FOSFOMYCIN AGAINST CARBAPENEM-RESISTANT *KLEBSIELLA PNEUMONIAE* BLOODSTREAM INFECTION: *IN VITRO* ACTIVITY, CORRELATION OF SUSCEPTIBILITY TESTING METHODS AND TREATMENT CHARACTERISTICS

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Abstract. Fosfomycin is an adjunctive therapy for carbapenem-resistant *Klebsiella pneumoniae* (CRKP) infection, despite the lack of defined breakpoints. Treatment decisions rely on pharmacokinetics/pharmacodynamics (PK/PD) targets. The study evaluated fosfomycin efficacy against CRKP isolates harboring various carbapenemase genotypes, the association between disc diffusion zone diameters and Etest MIC values, and treatment regimens of patients ≥20 years of age with CRKP bloodstream infection from four hospitals across Thailand. The most prevalent carbapenemase genotype identified by PCR was $bla_{OXA\text{-}48\text{-}like}$ (62%). Of the CRKP isolates (n = 133), 19 and 34% demonstrated fosfomycin MIC values ≤32 and ≥128 μg/ml, respectively. A categorical agreement of 99% (1% error rate) between the two sensitivity assay methods was achieved for isolates with MIC ≤ 128 μg/ml and zone diameter ≥16 mm. Over half of the patients received a combination therapy of fosfomycin plus colistin and the most prevalent comorbidity was chronic kidney disease (56%).

The 14-day all-cause mortality rate was 32%. Fosfomycin is still considered necessary in situations where alternative effective drugs are not available, highlighting the need for standardized susceptibility testing methods.

Keywords: *Klebsiella pneumoniae*, bloodstream infection, carbapenem-resistance, disk diffusion test, Etest, fosfomycin

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INTRODUCTION

Carbapenem-resistant *Enterobacterales* (CRE) has been listed among the bacterial priority pathogens by the World Health Organization (WHO) since 2017 and has remained on the latest (2024) list (WHO, 2017; WHO, 2024; Wise *et al*, 2024). Carbapenem-resistant *Klebsiella pneumoniae* (CRKP) and carbapenem-resistant *Escherichia coli* are responsible for over 90% of CRE cases (Ma *et al*, 2023). CRE infection, particularly bloodstream infection (BSI), has a higher mortality rate compared to that of carbapenem-susceptible *Enterobacterales* infection (Zhou *et al*, 2021).

Antibiotic treatment for CRE depends on the type(s) of carbapenemase expressed by the microorganism (Tamma *et al*, 2024), which varies with geographical regions. As β-lactam antibiotics targeting specific carbapenemases may not be available in all affected countries (Wise *et al*, 2024), the European Society of Clinical Microbiology and Infectious Diseases guidelines suggest treatment with a combination of available antibiotics shown to be active *in vitro* (Paul *et al*, 2022). Non-β-lactam antibiotics, such as aminoglycosides, colistin, fosfomycin, and tigecycline, may still be needed for CRE treatment in some countries, although

these drugs are generally not recommended by the Infectious Diseases Society of America (Tamma *et al*, 2024).

Fosfomycin ([(2R,3S)-3-methyloxiran-2-yl] phosphonic acid) is a last-resort treatment for drug-resistant organisms with *in vitro* activity against CRE and is not degraded by carbapenemases, regardless of type (Falagas *et al*, 2016; Hashemian *et al*, 2019). Susceptibility break-points have been established for intravenous (IV) fosfomycin treatment of *E. coli*, which include zone diameter and minimum inhibitory concentration (MIC), but zone diameter break-point of fosfomycin for *K. pneumoniae* remains undefined (EUCAST, 2023). In 2024, the European Committee on Antimicrobial Susceptibility Testing (EUCAST) did not propose an IV fosfomycin break-point for *Enterobacterales* other than *E. coli* for several reasons, *viz* high epidemiological cut-off values (ECOFFs), inability to achieve preclinical pharmacokinetic/pharmacodynamic (PK/PD) targets and limited clinical evidence supporting fosfomycin as a monotherapy (EUCAST, 2024a). Although fosfomycin is frequently used in combination therapy, no observed benefits from its addition to treatment regimens were reported, and no clinical data indicate practical MIC values (EUCAST, 2023; EUCAST, 2024a); however, when fosfomycin is prescribed, its clinical dosage is guided by PK/PD target attainment analysis derived from Monte Carlo simulation (provided an MIC value is available) (Leelawattanachai *et al*, 2020). Testing methods for determining MICs are unavailable in microbial laboratories in most countries. Although a disk diffusion test is employed in most hospital laboratories as the method is simple and inexpensive, it cannot determine MICs (Gajic *et al*, 2022).

