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The potential of fosfomycin for multi-drug resistant sepsis: an analysis of *in vitro* activity against invasive paediatric Gram-negative bacteria

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Abstract

Purpose. Antimicrobial resistance (AMR) is of increasing global concern, threatening to undermine recent progress in reducing child and neonatal mortality. Repurposing older antimicrobials is a prominent strategy to combat multidrug-resistant sepsis. A potential agent is fosfomycin, however, there is scarce data regarding its *in vitro* activity and pharmacokinetics in the paediatric population.

Methodology. We analysed a contemporary, systematically collected archive of community-acquired (CA) and hospital-acquired (HA) paediatric Gram-negative bacteraemia isolates for their susceptibility to fosfomcyin. MICs were determined using agar serial dilution methods and validated by disk diffusion testing where breakpoints are available. Disk diffusion antimicrobial susceptibility testing was also conducted for current empirical therapies (ampicillin, gentamicin, ceftriaxone) and amikacin (proposed in the literature as a new combination empirical therapeutic option).

Results. Fosfomycin was highly active against invasive Gram-negative isolates, including 90 % (202/224) of Enterobacteriaceae and 96 % (22/23) of *Pseudomonas* spp. Fosfomycin showed high sensitivity against both CA isolates (94 %, 142/151) and HA isolates (81 %, 78/96; P =0.0015). CA isolates were significantly more likely to be susceptible to fosfomycin than the current first-line empirical therapy (96 % vs 59 %, P <0.0001). Extended spectrum β-lactamases (ESBL) production was detected in 34 % (85/247) of isolates with no significant difference in fosfomycin susceptibility between ESBL-positive or -negative isolates [73/85 (86 %) vs 147/162 (91 %) respectively, P =0.245]. All isolates were susceptible to a fosfomycin-amikacin combination.

Conclusion. Gram-negative paediatric bacteraemia isolates are highly susceptible to fosfomycin, which could be combined with aminoglycosides as a new, carbapenem-sparing regimen to achieve excellent coverage to treat antimicrobial-resistant neonatal and paediatric sepsis.

INTRODUCTION

Antimicrobial resistance (AMR) is of increasing concern to global health, threatening to undermine the significant progress made in combating child mortality over the past two decades [1]. Sepsis remains an important cause of mortality in children and neonates – responsible for almost one-quarter of all neonatal deaths – with the international literature now concluding that up to one-third of these deaths are directly attributable to AMR [2]. For the treatment of sepsis in neonates, infants and children the World Health Organization (WHO) guidelines currently recommend first-line treatment with ampicillin (or penicillin)

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Abbreviations: AMR, antimicrobial resistance; API, analytical profile index; ATCC, American-type culture collection; CA, Community-acquired; CLSI, Clinical Laboratory Standards Institute; CTX-M, cefotaxime extended-spectrum beta-lactamase enzyme first isolated in Munich (M); ECOFF, epidemiological cut-off value; ESBL, extended-spectrum beta-lactamases; EUCAST, European committee on antimicrobial susceptibility testing; GCLP, Good Clinical Laboratory Practice; HA, hospital-acquired; KCH, Kilifi County Hospital; KEMRI, Kenya Medical Research Institute; MDR, multi-drug resistant; MIC, minimum inhibitory concentration; MRSA, multi-resistant *Staphylococcus aureus*; NEQAS, National External Quality Assessment Service; VRE, vancomycin-resistant enterococci.

plus gentamicin, with third-generation cephalosporins recommended as second-line therapy [3].

However, recent systematic reviews have reported high rates of AMR to the current regimen, documenting non-susceptibility to penicillin/gentamicin and third-generation cephalosporins of 44 and 43 % (respectively) with overall rates of non-susceptibility to ampicillin (80 %), gentamicin (22 %) and ceftriaxone (74 %) among Enterobacteriaceae [1, 4, 5], reflecting the global emergence and spread of extended spectrum β-lactamases (ESBLs). Simultaneously, carbapenem-resistant Enterobacteriaceae (CRE) are responsible for an increasing number of hospital-acquired outbreaks and substantial morbidity and mortality [6, 7].

