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Repurposing the anti-viral drug zidovudine (AZT) in combination with meropenem as an effective treatment for infections with multi-drug resistant, carbapenemase-producing strains of *Klebsiella pneumoniae.*

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Abstract

Multi-drug resistant (MDR) *Klebsiella pneumoniae* represent a global threat to healthcare due to lack of effective treatments and high mortality rates. The aim of this research was to explore the potential of administering zidovudine (AZT) in combination with an existing antibiotic to treat resistant *K. pneumoniae* infections. Two MDR *K. pneumoniae* strains were employed, producing either the NDM-1 or KPC-3 carbapenemase. Efficacy of combinations of AZT with meropenem were compared with monotherapies against infections in *Galleria mellonella* larvae by measuring larval mortality and bacterial burden. The effect of the same combinations *in vitro* was determined via checkerboard and time-kill assays. *In vitro*, both *K. pneumoniae* strains were resistant to meropenem but were susceptible to AZT. In *G. mellonella*, treatment with either AZT or meropenem alone offered minimal therapeutic benefit against infections with either strain. In contrast, combination therapy of AZT with meropenem presented significantly enhanced efficacy compared to monotherapies. This was correlated with prevention of bacterial proliferation within the larvae but not elimination. Checkerboard assays showed that the interaction between AZT and meropenem was not synergistic but indifferent. In summary, combination therapy of AZT with meropenem represents a potential treatment for carbapenemase-producing MDR *K. pneumoniae* and merits further investigation.

Introduction

Klebsiella pneumoniae belongs to the family *Enterobacteriaceae* and is part of the normal intestinal flora of healthy humans. However, *K. pneumoniae* is also an important opportunistic pathogen and is a major cause of community and healthcare associated infections (HAIs) such as bloodstream, urinary tract infections and pneumonias (Navon-Venezia 2017). Infection is often associated with invasive medical devices or surgery in patients that are immunocompromised [reviewed in (Peleg and Hooper 2010)]. Compounding the problem, *K. pneumoniae* is adept at acquiring genes encoding a range of antibiotic resistance mechanisms via either mutations or mobile genetic elements [Navon-Venezia 2017]. Acquisition of resistance to third generation cephalosporins via extended-spectrum beta-lactamases (ESBLs) led to increased use of the antibiotics of 'last-resort' – the carbapenem class of broad-spectrum beta-lactams – resulting in the inevitable selection of resistance to these also. With increasing incidence of resistance to carbapenems, the prevalence of multi-drug, or extremely- drug resistant (MDR and XDR respectively), *K. pneumoniae* strains that are resistant to nearly all available antibiotics is rising globally (Sanchez 2013). Consequently, there are only a limited number of antibiotic treatments available (Morrill 2015) resulting in higher mortality rates (Munoz-Price 2013; Tamma 2017) and unsurprisingly, MDR or XDR *K. pneumoniae* are considered an urgent public health threat and a major challenge to the delivery of safe healthcare (World Health Organisation 2017).

The dearth of novel antibiotic treatments in the pipeline for Gram-negative bacteria means that the development of alternative treatments based on innovative use of existing drugs is an option. Clinically, the administration of antibiotic combinations to patients suffering from MDR or XDR *K. pneumoniae* infection is often employed as a solution to improve therapeutic outcomes. However, agreement on the best combinations to use, and evidence of any real benefit, is contested (Tamma 2012). Examples of combination therapies employed with some success include, dual carbapenem therapy (Souli 2017), ceftazidime with avibactam (Lagace-Wiens 2014), meropenem with vaborbactam (Lee and Baker 2018), and the polymyxin colistin combined with other antibiotics (Gutierrez-Gutierrez 2017). Unfortunately, none of these options are ideal particularly because of

76 the nephrotoxicity and rise of resistance associated with colistin (Capone 2013), and the inability of
77 either avibactam or vaborbactam to inhibit metallo-beta-lactamases such as NDM-1. Thus, there is
78 an urgent clinical need to identify new treatment approaches for MDR or XDR *K. pneumoniae*
79 strains that have acquired carbapenemases.

80 To improve options available for clinicians, the 'repurposing' of already approved drugs, whose
81 primary use is not as antibacterials, as antibiotics could speed up the introduction of new treatment
82 options, particularly if these compounds are administered in combinations with existing antibiotics
83 (Cheng 2019; Ejim 2011). One example of a drug that could be 'repurposed' as an antibiotic is the
84 anti-viral drug zidovudine (AZT). AZT is a nucleoside analogue with known bactericidal activity
85 against Gram-negative bacteria, including *K. pneumoniae* (Elwell 1987; Lewin and Aymes 1989;
86 Peyclit 2018). In addition, the drug was efficacious in a mouse model of systemic *Escherichia coli*
87 infection (Keith 1989). Upon entering Gram-negative bacterial cells, AZT is phosphorylated by
88 thymidine kinases, incorporated into DNA, and arrests replication by acting as a DNA chain
89 terminator (Doleans-Jordheim 2011). A number of recent studies that screened libraries of approved
90 drugs identified synergistic combinations against Gram-negative bacteria that included AZT (Hind
91 2019; Ng 2018; Wambaugh 2017). Synergistic combinations of AZT that inhibited MDR, or XDR, *K.*
92 *pneumoniae in vitro* included combinations with the lipopeptide antibiotics colistin (Hu 2019) or
93 polymyxin B (Lin 2019) that also showed enhanced efficacy compared to monotherapy in murine
94 infection models. Despite these studies with the lipopeptide antibiotics, most studies of the effect of
95 AZT in combination with existing antibiotics have been performed *in vitro*.
96 The aim of this research was to explore further the potential of administering AZT in combinations
97 with existing antibiotics to treat infections with MDR pathogens using the *Galleria mellonella* larvae
98 infection model. This system permits screening of antibacterial activity *in vivo* against real infections,
99 in the presence of a functioning immune system, without the high costs and ethical issues
100 associated with mammalian infection models. Thus, combination treatments consisting of AZT with
101 the carbapenem beta-lactam, meropenem, were screened for enhanced efficacy compared to

102 monotherapies in *Galleria mellonella* larvae infected with carbapenemase-producing strains of *K.*
103 *pneumoniae*.