Fosfomycin remains vital in several countries, including Thailand. Therefore, using CRKP bloodstream isolates, we investigated fosfomycin activity and carriage of carbapenemase gene(s), correlation between fosfomycin zone diameters and MICs, and

fosfomycin combination treatment regimens and characteristics of infected patients. Expected outcomes include better insight into the role of fosfomycin against Thai CRKP isolates, assessment of susceptibility testing concordance, and evidence to support its use in combination therapy.

MATERIALS AND METHODS

K. pneumoniae isolates collection

K. pneumoniae isolates were obtained from blood samples collected from patients (n = 215) admitted to four tertiary hospitals across Thailand: Phramongkutklao and Nopparat Rajathanee Hospitals, Bangkok (central region), Buriram Hospital, Buriram Province (northeastern region) and Ratchaburi Hospital, Ratchaburi Province (western region), between January 2020 and March 2024. *K. pneumoniae* resistant to ertapenem, imipenem or meropenem was defined as a CRKP isolate (Tamma *et al*, 2024).

Fosfomycin susceptibility tests

In vitro fosfomycin susceptibility was determined by disk diffusion and gradient (Etest) methods (CLSI, 2024). In the disk diffusion method, each disk contained 200 µg of fosfomycin and 50 µg of glucose-6-phosphate (Thermo Scientific Oxoid, Waltham, MA), and in the Etest, each strip contained a gradient of fosfomycin from 0.016 to 256 µg/ml with glucose-6-phosphate (Liofilchem, Teramo, Italy). Susceptibility was assessed using a lawn of the test isolate on a Mueller-Hinton agar plate and incubation for 16-18 hours at ambient temperature (35±2 °C) (CLSI, 2024). In the disk diffusion method, MIC was determined based on the inhibition zone diameter measured with a caliper according to CLSI (2024) recommendations, and in the Etest, MIC was based on the inhibition zone at the calibrated strip position. A scatter plot was used to display MIC values and inhibition zone diameters from all isolates to determine MIC at the 50 percentile (MIC_{50}), MIC at the 90 percentile (MIC_{90}) and MIC range. Interpretive criteria for *K. pneumoniae* fosfomycin

susceptibility were determined using EUCAST break-point version 13.1 for *Enterobacterales* (MIC ≤32 μg/ml) and ECOFFs of *K. pneumoniae* (MIC ≤128 μg/ml) (EUCAST, 2023; EUCAST, 2024b).

Carbapenemase genes identification

Genomic DNA from *K. pneumoniae* isolates was extracted using a commercial kit (Thermo Fisher Scientific, Waltham, MA), and carbapenemase genes bla_{IMP}, bla_{VIM}, bla_{KPC}, bla_{NDM}, and $bla_{OXA\text{-}48\text{-}like}$ were amplified using gene-specific primer sets (Poirel *et al*, 2011). Amplifications were performed in a Biometra TGradient Thermocycler (Biometra, Gottingen, Germany) as follows: 94 °C for 3 minutes; 35 cycles of 94 °C for 30 seconds, 57 °C for 35 seconds and 72 °C for 45 seconds; followed by a final step of 72 °C for 5 minutes. Amplicons were separated by 1% agarose gel electrophoresis and stained with ethidium bromide. Carbapenemase genes were identified based on their amplicon size (Poirel *et al*, 2011).

