Ther Adv Infectious Dis
2017, Vol. 4(1) 10–17
DOI: 10.1177/2049936116684477

© The Author(s), 2017.
Reprints and permissions:
http://www.sagepub.co.uk/journalsPermissions.nav

Oral mucositis caused by *Candida glabrata* biofilms: failure of the concomitant use of fluconazole and ascorbic acid

Célia F. Rodrigues and Mariana Henriques

Abstract

Objectives: *Candida glabrata* is becoming one of the most prevalent pathogenic yeasts in cases of oral diseases. Mucositis is an recurrent oral infection in immunocompromised patients, and the actual guidelines recommend the use of fluconazole (Flu) for many cases. However, the azole resistance by *C. glabrata* is renowned, causing a reduced therapeutic response, especially when it occurs in biofilms. In this study, we performed an *in vitro* evaluation of an alternative pharmacotherapy for *C. glabrata* biofilm infections, combining ascorbic acid (AA) with Flu. AA is recognized for degrading β-glucans, an important compound of the biofilm matrices, which prevent drug diffusion.

Materials and Methods: Routine clinical 30 or 40 mg/l doses of Flu were applied to *C. glabrata* biofilms simultaneously with 200 or 300 mg/l of AA.

Results: The results showed that this combination effectively promoted the degradation of the biofilm network, but unfortunately, also stimulated the growth of the yeasts population due to release of several glucose monomers during β-glucans hydrolysis.

Discussion: AA lead to the hydrolysis of the β-glucans of the matrix, liberating glucose molecules which are used as carbon souce by the yeasts, thus suppressing the desired antifungal effect of the drug combination with Flu.

Conclusions: Unlike to what happens in treatment of bacterial infection, AA should not be used together with Flu in the treating oral mucositis caused by *Candida*.

Keywords: ascorbic acid, biofilm matrix, *Candida glabrata*, fluconazole, infection, resistance

Correspondence to:
Mariana Henriques, PhD
Centre of Biological Engineering (CEB), Laboratório de Investigação em Biofilmes Rosário Oliveira (LIBRO), University of Minho, 4710-057 Braga, Portugal.
mcrh@deb.uminho.pt

Célia F. Rodrigues, PharmD, MSc Mariana Henriques, PhD
Centre of Biological Engineering (CEB), Laboratório de Investigação em Biofilmes Rosário Oliveira (LIBRO), University of Minho, Braga, Portugal

Introduction

Fungal infections are a major clinical health problem that affects more than 300 million people annually.[1] *Candida glabrata* is a commensal yeast living in human mucosal surfaces (e.g. mouth, esophagus, intestine) but can easily turn into a pathogen, especially in immunocompromised individuals, instigating a high rate of morbidity and mortality.[2–5] In clinical practice *C. glabrata* is the second most prevalent pathogen in humans in the United States, and the third in Europe, after *Candida albicans* and *Candida parapsilosis*.[3,6] This species is highly resistant to antifungal agents[7–9] and has high capacity to produce biofilms,[10–12] which are extremely refractory to antimicrobial therapy, thus being very difficult to treat with the traditional antifungal therapies.[10,13–20] Formation of bioflims by *Candida* species in mouth and/or esophageal mucosae is very common, with *C. glabrata*[21–23] being a very important contributor to human oral disease due to a high level of antifungal resistance.[24–26] Biofilms are biological communities with an extraordinary degree of organization, in which microorganisms form structured, coordinated, and functional communities, embedded in an extracellular matrix.[24–26] These structures are known to induce high rates of morbidity and mortality, especially in immunocompromised patients,[27–29] creating a dangerous prospect of ineffective therapies against infectious diseases caused by *Candida*. The National Institutes of Health signposts that biofilms are directly or indirectly responsible for over 80% of all microbial infections.[30–32] Therefore, there is an urgent need to overcome the numbers related to biofilm-associated *C. glabrata* infections and to

understand the mechanisms behind the response of *C. glabrata* biofilms infections to antifungal treatment.

