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## **Activity of innate antimicrobial peptides and ivacaftor against clinical cystic fibrosis respiratory pathogens**

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1 **Activity of innate antimicrobial peptides and ivacaftor against clinical cystic**  
2 **fibrosis respiratory pathogens**

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## 29 ABSTRACT

30 There is a clear need for new antimicrobials to improve current treatment of chronic  
31 lung infection in people with cystic fibrosis (CF). This study determined the activity of  
32 antimicrobial peptides (AMPs) and ivacaftor, a novel CF transmembrane regulator  
33 potentiator for treatment of CF. Antimicrobial activity of AMPs (LL37, Human  $\beta$ -  
34 Defensins [H $\beta$ D] 1-4 and SLPI) and ivacaftor against clinical respiratory isolates  
35 (*Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Streptococcus spp.*,  
36 *Achromobacter spp.* and *Stenotrophomonas maltophilia*) were determined using radial  
37 diffusion and time-kills assays, respectively. Synergy of LL37 and ivacaftor with  
38 tobramycin was determined by time-kill with *in vivo* activity of ivacaftor and tobramycin  
39 compared using a murine infection model. LL37 and H $\beta$ D3 were the most active AMPs  
40 tested with MICs for genera ranging from 1.1-51.9 mg/L and 1-35.4 mg/L, respectively,  
41 with the exception of *Achromobacter* which was resistant. H $\beta$ D1 and SLPI  
42 demonstrated no antimicrobial activity. LL37 demonstrated synergy with tobramycin  
43 against 4/5 *S. aureus* and 2/5 *Streptococcus spp.* isolates. Ivacaftor demonstrated  
44 bactericidal activity against *Streptococcus spp.* (mean log<sub>10</sub> decrease 3.31 CFU/ml),  
45 bacteriostatic activity against *S. aureus* (mean log<sub>10</sub> change 0.13 CFU/ml) but no  
46 activity against other genera. Moreover, ivacaftor demonstrated synergy with  
47 tobramycin with a mean log<sub>10</sub> decrease of 5.72 CFU/ml and 5.53 CFU/ml at 24 hours  
48 for *S. aureus* and *Streptococcus spp.*, respectively. Ivacaftor demonstrated  
49 immunomodulatory but no antimicrobial activity in a *P. aeruginosa in vivo* murine  
50 infection model. Following further modulation to enhance activity, AMPs and ivacaftor  
51 offer real potential as therapeutics to augment antibiotic therapy of respiratory infection  
52 in CF.

53 **Key words:** Ivacaftor, Cystic Fibrosis, antimicrobial, *Pseudomonas aeruginosa*,

54 innate antimicrobial peptides

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## 58 **1. Introduction**

59 Cystic fibrosis (CF) is a hereditary disease caused by mutations in the cystic fibrosis  
60 transmembrane conductance regulator (CFTR) gene. Respiratory failure caused by  
61 repeated cycles of infection and inflammation is the leading cause of morbidity and  
62 mortality in people with CF and is responsible for 80% of deaths [1]. Although  
63 *Pseudomonas aeruginosa* is the most frequently isolated CF respiratory pathogen [2],  
64 infection caused by other bacteria including *Staphylococcus aureus*, *Burkholderia*  
65 *cepacia* complex, *Stenotrophomonas maltophilia*, *Achromobacter spp.* and  
66 *Streptococcus spp.* [3,4] also occur. As a result, primarily due to increased use of  
67 antibiotics, bacteria causing respiratory infection are becoming progressively more  
68 resistant to conventional antibiotics with up to 45% of CF patients colonised with  
69 multidrug resistant pathogens [5–7]. Furthermore, when chronic infection is  
70 established, pathogens such as *P. aeruginosa* grow within polymicrobial biofilms in  
71 the CF lung and exhibit increased resistance to antibiotics [8,9]. Tobramycin is the  
72 most frequently prescribed inhaled antibiotic for CF patients with chronic *P. aeruginosa*  
73 infection [2], with ciprofloxacin frequently prescribed as an oral antibiotic [10].  
74 However, in a recent study of CF *P. aeruginosa* isolates in Northern Europe, 60% were  
75 multidrug resistant with 28% and 56% resistant to tobramycin and ciprofloxacin,  
76 respectively [11]. Therefore, there is a clear need for novel antimicrobial agents or  
77 combinations of antimicrobials to treat respiratory infection in CF patients.

78 Antimicrobial peptides (AMPs) form part of the non-specific innate immune response  
79 and have been shown to have antibacterial activity [12]. Some of the most well  
80 characterised AMPs include the human cathelicidin LL37, Human  $\beta$ -defensins 1-4  
81 (H $\beta$ D1-4) and secretory leukocyte protease inhibitor (SLPI) which are all produced by  
82 the lung epithelium [13–15]. The antibacterial properties of these peptides have been

83 previously demonstrated [16–19], but only LL37 has been specifically tested against  
84 clinical CF respiratory isolates.

85 Ivacaftor is a first-in-class CFTR potentiator that potentiates defective CFTR at the  
86 apical membrane of lung epithelial cells, thus increasing the probability of successful  
87 chloride transport across the membrane [20]. Treatment results in sweat chloride  
88 correction, decreased exacerbation frequency and an improvement in lung function  
89 and quality of life [21]. Furthermore, Reznikov et *al.* reported that ivacaftor  
90 demonstrated some antimicrobial activity against laboratory and non-CF clinical  
91 methicillin susceptible and resistant *S. aureus* (MSSA and MRSA) and *Streptococcus*  
92 *pneumoniae* isolates [22] and suggested that this may be due to the presence of a  
93 quinolone ring.

94 The aim of this study was to determine the antimicrobial activity of a number of AMPs  
95 and ivacaftor against clinical CF respiratory isolates from a range of genera.  
96 Furthermore, we investigated if there was synergy between LL37 or ivacaftor and  
97 tobramycin. Finally, a murine infection model was used to compare the *in vivo*  
98 antimicrobial and immunomodulatory activity of ivacaftor and tobramycin.

99

## 100 **2. Materials and Methods**

### 101 *2.1. Bacterial isolates*

102 Eighteen clinical bacterial isolates [*P. aeruginosa*, n=4; *S. aureus* n=4 (MRSA, n=3;  
103 MSSA n=1); *Streptococcus* spp., n=4; *Achromobacter* spp., n=3 and *S. maltophilia*,  
104 n=3] were used for susceptibility testing. The isolates were cultured from sputum  
105 samples or bronchoalveolar lavage fluid collected from CF patients attending CF  
106 clinics in Belfast and the University of North Carolina at Chapel Hill. Samples were  
107 cultured on non-selective agar plates and individual colonies sub-cultured to obtain  
108 pure bacterial culture. Bacterial isolates were identified using 16S rRNA sequencing  
109 as described previously [23]. Bacterial isolates were stored at -80°C. *P. aeruginosa*  
110 ATCC 27853, *S. aureus* ATCC 29213 and *Streptococcus anginosus* NCTC 10713  
111 were included as quality control and reference strains. In biofilm assays, *S.*  
112 *epidermidis* ATCC 35984 was used as a strong biofilm former (positive control) and a  
113 laboratory *S. capitis* isolate was used as a weak biofilm former (negative control). *P.*  
114 *aeruginosa* strain PAO1 was used for *in vivo* experiments.

### 115 *2.2. Antimicrobials and reagents*

116  
117 Etest® strips were purchased from bioMérieux (North Carolina, USA). Ivacaftor was  
118 purchased from Selleckchem (Houston, USA), synthetic LL37 and synthetic HβD1-4  
119 from Innovagen (Lund, Sweden) and recombinant human SLPI from R&D Systems  
120 (Minneapolis, USA); All AMPs were active. Tobramycin was obtained from Hospira  
121 (Warwickshire, UK). Ciprofloxacin (≥98% HPLC grade), monobasic and dibasic  
122 sodium phosphate, agarose (Type 1, low EEO), methanol (≥99.9% HPLC grade),  
123 crystal violet (for biofilm staining), Dulbecco's phosphate buffered saline (PBS)  
124 (endotoxin tested) and trypan blue were all purchased from Sigma-Aldrich (Gillingham,



125 UK). Mueller Hinton agar (MHA), Mueller Hinton broth (MHB), Ceftrimide agar,  
126 Anaerobe Basal agar (ABA) and Brain Heart infusion (BHI) broth were all purchased  
127 from Oxoid Ltd (Basingstoke, UK). Sterile, defibrinated horse blood was purchased  
128 from TCS Biosciences (Buckingham, UK). Xylazine (Xylacare 2% w/v) and Ketamine  
129 (Narketan 10, 100g/L) were obtained from Animalcare (York, UK) and Ventoquinol  
130 (Buckinghamshire, UK), respectively. IL-6 and KC enzyme-linked immunosorbent  
131 assay (ELISA) kits were purchased from eBioscience (Hatfield, UK) and R&D Systems  
132 (Minneapolis, USA), respectively.

### 133 2.3. MIC testing

134 The MICs of antibiotics (Supplementary Table 1) routinely used in the treatment of CF  
135 lung infection were determined by Etest<sup>®</sup> according to the manufacturer's instructions.  
136 The MICs of AMPs (LL37, H $\beta$ D1-4 and SLPI) were determined using a radial diffusion  
137 assay (RDA) as previously described [24]. BHI supplemented agar was used with  
138 *Streptococcus* spp., *S. maltophilia* and *Achromobacter* spp. with MHA used for *P.*  
139 *aeruginosa* and *S. aureus*. Peptides were tested at concentrations of 200, 150, 100  
140 and 50 mg/L.