Susceptibility correlation analysis

The disk diffusion and Etest results were compared using a categorical agreement (CA) method (US FDA, 2009). An acceptable CA value is ≥90%. CA was further divided into very major error (VME) and major error (ME). VME occurs when the Etest method indicates resistance and the disk diffusion method susceptibility (a false susceptible result). ME occurs when the Etest method indicates susceptibility and the disk diffusion method resistance (a false resistant result); the acceptable limit for VME and ME is ≤1.5 and ≤3%, respectively (US FDA, 2009).

Clinical characteristics, fosfomycin regimens and outcomes analyses

A descriptive method was applied in the analysis of clinical characteristics, fosfomycin regimens and treatment outcomes of patients ≥20 years of age who received definitive treatment for CRKP BSI with a fosfomycin regimen for at least 24 hours. Data were retrieved from electronically stored medical records of the 4 participating hospitals.

Ethical approval

The study protocols were approved by the respective Ethics Review Committee of the Royal Thai Army Medical Department and Phramongkutklao Hospital (approval no. Q002h/66), Ratchaburi Hospital (approval no. COA-RBHEC 006/66), Nopparat Rajathanee Hospital (approval no. 15/2566), and Buriram Hospital (approval no. 00331021). Patient informed consent was not required because the ethics review committees granted a waiver of consent for the use of archived bacterial isolates and retrospective chart reviews involving confidential and anonymized data.

RESULTS

***In vitro* studies**

Among 133 CRKP isolates obtained from blood samples, 79% (n = 105), 10% (n = 14), 10% (n = 13), and 1% (n = 1) were from Phramongkutklao Hospital, Ratchaburi Hospital, Buriram Hospital, and Nopparat Rajathanee Hospital, respectively. Of these isolates, 19% (n =26) demonstrated MIC values ≤32 μg/ml, while 34% (n = 45) MIC values ≤128 μg/ml. The MIC values and disc zone diameters are presented as a scattergram (Fig 1), with a correlation coefficient of -0.85. The horizontal boxes represent fosfomycin MIC thresholds at 32 μg/ml and 128 μg/ml, which are commonly used as reference points in susceptibility studies. The vertical box represents the zone diameter cut-off at 16 mm, a proposed screening threshold for categorizing susceptibility by disk diffusion.

The most prevalent carbapenemase gene carried by the clinical CRKP isolates was $bla_{OXA\text{-}48\text{-}like}$ (n = 82, 62%), followed by $bla_{OXA\text{-}48\text{-}like}$ + bla_{NDM} (n = 39, 26%) and bla_{NDM} (n = 8, 6%) (Table 1). Only one isolate carried $bla_{OXA\text{-}48\text{-}like}$ + bla_{NDM} + bla_{KPC} and three isolates unidentifiable *bla*. The MIC_{50} and MIC_{90} of $bla_{OXA\text{-}48\text{-}like}$ was >256 and >256 μg/ml, $bla_{OXA\text{-}48\text{-}like}$ + bla_{NDM} >256 and >256 μg/ml, and bla_{NDM} 32 and >256 μg/ml, respectively (Table 1).

Using an MIC cutoff of ≤128 μg/ml, the corresponding fosfomycin zone diameters of ≥14, ≥15, ≥16,

