# In vitro pharmacodynamic modelling of anidulafungin against *Candida* spp.

Sandra Gil-Alonso [a,b], Nerea Jauregizar [b], Ignacio Ortega [c], Elena Eraso [a], Elena Suárez [b], Guillermo Quindós [a,*]

[a] *Departamento de Inmunología, Microbiología y Parasitología, Unidad de formación e investigación multidisciplinar 'Microbios y Salud' (UFI 11/25), Facultad de Medicina y Odontología, Universidad del País Vasco/Euskal Herriko Unibertsitatea, Bilbao, Spain*

[b] *Departamento de Farmacología, (UFI 11/25), Facultad de Medicina y Odontología, Universidad del País Vasco/Euskal Herriko Unibertsitatea, Bilbao, Spain*

[c] *Departamento de Investigación, Desarrollo e Innovación, Faes Farma S.A., Bilbao, Spain*

ARTICLE INFO

*Article history: Received 6 May 2015 Accepted 16 December 2015*

*Cite: Gil-Alonso S, Jauregizar N, Ortega I, Eraso E, Suárez E, Quindós G. In vitro pharmacodynamic modelling of anidulafungin against Candida spp. Int J Antimicrob Agents. 2016 Mar;47(3):178-83. doi: 10.1016/j.ijantimicag.2015.12.011. Epub 2016 Jan 15. PMID: 26857078*

*Keywords::Pharmacodynamic, In vitro, Modelling, Anidulafungin, Candida*

* Corresponding author. Present address: Laboratorio de Micología Médica, Departamento de Inmunología, Microbiología y Parasitología (UFI 11/25 'Microbios y Salud'), Facultad de Medicina y Odontología, Universidad del País Vasco/Euskal Herriko Unibertsitatea, Apartado 699, E-48080 Bilbao, Spain. Tel.: +34 94 601 2854; fax: +34 94 601 3495.

*E-mail address*: guillermo.quindos@ehu.es (G. Quindós).

ABSTRACT

The aim of this study was to fit anidulafungin in vitro static time–kill data from nine strains of *Candida* with a pharmacodynamic (PD) model in order to describe the antifungal activity of this drug against *Candida* spp. Time–kill data from strains of *Candida albicans*, *Candida glabrata* and *Candida parapsilosis* clades were best fit using an adapted sigmoidal $E_{max}$ model and resulted in a set of PD parameters ($E_{max}$, $EC_{50}$ and Hill factor) for each fungal strain. The data were analysed with NONMEM 7. Anidulafungin was effective in a species- and concentration-dependent manner against the strains of *C. albicans*, *C. glabrata* and *C. parapsilosis* clades as observed with the $EC_{50}$ estimates. Maximum killing rate constant ($E_{max}$) values were higher against *C. glabrata* and *C. parapsilosis* complex strains. In conclusion, we demonstrated that the activity of anidulafungin against *Candida* can be accurately described using an adapted sigmoidal $E_{max}$ model.

## 1. Introduction

Invasive candidiasis remains a significant cause of global morbidity and mortality, especially among patients with underlying immunosuppression. *Candida albicans* remains the predominant cause of candidaemia and invasive candidiasis, accounting for 50% of all cases. However, the incidence of infections due to non-*albicans Candida* spp., such as *Candida parapsilosis* and *Candida glabrata*, is increasing [1]. These species are closely related to two phenotypically similar cryptic species. *Candida dubliniensis* and *Candida africana* are within the *C. albicans* clade; *Candida bracarensis* and *Candida nivariensis* are two species closely related to *C. glabrata*, and *Candida orthopsilosis* and *Candida metapsilosis* are newly recognised members of the *C. parapsilosis* complex of species.

The use of anidulafungin, a new class of antifungal agent, to treat serious *Candida* infections is increasing. Anidulafungin inhibits 1,3-β-D-glucan synthase, an enzyme that is necessary for synthesis of an essential component of the cell wall of several fungi. There are reports of species with decreased susceptibility to anidulafungin, such as isolates of *C. parapsilosis* and *C. glabrata* [2–4].

The parameter most commonly used to quantify the antifungal activity of a drug is the minimum inhibitory concentration (MIC). Although the MIC is a well-established in vitro pharmacodynamic (PD) parameter routinely determined in microbiology, this parameter has several disadvantages. For instance, the MIC does not provide information on the rate of fungal kill, and since MIC determination depends on the number of micro-organisms at a single time point, many different combinations of growth and kill rates can result in the same MIC.

Antifungal activity is a dynamic process, whereas the MIC is only a threshold value, a one-point measurement with poor precision determined in two-fold dilution steps. An alternative PD approach, namely microbial time–kill curves, has been proposed to offer detailed information about the antimicrobial efficacy as a function of both time and drug concentration [5,6].