### 141 2.4. LL37 and tobramycin synergy

142 A modified time-kill assay was used to determine if there was synergy between LL37  
143 and tobramycin against selected isolates (*P. aeruginosa* [n=5]; *S. aureus* [n=5] and  
144 *Streptococcus* spp. [n=5]). Overnight cultures were washed with 10mM sodium  
145 phosphate and adjusted to approximately  $1 \times 10^5$  CFU/ml. In a 96-well plate, the  
146 bacterial suspension was incubated with LL37 (64–1 mg/L) alone or in combination  
147 with tobramycin (0.5 MIC for each isolate) for 3 hours at 37°C under aerobic or  
148 microaerophilic conditions. Killing activity was assessed by enumerating on MHA or

149 ABA following serial dilution ( $10^{-1}$  to  $10^{-3}$ ) in sterile saline. The MBC of LL37 was  
150 determined as the lowest concentration at which there was no growth on the plate.  
151 Synergy was defined as a  $\geq 2 \log_{10}$  decrease in total viable count (TVC) compared to  
152 the starting inoculum and as a  $\geq 2 \log_{10}$  decrease in TVC by the combination compared  
153 to the most active single agent [25]. Results are expressed as mean CFU/ml  $\pm$ SD.

#### 154 2.5. Time-kill studies

155 All time-kill experiments were performed according to CLSI standards [25]. Initial time-  
156 kill studies were performed using a range of ivacaftor concentrations (32, 16, 8, 4 and  
157 1mg/L) against selected isolates (*P. aeruginosa* [n=5]; *S. aureus* [n=5] and  
158 *Streptococcus spp.* [n=5]); the highest concentration of ivacaftor tested (32 mg/L) was  
159 that previously used by Reznikov *et al.* [22]. Ivacaftor was dissolved in DMSO and  
160 time-kills were also performed using DMSO (0.32%) as a reagent control.  
161 Subsequently, time-kill assays were performed with these isolates to compare the  
162 activity of ivacaftor (32 mg/L) with ciprofloxacin (5 mg/L; concentration above MIC for  
163 the majority of isolates), a comparator fluoroquinolone. Time-kill assays were  
164 performed in MHB for *P. aeruginosa* and *S. aureus* isolates and BHI broth for  
165 *Streptococcus spp.*

166 For synergy studies, ivacaftor was used at a concentration of 32 mg/L in combination  
167 with tobramycin at 0.5 MIC. Tobramycin was chosen for synergy studies as it is the  
168 most frequently prescribed inhaled antibiotic in CF and is also frequently prescribed  
169 for treatment of acute infective exacerbations [2]. A no drug control was used in each  
170 assay.

171 Bacterial cultures and antibiotics were incubated at 37°C under aerobic or  
172 microaerophilic (5% CO<sub>2</sub> for *Streptococci*) conditions. Killing activity was assessed at

173 0, 2, 4, 6 and 24 h by carrying out serial dilutions in sterile saline ( $10^{-1}$  to  $10^{-6}$ ) followed  
174 by enumerating on MHA or ABA agar. Bactericidal activity was defined as a  $\geq 3 \log_{10}$   
175 reduction in colony forming units (CFU/ml) in the original inoculum [25]. Synergy was  
176 defined as described above [25] with results for time-kill assays expressed as mean  
177 CFU/ml  $\pm$ SD.

## 178 2.6. *Biofilm Studies*

179 The effect of ivacaftor on bacterial adherence and biofilm formation by all isolates  
180 (clinical isolates n=18; reference strains, n=3) was determined using a previously  
181 described method that quantifies the adherence of bacteria to microtitre plates  
182 (ThermoFisher Scientific, Waltham, USA) [26]. An overnight culture of bacteria was  
183 adjusted to  $1 \times 10^6$  CFU/ml with 200  $\mu$ l added to each well. Ivacaftor (32 mg/L),  
184 ciprofloxacin (5 mg/L) or DMSO (0.32%) were added to the wells and the plates were  
185 incubated for 24 hours at 37°C. The contents of the wells were aspirated, washed  
186 three times with 200  $\mu$ l sterile PBS and adherent bacteria stained using crystal violet.  
187 Bacterial adherence was quantified by measurement of OD<sub>570</sub> (FLUOstar Omega  
188 microplate reader) and compared with an untreated control. Limits for non, weak,  
189 moderate and strong biofilm formation were defined as previously described [26].

## 190 2.7. *In vivo activity using a mouse model of intraperitoneal infection*

191 The *in vivo* activity of ivacaftor was determined using a systemic sepsis model of  
192 infection in mice. Age and sex matched B6 mice (B6N-Tyrc-Brd/BrdCrCrI [Charles River])  
193 mice (n=5 per group; 4 male, 1 female) were inoculated intraperitoneally (IP) with 100  
194  $\mu$ l of *P. aeruginosa* (PAO1) ( $6 \times 10^8$  CFU/ml). PAO1 was used as our group and others  
195 have shown that it reproducibly produces intraperitoneal infection in mice [27].  
196 Ivacaftor (60  $\mu$ g/mouse), negative control (endotoxin free PBS) or tobramycin (140

197  $\mu\text{g}/\text{mouse}$ ), were administered IP immediately following infection. The dose of  
198 ivacaftor used in this model was calculated based on a single adult dose (150mg)  
199 adjusted for mouse weight (20 g) and the assumption that an adult with CF weighs 50  
200 kg. The dose of tobramycin used was calculated based on the intravenous once daily  
201 dose paediatric regimen of 7 mg/kg adjusted for mouse weight (20 g). The bacterial  
202 load inoculated was chosen to ensure systemic infection was achieved with mice  
203 sacrificed after 4 hours to prevent significant deterioration in health. A peritoneal  
204 lavage was performed with 5 ml of ice-cold sterile PBS, with collected samples stored  
205 on ice. Total viable count of PAO1 from the lavage was determined by enumerating  
206 on cetrimide agar, a *P. aeruginosa* selective agar. Total cell count and cell viability  
207 were determined following staining with trypan blue using the Countess™ Automated  
208 Cell Counter (Invitrogen). The lavage fluid was centrifuged at 600 xg and the  
209 supernatant used to determine IL-6 and KC (chemokine CXCL1, a functional  
210 homologue of human IL-8) levels by ELISA. Housing and experimentation was carried  
211 out in accordance with the Animal (Scientific Procedures) Act 1986 and current  
212 guidelines approved by the Queen's University Ethical Review Committee.

### 213 *2.8. Statistical analysis*

214 One-way ANOVA and Bonferroni's Multiple Comparison Tests were used to determine  
215 statistical significance in the biofilm assays and in the *in vivo* experiments. All analysis  
216 was performed using GraphPad software with a P-value of <0.05 considered  
217 statistically significant.

218

219 **3. Results**

220 *3.1. MIC testing*

221 The MICs of antibiotics routinely used in the treatment of CF lung infection were  
222 determined for all isolates (Supplementary Table 1) with differences in susceptibility  
223 apparent both within and between genera. The majority of *P. aeruginosa* (4/5; 80%)  
224 and *S. aureus* (4/5; 80%) isolates were susceptible to tobramycin; however, high  
225 tobramycin MICs were demonstrated for *Streptococcus* (3/5; 60%), *Achromobacter*  
226 (3/3; 100%) and *S. maltophilia* (4/5; 80%) isolates. With the exception of *Streptococci*,  
227 where all isolates displayed intermediate resistance, based on the breakpoint for *S.*  
228 *pneumoniae*, resistance and susceptibility to ciprofloxacin was apparent within each  
229 genera.

230 The MIC for each antimicrobial peptide against clinical isolates from each genus is  
231 summarised in Table 1 with the MICs of type strains presented for comparison. The  
232 MICs for individual isolates are also presented in Supplementary Table 2. Overall,  
233 LL37 and H $\beta$ D3 were the most active peptides tested with MICs for genera ranging  
234 from 1.1-51.9 mg/L and 1-35.4 mg/L, respectively, with the exception of  
235 *Achromobacter* which was resistant. In contrast, H $\beta$ D1 and SLPI demonstrated no  
236 activity against any isolates at the concentrations tested. Peptide MICs for type strains  
237 were within the range of those for clinical isolates, with the exception of *S. anginosus*  
238 (NCTC 10713) which was resistant to both H $\beta$ D2 and H $\beta$ D4.

239 *3.2. Synergy between LL37 and tobramycin*

240 When combined with tobramycin, LL37 demonstrated no synergistic activity against  
241 *P. aeruginosa* (Table 2). In contrast, LL37 demonstrated synergistic activity in  
242 combination with tobramycin against 4/5 *S. aureus* and 2/5 *Streptococcus* spp.

243 isolates (Table 2). Change in CFU/ml for individual isolates is presented in  
244 supplementary Table 3.

### 245 3.3. Bactericidal activity of ivacaftor

246 When tested alone, ivacaftor demonstrated no antimicrobial activity against *P.*  
247 *aeruginosa* at any concentration tested (Fig. 1A). In contrast, at the highest  
248 concentration tested, 32 mg/L, bacteriostatic activity was apparent against all *S.*  
249 *aureus* isolates tested (Fig. 1B). Similarly, bacteriostatic (n=3 isolates) and bactericidal  
250 (n=2 isolates) activity was apparent against *Streptococcus* spp. isolates (Fig. 1C) at  
251 this concentration with no effect apparent against any genera at lower concentrations.  
252 DMSO (0.32%) had no effect on bacterial growth in any assay. Individual time-kill  
253 curves are presented for *P. aeruginosa* (Supplementary Fig. 1), *S. aureus*  
254 (Supplementary Fig. 2) and *Streptococcus* spp. isolates (Supplementary Fig. 3.).

255 The change in CFU/mL in the presence of ivacaftor, ciprofloxacin or untreated control  
256 at 24 hours for isolates within each genus is summarised in Table 3. Ciprofloxacin  
257 demonstrated bactericidal activity against 16/21 isolates tested with no growth  
258 detected at 24 hours. In contrast, ivacaftor only demonstrated bactericidal activity  
259 against 2/21 isolates tested, both of which were clinical *Streptococcus* spp.

260 There was no synergy between ivacaftor and tobramycin against *P. aeruginosa* (Fig.  
261 2A, Table 4). In contrast, synergy was apparent for 4/5 *S. aureus* and 4/5  
262 *Streptococcus* spp. isolates (Fig. 2B & 2C, Table 4). Tobramycin (0.5 MIC) had no  
263 effect on growth of the isolates.