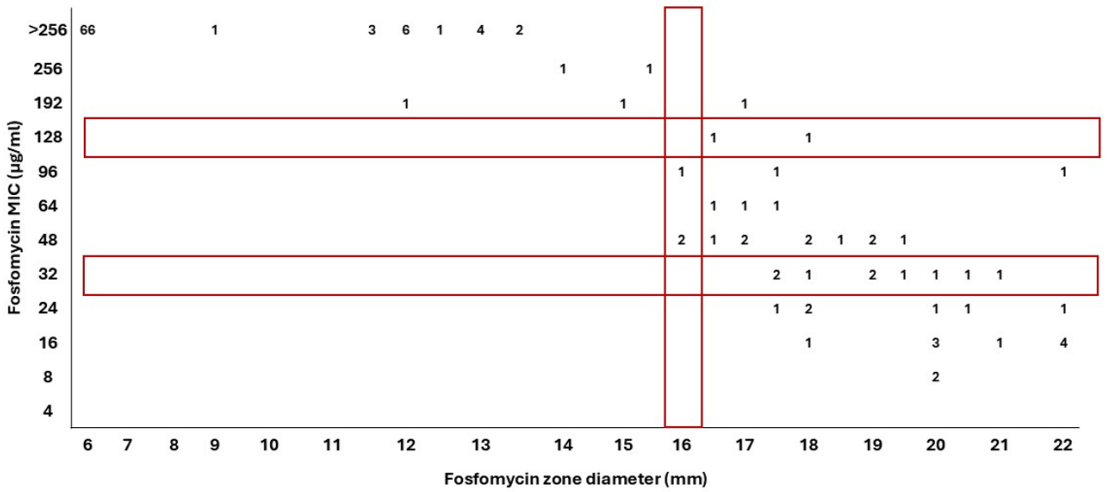

Fig 1 - Scattergram of fosfomycin minimum inhibitory concentrations (MICs) and fosfomycin (200 µg) disk zone diameters of carbapenem-resistant *Klebsiella pneumoniae* clinical isolates (N = 133)

Note: Each numeral indicates the number of isolates with the same MIC value and corresponding zone diameter.

Horizontal boxes mark MICs of 32 and 128 µg/ml; the vertical box indicates a 16 mm zone diameter cut-off proposed for disk diffusion screening.

mm: millimeters; µg: micrograms; µg/ml: micrograms per milliliter

and ≥17 mm had inter-method CA of 97, 98, 99, and 95%, respectively (Table 2). The zone diameter of ≥16 mm showed the highest CA with a VME rate of 1% and no ME, indicating an acceptable cutoff. Using an MIC cutoff of ≤32 µg/ml, the zone diameter threshold of ≥18, ≥19 and ≥20 mm showed lower inter-method CA value of 92, 92 and 93%, respectively and the error rates were not in an acceptable range (Table 2).

The CA between ECOFFs and ≥16 mm zone diameters for $bla_{OXA\text{-}48\text{-}like}$, $bla_{OXA\text{-}48\text{-}like}$ + bla_{NDM} and bla_{NDM} was 99, 100 and 100%, respectively. The VME rate for $bla_{OXA\text{-}48\text{-}like}$ was 2%.

Table 1

Minimum inhibitory concentrations (MICs) of fosfomycin against carbapenem-resistant *Klebsiella pneumoniae* (CRKP) isolates carrying carbapenemase genes

Carbapenemase gene carriage	MIC range (μg/ml)	MIC_{50} (μg/ml)	MIC_{90} (μg/ml)	Frequency, *n* (%)			
				MIC (32 μg/ml)	MIC[a] (≤32 μg/ml)	MIC[b] (≤128 μg/ml)	MIC (>256 μg/ml)
CRKP isolate[c] (N = 133)	8 - >256	>256	>256	9 (7)	26 (19)	45 (34)	83 (62)
$bla_{OXA\text{-}48\text{-}like}$ (N = 82)	8 - >256	>256	>256	4 (5)	12 (15)	22 (27)	56 (68)
bla_{NDM} (N = 8)	24 - >256	32	>256	1 (12)	4 (50)	6 (75)	2 (25)
$bla_{OXA\text{-}48\text{-}like}$ + bla_{NDM} (N = 39)	16 - >256	>256	>256	4 (10)	7 (18)	14 (36)	24 (61)