Although time–kill curves can be studied using animal models of infection, in vitro models offer significant advantages in cost, convenience and time, as well as allowing direct investigation of the drug–microbe interaction in a controlled and reproducible manner [7]. Once the specific time–kill experiments have been performed, the results can be accurately described using PD mathematical models and the respective PD parameters can be calculated.

The aims of this study were: (i) to establish a general mathematical model that is appropriate for describing the in vitro pharmacodynamics of anidulafungin in static time–kill curve experiments, and to obtain model parameters such as the concentration producing 50% of the maximal effect ($EC_{50}$) and the maximal effect ($E_{max}$); and (ii) to apply this model in order to compare the in vitro PD features of anidulafungin against different *Candida* strains.

## 2. Materials and methods

### *2.1. Fungal strains*

Nine reference strains were included in this study, including *C. albicans* NCPF 3153, *C. dubliniensis* NCPF 3949, *C. africana* ATCC 2669, *C. glabrata* ATCC 90030, *C. nivariensis* CECT 11998, *C. bracarensis* NCYC 3133, *C. parapsilosis* ATCC 22019 (QC strain), *C. metapsilosis* ATCC 96143 and *C. orthopsilosis* ATCC 96139.

### *2.2. Antifungal agents*

Anidulafungin (Pfizer SLU, Madrid, Spain) was dissolved in dimethyl sulfoxide (DMSO) to obtain a stock solution of 5120 mg/L. The dilutions were prepared in RPMI 1640 medium with L-glutamine and without $NaHCO_2$ buffered to pH 7 with 0.165 M morpholinepropanesulfonic acid (MOPS) (Sigma-Aldrich, Madrid, Spain). Stock solutions were stored at – 80 °C until use.

### *2.3. Determination of minimum inhibitory concentrations (MICs) and minimum fungicidal concentrations (MFCs)*

The MIC, defined as the minimum concentration producing ≥50 growth reduction, was determined following Clinical and Laboratory Standards Institute (CLSI) guidelines [8,9]. The MFC, defined as the lowest drug concentration that killed ≥99.9% of the final inoculum, was determined as described by Cantón et al. [10]. All MICs and MFCs were measured in RPMI 1640 medium buffered to pH 7.0 with 0.165 M MOPS and the results were read after 24 h of incubation.

*2.4. Time–kill procedures*

Before time–kill curve studies were performed, the antifungal carryover effect was determined as previously described by Cantón et al. [11]. Time–kill studies were performed as previously described [12–15]. Time–kill studies were carried out on microtitre plates for the computer-controlled microbiological incubator BioScreen C MBR (LabSystems, Vantaa, Finland) in RPMI (final volume 200 μL) using an inoculum size of 1–5 $10^5$ CFU/mL. Anidulafungin concentrations assayed ranged from 0.015–32 mg/L. These concentrations were selected based on the MIC determined for each species complex. When these concentrations did not lead to the maximum effect, additional higher concentrations were tested to achieve this target. Once the $E_{max}$ was attained, the concentration range was considered acceptable for parameter estimation. Plates were incubated for 48 h at 36 ± 1 °C (30 ± 1 °C for *C. africana*) without agitation. At predetermined time points (0, 2, 4, 6, 24 and 48 h), 10 μL (0–6 h) or 6 μL (24–48 h) was removed from both the control well (without drug) and each test solution well and was serially diluted in phosphate-buffered saline to determine the number of CFU/mL. Technical variability for the ranges of volumes used was CV = 0.5–0.8%. Volumes of 5, 10, 50 or 100 μL (depending on the dilution and concentration of the drug) were plated onto Sabouraud dextrose agar and were incubated at 36 ± 1 °C (30 ± 1 °C for *C. africana*) for 24–48 h. When the colony counts were expected to be <200 CFU/mL, samples of 5 μL were taken directly from the test solution and were plated. The lower limit of accurate and reproducibly detectable colony counts was 200 CFU/mL. Time–kill curve studies were conducted in duplicate and on two different days.

*2.5. Mathematical modelling of time–kill data*

Time–kill curve analysis and mathematical modelling of the time–kill curve data were performed using a non-linear mixed-effects approach as appropriate with NONMEM 7 (ICON Development Solutions, USA).

