J Med Sci
Volume 44, No.2, June 2012: 1 - 9

Candida albicans biofilm: formation and antifungal agents resistance

Tri Wibawa
Department of Microbiology, Faculty of Medicine, Universitas Gadjah Mada, Yogyakarta, Indonesia

ABSTRACT

Candida sp are the most common fungal pathogens causing fatal health care associated infections. Among the genus of Candida, *Candida albicans* is the most frequent species isolated from patients. The notorious *C. albicans* infection is the ability of this dimorphic fungus to form biofilm. Biofilm has been pointed as a dynamic phenotypic switching in bacteria and fungi, which may result in higher morbidity and mortality in human beings. This review addresses the basic explanation of biofilm formation which is characterized by the antifungal agents resistance. The factors that influence *C. albicans* biofim formation and antifungal agents resistance are discussed.

ABSTRAK

Candida sp merupakan jamur patogen yang paling sering menyebabkan infeksi yang fatal di rumah sakit. Diantara genus Candida, *Candida albicans* merupakan spesies paling sering yang dijumpai pada pasien. *C. albicans* adalah jamur dimorfik yang dapat membentuk biofilm. Biofilm adalah suatu rangkaian perubahan fenotipik pada bakteri maupun jamur, yang dapat menimbulkan peningkatan morbiditas dan mortalitas pada manusia. Review ini membahas tentang pembentukan biofilm yang disertai dengan adanya resistensi terhadap obat antijamur. Hal-hal yang berpengaruh terhadap pembentukan biofilm dan sifat resistensi terhadap antijamur yang menyertainya dibahas di dalam review ini.

Key words: Candida sp – antifungal – resistance – biofilm - pathogenecity

INTRODUCTION

Candida sp are documented as a causative agent of plenty fungal infection in human beings. *Candida sp* are the most common fungal pathogens causing fatal health care associated infections, especially in patients admitted to intensive care units and the fourth most frequent pathogen isolated and accounted for 9% of blood stream infection.[1] Colonization and biofilm formation of *Candida sp* has been reported in the biomaterials, such as: shunts, stents, prostheses, implants, catheters, and other indwelling medical devices.[2,3] The *Candida sp* isolated from patients suffering from nosocomial blood stream infection in the Surveillance and Control of Pathogens of Epidemiological Importance (SCOPE) study in the United States were *Candida albicans* (54%), followed by *C. glabrata* (19%), *C. parapsilosis* (11%), and *C. tropicalis* (11%) respectively.[4] *Candida albican*s has been reported as a common causative agent for

* corresponding author: twibawa@ugm.ac.id

nosocomial infection transmitted among patients in burn units,[5] and was documented as the most common cause of recurrent and non-recurrent vulvovaginitis.[6]

Candida albicans is a yeast form fungi which is well known as a classic example of opportunistic fungi which may be responsible for various superficial and systemic infection in human beings. *Candida albicans* is frequently found as the normal microbiota of humans and does not regularly cause disease in immunocompetent hosts. It exists as commensal in the skin and mucosal surfaces, as well as genital and gastrointestinal tracts. However, in immunocompomised patients it may be responsible for mild to severe clinical manifestation.[7]

Candida albicans becomes pathogen if the host's immune response is impaired. The impaired local or systemic immune response may contribute to the alteration of commensal-pathogen characteristic of *C. albicans*. Neutropenia, neutrophil dysfunction, disruption of mucosal barriers, patients receiving chemotherapy for neoplasm, and immuno-suppressants after organ transplantation or patients with HIV/AIDS are the risk factors for disseminated opportunistic fungal infections.[8] In an immunocompromised host, translocation from the gastrointestinal tract and intravascular catheters are the two main sites of entry for disseminated candida infection.[7] In HIV/AIDS patients, *C. albicans* was observed as the leading fungi that causes opportunistic infection.[9]

Candida albicans is unicellular, reproduced by budding, and grows well in routine automated blood culture bottles and on agar plates. *Candida albicans* is dimorphic fungi that is characterized by its ability to grow alternately as yeast and filamentous forms fungi. In specific conditions it can be found in where all the cells grow as yeast and in other conditions it can be found in where most cells grow as hyphae or filamentous form.[10] The morphological change of *C. albicans* is rapid and in response to the external signals. These dynamic changes are associated with the pathogenecity and virulence of the microorganism.[11]

