1 **Semi-mechanistic PK/PD modelling of fosfomycin and sulbactam combination against**
2 **carbapenem-resistant *Acinetobacter baumannii***

3
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13 Running title: Fosfomycin-sulbactam against *A. baumannii*

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18

19 **Abstract**

20 Due to limited treatment options for carbapenem-resistant *Acinetobacter baumannii* (CR-AB)
21 infections, antibiotic combinations are now considered potential treatments for CR-AB. This
22 study aimed to explore the utility of fosfomycin-sulbactam combination (FOS/SUL) therapy
23 against CR-AB isolates.

24 Synergism of FOS/SUL against 50 clinical CR-AB isolates were screened using the
25 checkerboard method. Thereafter, time-kill studies against two CR-AB isolates were
26 performed. The time-kill data were described using a semi-mechanistic
27 pharmacokinetic/pharmacodynamic (PK/PD) model. Monte Carlo simulations were then
28 performed to estimate the probability of stasis, 1-log kill and 2-log kill after 24-hours with
29 combination therapy.

30 The FOS/SUL combination demonstrated a synergistic effect against 74% of isolates. No
31 antagonism was observed. The MIC₅₀ and MIC₉₀ of FOS/SUL were decreased four- to eight-
32 fold, compared to the monotherapy MIC₅₀ and MIC₉₀. In the time-kill studies, the
33 combination displayed bactericidal activity against both isolates and synergistic activity
34 against one isolate, at the highest clinically achievable concentrations. Our PK/PD model was
35 able to describe the interaction between fosfomycin and sulbactam *in vitro*. Bacterial kill was
36 mainly driven by sulbactam, with fosfomycin augmentation. FOS/SUL regimens that
37 included sulbactam 4 g every 8 hours, demonstrated a probability of target attainment of 1-
38 log₁₀ kill at 24 h of ~69-76%, as compared to ~15-30% with monotherapy regimens at the
39 highest doses.

40 The reduction in the MIC values and the achievement of a moderate PTA of a 2-log₁₀
41 reduction in bacterial burden demonstrated that FOS/SUL may potentially be effective
42 against some CR-AB infections.

43

44 **Keywords**45 *Acinetobacter baumannii*; synergy; PK/PD model; Monte Carlo simulation; fosfomycin;46 sulbactam; *in vitro*.

47

48 Introduction

49 *Acinetobacter baumannii* possesses intrinsic resistance mechanisms and the ability to
50 acquire new resistance genes proficiently (1). These abilities render it resistant to many
51 antibiotics, thus restricting its treatment repertoire. Due to the limited treatment options for
52 carbapenem-resistant *A. baumannii* (CR-AB) infections, antibiotic combinations have been
53 used empirically. Antibiotics such as fosfomycin and sulbactam, in combination with other
54 antibiotics, are now considered potential treatment options for CR-AB (2, 3). *In vitro* studies
55 have reported synergy rates between 50-75% for fosfomycin in combination with colistin,
56 imipenem or sulbactam, against multidrug-resistant (MDR) *A. baumannii* (4-6). Deveci *et al.*
57 reported *in vitro* synergy rates ranging between 30-80% for sulbactam in combination with
58 meropenem, gentamicin, colistin, or cefepime (7). Despite promising outcomes from *in vitro*
59 studies, the role of combination therapy in clinical practice, particularly of fosfomycin-
60 sulbactam combination, in the treatment CR-AB is still unclear.

61 Fosfomycin is a bactericidal broad-spectrum antibiotic that hinders bacterial cell wall
62 synthesis via inhibition of enolpyruvyl transferase, an essential enzyme in peptidoglycan
63 biosynthesis leading to bacterial cell lysis (8-10). On the other hand, sulbactam is a
64 semisynthetic β -lactamase inhibitor which, when combined with certain β -lactams, extends
65 their activity against bacteria that are usually resistant to the antibiotic due to the production
66 of β -lactamases (11). Interestingly, sulbactam can bind to penicillin-binding proteins (PBP)
67 of *Acinetobacter spp.* (12). It has a strong inclination to bind to PBP2 in *A. baumannii* (13).
68 *In vitro* and *in vivo* studies have demonstrated clinically significant activity of sulbactam
69 against *Acinetobacter spp.*, making it unique from other β -lactamase inhibitors such as
70 tazobactam and clavulanic acid that do not directly exhibit bacterial cell killing (14, 15).