A previously described adapted $E_{max}$ model [16] was tried to fit to the log-transformed data of the static time–kill curve experiments of anidulafungin. This model accounts for delays in *Candida* growth and onset of killing as well as the maximum number of *Candida:*

$$\frac{\mathrm{d}N}{\mathrm{d}t} = \left[K_{\mathrm{g}}\left(1 - \frac{N}{N_{\max}}\right).\left(1 - \mathrm{e}^{-\alpha t}\right) - \left(\frac{E_{\max}.C^{h}}{\mathrm{EC}_{50}^{h} + C^{h}}\right).\left(1 - \mathrm{e}^{-\beta t}\right)\right].N$$

In this model, d$N$/d$t$ is the change in the number of *Candida* as a function of time; $K_g$ ($h^{-1}$) is the cell growth rate constant in the absence of drug; $E_{max}$ ($h^{-1}$) is a model estimated parameter that accounts for the maximum killing rate constant, it is not the maximum observed effect; $EC_{50}$ (mg/L) is the drug concentration necessary to produce 50% of the maximum effect; $C$ (mg/L) is the concentration of antifungal drug at any time ($t$); and $N$ is the number of viable *Candida* (CFU/mL).

This model also took into account the following factors: (i) in in vitro systems, available space and nutrients are limited. The factor that accounts for the resulting saturation of growth is the maximum number of fungi ($N_{max}$); (ii) isolates have not yet reached the logarithmic growth phase at time zero, i.e. delay in growth: $(1 - \mathrm{e}^{-\alpha t})$; (iii) delay in the

onset of killing: $\left(1-\mathrm{e}^{-\beta t}\right)$; and (iv) a Hill factor or sigmoidicity factor (*h*) modified the steepness of the slopes and smoothed the curves with a concentration increase.

Since anidulafungin concentrations did not change during the time–kill experiments, *C* was constant for the entire fitted time period. For each fungal strain, the initial estimates of $K_g$, $N_{max}$ and $\alpha$ were determined using the data for the growth rate in the absence of anidulafungin (control data). Thereafter, $K_g$, $N_{max}$ and $\alpha$ were fixed in each model at their determined values in the initial fit, whereas the drug parameters $E_{max}$, $EC_{50}$, $\beta$ and *h* were fitted simultaneously to the experimental data. The correlation between growth rate and $E_{max}$ was analysed by non-parametric correlation test of Spearman.

A first-order conditional estimation method algorithm was used, as implemented in the non-linear mixed-effects modelling software NONMEM 7. Interindividual variability of the model parameters was not included in the final model because the fungal inocula were obtained from a pure culture so that all of the experimental fungal cultures were assumed to be genetically identical. The residual variability was estimated by using an additive model. Evaluation of the model performance included analysis of standard diagnostic plots, objective function value and the precision of the parameter estimates, as well as visual inspection of the data for the quality of fit.

## 3. Results

### *3.1. Determination of minimum inhibitory concentrations and minimum fungicidal concentrations*

Anidulafungin MICs ranged from 0.03 mg/L for *C. albicans* complex strains to 1 mg/L for *C. parapsilosis* complex strains. Anidulafungin MFCs ranged from 1 mg/L for *C. nivariensis* to >16 mg/L for *C. albicans* complex strains (Table 1).

### *3.2. Static time–kill curves and pharmacodynamic model*

The fitted time–kill curves for the described model against the nine strains of *Candida* in the absence of anidulafungin (control) and in the presence of constant anidulafungin concentrations are shown in Fig. 1.

The determined PD parameters and their standard error of the estimate values (SEE%) for each individual strain are listed in Table 1. The results indicate that the model chosen is appropriate for fitting the data (Table 1; Fig. 2). Summarising the data, growth rates in the absence of antifungal were similar for the studied isolates (0.27–0.4 $h^{-1}$), except for *C. africana* ATCC 2669 and *C. parapsilosis* ATCC 22019, which showed slower growth rates in the absence of anidulafungin (0.12 $h^{-1}$ and 0.13 $h^{-1}$, respectively). No statistical correlation was obtained between fungal growth rate and anidulafungin maximum effect (maximum killing rate constant) ($P > 0.05$). Similarly, no differences in the $N_{max}$ fungal parameter was detected (7.23–7.63 CFU/mL). The $EC_{50}$ values estimated in the present study demonstrated that anidulafungin was effective in a species-dependent manner against the strains of *C. glabrata* and *C. parapsilosis* clades. $EC_{50}$ values ranged from 0.001 mg/L to 2.53 mg/L. The highest $EC_{50}$ value

was obtained against *C. parapsilosis* ATCC 22019, whilst the lowest value was reached against *C. africana* ATCC 2669.

Maximum killing rate constant ($E_{max}$) values were higher against strains of *C. glabrata* complex and *C. parapsilosis* complex, with the highest $E_{max}$ value against *C. parapsilosis* ATCC 22019 (Table 1).

In the presence of anidulafungin, the growth of *C. albicans* complex strains was delayed but no decay was observed from the starting inoculum, even at the highest concentrations tested, whereas in the presence of a sufficiently high concentration of drug decays of *C. glabrata* and *C. parapsilosis* complex over time were observed. Accordingly, the sigmoid $E_{max}$ model adequately described anidulafungin data.

