



# Potential Antibiotics for the Treatment of Neonatal Sepsis Caused by Multidrug-Resistant Bacteria

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## Abstract

Neonatal sepsis causes up to an estimated 680,000 deaths annually worldwide, predominantly in low- and middle-income countries (LMICs). A significant and growing proportion of bacteria causing neonatal sepsis are resistant to multiple antibiotics, including the World Health Organization-recommended empiric neonatal sepsis regimen of ampicillin/gentamicin. The Global Antibiotic Research and Development Partnership is aiming to develop alternative empiric antibiotic regimens that fulfil several criteria: (1) affordable in LMIC settings; (2) activity against neonatal bacterial pathogens, including extended-spectrum  $\beta$ -lactamase producers, gentamicin-resistant Gram-negative bacteria, and methicillin-resistant *Staphylococcus aureus* (MRSA); (3) a licence for neonatal use or extensive experience of use in neonates; and (4) minimal toxicities. In this review, we identify five antibiotics that fulfil these criteria: amikacin, tobramycin, fosfomycin, flomoxef, and cefepime. We describe the available characteristics of each in terms of mechanism of action, resistance mechanisms, clinical pharmacokinetics, pharmacodynamics, and toxicity profile. We also identify some knowledge gaps: (1) the neonatal pharmacokinetics of cefepime is reliant on relatively small and limited datasets, and the pharmacokinetics of flomoxef are also reliant on data from a limited demographic range and (2) for all reviewed agents, the pharmacodynamic index and target has not been definitively established for both bactericidal effect and emergence of resistance, with many assumed to have an identical index/target to similar class molecules. These five agents have the potential to be used in novel combination empiric regimens for neonatal sepsis. However, the data gaps need addressing by pharmacokinetic trials and pharmacodynamic characterisation.

## Key Points

Amikacin, tobramycin, fosfomycin, flomoxef, and cefepime are five safe and off-patent antibiotics with experience of use in neonates that can be potentially used as empiric treatment of neonatal sepsis in low- and middle-income settings where antimicrobial resistance complicates current standard-of-care regimens.

The neonatal pharmacokinetics are well characterised for most, with cefepime and flomoxef needing some additional data.

All agents have data gaps in their pharmacodynamic characterisation in terms of bacterial killing and emergence of resistance

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## 1 Introduction

Over the last few decades, the global neonatal mortality rate has improved, with a fall of 37% since 1990 [1]. Despite this, neonatal infection continues to contribute to considerable mortality, with neonatal sepsis accounting for 15.6% of neonatal deaths (an estimated 430,000–680,000 deaths per annum), which predominantly occur in low- and middle-income countries (LMICs) [2–4].

For the past few decades, the World Health Organization (WHO) has recommended a relatively narrow-spectrum  $\beta$ -lactam (e.g., amoxicillin, benzylpenicillin, or, if at risk of staphylococcal infections, cloxacillin) in combination with gentamicin as first-line empiric treatment of neonatal sepsis, with cefotaxime/ceftriaxone as second line [5, 6]. The rationale behind this is to provide regimen options that are relatively narrow spectrum, effective against the main causative bacteria (*Streptococcus agalactiae*, *Staphylococcus aureus*, and Enterobacteriales [7]), have minimal toxicities, and are both inexpensive and practical to administer.

However, as with other bacterial infections, antimicrobial resistance (AMR) is an increasing concern in the treatment of neonatal sepsis [8]. A recent prospective multi-centre epidemiological neonatal sepsis study demonstrated 95%, 80%, and 60% resistance rates to amoxicillin, ceftriaxone, and gentamicin, respectively, in Gram-negative bacteria causing neonatal sepsis, with widespread carriage of extended-spectrum  $\beta$ -lactamase (ESBL) genes [9]. Another similar observational neonatal sepsis study in New Delhi [10] presented a similar picture, with over 56% of causative Gram-negative bacteria resistant to three or more classes of broad-spectrum antibiotics (defined in the study as extended-spectrum cephalosporins, piperacillin–tazobactam, fluoroquinolones, aminoglycosides, and carbapenems), and 38% of *S. aureus* infections were methicillin resistant. These observed resistance patterns have been replicated in other regional retrospective observational studies [11–16]. With such high rates of  $\beta$ -lactam and gentamicin resistance, it is increasingly clear that the current WHO-recommended regimen for neonatal sepsis is no longer optimal in the context of neonatal sepsis, and so an alternative first-line empiric regimen is needed.

Such a regimen should have activity against the main causative bacteria and resistance motifs and be suitable for use in an LMIC setting. Although newly developed agents may have the required spectrum of activity, they are unlikely to be available in the near future because of licensing and cost limitations and the well-recognised delay in obtaining a paediatric and neonatal licence (which can be up to 10 years after the adult licence) [17]. However, a number of older off-patent antimicrobials retain the requisite spectrum of

antimicrobial activity and could be repurposed for use in neonatal sepsis.

The identification of new antimicrobial regimens for treatment of neonatal sepsis is one of the goals of the Global Antibiotic Research and Development Partnership (GARDP). In particular, the GARDP aims to develop an antimicrobial regimen for use in LMICs for the empiric treatment of neonatal sepsis in locations with increasing resistance to current WHO-recommended treatments [18].

Criteria were developed for selecting antimicrobial agents that fulfilled the needs of such a treatment (Table 1) [18]. These criteria were applied to a list of antibiotics extracted from Kucers' [19], supplemented by additional agents licensed by the European Medicines Agency/US FDA after 2017. Five agents fulfilled these criteria and were identified as candidates with potential utility (either alone or in combination) for the treatment of neonatal sepsis in LMIC settings: amikacin, tobramycin, fosfomycin, flomoxef, and cefepime. This review examines the pharmacological characteristics of each, with a focus on neonatal use.

## 2 Literature Review Methodology

The literature was reviewed for each component of the review by searching MEDLINE (via PubMed) with the following general search term strategy: [antibiotic name] + [pharmacological characteristic]  $\pm$  [demographic qualifier]. For the term [antibiotic name], individual antibiotic names were used, along with development names (e.g., 6513-S for flomoxef) where known. For the term [pharmacological characteristic], broad terms were used for the domain of interest, with further narrower terms for specific components. For example, 'toxicity' was used for a broad initial search for the toxicity characteristics of each drug, with subsequent narrower search terms for specific identified toxicities (e.g., 'nephrotoxicity' for the aminoglycosides). A similar search term strategy (i.e., an initial broad search

**Table 1** Criteria for selection of candidate antibiotics for treatment of neonatal sepsis in low- and middle-income countries [18]

### Criteria for antimicrobial selection

1. Antimicrobial should be inexpensive to manufacture (i.e., off patent)
2. Clinically relevant activity against multidrug-resistant bacteria, particularly Gram-negative bacteria with gentamicin resistance or extended-spectrum  $\beta$ -lactamases and methicillin-resistant Gram-positive organisms
3. Licensed for use in neonatal infection by a stringent regulatory authority (e.g., the European Medicines Agency); or extensive experience of use in the neonatal context where no licence
4. Limited toxicity

term, with subsequent narrower search terms for components specific to each domain) was used for each pharmacological section (e.g., clinical pharmacokinetics, resistance mechanisms, pharmacodynamics, etc.).

These searches were performed with and without the [demographic qualifier] term for relevant pharmacological characteristics (i.e., toxicity, clinical pharmacokinetics) using the terms ‘neonate’, ‘neonatal’, and ‘infant’ to capture and identify published data in the neonatal age group, where these were available.

Literature retrieved by the searches was screened for relevance. The primary author extracted data for incorporation into the manuscript, and the senior authors provided expert critical review and feedback.