264

265

266 3.4. Effect of ivacaftor on biofilm formation

267 Biofilm formation was classified as non-adherent, weak, moderate or strong; 2/5 *P.*  
268 *aeruginosa*, 1/5 *Streptococcus* and 1/3 *Achromobacter* species were non-adherent  
269 and were excluded from further analysis. Of the remaining 17 isolates, 10,  
270 demonstrated weak adherence, 4 demonstrated moderate adherence and 3 were  
271 strong biofilm formers. Treatment with ivacaftor resulted in a significant decrease ( $P$   
272  $<0.001$ ) in biofilm formation for 1/3 *P. aeruginosa* and 2/4 *Streptococcus* spp. biofilm  
273 forming isolates. However, ivacaftor had no effect on biofilm formation by the  
274 remaining isolates tested ( $n=14$ ; Fig. 3). Similarly, ciprofloxacin did not decrease  
275 biofilm formation by *S. aureus* isolates ( $n=5$ ). However, ciprofloxacin caused a  
276 significant decrease ( $P <0.001$ ) in biofilm formation for 7/12 isolates (*P. aeruginosa*,  
277  $n=2/3$ , *Streptococcus* spp.,  $n=1/4$ , *Achromobacter* spp.,  $n=1/2$ ; *Stenotrophomonas*  
278 spp.,  $n=3/3$ ) across the other genera tested (Fig. 3). DMSO (0.32%) had no effect on  
279 bacterial adherence in any assay.

280 3.5. In vivo activity of ivacaftor

281 There was no significant difference between the control group (PBS) and any of the  
282 treatment groups in the total number of cells recovered from the IP lavage or cell  
283 viability (Fig. 4A & 4B). However, compared with control, treatment with tobramycin  
284 caused a significant decrease in the TVC of *P. aeruginosa* recovered from the  
285 peritoneal lavage (Fig. 4C). In contrast, there was no significant difference when the  
286 mice were treated with ivacaftor. Both treatments caused a significant reduction in IL-  
287 6 levels (Fig. 4D); however, KC was only significantly reduced in the group treated  
288 with tobramycin (Fig. 4E).

289

#### 290 4. Discussion

291 As bacteria causing pulmonary infection in CF become progressively more resistant  
292 to conventional antibiotics, interest in the use of AMPs as antimicrobials for treatment  
293 has increased considerably. In the present study, we have shown that LL37 and H $\beta$ D3  
294 possess antibacterial properties against CF respiratory pathogens such as *P.*  
295 *aeruginosa*, MRSA and *S. maltophilia*. Moreover, both of these AMPs demonstrated  
296 antibacterial activity against clinical isolates which were resistant to antibiotics  
297 routinely used in the treatment of CF pulmonary infection such as ciprofloxacin,  
298 tobramycin and meropenem. SLPI had no activity against any genera tested in the  
299 present study. These results contrast to those previously published which reported  
300 activity of SLPI against both *P. aeruginosa* and *S. aureus* [16]. However, in the study  
301 by Wiedow et al. [16], antibacterial activity of SLPI was assessed using a time-kill  
302 assay with a single dermatological *P. aeruginosa* and *S. aureus* isolate. No data were  
303 provided with respect to the antibiotic susceptibility of these two isolates. It is likely  
304 that the isolates used in the present study demonstrated greater inherent antimicrobial  
305 resistance due to prolonged and repeated exposure to antibiotics, which may account  
306 for the lack of concordance between studies.

307 If used clinically, it is likely that AMPs would need to be administered by inhalation in  
308 combination with an antibiotic to directly target the site of infection. Therefore, we  
309 determined the activity of the most potent AMP, LL37, in combination with tobramycin,  
310 the most frequently prescribed inhaled antibiotic for treatment of *P. aeruginosa*  
311 pulmonary infection in CF [2]. This combination demonstrated greater antimicrobial  
312 activity than either agent alone against both clinical *S. aureus* and *Streptococcus* spp.  
313 isolates. However, no synergistic activity was apparent against *P. aeruginosa*. In



314 contrast to our findings, it has been previously reported that LL37 and tobramycin in  
315 combination demonstrate enhanced killing of *P. aeruginosa* biofilms [28]. However,  
316 the concentration of both LL37 (640 mg/L) and tobramycin (160-2560 mg/L) used in  
317 this biofilm killing study were considerably higher than those used in the present study  
318 which ranged from 1-64 mg/L and 0.25-3 mg/L for LL37 and tobramycin, respectively.  
319 Synergy may also have been apparent in our study if we had used both LL37 and  
320 tobramycin at higher concentrations. Despite the excellent antimicrobial activity  
321 demonstrated by LL37 and H $\beta$ D-3, we were unable to perform further work such as  
322 biofilm assays due to the high cost of these AMPs.

323 As previous studies have reported that ivacaftor, a first-in-class CFTR potentiator, has  
324 some antimicrobial activity against non-CF clinical isolates such as *S. aureus*, we  
325 determined its antimicrobial activity against clinical CF respiratory isolates from a  
326 range of genera. Using quantitative culture time-kill assays, we demonstrated activity,  
327 at a concentration of 32 mg/L, against MSSA, MRSA, and *Streptococcus* spp. isolates  
328 but no activity against *P. aeruginosa*, *S. maltophilia* and *Achromobacter* species. This  
329 result is consistent with the findings of Reznikov et al. who reported that ivacaftor had  
330 some antimicrobial activity against *Streptococcus* spp. and *S. aureus*, but was not  
331 active against *P. aeruginosa* [22]. Similar to Reznikov et al., who reported enhanced  
332 antimicrobial activity when ivacaftor was used in combination with vancomycin or  
333 ciprofloxacin, we also found a synergistic effect against *S. aureus* and *Streptococcus*  
334 spp. when ivacaftor was combined with tobramycin.

335 Reznikov et al. suggested that the antimicrobial activity of ivacaftor may be due to the  
336 presence of a quinolone ring in its structure, similar to that of fluoroquinolone  
337 antibiotics such as ciprofloxacin [22]. Quinolones are broad spectrum antibiotics and

338 are typically more active against Gram negative bacteria. In the present study,  
339 ciprofloxacin demonstrated bactericidal activity against both Gram-positive bacteria  
340 (*S. aureus* and *Streptococcus* spp.) and Gram-negative bacteria (*P. aeruginosa*, *S.*  
341 *maltophilia* and *Achromobacter* spp.), consistent with broad-spectrum activity  
342 expected from a conventional quinolone antibiotic. In contrast, ivacaftor had no activity  
343 against any of the Gram negative isolates tested, suggesting that its antimicrobial  
344 effect may not be directly related to the quinolone ring in the structure. Alternatively,  
345 lack of activity against Gram negative bacteria could be due to a number of other  
346 potential mechanisms such as inability to cross the outer membrane and enzymatic  
347 inactivation.

348 In addition to clinical outcomes, the effect of ivacaftor treatment on the CF lung  
349 microbiota was also determined in the GOAL study, a longitudinal cohort study of 151  
350 CF patients before and up to 6 months after ivacaftor initiation. A significant reduction  
351 in the number of patients from whom *P. aeruginosa* was cultured from sputum samples  
352 either through a decrease in *P. aeruginosa* culture positivity over 6 months [29] or  
353 change from *P. aeruginosa* culture positive to negative over the course of a year [30]  
354 was reported. In contrast, there was no significant change in sputum culture positivity  
355 for MRSA, MSSA, *Stenotrophomonas* spp. or *Achromobacter* spp. [29,30]. Similarly,  
356 in a small study of three paediatric CF patients, a reduction in the relative abundance  
357 of *Streptococcus* spp. was reported following treatment with ivacaftor. The results of  
358 our study suggest that these changes in *Streptococcus* relative abundance could be  
359 due to the antimicrobial activity of ivacaftor on this genus; however, the increased  
360 abundance of other genera detected could also account for this change [31]. In  
361 contrast, our results, which clearly show that ivacaftor has no direct antimicrobial  
362 activity against *P. aeruginosa*, growing planktonically or in biofilm, suggest that the

363 change in *P. aeruginosa* culture positivity reported in the GOAL study is more likely  
364 attributed to increased mucociliary clearance resulting in increased clearance of  
365 biofilm from the airways. The concentration of ivacaftor achieved in sputum following  
366 oral administration has not been reported to date; however, it is likely to be significantly  
367 lower than the reported serum levels of ~1.4 mg/L [32]. Therefore, the concentration  
368 of ivacaftor (32 mg/L) which demonstrated antimicrobial activity in both our study and  
369 that of Reznikov et al. [22] is likely to be considerably higher than that achievable in  
370 sputum following oral administration. This further supports the hypothesis that  
371 changes in pathogen culture positivity reported post-ivacaftor treatment are not as a  
372 result of a direct antimicrobial effect.

373 Given that pulmonary infection in the CF airways involves bacterial growth in biofilms  
374 [8,9], we also determined the effect of ivacaftor on biofilm formation. The effect of  
375 ciprofloxacin on biofilm formation was also determined to enable comparison between  
376 ivacaftor and a fluoroquinolone antibiotic with a related chemical structure. In general,  
377 ivacaftor had limited effect on biofilm formation. However, as the majority of isolates  
378 demonstrated weak adherence, any change attributable to the use of ivacaftor was  
379 difficult to detect. The decrease in adherence for 2/5 *Streptococcus* spp. isolates is  
380 likely due to the bactericidal activity of ivacaftor against these isolates. In contrast,  
381 ivacaftor had no antimicrobial activity against *P. aeruginosa* and thus the decrease in  
382 biofilm formation with one *P. aeruginosa* isolate is indicative of inhibition of adherence.  
383 Ciprofloxacin demonstrated bactericidal activity against the majority of isolates tested;  
384 therefore, inhibition of biofilm formation could be as a result of either a direct effect on  
385 bacterial adherence or bacterial cell death before adherence could occur.

