Supplementary materials

Supplementary Table 1: NeoSep Severity Score: factors and overall score at presentation

	Cohort 1 fosfomycin/ amikacin N=21	Cohort 2 flomoxef/ amikacin N=21	Cohort 3 fosfomycin/ flomoxef N=20	Total N=62
Time in hospital (days)	1 [0, 23]	1 [0, 26]	1 [0, 7]	1 [0, 26]
Gestational age at birth [a]	31 [27, 38]	34 [26, 40]	32 [30, 42]	32 [26, 42]
Preterm	19 (90%)	14 (67%)	15 (75%)	48 (77%)
Birth weight (g)	1285 [875, 3105]	1670 [870, 3310]	1682 [1180, 2970]	1478 [870, 3310]
Congenital anomalies? [b]				
None	19 (90%)	19 (90%)	17 (85%)	55 (89%)
Minor	2 (10%)	2 (10%)	3 (15%)	7 (11%)
Temperature (°C)	36.6 [34.8, 37.2]	36.5 [34.3, 37.0]	36.7 [34.1, 37.7]	36.6 [34.1, 37.7]
<35.5	3 (14%)	6 (29%)	3 (15%)	12 (19%)
35.5-37.9 [c]	18 (86%)	15 (71%)	17 (85%)	50 (81%)
Respiratory support? [d]				
No	4 (19%)	2 (10%)	2 (10%)	8 (13%)
Oxygen supplementation only	6 (29%)	11 (52%)	6 (30%)	23 (37%)
CPAP	10 (48%)	6 (29%)	10 (50%)	26 (42%)
HFNC	1 (5%)	1 (5%)	0 (0%)	2 (3%)
Invasive ventilation	0 (0%)	1 (5%)	2 (10%)	3 (5%)
Abdominal distension?				
Yes	6 (29%)	2 (10%)	0 (0%)	8 (13%)
Difficulty in feeding?				
None	6 (29%)	4 (19%)	2 (10%)	12 (19%)
Yes, difficulty in feeding or feeding intolerance	3 (14%)	5 (24%)	4 (20%)	12 (19%)
Not fed at all [e]	12 (57%)	12 (57%)	14 (70%)	38 (61%)
Evidence of shock?				
Yes	0 (0%)	1 (5%)	1 (5%)	2 (3%)
Lethargy or reduced or no movement?				
None	7 (33%)	10 (48%)	14 (70%)	31 (50%)
Lethargy only	1 (5%)	5 (24%)	5 (25%)	11 (18%)
Movement only on stimulation	13 (62%)	6 (29%)	1 (5%)	20 (32%)
NeoSep Severity Score	6 [5,9]	6 [5,8]	6 [5,8]	6 [5,9]
NeoSep Severity Score:				
5	5 (24%)	10 (48%)	7 (35%)	22 (35%)
6	7 (33%)	5 (24%)	9 (45%)	21 (34%)
7	2 (10%)	3 (14%)	2 (10%)	7 (11%)
8	3 (14%)	3 (14%)	2 (10%)	8 (13%)
9	4 (19%)	0 (0%)	0 (0%)	4 (6%)

Note: numbers are N (%) or median [range]. CPAP: continuous positive airway pressure. HFNC: high-flow nasal cannula. [a] weeks; [b] Major congenital anomalies were an exclusion criterion; [c] No neonate had a temperature of 38°C or higher at presentation; [d] If a neonate received multiple ways of support, the highest level of support was reported; [e] not fed at all orally.