Other than dimorphic (yeast-hyphae) biological property, *C. albicans* has morphological characteristics which may occur naturally in its life cycle. These distinct morphologies include the pseudohyphal form, opaque form and chlamydospore. Pseudohyphae is related with its budding reproduction property. It is often found with yeast and hyphal forms in vegetative culture and during infection.[11] It is well known that *C. albicans* may produce pseudohyphae and true hyphae at the same time (FIGURE 1). The opaque form is associated with mating-competent cells. The white-opaque transition is associated to the sexual mating process in *C. albicans*.[12]

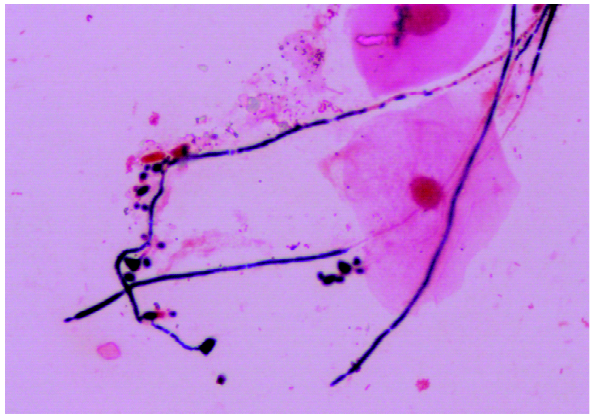

FIGURE 1. Microscopic appearance of Gram staining of sputum obtained from HIV/AIDS patient that showed colonization of *C. albicans*. The budding yeast, true hyphae and pseudohyphae were observed.

This review is addressing biofilm formaton of *C. albicans* and its implication to the antifungal resistant and factors that may be

useful to modify the deleterious impact of antifungal resistant.

DISCUSSION

Biofilm formation of *C. albicans*

Biofilm can be defined as a community of microorganisms that are irreversibly attached to a surface, containing exopolymeric matrix and exhibiting distinctive phenotypic properties.[13] The most important distinctive phenotypic property of biofilm forming microorganism is the antimicrobial resistance. This might result to a critical situation in patient setting, as in some circumstances it means the necessity of removing the prosthesis, implant or other medical device. The increases of morbidity and mortality as well as medical expenses are the inevitable consequences.

Candida albicans is not the only species included in the Candida genus which is able to develop biofilm. Indeed, *C. albicans* biofilm is the most studied biofilm formed by yeasts. Three stages of *C. albicans* biofilm formation are hypothesized as: the adherence of yeast cells to the surface (initial phase), formation of an extracellular matrix with dimorphic switching from yeast to hyphal forms (intermediate phase), and increase in the extracellular matrix material recruitment to form three dimensional structure of biofilm (maturation phase) (FIGURE 2).[3,14] It begins with adherence of yeast cells to a foreign substrate (host tissue or medical device) (A), followed by proliferation of the yeast cells across the substrate surface with hyphal development which may include the pseudohyphae and true hyphae (B). The final step of biofilm development is the maturation phase with recruitment of massive exctra-cellular matrix (brown) from the environmental substrate that may produced by the host or the microorganisms that contribute to the biofilm development (C). After entering maturation phase, a few planktonic cells may be released from the mature biofilm and transferred to the new surface to start the new biofilm formation cycle.