Mucositis (or oropharyngeal candidiasis) is a frequent infection among immunocompromised patients,[33,34] which is characterized by the presence of creamy, white plaques on the tongue and buccal mucosa that generally leave a raw, painful, and ulcerated surface when scraped. Although not being a severe infection normally, it can be uncomfortable and detrimental to the patient's nutritional status thanks to the diminished food and liquid intake. The latest 2016's guidelines recommend the treatment general mucositis infections with oral fluconazole.[35] However, the number of effective antifungal drugs is limited, and resistance to frequently used agents is emerging.[24] Specifically, the resistance of *C. glabrata* to the azole class of drugs has been well described.[25]

Candida biofilm matrix is mainly composed of exopolysaccharides, the most predominant being β-glucans. These D-glucose-based polysaccharides are present in different types of glycosidic bonds,[26,36–38] consisting of a repeating structure with the β-D-glucose units linked together in linear chains by β-bonds, forming homoglucans, which can extend either from carbon 1 of one saccharide ring to carbon 3 of the next (β1→3) or from carbon 1 to carbon 6 (β1→6). This net of polymers hampers the diffusion of azoles through the biofilms and, thus prevents the drug from reaching cells inside them.[39–41]

Ascorbic acid, also designated as vitamin C, has being used concomitantly in antibiotherapy for its capacity to boost the immune system and role as a helper in the treatment of several infections, for several years.[42] AA's concentration in phagocytes and lymphocytes is very high compared with the level in plasma, indicating that AA may have functional roles in these immune system cells, for example, increasing the functioning of phagocytes, the proliferation of T-lymphocytes and the production of interferon, and decreasing the replication of viruses.[43] Furthermore, its metabolism is affected by various infections (e.g. common cold, pneumonia resultant from many microorganisms, scurvy or *Helicobacter pylori* infections), proposing that there might be treatment benefits in the association of this compound to the regular pharmacotherapy.[44–47] Furthermore, AA's metabolism also has the capacity to induce formation of hydroxyl radicals ($OH^{\bullet}$), which are reactive oxygen species that may oxidize biomolecules such as proteins, DNA, and other biopolymers (e.g. β-glucans).

So, the goal of this study was to check the *in vitro* efficacy, already demonstrated on the use of AA with antibiotics, will also be the same with an antifungal agent. Therefore, AA will be used concomitantly with Flu to treat *C. glabrata* biofilm oral infections.

Materials and methods

Organisms and growth conditions

C. glabrata ATCC2001 (reference strain from the American Type Culture Collection) was used in the course of this study. For each experiment, *C. glabrata* ATCC2001 was subcultured on Sabouraud dextrose agar (SDA; Merck, Darmstadt, Germany) for 24 h at 37°C. Cells were then inoculated in Sabouraud dextrose broth (Merck, Darmstadt, Germany) and incubated for 18 h at 37°C under agitation at 120 rpm. After incubation, cells were harvested by centrifugation at 3000 g for 10 min at 4°C and washed twice with phosphate buffered saline (PBS, pH = 7.5). Pellets were then suspended in RPMI 1640 (Sigma-Aldrich, Roswell Park) and the cellular density was adjusted to 1×10^5 cells/ml using a Neubauer counting chamber.[48]

Flu and AA

Flu was kindly provided by Pfizer®. AA was purchased from Sigma-Aldrich (Roswell Park). Aliquots of 5000 mg/l were prepared using dimethyl-sulfoxide (DMSO), and the final concentrations used (30 and 40 mg/l for Flu and 200 and 300 mg/l for AA) were prepared with RPMI 1640. Controls were performed with DMSO in order to assure that the concentration used was not toxic (concentrations below 1% (v/v) DMSO).

Flu and/or AA effect on C. glabrata *biofilm*

Biofilm formation. Cell suspension was prepared and washed as described above and resuspended in RPMI 1640. Then, standardized cell suspensions (200 µl) were placed into selected wells of 96-well polystyrene microtiter plates (Orange Scientific, Braine-l'Alleud, Belgium). As a negative control, RPMI 1640 was used without cells and antifungal agent. As positive control, only cell suspensions were tested without antifungal agent. After 24 h,

100 µl of RPMI 1640 was removed and an equal volume of fresh RPMI 1640 plus the antifungal agent (2× concentrated) or the combination of flu and AA was used. The plates were incubated at 37°C for an additional 24 h period, a total of 48 h at 120 rpm. In addition, in order to assess the effect of glucose in the medium, the assay was performed in the same conditions explained above using RPMI supplemented with 2% of glucose.