386 It has been previously reported that ivacaftor has some immunomodulatory activity  
387 with Bratcher et al. (2015) demonstrating a decrease towards normalisation of blood  
388 leukocyte activation following ivacaftor treatment [33]. To establish whether ivacaftor  
389 exhibited any antimicrobial or immunomodulatory activity *in vivo*, an acute systemic  
390 mouse infection model was used; mice were administered ivacaftor or tobramycin at  
391 doses approximately reflecting those used in humans. Ivacaftor demonstrated no  
392 antimicrobial activity in this model; however, there was some indication of modulation  
393 of the innate immune response, based on the reduced production of cytokines and  
394 chemokines. The immunomodulatory effect of fluoroquinolones has been widely  
395 documented [34]; furthermore, in a mouse injury model, treatment with ciprofloxacin  
396 decreased production of IL-6 and KC [35]. A subset of fluoroquinolones with the  
397 cyclopropyl moiety have been linked to this immunomodulatory activity, although the  
398 mechanism has yet to be elucidated [34]. The similarity in structure between ivacaftor  
399 and fluoroquinolones could potentially explain the immunomodulatory activity  
400 demonstrated here.

401 There are a number of limitations to this study. Firstly, the AMPs used were expensive  
402 restricting the volumes that could be used for testing; therefore, we could not  
403 determine MIC by the preferred microbroth dilution method or bactericidal activity  
404 using time-kill assays. This also limited the number and range of isolates which could  
405 be tested. Physiological conditions may also be important when evaluating AMPs as  
406 potential novel antimicrobial therapies. It has been suggested that higher salt  
407 concentration in CF airway surface liquid could reduce the antimicrobial activity of  
408 AMPs [36] by affecting the ionic interaction between the AMPs and bacterial  
409 membranes. Moreover, It has also been demonstrated that in CF sputum, LL37 is  
410 inactivated by binding to DNA, F-actin and cell debris bundles [37]. In contrast, it has

411 also been demonstrated that the presence of carbonate, which is found in many  
412 microenvironments of the body including the respiratory tract, can greatly enhance  
413 bacterial susceptibility to AMPs under physiological ionic conditions [38]. Therefore,  
414 further work testing AMP activity under conditions more reflective of the environment  
415 present in the CF airways would be required if these compounds were being  
416 considered as potential therapeutics to treat CF pulmonary infection. Furthermore,  
417 tobramycin was the only antibiotic used in synergy studies with LL37 and ivacaftor.  
418 Given the wide range of antibiotics used in the prophylaxis and treatment of CF  
419 pulmonary infection, future work to determine synergy between an extended range of  
420 antibiotics and LL37/ivacaftor could be of potential clinical benefit.

421

422

423 **5. Conclusion**

424 In summary, we have shown that the AMPs, LL37 and H $\beta$ D3, demonstrate  
425 antimicrobial activity against CF pathogens from a range of genera with LL37 also  
426 demonstrating synergistic activity, in combination with tobramycin, against *S. aureus*  
427 and *Streptococcus* spp. isolates. Similarly, ivacaftor demonstrated bactericidal activity  
428 against *S. aureus* and *Streptococcus* spp. isolates but no activity against Gram-  
429 negative bacteria. There is a low propensity for the development of resistance to AMPs  
430 due to the interaction of the peptides with the cytoplasmic membrane of bacteria and  
431 their bactericidal nature [12]. Therefore, AMPs could potentially be developed as novel  
432 therapeutic options but further work is required to enhance their activity.

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442

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556

**Table 1**

Antimicrobial activity (MIC range) of antimicrobial peptides against CF respiratory isolates and reference strains *P. aeruginosa* (ATCC 27853), *S. aureus* (ATCC 29213) and *S. anginosus* (NCTC 10713).

Genus	MIC Range (mg/L)					
	LL37	HβD1	HβD2	HβD3	HβD4	SLPI
<b><i>Pseudomonas</i> (n=4)</b>	3.9-22.8	>200	43.4->200	6.4-17.6	39.1-49.3	>200
<b>ATCC 27853</b>	3.2	>200	28.6	13.9	17.8	>200
<b><i>Staphylococcus</i> (n=4)</b>	15.7-29.9	>200	>200	8.9-9.6	>200	>200
<b>ATCC 29213</b>	21.5	>200	>200	12.7	>200	>200
<b><i>Streptococcus</i> (n=4)</b>	2.4-33.2	>200	3.8-49.9	4.8-18.9	32.4-45.7	>200
<b>NCTC 10713</b>	28.5	>200	>200	18.6	>200	>200
<b><i>Achromobacter</i> (n=3)</b>	36.6->200*	>200	>200	50.6->200*	>200	>200
<b><i>S. maltophilia</i> (n=3)</b>	3.3-34.6	>200	47.7->200*	9.6-29.4	51.5->200*	>200

\*only active against 1 isolate

**Table 2.**

Mean log change in CFU/ml ( $\pm$ SD) at 3 hours to determine synergistic activity between LL37 and tobramycin.

Genus	Log change in CFU/ml (mean $\pm$ SD)			
	LL37 (0.5 MIC)	Tobramycin (0.5 MIC)	LL37 (0.5 MIC) + tobramycin (0.5 MIC)	Control
<i>Pseudomonas</i> (n=5)	-0.31 ( $\pm$ 0.22)	-0.58 ( $\pm$ 0.31)	-0.92 ( $\pm$ 0.37)	0.02 ( $\pm$ 0.02)
<i>Staphylococcus</i> (n=5)	-1.37 ( $\pm$ 0.26)	-0.04 ( $\pm$ 0.07)	-3.82 ( $\pm$ 1.05)	0.01 ( $\pm$ 0.01)
<i>Streptococcus</i> (n=5)	-0.13 ( $\pm$ 0.15)	-1.12 ( $\pm$ 1.51)	-2.53 ( $\pm$ 1.96)	0.03 ( $\pm$ 0.03)

**Table 3**

Mean log change in CFU/mL ( $\pm$  SD) at 24 hours following challenge with ivacaftor or ciprofloxacin.

<b>Genus</b>	<b>Log change in CFU/mL (mean <math>\pm</math> SD)</b>		
	Ivacaftor (32 mg/L)	Ciprofloxacin (5 mg/L)	Control
<b><i>Pseudomonas</i> (n=5)</b>	4.44 ( $\pm$ 0.30)	-5.83 ( $\pm$ 0.28)	3.84 ( $\pm$ 0.79)
<b><i>Staphylococcus</i> (n=5)</b>	-0.51 ( $\pm$ 0.61)	-2.06 ( $\pm$ 4.33)	3.37 ( $\pm$ 0.44)
<b><i>Streptococcus</i> (n=5)</b>	-3.16 ( $\pm$ 2.25)	-4.50 ( $\pm$ 1.35)	3.63 ( $\pm$ 0.51)
<b><i>Achromobacter</i> (n=3)</b>	2.83 ( $\pm$ 0.27)	-1.63 ( $\pm$ 3.84)	3.01 ( $\pm$ 0.17)
<b><i>Stenotrophomonas</i> (n=3)</b>	2.69 ( $\pm$ 0.16)	-5.83 ( $\pm$ 0.29)	3.11 ( $\pm$ 0.01)

**Table 4**

Mean log change in CFU/ml ( $\pm$  SD) at 24 hours to determine synergistic activity between ivacaftor and tobramycin.

<b>Genus</b>	<b>Log change in CFU/mL (mean <math>\pm</math> SD)</b>			
	Ivacaftor (32 mg/L)	Tobramycin (0.5 MIC)	Ivacaftor (32 mg/L) & Tobramycin (0.5 MIC)	Control
<b><i>Pseudomonas</i> (n=5)</b>	3.91 ( $\pm$ 1.57)	3.69 ( $\pm$ 0.66)	2.46 ( $\pm$ 1.61)	3.84 ( $\pm$ 0.49)
<b><i>Staphylococcus</i> (n=5)</b>	0.13 ( $\pm$ 0.45)	3.53 ( $\pm$ 0.15)	-5.72 ( $\pm$ 0.17)	3.51 ( $\pm$ 0.27)
<b><i>Streptococcus</i> (n=5)</b>	-3.31 ( $\pm$ 2.13)	2.38 ( $\pm$ 1.42)	-5.53 ( $\pm$ 0.61)	3.49 ( $\pm$ 0.59)