Supplementary Table 2: Laboratory values at baseline and Day 5 of antibiotics

	N	Baseline	N	On Day 5
Sodium (mmol/L)	56	140 (135, 142) [127, 150]	29	142 (139, 146) [127, 154]
Potassium (mmol/L)	54	5.0 (4.3, 5.4) [3.1, 8.3]	27	4.5 (3.8, 5.1) [2.8, 6.3]
BUN (mmol/L)	50	1.8 (1.3, 2.7) [0.6, 6.4]	27	1.2 (0.9, 2.0) [0.2, 4.7]
Creatinine (µmol/L)	54	78 (61, 92) [32, 151]	29	60 (48, 76) [32, 90]
CRP (mg/L)	55	1.0 (1.0, 3.0) [0.1, 89.0]	20	1.9 (1.0, 4.1) [0.1, 18.0]
ALT (U/L)	48	11 (7, 15) [5, 86]	13	11 (8,19) [7, 35]
AST (U/L)	44	64 (38, 77) [24, 319]	14	36 (27,40) [19, 59]
Total bilirubin (µmol/L)	54	70 (35, 96) [2, 238]	30	114 (73, 154) [12, 278]
Hemoglobin (g/dL)	60	16.2 (14.2, 17.2) [8.5, 21.5]	18	15.2 (12.5, 15.80) [9.3, 17.1]
RBC ($x10^{12}$ cells/L)	60	4.3 (3.9, 4.8) [2.6, 6.1]	18	4.1 (3.2, 4.6) [2.8, 5.5]
Platelets ($x10^{9}$ cells/L)	60	256 (186, 320) [30, 775]	18	181 (129, 282) [15, 369]
WBC ($x10^{9}$ cells/L)	60	11.4 (7.5, 15.3) [3.7, 36.7]	18	6.5 (5.1, 9.3) [2.4, 68.7]
Neutrophils ($x10^{9}$ cells/L)	58	5.7 (2.9, 8.1) [0.9, 26.8]	16	2.2 (1.3, 4.0) [0.7, 28.0]

Numbers presented are N (%) or median (IQR) [range].

Supplementary Table 3: Day 5 laboratory values by cohort

	Cohort 1 fosfomycin/ amikacin	**Cohort 2 flomoxef/ amikacin**	**Cohort 3 fosfomycin/ flomoxef**
Sodium (mmol/L)	N=7 139 [127, 147]	N=9 144 [131, 148]	N=13 143 [137, 154]
Potassium (mmol/L)	N=6 4.8 [3.7, 5.2]	N=8 4.6 [3.7, 6.3]	N=13 4.0 [2.8, 5.4]
Creatinine (µmol/L)	N=6 54 [36, 84]	N=9 51 [32, 90]	N=14 64 [37, 87]
Total bilirubin (µmol/L)	N=8 90 [12, 154]	N=9 97 [17, 255]	N=13 129 [20, 278]

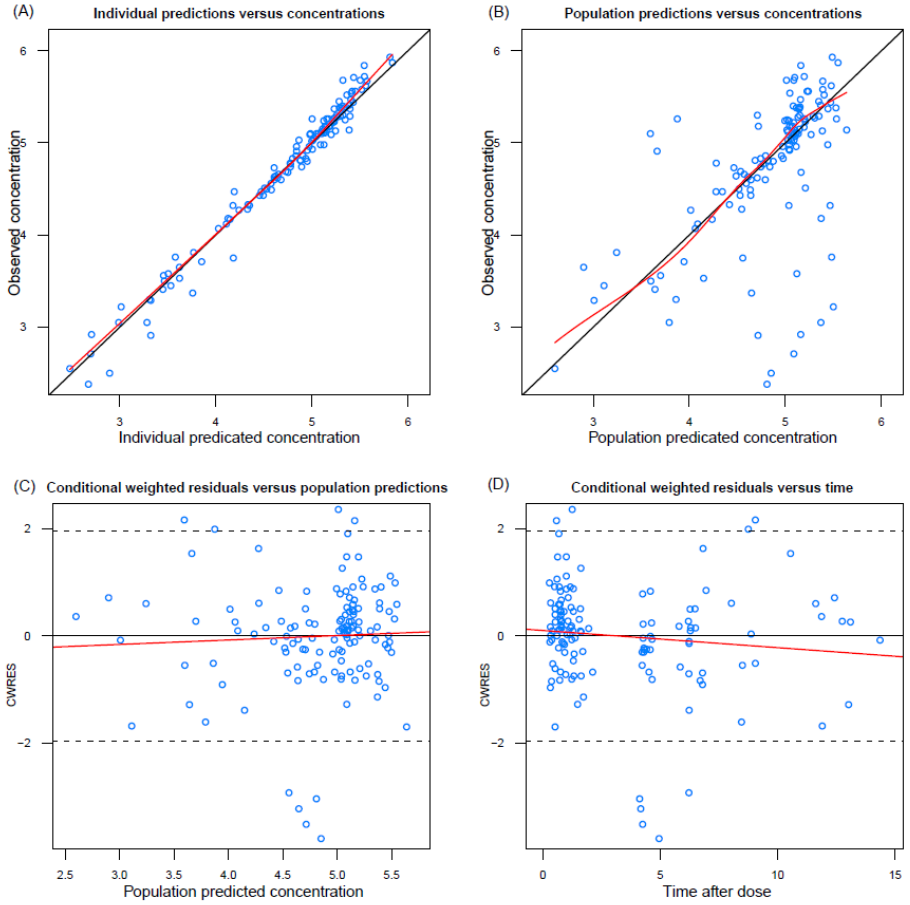
Supplementary Table 4: Neonates who started non-trial IV antibiotics or antifungals post-enrolment

