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## ► To cite this version:

Charles Baulier, Marion Giry, Manuel Etienne, Julien Kallout, Kévin Alexandre. Pharmacokinetic/pharmacodynamic targets of  $\beta$ -lactams associated with bacterial killing and suppression of the resistance emergence in the hollow fiber infection model: A systematic review. *Journal of Global Antimicrobial Resistance*, 2025, 45, pp.220 - 227. [⟨10.1016/j.jgar.2025.10.009⟩](https://doi.org/10.1016/j.jgar.2025.10.009). [⟨hal-05352140⟩](https://hal.science/hal-05352140)

**HAL Id: hal-05352140**

**<https://hal.science/hal-05352140v1>**

Submitted on 6 Nov 2025

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## Review

# Pharmacokinetic/pharmacodynamic targets of $\beta$ -lactams associated with bacterial killing and suppression of the resistance emergence in the hollow fiber infection model: A systematic review



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## ARTICLE INFO

## Article history:

Received 28 June 2025

Revised 3 October 2025

Accepted 10 October 2025

Available online 14 October 2025

Editor: Professor Athanasios Tsakris

## Keywords:

$\beta$ -lactam resistance

Pharmacokinetics

Models

Biological

Anti-bacterial agents

## ABSTRACT

**Introduction:** The hollow-fibre infection model (HFIM) is an in vitro model used to study pharmacokinetic/pharmacodynamic (PK/PD) interactions between antibiotics and bacteria. However, its external validity remains poorly assessed. This study aimed to identify PK/PD targets and experimental variables associated with bacterial killing and prevention of resistance emergence during  $\beta$ -lactam treatment in HFIM.

**Methods:** Systematic review of studies using HFIM and  $\beta$ -lactams, with literature search conducted in 4 databases, following PRISMA guidelines. After a quality assessment (modified ToxRtools scale) of the included studies, univariate and multivariate analyses identified factors associated with the prevention of resistance emergence. CART analysis determined values of PK/PD indices best predicting this outcome.

**Results:** Of the 497 screened studies, 41 were included. A total of 367 experiments was analysed, mainly involving *Pseudomonas aeruginosa* (51%) and carbapenems (46%). Antibiotic exposure through minimal or steady-state free concentrations ( $fC_{\min,ss}/MIC$ ) (OR 0.78, 95% CI [0.70–0.85],  $P < 0.001$ ), the initial inoculum size (OR 1.48, 95% CI [1.08–2.06],  $P = 0.017$ ) and the use of a combination therapy (OR 0.33, CI [0.17–0.64],  $P = 0.001$ ) remained significantly associated with resistance emergence in multivariate analysis. For carbapenems in monotherapy, the prevention of resistance emergence was likely with  $fC_{\min,ss}/MIC > 5.7$ .

**Conclusions:** Stringent PK/PD indices thresholds were necessary to prevent resistance emergence in HFIM experiments.

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## 1. Introduction

Pharmacokinetic/pharmacodynamic (PK/PD) studies investigate the relationship between antibiotic exposure and its pharmacological effects. These studies have a wide range of applications, including the preclinical drug evaluation, the determination of susceptibility breakpoints and the optimisation of doses and infusion regimens [1]. The efficacy of an antibiotic treatment is influenced

by various factors, with pharmacokinetics playing a crucial role. For time-dependent antibiotics, like  $\beta$ -lactams, bacterial killing is enhanced when the free drug concentration remains above the MIC for an extended period of time ( $\%T > MIC$ ) [2–4]. Conversely, for concentration-dependent antibiotics, the area under the free concentration-time curve ( $fAUC_{0-24h}/MIC$ ) and the unbound peak concentration divided by the MIC ( $fC_{\max}/MIC$ ) are the PK/PD indices most strongly associated with bactericidal activity [5]. Targeting specific values of PK/PD indices has two objectives: improving patient outcomes and preventing the emergence of antibiotic resistance [4,6,7]. Most PK/PD targets are derived from animal model data, which come with inherent limitations. Pharmacokinetic mod-

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elling in animals may be inaccurate, and extrapolating findings to humans introduce uncertainty due to interspecies differences in parameters like elimination half-life and volume of distribution [8]. Ethical considerations further constrain animal model use, driving growing interest in alternative approaches [9].

The hollow fibre infection model (HFIM) is a suitable alternative to animal models. HFIM is an *in vitro* PK/PD model with two compartments. Since its first description by Zinner et al. in 1981, the HFIM has been widely used to investigate bacteria-antibiotic interactions [10]. Its main advantage is accurately simulating dynamic antibiotic concentration changes to replicate realistic treatment conditions with various therapies, perfusion administration, and treatment durations [11]. It is also possible to simulate predefined pharmacokinetic conditions. In contrast to animal models, the lack of an immune system and the immediate distribution of the antibiotic to the bacterial inoculum are key parameters to consider when interpreting results of HFIM studies [11]. Indeed, hollow fibre model evaluate the primary driver of bacterial killing that is the interaction of the antibiotic with its target, hereby reducing confounding bias. However, its application remains not standardised, Sadouki et al. highlighting insufficient reporting of materials and methods among HFIM studies in a recent systematic review [12].