### *3.3. Model diagnostics*

The diagnostic plots of the final model presented in Fig. 2 show random uniform scatter around the line of identity (Fig. 2A) and suggested the absence of any trend or bias ($R^2$ = 0.92). Weighted residuals were randomly scattered around 0 (Fig. 2B). Although overall the model described the data well as shown in Fig. 2, there was a larger deviation for log CFU/mL < 3. It can be observed that some points present higher values for predictions than the real observed data, but these points represent only 10–15% of the observations with a log CFU/mL equal to 0.

## 4. Discussion

In vitro time–kill curves are attractive tools for studying the pharmacodynamics of antimicrobial agents as they provide detailed information on antimicrobial efficacy as a function of both time and concentration [5]. In this study, we have used a PD approach based on time–kill curves to evaluate the antifungal efficacy of the echinocandin anidulafungin against nine strains of the clades of *C. albicans*, *C. glabrata* and *C. parapsilosis*. Although widely used, MICs and MFCs do not provide very detailed characterisation of antimicrobial activity [7]. Therefore, the most sophisticated time–kill curve approach was used. To our knowledge, this is the first report of PD modelling based on time–kill curves of anidulafungin.

In this study, the obtained MICs and MFCs of anidulafungin were in the range of values typically observed with *C. albicans*, *C. glabrata* and *C. parapsilosis*. The in vitro antifungal spectrum of anidulafungin was described in a previous review and it could be observed that the anidulafungin MICs were 0.03–2 mg/L against *C. albicans* strains and ≤0.03–1 mg/L against *C. dubliniensis* [17]. Mariné et al. observed similar MICs and values of MFC > 16 mg/L against *C. dubliniensis* [18].

In the *C. albicans* clade, an increase in the effect could be observed with increasing initial anidulafungin concentrations (Fig. 1), reaching a maximum response with an initial concentration of 2 mg/L for *C. albicans* and 32 mg/L for *C. dubliniensis*. In each case, the two highest concentrations follow very similar profiles, indicating that the maximum response has been reached. Conversely, a similar effect could be observed for *C. africana* for the whole range of concentrations of anidulafungin tested, and the activity profiles of all of the concentrations were almost superimposable, indicating that

the $E_{max}$ was attained with the lowest concentrations. Accordingly, the $EC_{50}$ for *C. africana* was the lowest for all of the studies strains (Table 1).

In the case of *C. glabrata*, *C. nivariensis* and *C. bracarensis*, an increase in the effect could be observed with increasing anidulafungin concentrations. For *C. bracarensis*, a small difference was observed between the kill profiles for 0.5 mg/L and 2 mg/L, which indicates that the maximum kill rate is approached for this fungal strain at 0.5 mg/L.

Similarly, for *C. parapsilosis* an increase in the concentration of anidulafungin results in an increase in the killing effect, with a maximum at 8 mg/L. However, different kill profiles are observed with *C. metapsilosis* and *C. orthopsilosis* strains. The kill profiles for these two strains showed an absence of effect at 0.25 mg/L, whilst the maximum kill profile was attained with the following higher concentrations tested, with the kill profiles for all of these concentrations being almost superimposable.

We have previously reported different in vitro antifungal activity of anidulafungin depending on the studied strain [14,15]. The time–kill results in the current study corroborate and extend those previous studies. Nguyen et al. also observed lower activity of anidulafungin against *C. albicans* than *C. parapsilosis* in time–kill experiments, even MICs were smaller than *C. parapsilosis* [19].

The current data show activity against these susceptible strains of *C. albicans* when compared with the control growth of these strains. However, the activity was lower than that observed against strains from the other species of *Candida* tested. Cantón

et al. reported an antifungal activity of anidulafungin compared with control curves for *C. parapsilosis* and *Candida lusitaniae* [12,13]. Moreover, we have calculated the anidulafungin MFCs for the strains in this study; for *C. albicans*, *C. dubliniensis* and *C. africana* they were all >16 mg/L, contrasting with the lower MFCs for the strains from the other species (Table 1).

It should be noted that the lower activity of anidulafungin during time–kill experiments against *C. albicans* NCPF 3153, *C. dubliniensis* NCPF 3949 and *C. africana* ATCC 2669 cannot be extrapolated to the whole *C. albicans* complex. *Candida* isolates that were not killed by anidulafungin did not exhibit elevated MICs in this study, with the MFC being >16 mg/L.

The data obtained with the static time–kill curves were successfully fit using an adapted $E_{max}$ model. The $EC_{50}$ values estimated with the developed PD model demonstrated that anidulafungin was effective in a species-dependent manner. The $EC_{50}$ value estimated for *C. africana* was very low compared with the $EC_{50}$ for the other species. This may be related to the fact that even at the lowest concentration tested (0.015 mg/L) the maximal effect was achieved. The observed interstrain variability in $EC_{50}$ is an expected result, taking into account the MIC ranges among strains. A mathematical relationship exists between the $EC_{50}$ and the MIC [20]. Scarce studies on anidulafungin pharmacokinetics/pharmacodynamics with $EC_{50}$ estimation have been published. Gumbo et al. reported an anidulafungin $EC_{50}$ value (5.46 mg/L) for a strain of *C. glabrata* in a neutropenic murine model of disseminated candidiasis [21].