	Cohort 1 fosfomycin/ amikacin N=6	Cohort 2 flomoxef/ amikacin N=5	Cohort 3 fosfomycin/ flomoxef N=9	Total Enrolled N=20
Start of non-trial antibiotics				
Days after enrolment	9 [4, 20]	4 [1,7]	6 [2,10]	7 [1, 20]
Reason for starting non-trial antibiotic treatment				
Suspected new onset clinical sepsis	5 (83)	3 (60)	5 (56)	13 (65)
Not responding to empiric trial antibiotics	-	1 (20)	3 (33)	4 (20)
Necrotizing enterocolitis	1 (17)	-	-	1 (5)
Persistent pulmonary hypertension developed	-	-	1 (11)	1 (5)
Spontaneous intestinal perforation	-	1 (20)	-	1 (5)
Type of non-trial antibiotic and anti-fungal treatment				
Cefotaxime	-	1	1	2
Ceftazidime	-	-	1	1
Meropenem	1	1	2	4
Meropenem, colistin	-	1	-	1
Meropenem, vancomycin	1	-	1	2
Piperacillin/tazobactam, amikacin	4	2	4	10
Fluconazole	2	-	-	2
Amphotericin B	1	-	1	2
Pathogens isolated from sterile sites				
Candida non-albicans spp	2	-	2	4
Klebsiella pneumoniae	1	1	-	2
Pseudomonas stutzeri [1]	-	1	-	1
Serratia liquefaciens	-	1	-	1
Enterococcus faecium	-	-	1	1
Enterococcus faecalis	-	-	1	1
Staphylococcus aureus	-	-	1	1

Numbers are n (%) or median [min, max]; [1] all pathogens were identified from blood cultures, except this one identified from cerebrospinal fluid.

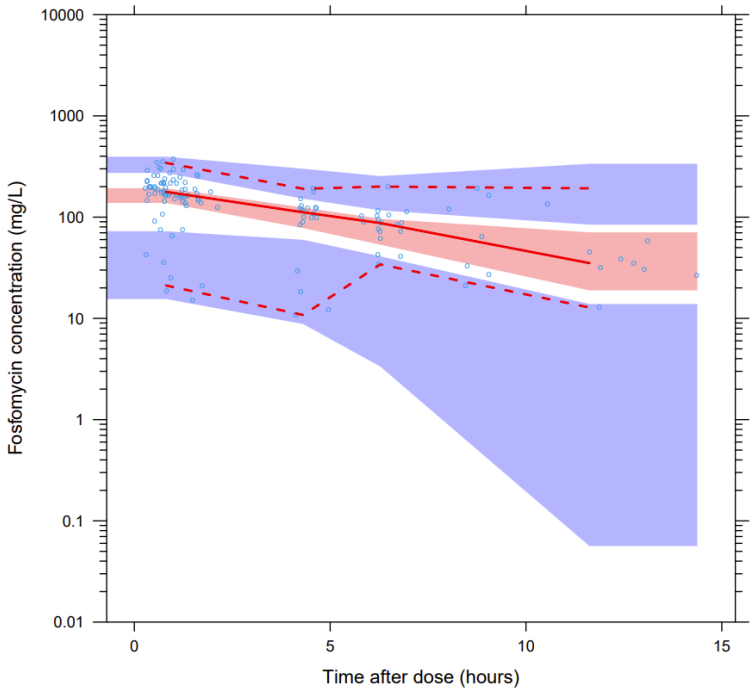
Supplementary Figure 1: Goodness of fit diagnostics of the final fosfomycin model

(A) Observed fosfomycin concentrations versus individual predicted concentrations (IPRED); (B) observed fosfomycin concentrations versus population predicted concentrations (PRED); (C) conditional weighted residuals (CWRES) versus PRED; (D) CWRES versus time after dose. The blue circles represent observed fosfomycin concentrations, and the solid red lines indicate the line of identity