[a]EUCAST breakpoint for *Enterobacterales* version 13.1 (EUCAST, 2023); [b]Epidemiological cut-off value of *K. pneumoniae* (EUCAST, 2024b); [c]Including one isolate with $bla_{OXA\text{-}48\text{-}like}$ + bla_{NDM} + bla_{KPC} (MIC = 16 μg/ml) and three isolates with unidentified fosfomycin resistance gene(s) (MIC = 8, 24 and >256 μg/ml)

bla_{KPC}: carbapenemase gene; bla_{NDM}: beta-lactamase New Delhi metallo-beta-lactamase like gene; $bla_{OXA\text{-}48\text{-}like}$: beta-lactamase oxacillinase-48 like gene; MIC_{50}: minimum inhibitory concentrations at 50 percentile for all tested isolates; MIC_{90}: minimum inhibitory concentrations at 90 percentile for all tested isolates; μg/ml: micrograms per milliliter

Table 2

Error rates between susceptibility results of disk diffusion and Etest methods of carbapenem-resistant *Klebsiella pneumoniae* clinical isolates (N = 133)[a]

Zone diameter (mm)		MIC ≤32 µg/ml[b]			MIC ≤128 µg/ml[c]		
		CA (%)	Error rate (%)		CA (%)	Error rate (%)	
Susceptible	Resistant		VME	ME		VME	ME
≥14	<14	83	21	0	97	4	0
≥15	<15	83	21	0	98	3	0
≥16	<16	85	19	0	99	1	0
≥17	<17	89	13	0	95	1	13
≥18	<18	92	7	11	89	0	31
≥19	<19	92	4	27	83	0	49
≥20	<20	93	1	31	79	0	62
≥21	<21	85	1	73	72	0	82
≥22	<22	83	1	81	71	0	87

[a]Disk diffusion and Etest results are shown in Fig 1; [b]EUCAST breakpoint for *Enterobacterales* version 13.1 (EUCAST, 2023); [c]Epidemiological cut-off values of *Klebsiella pneumoniae* (EUCAST, 2024b). An acceptable rate for CA ≥90%, VME ≤1.5% and ME = 3% (US FDA, 2009).

CA: categorical agreement; ME: major error; MIC: minimum inhibitory concentration; mm: millimeters; VME: very major error; µg/ml: micrograms per milliliter

Clinical characteristics, fosfomycin regimens, and outcomes

Twenty-five percent (55/215) patients with CRKP BSI had received fosfomycin for at least 24 hours but patients (*n* = 41, 19%) who received fosfomycin as the definitive treatment were included in the study. The predominant site of infection was the respiratory tract (34%), followed by the urinary tract (19%) and then the intra-abdominal region (19%) (Table 3). Fosfomycin was administered as a combination therapy, with the most frequently used regimen being fosfomycin + colistin (*n* = 21, 51.2%). Fosfomycin was administered as a combination therapy, with the most frequently used regimen being fosfomycin + colistin (*n* = 21, 51.2%), followed by fosfomycin + amikacin (*n* = 7, 17.1%), fosfomycin + gentamicin (*n* = 4, 9.8%), and fosfomycin + colistin + meropenem (*n* = 3, 7.3%) (Table 3). Only CRKP BSI from 68% (*n* = 28) of patients were sensitive to fosfomycin, with 19 isolates evaluated using zone diameters and the remainder using MIC values. Fosfomycin dosage ranged from 3 to 24 g/day, adjusted according to the estimated glomerular filtration rate (Table 3).

Two patients had their fosfomycin regimens modified: one initially treated with fosfomycin + colistin but was switched to tigecycline + imipenem after CRKP bloodstream isolate was still detected on Day 7, and the other initially treated with fosfomycin + gentamicin was switched to tigecycline + gentamicin due to insufficient clinical improvement, increased vasopressor requirement and a fosfomycin MIC >256 μg/ml. The all-cause mortality at Day 14 was 32%.