71 Apart from the use of antibiotic combinations, optimization of dosing regimens of
72 existing antibiotics has become increasingly important to respond to the threat of MDR

73 bacterial infections. Optimal dosing strategies for antibiotics are essential to improve
74 outcomes against multidrug-resistant infections (16). In this study, we endeavoured to
75 explore the utility of fosfomycin and sulbactam in combination against CR-AB isolates and
76 describe the interaction between fosfomycin and sulbactam using a semi-mechanistic
77 pharmacokinetic/pharmacodynamic (PK/PD) model, based on time-kill experiments.

78

79 **Results**

80

81 **Minimum inhibitory concentrations (MIC), checkerboard and time-kill analyses**

82 All isolates were meropenem-resistant with a minimum inhibitory concentration
83 (MIC) ranging from 32 – 512 mg/L.

84 Figure **legend**

85

86 Figure 1: Time-kill curves of CR-AB isolate (A) #79 and (B) #80.

87

88 Figure 2: Observed versus individual and population fitted viable counts for fosfomycin-
89 sulbactam combination against four CR-AB strains.

90

91

Figure 1: Time-kill curves of CR-AB isolate (A) #79 and (B) #80.

Figure 2: Observed versus individual and population fitted viable counts for fosfomycin-sulbactam combination against four CR-AB strains.

98 Table 1 summarizes the MIC, MIC₅₀ and MIC₉₀ (the MIC required to inhibit 50% and
99 90% of the 50 isolates, respectively) of fosfomycin and sulbactam, in monotherapy and in
100 combination against each isolate, and the minimum (range) fractional inhibitory
101 concentration index (FICI) for each isolate. The ranges of MICs were 16 – 256 mg/L for
102 sulbactam alone and 128 – 2048 mg/L for fosfomycin alone. The range of MICs of
103 fosfomycin and sulbactam in combination were 8 – 256 mg/L and 0.5 – 64 mg/L,
104 respectively. The MICs of the antibiotics in combination were lower than the MICs of the
105 antibiotics in monotherapy for most isolates (49/50). The MIC₅₀ of fosfomycin and sulbactam
106 in combination were decreased by 4- and 8-fold and the MIC₉₀ were reduced by 8- and 4-
107 fold, respectively, compared to the MIC in the monotherapy setting. The fosfomycin-
108 sulbactam combination was synergistic against 74% (37/50), additive against 24% (12/50)
109 and indifferent against 2% (1/50) of the isolates. No antagonism was observed in the
110 checkerboard study.

111 The time-kill curves for both isolates are shown in Figure 1. The fosfomycin-
112 sulbactam combination displayed bactericidal activity against both isolates at the highest
113 clinically achievable concentrations (fosfomycin 128 mg/L and sulbactam 128 mg/L)
114 ($\Delta\log\text{CFU}_{0-24}$ -3.09 and -5.14 $\log_{10}$ CFU/mL for isolates #79 and #80, respectively).
115 Synergism was only observed against isolate #80 at concentration of 128 mg/L for both
116 fosfomycin and sulbactam ($\Delta\log\text{CFU}_{24}$ combination-monotherapy -2.95 $\log_{10}$ CFU/mL). At
117 concentrations equal to the fractional inhibitory concentration (FIC) (fosfomycin 64 - 128
118 mg/L and sulbactam 8 - 16 mg/L), there was a 2 to 3- $\log_{10}$ reduction in the bacterial density
119 by 6 to 8 hours, followed by regrowth, for both isolates #79 and #80. No bactericidal activity
120 was observed against both isolates in the presence of fosfomycin monotherapy. When
121 exposed to sulbactam monotherapy at a concentration of 128 mg/L, there was a 2.6- and 2.2-

122 $\log_{10}$ reduction in bacterial burden at 24 hours for isolates #79 and #80, respectively. No
 123 antagonism was observed in the time-kill study for both isolates.

124

125 **Semi-mechanistic PK/PD model**

126 Interaction between the fosfomycin and sulbactam and their effects on bacterial
 127 growth was best described using the general pharmacodynamic interaction (GPDI) model
 128 developed by Wicha *et al.* (17), with fosfomycin potentiating the effects of sulbactam, and
 129 two bacterial subpopulations, that are either (1) sensitive or (2) resistant to the combination
 130 therapy. The pharmacodynamic model is outlined by equations 1 and 2 that describe the
 131 bacterial growth and death, including the theoretical maximal bacterial density and drug-
 132 mediated killing of both bacterial subpopulations. The parameter estimates for each isolate
 133 are detailed in Table S1. The observed versus individual and population Bayesian posterior
 134 correlation coefficient (R^2) is shown in Figure 2.