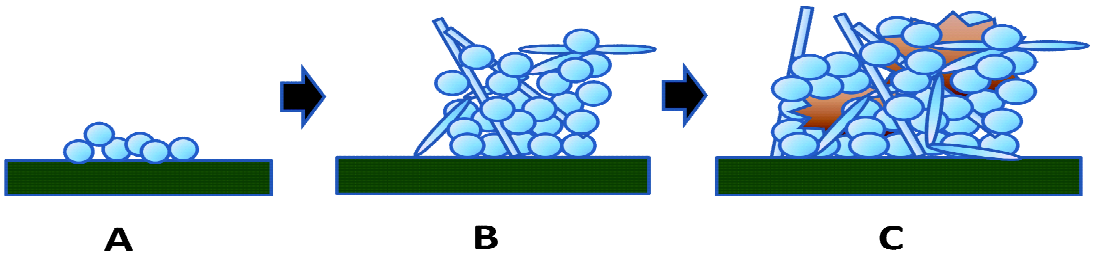

FIGURE 2. Cartoon of step-wise process of *C. albicans* biofilm development

In every step of biofilm formation, there are dynamic gene expressions that may facilitate the biofilm formation. Some genes are specifically expressed in planctonics, initial phase, intermediate phase or maturation phase.[15] There are plenty genes that have been identified involved in cell adhesion (42 genes), biofilm formation (122 genes), filementous growth (512 genes), and phenotypic switching (44 genes) respectively.[16] Very recently, Nobile *et al*[17] described a master circuit of six transcription regulators, sequence-specific DNA-binding proteins that regulate transcription, which controls biofilm formation by *C. albicans*. Exploring the regulation of genes expression in the biofilm formation phases is important to

understand the characteristic of candidal biofilm. It is even more important to find the molecular target to inhibit the biofilm formation.

The initial phase is started when *C. albicans* cells attached to the surface of living or unanimated material such as, mucosal layer, dental surface, or indwelling medical devices. It is important to consider that cells-surface adherence of the yeast is the key point of biofilm development. It needs to anchor the structure on the surface. On the other hand, the adherence among yeast cells itself has been also highlighted as key point of initial phase of biofilm formation properties.[13,18] The adherence of yeast cells to the surface were regulated by the environmental milieu. For example in oral cavity, the adherence was influenced by the dietary and salivary factors. The presence of two monosaccharides, glucose and galactose, has been extensively investigated for their effects on candidal adhesion.[19] This finding was in agreement with another report which compared the biofilm formation *in vitro* by using two difference media, RPMI and synthetic urine. The level of hyphal formation of yeast cells biofilms formed in synthetic urine medium was diminished compared to those grown in RPMI medium.[20]

The nature of medical devices surface is important to build a candida biofilm. The property of medical devices surface, contact angle of materials, and the index of hydrophobicity were found to be correlated positively with initial adhesion and biofilm formation of *C. albicans*.[21,22] Modification of medical devices surface has been showed as candidate approach to inhibit candida biofilm formation.[23] The surface support for biofilm formation is depending on the nature of biomaterial. It was showed in the *C. albicans* biofilm formation model that latex and silicone elastomer increased the biofilm formation but not the polyurethane or pure silicone.[24,25] Nonetheless, there was a negative correlation between mucin absorption with removability of candida biofilm.[21,22]

Intermediate phase was characterized by formation of an extracellular matrix with dimorphic switching from yeast to hyphal forms. However, switching of the yeast to hyphal form was not obligated for *C. albicans* biofilm. Two mutants of *C. albicans* derived from one parental strain were characterized as incapable to form hyphae or yeast. Experimental procedures using these two mutants showed that it still showed the ability to form biofilm.[26] It was observed that the characteristic of the biofilms of the two mutants was different. The hypha-negative mutant produced only the basal layer, and the yeast-negative mutant produced only the outer layer, which was more easily detached from the catheter disks. This suggests that dimorphism might be necessary for biofilm architecture and structure.[3] This result is in agreement with the report that found farnesol, a quorum-sensing molecule, inhibit filamentation in *C. albicans*, also inhibits its biofilm formation.[2,27] However, it should be noted that biofilm somehow absolutely does not depend to the morphological properties of the fungi. Either yeasts or hyphal morphology may contribute to the potential effect of biofilm formation.

Important to the maturation of candidal biofilm is recruitment and deposition of extracellular matrix. This complex extracellular material might function to defend against phagocytic cells of host immune response, to serve as a scaffold to maintain biofilm structure integrity, and to safe guard the biofilm from environmental exposure.[18] Environmental factor that may be important to the candida biofilm is the bacterial and fungal populations that may interact with *C. albicans* to modulate the nature of the biofilm extracellular matrix. The viability of candida biofilms was significantly decreased by the presence of *Pseudomonas aeruginosa* and *Escherichia coli*. Further, it was reported

that *Streptococcus mutans* increased *C. albicans* biofilm formation, and that *C. albicans* displayed synergism with *C. glabrata* when they developed biofilm.[28,29] The relationship between bacteria or other fungi with candidal biofilm is complex and not completely elucidated yet. It depends on the bacterial species and its numbers, and may affect the morphogenesis of the yeast.[29]