Biofilm cultivable cells and biomass determination. The number of cultivable cells in the bioflim was determined by the enumeration of colony forming units (CFUs). For that, after the period of biofilm formation, all medium was aspired and the biofilms were washed once with 200 µl of PBS to remove non-adherent cells. After biofilms were scraped from the wells and the suspensions were vigorously vortexed for 2 min to disaggregate cells from the matrix. Serial decimal dilutions in PBS were plated on SDA and incubated for 24 h at 37°C. The results were presented as total of CFUs per unit area (Log_{10} CFUs/cm^2). Total biofilm biomass was quantified by crystal violet (CV) staining. After the biofilm formation, the medium was aspirated and non-adherent cells removed by washing the biofilms with sterile ultra-pure water. Biofilms were then fixed with 200 µl methanol, which was removed after 15 min of contact. The microtiter plates were allowed to dry at room temperature, and 200 µl of CV (1% v/v) added to each well and incubated for 5 min. The wells were then gently washed twice with sterile, ultra-pure water and 200 µl of acetic acid (33% v/v) was added to release and dissolve the stain. The absorbance of the obtained solution was read in triplicate in a microtiter plate reader (Bio-Tek Synergy HT, Izasa, Lisbon, Portugal) at 570 nm. The results were presented as percentage of biomass.[48] All the assays were performed in triplicate and on three separate occasions.

Statistical analysis

Results were compared using one-way analysis of variance (ANOVA), Dunnett's and Bonferroni's multiple comparisons test, using GraphPad Prism 5 software. All tests were performed with a confidence level of 95%.

Results

The present study aimed to evaluate an alternative treatment for oral mucositis related to *C. glabrata* biofilms using an combination of Flu and AA, similar to what has been used for treatment of bacterial infections for years. To determine the influence of AA in the fungistatic activity of Flu, *C. glabrata* ATCC2001 biofilms were grown for 24 h and then different concentrations of these agents were added and allowed to incubate for an additional period of 24 h. The working concentrations, for both Flu and AA, were chosen according to their use in clinical practice, since the goal was to overtake the biofilm resistance of *C. glabrata*, using Flu in the same doses, but with another compound as an adjuvant.

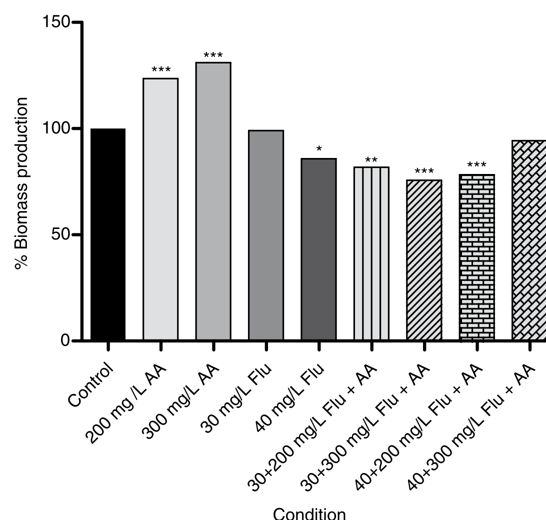

Figure 1. Percentage of biomass detected using CV staining with Flu and Flu + AA in biofilms of *Candida glabrata* ATCC2001 (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$). The control is considered to have 100% of biomass production.
AA, ascorbic acid; CV, crystal violet.

Figure 1 shows the percentage of biomass of *C. glabrata* ATCC2001 when exposed to different concentrations of AA combined with Flu, in a pre-formed 24-h-biofilm. Controls were performed with the two agents alone. After 24 h of exposure, AA alone had no effect on the reduction of the biofilm biomass, compared with the lower dose of Flu. Furthermore, AA showed to be an enhancer of *C. glabrata*'s biomass (Figure 1). On the other hand, combining 30 mg/l of Flu with both concentrations of AA, the biomass presented a statistically significant reduction ($p < 0.001$), compared with the use of Flu alone, however, in a very low percentage: 10–20%. Moreover, when the Flu concentration was higher, this effect was not noticed. An increase in biofilm biomass was also observed in the presence of the higher concentration of AA when used with the 40 mg/l of

Table 1. CFUs count (Log10 CFUs/cm^2) when using AA and Flu alone in biofilms of *Candida glabrata* ATCC2001 after 24 h and percentage of CFU reduction.

Condition	Log_{10} CFUs/ cm^2 ± SD	% CFU reduction
Control	6.22 ± 0.02	–
AA 200 mg/l	6.83 ± 0.25	−9.8
AA 300 mg/l	7.22 ± 0.05	−16.08
Flu 30 mg/l	5.65 ± 0.11	9.1
Flu 40 mg/l	5.78 ± 0.48	7.07

AA, ascorbic acid; CFU, colony forming units.