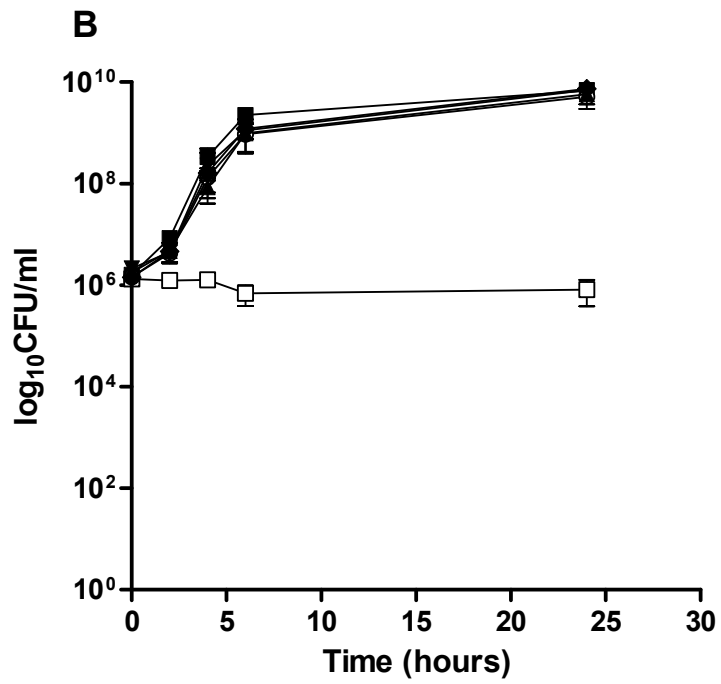
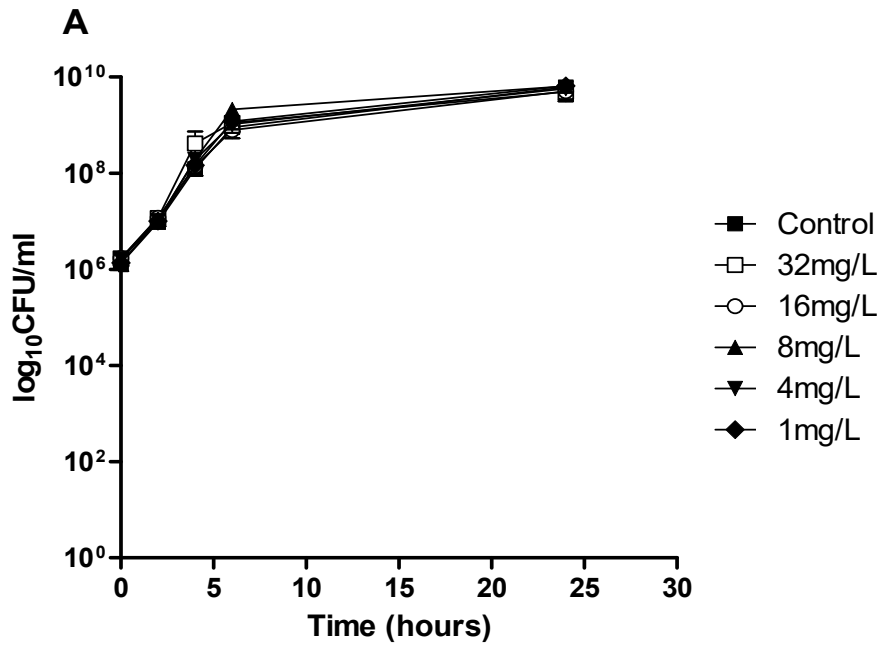
**Fig. 1.** Time-kill curves for A) *P. aeruginosa* (n=5), B) *S. aureus* (n=5) and C) *Streptococcus spp.* (n=5) challenged with ivacaftor at a range of concentrations (1-32 mg/L) plotted as the mean change in CFU/ml ( $\pm$ SD).

**Fig. 2.** Activity of ivacaftor and tobramycin (0.5 MIC) in combination against A) *P. aeruginosa* (n=5), B) *S. aureus* (n=5) and C) *Streptococcus spp.* (n=5); the mean change in CFU/ml ( $\pm$ SD) for each genera is shown.

**Fig. 3.** Effect of ivacaftor (32mg/L) and ciprofloxacin (5mg/L) on biofilm formation by *P. aeruginosa* (n=3), *S. aureus* (n=5), *Streptococcus spp.* (n=4), *Achromobacter spp.* (n=2) and *Stenotrophomonas spp.* (n=3). Adherence is plotted as the mean of 8 replicates ( $\pm$ SD). Non-adherent bacteria were not included in the analysis.  $P \leq 0.01^{**}$ ,  $P \leq 0.001^{***}$ .

**Fig. 4.** Effect of ivacaftor in comparison to tobramycin on intraperitoneal infection using *P. aeruginosa* (PAO1) in C57bl6 albino mice. A) total number of cells recovered from the peritoneal lavage, B) cell viability, C) Total viable count (TVC) of *P. aeruginosa* recovered from the peritoneal lavage, D) IL-6; E) KC. Results are plotted as the mean value from 5 mice ( $\pm$ SD).  $P \leq 0.05^*$ ,  $P \leq 0.01^{**}$ ,  $P \leq 0.001^{***}$ .

Fig. 1.



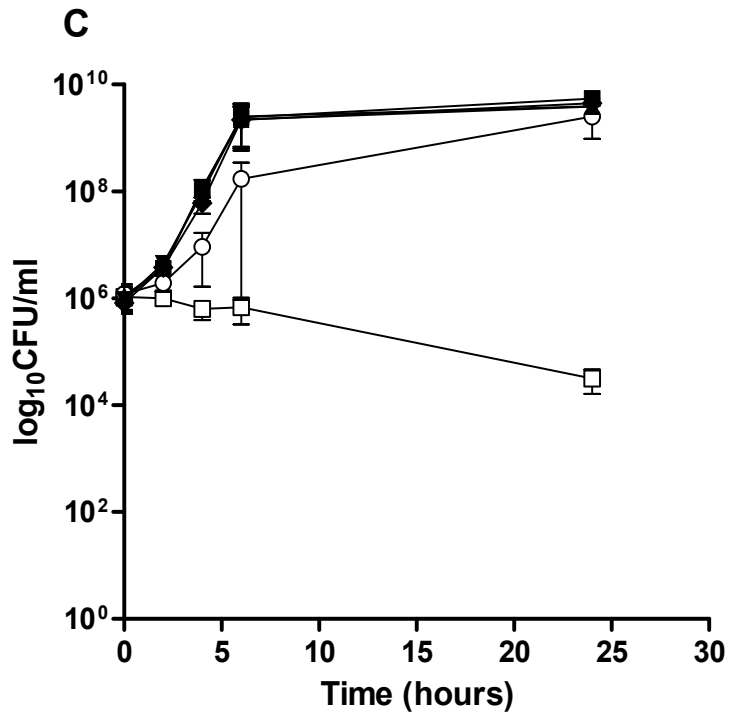
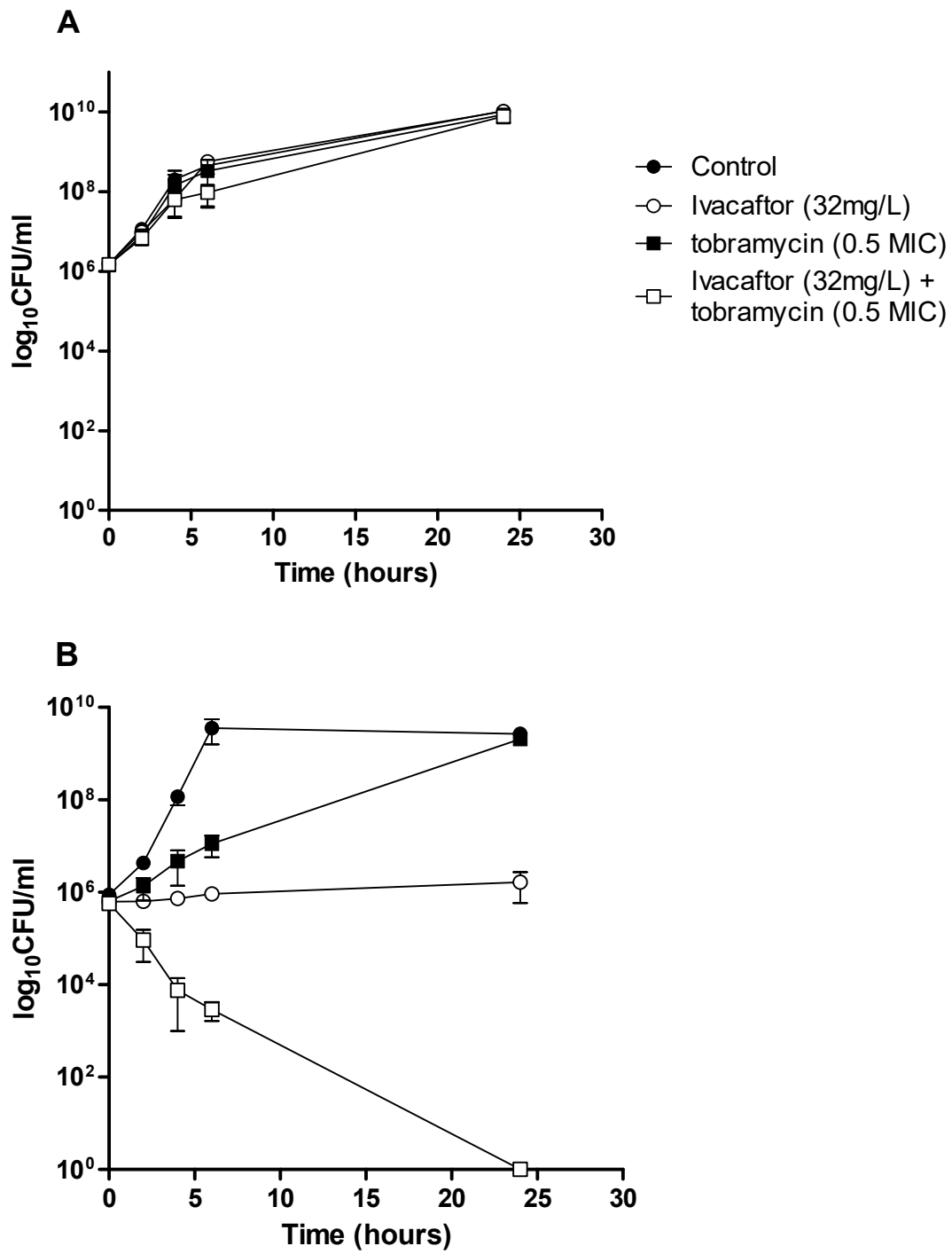


Fig. 2.