DISCUSSION

Several developing countries are unable to obtain new effective antibiotics for treating CRE infection due to the unavailability of drugs, medical treatment rights and cost; thus, fosfomycin is an alternative drug (Grabein *et al*, 2017; Paul *et al*, 2022). Fosfomycin exhibited poor *in vitro* activity against CRKP isolated from patients in 4 hospitals across Thailand, with a larger

Table 3

Characteristics of patients (N = 41) with carbapenem-resistant *Klebsiella pneumoniae* bloodstream infection treated with intravenous fosfomycin

Characteristic	Frequency* *n* (%)
Gender	
Female	12 (29)
Male	29 (71)
Age (years), median (IQR)	60 (48 - 74)
Comorbidity	
Chronic kidney disease	23 (56)
Hypertension	19 (46)
Diabetes	11 (27)
Cardiovascular disease	11 (27)
Hematologic malignancy	9 (22)
Solid tumor	8 (19)
Cerebrovascular disease	5 (12)
Hepatic disease	5 (12)
Charlson comorbidity index score, median (IQR)	4 (2 - 6.5)
Intensive care unit ward	24 (58)
Septic shock	19 (46)
Pitt bacteremia score at onset of bacteremia, median (IQR)	6 (4 - 7)

Table 3 (cont)

Characteristic	Frequency* *n* (%)
Site of infection	
Respiratory tract infection	14 (34)
Urinary tract infection	8 (19)
Intraabdominal infection	8 (19)
Catheter-related bloodstream infection	6 (15)
Skin and soft tissue infection	4 (10)
Unknown source	1 (2)
Antimicrobial resistance	
Meropenem	37 (90)
Imipenem	35 (85)
Gentamicin	14 (34)
Amikacin	13 (32)
Colistin (N = 37)[a]	14 (41)
Tigecycline (N = 22)[a]	3 (15)
Ceftazidime/avibactam (N = 18)[a]	10 (55)
Fosfomycin sensitive	28 (68)
Zone diameter ≥16 mm (N = 19)[b]	16 (84)
MIC ≤128 μg/ml (N = 9)[b]	3 (33)

Table 3 (cont)

Characteristic	Frequency* *n* (%)
Fosfomycin treatment regimen	
Fosfomycin + colistin	21 (51.2)
Fosfomycin + amikacin	7 (17.1)
Fosfomycin + gentamicin	4 (9.8)
Fosfomycin + colistin + meropenem	3 (7.3)
Fosfomycin + imipenem	2 (4.9)
Fosfomycin + tigecycline	1 (2.4)
Fosfomycin + amikacin + meropenem	1 (2.4)
Fosfomycin + amikacin + tigecycline	1 (2.4)
Fosfomycin + ceftazidime/avibactam + imipenem	1 (2.4)
Fosfomycin daily dose based on eGFR, g/day	
eGFR >60 ml/min/1.73 m^2	12 - 24
eGFR >30 - 60 ml/min/1.73 m^2	8 - 24
eGFR 0 - 30 ml/min/1.73 m^2	3 - 24

*Unless otherwise stated

[a]Susceptibility results of ceftazidime/avibactam, colistin and tigecycline were not reported for all isolates; [b]Susceptibility results of fosfomycin were not reported for all isolates.

eGFR: estimated glomerular filtration rate; IQR: interquartile range; MIC: minimum inhibitory concentration; ml/min: milliliters per minute; mm: millimeters; m^2: square meters; µg/ml: micrograms per milliliter

proportion with higher MIC levels than those previously reported (Leelawattanachai *et al*, 2020; Pudpong *et al*, 2022). An explanation is that fosfomycin has been used in the country since 1980 (Thai FDA, 2024), potentially leading to development of fosfomycin resistance. The fosfomycin MICs reported in the current study were higher than those earlier reported from Germany (Kaase *et al*, 2014) and India (Bakthavatchalam *et al*, 2020). The predominant carbapenemase identified was New Delhi metallo-β-lactamase (NDM), oxacillinase (OXA)-type, which was consistent with previous studies (Nulsopapon *et al*, 2022; Pudpong *et al*, 2022; Ma *et al*, 2023). CRKP isolates harboring only bla_{NDM} exhibited lower MIC values than those carrying $bla_{OXA\text{-}48\text{-}like}$, an observation consistent with previous studies reporting isolates harboring $bla_{OXA\text{-}48\text{-}like}$ are more likely to demonstrate elevated fosfomycin MICs (Pudpong *et al*, 2022; Zarakolu *et al*, 2022).