Although PK/PD targets identified through *in vitro* HFIM studies have provided valuable insights into antimicrobial efficacy, their direct applicability to clinical practice remains uncertain. This systematic review aims to comprehensively evaluate the PK/PD indices associated with optimal bacterial killing and prevention of the antibiotic resistance emergence during  $\beta$ -lactam treatment in HFIM studies.

## 2. Methods

Practical aspects regarding tubing and setting of hollow fibre model have been extensively described elsewhere [11]. This systematic review, registered in PROSPERO (CRD420251042321), was conducted in agreement with PRISMA 2020 guidelines.

### 2.1. Search strategy

A literature search was conducted on 2 September 2025, across four databases (PubMed, Web of Science, Lens.org, and Cochrane Library) by an expert librarian. The search strategies, including full query details, are provided in Supplementary Material. Two authors (CB and KA) independently screened titles, abstracts, and full texts for eligibility, including studies published in English or French.

### 2.2. Inclusion and exclusion criteria

To be considered for inclusion, the PK/PD studies in the HFIM needed to be based on  $\beta$ -lactams, have a minimal report of pharmacokinetic and PK/PD data ( $\%fT > MIC$  and/or  $fC_{min}$ ), and maintain experimental conditions for a duration of 72 h or longer.

Studies involving mycobacteria, viruses, fungi, parasites, intracellular bacteria, or *B. anthracis* were excluded.

### 2.3. Quality assessment

A quality assessment procedure was conducted independently by two authors (CB and KA), using an adapted version of the ToxRtools scale (Table S1). This procedure resulted in three levels of methodological robustness, depending on the mean attributed scores, as previously described [13]. Studies were classified as reliable without restriction (15–18 points), reliable with restrictions (11–14 points), or not reliable (<11 points). Studies with a mean

score of  $\geq 11$  points were classified as reliable quality and included in the analysis.

### 2.4. Data extraction and definition of outcomes

PK/PD indices included were the time during which the free antibiotic concentration exceeded the MIC ( $\%fT > MIC$ ) or 4 times the MIC ( $\%fT > 4MIC$ ), the  $fC_{min,ss}/MIC$  (minimal or steady-state concentration, depending on the intermittent or continuous infusion, respectively), and  $fC_{max}/MIC$  ratios, the area under the curve of the free antibiotic concentrations during the first 24 h of the experiment ( $fAUC_{0-24h}$ ), and its ratio to the MIC ( $fAUC_{0-24h}/MIC$ ).

As a secondary objective of our study, other variables were collected to analyse their impact on the outcome of HFIM experiments. These included the mechanisms of resistance, the type of bacteria studied (genus and species), and the simulated antibiotic treatment (infusion regimen, and combination therapy or not). The antibiotic infusion regimen was defined as intermittent if the infusion duration was less than half the interval between two doses, extended if it was longer, or continuous. When the antibiotics were administered as a combination therapy, the PK/PD indices reported were those of the  $\beta$ -lactam. If authors did not mention PK/PD indices, these were calculated with  $\beta$ -lactam dosages and primary PK parameters mentioned in the manuscript, using a one-compartment model in Monolix software (Monolix 2023R1, Lixoft SAS, a Simulations Plus company, Antony, France).

When precise bacterial counts were not available, the data were obtained through formal requests to the authors or visual extrapolation of the figures.

The threshold for optimal bacterial killing was chosen according to common definitions of bactericidal activity [14]. Based on the difference in bacterial population between the final day of the experiment and the initial inoculum, each experiment resulted in one of the three following outcomes:

