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Profile of Candida Resistancy to Fluconazole in Male Patient with Oral Candidiasis and HIV/AIDS

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Abstract

Background: Oral candidiasis is one of the most common opportunistic infections found in patients with Human Immunodeficiency Virus (HIV)/Acquired immune deficiency syndrome (AIDS). The number of HIV/AIDS patient increases every years. Past studies revelead the increment in the resistance of Candida species causing oral candidiasis against Fluconazole despite Fluconazole being the treatment of choice for this condition.

Objective: To evaluate the resistancy of Candida species to fluconazole in male patients with oral candidiasis and HIV/AIDS.

Methods: This was a descriptive observational study conducted in Dr. Soetomo General Hospital, Surabaya. The candida species was identified by using conventional methods. Resistancy against fluconazole were evaluated bydiskdiffusion method.

Results: There were 20 research subjects with 37 isolates of Candida species growing in culture. Candida species was found in 20 (54.1%) isolates, while 17 (45.9%) isolates showed non-albicans species. The resistance test of Candida species to fluconazole revealed that 18 (48.6%) isolates were resistant to fluconazole. Majority of the resistant isolates were of Candida non-albicans 13 isolates (72.2%).

Conclusion: The use of fluconazole drugs especially in patients with HIV/AIDS should be reevaluated as Candida species has developed high resistance towards the medication.

Keywords: Resistance test; Fluconazole; Candida species

Introduction

Oral candidiasis is one of the most common skin and mucosal manifestations found in patients with Human Immunodeficiency Virus (HIV) or Acquired Immune Deficiency Syndrome (AIDS). Oral candidiasis is an infection of the mucosa caused by Candida species. Nearly 90% of patients with HIV/AIDS have experienced oral candidiasis in the course of the illness. Cases of oral candidiasis are found in patients with CD4 T cell counts <200/mm^3 and high viral load numbers [1-3]. The most common etiology of oral candidiasis in patients with HIV/AIDS are *Candida albicans* in Malang, Indonesia of 86% [4] and in India, oral candidiasis cause by *Candida albicans* was 52% [5]. In other study conducted in Surabaya in 2008, there was also an increase in Candida non-albicans oral candidiasis [6].

World Health Organization (WHO) claimed that fluconazole and miconazole are the first-line therapy in cases of oral candidiasis in patients with HIV/AIDS. Fluconazole is a safe, inexpensive, effective, and available antifungal option in Indonesia. Topical drugs such as nystatin or other oral antifungal such as ketoconazole and itraconazole are also used as therapeutic options for oral candidiasis [4].

Extensive use of fluconazole in cases of oral candidiasis has been reported to be associated with candida species and *in vitro* sensitivity to fluconazole [7,8]. Some of the factors that influence the decrease in sensitivity in the treatment of oral candidiasis can be caused by two factors: changes in causative species from the albicans to non-albicans which have intrinsic resistance such as *C. glabrata* and *C. krusei*; and the emergence of resistance against azole stains [7,9].

A decrease in fluconazole sensitivity shows as the increment in drug dosage and resistance in cases of oral candidiasis. Resistance in cases of oral candidiasis were primarily reported at 1990 [10]. Research conducted in various places shows the resistance of *Candida* species to fluconazole ranging from 6-36%. Lattif et al. reported that 5% of isolates of Candida species were resistant to fluconazole [11]. Research in Malang showed resistance caused by Candida non-albicans to fluconazole by 8% [4].

In Indonesia the amount HIV/AIDS patients on 2017 reported as many as 242,699 patients. Data of Dr. Soetomo General Hospital Surabaya, Indonesiaon 2013 showed that 301 HIV/AIDS patients with 244 (81.1%) experienced candidiasis problem [11]. On 2017, 70-90% of patients HIV/AIDS in Dr. Soetomo General Hospital Surabaya, Indonesia experienced candidiasis. Ninety percents of those was male. On the other hand, the resistance pattern of Candida species sp against fluconazole in Indonesia is still minimally explored. The aim of this research is to evaluate the resistancy of Candida species to fluconazole on males oral candidiasi patient with HIV/AIDS.

Methods

Subject in this research is HIV/AIDS patients at Dr. Soetomo General Hospital Surabaya, Indonesia. Inclusion criteria of subject

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are patient diagnosed with HIV/AIDS using rapid test [12,13], have been diagnosed of candidiasis positive examined with potassium hydroxide (KOH) 10-20% [14,15], male, and >18 years old. Criteria exclusion aresubject with antifungal medications within 2 weeks prior to the study conducted and no growth of fungal colonies on culture examinationect. Subject who are willing to take part in the study get an explanation regarding the research and assign informed consent.