With a global shortage of newly discovered or effective and affordable alternative antibiotics available, the repurposing of older antimicrobials (with improved susceptibility profiles) to update empirical therapeutic guidelines has recently received increasing international attention [8]. One promising agent is fosfomycin, a bactericidal antibiotic that was first discovered in 1969. Its use was quickly overshadowed by the heavily promoted discovery of cephalosporins, resulting in infrequent international use and subsequently low global resistance rates [9].

The WHO has classified fosfomycin in the category of a 'critically important' antimicrobial for investigation in light of its efficacy against multi-resistant Gram-negative organisms [10]; and it has been identified as an antimicrobial which holds great promise worldwide for managing MDR Gram-negative infections, due to its affordable cost and efficacy as a carbapenem-sparing regimen [10, 11]. Fosfomycin's rapidly bactericidal activity is due to a unique mechanism of action of inhibition of the synthesis of peptidoglycan by blocking the formation of *N*-acetylmuramic acid at an earlier step than β-lactams, interfering with cell wall synthesis in both Gram-positive and Gram-negative bacteria [12]. This affords the opportunity for synergistic mechanisms with other antimicrobials [13, 14] and circumvents the resistance caused by β-lactamase-producing organisms, resulting in efficacy against many problematic pathogens such as methicillin-resistant *Staphylococcus aureus* (MRSA), glycopeptide-resistant Enterococci and multidrug-resistant (MDR) Enterobacteriaceae [15–17]. Fosfomycin is also able to penetrate biofilms, including those produced by vancomycin-resistant enterococci (VRE) [18]. However, activity against *Acinetobacter baumannii* and *Burkholderia* spp. and the anaerobic species Bacteroides is limited [17, 19].

Fosfomycin is well distributed within tissues and achieves clinically beneficial concentrations throughout serum, abscess fluid, and renal and cardiorespiratory systems (although it does not achieve sufficient concentrations in the CSF to be used as monotherapy for meningitis) [20–22]. Fosfomycin is not considered an appropriate empirical monotherapy option for sepsis due to the potential for the rapid emergence of resistance (and higher *in vitro* MICs observed in certain Gram-negative bacteria, such as *Pseudomonas aeruginosa*) [12]. For this reason, investigation of a broad-spectrum fosfomycin-amikacin combination therapy for the treatment of neonatal sepsis and meningitis is currently promoted in the international literature [8].

Previous research has identified fosfomycin as a relatively safe drug with high tolerability in children [12]. However, as fosfomycin's discovery occurred prior to the current international standard of drug development and approval procedures, significant knowledge gaps regarding its *in vitro* capacity exist. The minimal *in vitro* data that is available appears promising, revealing susceptibility to (predominantly urinary) Gram-positive and Gram-negative isolates (including ESBL-producing *E. coli* and MRSA) [23, 24]. However, there is a lack of published evidence for the *in vitro* efficacy of fosfomycin on isolates causing bacteraemia.

With the possibility that fosfomycin may be a promising candidate as an empirical therapeutic regimen in neonatal and paediatric sepsis, its potential efficacy in a paediatric population is particularly important. We therefore aimed to address this research gap by evaluating the susceptibility profiles of fosfomycin in contemporary invasive Gram-negative blood isolates of a neonatal and paediatric population, and to analyse its susceptibility profile in comparison to the currently recommended empirical therapy antibiotics (ampicillin, gentamicin and ceftriaxone). A second objective was to evaluate potential carbapenem-sparing fosfomycin-amikacin combination, currently under investigation as a new empirical therapeutic regimen for neonatal sepsis [8].

METHODS

Patients

The study examined isolates systematically collected from children aged 0–5 years admitted between 28 February 2012 to 25 May 2017 to Kilifi County Hospital (KCH). KCH is a first-level referral hospital situated in a rural coastal region of Kenya, with a 35-bed paediatric ward, five-bed research high dependency ward and a neonatal room, which serves approximately 4000 patients per year. As part of the routine admission investigations, blood cultures are systematically performed on admission for all children, unless admitted for elective surgery or minor trauma [25]. Blood cultures are repeated in the case of clinical deterioration whilst in hospital and the date of collection recorded, allowing classification of cultures as either community- or hospital-acquired infections.