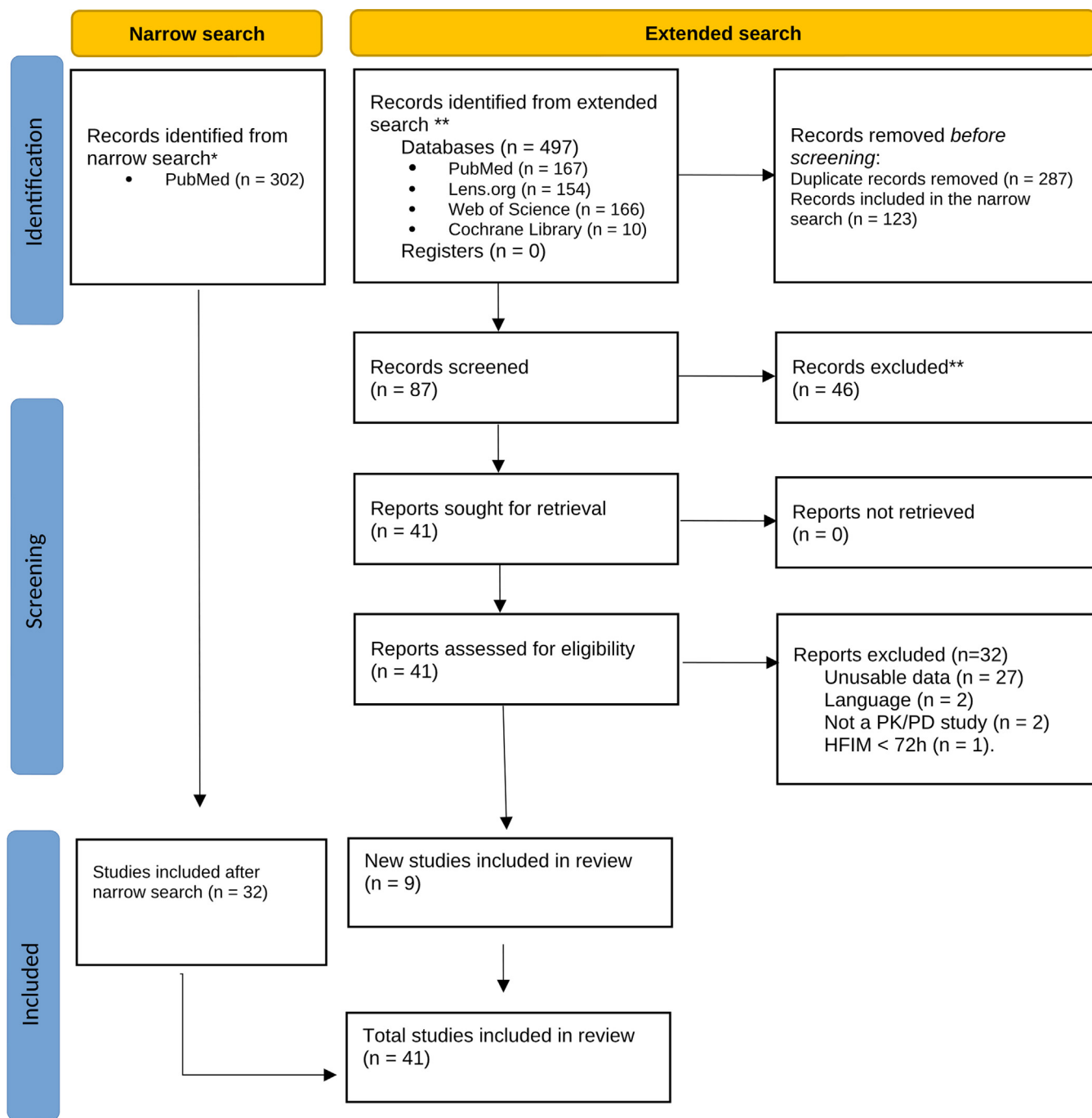
- Sustained bacterial suppression if the difference was  $\leq -3 \log_{10}$  CFU/mL.
- Regrowth without resistance emergence if the difference was  $> -3 \log_{10}$  CFU/mL, without growth on antibiotic-enriched agar.
- Regrowth with resistance emergence if the difference was  $> -3 \log_{10}$  CFU/mL, with growth on both standard and antibiotic-enriched agar.

### 2.5. Statistical analysis

The normality of the distribution was assessed using QQ-plots for each variable. Quantitative variables were presented as median and interquartile range (IQR), and analysed using ANOVA or the Kruskal-Wallis test, depending on their normal or non-normal distribution, respectively. Qualitative variables were presented as number and percentages, and analysed using the  $\chi^2$  test or Fisher's exact test, as appropriate.

Initially, a descriptive analysis was performed, based on the three outcomes. Subsequently, a logistic regression was conducted, with the binary dependent variable being the emergence of antibiotic resistance. The candidate and independent variables were chosen based on their association ( $P < 0.20$ ) with this binary outcome in univariate analysis (Table S2). Model selection was performed using a backward elimination procedure, retaining the model with the best goodness-of-fit according to the Akaike Information Criterion (AIC). Collinearity was assessed, with a variance inflation factor  $< 2.5$  considered acceptable [15].

We applied classification and regression tree analysis (CART) to the most common antibiotic-bacterial species combinations in our study to determine the PK/PD indices that effectively differentiate between the emergence and the prevention of antibiotic resistance. Only results with the least root node error are displayed.