135

136 Equation 1:

$$\begin{aligned} \frac{dCFU_s}{dt} = & Kg_s \times \left(1 - \frac{CFU_s + CFU_r}{Bmax}\right) \times CFU_s - \frac{Emax_F \times C_F^{H_F}}{EC50_{FS} + C_F^{H_F}} \times CFU_s \\ & - \frac{Emax_S \times C_S^{H_S}}{(EC50_{SS} \times \left(1 + \frac{INT_{FS} \times C_F^{HI_{FS}}}{EC50_{INT_{FS}} + C_F^{HI_{FS}}}\right))^{H_S} + C_S^{H_S}} \times CFU_s \end{aligned}$$

137

138 Equation 2:

$$\begin{aligned} \frac{dCFU_r}{dt} = & Kg_r \times \left(1 - \frac{CFU_s + CFU_r}{Bmax}\right) \times CFU_r - \frac{Emax_F \times C_F^{H_F}}{EC50_{Fr} + C_F^{H_F}} \times CFU_r \\ & - \frac{Emax_S \times C_S^{H_S}}{(EC50_{Sr} \times \left(1 + \frac{INT_{FS} \times C_F^{HI_{FS}}}{EC50_{INT_{FS}} + C_F^{HI_{FS}}}\right)^{H_S} + C_S^{H_S}} \times CFU_r \end{aligned}$$

139

140 CFU_s and CFU_r represent the bacterial burden for the sensitive and resistant bacterial
 141 subpopulations, respectively; Kg_s and Kg_r are the growth rate constant for the sensitive and
 142 resistant bacterial subpopulations, respectively; Bmax is the maximal bacterial burden;
 143 Emax_F and Emax_S represent the maximum rate of fosfomycin- and sulbactam-mediated
 144 bacterial killing, respectively (log₁₀ CFU/mL/h); C_F and C_S represent the concentration of
 145 fosfomycin and sulbactam, respectively; H_F and H_S are the power parameter (Hill coefficient)
 146 for fosfomycin and sulbactam effect on both subpopulations, respectively; EC50_{FS} and
 147 EC50_{Ss} represent the fosfomycin and sulbactam concentration for which effect is 50% on the
 148 sensitive subpopulation, respectively; EC50_{Fr} and EC50_{Sr} represent the fosfomycin and
 149 sulbactam concentration for which effect is 50% on the resistant subpopulation, respectively;
 150 INT_{FS} represents the maximum fractional change of the EC50_{FS} and EC50_{Fr} caused by
 151 sulbactam; HI_{FS} represents the power parameter (Hill coefficient) for sulbactam potentiation
 152 of fosfomycin effect; EC50_{INT_{FS}} represents the sulbactam concentration needed to achieve
 153 50% of INT_{FS}.

154 The effect of both fosfomycin and sulbactam was best described by a sigmoidal Emax
 155 model, with the same Emax of both drugs for both subpopulations but different EC50. The
 156 potentiation of the effect of sulbactam on both subpopulations by fosfomycin was best
 157 described by a modification of EC50_{Ss} and EC50_{Sr} by a sigmoidal Emax function of
 158 fosfomycin concentration. The interaction parameters were set to the same values for both
 159 subpopulations.

160

161 **Monte Carlo simulations**

162 The simulated time-concentration profiles of fosfomycin 4 g, 6 g and 8 g every 8
163 hours given as a 1 h-infusion and sulbactam 2 g, 3 g and 4 g every 8 hours given as a 4 h-
164 infusion are shown in Figure S1. The simulated bacterial killing of various fosfomycin-
165 sulbactam monotherapy and combination dosing regimens are shown in Figures S2 and S3.
166 The probability of target attainment of 2- $\log_{10}$ kill, 1- $\log_{10}$ kill and stasis at 24 h is
167 summarized in Table 2.