Bacterial isolates

Culture and antimicrobial susceptibility testing was undertaken at the KEMRI/Wellcome Trust Research Programme, which is co-located at KCH. All isolates were identified at the time of collection by conventional methods with confirmation provided by the API system (bioMérieux, Marcy l'Etoile, France), then stored at −80 °C in tryptic soy broth with 15 % glycerol. The KEMRI/Wellcome Trust laboratory is Good Clinical Laboratory Practice (GCLP) accredited and

participates in external quality assessment (including the UK National External Quality Assessment Service, NEQAS).

For this study, consecutive non-duplicate blood culture isolates were retrieved for the following Gram-negative organisms: *Escherichia coli*, *Klebsiella pneumoniae, Klebsiella oxytoca*, *Pseudomonas aeruginosa*, *Pseudomonas luteola, Enterobacter cloacae* complex, *Pantoea* spp., *Salmonella* spp., *Shigella flexneri* and *Serratia marcescens*. Upon retrieval, isolates were sub-cultured to blood agar and incubated for 18–24 h at 37 °C in air. Isolates were classified as community-acquired (CA) if they were collected within 48 h of admission, or hospital acquired (HA) if collected beyond 48 h of admission.

Antimicrobial susceptibility testing

Fosfomycin susceptibility was determined by the agar dilution method for all isolates, and additionally by disk diffusion methods [for *E. coli* only: as described by the Clinical Laboratory Standards Institute (CLSI); disk diffusion breakpoints have not yet been validated for other Enterobacteriaceae in regards to fosfomycin] [26].

Disk diffusion testing was performed using Mueller–Hinton agar (BBL Microbiology Systems, Cockeysville MD). For *E. coli* isolates, fosfomycin disks containing 200 µg of fosfomycin and 50 µg of glucose-6-phosphate (Sigma Aldrich, Dorset, UK) were used; for all other Enterobacteriaceae, disks containing amikacin (30 µg), ampicillin (10 µg), gentamicin (10 µg) and ceftriaxone (30 µg) (BBL Microbiology Systems, Cockeysville MD). *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27953 and *S. aureus* ATCC 25923 were utilized as control strains and compared to documented quality control reference ranges [27]. Following overnight incubation in aerobic conditions at 35 °C, zone diameters were measured and interpreted using CLSI guidelines [27].

For susceptibility testing by agar dilution, Mueller–Hinton agar (BBL Microbiology Systems, Cockeysville MD) plates were prepared by serial dilution using fosfomycin disodium salt powder supplemented with 25 µg ml^{-1} of glucose-6-phosphate (Sigma Aldrich, Dorset, UK) [14, 28]. For each isolate, 10 µl of a 0.5 ml McFarland suspension was inoculated on to the test plate using a transfer loop. Control strains, including *E. coli* ATCC 25922 and *S. aureus* ATCC 25923 were included in each set of tests. The inoculated plates were incubated in aerobic conditions at 35 °C for 18–24 h. The MIC of each antimicrobial agent was defined as the lowest concentration that inhibited visible growth of the organism.

Interpretation of susceptibility results

For the purpose of this study assessing Gram-negative bacteraemia, criteria for susceptibility categories by MIC were applied using the EUCAST interpretive criteria for all isolates of *Enterobacteriaceae*, as CLSI interpretive criteria are only available for urinary tract isolates of *E. coli* (Table 1) [27, 29]. For *P. aeruginosa*, although EUCAST/CLSI breakpoint criteria have not yet been established, we applied a breakpoint of ≤64 µg ml^{-1} to designate susceptibility, which was inferred from previous evaluation on clinical isolates [30], and is within the EUCAST epidemiological cut-off value (ECOFF) of <128 µg ml^{-1} [31]. Breakpoints for commonly used clinical antibiotic therapy (ampicillin, gentamicin, ampicillin/gentamicin combination therapy, ceftriaxone, amikacin and imipenem) were also analysed for isolates, which were found to be non-susceptible to fosfomycin (defined by EUCAST MIC criteria, ≥32 µg l^{-1}).

Statistical analysis of the results was performed using STATA statistical software for Windows (Data Analysis and Statistical Software, version 15.0).