104 **Materials and Methods**

105 Bacteria and growth media

106 *K. pneumoniae* strains were obtained from the National Collection of Type Cultures (NCTC;
107 https://www.phe-culturecollections.org.uk/collections/nctc.aspx): NCTC 9633T, an antibiotic
108 susceptible Type strain, NCTC 13443, producing the NDM-1 metallo-beta-lactamase, and NCTC
109 13438, producing the KPC-3 carbapenemase (Woodford 2008). NCTC 9633T was included as a
110 control to illustrate the resistance of the two carbapenemase strains to meropenem. All strains were
111 grown to stationary phase in Mueller–Hinton broth (MHB; Merck, Darmstadt, Germany) at 37°C with
112 shaking (at 200 rpm) overnight to prepare inocula for antibiotic efficacy testing *in vitro* or *in vivo*.

113 Drugs and *G. mellonella* larvae

114 Meropenem and zidovudine were purchased from Sigma–Aldrich Ltd (Dorset, UK). Stock solutions
115 of meropenem or AZT were prepared in sterile deionized water with 10% dimethylsulphoxide. Sub-
116 stocks of each drug for injection into larvae were prepared in deionized water. *G. mellonella* larvae
117 were obtained from UK Waxworms Ltd (Sheffield, UK).

118 Antibiotic susceptibility testing *in vitro*

119 Minimum inhibitory concentrations (MICs) of antibiotics against the *K. pneumoniae* strains were
120 determined in 96-well microplates as previously described (Hill 2014). Briefly, doubling dilutions of
121 meropenem or zidovudine were prepared in MHB and subsequently inoculated with 1.0 x 10^6
122 cfu/mL of either *K. pneumoniae* strain. Microplates were incubated at 37°C and the MIC was defined
123 as the concentration(s) present in the first optically clear well after 24 h.

124 Testing combinations for synergy *in vitro*

125 The effect of combinations of meropenem with AZT against both *K. pneumoniae* strains was carried
126 out using 96-well microplate assays prepared via doubling dilution of meropenem in MHB followed

127 by subsequent addition of AZT to form a combination checkerboard. Each well was then inoculated
128 with 1.0 x 10^6 cfu/mL of either *K. pneumoniae* strain and microplates were incubated at 37ºC. After
129 24 h, each well was scored for visible growth and fractional inhibitory concentration index (FICI)
130 values were calculated for each combination tested. Synergy was defined as FICI ≤0.5 (Eliopoulos
131 1996). Each *K. pneumoniae* strain was tested in duplicate.

132 The effect of meropenem and AZT combinations against both *K. pneumoniae* strains was also
133 measured using a time-kill assay. Briefly, tubes containing concentrations of AZT, meropenem or a
134 combination of both drugs were prepared in MHB broth. A control tube contained only sterile water
135 in MHB. Drug concentrations were prepared at MIC_{50} for each strain. Tubes were then inoculated
136 with 1.0 x 10^7 cfu/mL of either *K. pneumoniae* strain. Viability was determined after 0, 2, 4 and 6 h
137 incubation at 37°C by serial dilution in MHB and plating on Nutrient Agar (Merck, Darmstadt,
138 Germany). Each experiment was performed in duplicate and results expressed as mean ± standard
139 error of the mean (SEM).

140 *G. mellonella* infection model

141 Efficacy of meropenem or AZT alone or in combination versus *G. mellonella* larvae infected with the
142 *K. pneumoniae* strains was carried out exactly as described previously (Hill 2014; Krezdorn 2014;
143 Adamson 2015). *G. mellonella* at their final instar larval stage were kept at room temperature in
144 darkness. Larvae weighing within the range of 250 to 350 mg were selected for each experiment to
145 ensure consistency in subsequent drug administration and were used within 1 week of receipt.
146 Groups of 15 larvae were infected with inocula (10 µL) of either *K. pneumoniae* strain containing
147 increasing numbers of bacteria to determine an appropriate infectious dose for subsequent drug
148 efficacy studies. Control experiments using a heat-killed inoculum of each strain was carried out in
149 which the bacterial suspension was heated at 98ºC for 10 min prior to infection of a group of larvae.
150 For all studies of drug efficacy, an inoculum of 5.6 x 10^5 cfu, or 9.1 x 10^7 cfu, was used for *K.*
151 *pneumoniae* NCTC 13443 (NDM-1) and NCTC 13438 (KPC-3), respectively. A single treatment with
152 phosphate-buffered saline (PBS), meropenem, AZT or a combination of both drugs was
153 administered 2 h post-infection. The experiments were repeated in duplicate using larvae from a

154 different batch and the data from these replicate experiments were pooled to give *n*=30. Survival
155 data were plotted using the Kaplan–Meier method (Bland and Altman 1998) and comparisons made
156 between groups using the log-rank test (Bland 2004). In all comparisons with the negative control it
157 was the uninfected control (rather than the unmanipulated control) that was used. Holm's correction
158 was applied to account for multiple comparisons in all tests and $p \leq 0.05$ was considered significant
159 (Holm 1979).

160 Bacterial burden within larvae from each treatment group was measured exactly as described
161 previously (Krezdorn 2014; Adamson 2015; Ballard and Coote 2016). Groups of 30 larvae were
162 infected with either strain of *K. pneumoniae* using the same inoculum sizes as described above.
163 Meropenem, AZT or a combination treatment of both drugs were administered at 2 h post-infection.
164 Larvae were incubated in Petri dishes at 37°C. At 24 h intervals, five larvae were randomly selected
165 from each treatment group and surface decontaminated and anaesthetised by washing in absolute
166 ethanol. Each larva was then placed in an Eppendorf tube containing 1 mL of sterile PBS and
167 homogenised using a sterile pestle. Bacterial burden from individual caterpillars was then
168 determined by serial dilution of the homogenate in MHB and plating on MacConkey agar
169 (Formedium Ltd, Hunstanton, England). The detection limit for this assay was 100 cfu/mL of larval
170 homogenate.