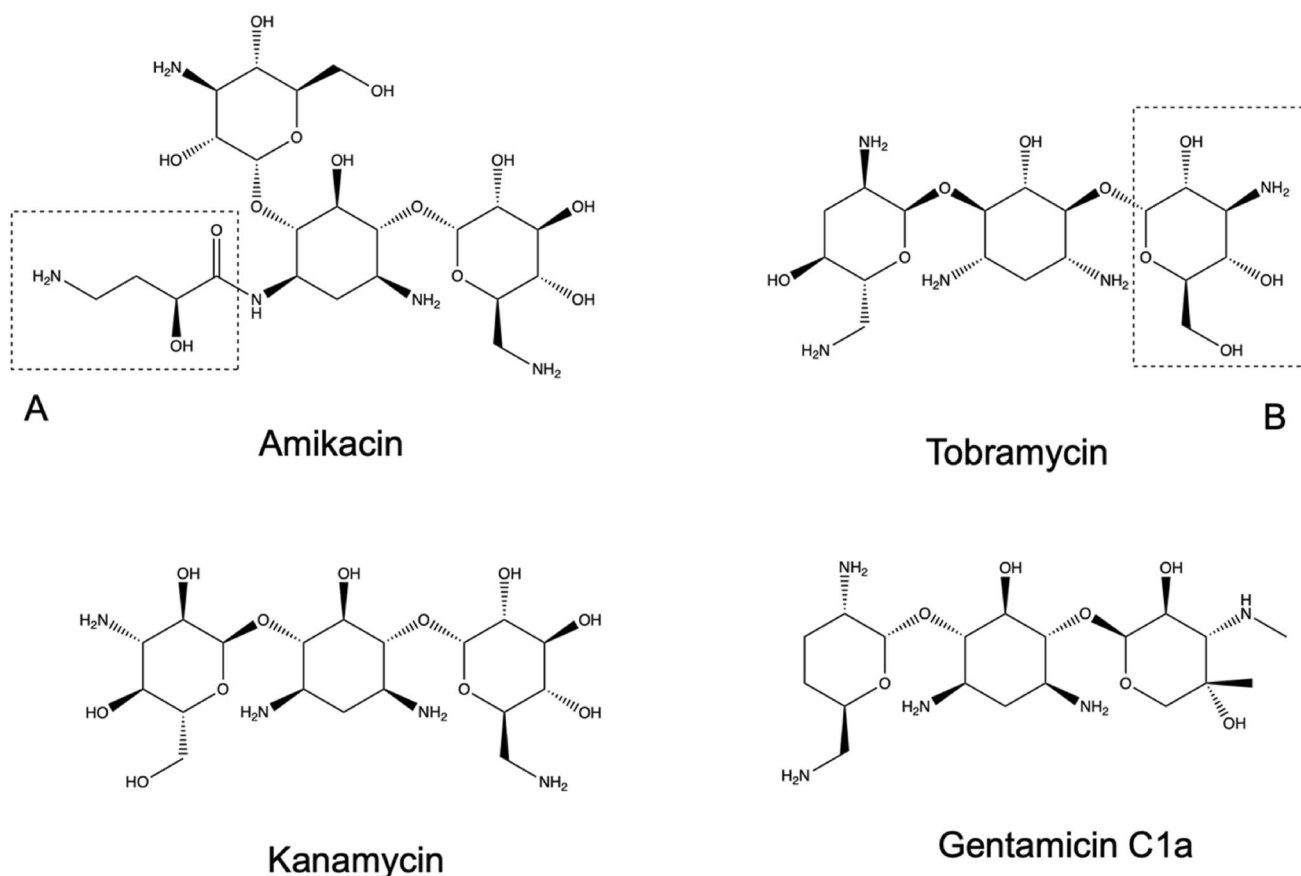
### 3 Amikacin and Tobramycin

Amikacin and tobramycin are aminoglycosides with in vitro activity against gentamicin-resistant bacteria. Aminoglycosides consist of a central dibasic aminocyclitol core (usually 2-deoxystreptamine) with one or two amino sugar moieties

connected via glycosidic links [20]. Resistance to first-generation aminoglycosides (e.g., kanamycin, gentamicin) became widespread with their use in clinical practice, with resistance largely caused by aminoglycoside-modifying enzymes (AMEs) [21]. Later-generation aminoglycosides have properties that circumvent some AMEs, increasing their clinical usefulness in infections caused by isolates resistant to first-generation aminoglycosides.

Amikacin is a semi-synthetic aminoglycoside derived from kanamycin by the addition of an L-(–)- $\gamma$ -amino- $\alpha$ -hydrobutyryl side chain to the 2-deoxystreptamine component of the molecule [22] (Fig. 1). This additional side chain interferes with the binding of many AMEs, which otherwise inactivate many other aminoglycosides, extending the activity of amikacin to most gentamicin-resistant bacteria.

Tobramycin is a naturally occurring aminoglycoside isolated from *Streptomyces tenebrarius* and is structurally similar to kanamycin and gentamicin C1a [23]. Compared with the latter, tobramycin has a 3-amino-3-deoxyglucose instead of a 3-deoxy-3-methylamino-4-C-methylxylose (Fig. 1). Tobramycin was developed because of its increased



**Fig. 1** Chemical structures of amikacin and tobramycin, with comparator molecules of kanamycin and gentamicin C1a. (A) L-(–)- $\gamma$ -amino- $\alpha$ -hydrobutyryl side chain, (B) 3-amino-3-deoxyglucose

activity against *Pseudomonas aeruginosa* compared with pre-existing aminoglycosides [24].

### 3.1 Mechanism of Action

Tobramycin and amikacin inhibit protein synthesis by binding to the 30S bacterial ribosome subunit, causing mistranslation of bacterial proteins. Specifically, they bind to the aminoacyl-transfer RNA recognition site of the 16S ribosomal RNA (rRNA) component of the 30S ribosome [20, 25]. Entry to the bacterial cell is via the ‘self-promoted uptake pathway’. Both agents are polycationic and must initially bind to anionic compounds, e.g., lipopolysaccharides in Gram-negative bacteria and teichoic acids and phospholipids in Gram-positive bacteria. This binding increases the permeability of the outer membrane, allowing penetration of the antibiotic into the periplasmic space [26]. Following this, an energy-dependent uptake process provides access to the cytoplasm [27], where it can engage with the ribosomal target. Protein mistranslation caused by ribosomal binding further increases membrane permeability, allowing further entry of the aminoglycoside into the cytoplasm and leading to further bactericidal effect.

As with other aminoglycosides, both agents have a spectrum of activity against Gram-negative bacteria, including *P. aeruginosa* [20]. Aminoglycosides also have activity against staphylococci but are largely ineffective against other Gram-positive bacteria as monotherapy. The need to use the active electron transport process to enter the bacterial cell means anaerobic bacteria are intrinsically resistant [20].

### 3.2 Resistance Mechanisms

Resistance to amikacin and tobramycin, like other aminoglycosides, occurs by one of the five following mechanisms.

#### 3.2.1 Inactivation by Aminoglycoside-Modifying Enzymes

AMEs act by modifying specific hydroxyl or amino groups on either the 2-deoxystreptamine nucleus or the sugar moieties. There are three main types: acetyltransferases (AACs), nucleotidyltransferases (ANTs), and phosphotransferases (APHs). Individual AMEs are named as the acronym of their type, with the site of enzymatic modification within parenthesis, e.g., AAC(2'). Subsequent roman numerals refer to the resistance profile they confer, with further lower-case letters as individual identifiers [28].

