

1 ***In vitro* and *in vivo* interaction of caspofungin with isavuconazole against *Candida auris***
2 **planktonic cells and biofilms**

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5 Short title: Isavuconazole with caspofungin against *C. auris*

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28

29 **Abstract**

30 The *in vitro* and *in vivo* efficacy of caspofungin was determined in combination with
31 isavuconazole against *Candida auris*. Drug–drug interactions were assessed utilising the
32 fractional inhibitory concentration indices (FICIs), the Bliss independence model and an
33 immunocompromised mouse model. Median planktonic minimum inhibitory concentrations
34 (pMICs) of 23 *C. auris* isolates were between 0.5 and 2 mg/L and between 0.015 and 4 mg/L
35 for caspofungin and isavuconazole, respectively. Median pMICs for caspofungin and
36 isavuconazole in combination showed 2–128-fold and 2–256-fold decreases, respectively.
37 Caspofungin and isavuconazole showed synergism in 14 out of 23 planktonic isolates (FICI
38 range 0.03–0.5; Bliss cumulative synergy volume range 0–4.83). Median sessile MICs
39 (sMIC) of 14 biofilm-forming isolates were between 32 and >32 mg/L and between 0.5 and
40 >2 mg/L for caspofungin and isavuconazole, respectively. Median sMICs for caspofungin and
41 isavuconazole in combination showed 0–128-fold and 0-512-fold decreases, respectively.
42 Caspofungin and isavuconazole showed synergistic interaction in 12 out of 14 sessile isolates
43 (FICI range 0.023–0.5; Bliss cumulative synergy volume range 0.13–234.32). In line with the
44 *in vitro* findings, synergistic interactions were confirmed by *in vivo* experiments. The fungal
45 kidney burden decreases were more than 3 log volumes in mice treated with combination of 1
46 mg/kg caspofungin and 20 mg/kg isavuconazole daily; this difference was statistically
47 significant compared with control mice ($p < 0.001$). Despite the favourable effect of
48 isavuconazole in combination with caspofungin, further studies are needed to confirm the
49 therapeutic advantage of this combination when treating an infection caused by *C. auris*.

50

51 Keywords: *Candida auris*, biofilms, synergy, isavuconazole, mouse, echinocandin,

52

53 **1. Introduction**

54 Since its first identification more than 10 years ago, *Candida auris* has emerged as a global
55 public health threat due to its ability to cause nosocomial outbreaks of invasive infections in
56 health care facilities worldwide [1]. Previously, four major phylogenetically distinct lineages
57 (South Asian, East Asian, South African and South American) emerged simultaneously, a
58 phenomenon that highlights the global dissemination of this pathogen. In addition, a potential
59 fifth clade (Iranian origin) has also been described in the recent past [2-3].

60 *C. auris* can colonise a variety of body sites and medical implants such as central venous
61 catheters, where biofilm development is one of the most important complications [4]. Clinical
62 studies have shown that indwelling devices were the source in 89% of *C. auris* bloodstream
63 infections; these data emphasise the clinical importance of these sessile communities [5-6]. It
64 is clear that *C. auris* has exceptionally high minimum inhibitory concentrations (MICs)
65 against the three main classes of antifungals [7-9]; therefore, the potential biofilm-forming
66 ability further complicates treatment [10]. For example, echinocandins – including
67 caspofungin – are frequently administered for the treatment of invasive *C. auris* infections
68 [11-12]. However, these drugs are not expected to be effective in biofilm-related *C. auris*
69 diseases due to the 2–512-fold higher sessile MIC values to echinocandins [6]. The need for
70 novel therapeutic approaches against *C. auris* is increasing, but the development of new
71 antifungal drugs has decelerated. Therefore, a promising treatment strategy would be to
72 administer antifungals in combination, an approach that can reduce the toxicity and improve
73 the pharmacokinetics and the antifungal effect of drugs used, ultimately improving the
74 prognosis of patients [13, 14].

75 In 2016, a new broad-spectrum antifungal drug, isavuconazole, was introduced in clinical
76 practice; it has a favourable safety profile with high activity against a wide variety of fungal
77 pathogens, but the activity of isavuconazole against *C. auris* is variable [15]. Nevertheless, a

78 multicenter study revealed that isavuconazole was not inferior relative to caspofungin for the
79 primary treatment of candidaemia and invasive candidiasis [16]. Whether combinations of
80 isavuconazole with echinocandins possess synergistic interactions against *C. auris*, especially
81 against biofilms, has been poorly studied. Hence, we examined *in vitro* and *in vivo*
82 combinations of isavuconazole and caspofungin against *C. auris* isolates derived from the
83 four main clades.

84

85 **2. Material and Methods**

86 **2.1. Isolates**

87 Isolates of four different *C. auris* clades (South Asian, n = 9; East Asian, n = 4; South
88 African, n = 5; South American, n = 5) were tested; their origin is listed in Supplementary
89 Table 1. All isolates were identified to the species level by matrix-assisted laser
90 desorption/ionisation time-of-flight mass spectrometry. Clade delineation was conducted by
91 polymerase chain reaction (PCR) amplification and sequencing of the 28S ribosomal DNA
92 (rDNA) gene and the internal transcribed spacer region 1, as described previously [17-18].