\* Algorithm 1 : Adapted from Sadouki et al. (PubMed-only query)

\*\* Algorithm 2 : Homemade comprehensive query (PubMed, Embase, Scopus, Web of Science)

Fig. 1. PRISMA flowchart.

Finally, we conducted two subgroup univariate analyses to address treatment group heterogeneity: one for experiments using carbanem monotherapy and another for those involving Pseudomonadales.

No approach was applied to handle missing data. The significance threshold was set at  $P < 0.05$ . Statistical analyses were conducted using R (R version 4.3.1, R Foundation for Statistical Computing, Vienna, Austria). Classification and regression trees were performed with *rpart* package (version 4.1.19).

### 3. Results

Our search retrieved 497 eligible records (PRISMA flowchart, Fig. 1). Of these, 410 were excluded outright (287 were duplicates, and 123 records were ineligible). The title/abstract and full text screening procedures resulted in the final inclusion of 41 studies. Reasons for exclusion mainly relied on the lack of pharmacokinetic data, the use of antibiotics other than  $\beta$ -lactams, or the study of pathogens out of the scope of this review. Using the modified

**Table 1**  
Microbiological characteristics of the HFIM experiments.

	Final outcome				P-value <sup>1</sup>
	Overall N = 367	Sustained bacterial suppression N = 158	Regrowth N = 27	Regrowth with resistance N = 182	
<b>General characteristics</b>					
Experimental duration (h), Median (IQR)	168 (167 to 240)	168 (168 to 240)	167 (120 to 192)	168 (168 to 240)	0.035
<b>Bacterial strains</b>					
Bacterial order, n (%)					<0.001
<i>Bacillales</i>	41 (11)	22 (14)	6 (22)	13 (7.1)	
<i>Enterobacterales</i>	109 (30)	64 (41)	5 (19)	40 (22)	
<i>Pseudomonadales</i>	217 (59)	72 (46)	16 (59)	129 (71)	
<b>Bacterial species, n (%)</b>					
<i>A. baumannii</i>	28 (7.6)	8 (5.1)	0 (0)	20 (11)	
<i>E. coli</i>	64 (17)	46 (29)	0 (0)	18 (9.9)	
<i>K. pneumoniae</i>	45 (12)	18 (11)	5 (19)	22 (12)	
<i>P. aeruginosa</i>	189 (51)	64 (41)	16 (59)	109 (60)	
<i>S. aureus</i>	41 (11)	22 (14)	6 (22)	13 (7.1)	
Initial inoculum (log <sub>10</sub> ), Median (IQR)	7.00 (7.00 to 8.00)	7.00 (7.00 to 8.00)	7.00 (7.00 to 7.00)	7.20 (7.00 to 8.00)	0.002
Bacterial count difference (log <sub>10</sub> ), Median (IQR)	-1.0 (-5.0 to 2.0)	-5.2 (-6.2 to -4.5)	-1.2 (-2.4 to 1.8)	2.0 (1.1 to 2.8)	<0.001
<b>Baseline resistance mechanisms</b>					
Altered target, n (%)	62 (17)	22 (14)	6 (22)	34 (19)	0.32
β-lactamase, n (%)	152 (41)	67 (42)	9 (33)	76 (42)	0.68
- Carbapenemase, n (%)	56 (15)	25 (16)	3 (11)	28 (15)	0.92
- ESBL, n (%)	72 (20)	40 (25)	3 (11)	29 (16)	0.057
- AmpC, n (%)	60 (16)	16 (10)	4 (15)	40 (22)	0.010
Efflux pump, n (%)	31 (8.4)	4 (2.5)	1 (3.7)	26 (14)	<0.001
Decreased permeability, n (%)	63 (17)	31 (20)	5 (19)	27 (15)	0.49
Baseline MIC, Median (IQR)	3 (1 to 8)	2 (1 to 4)	4 (1 to 4)	4 (2 to 16)	<0.001
<b>Susceptibility, n (%)</b>					
Resistant	114 (31)	33 (21)	10 (37)	71 (39)	<0.001
Susceptible	253 (69)	125 (79)	17 (63)	111 (61)	<0.001

<sup>1</sup> Kruskal-Wallis rank sum test; Fisher's exact test. IQR = Interquartile range.