RESULTS

A total of 247 consecutive non-duplicate archived Gram-negative aerobic blood culture were retrieved (Table 2): 132 isolates (53 %) from neonates (aged <28 days); 88 (36 %) from children aged 28 days to <2 years; and 27 (11 %) from children aged 2 to 5 years. Fifty-eight (23%) were isolated from children outside the neonatal age with severe acute malnutrition.

Table 1. EUCAST interpretive criteria of fosfomycin and amikacin for Enterobacteriaceae [32]

	MIC (µg ml^{-1})			Zone diameter breakpoints (mm)		
	Susceptible	Intermediate	Resistant	Susceptible	Intermediate	Resistant
Fosfomycin	≤32		>32	≥24*	NA	<24*
Amikacin	≤16	32	≥64	≥17	15–16	≤14
Gentamicin	≤4	8	≥16	≥15	13–14	≤12
Ampicillin	≤8	16	≥32	≥17	14–16	≤13
Ceftriaxone	≤1	2	≥4	≥23	20–22	≤19
Imipenem	≤1	2	≥4	≥23	20–22	≤19

*For *E. coli* only.
NA, not available.

Table 2. Overview of species tested and ESBL status

Species	Total	Community-acquired	Hospital-acquired	ESBL positive	ESBL negative
Escherichia coli	56	89 % (50/56)	11 % (6/56)	14 % (8/56)	86 % (48/56)
Klebsiella spp.	87	26 % (23/87)	74 % (64/87)	78 % (68/87)	22 % (19/87)
Enterobacter cloacae complex	17	53 % (9/17)	47 % (8/17)	24 % (4/17)	76 % (13/17)
Pantoea spp.	10	70 % (7/10)	30 % (3/10)	10 % (1/10)	90 % (9/10)
Pseudomonas spp.	23	61 % (14/23)	39 % (9/23)	0 % (0/23)	100 % (23/23)
Salmonella spp.	35	91 % (32/35)	9 % (3/35)	5 % (2/35)	94 % (33/35)
Serratia marcescens	15	87 % (13/15)	13 % (2/15)	13 % (2/15)	87 % (13/15)
Shigella flexneri	3	67 % (2/3)	33 % (1/3)	0 % (0/3)	100 % (3/3)
Salmonella Typhi	1	100 % (1/1)	0 % (0/1)	0 % (0/1)	100 % (1/1)
Total	247	61 % (151/247)	39 % (96/247)	35 % (85/247)	66 % (162/247)

MIC distributions – Enterobacteriaceae and *Pseudomonas* spp.

The MIC distributions of the 247 Gram-negative isolates are presented in Fig. 1, with analysis by species presented in Fig. 2. Fosfomycin was highly active against the Enterobacteriaceae isolates examined, with 90 % (202/224) of all isolates classified as sensitive (MIC ≤32 µg ml^{-1}) as per EUCAST criteria. Among *Pseudomonas* spp., 96 % (22/23) were sensitive as per the ECOFF criteria (≤64 µg ml^{-1}) [31].

Among the Enterobacteriaceae, *K. pneumoniae* exhibited the highest frequency of non-susceptibility (20 %, 17/83), followed by (in order of non-susceptibility) *E. coli* (5 %, 3/55) and *E. cloacae* (6 %, 1/17), while all *Salmonella* spp. and *S. flexneri* samples were fully sensitive.

Community-acquired versus hospital-acquired isolates

In total, 151 (61 %) isolates were CA samples while 96 were HA (collected >48 h after admission), of which 64/96 (67 %) were isolated from neonates. *E. coli* was the most frequently isolated CA sample (51/151, 34 %), followed by *Klebsiella* spp. (22/151, 15 %). *Klebsiella* spp. (*n*=64) were