93

94 **2.2. Determination of the planktonic minimal inhibitory concentration**

95 The planktonic MIC (pMIC) was determined according to the recommendations proposed by
96 the Clinical Laboratory Standards Institute M27-A3 protocol [19]. Susceptibility to
97 caspofungin pure powder (Molcan, Toronto, Canada) and isavuconazole pure powder (Merck,
98 Budapest, Hungary) was determined in RPMI-1640 (with L-glutamine and without
99 bicarbonate, pH 7.0, and with MOPS; Merck, Budapest, Hungary). The drug concentrations
100 tested ranged from 0.008 to 4 mg/L for isavuconazole and from 0.03 to 2 mg/L for
101 caspofungin. pMICs were determined as the lowest drug concentration that produces at least
102 50% growth reduction compared with the growth control. pMICs represent three independent
103 experiments per isolate and are expressed as the median. *Candida parapsilosis* ATCC 22019
104 and *Candida krusei* ATCC 6258 were used as quality control strains.

105

106 **2.3. Biofilm development**

107 *C. auris* isolates were subcultured on Sabouraud dextrose agar (Lab M Ltd., Bury, United
108 Kingdom). After 48 hours, fungal cells were harvested by centrifugation (3000 g for 5 min)
109 and were washed three times in sterile physiological saline. After the final washing step,

110 pellets were resuspended in physiological saline (ca. 5–6 mL) and were counted using a
111 Bürker chamber (Hirschmann Laborgeräte GmbH & Co. KG, Eberstadt, Germany). The
112 final density of inoculums was adjusted in RPMI-1640 broth to 1×10^6 cells/mL and 100 μ L
113 aliquots were inoculated onto flat-bottom 96-well sterile microtitre plates (TPP, Trasadingen,
114 Switzerland) and then incubated statically at 37°C for 24 hours to produce one-day-old
115 biofilms [20–22].

116

117 **2.4. Determination of the minimal inhibitory concentration of one-day-old biofilms**

118 The examined caspofungin concentrations for sessile MIC (sMIC) determination ranged from
119 1 to 32 mg/L, while the examined isavuconazole concentrations ranged from 0.008 to 2 mg/L.
120 One-day-old biofilms were washed three times with sterile physiological saline.
121 Subsequently, sMICs were determined in RPMI-1640 using a metabolic activity change–
122 based XTT assay. The percentage change in metabolic activity was calculated based on
123 absorbance (A) at 492 nm as $100\% \times (A_{\text{well}} - A_{\text{background}}) / (A_{\text{drug-free well}} - A_{\text{background}})$. sMICs were
124 defined as the lowest drug concentration resulting in at least a 50% metabolic activity
125 decrease compared with untreated control cells [20–22]. sMICs represent three independent
126 experiments per isolate and are expressed as the median.

127

128 **2.5. Evaluation of interactions by fractional inhibitory concentration index and the Bliss** 129 **independence model**

130 Interactions between caspofungin and isavuconazole were assessed by a two-dimensional
131 broth microdilution chequerboard assay [20–22]. Interactions were then analysed by
132 determining the fractional inhibitory concentration index (FICI) and using the Bliss
133 independence model [14, 20–23]. In the case of planktonic cells, the tested concentration
134 ranged from 0.008 to 2 mg/L for isavuconazole and from 0.015 to 1 mg/L for caspofungin.

135 For biofilms, the examined caspofungin concentrations ranged from 1 to 32 mg/L, while the
136 tested isavuconazole concentrations ranged from 0.008 to 2 mg/L. FICIs were calculated with
137 the widely used following formula: $\Sigma\text{FIC} = \text{FIC}_A + \text{FIC}_B = [(\text{MIC}_A^{\text{comb}}/\text{MIC}_A^{\text{alone}})] +$
138 $[(\text{MIC}_B^{\text{comb}}/\text{MIC}_B^{\text{alone}})]$, where $\text{MIC}_A^{\text{alone}}$ and $\text{MIC}_B^{\text{alone}}$ stand for MICs of drugs A and B
139 when used alone, and $\text{MIC}_A^{\text{comb}}$ and $\text{MIC}_B^{\text{comb}}$ represent the MICs of drugs A and B in
140 combination at isoeffective combinations, respectively [14, 20–23]. FICIs were determined as
141 the lowest ΣFIC . MICs of the drugs alone and of all isoeffective combinations were
142 determined as the lowest concentration resulting in at least 50% metabolic activity reduction
143 compared with the untreated control biofilms. If the obtained MIC was higher than the highest
144 tested drug concentration, the next highest twofold concentration was considered the MIC.
145 FICIs ≤ 0.5 were defined as synergistic, between > 0.5 and 4 as indifferent, and > 4 as
146 antagonistic [14, 20–23]. FICIs were determined in three independent experiments and are
147 presented as the median.