This study used descriptive observational which was be held from june to October 2018. The study was conducted in two places, namely Dr. Soetomo General Hospital Surabaya, Indonesia and Surabaya Health Laboratory, Indonesia. Culture taking was carried out at Dr. Soetomo General Hospital Surabaya, Indonesia. Examination of culture was carried out at the Surabaya Health Laboratory, Indonesia. The number of subjects was 20 HIV/AIDS patients who went through the identification process can be seen in Figure 1. Before the research was conducted we had already undergone ethical tests (0231/KEPK/IV/2018).

The procedure in this study began with patients culture checks [14,15] taken from oral tissue swabs implanted into the CHROMagar Candida media (CHROMagar Candida, France). Furthermore, the culture was grown for 36-48 hours and followed by carbohydrate fermentation test to determine the fungal morphology in the period of 48-72 hours. Carbohydrate fermentation test using microtitre plate [16]. After successfully identifying Candida species, a resistance test was performed using the disk diffusion method on Mueller Hinton agar with 2% glucose and methylene blue. Candida species isolates were implanted in the agar, then paper disks containing the potential of nystatin, ketoconazole and fluconazole were placed on top of it. Observe in 24-48 hours the appearance of a resistance zone around the disk paper. Measurement of the diameter of the inhibition zone was accurately done with the calipers. Then the interpretation was done usingguideline of Clinical and Laboratory Standart Institude (CLSI) and Rosco Diagnostica Company. The results were documented, which then be evaluated and presented in the form of tables and graphs.

Results

From June to October 2018, the number of HIV/AIDS patients in Dr. Soetomo General Hospital Surabaya, Indonesia were found 59 male patients who were clinically diagnosed with oral candidiasis but 19 of them had already taken antifungal treatment formerly so that they were excluded from this study. Then 40 patients were tested for 10-20 KOH%, as much as 24 male patients had positive result of fungal infection. When fungal culture was performed, only 20 male patients with oral candidiasis and HIV/AIDS patients met the subject criteria. Twenty HIV/AIDS patients produced 37 isolates of Candida species. The age characteristics of subjects mostly late adult and early elderly as much as 25% each group, then young adult with 20.00%. Most subjects had a final education at the high school as much as 45% followed by elementary/junior high school level as much as 25% (Table 1).