A large number of AMEs have been identified [21], encoded on transmissible elements (e.g., plasmids), transposons, and bacterial chromosomes. Aminoglycoside susceptibility to individual AMEs is linked to the specific configuration of their side chains.

Amikacin is refractory to inactivation by most AMEs because of its amino- $\alpha$ -hydrobutyryl side chain. However, multiple AMEs with activity against amikacin have emerged, mostly by acetylation of 6'-N position. These AAC(6') enzymes have activity against amikacin but not normally gentamicin [21].

Tobramycin is resistant to the AAC(1), AAD(4'), and APH(2'') enzymes that inactivate gentamicin. However, several enzymes confer cross-resistance to both gentamicin and tobramycin (but not amikacin), including AAC(2), AAC(3), AAC(2'), AAC(6'), and AAD(2''). Additionally AAD(4') deactivates tobramycin but not gentamicin [25].

Although AMEs usually have limited cross-reactivity between aminoglycosides, bacteria can accumulate multiple AME genes, conferring pan-aminoglycoside resistance.

#### 3.2.2 Modification of Target Site by 16S Methylation

Post-transcriptional methylation of the 16S rRNA subunit inhibits the binding of aminoglycosides to the ribosome. It is commonly observed in aminoglycoside-producing Actinobacteria to provide autoprotection against endogenous aminoglycoside production [29]. However, nine plasmid-mediated 16S rRNA methyltransferases have been identified in non-Actinobacteria species: ArmA, RmtA–H, and NpmA [29]. These enzymes give high-level resistance to all 4,6-disubstituted 2-deoxystreptamine aminoglycosides (which include gentamicin, tobramycin, and amikacin) as well as newer aminoglycosides [30]. The spread of plasmid-mediated 16S rRNA methylases in Gram-negative bacteria is of particular concern because they also usually carry genes that encode ESBLs and carbapenemases [31].

#### 3.2.3 Target Site Modification

Chromosomal mutation of the 16S rRNA gene in *Mycobacterium tuberculosis* confers resistance to kanamycin [32] and streptomycin [33]. Mutations conferring resistance to amikacin have been reported in atypical mycobacteria [34]. To date, no chromosomal 16S rRNA gene mutation has been reported in non-mycobacterial bacteria.

#### 3.2.4 Alteration of Uptake Mechanisms

Given the complexity of aminoglycoside uptake into Gram-negative bacteria, resistance mediated by changes to self-promoted uptake is poorly understood. There is little evidence for porin involvement in uptake, and specific mutations in porin genes conferring clinically relevant levels of resistance have not been described [35]. Disruption of uptake into cells is thought to also be a mechanism of resistance in staphylococci [36].

### 3.2.5 Enhanced Drug Efflux

A wide range of bacterial efflux pumps have been characterised [37, 38]. Some pumps export aminoglycosides; for example, *P. aeruginosa*-expressing MexXY pumps have been associated with amikacin resistance [39].

### 3.3 Clinical Pharmacokinetics

Like other aminoglycosides, amikacin and tobramycin have poor oral bioavailability, requiring parenteral administration. Peak plasma concentration ( $C_{\max}$ ) occurs approximately 30 min post-infusion for both drugs in adults and neonates [40–43].

Both drugs are strongly hydrophilic and distribute primarily into extracellular fluid with a volume of distribution ( $V_d$ ) of 0.5–0.6 L/kg in neonates for both agents [44, 45]. Aminoglycosides do not significantly penetrate the cerebrospinal fluid (CSF) in adults [46], even when the meninges are inflamed. However, in neonates, a CSF partition coefficient of 0.103 for amikacin has been estimated [47], although there is limited evidence of drug penetration with tobramycin [48] (for comparison, gentamicin penetrance is negligible [49, 50]). Protein binding is minimal for both drugs [51, 52].

Amikacin and tobramycin are renally excreted unmetabolised. The adult elimination half-life is ~2 h for both agents, with > 90% of drug recovered from urine within 24 h [51, 53]. However, the terminal elimination phase after cessation of therapy is prolonged, with 100% renal excretion requiring 10–20 days for both drugs [51, 54]. In neonates, the half-life can be significantly longer because of renal ontogenesis:  $7.6 \pm 4.4$  h for amikacin [55] and 2–7.3 h for tobramycin [43].

Renal tubular accumulation of aminoglycosides, mediated by several transporters [56], is thought to be the mechanism of nephrotoxicity and the prolonged terminal phase of elimination. Identified transporters include Megalin, Cubilin, TRPV1, and TRPV4 [57–60]; all have affinity for most aminoglycosides, but the relative affinity varies between individual molecules, likely reflecting the variable nephrotoxicity of different aminoglycosides.

### 3.4 Toxicity

There are three main aminoglycoside toxicities. First, aminoglycoside use can cause a reversible non-oliguric renal impairment due to aminoglycoside accumulation in proximal tubular epithelial cells, leading to tubular necrosis [61]. Once-daily dosing in adults reduces this toxicity compared with multiple daily dosing [62], implying a time above plasma concentration threshold relationship for nephrotoxicity (in contrast to a peak concentration relationship for

bacterial killing). Comparable data are scarce in neonates [63, 64], although the relationship to dosing schedule is presumed to be consistent between age groups, leading to predominant use of once-daily dosing in neonates [65–67].

Aminoglycosides can cause ototoxicity via damage to the sensory hair cells of the inner ear, particularly the high-frequency outer hair cells [68]. This can produce irreversible cochlear function impairment. Vestibular impairment can also occur, but this is reversible on cessation of the drug. The exact mechanism is not understood; the dose–effect relationship seems to be idiosyncratic and possibly associated with certain mitochondrial genetic variations [68, 69]. Detailed neonatal data are absent, but neonatal amikacin use may be associated with a 3% ototoxicity incidence [63]. Tobramycin does not appear to be associated with significant ototoxicity [70].

Neuro-muscular blockade is a rare but serious toxicity associated with aminoglycoside use. However, cases have only been reported in adults [71–73]; there have been no reported cases in neonates.

### 3.5 Pharmacodynamics

Specific pharmacodynamic targets for tobramycin and amikacin have not been established. However, there are considerable published in vitro and in vivo pharmacodynamic data for other aminoglycosides.

Multiple aminoglycoside dose fractionation studies in neutropenic mouse models [74–77] suggest that the pharmacodynamic index that best links drug exposure with antimicrobial activity is  $C_{\max}$ /minimum inhibitory concentration (MIC). However, other experimental platforms suggest the relevant pharmacodynamic index is area under the concentration–time curve (AUC)/MIC [78, 79].

In multiple adult clinical trials, the  $C_{\max}$ /MIC ratio has repeatedly been related to clinical success of aminoglycosides [80–85], with a pharmacodynamic target of  $C_{\max}$ /MIC  $\geq 10$  for a > 90% successful clinical outcome where this was calculated [80, 84]. Interestingly, where the AUC/MIC was also calculated, it was equally related with patient outcomes [84, 85].

Because of the limited human dosing schedules of aminoglycosides (once daily in most published trials),  $C_{\max}$  and AUC are co-linear [86], a likely reason for the inability to distinguish  $C_{\max}$ /MIC and AUC/MIC as the relevant pharmacodynamic index. Therefore, pragmatically,  $C_{\max}$  (rather than AUC) is used in therapeutic drug monitoring in clinical contexts [84, 86].

### 3.6 Potential Utility in Neonatal Sepsis

Both aminoglycosides have potential utility in neonatal sepsis given their spectra of activity against Gram-negative



bacteria that are gentamicin resistant and staphylococci. Given its stability to a wider range of AMEs, amikacin is the more promising choice in an AME-prevalent environment. Both agents would rely on another antibiotic in a potential combination regimen to provide activity against streptococci.