The Hill sigmoidicity factor (*h*) was estimated to be very high when anidulafungin had a very steep concentration–effect relationship, indicating an all-or-nothing effect. This could be observed in *C. orthopsilosis* and *C. africana* (Fig. 1). To avoid mathematical problems in the iteration process with such high values, this parameter was fixed to the lowest value that did not have a detrimental effect on the fit.

In this study, the lack of correlation between the $E_{max}$ parameter of anidulafungin for each strain and the growth rates of the isolates suggests that the highest kill rates were not achieved against the most rapidly proliferating cells. Conversely, a significant correlation was obtained for voriconazole in a previous study [16]; these differences can be related to the mechanisms of action of the two drugs, as voriconazole inhibits the biosynthesis of cell membrane ergosterol and anidulafungin inhibits cell wall 1,3-β-D-glucan biosynthesis.

Besides allowing a good summarisation and description of the data, these kinds of models may be used to make predictions and simulations of untested scenarios. It was previously demonstrated that the exposure magnitudes associated with efficacy both in vitro and in animal models are in accordance with those required for efficacy in humans [22]. Pharmacokinetic (PK) parameters from human data sets may be used in the model to obtain expected kill curves for a selected dosing schedule. Approaches that combine in vitro time–kill data with PK data collected in vivo have been used for antibacterial agents such as cefaclor [23], cefpodoxime and cefixime [5], azithromycin [24] and more recently for vertilmicin and ceftazidime [25] and to a lesser extent for antifungal drugs such as voriconazole [16], fluconazole and caspofungin [26].

The present study showed the anidulafungin free concentrations required to achieve optimal fungal kill. Further studies should be defined to establish the relationship between target tissue pharmacokinetics and plasma pharmacokinetics of anidulafungin.

Human PK parameters were used to simulate anidulafungin free concentration–time profiles assuming plasma protein binding of 99.9% (multiply total concentrations by 0.01 [27]) and then applied to the sigmoid $E_{max}$ model in order to obtain expected time–kill curves for a certain dosing regimen. However, free concentration values obtained with these simulations were very low (results not shown), quite lower than the $EC_{50}$ estimated by the models, and consequently would wrongly suggest a lack of antifungal effect of anidulafungin for several *Candida* strains.

This fact would be an expected result on the basis that anidulafungin is considered a concentration-dependent antifungal [28]. Moreover, the reduced activity related to free drug plasma concentrations would be corroborated by previous studies in which the antifungal effect of echinocandins was more closely related to tissue concentrations than plasma concentrations [29]. Anidulafungin distributes rapidly and extensively into organs affected by invasive candidiasis such as the kidneys, liver, lungs and spleen, reaching higher concentrations than in plasma. Furthermore, in animal studies it was seen that anidulafungin appears to persist longer in these tissues than in plasma, and tissue concentrations can reach ten times that of plasma [21,30]. These studies in rats suggested that anidulafungin tissue concentrations will be in the order of the $EC_{50}$ parameters estimated by the model and consequently sufficient to produce the antifungal activity observed in clinical practice. However, the relationship between the

pharmacokinetics of anidulafungin in target tissues and that in serum is not yet well understood [21]. No specific tissue distribution studies of anidulafungin have been performed in humans. In future, in vivo PK data of anidulafungin obtained from human tissues might be employed to simulate the target-site PD profile of anidulafungin, as has been previously reported for other drugs [23,31].

Although PD studies comparing the efficacy of various regimens of antifungal drugs are feasible in animal models [32], they are complicated, laborious and expensive. Although many investigators have confirmed that the behaviour of micro-organisms in an in vitro environment is not equivalent to that in vivo (e.g. changes in growth characteristics, micro-organism viability, effects of protein binding on antimicrobial activity, immune defence system, etc.), in vitro time–kill assays permit direct, controlled and reproducible studies of the interaction between antifungal drugs and fungi, and they allow comparisons among different agents and dosing strategies in a more convenient, faster and cheaper way without expending animal lives [5,16].

A limitation of this study design was that anidulafungin concentrations were constant. In future we will model anidulafungin dynamic time–kill data, with anidulafungin concentrations changing over time in a manner consistent with the PK profiles in humans.

In summary, it was demonstrated that it is possible to fit anidulafungin in vitro static time–kill data accurately using an adapted $E_{max}$ sigmoid mathematical model. The model structure may be applied to other strains and echinocandins and might provide

a tool, after further refinement and complementary tissue/plasma PK studies, for the development of improved dosing regimens.