**Table 2**  
Treatment characteristics and antibiotic exposure in HFIM experiments.

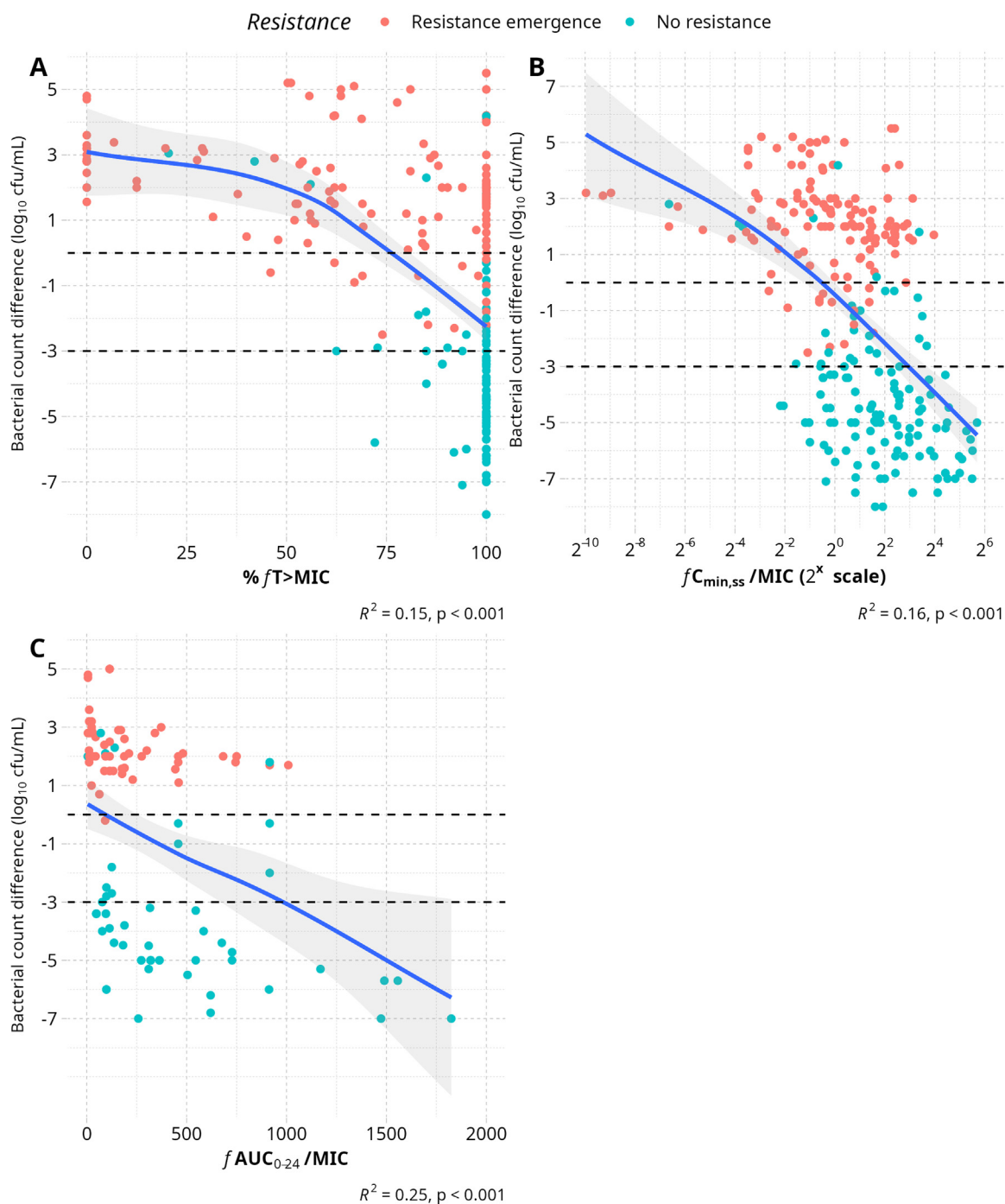
	Final outcome				P-value <sup>1</sup>
	Overall N = 367	Sustained bacterial suppression N = 158	Regrowth N = 27	Regrowth with resistance N = 182	
<b>Antibiotics</b>					
Infusion regimen, n (%)					0.017
Continuous	68 (19)	28 (18)	1 (3.7)	39 (21)	
Intermittent	281 (77)	126 (80)	22 (81)	133 (73)	
Prolonged	18 (4.9)	4 (2.5)	4 (15)	10 (5.5)	
<b>Protocol, n (%)</b>					
Combination therapy	58 (16)	37 (23)	0 (0)	21 (12)	<0.001
Monotherapy	309 (84)	121 (77)	27 (100)	161 (88)	
<b>β-lactam subtype, n (%)</b>					
Carbapenem	170 (46)	62 (39)	4 (15)	104 (57)	
Cephalosporin	39 (11)	22 (14)	6 (22)	11 (6.0)	
Cephalosporin+BLi	84 (23)	42 (27)	6 (22)	36 (20)	
Penicillin	15 (4.1)	4 (2.5)	4 (15)	7 (3.8)	
Penicillin+BLi	59 (16)	28 (18)	7 (26)	24 (13)	
<b>PK/PD indices</b>					
% fTime > MIC, Median (IQR)	100 (64 to 100)	100 (100 to 100)	100 (85 to 100)	84 (52 to 100)	<0.001
fC <sub>min,ss</sub> /MIC ratio, Median (IQR)	2 (1 to 5)	3 (1 to 10)	2 (1 to 4)	1 (0 to 2)	<0.001
fC <sub>max</sub> /MIC ratio, Median (IQR)	10 (5 to 24)	14 (5 to 34)	71 (10 to 182)	7 (3 to 12)	0.022
fAUC <sub>0-24h</sub> , Median (IQR)	458 (195 to 1,033)	620 (456 to 1,033)	1,831 (373 to 3,663)	375 (115 to 707)	0.009
fAUC <sub>0-24h</sub> /MIC, Median (IQR)	177 (63 to 458)	317 (114 to 676)	125 (98 to 458)	114 (28 to 211)	<0.001

