BSI in neonates cannot be made at the moment, with the few existing data too weak to be used for clinical decision-making.

The optimum choice of drug, dose, and duration to treat neonatal sepsis in settings of high resistance to WHO first-line empirical therapy is still unknown. The evidence-base for the use of new (and older) antibiotics needs to be developed through both strategic and regulatory clinical trials in order to define the best available treatment and improve the management of these severe infections in neonates, especially in LMIC setting. Harmonisation of surveillance definitions, including age groups and outcome measures, would also allow pooling data from different datasets.

Key points:

- ESBL-producing and Carbapenem-resistant *Enterobacteriaceae* are of great concern in the neonatal population, with a worldwide rise in the reported incidence and with very limited therapeutic options.
- Alarming proportions of pathogens causing SBI in neonates are now reported to be resistant to the WHO first-line and second-line recommended treatments.
- The spread of CRE among NICU patients has resulted in the increasing use of old antibiotics in neonates, despite the paucity of available data on safety and appropriate dosage.
- The evidence-base for the use of new (and older) antibiotics needs to be developed through both strategic and regulatory clinical trials in order to define the best available treatment for MDR-GN infections in neonates.

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Table 1: Summary of resistance mechanisms and phenotypes for clinically important β-lactamases according to Ambler classification

Ambler classification	Resistance mechanism	Representative enzymes	Genetic basis	Relevant organisms	Substrates
Class A	ESBLs	TEM, SHV, CTX-M	Plasmid	*Escherichia coli, Klebsiella spp, Proteus mirabilis*	Penicillins, 3rd gen-cephalosporins
	Carbapenemases	KPC	Plasmid	*Klebsiella pneumoniae, E. coli, Klebsiella oxytoca, Serratia marcescens, Enterobacter spp, Citrobacter freundii*	All β-lactams
Class B	Carbapenemases, metallo-β-lactamases	VIM, IMP, NDM-1	Plasmid	*K. pneumoniae, E. coli, K. oxytoca, S. marcescens, Enterobacter spp, C. freundii*	All β-lactams, except monobactams
Class C	Cephalosporinases	AmpC	Chromosomal	*K. pneumoniae, E. coli, Enterobacter spp, Salmonella enteritidis, C. freundii, S. marcescens*	Cephamycins, 3rd gen-cephalosporins
Class D	Carbapenemases	OXA	Plasmid	*Acinetobacter baumannii, Pseudomonas aeruginosa, E. Coli, K. Pneumoniae, P. Mirabilis, C. freundii*	All β-lactams

Table 2: Currently available treatments for Gram-negative neonatal sepsis

Drug	Recommended IV dosage*		CSF penetration	Notes
	0-6 days	7-28 days		
Ampicillin	30–60 mg/kg 12 hourly	30–60 mg/kg 6–8 hourly	Poor if meninges are not inflamed	High doses can be administered because of low toxicity
Amoxicillin	50–100 mg/kg 12 hourly	50–100 mg/kg 8 hourly	Poor if meninges are not inflamed	High doses can be administered because of low toxicity
Piperacillin-tazobactam	90 mg/kg 8 hourly	90 mg/kg 8 hourly	Poor	**Active against most ESBL-producing Enterobacteriaceae but limited data in neonates**
Cefotaxime	25–50 mg/kg 12 hourly	25–50 mg/kg 6–8 hourly	Adequate when meninges are inflamed	High doses can be administered because of low toxicity
Ceftazidime	25–50 mg/kg once daily	25–50 mg/kg 8–12 hourly	Adequate when meninges are inflamed	High doses can be administered because of low toxicity
Meropenem	20-40 mg/kg 12 hourly	20-40 mg/kg 8 hourly	Adequate	**Active against ESBL-producing Enterobacteriaceae**
Gentamicin	5 mg/kg 36 hourly	5 mg/kg 24 hourly	Poor	TDM** required
Ciprofloxacin	10 mg/kg 12 hourly	10 mg/kg 12 hourly	Good	Consider TDM in serious infections
Colistin	25,000 units/kg 8 hourly	25,000 units/kg 8 hourly	Inadequate	TDM recommended. **Active against Carbapenem-resistant Enterobacteriaceae (CRE)**
Fosfomycin	50 mg/kg 12 hourly	100 mg/kg 12 hourly	Good	To use in combination to avoid development of resistance. **Active against ESBL-producing Enterobacteriaceae and most CRE**
Tigecycline	Insufficient data available		Inadequate	**Active against ESBL-producing Enterobacteriaceae and CRE**

Modified from: Gray JW et al [40]. *Dosages according to those recommended in the British National Formulary for Children (BNFC) [41]. **TDM: Therapeutic Drug Monitoring

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