168 Against an initial inoculum of 10^7 CFU/mL, both fosfomycin and sulbactam
169 monotherapy resulted in no or limited bacterial killing at 24 h (50th percentile) (Figure S2A-
170 B). At the 50th percentile, there was a >3.5- $\log_{10}$ reduction in bacterial burden by 24 h in
171 combination regimens including sulbactam 4 g every 8 hours (Figure S3D-F), with the
172 combination of fosfomycin 8g every 8 hours and sulbactam 4 g every 8 hours displaying the
173 most extensive bacterial killing by 24 h (~5.5- $\log_{10}$ reduction in colony count) (Figure S3F).
174 For combination regimens comprising of sulbactam 3 g every 8 hours with 4 g, 6 g or 8 g
175 fosfomycin every 8 hours, bacterial killing ranged between ~1- to 3.5- $\log_{10}$ (Figure S3A-C).
176 Limited or no bacterial killing was observed for combinations regimens containing sulbactam
177 2 g every 8 hours (Figure S2C-E).

178

179 **Discussion**

180 To the best of our knowledge, this is the first study to describe the interaction between
181 fosfomycin and sulbactam in combination against CR-AB isolates using semi-mechanistic
182 pharmacodynamic modelling with Monte Carlo simulations. As mentioned above, we found
183 the GPDI model (17) to best describe the interaction between the two antibiotics. In general,
184 this model allows for measurement of the interaction between two drugs by measuring the

185 change in Emax or EC50, which provides a quantitative and statistically interpretable (point)
186 estimate of a pharmacodynamic interaction (17). The GPDI model is also able to identify the
187 perpetrator and victim of a pharmacodynamic interaction, whereby a perpetrator alters the
188 Emax or EC50 of the victim drug leading to either synergism or antagonism (17).

189 Our pharmacodynamic model indicated that fosfomycin (perpetrator) enhanced the
190 bacterial killing of sulbactam despite the lack of susceptibility to fosfomycin in these isolates.
191 This observation is illustrated in Figure S3, whereby, the increment of sulbactam from 3 g to
192 4 g improved bacterial killing by 2- $\log_{10}$. This observation also suggests that when sulbactam
193 is used at a sufficiently high dose, the fosfomycin dose could be reduced to achieve a similar
194 decrease in the colony count. As shown in Figure S3C and S3D, the combination regimen of
195 sulbactam 4 g every 8 hours with fosfomycin 4 g every 8 hours was able to achieve bacterial
196 kill of $\sim 3.5\text{-}\log_{10}$, similar to that of the regimen containing sulbactam 3 g every 8 hours with
197 fosfomycin 8 g every 8 hours.

198 The potentiation of one antibiotic by another is not uncommon. Fosfomycin has been
199 previously described to enhance the uptake of tobramycin in *Pseudomonas aeruginosa*,
200 leading to greater repression of bacterial protein synthesis (18). Unfortunately, the
201 mechanism underlying the enhancement of the *in vitro* activity of sulbactam by fosfomycin
202 has not been previously described. Furthermore, we still do not fully understand the
203 mechanisms by which sulbactam acts against *A. baumannii* (19). At this juncture, we know
204 that both sulbactam and fosfomycin act by disrupting the biosynthesis of bacterial cell wall
205 via two distinct pathways (10, 19). Fosfomycin acts by targeting mucopeptide synthesis,
206 thereby inhibiting phosphoenolpyruvate transferase, which is the first enzyme involved in
207 peptidoglycan synthesis (10). It therefore acts on the first stage of peptidoglycan synthesis,
208 inhibiting bacterial cell wall production at an earlier stage than most antibiotic classes (10),
209 which could potentially lead to an increased uptake of sulbactam by fosfomycin.

210 In addition, our simulation illustrated that clinically achievable plasma fosfomycin
211 and fosfomycin concentrations may be able to attain synergistic killing (≥ 2 -log₁₀ kill) when
212 used in combination in ~60 to 70% of the simulated patients, compared to ~15 to 30% with
213 fosfomycin or sulbactam monotherapies at the highest doses (Table 2), against CR-AB
214 isolates. Our simulations suggest that a combination of fosfomycin 4 g every 8 hours (1 h-
215 infusion) with sulbactam 4 g every 8 hours (4 h-infusion), as a minimum, may provide a
216 favourable effect in lowering the bacterial load to a level where the immune system in
217 immunocompetent patients can eradicate remaining bacteria, as exhibited in a murine
218 pneumonia model by Louie *et al.*(20).

219 Through the use of mathematical modelling and Monte Carlo simulations, we were
220 able to appraise a range of fosfomycin-sulbactam dosing regimens, also incorporating
221 between-patient pharmacokinetic variability that is often seen in critically ill patients (21).
222 With the absence of an immune response *in vitro*, our pharmacodynamic model and
223 simulations may represent an immunocompromised patient population. Moreover, to emulate
224 a worst-case scenario, we simulated a high inoculum of 10⁷ CFU/mL of an extremely
225 difficult-to-treat isolate (fosfomycin MIC 2048 mg/L, sulbactam MIC 128 mg/L and
226 meropenem MIC 128 mg/L).