**Acknowledgment:** SG-A received a scholarship from the Universidad del País Vasco-Euskal Herriko Unibertsitatea (Bilbao, Spain).

**Funding:** This work was supported by Consejería de Educación, Universidades e Investigación [GIC12 210-IT-696-13] and Departamento de Industria, Comercio y Turismo [SPR12UN002, S-PE13UN025] of Gobierno Vasco–Eusko Jaurlaritza, and UPV/EHU [UFI 11/25].

**Competing interests:** IO works for Faes Farma S.A; EE has received grant support from Astellas Pharma and Pfizer; GQ has received grant support from Astellas Pharma, Gilead Sciences, Pfizer and Merck Sharp & Dohme, has been an advisor/consultant to Merck Sharp & Dohme, and has been paid for talks on behalf of AbbVie, Astellas Pharma, Esteve Hospital, Gilead Sciences, Merck Sharp & Dohme and Pfizer. All other authors declare no competing interests.

**Ethical approval:** Not required.

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**Fig. 1.** Fitted time–kill curves derived from the mathematical model for constant concentrations of anidulafungin. The plots show the number of CFU/mL (mean ± standard deviation) of the different *Candida* strains for the control experiments without anidulafungin and after exposure to the different initial anidulafungin concentrations. The lines represent the curve fits from the respective data analysis. Horizontal lines indicate the quantification limit.

**Fig. 2.** Diagnostic plots: (A) observations versus predictions and (B) weighted residuals versus predictions for anidulafungin against nine reference strains of *Candida.*

**Table 1**

MIC and MFC values, pharmacodynamic (PD) parameter estimates, and residual variability against *Candida* strains

| Parameter | *C. albicans* NCPF 3153 | | *C. dubliniensis* NCPF 3949 | | *C. africana* ATCC 2669 | | *C. glabrata* ATCC 90030 | | *C. nivariensis* CECT 11998 | | *C. bracarensis* NCYC 3133 | | *C. parapsilosis* ATCC 22019 | | *C. metapsilosis* ATCC 96143 | | *C. orthopsilosis* ATCC 96139 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| MIC (mg/L) | 0.03 | | 0.03 | | 0.03 | | 0.12 | | 0.12 | | 0.12 | | 1 | | 1 | | 1 | |
| MFC (mg/L) | >16 | | >16 | | >16 | | 2 | | 1 | | 4 | | 8 | | 4 | | 2 | |
| PD parameter | Value | SEE (%) | Value | SEE (%) | Value | SEE (%) | Value | SEE (%) | Value | SEE (%) | Value | SEE (%) | Value | SEE (%) | Value | SEE (%) | Value | SEE (%) |
| $K_g$ ($h^{-1}$) [a] | 0.3 | 30.1 | 0.37 | 7.4 | 0.12 | 25.5 | 0.29 | 13.58 | 0.40 | 13.22 | 0.27 | 10.69 | 0.13 | 1.7 | 0.39 | 0.31 | 0.38 | 10.44 |
| $N_{max}$ (CFU/mL) [a] | 7.4 | 0.24 | 7.23 | 0.23 | 7.57 | 1.56 | 7.54 | 1.32 | 7.52 | 1.61 | 7.63 | 0.76 | 7.52 | 0.02 | 7.41 | 1.01 | 7.34 | 2.3 |
| $\alpha$ [a] | 0.29 | 16.85 | 0.15 | | 0.15 | | 0.1 | | 0.1 | | 0.15 | | 0.38 | 19.12 | 0.1 | | 0.15 | |
| $E_{max}$ ($^{h-1}$) | 0.08 | 7.01 | 0.11 | 5.45 | 0.03 | 3.35 | 0.24 | 13.53 | 0.23 | 3 | 0.2 | 2.98 | 0.82 | 14.48 | 0.26 | 0.98 | 0.25 | 1.36 |

| | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $EC_{50}$ (mg/L) | 0.11 | 54.39 | 0.19 | 11.34 | 0.001 | 50.68 | 0.23 | 4.63 | 0.24 | 6.54 | 0.05 | 4.18 | 2.53 | 13.44 | 0.62 | 12.38 | 1.49 | 4.4 |
| β | 0.3 | 40.67 | 0.28 | 24.35 | 0.46 | 65.13 | 0.07 | 51 | 0.32 | 25.84 | 0.13 | 31.91 | 0.7 | | 0.15 | 7.66 | 0.1 | 12.38 |
| *h* | 0.69 | 34.93 | 1.18 | 13.9 | 16 | | 2.22 | 7.84 | 2.33 | 15.45 | 2.74 | 12.92 | 3.07 | 27.04 | 3.44 | 13.28 | 10 | |
| RV | 24.50 | 28.52 | 24.50 | 18.49 | 28.28 | 32.62 | 46.90 | 35.61 | 44.38 | 17,71 | 58.05 | 30.56 | 35.50 | 43.65 | 17.75 | 24,13 | 24.08 | 39.14 |