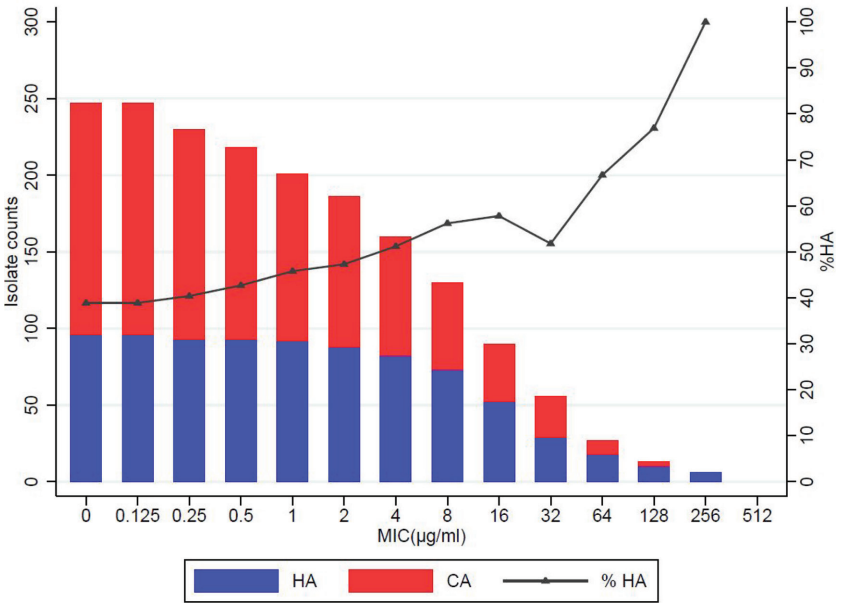

Fig. 1. Frequency histogram showing the number of isolates exhibiting growth at each fosfomycin agar dilution concentration level, identifying those classified as sensitive (MIC ≤32 µg ml^{-1} for Enterobacteriaceae, or ECOFF <128 µg ml^{-1} for *Pseudomonas* spp.). CA=community-acquired, HA=hospital-acquired.

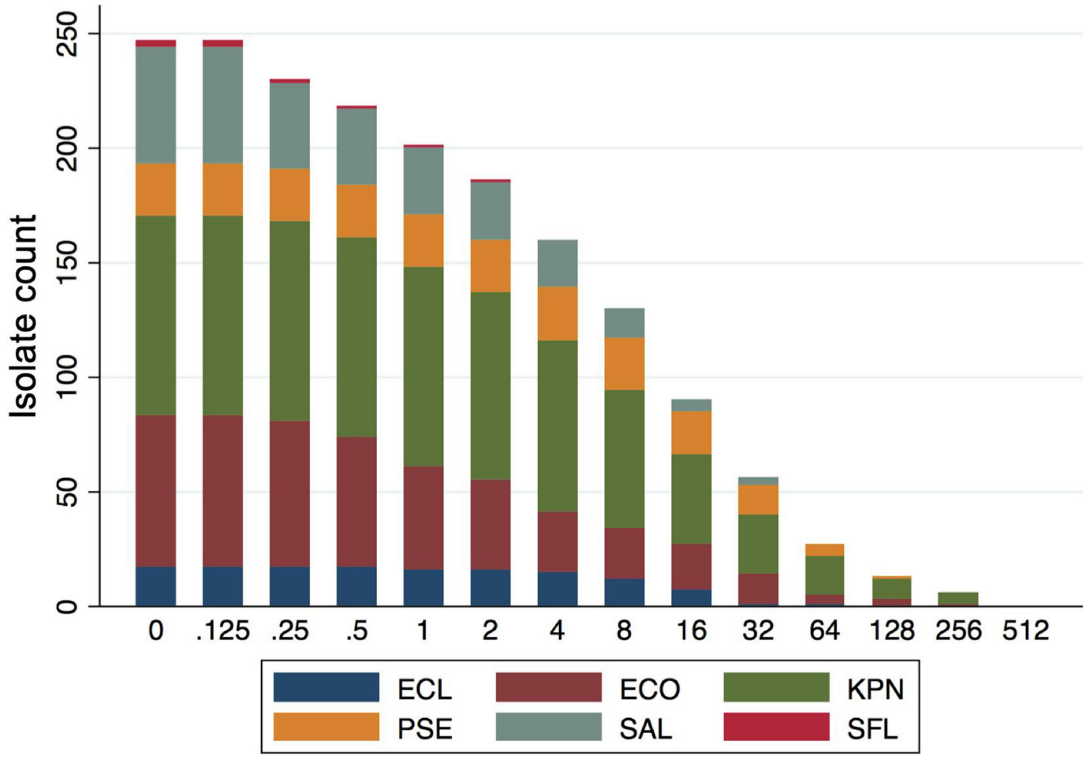

Fig. 2. Frequency histogram of the number of isolates exhibiting growth at each fosfomycin dilution level, by species. ECL=*E. cloacae;* ECO=*E. coli;* KPN=*K. pneumonia;* PSE=*Pseudomonas* spp.; SAL=*Salmonella* spp.; SFL=*S. flexneri.*