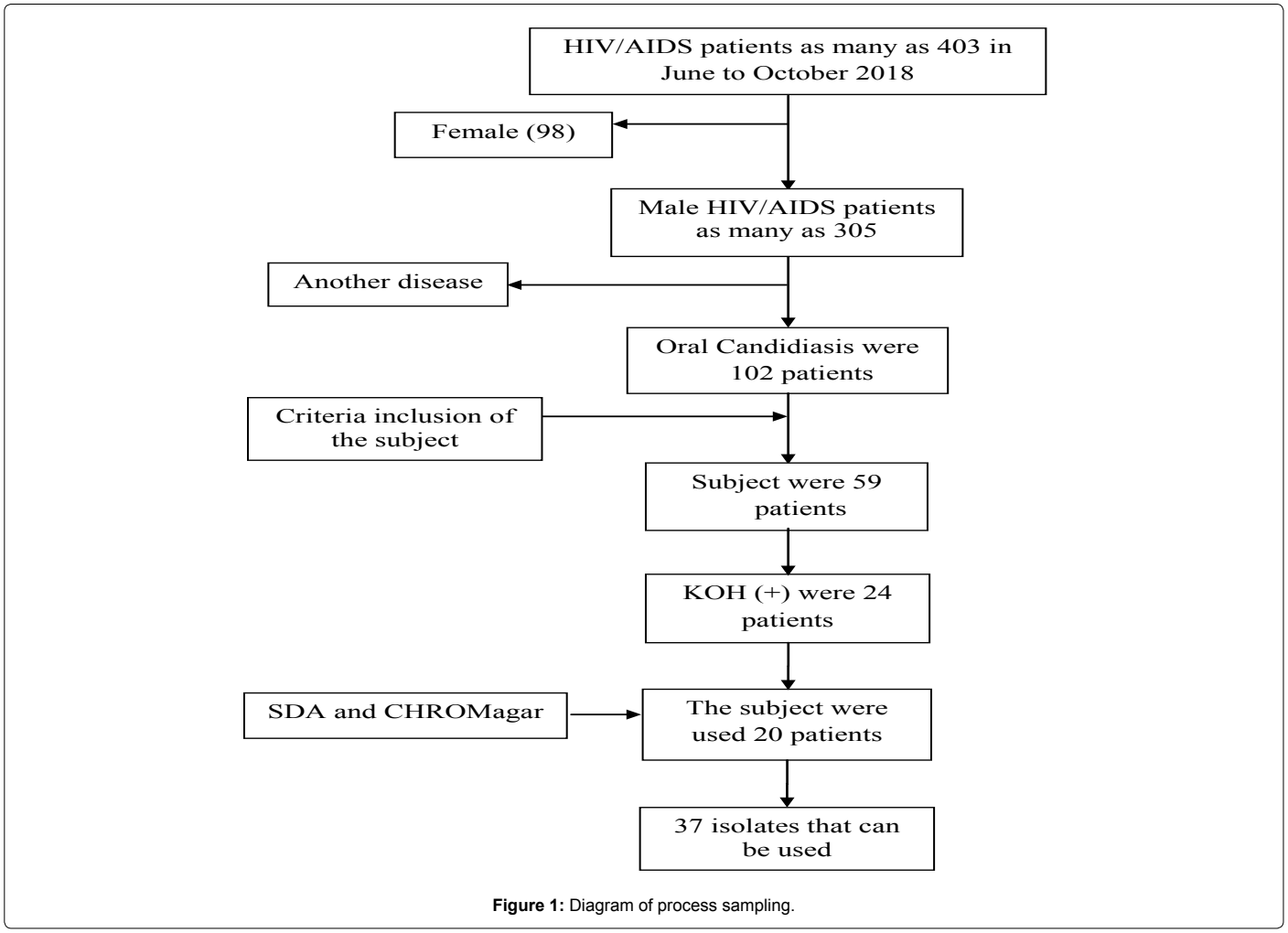

Figure 1: Diagram of process sampling.

The chief complaint of oral candidasis in this study is white spots in the oral cavity, found in all subjects of 20 people (100%). The lesion were found on the tongue in all subjects of 20 (100%). Approximately 11 subjects (55%) experienced oral candidiasis for the first time. Previous history of systemic and topical anti-fungal treatment was found in 12 people, 4 of whom had received systemic antifungals and 8 had received topical antifungals. One study subject can have more than one main complaint, location of the disease and antifungal treatment (Table 2).

Our subjects had diverse HIV/AIDS status, HIV/AIDS was most commonly transmitted through heterosexual or free sex behavior as many as 13 (65%). Each subject could have more than one type of HIV transmission pathway. Most subjects experienced HIV/AIDS for <1 year period as much as 50% and most of the subjects were in AIDS stage III counted as 65%. Dominantly subjects received routine antiretroviral (ARV) therapy as much as 80%. The absolute CD4 value of the subject was mostly <200 cell/μL as much as 65% (Table 3). Based on the results of the examination it was found that the majority of subjects diagnosed with pseudomembranous oral candidiasis as much as 80%, followed by oral candidiasis of plaque hyperplasia of 25%, and 5% respectively for atrophic oral candidiasis and cheilitis candidiasis. This condition can be caused by one subject possibly have two different types of fungi.

In 10 research subjects, there were more than one species of *Candida*. *C. albicans* is still the main cause of oral candidiasis in patients with HIV/AIDS in Dr. Soetomo General Hospital Surabaya, Indonesia. The fungi was found in 54.1% (Table 4). The non-albicans group was found in 17 isolates (45.9%). The resistance test against fluconazole showed that the number of *Candida* species that is still sensitive to fluconazole (51.4%) was relatively equal to the number of *Candida* species that is resistant (18 isolates) (48.6%). Amongst the resistant group was mainly the *non-albicans Candida* of 76.4% (Table 5).

No	Basic Data		N	%
	Category	Group		
1	Age	Late teens 18-25 year	3	15%
		Early adult 26-35 year	4	20%
		Late adult 36-45 year	5	25%
		Early senior adult 46-55 year	5	25%
		Late senior adult 56-65 year	3	15%
		Geriatric >66 year	0	0
2	Education	No prior education	3	15%
		Graduated from elementary/junior high school	5	25%
		Graduated from high school	9	45%
		Graduated from S1/diploma	3	15%
3	Occupation	Unemployed	9	45%
		Civil servants	2	10%
		Private employees	8	40%
		Student	1	5%

Male HIV/AIDS patient

Table 1: Demographic data on research subjects.