148 To further evaluate caspofungin–isavuconazole interactions, MacSynergy II analysis was
149 applied; this approach employs the Bliss independence algorithm in a Microsoft Excel–based
150 interface to determine synergy [20–24]. The Bliss independence algorithm is a well-described
151 method for the examination of the nature of drug–drug interactions. Briefly, the Bliss
152 independence algorithm calculates the difference (ΔE) in the predicted percentage of growth
153 (E_{ind}) and the experimentally observed percentage of growth (E_{exp}) to define the interaction of
154 the drugs used in combination. E_{ind} is calculated with the equation $E_{\text{ind}} = E_A \times E_B$, where E_{ind}
155 is the predicted percentage of growth that defines the effect of combination when the drugs
156 are acting alone. E_A and E_B are the experimental percentages of growth with each drug acting
157 alone. The MacSynergy II model uses interaction volumes and defines positive volumes as
158 synergistic and negative volumes as antagonistic. The obtained E values of each combination
159 are presented on the z-axis in the three-dimensional plot. Synergy or antagonism is significant

160 if the interaction log volumes are higher than 2 or lower than 2, respectively [14, 24].
161 Log volume values between > 2 and 5, between > 5 and 9, and > 9 should be considered as
162 minor synergy, moderate synergy and strong synergy, respectively. The corresponding
163 negative values define minor, moderate and strong antagonism, respectively. The synergy
164 volumes were calculated at the 95% confidence level [24].

165

166 **2.6. Infection model**

167 Pathogen-free female BALB/c mice weighing 22 to 24 g were used for the *in vivo*
168 experiments. The Guidelines for the Care and Use of Laboratory Animals were strictly
169 followed during the maintenance of mice. Animals were allowed access to food and water *ad*
170 *libitum*. *In vivo* experiments were approved by the Animal Care Committee of the University
171 of Debrecen (permission number is 12/2014). An immunocompromised mouse disseminated
172 model was used for the studies. The animals were rendered neutropenic by intraperitoneal
173 injection of cyclophosphamide (Endoxan, Baxter, Deerfield, IL, United States) 4 days (150
174 mg/kg body weight) and 1 day (100 mg/kg body weight) before infection and then 2 and 4
175 days postinfection (100 mg/kg body weight) [25]. Mice were infected intravenously through
176 the lateral tail vein with $1-1.3 \times 10^7$ colony-forming units (CFU) in 200 μ L physiological
177 saline [25]. The inoculum density was confirmed by plating serial dilutions on Sabouraud
178 dextrose agar. Mice were divided into four groups (8 mice per group): (i) untreated control;
179 (ii) 1 mg/kg/day caspofungin; (iii) 20 mg/kg/day isavuconazole; and (iv) 1 mg/kg/day
180 caspofungin + 20 mg/kg/day isavuconazole. Cresemba[®] intravenous formulation (Basilea
181 Pharmaceutica Ltd., Basel, Switzerland) was used for isavuconazole treatment. All treatment
182 arms were given intraperitoneally and started 24 hours postinfection. In the case of
183 caspofungin–isavuconazole combination, isavuconazole doses were administered 1 hour after
184 the caspofungin treatments. Control mice were given 0.5 mL sterile physiological saline

185 intraperitoneally. At 6 days postinfection, animals were euthanised; subsequently, the kidneys
186 of each mouse were removed, weighed and homogenised aseptically. Homogenates were
187 serially diluted tenfold and 100 μ L aliquots were plated onto Sabouraud dextrose agar for
188 viable fungal colony counts after incubation for 48 hours at 37°C [25]. The lower limit of
189 detection was 500 CFU/kidney. The kidney burden was analysed using the Kruskal–Wallis
190 test with Dunn’s post-test (GraphPad Prism 6.05.). Significance was defined as $p < 0.05$.

191

192

193 3. Results

194 The median and the range of MICs for planktonic and sessile *C. auris* isolates are shown in
195 Table 1. The planktonic form of the tested isolates was considered to be susceptible to
196 caspofungin based on the tentative MIC breakpoint recommended by the Centers for Disease
197 Control and Prevention (≥ 2 mg/L) [26]. By the microdilution method, the 23 isolates
198 exhibited pMICs for caspofungin alone from 0.5 to 2 mg/L, with a pMIC₅₀, pMIC₉₀ and
199 geometric mean pMIC of 1, 2 and 1.13 mg/L, respectively. In the case of isavuconazole,
200 pMICs were from 0.015 to 4 mg/L, with a pMIC₅₀, pMIC₉₀ and geometric mean pMIC of 0.5,
201 2 and 0.33 mg/L, respectively. Fourteen out of 23 isolates formed biofilms, which showed
202 significantly higher resistance to caspofungin and isavuconazole compared with planktonic
203 cells (Table 1). sMICs for caspofungin alone were from 2 to > 32 mg/L, with a sMIC₅₀,
204 sMIC₉₀ and geometric mean sMIC of > 32 , > 32 and 45.25 mg/L, respectively (64 mg/L was
205 used for geometric mean sMIC analysis in the case of sMIC > 32 mg/L). The biofilm-forming
206 isolates exhibited sMICs for isavuconazole alone from 0.5 to > 2 mg/L, with a sMIC₅₀,
207 sMIC₉₀ and geometric mean sMIC of > 2 , > 2 and 3.12 mg/L, respectively (4 mg/L was used
208 for geometric mean sMIC analysis in the case of sMIC > 2 mg/L) (Table 1).