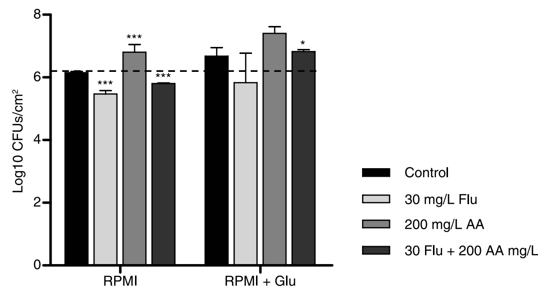

Figure 2. Cell production with Flu and Flu + AA in biofilms of *Candida glabrata* ATCC2001 (*$p < 0.05$; ***$p < 0.001$), by CFUs count (Log10 CFUs/cm^2).
AA, ascorbic acid; CFU, colony forming unit.

Flu. In order to explain the results obtained regarding the lack in the drug activity of Flu, viability of cells after treatment was also assessed (Table 1) for the drugs alone. It was verified that using AA, the viable cells increased (around 10% and 15% for 200 and 300 mg/l of AA, respectively), when compared with the controls. Flu was unable to eradicate 50% of *C. glabrata* viable cells even at higher concentrations (Table 1). In fact, when the highest concentration of Flu (40 mg/l) was used, it was only possible to reach a reduction of 7.0% in terms of biofilm cell viability (Table 1). With 30 mg/l, the values were rather superior (9.1%), but with no biomass reduction (Figure 1), thus not therapeutically interesting.

To verify if the possibility of whether the increased glucose concentration resulting from the β-glucans hydrolysis was contributing to the increase in the *C. glabrata* population, another assay was performed. The RPMI was supplemented with 2% of glucose for three selected conditions: 200 mg/l of AA, 30 mg/l of Flu, and 30 mg/l of Flu + 200 mg/l of AA (Figure 2). With extra glucose in the medium, the growth in control was 10% more than the control without glucose. Also, significant results were obtained when comparing the use of 200 mg/l of AA with 30 and 40 mg/l of Flu alone ($p < 0.01$ and $p < 0.001$, respectively). No significant differences were obtained when comparisons with the drug combinations were made (Figure 2).

Lastly, concerning the drug combination (30 mg/l Flu + 200 mg/l AA), results show that the presence of glucose (Figure 2) in the medium was clearly harmful, since there was no variation in CFU count (thus, no cell death) comparing with the respective control. Additionally, when comparing this result with the control without glucose, there was an increase in cell population (Figure 2).

Discussion

Mucositis associated to erythematous ulcerations in the oral cavity causes pain, xerostomia, dysphagia, and lastly septicaemia.[49] It disturbs functions such as drinking, eating, speaking, dental and other mouth care practices, and it affects not only nutrition and quality but also can be life threatening.[23,49]

Despite the recognized resistance profile of *Candida* biofilm cells to antifungal agents,[15,17,18] mucositis still has good outcomes with the use of azoles, particularly Flu.[23,33,35,50] The knowledge that the therapeutic response of *C. glabrata* oral biofilms to Flu is lower than the other *Candida* species (e.g. *C. parapsilosis* or *Candida guillermondii*)[24–26] is the main reason for the development of the present study, in which clinical doses of Flu are associated with AA.

It was verified (Figure 1) that AA alone had no effect on the reduction of the biofilm biomass, compared with 30 mg/l of Flu, and it showed an increase in the growth of *C. glabrata*'s biomass. Generally, no significant differences were obtained when comparisons with the drug combinations were made. With the aim of explaining these results, cell viability after treatment with the drugs alone was also assessed (Table 1). It was then possible to observe, contrarily to what was expected, an increase in the cell viability, showing a possible degradation and consumption of β-glucans.

In fact, other researchers described that during the degradation of AA, the hydrolysis into glucose monomers occurred.[51,52] Xu *et al.*,[53] have also showed a similar situation. The authors tested a considered antifungal drug compound, β-1,3 glucanase, in a *C. albicans* strain, but instead of having a reduced yeast count, there was a proliferation in *C. albicans*, which can be explained by the same reasons mentioned above. Similar to the goal of this work, Al-Fattani and Douglas[54] tested various enzymes to degrade biofilms and noted that they were also easily detached from biofilms, but as specific hydrolase enzymes, they released many glucose monomers. Despite this fact and since the authors used amphotericin B as the antifungal agent, the results for the CFU count were favorable.