<sup>1</sup> Kruskal-Wallis rank sum test; Fisher's exact test. BLi = Beta-lactamase inhibitor, IQR = Interquartile range.

ToxRtools scale for quality assessment of the 41 included studies, 37 were rated as reliable without restrictions, 4 as reliable with restrictions, and none were classified as unreliable (Table S1, Supplementary).

The species studied were mainly *Pseudomonas aeruginosa* in 189 of 367 experiments (51%) (Table 1). A substantial proportion of bacterial strains (31%) exhibited baseline resistance to the studied β-lactam. Combination therapy (16%) and continuous infusion of β-lactams (19%) were not frequently employed (Table 2).

Several factors were linked to the final outcome in univariate analysis. HFIM experiments were significantly more likely to result in regrowth with resistance, compared to regrowth without resistance or sustained bacterial suppression, when involving Pseudomonadales (129 (71%) vs. 16 (59%) vs. 72 (46%),  $P < 0.001$ ), bacterial strains harbouring efflux pump as a baseline resistance mechanism (26 (14%) vs. 1 (3.7%) vs. 4 (2.5%),  $P < 0.001$ ), or in presence of high initial inoculum size (7.20 (7.00–8.00) vs. 7.00 (7.00–7.00) vs. 7.00 (7.00–8.00) log<sub>10</sub> CFU/mL,  $P = 0.002$ ) (Table 1).



**Fig. 2.** Bacterial count difference between end and start of all HFIM experiments ( $\log_{10}$  UFC/mL) with  $\beta$ -lactam monotherapy, according to % $T > CMI$  (A),  $fC_{min,ss}/MIC$  (B,  $\log_2$  scale) and  $fAUC_{0-24h}/MIC$  (C). Regression line (blue) and 80% confidence interval (grey shaded area) represent the relationship between bacterial count and the respective PK/PD indices. Horizontal, dashed lines for  $y = 0$ , and  $-3\log$  of bacterial killing are also displayed.

Conversely, sustained bacterial suppression was significantly associated with combination therapy (37 (23%) vs. 0 (0%) vs. 21 (12%),  $P < 0.001$ ) (Table 2).

Continuous infusion yielded significantly higher  $\beta$ -lactam exposure compared with intermittent or extended regimens (median  $fC_{min,ss}/MIC$  4.3 vs. 1.1,  $P < 0.001$ ). However, when resistance suppression was analysed as a binary outcome, the association between infusion mode and resistance suppression did not reach statistical significance (Table S2, Supplementary).

Higher antibiotic exposure was strongly associated with both resistance suppression and enhanced bacterial killing (Fig. 2). Me-

dian  $fC_{min,ss}/MIC$  values progressively increased across outcomes categories: 1 (0–2) for regrowth with resistance, 2 (1–4) for regrowth without resistance, and 3 (1–10) for sustained bacterial suppression ( $P < 0.001$ ) (Table 2). These findings were confirmed in subgroup analyses of carbapenem monotherapy (1 (0–2) vs. 1 (0–1) vs. 6 (2–17),  $P < 0.001$ ) and Pseudomonadales (0.9 (0.2–2.1) vs. 1.9 (0.3–7.6) vs. 3.8 (1–8.7),  $P < 0.001$ ) (Tables S3 and S4, Supplementary).

Multivariate analysis confirmed the key determinants of resistance emergence (Table 3). Higher  $fC_{min,ss}/MIC$  (OR 0.78, 95% CI [0.70–0.85],  $P < 0.001$ ) and combination therapy (OR

**Table 3**  
Generalised linear model for the emergence of antibiotic resistance.

Characteristic	OR (95% CI)	P-value
(Intercept)	0.05 (0.00–0.51)	0.014
$fC_{\min}/MIC$	0.78 (0.70–0.85)	<0.001
Initial inoculum ( $\log_{10}$ )	1.48 (1.08–2.06)	0.017
Protocol		
Monotherapy	–	
Combination therapy	0.33 (0.17–0.64)	0.001
Bacterial order		
Other	–	
Pseudomonadales	3.24 (1.74–6.18)	<0.001
ESBL	2.99 (1.39–6.58)	0.006
Efflux pump	3.43 (1.30–10.8)	0.020

CI = confidence interval, OR = odds ratio.