227 Nevertheless, this study has some limitations. To avoid over- or underestimation of
228 the drugs' plasma concentration and for ease of comparison, we fixed the simulated patient
229 weight to 70 kg and creatinine clearance to 100 mL/min. Hence, weight and renal clearance
230 should be taken into account when extrapolating the results of the simulation, as they were
231 essential covariates in the pharmacokinetic model (22, 23). Nonetheless, these parameters can
232 be easily modified in the simulation, allowing for the possibility of various weight and renal
233 function combination, depending on the patient of interest in the clinical setting. Furthermore,

234 the pharmacodynamic model utilized data from static time-kill studies, which does not mirror
235 the changing drug concentrations *in vivo*.

236 Also, in this model, a fixed, non-concentration dependent interaction term (INT_{FS})
237 was used, whereby the mean INT_{FS} estimate was derived from the best-case scenario, and
238 applied for subsequent simulations. We acknowledge that the time-kill data used for the
239 pharmacodynamic simulation can restrict the application unless it is inclusive of the best- and
240 worst-case scenarios. We have used time-kill data from four isolates with varying response to
241 the combination in the model building and used the time-kill data of isolate #79 to derive the
242 final model parameter estimates used for the simulation, as this isolate had the highest
243 fosfomycin, sulbactam and meropenem MICs (therefore, the most resistant isolate) and
244 synergism was not observed when exposed to the combination therapy, to mirror a worst-
245 case-scenario. Further evaluation of this promising combination in across various CR-AB
246 isolates, and testing using dynamic infection models should be pursued to provide a clearer
247 picture of the potential utility of this antibiotic combination in the treatment of CR-AB.

248 Screening of synergistic activity for the fosfomycin-sulbactam combination was done
249 using broth microdilution, instead of agar dilution method. Nonetheless, the broth
250 microdilution and agar dilution method have been shown to correlate reasonably well (24,
251 25). One study demonstrated that MIC values were within one 2-fold dilution when tested in
252 broth microdilution compared to those tested in agar dilution for 86 of 106 isolates (81.1%)
253 (24). Additionally, there are no studies which investigated the PK/PD target attainment of
254 fosfomycin and sulbactam in combination. The pharmacodynamic targets (stasis, 1- $\log_{10}$ and
255 2- $\log_{10}$ reduction) used in this study were based on the European Medicines Agency's
256 guideline on the use of pharmacokinetics and pharmacodynamics in the development of
257 antimicrobial medicinal products (26).

258 As mentioned above, simulation of a different patient weight and renal function
259 combination, according to the patient of interest, is possible, by adjusting these parameters
260 during the simulation. Hence, we propose that the model may have potential use in the
261 clinical setting, particularly when dealing with critically-ill patients with multidrug-resistant
262 *A. baumannii* infections, as these patients are at heightened risk of suboptimal drug dosing
263 due to altered pharmacokinetics (27). The use of the PK/PD model and simulation in the
264 clinical setting, would allow clinicians to predict the probability of attaining a particular
265 pharmacodynamic target (stasis, 1-log₁₀ and 2-log₁₀ reduction) for a patient of interest, based
266 on the individual's weight and renal function. However, as this combination is only
267 synergistic in ~70% of isolates, as observed in our checkerboard study, we are unable to
268 extrapolate the use of this model to all patients with CR-AB infection, particularly those with
269 CR-AB isolates against which the fosfomycin and sulbactam combination lacks synergism.
270 Therefore, we suggest the application of this PK/PD model in combination with *in vitro*
271 antibiotic combination testing e.g. the checkerboard assay, to screen for synergism of the
272 fosfomycin and sulbactam combination. The model would provide better quantification of the
273 effect of the combination compared to the checkerboard testing alone. *In vitro* antibiotic
274 combination testing in the clinical setting has been previously reported and appears to hold a
275 promising role in improving patient outcome and judicious antibiotics use (28).

276

277 Conclusion

278 Favourable results for fosfomycin and sulbactam combination were observed through
279 synergism analysis and PK/PD evaluation. Our study demonstrated that the fosfomycin and
280 sulbactam combination could be a plausible option to treat CR-AB infections for a number of
281 clinically relevant isolates. Further dynamic infection models, *in vivo* preclinical or clinical
282 studies are needed to validate these *in vitro* and *in silico* results.