Supplementary Figure 2: Visual predictive check of final fosfomycin model

Open circles represent the observed data. The lower, middle and upper lines are the 5th, 50th and 95th percentiles of the observed data. The shaded areas are the 95% confidence intervals of the 5th, 50th and 95th percentiles of the simulated data (n=1,000)



Supplementary Table 5: Population PK parameter estimates from the final fosfomycin PK model

Fosfomycin PK parameters	Final model		SIR	
	Estimates	RSE (%)	Median	2.5th - 97.5th percentile
CL (L/hr/70 kg)[a]	10.6	17.7	10.6	8.7 - 12.7
θ_{WT}	0.75 FIX	-	-	-
Vc (L/70 kg) [a]	14.8	33.2	15.0	8.2 - 24.6
θ_{WT}	1.0 FIX	-	-	-
Q (L/hr/70 kg) [a]	29.4	23.3	30.2	18.9 - 46.8
Vp(L/70 kg) [a]	22.0	17.0	22.2	17.9 - 25.7
Fraction of CL on the 1st day of life (θ_M)	0.542	20.3	0.539	0.316 - 0.752
Post-natal maturation rate constant (/day) (θ_N)	0.361	92.0	0.367	0.186 - 0.708
Inter-individual variability (IIV)				
IIV CL, %CV	44.4	14.8	44.7	34.5 - 58.4
IIV Vc, %CV	230.1	24.5	232.0	149.5 - 375.5
IIV Q, %CV	-	-	-	-
IIV Vp, %CV	-	-	-	-
Residual variability	0.0293	27.8	0.0299	0.0217 - 0.0383

*[a]Parameter estimates are scaled to typical patient with body weight of 70 kg. Abbreviations: CL, clearance; Vc, volume of distribution of central compartment; Q, inter-compartment clearance; Vp, volume of distribution of peripheral compartment; RSE%: relative standard error (standard error of estimate / estimate*100); SIR, sampling importance resampling*

Final Pharmacokinetic Parameter Equations:

$$CL\ (L/hr) = (10.6).\left(\frac{BW_i}{70}\right)^{0.75}.\left(\frac{PMA_i^{3.4}}{47.7^{3.4} + PMA_i^{3.4}}\right).\left[0.542 + (1 - 0.542).\left(1 - e^{(-PNA_i)(0.361)}\right)\right].e^{\eta_i}$$

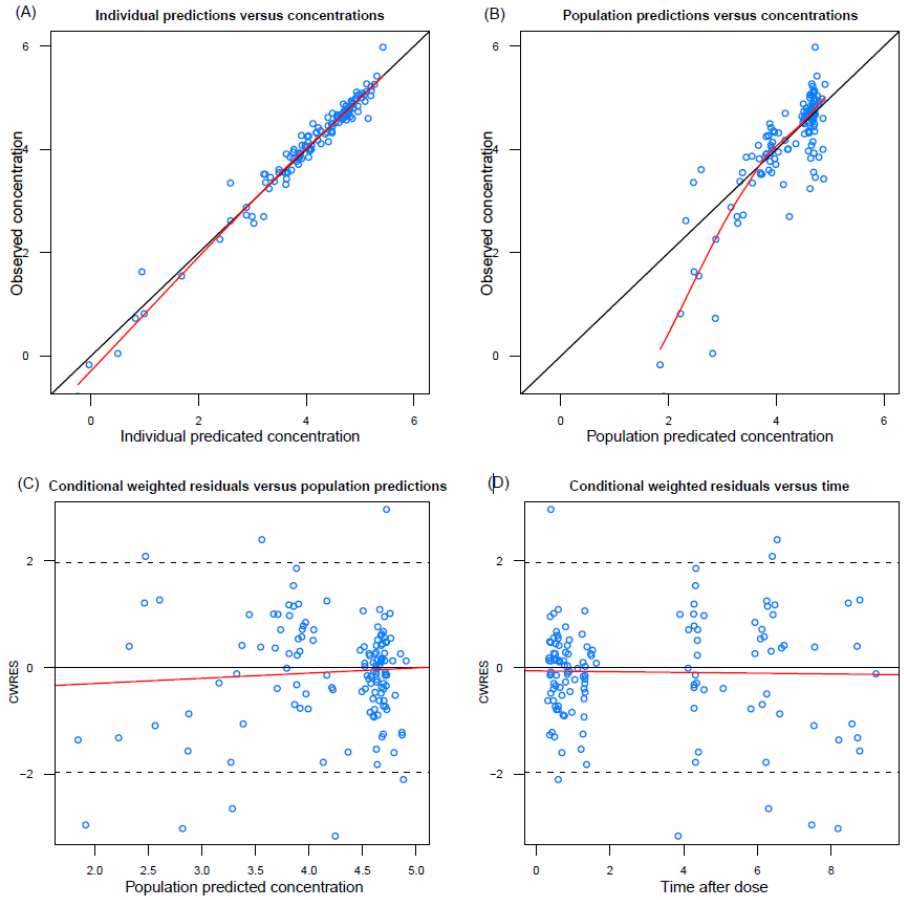
$$Vc\ (L) = (14.8).\left(\frac{BW_i}{70}\right).e^{\eta_i}$$