Both aminoglycosides have a long history of neonatal use, with pharmacokinetics and pharmacodynamics well understood. Although toxicities are associated with their use, they can be mitigated.

## 4 Fosfomycin

Fosfomycin is a phosphoric acid derivative isolated from various *Streptomyces* species [87]. Fosfomycin is a small molecule (Fig. 2), with a molecular weight of 138 g/mol [88] and three different formulations: a disodium compound, used for intravenous administration, and tromethamine and calcium compounds, used for oral administration. The following discussion is focused on the disodium formulation given the typical requirement for parenteral treatment of neonates.

### 4.1 Mechanism of Action

Fosfomycin inhibits the enzyme MurA, which catalyses the reaction of UDP-*N*-acetylglucosamine (UDP-GlcNAc) with phosphoenolpyruvate (PEP) to form UDP-GlcNAc-enolpyruvate plus inorganic phosphate [89]. Specifically, fosfomycin acts as a PEP analogue to inhibit the enzyme [90]. This reaction is the first step in peptidoglycan synthesis, with inhibition of this process inhibiting bacterial cell wall synthesis, leading to cell death.

Uptake of fosfomycin into the bacterium is dependent on two bacterial transporters. The first, GlpT, is an antiporter that normally transports glycerol-3-phosphate (G3P) in exchange for a phosphate molecule [91]. This uptake system is found amongst many species of bacteria [90].

Alternatively, the hexose-phosphate uptake system (UhpT) can also transport fosfomycin. This mechanism is glucose-6-phosphate (G6P) dependent and limited to Enterobacterales (with the exception of *Proteus* species) and *S. aureus* species [92]. This transporter is only induced in the presence of G6P, so fosfomycin uptake via this pathway

usually requires the presence of G6P. As a result, in vitro assays involving fosfomycin require G6P supplementation.

Fosfomycin has a broad spectrum of activity, with potential activity against most Gram-positive and Gram-negative bacteria, including *Pseudomonas* species and anaerobes [93], with *Acinetobacter* and *Listeria* species being important exceptions [89, 94].

### 4.2 Resistance Mechanisms

There are three main resistance mechanisms against fosfomycin.

#### 4.2.1 Reduced Permeability to Fosfomycin

Fosfomycin uptake is dependent on the presence of either GlpT or UhpT transporters. Mutations in the genes encoding either can confer resistance. Species that lack both transporters (e.g., *Listeria monocytogenes*) are inhibited by intrinsically higher fosfomycin MICs [94, 95]. Some species intrinsically lack one pathway, with development of resistance requiring only a single gene mutation, e.g., *glpT* in the UhpT-lacking *P. aeruginosa*.

Both transport systems are cyclic adenosine monophosphate (cAMP) dependent. Therefore, mutations that reduce cAMP levels (e.g., *ptsI* or *cyaA* mutations) will prevent cell penetration of fosfomycin [96, 97], albeit at a survival cost due to alterations in carbohydrate catabolism. This survival cost may explain the discrepancy between the high rate of resistance ( $> 10^{-2}$ ) observed in vitro and the rare occurrence of fosfomycin-resistant clinical isolates where fosfomycin use is prevalent [98].

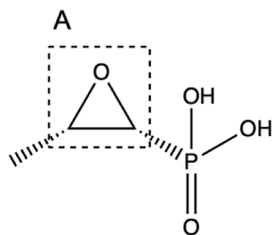
#### 4.2.2 Modification of MurA Target

Mutations of the *murA* gene encoding the target MurA protein can occur. In vitro mutagenesis of *MurA* altering Cys 115 to Asp confers resistance [99], but this has not been seen in clinical isolates. A number of other mutations in *murA* (Asp369Asn, Leu370Ile, Asp3890Ala, Gln59Lys, GIU139Lys, Val389Ile) have been identified in fosfomycin-resistant clinical samples [100–102], but these mutations are infrequent compared with other resistance mechanisms. Interestingly, in vitro overexpression of *murA* also increases the fosfomycin MIC [103], although this mechanism has not been identified in clinical isolates.

#### 4.2.3 Modification by Fosfomycin-Modifying Enzymes

Fosfomycin-modifying enzymes are the most frequently identified cause of resistance in clinical isolates, with three metalloenzymes identified: FosA, FosB, and FosX. All modify the fosfomycin molecule by opening the oxirane ring of

Fig. 2 Molecular structure of fosfomycin. A oxirane ring



the fosfomycin molecule (Fig. 2), using different substrates to add chemical groups to the molecule:

- FosA is a  $Mn^{2+}$ - and  $K^{+}$ -dependent glutathione-S-transferase, originally identified on a plasmid in a *Serratia marcescens* strain [104, 105]. FosA-type enzymes have been identified in multiple different clinically resistant isolates [101] and are often co-expressed with other AMR genes (e.g., *bla*<sub>KPC</sub> and *bla*<sub>CTX-M</sub>) in some geographic regions [106, 107]. *fosA* homologues have also been identified in the chromosome of many Gram-negative bacteria strains, including *Klebsiella*, *Enterobacter*, *Serratia*, and *Pseudomonas* species [108, 109].
- FosB is a  $Mg^{2+}$ -dependent thiol-S-transferase, originally identified in *Staphylococcus epidermidis* [110] but found in many other low G+C Gram-positive bacteria (e.g., *Bacillus* species and *S. aureus*) that do not synthesise glutathione. This is the most prevalent fosfomycin-resistance mechanism in *S. aureus* [111].
- FosX is a  $Mn^{2+}$ -dependent epoxide hydrolase that uses water to hydrolyse the oxirane ring. The *fosX* gene has been identified in the chromosomes of several species, including *L. monocytogenes* [112].

In addition, there are two fosfomycin kinases: FomA and FomB. These sequentially add phosphate groups to the phosphonate moiety of fosfomycin from adenosine triphosphate with  $Mg^{2+}$  as a cofactor [113]. These have only been found in fosfomycin-producing *Streptomyces* species, providing autoprotection to these bacteria.

### 4.3 Clinical Pharmacokinetics

Given its small molecular weight, fosfomycin distributes widely into most tissues, including bone and soft tissue [114, 115], with an adult  $V_d$  of 9–30 L [116], and protein binding is negligible [117]. CSF penetration is partial, with a partition coefficient of ~0.15–0.2 in adults [118] and 0.32 in neonates [119]. Fosfomycin is almost entirely cleared by renal excretion, with ~90% of the drug recovered unchanged in the urine by 48 h [120, 121] and an adult half-life of 1.9–3.9 h [116]. Fosfomycin is not metabolised. Significant levels of fosfomycin have been detected in the bile [116, 122], suggesting that biliary excretion accounts for the remaining clearance. However, as studies measuring faecal fosfomycin levels used incompletely bioavailable oral formulations, this has yet to be confirmed.

A recent fosfomycin pharmacokinetic study in neonates ( $n = 61$ ) examining 100 mg/kg bolus doses gave a median  $C_{max}$  of approximately 350 mg/L (with large inter-individual variability) with a median  $\beta$ -phase half-life of 5.2 h [119]. This is concordant with the limited published pharmacokinetic data in neonates, with two small pharmacokinetic

studies (total  $n = 22$ ) giving  $C_{max}$  values of ~96–98 mg/L and ~135 mg/L following 50 mg/kg intravenous bolus and 200 mg/kg infusion (30–120 min), respectively, and a mean half-life of ~7 h, with a half-life of 4.9 h in a subset of older neonates (3–4 weeks old;  $n = 6$ ) [123, 124].