MIC, minimum inhibitory concentration; MFC, minimum fungicidal concentration; SEE, standard error of estimate; $K_g$, fungal growth rate constant in the absence of anidulafungin; $N_{max}$, maximum number of *Candida*; α, constant used to fit the initial lag phase for growth; $E_{max}$, maximum killing rate constant (maximum effect); $EC_{50}$, concentration of anidulafungin necessary to produce 50% of the maximum effect; β, constant used to fit the initial lag phase for inhibition or killing; *h*, Hill factor; RV, residual variability.

[a] These parameters have been estimated with control data of each strain.

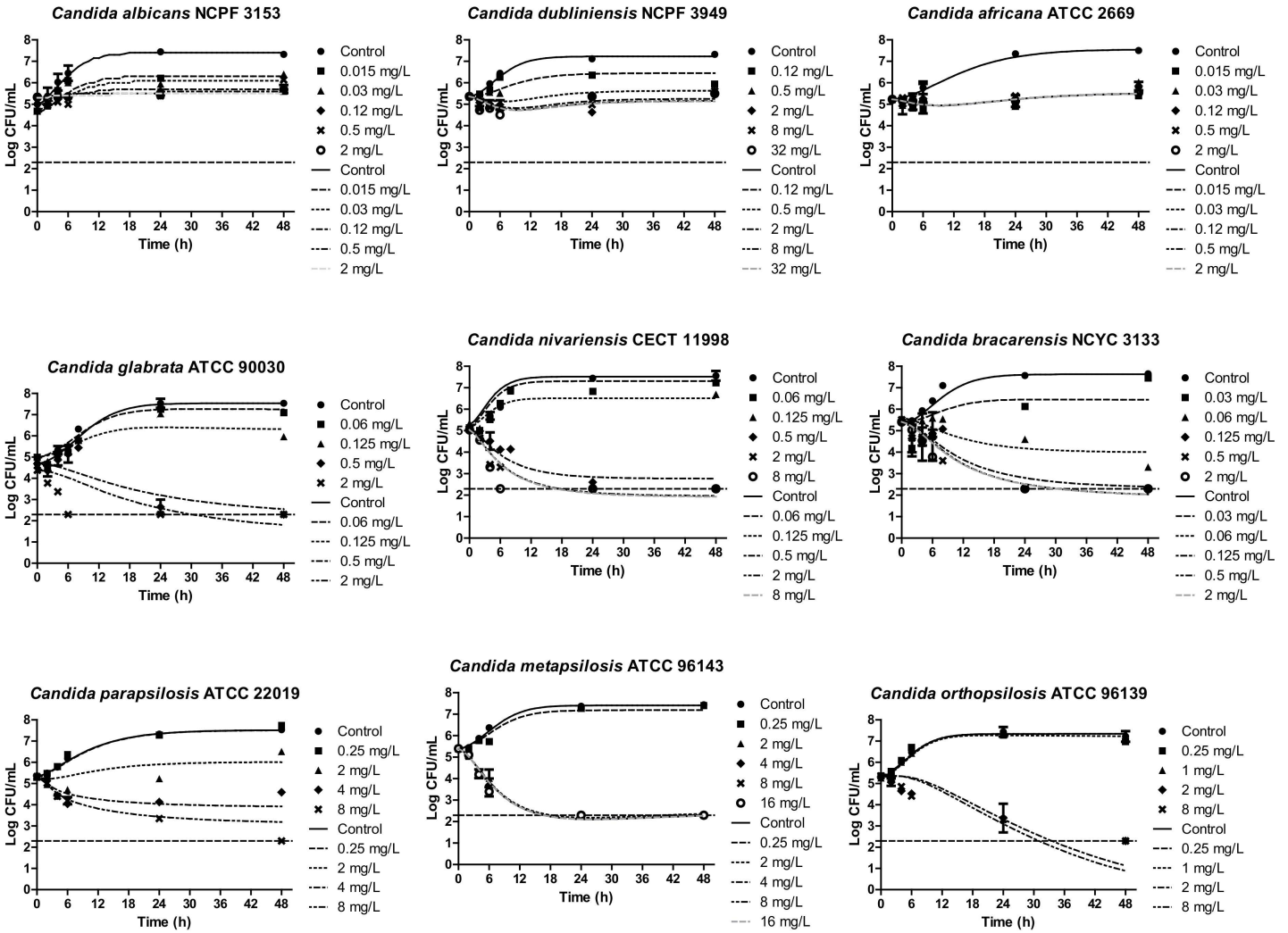
*Candida albicans* NCPF 3153
Log CFU/mL
Time (h)
Control
0.015 mg/L
0.03 mg/L
0.12 mg/L
0.5 mg/L
2 mg/L
*Candida dubliniensis* NCPF 3949
Log CFU/mL
Time (h)
Control
0.12 mg/L
0.5 mg/L
2 mg/L
8 mg/L
32 mg/L
*Candida africana* ATCC 2669
Log CFU/mL
Time (h)
Control
0.015 mg/L
0.03 mg/L
0.12 mg/L
0.5 mg/L
2 mg/L
*Candida glabrata* ATCC 90030
Log CFU/mL
Time (h)
Control
0.06 mg/L
0.125 mg/L
0.5 mg/L
2 mg/L
*Candida nivariensis* CECT 11998
Log CFU/mL
Time (h)
Control
0.06 mg/L
0.125 mg/L
0.5 mg/L
2 mg/L
8 mg/L
*Candida bracarensis* NCYC 3133
Log CFU/mL
Time (h)
Control
0.03 mg/L
0.06 mg/L
0.125 mg/L
0.5 mg/L
2 mg/L
*Candida parapsilosis* ATCC 22019
Log CFU/mL
Time (h)
Control
0.25 mg/L
2 mg/L
4 mg/L
8 mg/L
*Candida metapsilosis* ATCC 96143
Log CFU/mL
Time (h)
Control
0.25 mg/L
2 mg/L
4 mg/L
8 mg/L
16 mg/L
*Candida orthopsilosis* ATCC 96139
Log CFU/mL
Time (h)
Control
0.25 mg/L
1 mg/L
2 mg/L
8 mg/L