$$Q\ (L/hr) = (29.4).\left(\frac{BW_i}{70}\right)^{0.75}.e^{\eta_i}$$

$$Vp(L) = (22.0).\left(\frac{BW_i}{70}\right).e^{\eta_i}$$

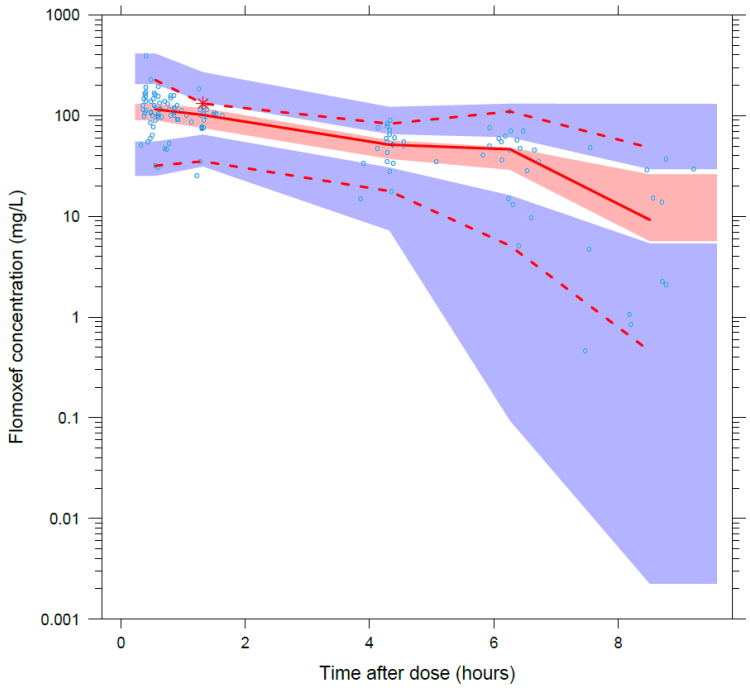
Supplementary Figure 3: Goodness of fit plots of the final flomoxef model

(A) Observed flomoxef concentrations versus individual predicted concentrations (IPRED); (B) observed flomoxef concentrations versus population predicted concentrations (PRED); (C) conditional weighted residuals (CWRES) versus PRED; (D) CWRES versus time after dose. The blue circles represent observed flomoxef concentrations, and the solid red lines indicate the line of identity



Supplementary Figure 4: Visual predictive checks for the final flomoxef model

Open circles represent the observed data. The lower, middle and upper lines are the 5th, 50th and 95th percentiles of the observed data. The shaded areas are the 95% confidence intervals of the 5th, 50th and 95th percentiles of the simulated data (n=1,000)



Supplementary Table 6: Population PK parameter estimates from the final flomoxef PK model

Flomoxef PK parameters	Final model		SIR	
	Estimates	RSE (%)	Median	2.5th - 97.5th percentile
CL (L/hr/70 kg)[a]	12.6	5.6	12.6	11.2 - 14.0
θ_{WT}	0.75 FIX	-	-	-
Vd (L/70 kg)[a]	23.9	8.4	23.8	20.3 - 28.1
θ_{WT}	1.0 FIX	-	-	-
Fraction of CL on the 1st day of life (θ_M)	0.449 *fixed*[b]	-	-	-
Post-natal maturation rate constant (/day) (θ_N)	0.117 *fixed*[b]	-	-	-
Inter-individual variability (IIV)				
IIV CL, %CV	27.8	14.7	27.9	22.4 - 32.1
IIV Vd, %CV	52.3	18.2	52.6	42.9 - 70.6
Residual variability	0.0634	31.7	0.0642	0.0475 - 0.0926