### 4.4 Toxicity

Fosfomycin is associated with no common serious adverse events and limited common adverse events, most notably hypokalaemia [125]. Phlebitis, rash, and gastrointestinal upset occur commonly but are no more frequent than with comparator antibiotics. There is potential concern about the inherent sodium load with intravenous fosfomycin disodium administration, with adverse outcomes related to sodium overload noted in two recent adult trials, both involving cardiac patients at risk of overload and prolonged parental treatment [126, 127]. This may be relevant to neonates given their risk of fluid overload [128], but such adverse events have yet to be demonstrated in this context.

### 4.5 Pharmacodynamics

Studies examining the most relevant pharmacodynamic index for fosfomycin have yielded conflicting results. Different animal models have identified the relevant pharmacodynamic index as  $C_{max}/MIC$  [129] or AUC/MIC (with ratios of 23 and 83 required for stasis and 1-log kill in Enterobacteriales, respectively) [130], and an in vitro dynamic one-compartment experiment assessing *P. aeruginosa* suggested %time>MIC is the relevant index [131] (although the possibility of AUC/MIC as the index was not assessed). Several other non-dynamic in vitro studies have come to different conclusions about the index for bacterial kill [118, 132, 133], but it is difficult to give weight to these conclusions over those from dynamic models. With regards to emergence of resistance, a single hollow fibre infection model experiment suggested that the AUC/MIC ratio is the relevant pharmacodynamic index [134].

### 4.6 Potential Utility in Neonatal Sepsis

Fosfomycin has been underused since its discovery in the 1960s, despite its broad spectrum of activity, and it has activity against most neonatal sepsis pathogens. Additionally, its unique mechanism of action limits potential cross-resistance from other antimicrobial classes. These factors mean that fosfomycin-resistance rates are low globally and make this agent suitable for treatment of neonatal sepsis in AMR-prevalent settings [128].

Previous data gaps in neonatal pharmacokinetics have largely been answered by the recent large NeoFOSFO pharmacokinetic study [119]. However, the pharmacodynamic

characterisation of fosfomycin is incomplete, with the exact pharmacodynamic indices uncertain. Finally, regulatory approval for fosfomycin is not universal, with a licence not yet available in many LMICs.

## 5 Flomoxef

Flomoxef is an oxacephem class  $\beta$ -lactam antibiotic first synthesised in Japan in the 1980s [135, 136].  $\beta$ -lactams are amongst the most widely used class of antibiotic globally [137, 138], characterised molecularly by a common  $\beta$ -lactam ring [137]. The predominant mechanism of  $\beta$ -lactam resistance is degradation by  $\beta$ -lactamases, with increasing prevalent ESBLs being a significant cause of  $\beta$ -lactam resistance globally [139].

Oxacephems are derivatives of cephalosporins, originally isolated from *Streptomyces* species [135]. The molecular configuration of oxacephem sidechains confer particular activity against Gram-negative bacteria and stability against degradation by  $\beta$ -lactamases [140], albeit normally at the expense of activity against Gram-positive bacteria [141].

However, flomoxef has an additional 7- $\beta$ -difluoromethylthioacetamido side chain substitution that enables high levels of activity against both Gram-positive and Gram-negative bacteria (including anaerobes) and a reduced toxicity profile [142, 143] (Fig. 3). Additionally, the *N*-methyltetrazolethiol (NMTT) group attached to the 3'-position of the oxacephem nucleus (thought to be responsible for the disulfiram- and coumarin-like side effects seen in NMTT-containing oxacephems, e.g., latamoxef) is substituted with a methylthiadiazolethiol (MTDT) group [144, 145].

Despite the benefits of this molecule over other  $\beta$ -lactams and its widespread use in Japan [136], flomoxef is currently licensed and used only in East Asia.

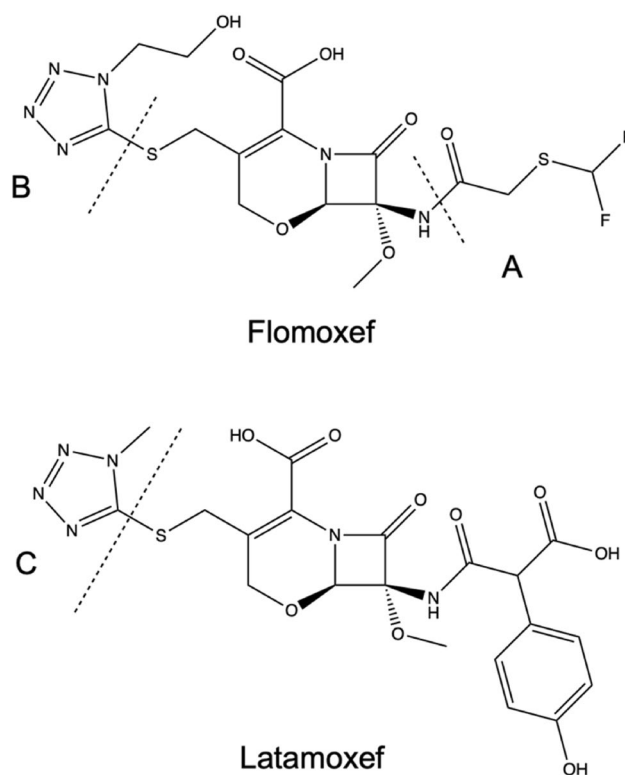
### 5.1 Mechanism of Action

Like other  $\beta$ -lactams, flomoxef acts by binding to penicillin-binding proteins (PBPs) (specific flomoxef affinities to PBP types are unknown), inhibiting cell wall synthesis and leading to bacterial cell death.

Unlike many other cephalosporins, flomoxef demonstrates high activity against staphylococci (including methicillin-resistant strains), most Enterobacterales and almost all anaerobes [142, 146, 147]. Important gaps in the spectrum of activity include enterococci, *Pseudomonas*, and *Acinetobacter* species [142, 143, 147].

### 5.2 Resistance Mechanisms

As a  $\beta$ -lactam, flomoxef resistance is predominantly via one of four mechanisms.



**Fig. 3** Molecular structures of flomoxef and latamoxef. **A** 7- $\beta$ -difluoromethylthioacetamido side chain, **B** methylthiadiazolethiol group, **C** *N*-methyltetrazolethiol group

#### 5.2.1 Hydrolysis by $\beta$ -Lactamase Enzymes

The predominant resistance mechanism for  $\beta$ -lactams is hydrolysis of the active  $\beta$ -lactam ring by bacterial  $\beta$ -lactamase enzymes. Oxacephems, including flomoxef, are stable to many  $\beta$ -lactamases, including ESBLs such as the CTX-M enzymes [143, 148–150], an attractive feature in the context of the rising incidence of ESBL-mediated AMR. However, flomoxef is susceptible to AmpC-like (i.e., Ambler class C)  $\beta$ -lactamases and carbapenems [150, 151]. This is potentially of concern because of the emergence of *Klebsiella* strains producing plasmid-mediated AmpC-like  $\beta$ -lactamases, which confer high flomoxef MICs [152].

Epidemiological studies have demonstrated that, because of flomoxef's overall stability to ESBLs, flomoxef susceptibility rates in bacteria are comparable to those of carbapenems, even in geographical regions where it is used widely (e.g., China) [148, 149, 153–157]. Retrospective studies have suggested that treatment of bloodstream infections caused by ESBL-producing bacteria is equally successful with either flomoxef or carbapenems [158–160], highlighting the potential use of flomoxef as a carbapenem-sparing agent.