No	Clinical picture		N	%
	Category	Subject		
1	Chief complaint	White spot in oral cavity	20	100
		Red spot in oral cavity	1	5
		Spot and wound on the corner of the lips	1	5
		Spot or wound accompanied by pain on swallowing or burning sensation in the throat	4	20
2	Location	Mucosal tissue	1	5
		Tongue	20	100
		Corner of the lips	1	5
3	Frequency	Recurrent / relapse	9	45
		First onset	11	55
4	Previous medication	Systemic antifungal Yes No	 4 16	 20 80
		Topical antifungal Yes No	 8 12	 40 60

Table 2: Clinical features of the study subjects.

No	HIV Status		N	%
	Category	Group		
1	HIV Transmission	Homosexual	6	30
		Heterosexual/*Free sex*	13	65
		Bisexsual	1	5
		Needle syringe/Tattoo/Transfusion	4	20
2	Duration of HIV infection	<1 year	10	50
		1-3 year	8	40
		>3 year	2	10
3	Clinical Stadium of HIV	Stadium I	0	0
		Stadium II	1	5
		Stadium III	11	55
		Stadium IV	8	40
4	Absolute CD4 Count	>500 sel/ µL	0	0
		200-500 sel/µL	7	35
		<200 sel/µL	13	65
5	ARV Therapy	Yes No	16 4	80 20

Table 3: HIV status of research subjects.

Discussion

Demographic data of this study revelead the age, education level and employment status. Most of our subjects were in adult age group (63%). In 2016 Report of the Directorate General of Disease Control and Environmental Health, Ministry of Health Republic of Indonesia, found that more than 50% of HIV/AIDS patients were young adult and productive age groups with age groups 25-49 years [12]. The finding supports that adults who are productive and sexually active are more likely to engage in unsafe sexual behavior that is at risk for HIV transmission [13].

HIV/AIDS infection is a disease that has a large social impact. Nearly 90% of patients patients with HIV/AIDS may develop symptoms in the oral cavity. This condition might affect the quality of life, including the employment sector. In this study, 45% of the research subjects were unemployed with the other 45% of the population graduated from high school or equivalent. HIV/AIDS patients with poor educational backgrounds and socioeconomics condition have poor oral health, leading to higher change to developdiseases in the oral cavity [14].

The most prevalent HIV transmission in this study was through heterosexual intercourse (65%). This is similar to the general overview of HIV/AIDS in East Java where the main transmission of HIV/AIDS, mainly through heterosexual intercourse (69.6%) while others were trasmitted mostly through drugs and narcotics(21.9%) [15]. The most common patints complaintwas white spots in the oral cavity (100%) with a diagnosis of pseudomembranous candidiasis. The results of the same study were carried out in India in 2013 where pseudomembranous oral candidasis was found in 72% of research subjects [16]. Administration of ARV in patients with HIV/AIDS can significantly reduce the incidence of oral candidiasis. In this study, some research subjects had received antiretroviral therapy but in some other subjectswere new HIV/AIDS patients who had not received ARV [17]. Most patients with a history of both systemic and topical antifungal therapy still have fungal infections [4].

In 10 research subjects showed growth of more than one type of *Candida* species. The most common causes of oral candidiasis in patients with HIV/AIDS infection found in this study were *Candida albicans*, which amounted to 54.1%. While *non-albicans Candida* were obtained as many as 45.9% consisting of *C. glabarata, C. parapsilosis, C. tropicalis* and *C. krusei* species. Research by Reza in 2017 also shows that *Candida albicans* is still the main cause of oral candidiasis [4]. Identification of *Candida* species in this study using the conventional method of combining CHRO Magar *Candida*, *Cornmealagar* and carbohydrate tests. Identification of species using the method also has the advantage, especially for areas with limited facilities, and this method also has good accuracy compared with the latest methods such as Vitek-II which are more expensive [18].