The chemical events underlying this process were explained through the β-glucan degradation pathways.[55–58] Thereby, there are, mainly, two paths: (1) the oxidative cleavage of β-glucan is initiated by the removal of a hydrogen atom from the anomeric carbon (C1) of the polysaccharide inducing the formation of an alkyl radical, and there are two possible courses for the cleavage of the glycosidic bond with the generation of a lactone in C1. The glycosidic bond may fragment due to delocalization of the unpaired electron, leading to the release of a β-glucan fragment with a lactone, and of a β-glucan fragment with an alkyl radical, which may react with O_2 to form the corresponding peroxyl radical, which can further undergo transformations. Also, the glucan with an alkyl radical on the C1 can also suffer hydrolysis, which would point to the release of a nonradical β-glucan fragment (glucose monomers) and a β-glucan fragment containing a radical at C1; (2) the formation of peroxyl radical following the alkyl radical in C1. The carbon-centered radical in C1 would react rapidly with O_2 to give a peroxyl radical, which can further combine with another peroxyl radical and fragment *via* an alkoxyl radical (R–O$^{\bullet}$).[46] The alkoxyl radical formed would undergo a β-fragmentation, liberating a β-glucan fragment with a lactone in C1 and a β-glucan fragment with an alkoxyl radical at C3. Other pathways can contribute to the scission of β-glucan, since the non-selective attack of $^{\bullet}$OH radicals most likely also generate radicals at other locations besides C1.[44,59]

In fact, a loss of viscosity in the biofilm was observed during the biofilm manipulation whenever AA was used. The biofilm was more flexible and easily breakable, which is related to the formation of hydroxyl radicals during β-glucan degradation.[44,57,60,61] Actually, this glucose hydrolysis reaction is used as an alternative method to the DuBois *et al.*[62] for carbohydrate quantification.[63,64]

In the concomitant use of Flu and AA (30 mg/l Flu + 200 mg/l AA), the addition of glucose to the RPMI was very unfavorable. No cell death was achieved (there was no variation in the CFU count), compared with the respective control. In fact, there was even a small increase in cell population (Figure 2) because of the consumption of the free glucose derived from the hydrolysis of the β-glucans and/or the supplementation of the medium, which disrupted the Flu fungistatic activity.

To conclude, Flu is a drug that is significant in the treatment of mucositis. Although with certain side effects, it is generally well tolerated, it has low toxicity, and the usual therapeutic regimen is very appealing to the patient. Unfortunately, once more, the final data presented indicate that this drug might not work for infections derived from *C. glabrata* biofilms, as previous work from our group have demonstrated. The matrix of *Candida* biofilms is a solid barrier to the diffusion of drugs into the cells. Thus, the use of a drug combination in which one drug would degrade this matrix and the other drug would be fungicidal or fungistatic could be a good strategy and improve the therapeutic response in fungal infections. Flu has been shown to be recalcitrant *in vitro* due to the lack of drug penetration.[41]

AA leads to hydrolysis of β-glucan which forms glucose that is later used as carbon source by *C. glabrata*, enabling the cells to colonize and infect. Thus, their virulence was not annulled by Flu in this drug combination. Though this outcome might be possible in other *Candida* strains and/or species, this work has a clear limitation because of the use of a single strain and thus the results cannot be directly generalized to all *C. glabrata* strains.

Acknowledgements

The authors thank the Project 'BioHealth – Biotechnology and Bioengineering approaches to improve Programa Operacional Regional do Norte' (ON.2 – O Novo Norte), QREN, FEDER. The authors would also like to thank Pfizer(R) for the kind donation of Fluconazole.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship,

and/or publication of this article: This work was supported by the Programa Operacional, Fatores de competitividade – COMPETE and by national funds through FCT – Fundação para a Ciência e a Tecnologia on the scope of the projects FCT PTDC/SAU-MIC/119069/2010, RECI/EBB-EBI/0179/2012 and PEst-OE/EQB/LA0023/2013 and Célia F. Rodrigues' SFRH/BD/93078/2013 PhD grant.