responsible for 67 % (64/96) of the HA isolates, followed by *Pseudomonas* spp. (*n*=9). HA isolates were more likely to exhibit fosfomycin non-susceptibility (19 %, 18/96) than CA isolates (6 %, 9/151, *P*=0.0015).

ESBL-producing isolates

Overall, 85 (34 %) of the 247 isolates tested were ESBL-producing organisms, of which the majority (*n*=67, 79 %) were *Klebsiella* spp. Fosfomycin was highly active against both ESBL-positive and -negative isolates, revealing susceptibility in 91 % (147/162) of ESBL-negative isolates and 86 % (73/85) of ESBL-positive isolates (*P*=0.2).

Zone diameter interpretive criteria

Fig. 3 documents the MIC versus zone diameters for *E. coli*, illustrating high concordance between agar dilution sensitivity MIC and zone diameter breakpoints by disk diffusion.

Comparison of fosfomycin susceptibility with currently used first-line agents

The analysis of fosfomycin susceptibility to Gram-negative infections for commonly used antimicrobials is presented in Table 3. Of 224 Enterobacteriaceae, 92 (41 %) were non-susceptible to the current WHO first-line therapy (ampicillin and gentamicin), including 16 % of CA isolates (24/151) and 71 % (68/96) of HA isolates. Altogether, 80 of these 92 isolates (87 %) were susceptible to fosfomycin (alone), and all (100 %) were susceptible to either fosfomycin or amikacin (Table 3). Similarly, of the 93 isolates (26 CA, 67 HA) not susceptible to ceftriaxone, 79 (85 %) were susceptible to fosfomycin and all (100 %) were susceptible to the combination of fosfomycin and amikacin.

Fosfomycin-amikacin combination therapy

Fig. 4 illustrates the fosfomycin MIC against amikacin zone diameter breakpoints. All 247 Gram-negative isolates were sensitive to either fosfomycin or amikacin, the potential empirical combination therapy for neonatal sepsis [33].

Fosfomycin sensitivity and mortality

Of the 247 isolates tested 87 (35 %) were from children who died during that hospital admission; 47 (54 %) from neonates aged <28 days, with the remaining mortality cases from children aged <48 months. Of the 87 isolates associated with mortality, 42 (48 %) had community-acquired infections; 31 % of these (13/42) were resistant to ampicillin and gentamicin, yet all of these isolates exhibited fosfomycin susceptibility.

Of the 87 isolates from children who died, 45 were associated with hospital-acquired infections, of which 49 % (31/45) were non-susceptible to second-line therapy (ceftriaxone), yet 22 (71 %) of these resistant HA isolates were susceptible to fosfomycin. Overall,