The innate immune system has been identified as the principle protection against candidiasis. Polymorphonuclear leukocytes (PMNs) are the primary components of the innate immune response against *Candida* infections.[30] Macrophages and neutrophiles are the cells that are most commonly associated with the innate immune response against *C. albicans* infection. Macrophages produce a variety of soluble factors, including cytokines and chemokines, in response to specific microorganism, including *C. albicans*.[31] Little is known about the ways in which macrophages and neutrophils recognize *C. albicans* as a pathogenic microorganism, or how the fungal–leukocyte interaction triggers an inflammatory response.[8]

It was reported that viable peripheral blood mononuclear cells (PBMC) did not phagocytes the fungal cells in the biofilm form. This phenomenon is in contrast with the finding that PBMCs phagocytes the planctonic *C. albicans.* Indeed, the host innate immune response influences the biofilm formation of *C. albicans*. The biofilm formation enhancing effect of PBMCs is mediated by soluble factors, which consist of pro- and anti- inflammatory cytokines, and released into the co-culture medium of PBMCs with *C. albicans*.[31]

Antifungal agents resistance

As opportunistic fungi, *C. albicans* might be found either as commensals or pathogens. The factors that facilitate its switching are considered as virulence factors. Several virulence factors were suggested, such as genes and proteins that regulate adhesion, hyphal formation, proteinase protein, phenotypic switching and biofilm formation.[16] There is a positive association between the levels of virulence of *C. albicans* with the ability to form biofilm.[24]

In clinical setting, biofilm forming *C. albicans* has a significant difference characteristic compared to non biofilm forming. This condition is believed as the result of distinct diseases pathogenesis. The most discussed characteristic is its resistance to antifungal treatment. It has been reported that 50% inhibition of [^{3}H]leucine incorporation (IL_{50}) and 50% inhibition of MTT-formazan formation (IF_{50}) for biofilms were 5 to 8 times higher than the observed values for planktonic cells and 30 to 2,000 times higher than the relevant minimum inhibitory concentrations (MIC) for five antifungals studied, i.e: amphotericin B, fluconazole, flucytosine, intraconazole, and ketoconazole. Among antifungal drugs tested in this study, fluconazole is the most effective for *C. albicans* biofilm.[32] *C. albicans* biofilm was documented as highly resistant to antifungal agents: fluconazole, nystatin, amphotericin B, voriconazole, ravuconazole, terbenafine, and chlorhexidine in other reports.[14,33] The resistance of *C. albicans* to antifungal agents, as reflected by its MIC was reported to increase during the development of biofilm in progress. The drug resistance develops over time, and corresponds to the development of biofilm itself, which was associated with the increase of metabolic activity of developing biofilm.[14]

The mechanism of antifungal agents resistance in *C. albicans* biofilm is not well known yet. However, there are several reports that serve data for hypothetical mechanism of antifungal agents resistance in *C. albicans* biofilm: (1) biofilm slow growth; (2) decrease of the concentration of antifungal agents in the depth of biofilm because of penetration barrier; and (3) expression of resistant regulator genes

that associated with surface contact.[25] One possible resistance mechanism is related to the slow growth rate of *C. albicans* biofilm cells as a result of the limited availability of nutrients.[25] Slow growth is the main difference of the characteristic of biofilm forming yeast cells compared to the planktonic cells. However, the resistance to amphotericin B of *C. albicans* biofilm was not dependent to growth rate. It was in contrast with the susceptibility of planktonic cells to the drug that was highly dependent on growth rate. In the very low growth rate, planktonic cells were resistant to amphotericin B, but they became susceptible when the growth rate increased.[26] This data suggested that drug resistance to antifungal drugs is not simply resulted from slow growth of *C. albicans* biofilm.

It was hypothesized that the biofilm's three dimensional structures can physically prevent the entrance of antifungal agents inside the depth of biofilm, that eventually diminishes the susceptibility of biofilm against antifungal agents. Physical barrier of biofilm is mainly the consequence of deposition of extracellular matrix among the microorganisms cellular body which contributes to the biofilm formation. *C. albicans* biofilms which were grown in static condition and gentle shaking were compared regarding to their susceptibility to antifungal agents. Static condition is mimicking the condition with limited extracellular matrix and shaking condition is similar with condition that induces more extracellular matrix. By using this experiment, Baillie and Douglas[34] showed that the biofilm susceptibility against antifungal agents between the two growing conditions was not significantly different. It was suggested that the extracellular matrix did not associate with the resistance of biofilm to the antifungal agents. It was also documented that the surface on which the biofilm was developed did not affect the drug susceptibility profile. However, the role of cell density in the phenotypic resistance of biofilm is in debate. There was contribution of cell density to the phenotypic resistant.[35,36] However, recently it was reported that the higher number of cells does not explain the higher resistance of biofilm against biocides.[37]

