The Contribution of Growth Rate to the Pathogenicity of Candida spp.

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Abstract—Fungal infections are becoming more common and the range of susceptible individuals has expanded. While Candida albicans remains the most common infective species, other Candida spp. are becoming increasingly significant. In a range of large-scale studies of candidaemia between 1999 and 2006, about 52% of 9717 cases involved C. albicans, about 30% involved either C. glabrata or C. parapsilosis and less than 15% involved C. tropicalis, C. krusei or C. guilliermondii. However, the probability of mortality within 30 days of infection with a particular species was at least 40% for C. parapsilosis cases involved studies of candidaemia between 1999 and 2006, about 52% of 9717 spp. are becoming increasingly significant. In a range of large-scale epidemiological reports of Candida spp. infection and the mortality associated with such infection. Of course, our work represents a first attempt, and it would be necessary to relate growth rate to the outcome of infections with each of many strains of each of the Candida spp. to substantiate our approach.

Keywords—Candida spp., candidiasis, growth, pathogenicity.

I. INTRODUCTION

CANDIDIASIS is becoming more common for at least two reasons. First, species other than Candida albicans are more frequently encountered [3] and widespread resistance to current antifungal agents (such as fluconazole) has developed. Second, the range of susceptible individuals has expanded; for example those living with HIV-AIDS or recovering from major surgery, premature babies and the elderly are particularly at risk of infection [6]. It has been estimated that invasive candidiasis (IC) affects 72-228 per million population each year and the excess mortality rate attributable to IC has been estimated to be 10-49% [8].

Early antifungal treatment may reduce mortality, but if delayed the risk increases [11, 12], which has prompted the suggestion that prophylactic antifungal treatment may be justified in some circumstances. It is also likely that undiagnosed IC contributes to mortality, but identifying the infective species may delay treatment by 2 to 3 days [15-17] which also contributes to mortality.

At least two factors contribute to the mortality associated with Candida spp. infection. First, the more commonly a particular species is encountered, the more likely it is that infection with that species will be reported. Second, the greater the burden of the pathogen carried by an individual, the greater the risk of mortality [11]. Both of these factors relate to the growth of the pathogen.

Growth is characterised by a growth rate (μ), a lag time (λ) and a maximum amount (Amax), which are themselves interdependent [21]. From this we infer that the more rapidly a pathogen grows, the more likely it is that an infection will eventually prove fatal. This inference is supported by both in vitro and in vivo Candida spp. infections [24-26] and by experiments with pathogenic bacteria [29, 30]. However, in a study of 43 strains of C. albicans, MacCallum et al. [32] observed no correlation between growth rate in the cell culture medium RPMI and any measure of pathogenicity. Candida spp. differ considerably in pathogenicity. The results of several experimental studies [25, 33-38] can be summarised by specifying the relative pathogenicity of Candida spp. as C. albicans ≥ C. tropicalis > C. glabrata ≥ C. krusei > C. parapsilosis ≥ C. guilliermondii.

Here we develop a means of quantifying the contribution of growth rate to the pathogenicity of Candida spp. We use experimental measures of growth and relate them to large-scale epidemiological reports of Candida spp. infection and the mortality associated with such infection. Of course, our work represents a first attempt, and it would be necessary to relate growth rate to the outcome of infections with each of many strains of each of the Candida spp. to substantiate our approach.

II. MATERIALS AND METHODS

Clinical isolates of Candida spp. were obtained from the culture collections of the Launceston General Hospital or the University of Tasmania and their identity was confirmed as previously described [39]. Candida spp. were grown in liquid YPD (2% (w/v) glucose, 1% (w/v) yeast extract, 2% (w/v) bacteriological peptone) in an orbital incubator (200 min⁻¹) at 37°C as described previously [40]. As the cultures were monitored repeatedly, exit cultures on MacConkey agar, blood agar and YPD agar plates were prepared to check for possible
contamination. Growth was monitored by measuring the absorbance of the cell suspension at 600 nm ($A_{\text{600}}$) and the standard Gompertz model [41] was fitted to the growth data by nonlinear regression [42].

Epidemiological data were obtained from studies of candidaemia published between 1989 and 2006. For a study to be included we required that both the number of isolates and the mortality for several Candida spp. were reported.