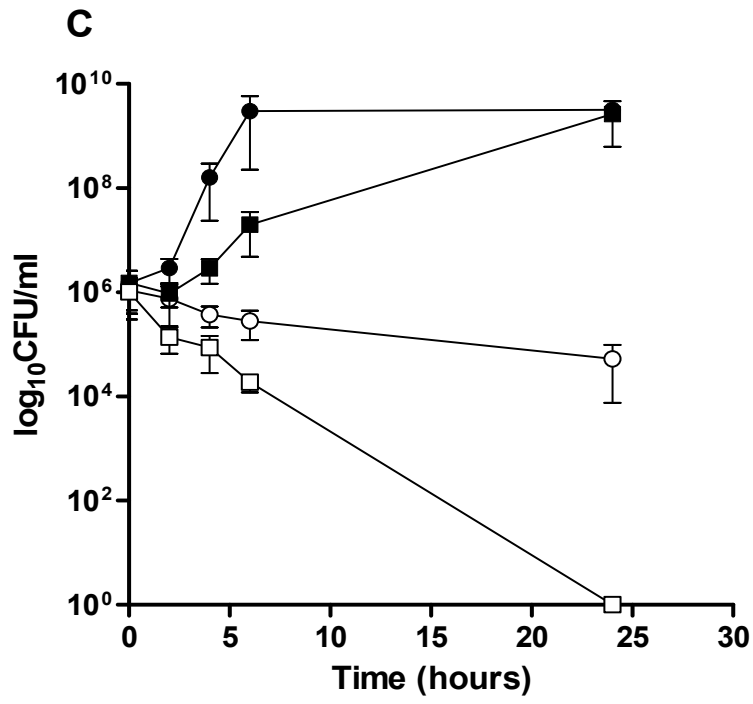


Fig. 3.

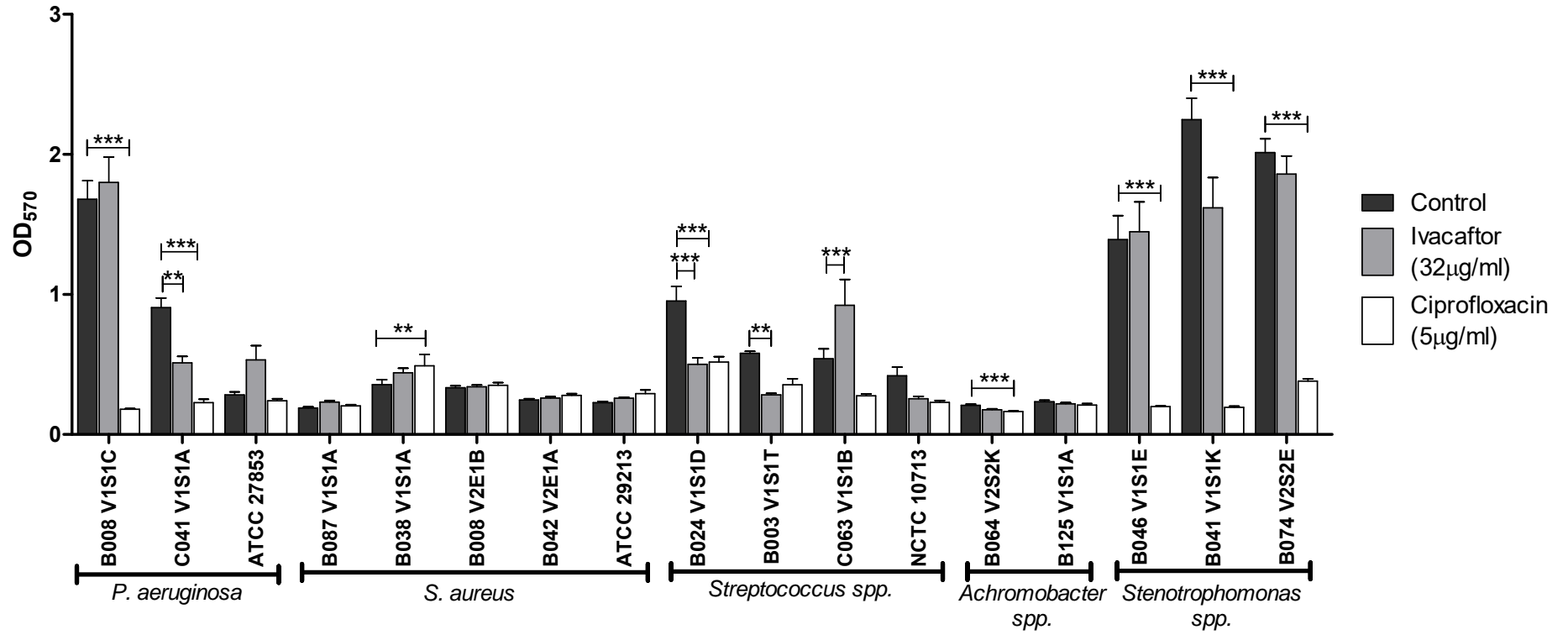
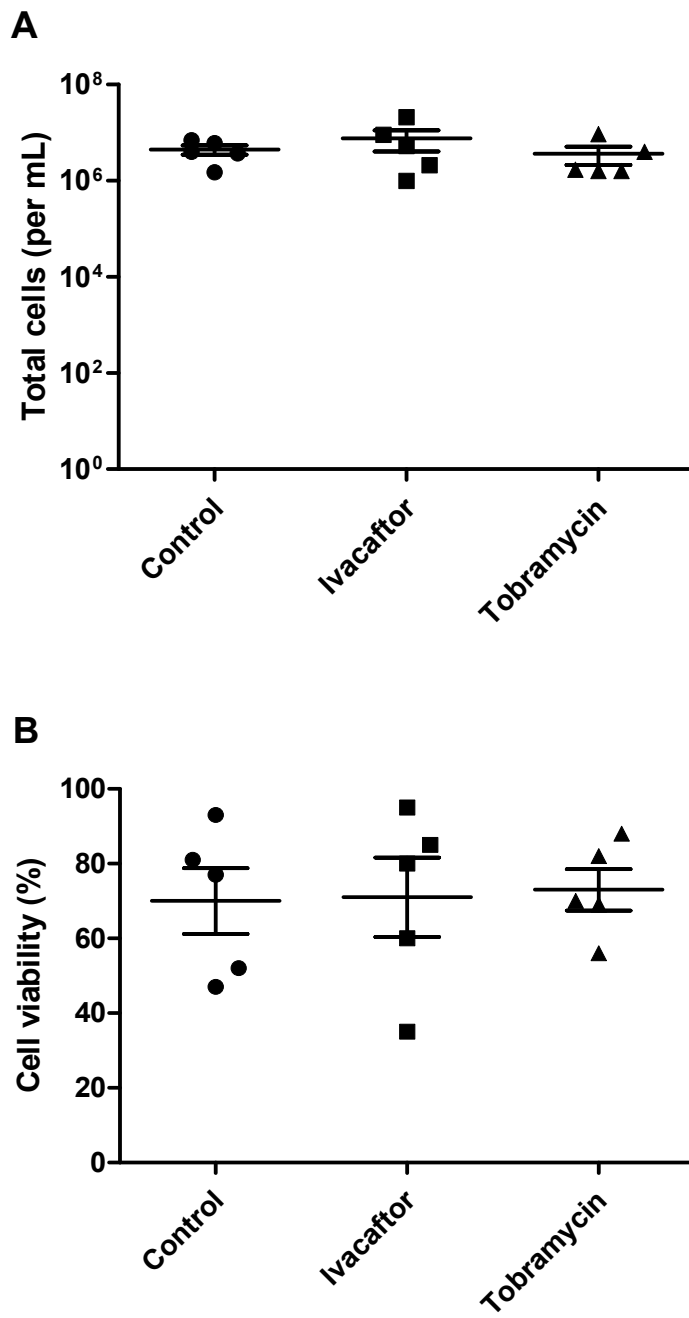
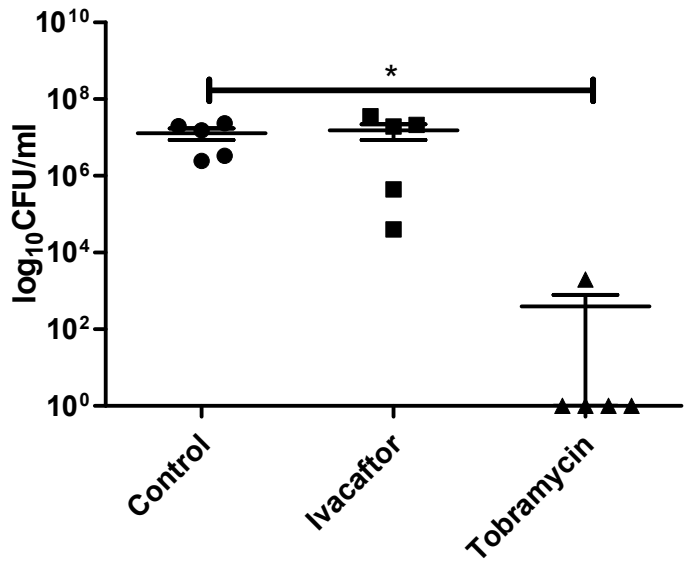


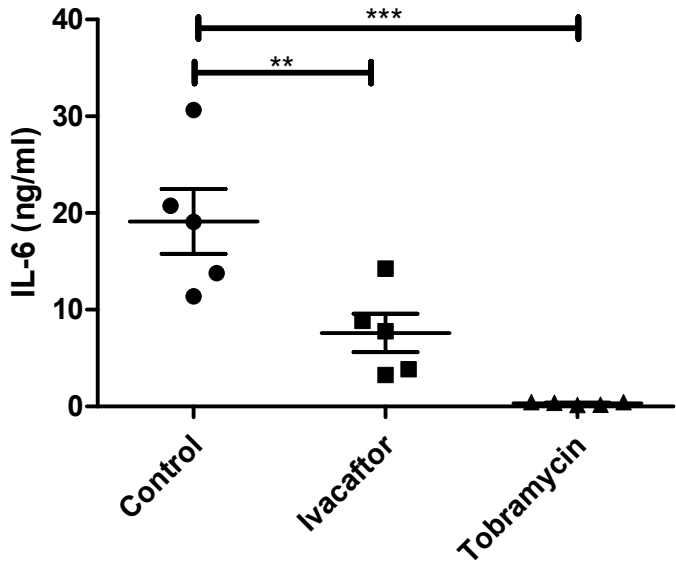
Fig. 4.