The all-cause 14-day mortality rate was comparable with previous studies (Pudpong *et al*, 2022; Karnmueng *et al*, 2024). Fosfomycin is commonly used with other antibiotics (particularly when the preferred drugs are unavailable) to enhance efficacy and reduce the risk of resistance development with fosfomycin monotherapy (Grabein *et al*, 2017; So-Ngern *et al*, 2023). The most common combination used in the current study was fosfomycin + colistin, followed by fosfomycin + aminoglycosides (gentamicin or amikacin). Chukamnerd *et al* (2021) investigating *in vitro* synergistic effects of fosfomycin combinations against $bla_{NDM\text{-}1}$-positive CRKP isolates, 67% of which co-harbored $bla_{OXA\text{-}48\text{-}like}$, reported that among the isolates (n = 21) tested, fosfomycin + gentamicin has the highest synergistic effect, and approximately 25% of the isolates demonstrate a synergistic effect when fosfomycin is combined with either amikacin or colistin. Erturk Sengel *et al* (2020) reported synergistic effects in 14.3 and 28.6% of $bla_{OXA\text{-}48\text{-}like}$-carrying CRKP isolates treated with fosfomycin combined with colistin and with amikacin respectively; however,

an antagonistic effect is observed in one $bla_{OXA\text{-}48\text{-}like}$- and one bla_{NDM}-carrying CRKP isolate treated with fosfomycin plus colistin. Liao *et al* (2017) demonstrated that fosfomycin combination therapy improves the survival rate of patients with CRKP infections. Similarly, Karnmueng *et al* (2024) found that fosfomycin combination regimens, usually with aminoglycosides, improve survival rate (51.7%) in cases of $bla_{NDM\text{-}1}$ + $bla_{OXA\text{-}48}$-carrying CRE BSI, followed by $bla_{OXA\text{-}48}$- and $bla_{NDM\text{-}1}$-carrying isolates (31.7 and 15.0% respectively).

In vitro data indicate that fosfomycin + colistin may not be an optimal choice for treating $bla_{OXA\text{-}48}$-harboring CRKP infections, and the use of ceftazidime/avibactam should be prioritized (Erturk Sengel *et al*, 2020). However, in the current study, more than half of the patients who received fosfomycin combination treatment had CRKP isolates lacking susceptibility to ceftazidime/avibactam. Among 18 patients with reported CRKP susceptibility data, only 44% (n = 8) of the isolates were susceptible to ceftazidime/avibactam. Nasomsong *et al* (2021) noted that only 47.7% of tested CRKP isolates carrying various carbapenemase genotypes are susceptible to ceftazidime/avibactam compared to 90.5% of those harboring $bla_{OXA\text{-}48}$. These findings highlight the need for maintaining regular antibiogram profiles of CRKP, particularly in healthcare facilities treating patients with high risk for infection of multidrug-resistant microorganisms (CLSI, 2024).

Aztreonam and ceftazidime/avibactam are available in Thailand; however, treatment eligibility criteria restrict access. At present, there is insufficient clinical evidence to recommend an optimal drug combination regimen for the treatment of CRKP infections (Pudpong *et al*, 2022; So-Ngern *et al*, 2023; Karnmueng *et al*, 2024). However, when prescribing fosfomycin knowledge of its activity against the target CRKP isolates is essential. More than half of the cases utilized susceptibility tests to confirm CRKP sensitivity to fosfomycin.

Etest and disk diffusion are the methods employed. While both methods are user-friendly, the Etest is more expensive than the disc diffusion method, as it effectively combines the disk diffusion principle and dilution technique (EUCAST, 2023; CLSI, 2024). Studies have reported that the disk diffusion assay fails to establish a precise zone breakpoint that minimizes error to an acceptable level (Kaase *et al*, 2014; Karlowsky *et al*, 2021). Both of the latter studies used agar dilution as the reference method; however, the Etest and disk diffusion method is deemed unacceptable for *Enterobacterales* other than *E. coli* due to the frequent occurrences of scattered colonies within the inhibition zones. Interpreting scattered colonies within inhibition zones as areas of bacterial growth could improve the accuracy of the analysis and address some limitations of the Etest.