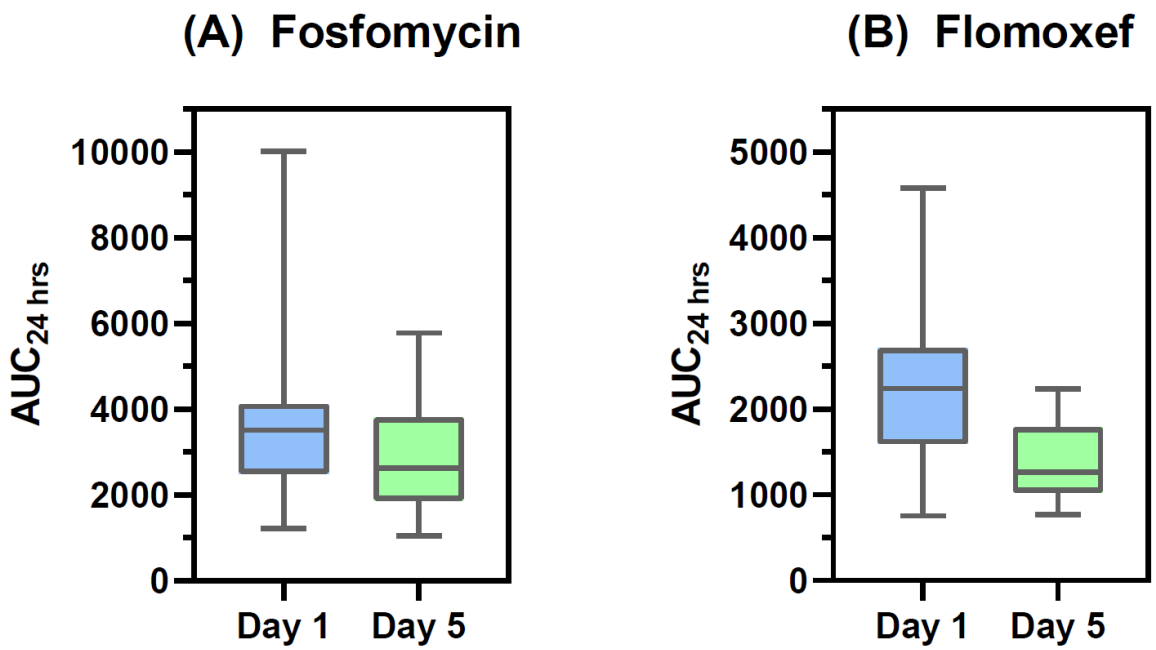
*[a]Parameter estimates are scaled to typical patient with body weight of 70 kg. [b]The values of q_M and q_N were fixed to reported values from the NeoFos study.(14) Abbreviations: CL, clearance; Vd, volume of distribution; RSE%: relative standard error (standard error of estimate / estimate*100); SIR, sampling importance resampling*

Final Pharmacokinetic Parameter Equations:

$$CL\ (L/hr) = (12.6).\left(\frac{BW_i}{70}\right)^{0.75}.\left(\frac{PMA_i^{3.4}}{47.7^{3.4} + PMA_i^{3.4}}\right).\left[0.449 + (1 - 0.449).\left(1 - e^{(-PNA_i)(0.117)}\right)\right].e^{\eta_i}$$

$$Vd\ (L) = (23.9).\left(\frac{BW_i}{70}\right).e^{\eta_i}$$

Supplementary Figure 5: Box-plot of predicted AUC on Days 1 and Day 5 for (a) fosfomycin and (b) flomoxef



Supplementary Table 7. Probability of target attainment (PTA) in neonates with different PNA administered fosfomycin every 12 hours achieving target $AUC_{24\ hr}$/MIC ratios of 83