171

172 **Results**

173 **Two carbapenemase-producing strains of *K. pneumoniae* are resistant to meropenem but**
174 **sensitive to the antiviral drug AZT**. In comparison to the antibiotic-susceptible Type strain, the two
175 strains producing the carbapenemases displayed resistance to meropenem as expected (Table 1).
176 The MICs of meropenem were the same for both carbapenemase-producing strains but *K.*
177 *pneumoniae* NCTC 13438, carrying the KPC-3 carbapenemase, was more resistant to AZT (4 mg/L)
178 than NCTC 13443 with NDM-1 (1 – 2 mg/L). These results confirmed previous studies with different

179 strains of *K. pneumoniae* that showed sensitivity to AZT (Elwell 1987; Lewin and Aymes 1989;
180 Peyclit 2018).

181 ***G. mellonella* larvae display dose-dependent lethality in response to infection by either strain**
182 **of MDR *K. pneumoniae***. The effect of infection with either *K. pneumoniae* strains on survival of *G.*
183 *mellonella* is shown in Figure 1. In both strains, the heat-killed inoculum had no significant effect on
184 larval survival (p>0.05). With live inocula, larval survival was affected in a dose-dependent manner
185 during 96 h incubation (Figure 1). Together, these data indicate that infection with live *K.*
186 *pneumoniae* is required to cause larval death and support previous studies that have utilised *G.*
187 *mellonella* to study infection by different *K. pneumoniae* strains (Insua 2013; Wand 2013;
188 McLaughlin 2014; Benthall 2015).

189 *K. pneumoniae* NCTC 13443 (NDM-1) displayed greater virulence than NCTC 13438 (KPC-3),
190 requiring a smaller inoculum of viable bacteria to induce a similar degree of lethality to infected
191 larvae. Infectious doses for each strain were selected for use in subsequent studies on antibiotic
192 efficacy (5.6 x 10^5 cfu for *K. pneumoniae* NCTC 13443, and 9.1 x 10^7 cfu for NCTC 13438) that
193 resulted in the death of approximately 90% of larvae after 96 h incubation at 37ºC.

194 **Efficacy of AZT or meropenem monotherapy in *G. mellonella* larvae infected with either**
195 **carbapenemase-producing strain of *K. pneumoniae* is poor**. The effect of single doses of AZT
196 or meropenem, 2 h post-infection (p.i) with either *K. pneumoniae* strain, on survival of *G. mellonella*
197 larvae is shown in Figure 2. Both drugs increased larval survival in a dose-dependent manner
198 regardless of the *K. pneumoniae* strain. However, neither drug induced high levels of therapeutic
199 benefit, with the exception of AZT versus larvae infected with *K. pneumoniae* NCTC 13443 (NDM-1)
200 treated with the highest doses of 6.25 or 12.5 mg/kg. In contrast, treatment of larvae infected with
201 the KPC-3-producing strain with the same doses of AZT had no therapeutic benefit after 120 h. As
202 would be expected with these strains, treatment with meropenem induced only low levels of
203 therapeutic benefit even at the highest doses tested, particularly against infections with *K.*
204 *pneumoniae* NCTC 13438 (KPC-3).

205 In summary, neither AZT or meropenem monotherapy offered high levels of therapeutic benefit
206 versus infections with either strain of carbapenemase-producing *K. pneumoniae*, although both
207 drugs were moderately more effective against the NMD-1-producing strain than the KPC-3 strain.

208 **Combination therapy with AZT plus meropenem shows enhanced efficacy compared to**
209 **monotherapy versus *G. mellonella* infections with carbapenemase-producing MDR strains of**
210 ***K. pneumoniae***. The effect of monotherapy, with AZT or meropenem, compared with combination
211 therapy on survival of *G. mellonella* larvae, and burden of infecting bacteria, for both
212 carbapenemase-producing strains of *K. pneumoniae* is shown in Figure 3. Pilot experiments were
213 carried out testing many different doses of AZT or meropenem in combination to identify the most
214 effective doses to employ against larval infections with either strain of *K. pneumoniae* (data not
215 shown). From these initial studies, optimal combination dosing regimens were selected that offered
216 the best therapeutic benefit and subsequently studied in detail – NCTC 13443 (AZT - 0.78 mk/kg +
217 meropenem - 6.25 mg/kg) and NCTC 13438 (AZT - 6.25 mg/kg + meropenem - 12.5 mg/kg) (Figure
218 3).

219 As shown previously (Figure 2), a single dose 2 h p.i of either AZT or meropenem had minimal
220 therapeutic benefit on larvae infected with either strain of *K. pneumoniae* (Figure 3). This was
221 reflected in large increases over 24 h in the internal burden of either infecting *K. pneumoniae* strain
222 within individual larvae. For larvae infected with NCTC 13443 (NDM-1), the increase in bacterial
223 numbers after treatment with either meropenem or AZT was the same as larvae mock treated with
224 PBS. With strain NCTC 13438 (KPC-3), larvae treated with either meropenem or PBS alone also
225 displayed an identical increase in bacterial burden after 24 h. Notably, the increase in bacterial
226 numbers after treatment with AZT was reduced (approximately 0.5 $\log_{10}$ cfu/mL), indicating some
227 inhibitory effect of the drug on proliferation of strain NCTC 13438 within the larvae. However, this
228 smaller increase in bacterial numbers post AZT therapy was not reflected in any significant
229 reduction in larval death compared to treatment with meropenem or PBS (Figure 3).