Conflict of interest statement

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

References

1. Brown GD, Denning DW, Gow NA, *et al.* Hidden killers: human fungal infections. *Sci Transl Med* 2012; 4: 165rv13.
2. Almirante B, Rodriguez D, Park BJ, *et al.* Epidemiology and predictors of mortality in cases of Candida bloodstream infection: results from population-based surveillance, Barcelona, Spain, from 2002 to 2003. *J Clin Microbiol* 2005; 43: 1829–1835.
3. Fidel P, Vazquez J and Sobel J. Candida glabrata: review of epidemiology, pathogenesis, and clinical disease with comparison to C. albicans. *Clin Microbiol Rev* 1999; 12: 80–96.
4. Kao AS, Brandt ME, Pruitt WR, *et al.* The epidemiology of Candidemia in two United States cities: results of a population-based active surveillance. *Clin Infect Dis* 1999; 29: 1164–1170.
5. Kusne S, Tobin D, Pasculle AW, *et al.* Candida carriage in the alimentary tract of liver transplant candidates. *Transplantation* 1994; 57: 398–402.
6. De Groot PW, Kraneveld EA, Yin QY, *et al.* The cell wall of the human pathogen Candida glabrata: differential incorporation of novel adhesin-like wall proteins. *Eukaryot Cell* 2008; 7: 1951–1964.
7. Lass-Flörl C. The changing face of epidemiology of invasive fungal disease in Europe. *Mycoses* 2009; 52: 197–205.
8. Krogh-Madsen M, Arendrup MC, Heslet L, *et al.* Amphotericin B and caspofungin resistance in Candida glabrata isolates recovered from a critically ill patient. *Clin Infect Dis* 2006; 42: 938–944.
9. Ellis D. Amphotericin B: spectrum and resistance. *J Antimicrob Chemother* 2002; 49(Suppl. 1): 7–10.
10. Rodrigues CF, Silva S and Henriques M. Candida glabrata: a review of its features and resistance. *Eur J Clin Microbiol Infect Dis* 2014; 33: 673–688.
11. Sánchez-Vargas LO, Estrada-Barraza D, Pozos-Guillen AJ, *et al.* Biofilm formation by oral clinical isolates of Candida species. *Arch Oral Biol* 2013; 58: 1318–1326.
12. Martins CHG, Pries RH, Cunha AO, *et al.* *Candida*/*Candida* biofilms. First description of dual-species *Candida albicans*/*C. rugosa* biofilm. *Fungal Biology* 2016; 120: 530–537.
13. Sardi JC, Scorzoni L, Bernardi T, *et al.* Candida species: current epidemiology, pathogenicity, biofilm formation, natural antifungal products and new therapeutic options. *J Med Microbiol* 2013; 62: 10–24.
14. d'Enfert C and Janbon G. Biofilm formation in Candida glabrata: what have we learnt from functional genomics approaches? *FEMS Yeast Res* 2016; 16: fov111.
15. Ferrari S, Sanguinetti M, De Bernardis F, *et al.* Loss of mitochondrial functions associated with azole resistance in Candida glabrata results in enhanced virulence in mice. *Antimicrob Agents Chemother* 2011; 55: 1852–1860.
16. Al-fattani MA and Douglas LJ. Penetration of Candida biofilms by antifungal agents. *Antimicrob Agents Chemother* 2004; 48: 3291–3297.
17. De Luca C, Guglielminetti M, Ferrario A, *et al.* Candidemia: species involved, virulence factors and antimycotic susceptibility. *New Microbiol* 2012; 35: 459–468.
18. Grandesso S, Sapino B, Mazzuccato S, *et al.* Study on in vitro susceptibility of Candida spp. isolated from blood culture. *Infez Med* 2012; 20: 25–30.
19. Lewis RE, Kontoyiannis DP, Darouiche RO, *et al.* Antifungal activity of amphotericin B, fluconazole, and voriconazole in an in vitro model of Candida catheter-related bloodstream infection. *Antimicrob Agents Chemother* 2002; 46: 3499–3505.
20. Donlan R and Costerton J. Biofilms: survival mechanisms of clinically relevant microorganisms. *Clin Microbiol Rev* 2002; 15: 167–193.
21. Kamikawa Y, Mori Y, Nagayama T, *et al.* Frequency of clinically isolated strains of oral Candida species at Kagoshima University Hospital, Japan, and their susceptibility to antifungal drugs in 2006-2007 and 2012-2013. *BMC Oral Health* 2014; 14: 14.
22. Tati S, Davidow P, McCall A, *et al.* Candida glabrata binding to Candida albicans hyphae

enables its development in oropharyngeal Candidiasis. *PLoS Pathog* 2016; 12: e1005522.