III. RESULTS

A. Growth of Candida spp.

The maximum growth rate ($\mu_m$) of the seven Candida spp. ranged from 4.9 h$^{-1}$ for C. glabrata to 1.5 h$^{-1}$ for C. parapsilosis (Fig 1). The the lag time ($\lambda$) ranged from 2.37 h for C. glabrata to 6.2 h for C. parapsilosis (Fig 1). The extent of the growth ($A_{\text{max}}$) was about 17 for C. albicans and C. glabrata, but only reached about 12 for C. guilliermondii and C. parapsilosis (Fig 1). However, the early lag phase of the C. guilliermondii growth curve was not especially well modelled by the Gompertz function (Fig 1) and the lag time appeared to be underestimated. Despite this, those species sometimes reported as less pathogenic (such as C. glabrata and C. parapsilosis) had and lower $\mu_m$ than those usually regarded as pathogens (C. glabrata and C. albicans, for example), as is apparent from Fig 2. As expected [21], the values of $\mu_m$ and $\lambda$ varied inversely ($r = -0.954, p = 0.003$), excluding the data for C. guilliermondii. The data for C. guilliermondii did not lie on the same line as those of the other six species (Fig 2), perhaps because of the underestimation of $\lambda$. The values of $A_{\text{max}}$ and $\mu_m$ were more weakly correlated ($r = 0.44, p = 0.32$).

![Fig. 1. Representative growth curves of seven Candida spp. The solid curves represent the fit of the Gompertz function to the data and the error bars represent ± SE for at least 3 replicates and where they are not visible they are hidden by the symbol. CA: C. albicans; CD: C. dubliniensis; CGl: C. glabrata; CGu: C. guilliermondii; CK: C. krusei; CP: C. parapsilosis; CT: C. tropicalis.](image)

![Fig. 2. Relationship between lag time ($\lambda$) and maximum growth rate ($\mu_m$) for the seven Candida spp. Parameter estimates were obtained from the growth curves shown in Fig 1. Closed symbols indicate those species usually taken to be pathogenic and open symbols indicate those sometimes said to be non-pathogens. Error bars are ± 95% CI of the parameter estimate. The line is the least squares fit ($r = -0.954, p = 0.003$) to the data without considering the data for C. guilliermondii (CGu). The other species abbreviations are: CA – C. albicans; CD – C. dubliniensis; CGl – C. glabrata; CK – C. krusei; CP – C. parapsilosis; CT – C. tropicalis.](image)

B. Epidemiology of Candida spp. Infection

The pathogenicity of Candida spp. was assessed from seventeen large-scale epidemiological studies of candidaemia (referenced in Table I) involving a total of 9717 cases. As can be seen in Table I, 52% (range: 37.1–68.9%) of these isolates were C. albicans and the next two most significant species were C. glabrata (17%, range: 4.7–25%) and C. parapsilosis (14%, range: 5.4–33.1%). However, the overall 30-day mortality rate among those infected with C. albicans or C. glabrata was about 40%, whereas only 22% of C. parapsilosis infections resulted in mortality within 30 days (Table II). The number of cases involving C. dubliniensis or C. guilliermondii was very low (Table I) and so these data were combined with the ‘other’ species in the subsequent analysis (in Table II and Fig 3). These data were obtained in many countries, over more than a decade and no account was taken of underlying morbidity or antifungal resistance. Despite this, the data for each species are remarkably consistent, presumably reflecting the pathogenicity of each species.

IV. ANALYSIS

The data in Table II represent the probability that an instance of candidaemia associated with an infection with species ($i$) of Candida is fatal ($P(\text{mortality}|i)$) and those in Table I are the probability that an instance of candidaemia is associated with species ($i$) ($P(i|$ candidaemia$)$). The probability of mortality associated with candidaemia is
where the summation is taken over all the Candida spp. and the individual terms on the right hand side are the contributions of each species to the mortality associated with candidaemia.

The log of the odds of mortality given infection with species $i$ increased linearly with growth rate (Fig 3). The slope and intercept of this relationship can be related to the contribution of growth rate to the mortality associated with candidaemia and the probability mortality associated with the underlying condition (see the Appendix). From Fig 3, a unit increase in the growth rate of Candida sp. is associated with
an increase in the probability of mortality of 0.09 ± 0.02 and
the underlying probability of mortality is 0.13 (95%
confidence interval: 0.10, 0.17).