Previous fosfomycin treatment outcomes were guided by susceptibility results from the Etest (Kanchanasurakit *et al*, 2020; Karnmueng *et al*, 2024). In the current study with fosfomycin having an MIC ≤32 µg/ml, the previously used breakpoint, the inter-method CA and error rates were deemed unacceptable; however, for fosfomycin MIC values ≤128 µg/ml, which aligns with that of EUCAST (2024b), the inter-method CA and error rates were deemed acceptable when the inhibition zone diameter was ≥16 mm. ECOFF identifies bacterial strains from the wild-type group, which do not exhibit phenotypically detectable resistance mechanisms (Kahlmeter and Turnidge, 2022). Leelawattanachai *et al* (2020) performed a Monte Carlo simulation, which indicates that a maximum fosfomycin dose of 24 g/day achieves at least a 90% probability of attaining a ratio of the area under the curve to MIC >21.5 for critically ill patients with fosfomycin MIC values ≤128 µg/ml. Given the limitations of testing methods, further investigation is required to enhance the interpretation's accuracy. Agar dilution remains the reference method for assessing CRKP susceptibility to fosfomycin;

however, if the agar dilution assay is not feasible, the Etest and disk diffusion method may provide data to support fosfomycin use as an adjunctive antibiotic in combination therapies.

The study has several limitations: i) non-standardized susceptibility testing methods were employed; ii) resistance mechanisms, such as the presence of extended-spectrum beta-lactamase, AmpC beta-lactamase, efflux pumps, and porin loss, were not investigated beyond the identification of carbapenemase genes present in the clinical CRKP isolates; iii) this was a retrospective study and descriptive in nature, thereby inherently restricting the evaluation of critical clinical outcomes, such as rates of clinical cure, microbiological eradication and safety profiles associated with the treatment regimens; and iv) small numbers of clinical CRKP isolates and patients' biodata were obtained from Buriram, Nopparat Rajathanee and Ratchaburi Hospitals, which may not be representative of the antibiograms and treatment regimens of the whole geographical region in which the hospitals are located. These limitations highlight the lack of standardized fosfomycin MIC breakpoints for *K. pneumoniae* required for clinical interpretation. Further comprehensive studies are required to provide a more robust understanding of the efficacy and safety of fosfomycin combination therapy.

In conclusion, the study revealed that fosfomycin did not exhibit consistent effectiveness against carbapenem-resistant *K. pneumoniae* (CRKP). The majority of clinical CRKP isolates carried $bla_{OXA\text{-}48\text{-}like}$ that was associated with high fosfomycin MIC values. Although standardized MIC breakpoints for fosfomycin use against CRKP remain unavailable, thereby making clinical interpretation difficult, in situations where no other effective antibiotics are available fosfomycin was still a useful adjunctive drug in combination regimens for CRKP bloodstream infection, when fosfomycin disc diffusion inhibition zone diameter was ≥16 mm (corresponding to an MIC of ≤128 μg/ml by the Etest). Further

research is necessary to explore CRKP resistance mechanisms, the relationship between resistance and carbapenemase types, the correlation between fosfomycin susceptibility test methods and standard reference techniques, and the therapeutic efficacy of the various fosfomycin combination regimens for CRKP bloodstream infection.

ACKNOWLEDGEMENTS

The authors thank all participants, infectious disease physicians, clinical pharmacists, and clinical microbiology laboratory staff for their contributions to the bacterial isolations and standard antimicrobial susceptibility tests.

The research was supported by Phramongkutklao, Nopparat Rajathanee, Ratchaburi, and Buriram Hospitals, and Dr. Kasem Pangsrivongse Foundation, Bangkok Thailand (grant no. 111/2566).

CONFLICT OF INTEREST DISCLOSURE

The authors declare no conflict of interest.

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