### 5.2.2 Modifications of Penicillin-Binding Proteins (PBPs)

Modification of target PBPs is another important resistance mechanism to  $\beta$ -lactams, e.g., PBP2a confers  $\beta$ -lactam resistance in methicillin-resistant *S. aureus* (MRSA). Flomoxef retains activity against MRSA [142] and appears to induce production of PBP2a to a lesser extent than other  $\beta$ -lactams [161]. *Streptococcus pneumoniae* also develops  $\beta$ -lactam resistance by a variety of PBP gene mutations, although many penicillin non-susceptible pneumococcal strains remain susceptible to flomoxef [149].

### 5.2.3 Mutations in Porin Genes

Mutations in porin genes can affect the antimicrobial penetration of  $\beta$ -lactams, although these have not been characterised in the context of flomoxef. However, some porin mutations (e.g., *ompF*) seem to produce small changes in the flomoxef MIC [150].

### 5.2.4 Efflux

As discussed in Sect. 3.2, many efflux mechanisms have been identified [37, 38], several of which affect  $\beta$ -lactams and may affect flomoxef, although no particular associations with flomoxef have been described.

## 5.3 Clinical Pharmacokinetics

Flomoxef is only formulated as an intravenous injection, with  $C_{\max}$  occurring about 1 h post administration [136]. Protein binding is minimal at < 10% [162]. CSF penetration is limited, with a partition coefficient of  $\sim 0.05$  in active meningitis in children [163].

Flomoxef is predominantly cleared by renal excretion, with 85% of the compound excreted unchanged within 6 h [136]. Involvement of active excretion via an organic anion transporter, possibly OAT1, is implied by competitive inhibition of renal clearance by co-administered probenecid [164, 165], although specific transporter associations have not been identified. However, there is some metabolic clearance, although the enzymatic mechanisms are poorly characterised. Flomoxef oxide is an active metabolite with approximately 25% of the activity of flomoxef [166]. However, clearance via this route only accounts for  $\sim 0.1\%$  of the total [167]. Metabolism to the inactive metabolite hydroxyethyl-tetrazoletinol accounts for approximately 10–15% of total clearance [167].

The adult  $\beta$ -phase half-life of flomoxef is approximately 50 min [136]. No single large pharmacokinetic study involving neonates has been conducted. However, many small neonatal studies involving flomoxef have estimated pharmacokinetic parameters for each dataset [168–177]. The

parameters from these studies vary widely because of the high degree of inter-subject variability in age, weight, and gestation, with a median half-life of 2.21 h (range 0.68–6.6). However, all pharmacokinetic studies (adult and neonatal) were performed in a Japanese population, and validation of pharmacokinetic characteristics in non-Japanese populations is ideally required.

## 5.4 Toxicity

Other NMTT-containing oxacephems (e.g., latamoxef) exhibit coagulopathic side effects due to inhibition of vitamin K<sub>1,2,3</sub>-epoxide reductase. Flomoxef inhibits this enzyme at a lower affinity than other oxacephems, with coagulopathic toxicity not manifesting in clinical use [144, 145]. Additionally, many oxacephems induce a disulfiram-like reaction with co-administration of alcohol due to inhibition of aldehyde dehydrogenase [178, 179], but this does not appear to occur with flomoxef, because of its MTD side chain substitution.

Flomoxef has only mild common side effects, with frequent gastrointestinal disturbance (possibly due to the effects on anaerobes in the gut), rash, eosinophilia, neutropenia, and drug fever [145, 180]. Although most toxicity data have been determined from studies in adults, the limited toxicity data from neonatal pharmacokinetic studies [168–177] suggest a similar toxicity profile in neonates.

Rare side effects such as pneumonitis have been reported, although only in adults [181].

## 5.5 Pharmacodynamics

Previously, an assumption was made that the pharmacodynamics of flomoxef were similar to those of cephalosporins, with assumed pharmacodynamic targets of 40% (bacteriostatic) or 70% (bactericidal) time > MIC [182–184]. However, a recent mouse thigh model determined targets of 40% and 25% time > MIC for 1-log bacterial kill and stasis, respectively [185]. No pharmacodynamic target for prevention of emergence of resistance has been identified.

## 5.6 Potential Utility in Neonatal Sepsis

Flomoxef is an attractive option for neonatal sepsis in AMR-prevalent settings. It has a broad spectrum of activity and intrinsic stability to non-AmpC-type ESBLs, with low resistance rates epidemiologically. Significant neonatal pharmacokinetic data are also available (albeit only in Japanese populations), and it has a safe toxicity profile. The pharmacodynamics of bactericidal killing have also recently been described, although pharmacodynamic characterisation for emergence of resistance is still lacking. The main barrier to

the use of flomoxef is the lack of regulatory approval outside of East Asia.

## 6 Cefepime

Cefepime is a  $\beta$ -lactam and fourth-generation cephalosporin. Cephalosporins are  $\beta$ -lactam antibiotics derived from the prototypical molecule cephalosporin C, isolated from a *Cephalosporium* (now referred to as *Acremonium*) species of fungi in the 1950s [186]. Subsequently, dozens of derivatives of this molecule have been created [138]. These are broadly categorised into generations, with successive generations generally having broader spectra of activity and greater activity in the presence of resistance mechanisms than earlier generations.

Cefepime is an aminothiazolyl methoxyimino cephalosporin that is structurally similar to third-generation cephalosporins such as ceftriaxone and cefotaxime (Fig. 4). It has an *N*-methyl-pyrrolidine moiety at the 3' position that confers zwitterionic properties [187]. This feature allows the drug to penetrate the Gram-negative bacterial outer membrane more rapidly than other  $\beta$ -lactams [188, 189].

### 6.1 Mechanism of Action

Like other  $\beta$ -lactams, cefepime inhibits cell wall synthesis by binding to PBPs (with particular affinities demonstrated for PBPs 2 and 3 in *Escherichia coli* [190]), inhibiting the peptidoglycan synthesis pathway and leading to cell lysis and death. Cefepime has a broad spectrum of activity affecting both Gram-positive and Gram-negative bacteria, including pseudomonads [191]. However, like other cephalosporins, it lacks activity against anaerobic bacteria [192].

## 6.2 Resistance Mechanisms

Like other  $\beta$ -lactams, resistance to cefepime occurs by one of four main mechanisms.

### 6.2.1 Hydrolysis by $\beta$ -Lactamase Enzymes

As discussed in Sect. 5.2, the most common resistance mechanism to  $\beta$ -lactams is hydrolysis by  $\beta$ -lactamases. Compared with other  $\beta$ -lactams, cefepime is significantly more stable to  $\beta$ -lactamases [193]. Compared with other cephalosporins, such as cefotaxime and ceftazidime, AmpC-like ESBLs have a lower affinity and hydrolysis rate to cefepime [194, 195], albeit with an associated inoculum effect [196, 197]. However, cefepime has affinity and lability to non-AmpC ESBLs [193, 195, 198]. Although cefepime MICs for ESBL-producing bacteria may fall below the European Committee on Antimicrobial Susceptibility Testing/Clinical Laboratory Standards Institute (CLSI) breakpoints for this drug [199], the use of cefepime against these bacteria has been associated with clinical failure [199, 200].

### 6.2.2 Modification of Target PBPs

Modification of the target PBP is a common resistance mechanism in Gram-positive bacteria. As with most other  $\beta$ -lactams, production of PBP2a in MRSA confers resistance to cefepime [201].