Biofilm formation results to the phenotypic change of *C. albicans*, which is believed to be associated with the dynamic of genes expression. Particular interest is highlighted to the genes which regulate the antifungal resistant phenotypes (TABLE 1). Understanding the genes regulation may open a new insight to the mechanisms of antifungal agents resistance associated with biofilm formation.

TABLE 1. Genes contribute to the *C. albicans* biofilm antifungal agents resistance

Genes	Function	References
CDR1, CDR2, MDR1	drug efflux activity	[38]
Kre1, Skn1	β-1,6-glucan biosynthesis	[39]
Erg 1, 3, 6, 11, and 25	ergosterol biosynthesis	[39] [40]
Fks1p	β-1,3-glucan biosynthesis	[41]
SMI1, RLM1	β-1,3-glucan biosynthesis	[42]
Hsp90	stabilizing regulators of cellular signaling	[43]
OBP, PIK, PAP	non sex genes	[44]
BGL2, PHR1, and XOG1	glucan transferases	[45]
Gup1p	lipid metabolism	[46]
PIL1	the hyphal-specific echinocandin-binding protein	[47]

CONCLUSION

Candida albicans is the most frequent fungal biofilm forming yeast in the clinic. Fungal biofilm has been studied for a long time by many workers; nevertheless many questions still need to be addressed. Fungal biofilm is an inducible phenotype that may result to the antifungal agents resistance. The actual environmental and genetics make up of the yeast that plays pivotal role in this inducement is not clearly elucidated yet. Antifungal agent resistance is the most important topic, since the implication of fungal biofilm in the clinics is obvious. Molecular mechanism of antifungal resistant in biofilm is a complex pathway. We need more additional data that provide insight to find the molecular targets of drugs that may inhibit the development of biofilm in the clinical setting.

ACKNOWLEDGEMENT

Author would like to thank Ms. Mulyani for technical assistance of preparing *C. albicans* figure.

REFERENCES

1. Alangaden GJ. Nosocomial fungal infections: epidemiology, infection control, and prevention. Infect Dis Clin North Am 2011; 25(1):201-25.
2. Ramage G, Saville SP, Wickes BL, Lo´pez-Ribot JL. Inhibition of *Candida albicans* biofilm formation by farnesol, a quorum-sensing molecule. Appl Environ Microbiol 2002; 68(11):5459-63.
3. Kojic EM, Darouiche RO. Candida infections of medical devices. Clin Microbiol Rev 2004; 17(2):255-67.
4. Wisplinghoff H, Bischoff T, Tallent SM, Seifert H,Wenzel RP, Edmond MB. Nosocomial bloodstream infections in US hospitals: analysis of 24.179 cases from a prospective nationwide surveillance study. Clin Infect Dis 2004; 39(3):309-17.
5. Gupta N, Haque A, Lattif AA, Narayan RP, Mukhopadhyay G, Prasad R. Epidemiology and molecular typing of *Candida* isolates from burn patients. Mycopathologia 2004; 158(4):397-405.
6. Mahmoudi Rad M, Zafarghandi S, Abbasabadi B, Tavallaee M. The epidemiology of *Candida* species associated with vulvovaginal candidiasis in an Iranian patient population. Eur J Obstet Gynaecol Reprod Biol 2011; 155(2):199-203.
7. Miceli MH, Díaz JA, Lee SA. Emerging opportunistic yeast infections. Lancet Infect Dis 2011; 11(2):142-51.
8. Netea MG, Brown GD, Kullberg BJ, Gow NA. An integrated model of the recognition of *Candida albicans* by the innate immune system. Nat Rev Microbiol 2008; 6(1):67-78.
9. Onyancha OB, Ocholla DN. An informetric investigation of the relatedness of opportunistic infections to HIV/AIDS. Inform Process Manag 2005; 41:1573-88.
10. Whiteway M, Bachewich C. Morphogenesis in *Candida albicans.* Annu Rev Microbiol 2008; 61:529-53.
11. Sudbery P, Gow N, Berman J. The distinct morphogenic states of *Candida albicans*. Trends Microbiol 2004; 12(7):317-24.
12. Soll DR. Mating-type locus homozygosis, phenotypic switching and mating: a unique sequence of dependencies in *Candida albicans.* Bioessays 2004; 26:10-20.
13. Kumamoto CA. Candida biofilms. Curr Opin Microbiol 2002; 5(6):608-11.
14. Chandra J, Kuhn DM, Mukherjee PK, Hoyer LL, McCormick T, Ghannoum MA. Biofilm formation by the fungal pathogen *Candida albicans:* development, architecture, and drug resistance. J Bacteriol 2001; 183(18):5385-94.
15. Mukherjee PK, Mohamed S, Chandra J, Kuhn D, Liu S, Antar OS, *et al.* Alcohol dehydrogenase restricts the ability of the pathogen *Candida albicans* to form a biofilm on catheter surfaces through an ethanol-based mechanism. Infect Immun 2006; 74(7):3804-16.
16. Inglis DO, Skrzypek MS, Arnaud MB, Binkley J, Shah P, Wymore F, *et al.* Improved gene ontology annotation for biofilm formation, filamentous growth, and phenotypic switching in *Candida albicans*. Eukaryot cell 2013; 12(1):101-8.
17. Nobile CJ, Fox EP, Nett JE, Sorrells TR, Mitrovich QM, Hernday AD, *et al.* A recently evolved transcriptional network controls biofilm development in *Candida albicans.* Cell 2012; 148(1-2):126-38.