Fig. 1.

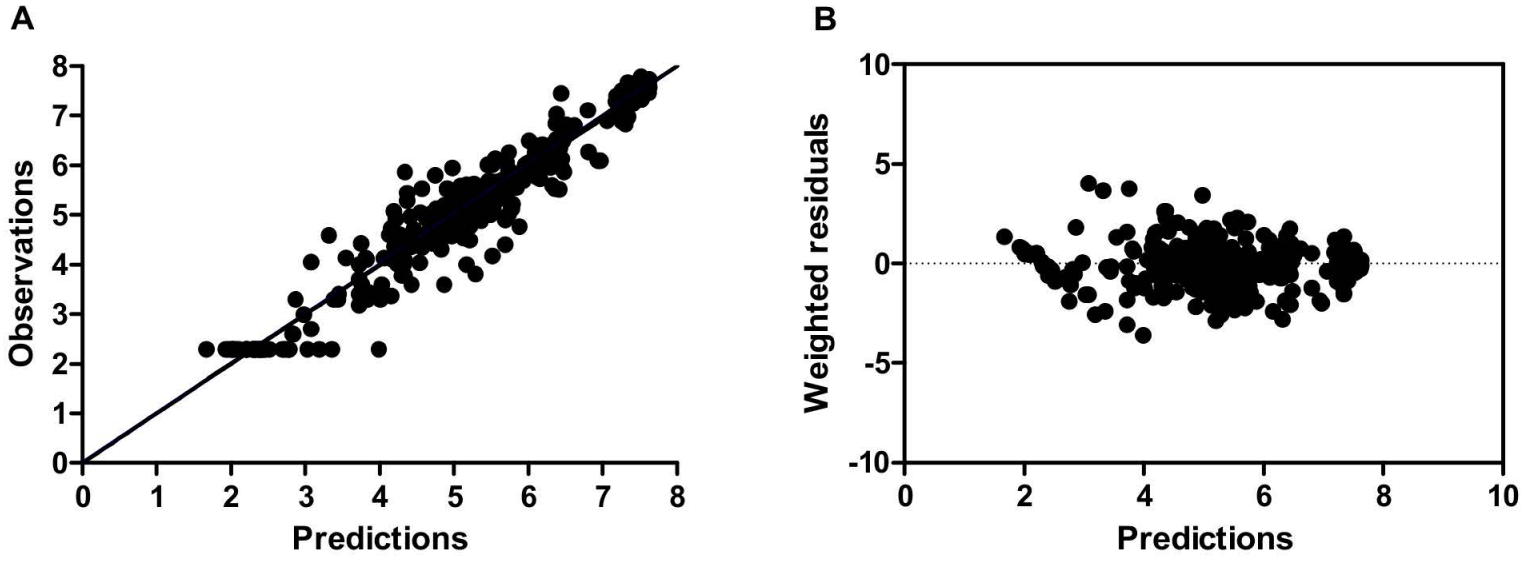
A
Observations
Predictions
0
1
2
3
4
5
6
7
8
B
Weighted residuals
Predictions
10
5
0
-5
-10
0
2
4
6
8
10

Fig. 2.