209 The median pMICs observed in combination showed a 2–128-fold and a 2–256-fold reduction
210 for caspofungin and isavuconazole, respectively. A similar marked reduction in median
211 sMICs was observed for biofilms (a 0–128-fold and a 0–512-fold decrease for caspofungin
212 and isavuconazole, respectively) (Table 1).

213 Table 2 summarises the *in vitro* interactions between caspofungin and isavuconazole based on
214 the median FICIs. An antagonistic interaction was never observed (all FICIs ≤ 4). Using a
215 two-dimensional broth microdilution chequerboard assay and FICI calculation, the nature of
216 the caspofungin–isavuconazole interaction was synergistic for 61% of the planktonic isolates,
217 with median FICIs from 0.03 to 0.5 and a mean of the median FICI of 0.34. In the case of

218 sessile cells, synergism was observed for 86% of the 14 biofilm-forming isolates, with median
219 FICIs from 0.029 to 0.5 and a mean of the median FICI of 0.14 (Table 2).
220 FICI calculation involves Loewe additivity-based analysis assuming that both drugs have the
221 same mechanism of action, while the Bliss independence-based MacSynergy II program does
222 not have this assumption. Figure 1 shows the dose-response surfaces for caspofungin-
223 isavuconazole generated with MacSynergy II. Based on clade-specific cumulative log
224 volumes, the combination of caspofungin and isavuconazole exerted minor synergy (the
225 synergy log volume was 4.83) against planktonic isolates derived from the South African
226 clade (Figure 1C). For the South Asian, South American and East Asian clades, the synergy
227 log volumes were zero, indicating indifferent interactions (Figure 1A, B and D). In the case of
228 biofilms, 77.2, 23.21 and 234.32 cumulative synergy log volumes were observed for South
229 African, South Asian and East Asian clades, respectively, indicating strong synergistic
230 interactions (Figure 1E, G and H). By contrast, the South American clade exhibited an
231 indifferent interaction, with a cumulative synergy log volume of 0.13 (Figure 1F). Based on
232 the evaluation of *in vitro* combinations, the data derived from the FICI calculation correlate
233 with the MacSynergy analysis primarily in the case of biofilms. Although the combination of
234 caspofungin and isavuconazole was synergistic or considerably reduced the amount of drug
235 needed in some instances, the observed results may show strain specificity within clades,
236 especially in the case of planktonic cells.

237 To further evaluate the *in vivo* applicability of the caspofungin and isavuconazole
238 combination, representative isolates were chosen where synergistic and indifferent
239 interactions were observed *in vitro*, respectively. The results of the *in vivo* experiments are
240 shown in Figure 2. One mg/kg daily caspofungin treatment decreased the fungal kidney
241 burden in the case of the tested isolates; however, this therapeutic strategy was not
242 statistically different compared with untreated control mice ($p > 0.05$). The 20 mg/kg/day

243 isavuconazole treatment proved to be statistically ineffective against the tested *C. auris*
244 isolates, especially in the case of isolate 13112 ($p > 0.05$). It is noteworthy that the fungal
245 tissue burden decreases were higher than the three log decreases in mice treated with a daily
246 combination of 1 mg/kg caspofungin and 20 mg/kg isavuconazole, which was statistically
247 significant compare with control mice ($p < 0.001$) (Figure 2).

248

249

250 4. Discussion

251 The impending challenge of antifungal resistance and newly emerged fungal pathogens
252 necessitates bold and innovative therapeutic solutions [27]. In recent years, combination-
253 based antifungal treatments have become a promising therapeutic approach, especially against
254 multidrug-resistant fungal species such as *C. auris*. Based on previous susceptibility studies
255 against *C. auris*, the efficacy of *in vitro* combinations has shown high variability; in addition,
256 the degree of activity is highly strain – or rather clade – specific [28–30].

257 Isavuconazole is recommended primarily for the treatment of invasive aspergillosis and
258 mucormycosis; however, it has also exerted variable *in vitro* activity against several *Candida*
259 species [15]. Sanglard and Coste (2016) reported that the activity range of isavuconazole is
260 similar to that of voriconazole against the *Candida* strains they tested, findings that were
261 confirmed by Marcos-Zambrano *et al.* (2018), who showed high *in vitro* activity of
262 isavuconazole against clinically relevant *Candida* species, particularly against *C. albicans*
263 [31, 32]. Desnos-Ollivier *et al.* (2019) reported an isavuconazole MIC of 0.015 mg/L against
264 planktonic *C. auris*; however, they tested only two strains [33]. Regarding clinical findings,
265 the ACTIVE trial compared intravenous isavuconazole to intravenous caspofungin followed
266 by oral isavuconazole in a phase 3 randomised, double-blind clinical trial for patients with
267 *Candida* bloodstream infection. These results support the use of isavuconazole as a potential
268 therapy for candidiasis [16].