18. Blankenship JR, Mitchell AP. How to build a biofilm: a fungal perspective. Curr Opin Microbiol 2006; 9(6):588-94.
19. Jin Y, Samaranayake LP, Samaranayake Y, Yip HK. Biofilm formation of *Candida albicans* is variably affected by saliva and dietary sugars. Arch Oral Biol 2004; 49(10):789-98.
20. Uppuluri P, Dinakaran H, Thomas DP, Chaturvedi AK, Lopez-Ribot JL. Characteristics of *Candida albicans* biofilms grown in a synthetic urine medium. J Clin Microbiol 2009; 47(12):4078-83.
21. Bürgers R, Hahnel S, Reichert TE, Rosentritt M, Behr M, Gerlach T, *et al*. Adhesion of *Candida albicans* to various dental implant surfaces and the influence of salivary pellicle proteins. Acta Biomater. 2010; 6:2307-13.
22. Li J, Hirota K, Goto T, Yumoto H, Miyake Y, Ichikawa T. Biofilm formation of *Candida albicans* on implant overdenture materials and its removal. J Dent 2012; 40(8):686-92.
23. Chandra J, Patel JD, Li J, Zhou G, Mukherjee PK, McCormick TS, *et al.* Modification of surface properties of biomaterials influences the ability of *Candida albicans* to form biofilms. Appl Environ Microbiol. 2005; 71(12):8795-801.
24. Hawser SP, Douglas LJ. Biofilm formation by *Candida* species on the surface of catheter materials *in vitro*. Infect Immun 1994; 62(3):915-21.
25. Douglas LJ. Candida biofilms and their role in infection. Trends Microbiol 2003; 11(1):30-6.
26. Baillie GS, Douglas LJ. Effect of growth rate on resistance of *Candida albicans* biofilms to antifungal agents. Antimicrob Agents Chemother 1998; 42(8):1900-5.
27. Deveau A, Hogan DA. Linking quorum sensing regulation and biofilm formation by *Candida albicans*. Methods Mol Biol 2011; 692:219-33.
28. Pereira-Cenci T, Deng DM, Kraneveld EA, Manders EM, Del Bel Cury AA, Ten Cate JM, *et al*. The effect of *Streptococcus mutans* and *Candida glabrata* on *Candida albicans* biofilms formed on different surfaces. Arch Oral Biol 2008; 53(8):755-64.
29. Thein ZM, Samaranayake YH, Samaranayake LP. Effect of oral bacteria on growth and survival of *Candida albicans* biofilms. Arch Oral Biol 2006; 51(8):672-80.
30. Soloviev DA, Jawhara S, Fonzi WA. Regulation of innate immune response to *Candida albicans* infections by áMâ2-pra1p interaction. Infect Immun. 2011; 79(4):1546-58.
31. Chandra J, McCormick TS, Imamura Y, Mukherjee PK, Ghannoum MA. Interaction of Candida albicans with adherent human peripheral blood mononuclear cells increases *Candida albicans* biofilm formation and results in differential expression of pro-and anti-inflammatory cytokines. Infect Immun 2007; 75(5):2612-20.
32. Hawser SP, Douglas LJ. Resistance of *Candida albicans* biofilms to antifungal agents *in vitro*. Antimicrob Agents Chemother 1995; 39(9):2128-31.
33. Kuhn DM, George T, Chandra J, Mukherjee PK, Ghannoum MA. Antifungal susceptibility of Candida biofilms: unique efficacy of amphotericin B lipid formulations and echinocandins. Antimicrob Agents Chemother 2002; 46:1773-80.
34. Baillie GS, Douglas LJ. Matrix polymers of Candida biofilms and their possible role in biofilm resistance to antifungal agents. J Antimicrob Chemother 2000; 46(3):397-403.
35. Perumal P, Mekala S, Chaffin WL. Role for cell density in antifungal drug resistance in *Candida albicans* biofilms. Antimicrob Agents Chemother 2007; 51(7):2454-63.
36. Watamoto T, Samaranayake LP, Jayatilake JA, Egusa H, Yatani H, Seneviratne CJ. Effect of filamentation and mode of growth on antifungal susceptibility of *Candida albicans*. Int J Antimicrob Agents 2009; 34(4):333-9.
37. Leung CY, Chan YC, Samaranayake LP, Seneviratne CJ. Biocide resistance of *Candida* and *Escherichia coli* biofilms is associated with higher antioxidative capacities. J Hosp Infect 2012; 81(2):79-86.
38. Mukherjee PK, Chandra J, Kuhn DM, Ghannoum MA. Mechanism of fluconazole resistance in *Candida albicans* biofilms: Phase-specific role of efflux pumps and membrane sterols. Infect Immun 2003; 71(8):4333-40.
39. Nailis H, Vandenbosch D, Deforce D, Nelis HJ, Coenye T. Transcriptional response to fluconazole and amphotericin B in *Candida albicans* biofilms. Res Microbiol 2010; 161(4):284-92.
40. Yu LH, Wei X, Ma M, Chen XJ, Xu SB. Possible inhibitory molecular mechanism of farnesol on