230 In direct contrast to monotherapies, a single dose of a combination of AZT + meropenem resulted in
231 significantly ($p \leq 0.05$) enhanced survival of larvae infected with either MDR strain of *K. pneumoniae*

232 (Figure 3). For example, 120 h p.i with NCTC 13443 (NDM-1), populations treated with PBS, AZT
233 alone or meropenem alone showed survival of 20, 40 and 47% respectively. In contrast, 67% of
234 larvae treated with the combination survived. At the same time p.i with NCTC 13438 (KPC-3),
235 treatment with PBS, AZT alone or meropenem alone showed survival of 10, 13 and 17%
236 respectively compared to 47% for the combination. Correlating with this enhanced survival, the
237 burden of bacteria within the infected larvae did not show the large increase after 24 h observed
238 previously with the monotherapies, and, bacterial numbers remained significantly lower throughout
239 the duration of the experiment ($p \leq 0.05$). Whilst the combination treatment halted the proliferation of
240 infecting bacteria of either strain, numbers did not fall below the initial infecting inoculum size. Thus,
241 the therapeutic benefit conferred by AZT+ meropenem combination treatment appears to be due to
242 a bacteriostatic effect *in vivo*.

243 In summary, a combination treatment of AZT + meropenem offers a potential novel therapy for
244 treatment of MDR, carbapenemase-producing strains of *K. pneumoniae*.

245 **The inhibitory action of the combination of AZT plus meropenem versus *K. pneumoniae* is**
246 **not significantly synergistic *in vitro***. To help understand the nature of the inhibitory action of the
247 combination of AZT with meropenem that conferred enhanced efficacy *in vivo*, checkerboard and
248 time-kill experiments were conducted *in vitro* (Figure 4). A checkerboard assay showing the effect of
249 different AZT and meropenem combinations on growth of both carbapenemase-producing strains of
250 *K. pneumoniae* is shown in Figure 4a. For NCTC 13443 (NDM-1), there was some evidence of
251 minor synergy (FICI ≤ 0.5) at only two combinations tested. The majority of the other combinations
252 that inhibited growth did so in an indifferent or additive fashion. For strain NCTC 13438 (KPC-3),
253 none of the inhibitory combinations tested were synergistic, with all displaying indifference or
254 additivity.

255 The effect of exposure to the single drugs (at MIC_{50}) and a combination (also at MIC_{50} for each
256 drug) on viability of both strains is shown in Figure 4b. Bacterial viability was measured over a
257 period of 6 h at 37°C. Control populations of both strains, mock treated with PBS increased in cell
258 number over the duration of the experiment. Exposure to AZT alone resulted in a loss of viability of

259 both strains of approximately 3 $\log_{10}$ cfu/mL after 6 h. In contrast, exposure to meropenem also
260 resulted in loss of viability, but after 6 h exposure, there was evidence from both strains that the
261 surviving population of bacteria was recovering and growth resuming. With both strains, the
262 combination treatment resulted in a steady decline in viability of approximately 4 $\log_{10}$ cfu/mL after 6
263 h. Despite the greater bactericidal effect of the combination compared to the individual drugs, the
264 loss of viability induced by the combination was only approximately 1 $\log_{10}$ cfu/mL more than AZT
265 alone. This supports the checkerboard results by showing that the inhibition of *K. pneumoniae*
266 induced by exposure to the combination of AZT with meropenem is not strongly synergistic. Notably,
267 despite being bactericidal, the combination did not eliminate all bacteria over the duration of the
268 experiment. This observation is supported by the *G. mellonella* larval burden assays (Figure 3)
269 where infecting *K. pneumoniae* were never eliminated and a reduced number of bacteria were
270 always detectable.

271 **Discussion**

272 The antibacterial properties of AZT have been well documented but the drug has never been
273 formally approved to treat bacterial infections (Elwell 1987; Lewin and Aymes 1989; Ng 2018). With
274 the emergence of untreatable infections by MDR, or XDR, Gram-negative bacteria, there has been
275 renewed interest in exploiting these properties and 'repurposing' the drug. In fact, a recent study
276 proposed using AZT alone as a salvage therapy for colistin-resistant infections (Peyclit 2018). In this
277 study, AZT was shown to inhibit two MDR, carbapenemase-producing strains of *K. pneumoniae* with
278 MICs between 1.0 and 4.0 mg/L, confirming the known inhibitory effect of AZT on this pathogen
279 (Elwell 1987; Lewin and Aymes 1989; Peyclit 2018). In addition, *G. mellonella* larvae infected with
280 the same strains displayed enhanced survival after monotherapy with AZT.

281 Despite many studies showing that AZT is antibacterial, one reason why the drug may not be highly
282 effective as a monotherapy is the induction of resistance after short-term exposure (Lewin 1990).
283 AZT only inhibits bacteria that possess a thymidine kinase that phosphorylates the AZT such that it
284 can then be incorporated into DNA and arrest DNA replication (Doleans-Jordheim 2011). In *E. coli*,
285 mutations, or the presence of insertion sequences, in the gene encoding thymidine kinase (that

286 could result in impaired function of the enzyme) correlated with AZT resistance (Doleans-Jordheim
287 2011). Furthermore, *E. coli* strains resistant to AZT have been isolated from patients undergoing
288 therapy with the drug and the activity of thymidine kinase in these strains was reduced (Lewin
289 1990). Thus, because of the issue of resistance to AZT, the most likely application of the drug as an
290 antibacterial therapy for MDR Enterobacteriaceae is in combination therapies with antibiotics that
291 could help reduce the onset of resistance.

292 A number of studies have highlighted effective combinations of AZT with various approved
293 antibiotics i*n vitro* including: tigecycline against MDR *E. coli* and *K. pneumoniae* (Ng 2018); colistin
294 versus colistin-resistant *K. pneumoniae* (Falagas 2019) or *E. coli* (Loose 2018; Peyclit 2018);
295 trimethoprim and/or sulfamethizole against trimethoprim-resistant *E. coli* and *K. pneumoniae* clinical
296 isolates (Wambaugh 2017); and the aminoglycosides, gentamicin and amikacin against *E. coli*
297 (Doleans-Jordheim 2011). Notably, two studies demonstrate enhanced efficacy *in vivo* of AZT in
298 combination with colistin (Hu 2019) or polymyxin B (Lin 2019), compared to their constituent
299 monotherapies, in murine infection models with NDM-producing *K. pneumoniae* and/or colistin-
300 resistant *E. coli*. In this study, a combination therapy consisting of AZT with meropenem resulted in
301 enhanced efficacy against infections by two MDR, carbapenemase-producing strains of *K.*
302 *pneumoniae* in *G. mellonella* larvae compared to each monotherapy. Supporting these findings, a
303 recent screen of Food and Drug Administration-approved drugs identified that AZT acted as an
304 antibiotic-resistance breaker (ARB) when combined with meropenem and potentiated the inhibitory
305 effect of the antibiotic against MDR *K. pneumoniae in vitro* (Hind 2019). Clearly, only two NCTC
306 carbapenemase-producing strains were used in this study and additional studies using a range of
307 carbapenemase-producing clinical isolates will be required to confirm the enhanced efficacy of this
308 combination.