269 To the best of our knowledge, this is the first study to examine the *in vitro* and *in vivo*
270 combined effect of isavuconazole and caspofungin against *C. auris* strains derived from four
271 different lineages focusing on both planktonic and sessile susceptibility. In the case of
272 *Aspergillus* spp., this combination showed synergistic interaction in 13% of tested strains
273 [34]. In our study, we found *in vitro* synergy for the caspofungin–isavuconazole combination
274 using chequerboard microdilution, especially based on FICI determination, which was

275 definitely pronounced in the case of one-day-old biofilms. Katragkou *et al.* [35] showed
276 synergistic interactions between isavuconazole and micafungin against *C. albicans*, *Candida*
277 *parapsilosis* and *Candida krusei* using the Bliss independence model (the degree of synergy
278 ranged from 1.8% to 16.7%), which was confirmed by time-kill curves, especially against *C.*
279 *albicans* and *C. parapsilosis*. Voriconazole exerted synergistic interaction with caspofungin
280 or other echinocandins against *C. auris* isolates using the FICI [36]. In a recent study, Pfaller
281 *et al.* [37] examined the *in vitro* activity of voriconazole or isavuconazole in combination with
282 anidulafungin; synergy or partial synergy was observed in 14% and 61% of the isolates with
283 the combination of anidulafungin plus voriconazole and in 19% and 53% of isolates for the
284 combination of anidulafungin plus isavuconazole. It is noteworthy that O'Brien *et al.* [29]
285 examined four pan-resistant *C. auris* strains derived from a New York outbreak to evaluate
286 whether they are susceptible to combinations of antifungals. Based on their results,
287 flucytosine combinations with either amphotericin B, azoles or echinocandins exhibited the
288 highest efficacy [29]. However, the combination of azoles with echinocandins had no superior
289 effect compared with monotherapy [29].

290 The number of *in vivo* experiments focusing on combination-based therapy against *C. auris* is
291 strongly limited. In the only published *in vivo* combination-based experiments, Eldesouky *et*
292 *al.* [38] observed that the examined sulfamethoxazole–voriconazole combination enhanced
293 the survival of *Caenorhabditis elegans* nematodes infected with *C. auris* by nearly 70%. Our
294 study is the first that has examined the effect of caspofungin in combination with
295 isavuconazole *in vivo* at clinically relevant concentrations using an immunocompromised
296 mouse model. Although caspofungin alone produced a remarkable reduction in the kidney
297 fungal burden, only its combination with isavuconazole was statistically superior compared
298 with the untreated control ($p < 0.001$).

299 The multidrug resistance phenotype is a well-known characteristic for *C. auris*; it may be
300 more pronounced in biofilms and further complicate treatment [6]. Based on previous
301 susceptibility testing, amphotericin B, fluconazole, voriconazole, anidulafungin, micafungin
302 and caspofungin could not completely eradicate *C. auris* biofilms *in vitro*, increasing the need
303 for effective combination therapies [39]. Certain non-antifungal agents in combination with
304 traditional antifungal drugs have been tested to eradicate *C. auris* biofilms with variable
305 efficacy [21, 22, 40]. However, to date there is no experimental evidence about the efficacy of
306 antifungal drug–drug combinations against *C. auris* biofilms. In our study, we found a
307 prominent synergistic interaction between caspofungin and isavuconazole against biofilms for
308 three out of the four clades examined. We observed indifferent interaction only in the case of
309 two hospital-derived isolates from the South American clade (13112, 13108). The origin of
310 these strains may explain the significantly higher resistance against drugs tested and the
311 observed indifferent interaction compared to other isolates.

312 It should be pointed out that our study had a limitation, namely the choice of the endpoint for
313 FICI-based assessment of antifungal combinations. To date, there is no a solid consensus
314 about which endpoint should be used [23, 24, 30]. In addition, for MacSynergy-based
315 evaluation, there is no endpoint at all, and the nature of the interaction is calculated only
316 based on the percentage of growth at given concentrations [24, 30]. Despite this limitation,
317 the therapeutic potential of the caspofungin and isavuconazole in combinations is
318 unquestionable, which was definitely confirmed against *C. auris* biofilms and our *in vivo*
319 experiments.

320 In summary, the presented synergistic combinations correspond to clinically achievable and
321 safe drug concentrations. Our findings suggest that administration of the caspofungin–
322 isavuconazole combination may help to expand the therapeutic options against *C. auris*.

323 Nevertheless, the more extensive *in vivo* correlation and significant clinical relevance of these
324 *in vitro* and *in vivo* results warrants further studies, especially in the case of biofilms.

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330

331 **Competing interests**

332 László Majoros has received conference travel grants from MSD, Astellas, Pfizer and Cidara.
333 All other authors declare no competing interests.

334

335 **Ethical approval**

336 Not required.

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516

517 **Table 1** Minimum inhibitory concentrations (MICs) of caspofungin alone and in combination
 518 with isavuconazole against *Candida auris* planktonic cells and one-day-old biofilms.