0.33, 95% CI [0.17–0.64],  $P = 0.001$ ) were independently associated with reduced resistance emergence. Conversely, resistance emerged more frequently with Pseudomonadales and efflux pump-harbouring strains or ESBL producers. Each tenfold increase in the initial inoculum size ( $+1\log_{10}$ ) increased the odds of resistance emergence by approximately 50% (OR 1.48, 95% CI [1.08–2.06],  $P = 0.017$ ). Multicollinearity precluded inclusion of multiple PK/PD indices in the logistic model.

CART analysis identified specific  $fC_{\min,ss}/MIC$  thresholds associated with microbiological outcomes for carbapenem monotherapy. Suppression of resistance emergence was most likely achieved with  $fC_{\min,ss}/MIC > 5.7$ , while bacteriostasis (0  $\log_{10}$  CFU/mL change) and 3- $\log_{10}$  bacterial killing were best predicted with  $fC_{\min,ss}/MIC > 1.0$  and  $>4.8$ , respectively. CART analyses performed with other  $\beta$ -lactam classes or bacterial strain subsets demonstrated poor discriminatory accuracy (Supplementary, Table S5).

#### 4. Discussion

This systematic review and meta-analysis evaluated 367 experimental results from 41 HFIM studies investigating the activity of  $\beta$ -lactams. Our analysis revealed that the PK/PD indices required to achieve bacterial suppression and prevent resistance emergence in HFIM are consistently higher than those reported in other preclinical models. These findings may underscore the relevance of HFIM as a robust preclinical tool for optimizing PK/PD parameters in the management of severe infections.

Animal models have traditionally guided PK/PD target selection for  $\beta$ -lactams, with bactericidal activity typically associated with  $\%fT > MIC$  values of 40% to 50%, and resistance suppression requiring 50% to 75% [16–23]. However, our meta-analysis of HFIM studies identifies significantly higher thresholds. Maintaining 100%  $fT > MIC$  alone was insufficient to distinguish between resistance emergence and suppression (Fig. 2A). Instead, resistance suppression was more likely achieved with  $fC_{\min,ss}/MIC$  ratio between 4 and 8 (Fig. 2B), findings corroborated by CART analysis. Notably, these HFIM-derived targets align closely with clinical observations in critically ill patients [18–24], where improved clinical cure and bacterial eradication rates have been reported when  $fC_{\min}/MIC$  exceeds five [6,24]. A recent meta-analysis by Gatti et al. demonstrated that achieving aggressive PK/PD targets ( $100\%T > 4xMIC$  or  $C_{\min}/MIC > 4-5$ ) improved clinical cure rates while reducing microbiological failure and resistance in ICU patients [25]. Consequently, current guidelines recommend maintaining free  $\beta$ -lactam trough concentration at 4- to 8-fold the MIC throughout the dosing interval in critically ill patients [5,26]. A translational case report by Lopez-Martesinos et al. further illustrates this concordance: in a patient with a ventilator-associated pneumonia (VAP) caused by extensively drug-resistant *P. aeruginosa*, bacterial suppression and

resistance prevention were achieved at  $fC_{\min,ss}/MIC$  ratio of 5–8 in both the clinical management (guided by therapeutic drug monitoring) and HFIM experiments using the same bacterial isolate [27].

Interestingly, our multivariate analysis identified two additional factors significantly associated with prevention of resistance emergence: combination therapy and initial inoculum size.

The beneficial effects of combination therapy in preventing resistance emergence are well-established in preclinical models [28], with synergistic interactions often incorporated into pharmacodynamic models [29]. For  $\beta$ -lactams, cell wall disruption enhances penetration of companion agents, such as aminoglycosides. However, meta-analyses have failed to demonstrate significant mortality or treatment success benefits for combination therapy compared to monotherapy, while consistently reporting increased nephrotoxicity with  $\beta$ -lactam-aminoglycosides combinations [30–32]. This preclinical-clinical discrepancy highlights the challenge of translating combination therapy findings into routine clinical practice and warrants cautious interpretation of our results.