MIC (mg/L)	%PTA			
	Preterm neonates (GA at birth < 37 weeks)		Term neonates (GA at birth ≥ 37 weeks)	
	PNA 1-7 days	PNA ≥ 8 days	PNA 1-7 days	PNA ≥ 8 days
$AUC_{24\ hr}$/MIC > 83				
1	100	100	100	100
2	100	100	100	100
4	100	100	100	100
8	99.91	99.73	99.95	99.72
16	96.11	91.54	96.58	88.68
32	63.40	47.78	63.22	36.72
64	14.40	6.48	12.32	2.89

$AUC_{24\ hr}$/MIC, 24-hours area under the concentration-time curve over the minimum inhibitory concentration; PTA, probability of target attainment; GA, gestational age; PNA, postnatal age

Supplementary Table 8. Probability of target attainment (PTA) in neonates with different PNA administered flomoxef every 8 hours achieving target *%f*T>MIC of 40.

MIC (mg/L)	%PTA			
	Preterm neonates (GA at birth < 37 weeks)		Term neonates (GA at birth ≥ 37 weeks)	
	PNA 1-7 days	PNA ≥ 8 days	PNA 1-7 days	PNA ≥ 8 days
0.06	100	99.99	100	99.94
0.12	100	99.99	100	99.86
0.25	100	99.98	100	99.77
0.5	99.99	99.95	99.99	99.61
1	99.99	99.88	99.98	99.28
2	99.99	99.72	99.96	98.58
4	99.99	99.31	99.86	96.95
8	99.94	98.18	99.52	92.35
16	99.55	93.53	97.54	78.02
32	94.93	71.74	78.55	32.87
64	50.67	17.95	12.84	1.09

PTA, probability of target attainment; GA, gestational age; PNA, postnatal age

Supplementary Table 9: Clinical features of the seven neonatal deaths

Death No	Cohort	GA (weeks)	Birth weight (grams)	Sex	Cause of Death	Died on trial antibiotics	Age at death (days)
1	Cohort 1	32	1320	F	Necrotizing enterocolitis	N	20
2	Cohort 1	31	1220	F	Culture negative sepsis	Y	17
3	Cohort 1	34	1500	M	Culture positive sepsis [1]	N	17
4	Cohort 2	39	2900	M	Culture negative sepsis	Y	4
5	Cohort 2	34	1160	M	Culture negative sepsis	N	5
6	Cohort 2	30	1120	F	Culture positive sepsis [2]	N	9
7	Cohort 3	31	1320	F	Persistent pulmonary hypertension	N	3

GA: gestational age; F=female; M=male; N=No; Y=Yes, 1= Enterobacter spp. and Klebsiella spp. and Candida non-albicans; 2 = Serratia liquefaciens

Supplementary Table 10: Adverse events related to trial antibiotics

Neonate No	Cohort	Event	Maximum grade	Related to fosfomycin	Related to flomoxef	Related to amikacin
1	Fosfomycin/amikacin	Neutropenia	Grade 1	yes		
2	Fosfomycin/amikacin	Blood urea increased	Grade 2			yes
	Fosfomycin/amikacin	Hypernatremia	Grade 2	yes		
3	Flomoxef/amikacin	Administration site complication	Grade 1		yes	
4	Flomoxef/amikacin	Sinus bradycardia	Grade 1		yes	yes
5	Flomoxef/amikacin	Blood creatinine increased	Grade 1			yes
	Flomoxef/amikacin	Blood urea increased	Grade 2		yes	yes
	Flomoxef/amikacin	Hypernatremia	Grade 2		yes	
6	Flomoxef/amikacin	Aspartate aminotransferase increased	Grade 1		yes	
	Flomoxef/amikacin	Blood creatinine increased	Grade 2		yes	yes
7	Flomoxef/amikacin	Blood creatinine increased	Grade 2		yes	yes
8	Fosfomycin/flomoxef	Hepatic function abnormal	Grade 2	yes	yes	
9	Fosfomycin/flomoxef	Hypokalemia	Grade 3	yes		
10	Fosfomycin/flomoxef	Hepatic function abnormal	Grade 3	yes	yes	
11	Fosfomycin/flomoxef	Hypokalemia	Grade 2	yes		
12	Fosfomycin/flomoxef	Hepatic function abnormal	Grade 2	yes	yes	
13	Fosfomycin/flomoxef	Acute kidney injury	Grade 2	yes	yes	
	Fosfomycin/flomoxef	Hepatic function abnormal	Grade 2	yes	yes	
	Fosfomycin/flomoxef	Hypernatremia	Grade 1	yes		