283

284 **Materials and methods**

285

286 **Bacterial isolates, antimicrobial agents, susceptibility testing, checkerboard assay and**
287 **static time-kill assay**

288 The methodology of the pharmacodynamic component of this study has been
289 published elsewhere (29). Briefly, the CR-AB isolates were obtained from The University of
290 Queensland Centre of Clinical Research (UQCCR). Fifty isolates were chosen from samples
291 previously analysed by Zowawi *et al.* (30). The study by Zowawi *et al.* suggested that the 107
292 isolates were from diverse clonal lineages. When choosing 50 out of the 117 isolates, they
293 were chosen randomly to avoid any systematic selection bias and to ensure the high genetic
294 diversity of the baseline population of isolates be represented in our study isolates.

295 Carbapenem resistance was determined in their study by the disk diffusion
296 susceptibility testing for imipenem (10 µg) and meropenem (10 µg) following the European
297 Committee on Antimicrobial Susceptibility Testing (EUCAST) methodology and in reference
298 to the updated breakpoint defined by EUCAST (31). To confirm carbapenem resistance, the
299 MIC of meropenem against the isolates were retested in this study using the broth
300 microdilution method (32).

301 Sulbactam (Toronto Research Chemicals) and fosfomycin (Sigma-Aldrich) were
302 obtained from their respective manufacturers. Stock solutions of sulbactam and fosfomycin
303 were prepared in sterile Milli-Q water, filter-sterilised with 0.22-µm polyvinylidene
304 difluoride (PVDF) syringe filter, aliquoted and stored at -80°C until required. Broth or agar
305 containing fosfomycin was supplemented with 25 mg/L of glucose-6-phosphate (Sigma-
306 Aldrich).

307 The MIC of sulbactam against the 50 *A. baumannii* isolates were determined by the
308 broth microdilution method, in quadruplicate, following the recommendations of the Clinical
309 and Laboratory Standards Institute (CLSI) as described in the CLSI M100 approved standard
310 (32). The mode MIC was reported for each isolate. Fosfomycin susceptibility testing was
311 performed by agar dilution (32). *Klebsiella pneumoniae* (ATCC 700603) and *Pseudomonas*
312 *aeruginosa* (ATCC 27853) strains were used as quality control strains for sulbactam and
313 fosfomycin, respectively. The MIC₅₀ and MIC₉₀ of monotherapy and combinations of the two
314 antimicrobial agents were the MIC required to inhibit 50% and 90% of the 50 isolates,
315 respectively.

316 The synergistic activities of fosfomycin and sulbactam in combination against the 50
317 CR-AB isolates were then screened using the checkerboard assay which was done twice for
318 each isolate. The range of concentrations of the antibiotics used was as follows: fosfomycin,
319 8 – 512 mg/L and sulbactam, 0.5 – 64 mg/L, with the highest concentration being those that
320 are clinically achievable in critically ill patients, based on previously published data (22, 23).
321 The FICI was calculated by the summation of $\text{MIC}_{\text{drug A in combination}}/\text{MIC}_{\text{drug A alone}}$ and MIC_{drug}
322 $\text{B in combination}/\text{MIC}_{\text{drug B alone}}$ (33). The interpretation of FICI was as follows: ≤ 0.5 = synergistic,
323 $0.5 < \text{FICI} \leq 1$ = additive, $1 < \text{FICI} \leq 4$ = indifferent and > 4 = antagonistic (34). The minimum
324 FICI was reported for each isolate. The mode MICs were reported.

325 Subsequently, static time-kill studies were conducted against two *A. baumannii*
326 isolates (#79 and #80,) which demonstrated synergistic activity in the checkerboard assay
327 (fosfomycin MIC 256-2048 mg/L and sulbactam MIC 128-256 mg/L). The concentrations of
328 the antibiotics equal to the FIC exhibiting synergy by the checkerboard and the highest
329 clinically achievable antibiotics concentrations, alone and in combination, were tested against
330 an inoculum of approximately 10^6 CFU/mL. A decrease of $\geq 3 \log_{10}$ at 24 h when compared
331 to the number of viable cells at the initial time point ($\Delta \log \text{CFU}_{0-24}$) was indicative of a

bactericidal effect (33). A synergistic effect was determined by a decrease of $\geq 2 \log_{10}$ in CFU/ml at 24 h when comparing the antibiotics in combination to the most active drug alone at that time point ($\Delta \log \text{CFU}_{24 \text{ combination-monotherapy}}$), while an increase of $> 2 \log_{10}$ was considered as antagonism (33). Indifference was interpreted as any other outcome that did not meet the criteria for either synergy or antagonism (35).