Our analyses demonstrated that inoculum size was a key determinant influencing both bacterial killing and resistance emergence in HFIM studies. The inoculum effect – an increase in MIC values observed at bacterial densities exceeding those specified in antimicrobial susceptibility testing guidelines [33] – is consistently observed in animal models, and can be driven by increased  $\beta$ -lactamase production, enhanced quorum sensing, and a bacterial transition into stationary growth phase [34]. Similarly, HFIM experiments are susceptible to inoculum effect; high bacterial loads within the extracapillary space have been associated with reduced  $\beta$ -lactam concentrations in the central compartment [35]. While the clinical implications of the inoculum effect remain uncertain, high bacterial burden, such as in VAP, has been linked to an increased risk of resistance development during therapy [36,37]. Conversely, infections characterised by lower bacterial loads, such as urosepsis with approximately 5  $\log_{10}$  CFU/mL, are often associated with more favourable clinical outcomes [38].

The concordance between HFIM-derived PK/PD targets and clinical outcomes in critically ill patients could be related to shared pathophysiological features. Sepsis, a life-threatening condition with a global mortality rate exceeding 30% [39], is characterised by both highly variable and unfavourable PK profiles – resulting in suboptimal antibiotic exposure [4,40] – and a profound immunosuppression affecting both innate and adaptive responses [41]. This impaired immune function, even in previously immunocompetent individuals, may necessitate higher antibiotic exposure to compensate for reduced host bacterial clearance. Additionally, critically ill patients frequently harbour MDR pathogens or susceptible strains with borderline MIC [42], necessitating PK/PD optimisation. HFIM replicates these challenging conditions by lacking immune-mediated bacterial clearance, thereby demanding higher antibiotic concentration for bacterial suppression. This feature could explain why HFIM may serve as a relevant preclinical model for severe infections [5]. Conversely, the applicability of these stringent PK/PD targets to less severe infections require careful consideration. In these cases, clinical and microbiological cure may be achieved with lower PK/PD thresholds [9].

This systematic review is the first, to our knowledge, to critically evaluate the external validity of HFIM as a preclinical PK/PD model. However, some limitations should be acknowledged. First, methodological heterogeneity and incomplete reporting limited our analyses. Several studies lacked bacterial counts and did not report  $fAUC_{0-24h}/MIC$ , consistent with a prior systematic review highlighting the inadequate of reporting of materials and methods in HFIM studies [12]. Imputation strategies for missing  $fAUC_{0-24h}/MIC$  values failed to improve classification, so we restricted our CART analysis to  $fC_{\min,ss}/MIC$ . Similarly, the  $\%fT > MIC$  index was not retained, as values were consistently censored be-

yond 100%, preventing discrimination between outcomes, as illustrated in Fig. 2. Second, combination therapy may have underestimated the antibiotic exposure required for resistance suppression. However, our CART analysis was restricted to monotherapies, and  $fC_{min,ss}/MIC$  remained an independent predictor of this outcome after adjustment for combination regimens. Finally, the complex interaction between antibiotic exposure, bacterial killing, and resistance suppression likely varies across bacterial strains (e.g., Gram-positive vs. Gram-negative, Pseudomonadales vs. Enterobacterales), the  $\beta$ -lactam used, and ultimately the specific  $\beta$ -lactam–bacterium pair. While we performed subgroup analyses, limited sample sizes prevented CART from defining such pair-specific thresholds. Our findings should therefore be interpreted with caution, given the substantial heterogeneity in bacterial species,  $\beta$ -lactam infusion regimen, experiments duration, and a high prevalence of baseline resistant phenotypes. Our global approach therefore provides preliminary insights, but further targeted studies are needed to account for heterogeneity across antibiotic–bacterium combinations in the HFIM. Nonetheless, the consistency between HFIM-derived PK/PD targets and clinical data from ICU patients supports the clinical relevance of our results and suggests that these findings may inform antibiotic dosing strategies in critically ill patients.

## 5. Conclusion

This systematic review addresses the external validity of the HFIM compared to other preclinical models and clinical data. HFIM may replicate PK/PD interactions observed in patients, particularly under critical conditions like sepsis or septic shock. However, the resulting PK/PD targets are consistently higher than those derived from animal models, as referenced in EUCAST rationale documents. Future research should aim to determine the most appropriate preclinical model for specific clinical context, while improved standardisation of methodological reporting is necessary to enhance reproducibility and reliability.

**Funding:** This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

**Author contributions:** Conceptualization: KA. Methodology: JK, CB and KA. Software: JK, CB and KA. Validation: KA and CB. Formal analysis: CB, MG and KA. Investigation: CB, MG and KA. Data curation: CB, MG and KA. Writing - Original Draft: CB and KA. Writing - Review & Editing: All authors. Supervision: KA.

**Declaration of competing interests:** The authors have nothing to declare.

**Acknowledgements:** The authors thank Gaëtan Kerdelhué for his invaluable help in the literature research.

**Data availability:** All data are available on request from the authors.

## Supplementary materials

Supplementary material associated with this article can be found, in the online version, at [doi:10.1016/j.jgar.2025.10.009](https://doi.org/10.1016/j.jgar.2025.10.009).

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