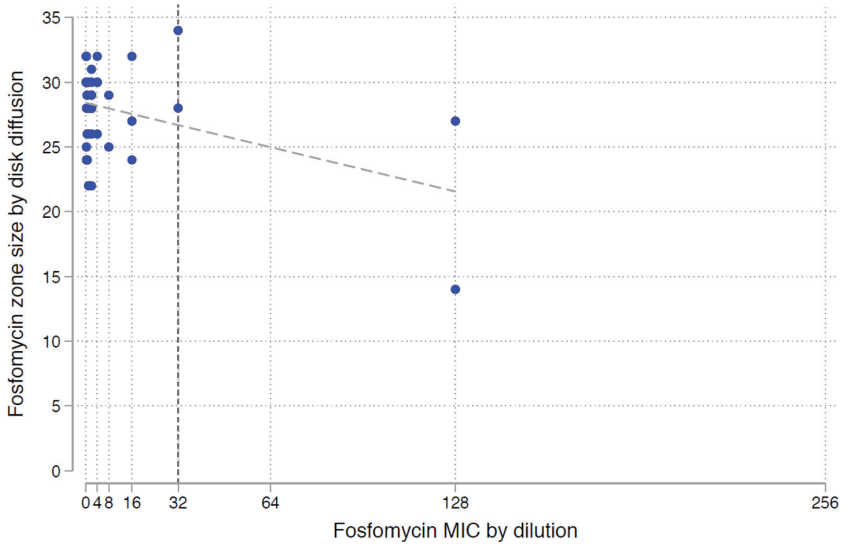

Fig. 3. Fosfomycin sensitivity of *E. coli* isolates, by MIC agar dilution and disk diffusion. The vertical line indicates the EUCAST breakpoint for Enterobacteriaceae (≤32μg ml^{-1}). The diagonal line is the fitted linear regression.

there was a significantly greater proportion of fosfomycin non-susceptible isolates among the children who died (17 %; 15/87) than among those who survived (7.5 %, 12/160, *P*=0.02).

DISCUSSION

Fosfomycin has potential as both a new empirical therapeutic regimen for clinical sepsis in neonates and children [8] and as a carbapenem-sparing treatment strategy in MDR sepsis [34, 35]. In light of renewed interest in fosfomycin's therapeutic potential, a range of studies have recently been published which acknowledge its promising *in vitro* activity [16, 32, 36–39] and our results, unique due to their presentation within clinical data, add strength to this literature.

Our analysis of 247 Gram-negative bacteremia isolates from children reveals high susceptibility among both Enterobacteriaceae and *Pseudomonas* spp., including MDR and ESBL-producing organisms, in both community- and hospital-acquired infections and across both neonates and older children. We have revealed that several Gram-negative invasive isolates, which were not susceptible to current empirical therapeutic regimens (ampicillin / gentamicin or ceftriaxone), were significantly more likely to be susceptible to fosfomycin. Our findings also revealed 100 % *in vitro* susceptibility to a combination of fosfomycin and amikacin, supporting the potential fosfomycin-amikacin empirical regimen for neonatal sepsis currently under discussion [8]. These two agents have the potential to provide broad-spectrum cover with adequate penetration of the meninges, with a promising safety profile, whilst minimizing the likelihood of resistance emerging [12]. Indeed, by circumventing the increasing global resistance challenges caused by ESBLs, this combination regimen would provide broad spectrum

Table 3. Proportion of Enterobacteriaceae resistant to individual antibiotics, with comparison to the likelihood of these isolates also being resistant to fosfomycin

Enterobacteriaceae *n*=224	No. of resistant isolates as per MIC testing [31]	Expressed as a % of isolates	No. of isolates also resistant to fosfomycin (as per MIC) [31]	% Resistant to both antibiotics	Difference in proportions (*P*-value)
Fosfomycin	22	9.8%	–	–	–
Gentamicin	92	41%	12	13%	28 % (*P*<0.001)
Amikacin	1	0.4%	0	0%	0.4 % (*P*>0.05)
Ampicillin	158	71%	18	11%	60 % (*P*<0.001)
Ceftriaxone	93	42%	14	15%	27 % (*P*<0.001)
Imipenem	1	0.4%	0	0%	0.4 % (*P*>0.05)
Ampicillin/gentamicin combination	92	41%	12	13%	28 % (*P*<0.001)