337

338 **Semi-mechanistic PK/PD modelling**

Semi-mechanistic PK/PD modelling was performed to quantify the exposure–effect relationships of both drugs provided alone or in combination, based on the static time-kill data. As mentioned above, we have performed static time-kill studies on two isolates (unpublished) and further enriched the simulation with data from two additional isolates from a previous publication (29), making it a total of four isolates. Parameter estimation was performed using the Pmetrics package (v1.52, Laboratory of Applied Pharmacokinetics and Bioinformatics, Los Angeles, California) (36) for R (v.3.6.2, R Core Team, Vienna, Austria). Model diagnostics including the Akaike-information-criteria, log-likelihood, coefficient of determination (R^2) from the observed vs. expected plots, plausibility of the parameter estimates and visual-predictive-checks were used to evaluate and compare models. Various structural models were tested to find the best-fitting model.

350

351 **Simulation of bacterial kill at clinically achievable concentrations**

To better understand fosfomycin and sulbactam pharmacodynamic interactions in a dynamic drug concentration as in the clinical setting, Monte Carlo simulations of various fosfomycin and sulbactam clinical dosing regimens were performed for 1,000 virtual patients. The final pharmacodynamic model was combined with previously reported human population pharmacokinetic models for fosfomycin (22) and sulbactam (23) in critically ill

356

357 patients. These Monte Carlo simulations accounted for between-patient variability in the
358 pharmacokinetics of each antibiotic. The mean parameter estimates and the coefficient of
359 variation of the parameters were also included in the simulation to account for between-
360 patient variability of the parameters estimates.

361 The simulated patients were assumed to have bacteremia caused by a difficult-to-treat
362 CR-AB isolate #79 against which the combination therapy did not exhibit synergism
363 (fosfomycin MIC of 2048 mg/L, sulbactam MIC of 128 mg/L and meropenem MIC of 128
364 mg/L) and lacked any aspect of the immune system, to paint a worst-case scenario. The
365 weight of the simulated patient was fixed at 70 kg to represent the weight of a typical adult
366 (37, 38), and the creatinine clearance (CL_{CR}) was fixed at 100 ml/min/1.73 m² to represent
367 patient with normal renal clearance. The simulated regimens included fosfomycin given as a
368 1 h-infusion of 4 g, 6 g or 8 g every 8 hours and sulbactam given as a 4 h-infusion of 2 g, 3 g
369 or 4 g every 8 hours. The probability of target attainment (PTA) of stasis, 1-log₁₀ kill and 2-
370 log₁₀ kill at 24 h for the simulated exposures of the 1,000 virtual patients were calculated.

371

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381 **Conflict of interest**

382 None to declare.

383

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385

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- 517

518 **Figure legend**

519

520 Figure 1: Time-kill curves of CR-AB isolate (A) #79 and (B) #80.

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522 Figure 2: Observed versus individual and population fitted viable counts for fosfomycin-
523 sulbactam combination against four CR-AB strains.

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Figure 1: Time-kill curves of CR-AB isolate (A) #79 and (B) #80.

530
531 Figure 2: Observed versus individual and population fitted viable counts for fosfomycin-sulbactam combination against four CR-AB strains.

532 Table 1: The MIC and the FICI of fosfomycin-sulbactam combination and monotherapy
533 against 50 carbapenem-resistant *A. baumannii*.
534