The log odds of species \( i \) infection in candidaemia is only
weakly related to \( \mu_m \), but is more strongly related to
\((A_{max} \lambda \mu_m) \) \( \) (Fig 4), which is the geometric mean of two
measures of overall growth: \( A_{max} \) and \( \lambda \mu_m \) \[21\]. From Fig 4, a
unit increase in the growth Candida sp. is associated with an
increase in the probability of infection of 0.2 ± 0.1 and the
underlying probability of infection in the absence of growth is
< 2.2 \times 10^{-6} \, (\text{which is the upper limit of the 95\% confidence}
interval estimated for this value). It is apparent from Fig 3 that
C. krusei grows rapidly and is associated with a high \( P(\text{mortality} \mid i) \), however it is involved in only about 2.5% of
infections (Table II, Fig 4) and so C. krusei infections are less
common than might be expected given its growth is greater
than C. albicans, C. glabrata and C. tropicalis (Fig 4).

The contribution of a Candida sp. to the log odds of
mortality given candidaemia is also related to \((A_{max} \lambda \mu_m) \) \( \) (Fig 5). A unit increase in the growth of Candida spp. is
associated with an increase in the probability of mortality of
0.2 ± 0.1 and the underlying probability of mortality is 0.01
(Fig 5). \[21, \text{Fig 4}\]]. From (1) and the estimates shown in Fig 5, the
overall \( P(\text{mortality} \mid \text{candidaemia}) \) can be estimated to be 0.37 ± 0.19 (± 95\% confidence interval), to which C. albicans
contributes about 0.2.

![Graph](image-url)  
**Fig. 3.** Log odds of mortality within 30 days given infection with a
particular Candida sp. (Table II) as a function of growth rate (\( \mu_m \); Fig 1). The solid line is the least squares fit to the data (\( r = 0.981, p < 0.001 \)) and has a slope and intercept of 0.16 ± 0.03 h and -0.8 ± 0.1 (± 95\% confidence intervals), respectively. Error bars are ± 95\% confidence interval. Closed symbols indicate those species usually taken to be pathogenic and ‘other’ includes C. guilliermondii and C. dubliniensis as described in the text. CA: C. albicans; CD: C. dubliniensis; CGl: C. glabrata; CGu: C. guilliermondii; CK: C. krusei; CP: C. parapsilosis; CT: C. tropicalis.

![Graph](image-url)  
**Fig. 4.** Log odds of infection with with a particular Candida sp. (Table I) as a function of growth. The dashed line is the least squares fit to the data (\( r = 0.895, p = 0.04 \)), neglecting that for C. krusei, and the solid line is the fit (\( r = 0.706, p = 0.12 \)) including the C. krusei data; for either fit the slope and intercept are 0.3 ± 0.2 h and -5 ± 1 (± SE), respectively. Error bars are ± 95\% confidence interval. Closed symbols indicate those species usually taken to be pathogenic and ‘other’ includes C. guilliermondii and C. dubliniensis as described in the text. CA: C. albicans; CD: C. dubliniensis; CGl: C. glabrata; CGu: C. guilliermondii; CK: C. krusei; CP: C. parapsilosis; CT: C. tropicalis.

![Graph](image-url)  
**Fig. 5.** Contribution of each Candida sp. to the log odds of mortality within 30 days given candidaemia (1) as a function of growth. The dashed line is the least squares fit to the data (\( r = 0.935, p = 0.02 \)), neglecting that for C. krusei, and the solid line is the fit (\( r = 0.814, p = 0.048 \)) including the C. krusei data; for either fit the slope and intercept are 0.3 ± 0.2 h and -6 ± 3 (± 95\% confidence interval), respectively. Error bars are ± 95\% confidence interval. Closed symbols indicate those species usually taken to be pathogenic and ‘other’ includes C. guilliermondii and C. dubliniensis as described in the text. CA: C. albicans; CD: C. dubliniensis; CGl: C. glabrata; CGu: C. guilliermondii; CK: C. krusei; CP: C. parapsilosis; CT: C. tropicalis.
V. DISCUSSION

The pathogenicity of Candida spp. depends on the rate of infection and on the virulence of the infecting species. We have shown that the log odds of mortality rises linearly with $\mu_{\text{max}}$ (Fig 3) and that the log odds of infection increases with the total growth (measured as the geometric mean of $A_{\text{max}}$ and $\lambda_{\mu}$, Fig 4). These observations are consistent with experimental reports [24-26]. However, MacCallum et al. [32] reported data for 43 strains of C. albicans from four different clades, that showed that there was no significant difference in the $\mu_{\text{max}}$ in RPMI of strains of differing pathogenicity or from different clades. It should be noted that the growth rates they reported were less than 10% (0.13-0.3 h$^{-1}$ for cultures in RPMI) of that reported here for C. albicans (Figs 1 and 2).