309 Despite the enhanced efficacy of the combination, the larval populations treated with the
310 combinations still suffered mortality for the duration of the experiments albeit less than those treated
311 with the monotherapies. This observation could be explained by the effect the different treatments
312 had on the burden of infecting bacteria within the larvae. For example, monotherapies had no

313 detrimental effect on the infecting bacteria of either strain because numbers increased rapidly over
314 the first 24 h in the same fashion as larvae sham-treated with PBS. However, combination therapy
315 had the effect of preventing infecting bacteria of either strain from proliferating in the larvae but,
316 notably, did not reduce bacterial burden. The fact that combination-treated larvae still contained
317 viable bacteria could account for these populations suffering mortality at a reduced rate compared
318 to populations treated with either monotherapy. These observations *in vivo* were supported by the
319 results from the *in vitro* time-kill experiments. For example, despite the combination of AZT with
320 meropenem showing a bactericidal effect, lethality slowed over a 6 h period of exposure and low
321 numbers of either strain survived. Furthermore, the enhanced efficacy of the combination treatment
322 *in vivo* was unlikely to be due to a synergistic interaction between AZT and meropenem because
323 the *in vitro* experiments largely showed an indifferent or additive effect. The lack of potent synergy
324 between AZT and meropenem versus the two *K. pneumoniae* strains could account for the
325 observed survival kinetics of infected larvae and the failure of the combination treatment to confer
326 full survival or eliminate all infecting bacteria at the doses tested. It is likely that the enhanced
327 efficacy of the combination observed *in vivo* can be explained by the FICI values observed *in vitro*.
328 For example, the best FICI value obtained for the NDM-1 strain was 0.5 and for the KPC-3 strain
329 0.62. These values represent a weak synergistic effect or, at the very least, an indifferent or additive
330 effect of the combination versus both strains. An additive effect is supported by the time-kill assay
331 whereby a 6 h exposure to the combination resulted in only approximately 1 $\log_{10}$ cfu/mL greater
332 reduction in cell numbers than either of the constituent drugs alone.

333 For therapy of Human Immunodeficiency Virus (HIV), AZT is administered orally at 300 mg twice a
334 day indicating that blood plasma concentrations above the MIC for *K. pneumoniae* could be reached
335 (Peyclit 2018; Falagas 2019). Furthermore, AZT is well-tolerated, and toxicity generally only
336 manifests after long-term use of the drug – a scenario that would be unlikely if antibiotic/AZT
337 combinations were used to treat acute bacterial infections.

338 In summary, this work has identified that combination of AZT with meropenem represents a
339 plausible alternative therapy to treat infections with MDR, carbapenemase-producing strains of *K.*
340 *pneumoniae* and merits further investigation.

341

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344

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455 Legends for Figures

456 **Figure 1**. Effect of increasing inoculum dose of live *K. pneumoniae* NCTC 13443 (NDM-1) or NCTC
457 13438 (KPC-3) on the survival of *G. mellonella* larvae during incubation at 37°C for 120 h. Numbers
458 in the legend indicate the inoculum size in bacterial cells per larva. For both strains, the effect of
459 heat-killed (h.k.) bacterial inocula is also shown. No significant mortality was observed in an
460 unmanipulated group (data not shown) or in the uninfected group mock 'infected' with sterile PBS.
461 For all infected groups, survival was significantly reduced compared to the mock 'infected' group
462 ($p<0.05$, log rank test with Holm correction for multiple comparisons); n=30 (pooled from replicate
463 experiments).

464 **Figure 2**. Effect of treatment with zidovudine (AZT) or meropenem (MEM) on survival of *G.*
465 *mellonella* larvae infected with *K. pneumoniae* strains. Groups of larvae were mock 'infected' with
466 sterile PBS, or 5.6×10^5 cells of the NDM-1 strain, or 9.1×10^7 cells of the KPC-3 strain. 2 h post-
467 infection (p.i), a single dose of either MEM or AZT was administered (dose in mg/kg is the number
468 shown on the figure). The mock 'treated' group represents infected larvae treated with sterile PBS.
469 Larval survival was measured over a period of 120 h at 37°C. * indicates significantly enhanced
470 survival compared to the mock 'treated' group ($p<0.05$, log rank test with Holm correction for
471 multiple comparisons); $n=30$ (pooled from duplicate experiments).

472 **Figure 3**. Effect of treatment with combinations of AZT and MEM on the survival and internal
473 bacterial burden of *G. mellonella* larvae infected with *K. pneumoniae* strains. Larvae were infected
474 with PBS (mock 'infected'), *K. pneumoniae* NCTC 13443 (NDM-1) – 5.6×10^5 cells, or NCTC 13438
475 (KPC-3) – 9.1×10^7 cells and treated with either PBS (mock 'treated'), or a single dose of each drug
476 individually, or a combination of MEM and AZT at 2 h p.i. (dose in mg/kg is the number shown on
477 the figure). Larvae were incubated at 37°C for 120 h and survival recorded every 24 h.
478 The larval burden of *K. pneumoniae* was determined from five individual larvae per treatment group
479 every 24 h for 96 h at 37°C. Error bars indicate ±SEM.