Strain #	MIC mg/L				Minimal FICI (range)	Interpretation
	MIC fosfomycin	MIC sulbactam	MIC FOS _{combination}	MIC SUL _{combination}		
73	512	64	128	8	0.38 (0.38-1.01)	Synergistic
74	128	64	32	16	0.50 (0.5-4.01)	Synergistic
75	128	64	≤8	32	0.56 (0.75-4.01)	Additive
76	128	64	32	16	0.50 (0.50-4.03)	Synergistic
77	2048	128	64	16	0.16 (0.16-0.50)	Synergistic
78	128	128	32	16	0.38 (0.38-4.01)	Synergistic
79	2048	128	128	8	0.13 (0.13-0.31)	Synergistic
80	256	256	64	16	0.31 (0.31-2.01)	Synergistic
81	256	64	32	32	0.63 (0.63-2.12)	Additive
82	512	32	128	8	0.50 (0.50-1.01)	Synergistic
83	256	128	128	8	0.56 (0.56-2.00)	Additive
84	1024	128	256	≤0.5	0.25 (0.25-0.51)	Synergistic
85	256	128	64	32	0.50 (0.50-2.00)	Synergistic
86	1024	128	128	16	0.25 (0.25-0.56)	Synergistic
87	256	256	128	32	0.63 (0.63-2.00)	Additive
88	256	128	64	32	0.50 (0.50-2.01)	Synergistic
89	128	128	≤8	64	0.56 (0.56-4.00)	Additive
90	256	128	64	32	0.50 (0.50-2.00)	Synergistic
91	128	128	≤8	64	0.56 (0.56-4.01)	Additive
92	512	128	≤8	32	0.27 (0.27-1.01)	Synergistic
93	128	128	≤8	32	0.31 (0.31-4.01)	Synergistic
94	256	128	32	32	0.38 (0.38-2.01)	Synergistic
95	512	16	128	4	0.50 (0.50-1.03)	Synergistic
96	1024	64	64	16	0.31 (0.31-1.01)	Synergistic
97	512	256	64	64	0.38 (0.38-1.25)	Synergistic
98	256	64	64	16	0.50 (0.50-2.01)	Synergistic
99	256	256	≤8	64	0.28 (0.28-2.13)	Synergistic
100	256	32	64	8	0.50 (0.50-2.02)	Synergistic
101	128	256	64	32	0.63 (0.63-4.00)	Additive
102	128	64	32	16	0.50 (0.50-4.06)	Synergistic
103	512	64	64	16	0.38 (0.38-1.25)	Synergistic
104	128	64	32	8	0.38 (0.38-4.02)	Synergistic
105	128	128	≤8	64	0.56 (0.56-4.00)	Additive
106	256	128	≤8	64	0.53 (0.53-2.25)	Additive
107	128	128	≤8	32	0.31 (0.31-4.01)	Synergistic
108	256	32	32	16	0.63 (0.63-2.25)	Additive
109	128	128	32	16	0.38 (0.38-4.02)	Synergistic
110	512	256	64	32	0.25 (0.25-1.06)	Synergistic

111	256	128	32	32	0.38 (0.38-2.02)	Synergistic
112	128	256	128	16	1.06 (1.06-4.00)	Indifferent
113	128	32	32	16	0.75 (0.75-4.02)	Additive
114	256	256	64	32	0.38 (0.38-2.00)	Synergistic
115	128	128	≤8	32	0.31 (0.31-4.03)	Synergistic
116	256	128	32	16	0.25 (0.25-2.02)	Synergistic
117	1024	256	64	32	0.19 (0.19-0.56)	Synergistic
118	128	256	32	8	0.28 (0.28-4.02)	Synergistic
119	512	128	32	16	0.19 (0.19-1.06)	Synergistic
120	1024	256	64	16	0.13 (0.13-0.56)	Synergistic
121	512	16	128	8	0.75 (0.75-2.02)	Additive
122	512	128	128	32	0.50 (0.50-1.12)	Synergistic
MIC ₅₀	256	128	64	16		
MIC ₉₀	1024	256	128	64		
MIC ₅₀ fold reduction			4	8		
MIC ₉₀ fold reduction			8	4		

MIC, minimum inhibitory concentration; FOS, fosfomycin; SUL, sulbactam; MIC FOS_{combination}, MIC of fosfomycin in combination with sulbactam; MIC SUL_{combination}, MIC of sulbactam in combination with fosfomycin.

538 Table 2: Probability of target attainment of 2-log₁₀ kill, 1-log₁₀ kill and stasis of various
539 dosing regimens at 24 h against carbapenem-resistant *A. baumannii* isolate #79 at an initial
540 inoculum of 10⁷ CFU/mL.
541

Fosfomycin (1 h-infusion) (g)	Sulbactam (4 h-infusion) (g)	Probability of target attainment (%)		
		2-log ₁₀ kill	1-log ₁₀ kill	Stasis
8	4	71.6	76.4	81.6
6	4	68.5	73.6	78.5
4	4	61.4	69.1	74.8
8	3	59.5	66.7	71.4
6	3	53.2	61	68.4
4	3	46.5	54.5	61.2
8	2	45.5	50.1	56.7
6	2	39.8	45.7	51.3
4	2	31	38.3	44.1
8	-	15.5	19.8	23.3
-	4	32.5	46.5	53.5

542