### 6.2.3 Mutations in Porin Genes

Many  $\beta$ -lactams, including cefepime, require porins to enter the bacterial cell [202]. *E. coli* and *Klebsiella* strains with loss or downregulation of genes encoding the porins OmpA, OmpC, and/or OmpF exhibit a two- to fourfold increase in the cefepime MIC [203]. Additionally, loss of the porin

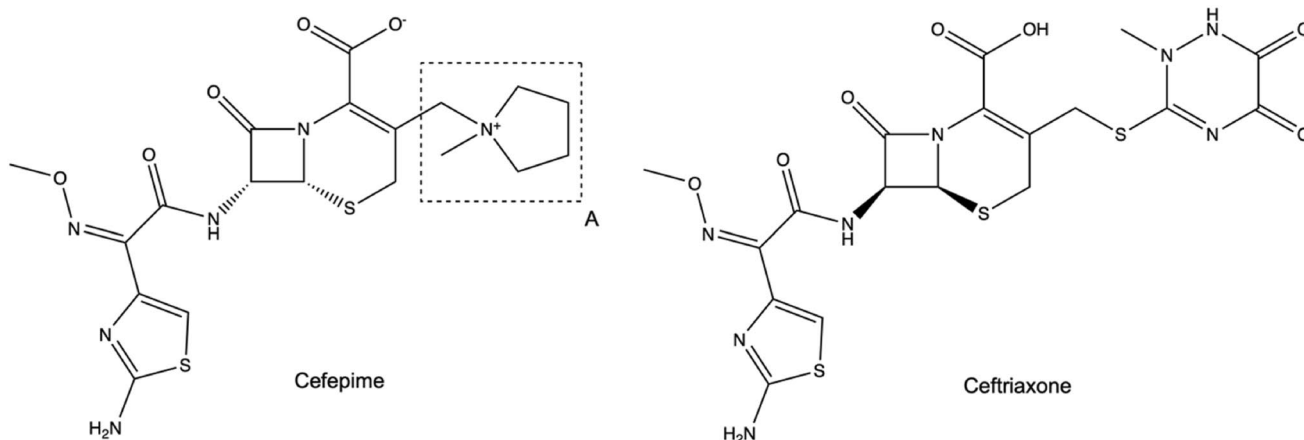


Fig. 4 Molecular structures of cefepime and ceftriaxone. A *N*-methyl-pyrrolidine moiety

Omp36 in *Klebsiella aerogenes* (homologous to OmpC in *E. coli* and OmpK36 in *Klebsiella pneumoniae*) has been associated with a fourfold increase in cefepime MIC [204].

#### 6.2.4 Efflux

As discussed in Sect. 3.2, many bacteria efflux pumps have been characterised [37, 38], several of which affect  $\beta$ -lactams. No comprehensive cataloguing of the efflux pumps that affect cefepime has been performed. However, *P. aeruginosa* strains overexpressing Mex-AB-OprM, Mex-XY-OprM, and/or Mex-CA-OprJ have been associated with an increased cefepime MIC [205, 206].

### 6.3 Clinical Pharmacokinetics

The half-life of cefepime in adults is approximately 2 h, with a  $C_{\max}$  increasing linearly with the administered dose [207, 208]. There is some protein binding, with binding rates of 16.2–19% to human serum proteins [191, 209]. Two cefepime neonatal pharmacokinetic trials have been performed with doses of 30 and 50 mg/kg [210, 211]. The calculated half-lives were  $4.9 \pm 2.1$  and  $4.32 \pm 1.8$  h with  $C_{\max}$  values of  $89 \pm 27$  and  $120.9 \pm 38.5$  mg/L, respectively.

Cefepime distributes readily into the soft tissue, with rapid penetration into inflammatory fluid, with close matching to plasma concentrations [212, 213]. Mean adult  $V_d$  is ~ 18–21 L [212, 214]; neonatal  $V_d$  is ~ 0.4 L/kg, although this increases with decreasing gestation below 30 weeks [211].

Adult CSF penetrance is variable, with partition coefficients of 0.05–0.34 [215]. A small neonatal study ( $n = 9$ ) suggested similarly variable CSF penetrance, with a median partition coefficient of 0.077 (range 0.030–0.876) [216].

Cefepime is primarily renally excreted, with ~ 80% of administered drug recovered unchanged in the urine [208]. Approximately 10–12% of administered cefepime is metabolised. Approximately 7% is metabolised first to *N*-methylpyrrolidine and then rapidly oxidised to *N*-methylpyrrolidine-*N*-oxide. Another 3% is metabolised to the 7-epimer of cefepime [217]. The overall rate of clearance in neonates is variable, ranging from 0.5 to 2.5 mL/min/kg (gestational age only marginally accounts for this variability) [211].

No specific transporters have been associated with cefepime transport; however, cefepime inhibits (but is not transported by) OCTN2 [218]. Unlike some other  $\beta$ -lactams, cefepime has no affinity to the intestinal transporter PEPT1, which likely explains its poor oral bioavailability [219].

### 6.4 Toxicity

Cefepime is a largely safe drug with common side effects being usually mild and similar to those affecting other

$\beta$ -lactams, e.g., gastrointestinal disturbance, rash, etc. [221, 222].

Cefepime is associated with rare neurotoxic side effects, which are heterogenous (presentations include encephalopathy, myoclonus, seizures, and coma) and usually start several days into treatment [223]. The neurotoxicity appears to be concentration dependent [223, 224] and associated with renal dysfunction and critical illness [223] and is thought to be due to concentration-dependent inhibition of GABA<sub>A</sub>-mediated neurotransmission [225]. This toxicity is mainly associated with increasing age and renal dysfunction [223], the latter of potential concern in neonates.

An initial meta-analysis of cefepime outcome data suggested a possible increased mortality associated with cefepime use compared with other  $\beta$ -lactams (including in paediatric populations) [226, 227]. However, two further meta-analyses (with combined adult and paediatric data, and with paediatric data alone, respectively) showed no statistically significant increase in mortality associated with cefepime use [228, 229]. Further retrospective studies of paediatric and neonatal patients receiving cefepime failed to show any statistically significant excess mortality [230–232].

### 6.5 Pharmacodynamics

Cefepime is presumed to have the same pharmacodynamic index as other cephalosporins, with a pharmacodynamic target of 60–70% time > MIC producing maximal effect [220]. A pharmacokinetic model and Monte Carlo simulation suggested that the widely used dose of 30 mg/kg administered 12 hourly would achieve a target 50% time > MIC for the CLSI-recommended breakpoint concentration for 99% of neonatal patients [211].

### 6.6 Potential Utility in Neonatal Sepsis

Cefepime has a broad spectrum of activity and, although not specifically licensed for neonatal use, experience with it in neonatal settings is extensive. Its reduced affinity for ESBLs render it potentially useful in AMR-prevalent settings, and it has a safe toxicity profile in neonates.

However, although it may be reasonable to assume characteristics similar to those of other cephalosporins, specific pharmacodynamic characterisation is lacking and, despite its reduced affinity to ESBLs, it remains labile to non-AmpC ESBLs in in vitro settings [195, 198] and has a poorer than expected clinical record for treatment of ESBL-producing bacteria [199, 200]. These characteristics may limit its utility in neonatal sepsis in ESBL-prevalent areas.