480 * combination treatment group with significantly enhanced survival compared with any of the
481 constituent monotherapies ($p < 0.05$, log-rank test with Holm's correction for multiple comparisons).
482 n=30 (pooled from duplicate experiments).
483 # significant difference in larval burden between groups treated with the combination of AZT + MEM
484 compared with each monotherapy ($p < 0.05$, the Mann–Whitney *U*-test compared the combination
485 therapy with each monotherapy). $n = 5$.
486 **Figure 4**. The effect of combination of AZT with MEM on the growth and viability of *K. pneumoniae*
487 NCTC 13443 (NDM-1) or NCTC 13438 (KPC-3) *in vitro*. Fractional inhibitory concentration indices
488 (FICI) of AZT combined with MEM versus NDM-1 and KPC-3 after 24 h in MHB at 37°C (A). Black
489 squares indicate FICI values where bacterial growth occurred. Grey squares indicate wells where
490 the FICI values were ≥ 0.5 (indicating inhibition was not synergistic). White squares show FICI
491 values of 0.5 or less where bacterial growth was inhibited and thus indicate synergistic inhibition of
492 growth. The experiment was performed in duplicate and a representative result is shown.
493 Time-kill curves of the effect of 6 h exposure to PBS or MIC_{50} of; MEM alone (128 mg/L for both
494 NDM-1 and KPC-3); AZT alone (0.5 mg/L NDM-1 and 2 mg/L KPC-3) or AZT + MEM (NDM-1 – 128
495 mg/L MEM + 0.5 mg/L AZT; KPC-3 – 128mg/L MEM + 2 mg/L AZT). Error bars indicate ±SEM from
496 duplicate experiments (B).
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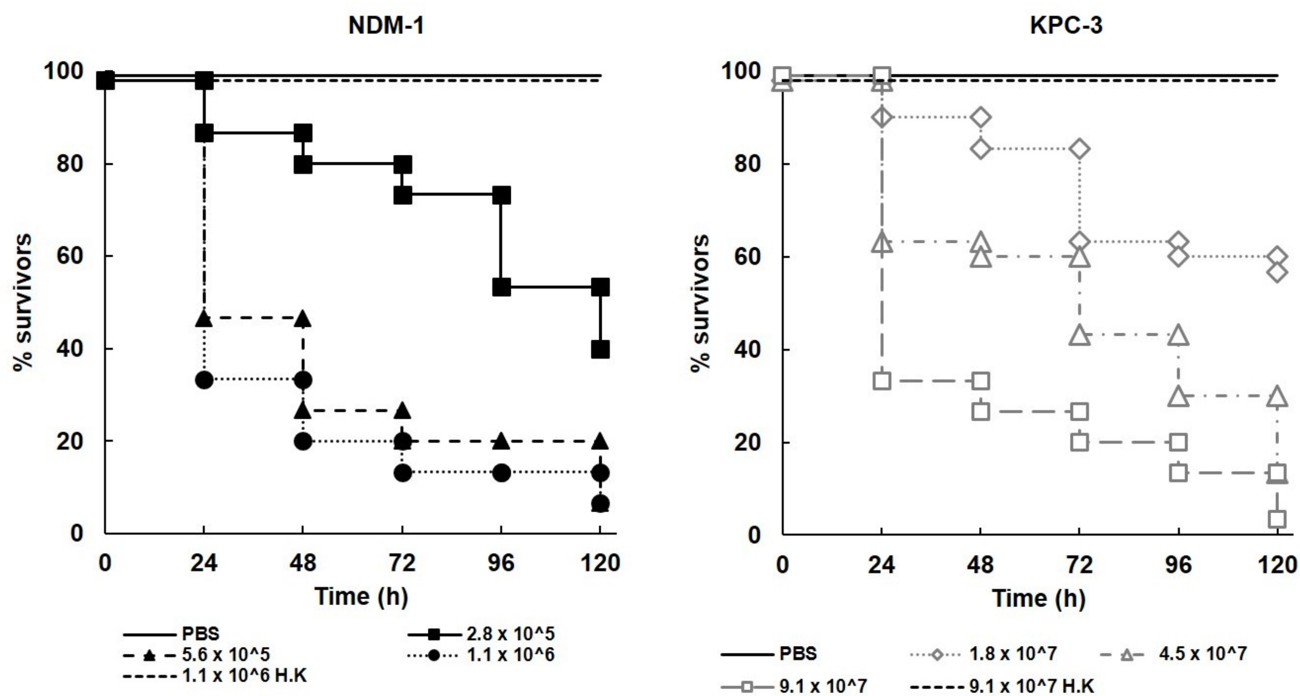
Table 1 – Minimum inhibitory concentration (MIC) of zidovudine (AZT) and meropenem (MEM) for the *K. pneumoniae* Type strain or strains possessing either the NDM-1 or KPC-3 carbapenemases.

Bacterial Strains	MIC (mg/L)	
	AZT	MEM
***K. pneumoniae* NCTC 9633T**	-	<0.0625
***K. pneumoniae* NCTC 13443 (NDM-1)**	1.0 – 2.0	128 - 256
***K. pneumoniae* NCTC 13438 (KPC-3)**	4.0	128 - 256