the development of fluconazole resistance in *Candida albicans* biofilm. Antimicrob Agents Chemother 2012; 56(2):770-5.

41. Nett JE, Sanchez H, Cain MT, Andes DR. Genetic basis of Candida biofilm resistance due to drug-sequestering matrix glucan. J Infect Dis 2010; 202(1):171-5.
42. Nett JE, Sanchez H, Cain MT, Ross KM, Andes DR. Interface of *Candida albicans* biofilm matrix-associated drug resistance and cell wall integrity regulation. Eukaryot Cell. 2011; 10(12):1660-9.
43. Robbins N, Uppuluri P, Nett J, Rajendran R, Ramage G, Lopez- Ribot JL *et al.* Hsp90 governs dispersion and drug resistance of fungal biofilms. PLoS Pathog. 2011; 7(9):1002257.
44. Srikantha T, Daniels KJ, Pujol C, Sahni N, Yi S, Soll DR. Nonsex genes in the mating type locus of *Candida albicans* play roles in a/á biofilm formation, including impermeability and fluconazole resistance. PLoS Pathog. 2012; 8(1):1002476.
45. Taff HT, Nett JE, Zarnowski R, Ross KM, Sanchez H, Cain MT *et al*. A Candida biofilm-induced pathway for matrix glucan delivery: implications for drug resistance. PLoS Pathog. 2012; 8(8):1002848.
46. Ferreira C, Silva S, Faria-Oliveira F, Pinho E, Henriques M, Lucas C. *Candida albicans* virulence and drug-resistance requires the O-acyltransferase Gup1p. BMC Microbiol 2010; 15(10):238.
47. Watamoto T, Samaranayake LP, Egusa H, Yatani H, Seneviratne CJ. Transcriptional regulation of drug-resistance genes in *Candida albicans* biofilms in response to antifungals. J Med Microbiol 2011; 60(9):1241-7.