Clades	Isolates	Planktonic cells Median MIC (range) of drug used (50% O.D. reduction in turbidity)				Biofilms Median MIC (range) of drug used (50% O.D. reduction in metabolic activity)			
		Alone		In combination		Alone		In combination	
		Caspofungin (mg/L)	Isavuconazole (mg/L)	Caspofungin (mg/L)	Isavuconazole (mg/L)	Caspofungin (mg/L)	Isavuconazole (mg/L)	Caspofungin (mg/L)	Isavuconazole (mg/L)
South Asian	10	>1 ^a	>2 ^b (2- >2)	0.25 (0.25-0.5)	0.015 (0.008-0.015)	>32 ^c	>2 ^b	0.5	0.015 (0.015-0.06)
	12	1	1 (0.5-1)	0.5 (0.015-0.5)	0.015 (0.015-0.25)	>32 ^c	>2 ^b	0.5	0.015 (0.008-0.015)
	20	>1 ^a	2 (2- >2)	1	0.015 (0.015-1)	>32 ^c	>2 ^b	0.5	0.03
	27	>1 ^a (1- >1)	1 (0.5- >2)	0.25 (0.25-0.5)	0.015 (0.015-0.06)	>32 ^c	>2 ^b	0.5	0.015 (0.008-0.015)
	33	>1 ^a	0.06	0.015 (0.015-0.03)	0.008	NA	NA	NA	NA
	82	>1 ^a	2	0.25 (0.25-1)	0.25 (0.03-0.25)	>32 ^c	>2 ^b	4 (4-8)	0.06 (0.008-0.06)
	164	>1 ^a	0.5	0.5 (0.5-1)	0.06 (0.03-0.06)	NA	NA	NA	NA
	174	>1 ^a	0.06	0.015 (0.015-0.25)	0.008	NA	NA	NA	NA
196	>1 ^a	0.06	0.06 (0.06-0.125)	0.008 (0.008-0.015)	NA	NA	NA	NA	
East Asian	15	0.5	0.5 (0.25-0.5)	0.25	0.25	>32 ^c	>2 ^b	0.5	0.125
	12372	1	0.5	0.125	0.008 (0.008-0.015)	>32 ^c	>2 ^b	4	1
	12373	0.5	0.5	0.25	0.25 (0.25-0.125)	NA	NA	NA	NA
	Type strain (NCPF 13029)	0.5	0.03	0.015	0.008	NA	NA	NA	NA
South African	2	1	0.125 (0.06-0.125)	0.015	0.008 (0.008-0.015)	NA	NA	NA	NA
	185	1	0.25 (0.125-0.25)	0.015	0.03 (0.03-0.015)	NA	NA	NA	NA
	204	0.5	0.125 (0.06-0.125)	0.015	0.03	32 (16-32)	>2 ^b	0.5	0.008
	206	1	0.25 (0.125-0.25)	0.015	0.008	NA	NA	NA	NA
	228	1	0.06	0.03 (0.015-0.06)	0.008 (0.008-0.015)	32	>2 ^b	0.5	0.008 (0.008-0.015)
South American	I-24	>1 ^a	1 (0.5-1)	0.015 (0.015-1)	0.008 (0.008-0.03)	>32 ^c	0.5	4	0.125
	I-172	1	0.5	0.5	0.25	>32 ^c	0.5 (0.5-2)	16	0.06
	13108	>1 ^a	2	1	0.008	>32 ^c	>2 ^b	>32 ^c	>2 ^b
	13112	0.5	2	0.015 (0.015-0.03)	0.5	>32 ^c	>2 ^b	>32 ^c	>2 ^b
	16565	0.5	0.015	0.015	0.008	2	1 (0.5-1)	0.5	0.06

519

520 ^aMIC is off-scale at >1 mg/L, 2 mg/L (one dilution higher than the highest tested concentration) was used for FICI analysis.

521 ^bMIC is off-scale at >2 mg/L, 4 mg/L (one dilution higher than the highest tested concentration) was used for FICI analysis.

522 ^cMIC is off-scale at >32 mg/L, 64 mg/L (one dilution higher than the highest tested concentration) was used for FICI analysis.

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528 **Table 2** *In vitro* interactions by fractional inhibitory concentration indices (FICIs) of
 529 caspofungin in combination with isavuconazole against *Candida auris* planktonic cells and
 530 one-day-old biofilms.
 531

Clades	Isolate	Planktonic cells		Biofilms	
		FICI		FICI	
		Median (range) of FICI	Interaction	Median (range) of FICI	Interaction
South Asian	10	0.28 (0.28-0.312)	Synergy	0.037 (0.037-0.068)	Synergy
	12	0.03 (0.03-1)	Synergy	0.076 (0.023-0.076)	Synergy
	20	0.51 (0.51-0.75)	Indifferent	0.023	Synergy
	27	0.5 (0.25-0.5)	Synergy	0.029 (0.029-0.038)	Synergy
	33	0.313 (0.258-0.375)	Synergy	NA	NA
	82	0.375 (0.25-0.51)	Synergy	0.155 (0.133-0.155)	Synergy
	164	0.62 (0.56-0.75)	Indifferent	NA	NA
	174	0.383 (0.375-0.5)	Synergy	NA	NA
	196	0.53 (0.5-0.625)	Indifferent	NA	NA
East Asian	15	1 (0.75-1)	Indifferent	0.5	Synergy
	12372	0.37 (0.31-0.5)	Synergy	0.375 (0.187-0.375)	Synergy
	12373	0.5 (0.5-1)	Synergy	NA	NA
	Type strain (NCPF 13029)	0.296	Synergy	NA	NA
South African	2	0.37 (0.255-0.49)	Synergy	NA	NA
	185	0.245 (0.245-0.255)	Synergy	NA	NA
	204	0.51 (0.49-0.54)	Indifferent	0.019	Synergy
	206	0.255 (0.255-0.3)	Synergy	NA	NA
	228	0.53 (0.5-0.625)	Indifferent	0.03 (0.03-0.06)	Synergy
South American	I-24	0.51	Indifferent	0.5 (0.5-0.56)	Synergy
	I-172	1	Indifferent	0.31 (0.28-0.37)	Synergy
	13108	0.5	Synergy	2	Indifferent
	13112	0.37 (0.37-0.5)	Synergy	2	Indifferent
	16565	0.563 (0.563-0.593)	Indifferent	0.31	Synergy