Butler et al. [43] reported that C. glabrata lacked, and C. guilliermondii had fewer copies of, several of the genes that appear to be enriched in the genomes of other pathogenic Candida spp. Since C. glabrata is more likely to be isolated (Table I) and grows faster (Fig 1) than C. guilliermondii, the absence of these genes may not be consistent with a significant role for them as determinants of pathogenicity if mortality is associated with growth (Fig 5) and growth rate (Fig 3). Unfortunately, no complete genome sequence is currently available for C. kruzei, which might provide further insight into the genetic basis for the disproportionately low rate of infection reported for this species (Table I and Fig 4).

Highly pathogenic Candida spp. are more commonly isolated from individuals with IC (Table I) and are more frequently associated with mortality than others species (Table II). Based on data from large-scale epidemiological studies (such as [22, 23, 27]), a relationship between the probability of mortality and the growth rate of Candida spp. can be established (Fig 3). One interpretation of these data is that the underlying probability of mortality is about 0.13 (95% confidence interval: 0.10-0.17), and that Candida infection increases the probability by 0.09 ± 0.02 for every unit increase in $\mu_{\text{max}}$ (Fig 3). As the most commonly isolated Candida spp. have growth rates of at least 2 h$^{-1}$ (Fig 1), the infection at least doubles the underlying P(mortality $|$ i). The probability of infection is also related to the growth of Candida spp. (Fig 4), but the amount, rather than the growth rate, of the pathogen is a more significant determinant of infection. Based on Fig 3, the relative pathogenicity of Candida spp. given an infection can be summarised as C. kruzei $>$ C. glabrata = C. albicans $\geq$ C. tropicalis $>$ C. parapsilosis, but the relative infectivity is C. albicans $>$ C. glabrata = C. parapsilosis $>$ C. tropicalis $>$ C. kruzei (Fig 4). The overall pathogenicity of the Candida spp. is a combination of these two sequences.

Naturally, our analysis has at least two important limitations. First, the growth of the clinical isolates reported here may not be representative of the many strains of each Candida sp. (there are hundreds of strains of C. albicans alone [44]). Second, we arbitrarily chose to grow the Candida spp. in conditions that are quite different from those in vivo, although this can be justified in part by the limited $\mu_{\text{max}}$ reported when grown in RPMI [32]. Despite these limitations, the relationship between growth rate and P(mortality $|$ i) (Fig 3) is highly significant ($p = 0.003$) prompting two tentative conclusions: First, the emergence of a faster growing Candida sp. would pose a significant threat. An example of this is the relatively recent emergence of C. kruzei [8], which has a growth rate only 15% greater than that of C. albicans (Fig 1) and is associated with a 10% greater P(mortality) (Table II). The low probability of mortality associated with C. kruzei candidaemia (Fig 5 and Table II) is due to the low probability of infection with this species (Fig 4 and Table I). Of course, C. kruzei has the potential to be a significant pathogen if more infectious strains were to develop. Second, this analysis confirms the suggestion that the excess mortality rate attributable to IC is 10-49% [8], but that the range in this estimate is likely to be related, at least in part, to growth rate.

VI. CONCLUSION

The pathogenicity of Candida spp. is directly related to the growth rate of the infecting species. Infection is more closely related to the extent of growth (measured as $(A_{\text{max}}\lambda_{\mu})^2$) of the infecting species. The probability of mortality given candidaemia is also related to the extent of growth. We have estimated that an increase in growth rate of 1 h$^{-1}$ is associated with an increase in the probability of mortality of 0.09 ± 0.02 over an underlying probability of mortality of 0.13 (95% confidence interval: 0.10, 0.17).

APPENDIX

Where the logarithm of the odds [45] is linearly related to a variable x (such as $x = \mu_{\text{max}}$ in Fig 3 and $x = (A_{\text{max}}\lambda_{\mu})^2$ in Figs 4 and 5), then

$$\log_{10}\left(\frac{P(\cdot)}{1-P(\cdot)}\right) = \alpha + \beta x,$$

where $P(\cdot)$ is the probability specified by the argument. This can be rearranged to yield

$$P(\cdot) = \left(1 + 10^{-(\alpha-\beta)}\right)^{-1}$$

from which the underlying $P(\cdot)$, corresponding to a situation in which $x = 0$, is given by

$$P(\cdot \mid x = 0) = \left(1 + 10^{-\beta}\right)^{-1}$$

and the contribution to mortality associated with a unit change in $x$ is related to the maximum slope which occurs at $x = -\beta/\alpha$

$$\frac{dP(\cdot)}{dx}\bigg|_{x=\beta/\alpha} = \frac{\ln(10)}{4} \alpha.$$  

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