23. Westbrook SD, Kirkpatrick WR, Wiederhold NP, *et al*. Microbiology and epidemiology of oral yeast colonization in hemopoietic progenitor cell transplant recipients. *Oral Surg Oral Med Oral Pathol Oral Radiol* 2013; 115: 354–358.
24. Samaranayake LP, Fidel PL, Naglik JR, *et al*. Fungal infections associated with HIV infection. *Oral Dis* 2002; 8(Suppl. 2): 151–160.
25. Kojic EM and Darouiche RO. *Candida* infections of medical devices. *Clin Microbiol Rev* 2004; 17: 255–267.
26. Usui S, Tomono Y, Sakai M, *et al*. Preparation and antitumor activities of beta-(1→6) branched (1→3)-beta-D-glucan derivatives. *Biol Pharm Bull* 1998; 18: 1630–1636.
27. Arnold TM, Dotson E, Sarosi GA, *et al*. Traditional and emerging antifungal therapies. *Proc Am Thorac Soc* 2010; 7: 222–228.
28. Alcazar-Fuoli L and Mellado E. Current status of antifungal resistance and its impact on clinical practice. *Br J Haematol* 2014; 166: 471–484.
29. West L, Lowman DW, Mora-Montes HM, *et al*. Differential virulence of Candida glabrata glycosylation mutants. *J Biol Chem* 2013; 288: 22006–22018.
30. Nobile CJ and Johnson AD. *Candida albicans* biofilms and human disease. *Annu Rev Microbiol* 2015; 69: 71–92.
31. Fox EP and Nobile CJ. A sticky situation: untangling the transcriptional network controlling biofilm development in Candida albicans. *Transcription* 2012; 3: 315–322.
32. Gulati M and Nobile CJ. Candida albicans biofilms: development, regulation, and molecular mechanisms. *Microbes Infect* 2016; 18: 310–321.
33. Lefebvrea J-L and Domengeb C. A comparative study of the efficacy and safety of fluconazole oral suspension and amphotericin B oral suspension in cancer patients with mucositis. *Oral Oncol* 2002; 38: 337–342.
34. Pasqualotto AC, Nedel WL, Machado TS, *et al*. Risk factors and outcome for nosocomial breakthrough Candidaemia. *J Infect* 2006; 52: 216–222.
35. Pappas PG, Kauffman CA, Andes DR, *et al*. Clinical practice guideline for the management of Candidiasis: 2016 update by the Infectious Diseases Society of America. *Clin Infect Dis* 2015; 62: e1–e50.
36. Synytsya A and Novák M. Structural diversity of fungal glucans. *Carbohydr Polym* 2013; 92: 792–809.
37. Mitchell KF, Zarnowski R and Andes DR. Fungal super glue: the biofilm matrix and its composition, assembly, and functions. *PLoS Pathog* 2016; 12: e1005828.
38. Zarnowski R, Westler WM, Lacmbouh GA, *et al*. Novel entries in a fungal biofilm matrix encyclopedia. *MBio* 2014; 5: 1–13.
39. Vannucci L, Kirzan J, Sima P, *et al*. Immunostimulatory properties and antitumor activities of glucans (Review). *Int J Oncol* 2013; 43: 357–364.
40. Johansson L, Virkki L, Antilla H, *et al*. Hydrolysis of β-glucan. *Food Chem* 2006; 97: 71–79.
41. Rodrigues CF, Silva S, Azeredo J, *et al*. Detection and quantification of fluconazole Within Candida glabrata biofilms. *Mycopathologia* 2015; 179(5–6): 391–395.
42. Hemilä H and Louhiala P. Vitamin C may affect lung infections. *J R Soc Med* 2007; 100: 495–498.
43. Hemila H. *Do vitamins C and E affect respiratory infections?* Helsinki: University of Helsinki, 2007.
44. Sezikli M, Cetinkaya ZA, Sezikli H, *et al*. Oxidative stress in helicobacter pylori infection: does supplementation with vitamins C and E increase the eradication rate? *Helicobacter* 2009; 14: 280–285.
45. Sezikli M, Çetinkaya ZA, Güzelbulut F, *et al*. Supplementing vitamins C and E to standard triple therapy for the eradication of Helicobacter pylori. *J Clin Pharm Ther* 2012; 37: 282–285.
46. Ma JL, Zhang L, Brown LM, *et al*. Fifteen-year effects of helicobacter pylori, garlic, and vitamin treatments on gastric cancer incidence and mortality. *J Natl Cancer Inst* 2012; 104: 488–492.
47. Sadeghpour A, Alizadehasl A, Kyavar M, *et al*. Impact of vitamin C supplementation on post-cardiac surgery ICU and hospital length of stay. *Anesth Pain Med* 2015; 5: e25337.
48. Silva S, Henriques M, Martins A, *et al*. Biofilms of non-Candida albicans Candida species: quantification, structure and matrix composition. *Med Mycol* 2009; 47: 681–689.
49. Markiewicz M, Dzierzak-Mietla M, Frankiewicz A, *et al*. Treating oral mucositis with a supersaturated calcium phosphate rinse: comparison with control in patients undergoing allogeneic hematopoietic stem cell transplantation. *Support Care Cancer* 2012; 20: 2223–2229.