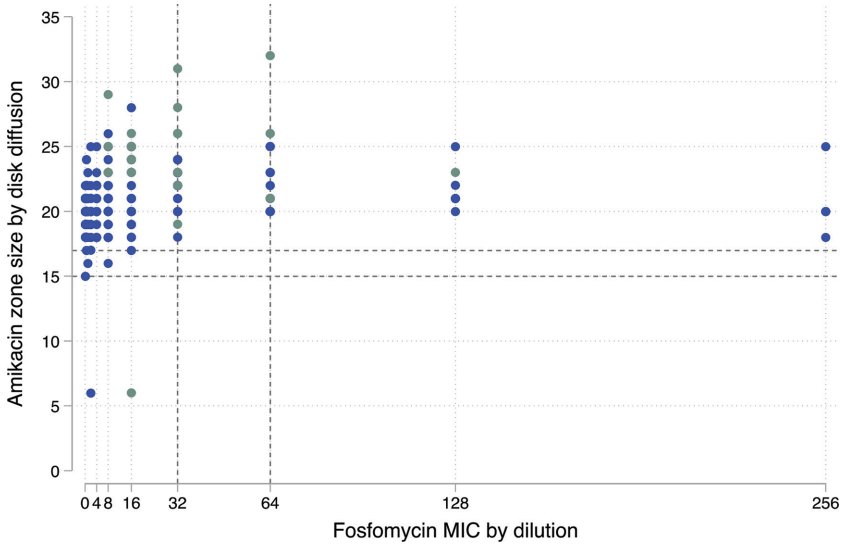

Fig. 4. Scattergram of fosfomycin MIC (μg ml^{-1}) and amikacin disk diffusion zone size (mm) of all (*n*=247) isolates, revealing susceptibility to either fosfomycin or amikacin in all pathogens; according to EUCAST breakpoints for Enterobacteriaceae (≤32 μg ml^{-1} by dilution and 15–17 mm by disk diffusion) and ECOFF criteria for *Pseudomonas* spp. (≤64 μg ml^{-1} by dilution) [31]. ●=Enterobacteriaceae, ●=*Pseudomonas* spp.

carbapenem-sparing cover, with a high likelihood of improved clinical efficacy.

Our results have some limitations. Firstly, the isolates evaluated in this study were collected from a single hospital in Kenya; expanding the geographic base to analyse further *in vitro* efficacy of fosfomycin would allow for improved generalizability. Secondly, isolates that were non-susceptible to fosfomycin were not sequenced to establish the mechanism of resistance, which would be informative for establishing regional fosfomycin susceptibility profiles. Enzymes conferring fosfomycin resistance have been categorized into four groups – FosA, FosB, FosC and FosX. The most important from an epidemiological point of view is FosA3 seen in *E. coli*, which is typically located on a conjugative plasmid that also carries the CTX-M-type ESBL – transmission of this plasmid-mediated resistance therefore causes both cephalosporin and fosfomycin resistance simultaneously [40]. While FosA3 was first reported in Japan in 2006, it has now spread across Asia in both *E. coli* and *K. pneumoniae*; although there are no published reports regarding its presence on other continents [41].

Our results have revealed very high susceptibility to fosfomycin in a range of common neonatal pathogens, including *E. coli* and *K. pneumonia,* and ESBL-producing Gram-negative infections. Furthermore, our research has provided a representative collection of invasive isolates due to the large community-acquired proportion tested, and importantly our analysis was broken down by the mode of acquisition (CA vs HA), which is commonly lacking in research pertaining to antimicrobial resistance [1]. Other strengths of this research include the generalizable population of children from whom the isolates were drawn, the systematic collection of cultures on admitted children to our unit (removing any potential bias caused by cultures only being collected on the most unwell children or those failing initial inpatient treatment), and the susceptibility testing was performed in an accredited laboratory, which participates in international external quality assurance.

This research therefore adds valuable data regarding the *in vitro* response of Gram-negative paediatric bacteremia to fosfomycin, with interesting clinical outcome associations, including revelations on the likelihood of improved efficacy compared to the current levels of non-susceptibility to first- and second-line antimicrobial therapy in CA and HA infections. While the results of our data reveal promising susceptibility profiles for fosfomycin, more clinical research is required before this therapy can be recommended as empiric therapy in a neonatal or paediatric population. An improved understanding of fosfomycin's pharmacology, pharmacodynamics and pharmacokinetics in a neonatal and paediatric population is required, and randomized trials comparing the efficacy of the currently recommended empirical regimens to fosfomycin (potentially in combination with amikacin) are also necessary, to determine clinical efficacy and toxicity outcomes.

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Author contributions
Phoebe C.M. Williams: investigation, formal analysis, writing – original draft preparation; Joseph Waichungo: investigation, validation; N. Claire Gordon: conceptualization, validation, writing – review and editing,

supervision; Mike Sharland: conceptualization; Sheila Murunga: data curation, formal analysis; Alice Kamau: data curation, formal analysis; Jay Berkley: conceptualization, writing – review and editing, visualization, supervision, project administration, funding.

Conflicts of interest
The authors declare that there are no conflicts of interest.

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