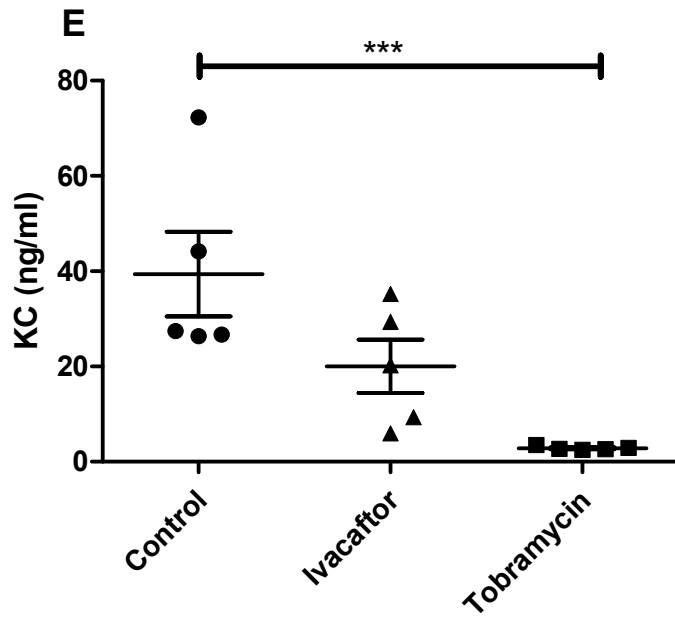
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Supplementary Table 1. MIC (mg/L) of a panel of antibiotics against the isolates tested. Breakpoints, where available, are taken from the EUCAST guidelines [39].

	Amoxicillin	Azithromycin	Ceftazidime	Chloramphenicol	Ciprofloxacin	Clindamycin	Co-amoxiclav	Colistin	Doxycycline	Meropenem	Tobramycin
<b><i>P. aeruginosa</i></b>											
B004 V2S2B	>256	>256	>256	12	2	>256	48	3	24	12	6
B008 V1S1C	3	48	1.5	16	3	>256	3	1	>256	0.094	1.5
C041 V1S1A	>256	48	1	>256	0.125	>256	>256	1.5	>256	0.38	0.75
C070 V1S1A	>256	128	1	>256	0.5	>256	256	1	>256	0.094	1
ATCC 27853	>128	32	1	>256	0.25	>256	128	4	48	0.25	0.5
<b>Breakpoints</b>			<b>R&gt;8</b>		<b>S≤0.5, R&gt;1</b>			<b>R&gt;4</b>		<b>S≤2, R&gt;8</b>	<b>R&gt;4</b>
<b><i>S. aureus</i></b>											
BO38 V1S1A <sup>#</sup>	24	>256	64	3	0.38	0.047	12	>256	4	0.5	32
BO87 V1S1A	1.5	>256	16	3	0.19	0.25	8	>256	0.094	0.38	0.38
BO42 V2E1A <sup>#</sup>	24	>256	>256	2	>32	8	48	96	0.094	>32	0.75
BO08 V2E1B <sup>#</sup>	8	>256	256	2	>32	0.047	12	32	0.064	1	0.094
ATCC 29213	0.5	0.12	8	2	0.5	0.06	0.25	>256	0.19	0.06	0.5
<b>Breakpoints</b>		<b>S≤1, R&gt;2</b>		<b>R&gt;8</b>	<b>R&gt;1</b>	<b>S≤0.25, R&gt;0.5</b>			<b>S≤1, R&gt;2</b>		<b>R&gt;1</b>
<b><i>Streptococcus sp.</i></b>											
B012 V1S1Q ( <i>S. anginosus</i> )	0.064	0.38	3	2	1.5	0.094	0.094	>256	3	0.064	96
B024 V1S1D ( <i>S. mitis</i> )	0.032	6	1	1.5	4	0.094	0.047	>256	1.5	0.064	96
B003 V1S1T ( <i>S. constellatus</i> )	0.5	>256	4	2	1	0.125	0.25	64	12	0.094	0.125
C063 V1S1B ( <i>S. constellatus</i> )	0.094	0.19	2	1.5	1.5	0.094	0.094	>256	1.5	0.094	3
NCTC 10713 ( <i>S. anginosus</i> )	0.19	0.25	3	2	1	0.064	0.38	>256	1	0.064	192
<b>Breakpoints</b>	<b>S≤0.5, R&gt;2</b>	<b>S≤0.25, R&gt;0.5*</b>		<b>R&gt;8*</b>	<b>S≤0.25, R&gt;0.5*</b>	<b>R&gt;0.5</b>			<b>S≤1, R&gt;2*</b>	<b>R&gt;2</b>	
<b><i>Achromobacter sp.</i></b>											
BO64 V2S2K ( <i>A. insolitus</i> )	4	32	2	2	1.5	>256	8	0.38	1.5	0.38	12
B125 V1S1A ( <i>A. insolitus</i> )	>256	>256	>256	3	>32	>256	>256	0.25	198	>32	>1024
BO32 V2E1D ( <i>A. spanius</i> )		>256	16	6	>32	>256	3	>256	16		>1024
<b>Breakpoints</b>	<b>No breakpoints available</b>										
<b><i>S. maltophilia</i></b>											
BO46 V1S1E	4	>256	0.75	2	4	>256	1.5	32	1.5	2	1.5
BO74 V2S2E	>256	32	32	3	2	>256	>256	4	3	>32	48
BO41 V1S1K		>256	96	8	>32	>256	64	0.38	48	>32	96
<b>Breakpoints</b>	<b>No breakpoints available</b>										

<sup>#</sup>MRSA; \*Indicates breakpoints for *S. pneumoniae* as no breakpoints available for *S. viridans* group

Supplementary Table 2. MIC (mg/L) of antimicrobial peptides against CF respiratory isolates

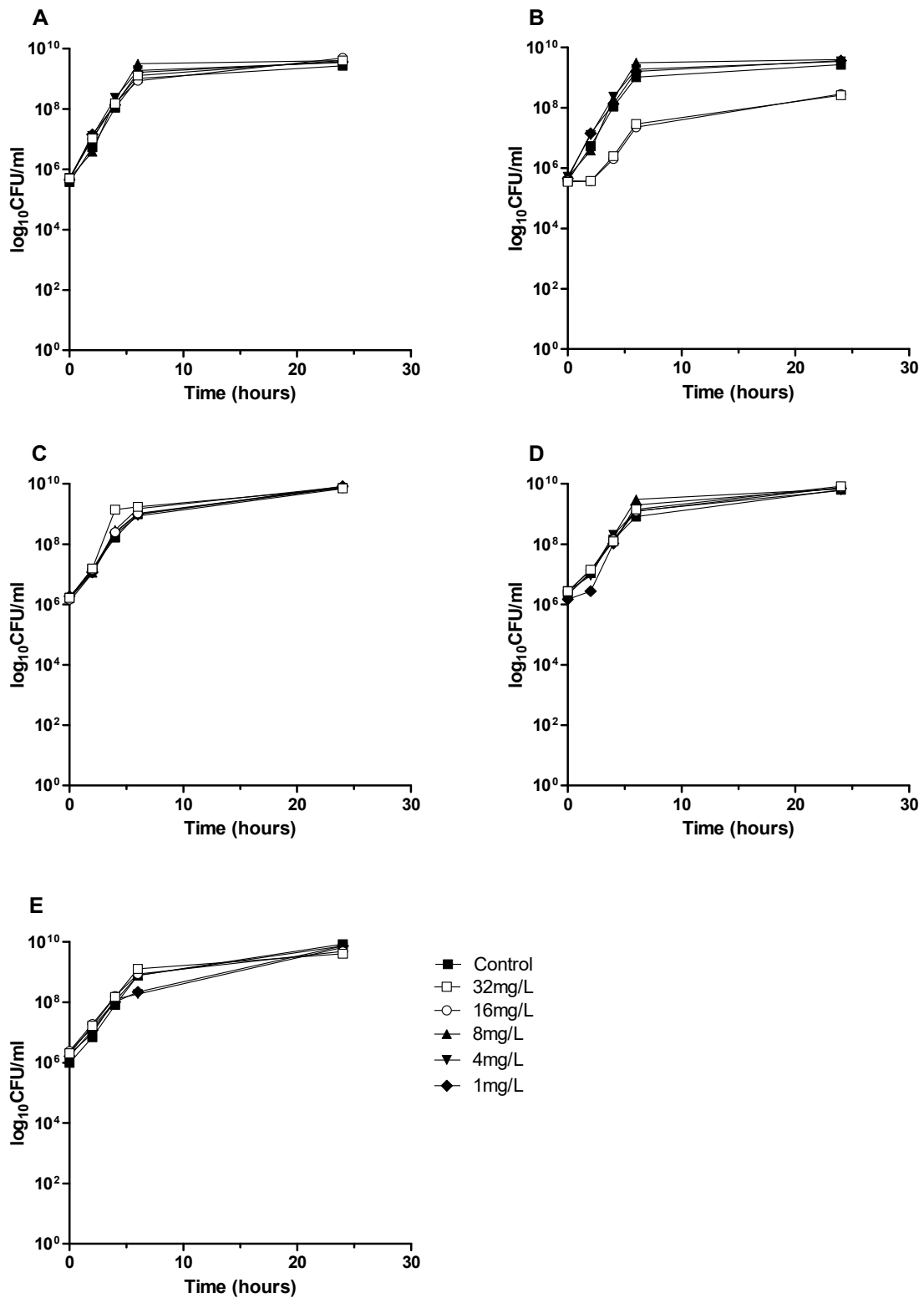
Isolate	LL37	H $\beta$ D1	H $\beta$ D2	H $\beta$ D3	H $\beta$ D4	SLPI
<b><i>P. aeruginosa</i></b>						
B004 V2S2B	22.8	$\geq 200$	$\geq 200$	8.1	49.3	$\geq 200$
B008 V1S1C	3.9	$\geq 200$	43.4	6.4	46.6	$\geq 200$
C041 V1S1A	9.5	$\geq 200$	51.3	9.6	44.1	$\geq 200$
C070 V1S1A	9.7	$\geq 200$	47.7	17.6	39.1	$\geq 200$
ATCC 27853	3.2	$\geq 200$	28.6	13.9	17.8	$\geq 200$
<b><i>S. aureus</i></b>						
BO38 V1S1A	29.9	$\geq 200$	$\geq 200$	9.2	$\geq 200$	$\geq 200$
BO87 V1S1A	17.8	$\geq 200$	$\geq 200$	8.9	$\geq 200$	$\geq 200$
BO42 V2E1A	22.4	$\geq 200$	$\geq 200$	9.4	$\geq 200$	$\geq 200$
BO08 V2E1B	15.7	$\geq 200$	$\geq 200$	9.6	$\geq 200$	$\geq 200$
ATCC 29213	21.5	$\geq 200$	$\geq 200$	12.7	$\geq 200$	$\geq 200$
<b><i>Streptococcus spp.</i></b>						
B012 V1S1Q ( <i>S. anginosus</i> )	2.4	$\geq 200$	49.9	8.9	45.7	$\geq 200$
B024 V1S1D ( <i>S. mitis</i> )	33.2	$\geq 200$	42.9	13.9	32.4	$\geq 200$
B003 V1S1T ( <i>S. constellatus</i> )	6.5	$\geq 200$	3.8	4.8	38.5	$\geq 200$
C063 V1S1B ( <i>S. constellatus</i> )	29.0	$\geq 200$	43.0	18.9	36.0	$\geq 200$
NCTC 10713 ( <i>S. anginosus</i> )	28.5	$\geq 200$	$\geq 200$	18.6	$\geq 200$	$\geq 200$
<b><i>Achromobacter sp.</i></b>						
BO64 V2S2K ( <i>A. insolitus</i> )	36.6	$\geq 200$	$\geq 200$	50.6	$\geq 200$	$\geq 200$
B125 V1S1A ( <i>A. insolitus</i> )	$\geq 200$	$\geq 200$	$\geq 200$	$\geq 200$	$\geq 200$	$\geq 200$
BO32 V2E1D ( <i>A. spanius</i> )	$\geq 200$	$\geq 200$	$\geq 200$	$\geq 200$	$\geq 200$	$\geq 200$
<b><i>S. maltophilia</i></b>						
BO46 V1S1E	3.3	$\geq 200$	$\geq 200$	23.6	$\geq 200$	$\geq 200$
BO74 V2S2E	34.6	$\geq 200$	$\geq 200$	29.4	$\geq 200$	$\geq 200$
BO41 V1S1K	18.6	$\geq 200$	47.7	9.6	51.5	$\geq 200$