50. Rao NG, Han G, Greene JN, *et al*. Effect of prophylactic fluconazole on oral mucositis and candidiasis during radiation therapy for head-and-neck cancer. *Pract Radiat Oncol* 2013; 3: 229–233.

51. Faure AM, Sánchez-Ferrer A, Zabara A, *et al*. Modulating the structural properties of β-D-glucan degradation products by alternative reaction pathways. *Carbohydr Polym* 2014; 99: 679–686.

52. Kivelä R, Nyström L, Salovaara H, *et al*. Role of oxidative cleavage and acid hydrolysis of oat beta-glucan in modelled beverage conditions. *J Cereal Sci* 2009; 50: 190–197.

53. Xu H, Nobile CJ and Dongari-Bagtzoglou A. Glucanase induces filamentation of the fungal pathogen Candida albicans. *PLoS ONE* 2013; 8: e63736.

54. Al-Fattani MA and Douglas LJ. Biofilm matrix of Candida albicans and Candida tropicalis: chemical composition and role in drug resistance. *J Med Microbiol* 2006; 55: 999–1008.

55. Kivelä R, Gates F and Sontag-Strohm T. Degradation of cereal beta-glucan by ascorbic acid induced oxygen radicals. *J Cereal Sci* 2009; 49: 1–3.

56. Kivelä R, Sontag-Strohm T, Loponen J, *et al*. Oxidative and radical mediated cleavage of beta-glucan in thermal treatments. *Carbohydr Polym* 2001; 85: 645–652.

57. Faure AM, Andersen ML and Nyström L. Ascorbic acid induced degradation of beta-glucan: hydroxyl radicals as intermediates studied by spin trapping and electron spin resonance spectroscopy. *Carbohydr Polym* 2012; 87: 2160–2168.

58. Ng TS, Desa MN, Sandai D, *et al*. Growth, biofilm formation, antifungal susceptibility and oxidative stress resistance of *Candida glabrata* are affected by different glucose concentrations. *Infect Genet Evol* 2015; 40: 331–338.

59. Von Sonntag C. *Advances in Carbohydrate chemistry and biochemistry*. New York: Academic Press, 1980.

60. De Moura FA, Pereiraa JM, da Silvab DO, *et al*. Effects of oxidative treatment on the physicochemical, rheological and functional properties of oat β-glucan. *Food Chem* 2011; 128: 982–987.

61. Faure AM, Münger LH and Nyström L. Potential inhibitors of the ascorbate-induced beta-glucan degradation. *Food Chem* 2012; 134: 55–63.

62. DuBois M, Gillies KA, Hamilton JK, *et al*. Colorimetric method for determination of sugars and related substances. *Anal Chem* 1956; 28: 350–356.

63. Freimund S, Janett S, Arrigoni E, *et al*. Optimised quantification method for yeast-derived 1,3-β-D-glucan and α-D-mannan. *Eur Food Res Technol* 2005; 220: 101–105.

64. Danielson ME, Dauth R, Elmasry NA, *et al*. Enzymatic method to measure β-1,3-β-1,6-glucan content in extracts and formulated products (GEM Assay). *J Agric Food Chem* 2010; 58: 10305–10308.

Visit SAGE journals online journals.sagepub.com/home/tai