524 Figure 1



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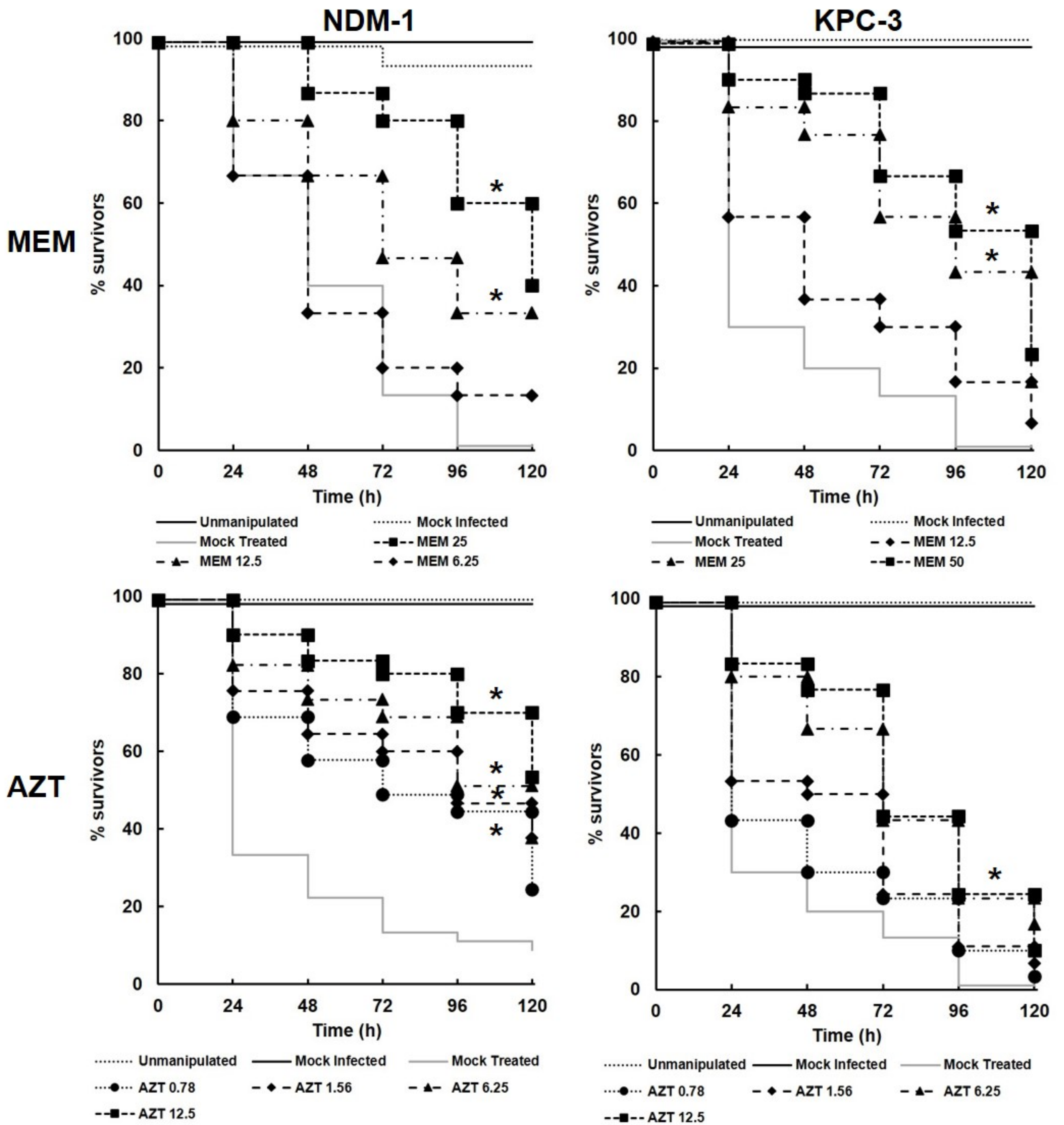
537 Figure 2