Table 2 Summary of pharmacological characteristics of candidate antimicrobials

Characteristic	Amikacin	Tobramycin	Fosfomycin	Flomoxef	Cefepime
Molecular target	16S ribosomal subunit	16S ribosomal subunit	MurA	PBP	PBP
Spectrum of activity	GN bacteria (including pseudomonads); some activity in staphylococci	GN bacteria (including pseudomonads); some activity in staphylococci	Enterobacteriales, pseudomonads, streptococci, and staphylococci	Enterobacteriales, streptococci, enterococci, and staphylococci	Enterobacteriales, pseudomonads, streptococci, and staphylococci
Important efficacy gaps	Streptococci	Streptococci; gentamicin-resistant GN bacteria with cross-reactive AMEs	Acinetobacter, listeria; GN bacteria with chromosomal FosA	AmpC-like ESBL-producing GN bacteria; enterococci; <i>Pseudomonas</i> species; <i>Acinetobacter</i> species	Enterococci; anaerobes; potentially non-class C ESBL-producing GN bacteria
Relevant resistance mechanisms	(1) AMEs; (2) methylation of 16S; (3) enhanced efflux	(1) AMEs; (2) methylation of 16S; (3) enhanced efflux	(1) fosfomycin-modifying enzymes; (2) MurA modification; (3) disruption to GlpT or UhpT uptake mechanisms	(1) AmpC-like ESBL; (2) modification of porins; (3) enhanced efflux (presumed)	(1) Non-AmpC ESBLs?; (2) modification of PBPs; (3) modification of porins; (4) enhanced efflux
Characterisation of neonatal pharmacokinetics	++	++	++	++	+
Significant toxicities	Dose-dependent renal impairment; non-dose-dependent ototoxicity (rare); neuromuscular blockade (very rare)	Dose-dependent renal impairment; non-dose-dependent ototoxicity (rare); neuromuscular blockade (very rare)	Sodium overload; hypokalaemia	Pneumonitis (very rare)	Neurotoxicity (rare)
CSF: plasma partition	0.103	Undefined	0.32	0.05	0.05–0.34
Presumed pharmacodynamic index (bacterial killing)	$C_{max}/MIC$ ratio	$C_{max}/MIC$ ratio	AUC/MIC ratio or %Time > MIC	%Time > MIC	%Time > MIC
Characterisation of exposure response (bacterial killing)	+	+	+	+	-
Characterisation of exposure response (emergence of resistance)	-	-	+	-	-

AME aminoglycoside-modifying enzymes, AUC area under the concentration–time curve,  $C_{max}$  peak plasma concentration, CSF cerebrospinal fluid, ESBL extended-spectrum  $\beta$ -lactamase, GN Gram negative, MIC minimum inhibitory concentration, PBP penicillin-binding protein, ++ indicates substantial data available, + indicates some data available but, ideally, more required, – indicates no data available

## 7 Summary

These five antimicrobials all have characteristics that make them potential candidates for an empiric antimicrobial regimen for neonatal sepsis (Table 2). All demonstrate spectra of activity against bacteria that cause neonatal sepsis with prevalent mechanisms of resistance that are problematic for the current WHO-recommended regimen: amikacin and tobramycin offer activity against gentamicin-resistant Gram-negative bacteria; flomoxef and cefepime have enhanced stability to ESBLs compared with other  $\beta$ -lactams; and fosfomycin still retains widespread activity against bacteria with resistance mechanisms to other classes. However, although they fulfil the selection criteria, both tobramycin and cefepime have a narrower spectrum of activity than the alternatives because of their lability to cross-reactive AMEs and non-AmpC ESBLs, respectively. This makes these agents less attractive than the other three agents.

All drugs are also off patent and have potential to be produced at an affordable rate for LMICs. Additionally, all drugs have favourable toxicity profiles, with toxicities being limited and predictable where they occur. All also have a licence for, or significant experience in the treatment of, neonatal infections (although licensing for fosfomycin and flomoxef remains geographically limited, which would need addressing via, for example, expedited local regulatory approval, should they be selected in an eventual empiric regimen).

Nevertheless, there are some key gaps in the pharmacological data available for neonates (Table 2). Although the clinical pharmacokinetics of all these drugs are well described for adults, the data are more limited in neonates. Neonatal pharmacokinetic data for fosfomycin are largely underpinned by a single large pharmacokinetic study [119]; those for cefepime rely on two relevant neonatal pharmacokinetic studies [210, 211]; and flomoxef pharmacokinetic data are absent in non-Japanese populations. Furthermore, knowledge of CSF penetration is limited in some agents. Neonatal CSF:plasma coefficients have not been determined for tobramycin [233], and the flomoxef coefficient is dependent on a single study [163]. The CSF penetration data available for the five agents compare well to those of the WHO regimen ( $\sim 0$  for gentamicin [49, 50]; 0.05–0.1 for amoxicillin [234]).

The other key knowledge gap is the pharmacodynamic characterisation of these drugs. Flomoxef has recently been characterised in terms of bactericidal killing, but the characterisation of other agents is more limited. For cefepime and the aminoglycosides, the exposure–response relationships and pharmacodynamic targets are presumed to be similar to those of the other molecules within the same class, but

these have not been determined independently. For fosfomycin, published evidence has definitively established neither the pharmacodynamic index nor the target. Furthermore, the exposure–response relationship and pharmacodynamic targets for prevention of emergence of resistance have been poorly characterised in all agents.

In addition to consideration of each agent's pharmacological characteristics, an eventual regimen selection is dependent on the epidemiology of resistance and manufacturing costs. As discussed in Sect. 1, high-quality observational studies of neonatal sepsis across multiple geographical locations are rare. Amongst these studies, reported resistance rates for these particular agents are rarer still. The single high-quality neonatal sepsis study reporting rates of resistance to these antibiotics indicated 63%, 55%, 25%, and 18% of Gram-negative bacteria (including non-Enterobacterales, e.g., *Acinetobacter* species) were resistant for tobramycin, cefepime, amikacin, and fosfomycin, respectively [9]. There are no data indicating the level of resistance to flomoxef in bacteria causing neonatal sepsis. However, as discussed in Sect. 5.2, flomoxef resistance is consistently seen in  $\leq 10\%$  isolates of Enterobacterales causing non-neonatal infections in geographic regions where flomoxef is currently used [149, 153, 155–157]. A further multi-centre neonatal sepsis observational cohort study, NeoOBS, is expected to report soon with AMR data for flomoxef, fosfomycin, and amikacin [235].

The current cost of each agent per vial is detailed in Table 3. Although more expensive than current WHO regimen antibiotics, the material costs of the five agents are potentially affordable in LMIC settings. Reformulation of vial size quantities relevant to paediatric or neonatal requirements could lower the material costs. Furthermore, many of the agents are only produced by a small number of generic manufacturers (e.g., intravenous fosfomycin); expansion of these could lower the costs further [236].

**Table 3** Comparative costs of the World Health Organization regimen antibiotics and the five reviewed antibiotics and typical neonatal regimen

Antibiotic	Material cost (\$US)	Typical neonatal regimen
Gentamicin	1.91/80 mg [237]	5 mg/kg q24h
Amoxicillin	0.45/250 mg [237]	30 mg/kg q8h
Amikacin	2.86/100 mg [237]	15 mg/kg q24h
Tobramycin	7.43/80 mg [237]	5 mg/kg q24h
Fosfomycin	20.75/2 g [237]	100 mg/kg q12h
Flomoxef	2.97/1 g [238]	20 mg/kg q8–12h
Cefepime	7.61/1 g [237]	30 mg/kg q8–12h

Costs converted to \$US using conversion rates on 15 July 2021  
 $qxh$  every  $x$  h,  $q8-12h$  every 8–12 h



An alternate first-line regimen for the empiric treatment of neonatal sepsis will likely have to be a combination of two of these agents to provide the necessary spectrum coverage to treat target pathogens and resistance motifs and protect against emergence of resistance to both agents. A positive pharmacodynamic interaction (e.g., synergy) is also desirable. Selection of an appropriate regimen from these agents will therefore be dependent on the AMR epidemiology in LMIC settings, characterisation of the pharmacodynamics of these agents (alone and in combination), and testing of candidate regimens in a clinical trial.

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