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534

535 **Figure legends**

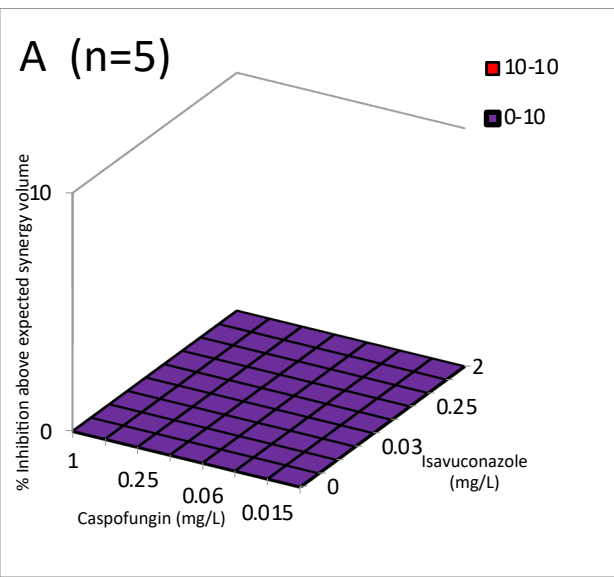
536 **Figure 1**

537 Effect of caspofungin in combination with isavuconazole against planktonic (A-D) and sessile
538 (E-H) *Candida auris* isolates using MacSynergy II analysis. Positive values show synergy,
539 while negative values indicate antagonism at given concentrations. The volumes are
540 calculated at the 95% confidence interval.

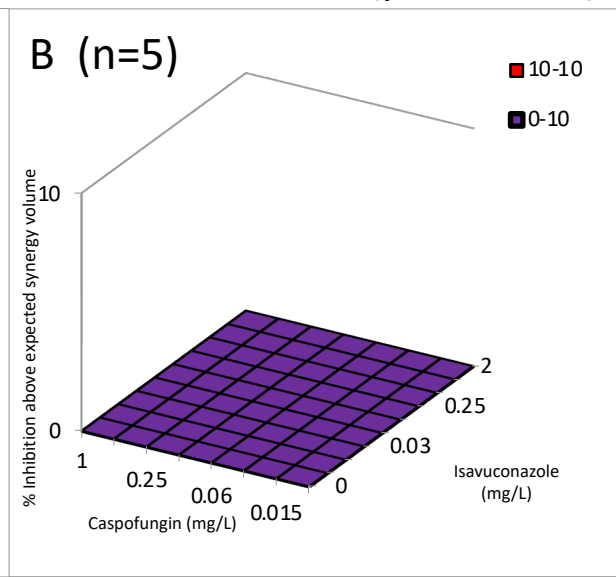
541 **Figure 2**

542 Kidney tissue burden of deeply neutropenic BALB/c mice infected intravenously with
543 *Candida auris* 12 (A) and 13112 (B) isolates. Daily intraperitoneal caspofungin (CAS)
544 (1mg/kg/day) and isavuconazole (ISA) (20 mg/kg/day) treatment was started 24 hours after
545 the infection. Tissue burden experiments were performed on day 6 post-infection. Bars
546 represent means \pm standard error of mean. *** corresponds to $p < 0.001$ compared with the
547 control population.

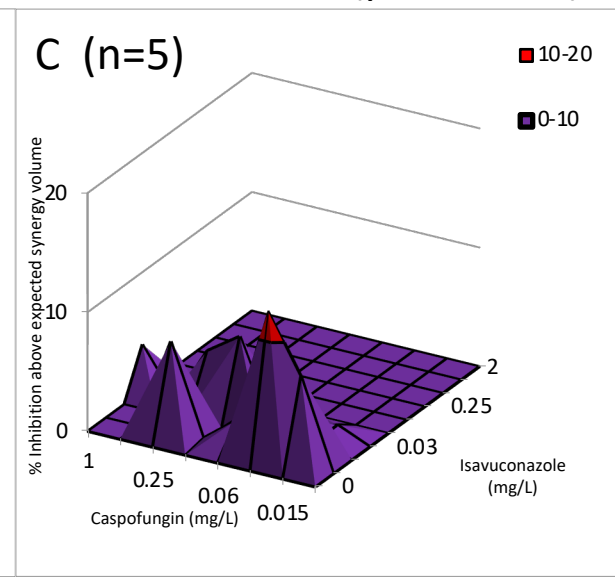
South Asian (planktonic)