Supplementary Table 3. Synergy between LL37 and tobramycin against CF respiratory isolates (change in log CFU/ml) at 3 hours.

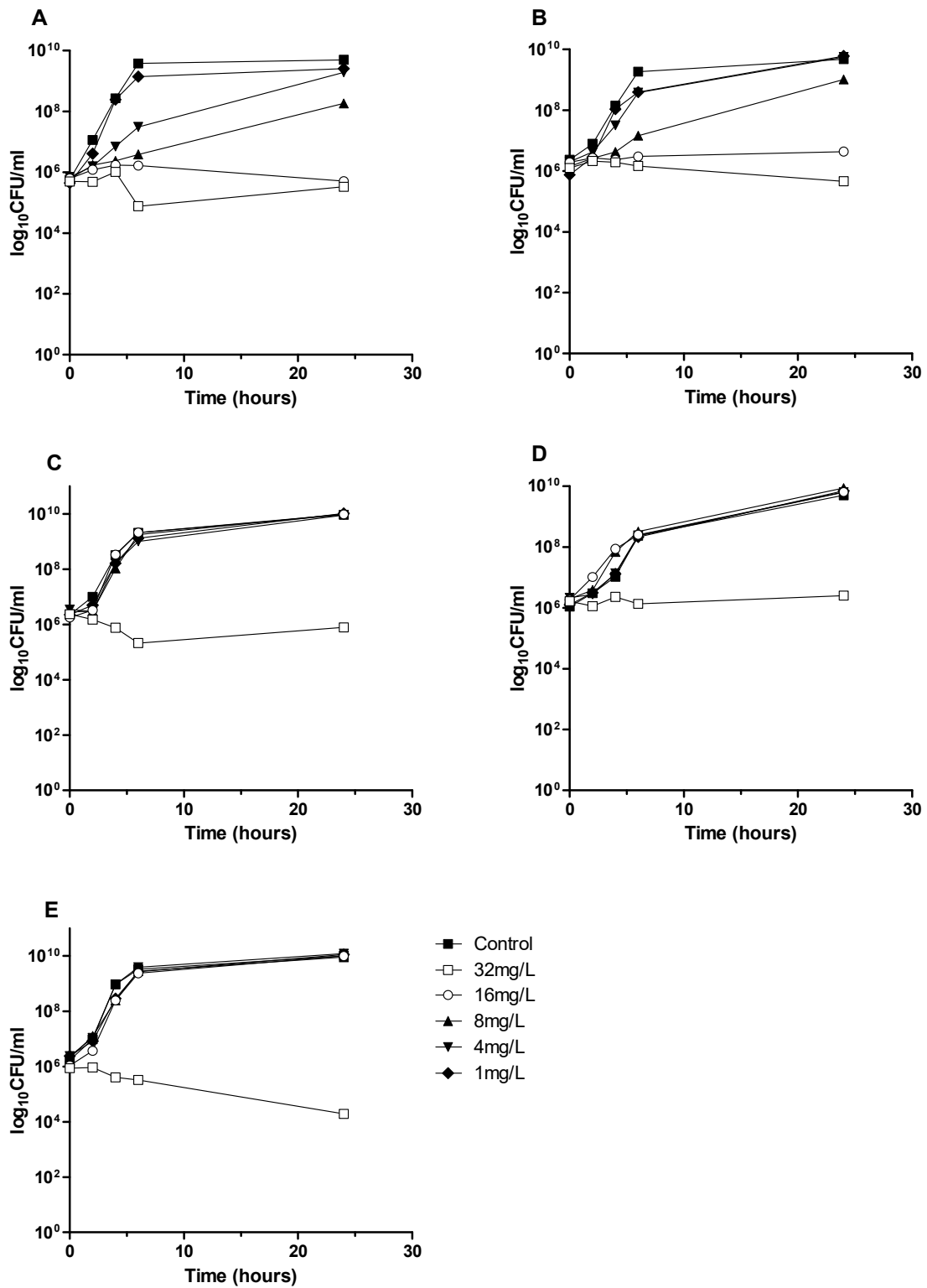
	<b>Log change in CFU/ml (<math>\pm</math>SD)</b>			
	Control	LL37 (0.5 MIC) + tobramycin (0.5 MIC)	LL37 (0.5 MIC)	Tobramycin (0.5 MIC)
<b><i>P. aeruginosa</i></b>				
B004 V2S2B	0.02	-1.17	-0.17	-0.90
B008 V1S1C	0.00	-0.70	-0.55	-0.46
C041 V1S1A	0.01	-1.52	0.05	-0.96
C070 V1S1A	0.07	-0.58	-0.41	-0.40
ATCC 27853	0.01	-0.62	-0.48	-0.16
<b>Mean</b>	0.02 ( $\pm$ 0.02)	-0.92 ( $\pm$ 0.37)	-0.31 ( $\pm$ 0.22)	-0.58 ( $\pm$ 0.31)
<b><i>S. aureus</i></b>				
BO38 V1S1A	0.01	-1.72	-0.91	-0.15
BO87 V1S1A	0.02	-4.30*	-1.61	0.02
BO42 V2E1A	0.02	-4.48*	-1.64	0.03
BO08 V2E1B	0.01	-4.30*	-1.40	0.00
ATCC 29213	0.00	-4.30*	-1.30	-0.08
<b>Mean</b>	0.01 ( $\pm$ 0.01)	-3.82 ( $\pm$ 1.05)	-1.37 ( $\pm$ 0.26)	-0.04 ( $\pm$ 0.07)
<b><i>Streptococcus spp.</i></b>				
B012 V1S1Q ( <i>S. anginosus</i> )	0.01	-4.18*	-0.10	-0.17
B024 V1S1D ( <i>S. mitis</i> )	0.02	-4.05*	-0.26	-4.05
B003 V1S1T ( <i>S. constellatus</i> )	0.08	0.21	0.14	0.03
C063 V1S1B ( <i>S. constellatus</i> )	0.01	-0.49	-0.18	-0.38
NCTC 10713 ( <i>S. anginosus</i> )	0.01	-4.14*	-0.24	-1.01
<b>Mean</b>	0.03 ( $\pm$ 0.03)	-2.53 ( $\pm$ 1.96)	-0.13 ( $\pm$ 0.15)	-1.12 ( $\pm$ 1.51)

\*Synergy (defined as a  $\geq 2$  log<sub>10</sub> decrease in total viable count (TVC) compared to the starting inoculum and as a  $\geq 2$  log<sub>10</sub> decrease in TVC by the combination compared to the most active single agent)

Supplementary Figure 1. Antimicrobial activity of ivacaftor at a range of concentrations against *P. aeruginosa* isolates; A) B004 V2S2B, B) B008 V1S1C, C) C041 V1S1A, D) C070 V1S1A and E) ATCC 27853.



Supplementary Figure 2. Antimicrobial activity of ivacaftor at a range of concentrations against *S. aureus* isolates; A) B038 V1S1A, B) B042 V2E1A, C) B087 V1S1A, D) B008 V2E1B and E) ATCC 29213.



Supplementary Figure 3. Antimicrobial activity of ivacaftor at a range of concentrations against *Streptococcus* spp. isolates; A) B012 V1S1Q (*S. anginosus*), B) C063 V1S1B (*S. constellatus*), C) B003 V1S1T (*S. constellatus*), D) B024 V1S1D (*S. mitis*), E) NCTC 10713 (*S. anginosus*).