Supplementary Table 11: NeoSep Severity Score for predicting 28-day mortality based on clinical information at the start of a sepsis episode

Factor (clinical signs in the 24h preceding start of clinical sepsis episode)	Score value if present
Time in hospital: ≤ 10 days	1
Gestational age: <37 weeks	1
Birth Weight: • >2 kg • 1-2 kg • <1 kg	 0 1 2
Congenital anomalies	2
Temperature • <35.5°C • 35.5 to 37.9 °C • 38 – 38.9 °C • ≥ 39 °C	 1 0 1 2
Maximum respiratory support: • None • Oxygen supplementation • CPAP, BiPAP, HFNC • Invasive ventilation	 0 2 3 3
Abdominal distension	1
Difficulty in feeding	1
Evidence of shock including cold peripheries	1
Lethargy / no or reduced movement: • Lethargy only • No movement or movement only on stimulation +/- lethargy	 1 2

Note: CPAP = continuous positive airway pressure, BiPAP = Bilevel Positive Airway Pressure, HFNC = high flow nasal cannula. The score was adapted from WHO possible serious bacterial infection (pSBI) criteria for hospitalised neonates with sepsis and based on the data generated from the NeoOBS study (Russell NJ, et al. PLoS Med. 2023;20(6):e1004179)

Supplementary Table 12: Trial Assessment Schedule (Part 1)

Visit type	Screening	Enrolment	Follow-up Treatment & Monitoring					TOC	StFU*
Timing (window)	**Day 0**	**Day 1**	**Daily while on IV antibiotics**	**Day 3 (±1 day)**	**Day 5 (±1 day)**	**Day 7 (±2 day)**	**EOT [8] (if not Day 7 or 14)**	**14 (± 4 days)**	**28 (± 5 days)**
Informed assent/consent	X[1]								
Verification of eligibility	X	X							
Enrolment to Part 1		X[2]							
Medical history	X	X							
Clinical review	X	X	X	X	X	X	X	X	X
C-reactive Protein	X[3]				X	X			
Full Blood Count (FBC)	X[3]				X	X[6]	X[6]	X[6]	X[6]
Urea & Electrolytes (U&Es)	X[3]				X	X[6]	X[6]	X[6]	X[6]
Liver function test (LFT)	X[3]				X	X[6]	X[6]	X[6]	X[6]
Creatinine	X[3]				X	X[6]	X[6]	X[6]	X[6]
Blood culture	X[4]			X[5]					
Administration of antibiotics		X	X	X	X	X	X		
Pharmacokinetic sample[7]		X			X				
Adverse event assessment		X	X	X	X	X	X	X	X
Concomitant medication		X	X	X	X	X	X	X	X

EOT= end of treatment, TOC = test of cure, StFU = short term follow-up visit. Last FU visit for Part 1 participants will be on Day 28.

* by telephone / if clinically indicated, then hospital visit.

[1] Written informed consent to be obtained from parent/guardian.

[2] Treatment allocation in Part 1 and treatment initiation may be on the same day as the screening visit.

[3] Laboratory results required within 48h before enrolment, but test can be done either at screening or randomisation or values from blood taken pre-screening.
FBC: Red blood count (RBC), white blood count (WBC) and differential, platelets. U&Es: including blood urea nitrate (BUN), sodium, potassium. LFTs: ALT, AST.

[4] Blood must be taken for culture within 48h before enrolment, but may precede screening visit by up to 48 hours if already taken for clinical management.

[5] Repeat blood culture only if neonate switches treatment (at the time of switch) due to clinical deterioration or lack of response. Blood for culture should be taken before switch of antibiotics except in exceptional circumstances outside the responsible clinician's control.

[6] Repeat blood tests only if abnormal at previous visit or baby's condition not stable.

[7] Pharmacokinetic samples for Part 1. PK sample from CSF may also be collected if lumbar puncture is clinically indicated and baby receiving fosfomycin.

[8] Planned duration of treatment at enrolment for blood culture-negative sepsis is to Day 7±2 days, for blood culture-positive sepsis is to Day 10 [-3,+4 days] if there is no switch to second-line. If antibiotics are switched to second-line, the total planned duration of antibiotic treatment including first and second line treatment is 14 ±7 days depending on the baby's condition.