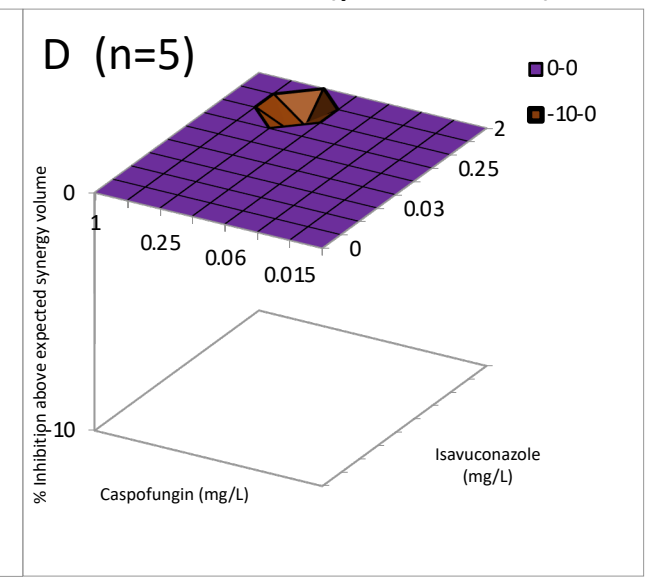
South American (planktonic)



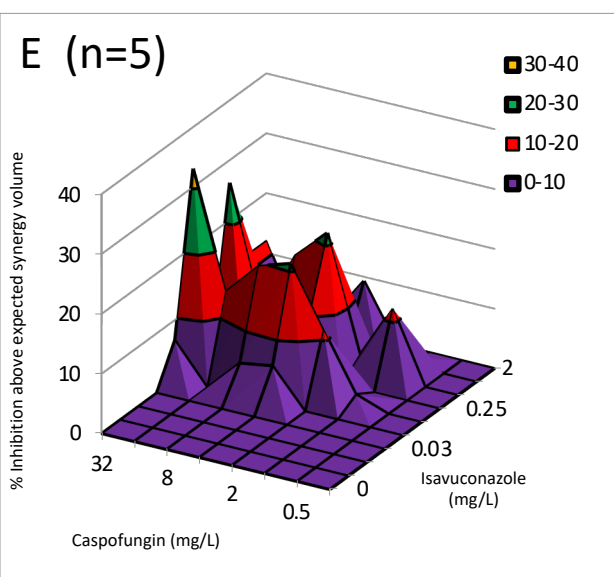
South African (planktonic)



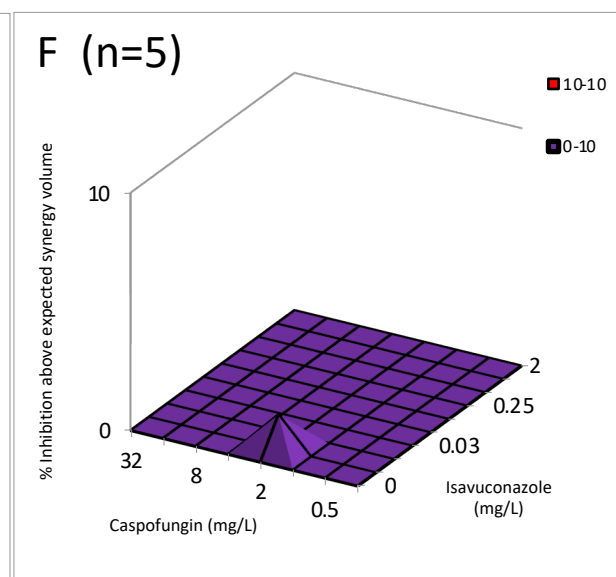
East Asian (planktonic)



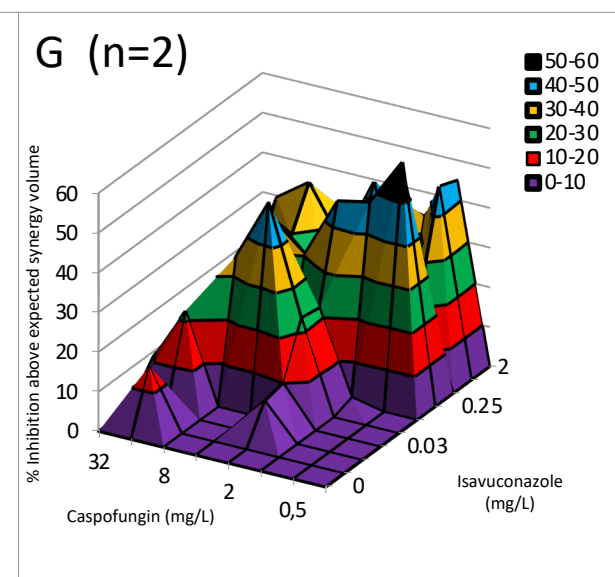
South Asian (biofilm)



South American (biofilm)



South African (biofilm)



East Asian (biofilm)