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542 Figure 3

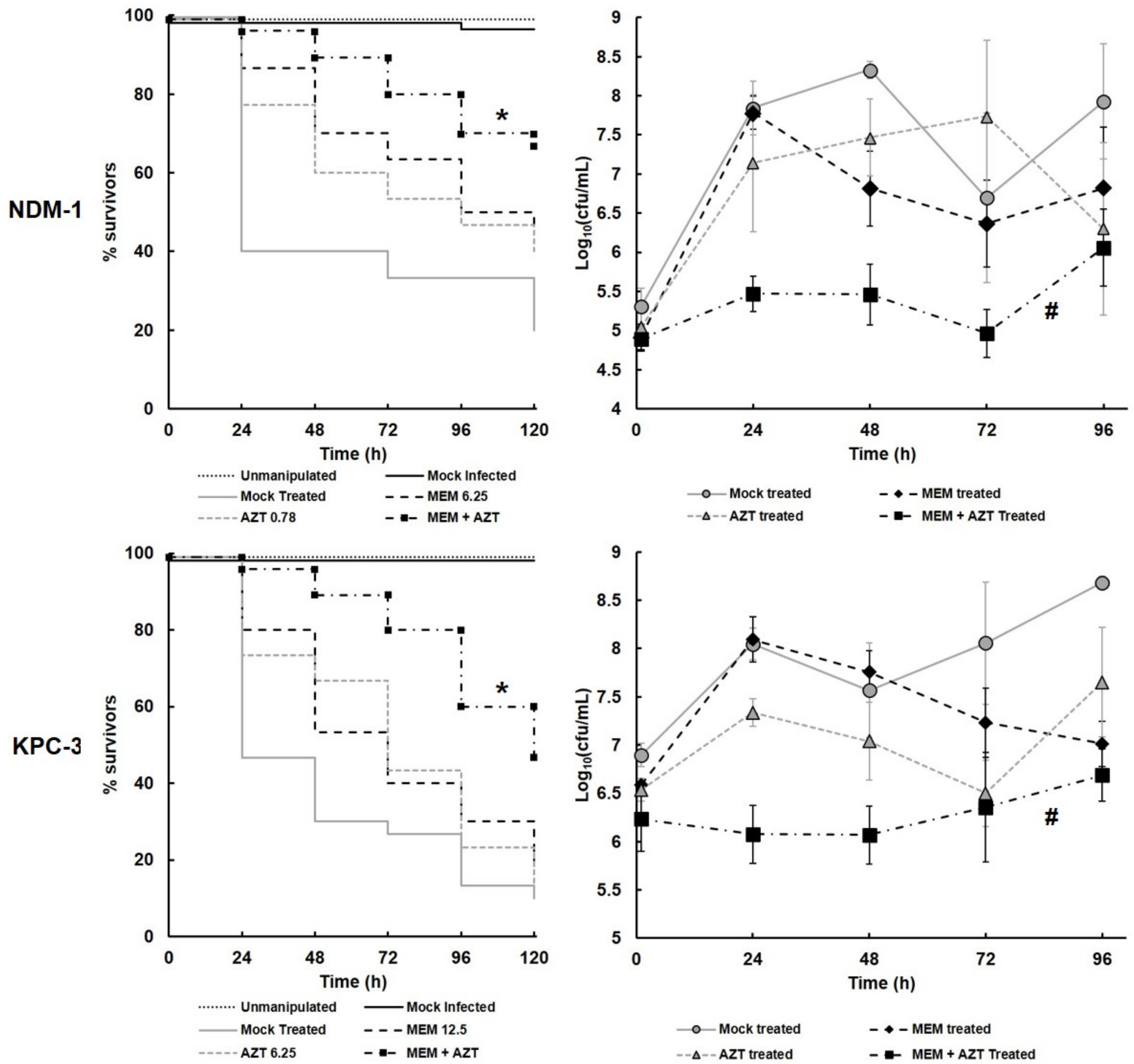

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Figure 4A

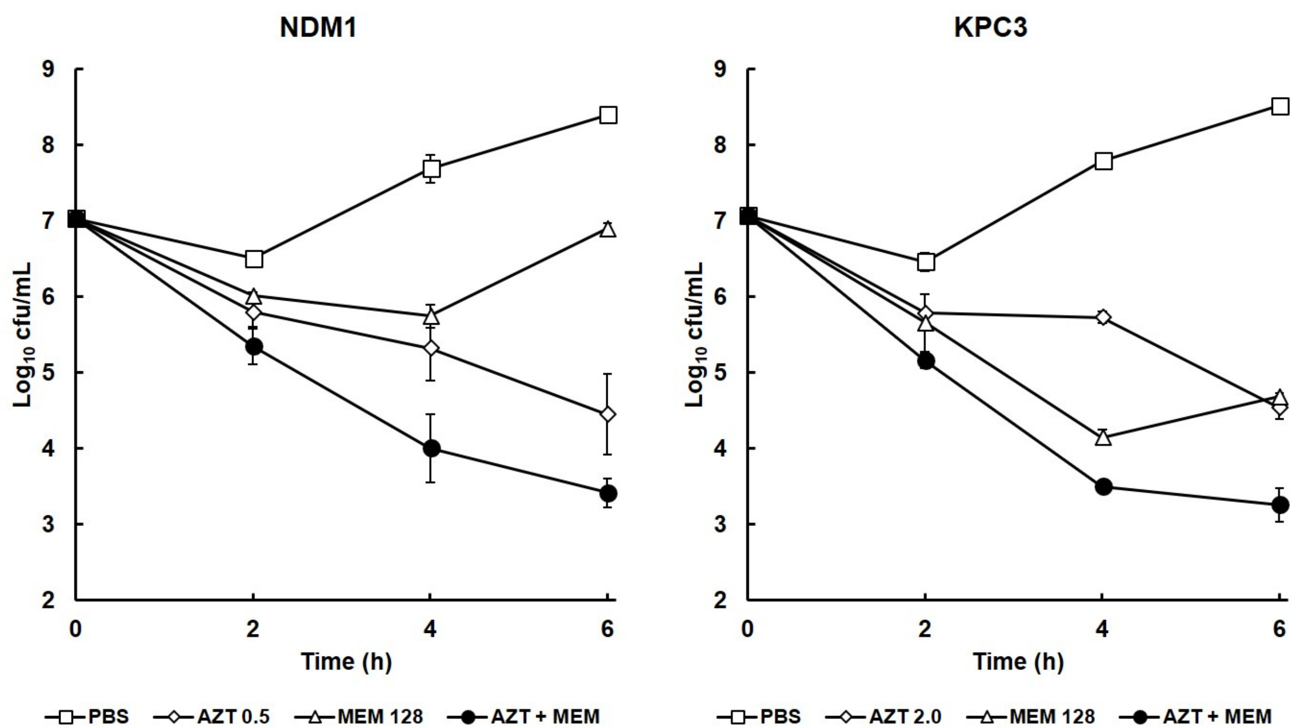
NDM-1

AZT (mg/L) \ Meropenem (mg/L)	0.25	0.5	1	2	4	8	16	32	64	128	256
2	1.0	1.0	1.0	1.01	1.01	1.03	1.06	1.12	1.25	1.5	2
1	0.5	0.5	0.5	0.51	0.51	0.53	0.56	0.62	0.75	1.0	1.5
0.5	0.25	0.25	0.25	0.26	0.26	0.28	0.31	0.37	0.5	0.75	1.25
0.25	0.13	0.13	0.13	0.13	0.14	0.15	0.19	0.25	0.37	0.62	1.12
0.125	0.06	0.06	0.07	0.07	0.08	0.09	0.12	0.19	0.31	0.56	1.06
0.062	0.03	0.03	0.03	0.04	0.05	0.06	0.09	0.15	0.28	0.53	1.03
0.031	0.02	0.02	0.02	0.02	0.03	0.05	0.08	0.14	0.26	0.51	1.01

KPC-3

AZT (mg/L) \ Meropenem (mg/L)	0.25	0.5	1	2	4	8	16	32	64	128	256
4	1.0	1.0	1.01	1.02	1.03	1.06	1.12	1.25	1.5	2.0	3.0
2	0.5	0.5	0.51	0.52	0.53	0.56	0.62	0.75	1.0	1.5	2.5
1	0.25	0.25	0.26	0.26	0.28	0.31	0.37	0.5	0.75	1.25	2.25
0.5	0.13	0.13	0.13	0.14	0.16	0.19	0.25	0.37	0.62	1.12	2.12
0.25	0.06	0.07	0.07	0.08	0.09	0.12	0.19	0.31	0.56	1.06	2.06
0.125	0.03	0.03	0.04	0.05	0.06	0.09	0.15	0.28	0.53	1.03	2.03
0.062	0.02	0.02	0.02	0.03	0.05	0.08	0.14	0.27	0.52	1.02	2.02

555 Figure 4B



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