All Candida samples were tested for sensitivity to fluconazole with the disk diffusion method. The sensitivity test results are obtained in the form of the diameter of the inhibition zone. The susceptibility and resistance criteria of antifungal agents were determined according to the interpretation of the diameter of the inhibition zone for fungi by Rosco Diagnostica Company as in Table 6 [19]. In this study, 5 (25%) isolates of *Candida albicans* were resistant to fluconazole, while 13 isolates of non-albicans (76,4%) were resistant to fluconazole. Fluconazole is currently the first line therapy for Oral Candidiasis in patients with HIV/AIDS.

Fluconazole resistance is grouped into, intrinsic resistance and extrinsic resistance. In extrinsic resistance there is a change in the sensitivity pattern of *Candida* species that were previously sensitive to antifungal therapy become resistant. While the intrinsic resistance of *Candida* species causing oral candidiasis has been happening since the beginning of the antifungal therapy. In this study, there was an intrinsic resistance in the form of an infection caused by *C. krusei*. Infection resistance of *C. krusei* species was found in the sample (8%). Fluconazole resistance in *C. krusei* was caused by a disruption of the 14a- demethylase enzyme which caused failure in the inhibition function of fluconazole [20].

Recurrent oralcandidiasis, history of antifungal medication may contribute to the difference in the Candida species. Moreover, these factors may change the patologic agent to non-albican species. These may happen due to the fact that patient with recurrect oral candidiasis is more likely to be exposed to antifungals, as supported by previous hypothesis. HIV/AIDS may worsen the condition and cause the expansion of fungal coloni [21].

No	Colony Colour*CHROMagar*	Hiydrolisis Urea	Fermentasi						Temperature 42-45°C	Microscopic apperance	Fungi	n (%)
			Glucose	Lactose	Sucrose	Maltose	Galactose	Trehalose				
1	**Green**	(-)	(+)	(-)	(-)	(+)	(-)	(-)	Grow	There is clamidospora terminalis, circular blastoconidia forming a unity on the pseudohifa	*Candida albicans*	20 (54.1)
2	**Blue Tosca**	(-)	(+)	(-)	(-)	(+)	(-)	(-)	Not grow	Abundant Clamidospora appear in pairs, or in groups. Pseudohyphae and hyphae in the blastoconidia cluster	*Candida dubliniensis*	0 (0.0)
3	**White**	(-)	(+)	(-)	(-)	(-)	(+)	(-)	-	Single blastoconidia formed small units along the pseudohiphae that are relatively short and curved. There is a "Giant Cell"	*Candida parapsilosis*	1 (2.7)
4	**Blue Prusi**	(-)	(+)	(-)	(+)	(+)	(+)	(+)	-	Small and single blastoconidia with pseudohyphae and true hyphae	*Candida tropicalis*	6 (16.2)
5	**Baby Purple**	(-)	(+)	(-)	(-)	(-)	(-)	(+)	-	Seen yeast cells without pseudohyphae	*Candida glabrata*	5 (13.6)
6	**Pink**	Variable	(+)	(-)	(-)	(-)	(-)	(-)	-	Pseudohyphae with oval to elongated blastoconidia which forms cross-match-sticks or resembles trees	*Candida krusei*	5 (13.6)
7	**White-Pink**	(-)	(+)	(-)	(+)	(-)	(+)	(+)		Small groups blastoconidia with short pseudohyphae and relatively few yeast cell	*Candida guiler-mondii*	0 (0.0)

In one subject can have>1 Candida isolate that grows

Table 4: *Candida* species identification results.

No	Species	n=37	%	Fluconazole					
				S	%	I	%	R	%
1	*Candida albicans*	20	54.1%	15	75	0	0	5	25
2	*Non-albicans Candida:*	17	45.9%	4	23.6	0	0	13	76.4
	-Candida dubliniensis	0	0	0	0	0	0	0	0
	-Candida glabrata	5	13.6%	2	40	0	0	3	60
	-Candida guilermondii	0	0	0	0	0	0	0	0
	-Candida krusei	5	13.6%	0	0	0	0	5	100
	-Candida parapsilosis	1	2.7%	1	100	0	0	0	0
	-Candida tropicalis	6	16.2%	1	16.6	0	0	5	73.3

S: sensitive, I: intermediate, R: resistance

Table 5: Resistance of each isolate of *Candida* species to fluconazole.

Drug	Potency	Diameter of inhibition zone (mm)		
		Sensitive	Intermediate	Resistance
Ketokonazole	10 μg/disk	≥ 28	21-27	≤ 20
Nystatin	50 μg/disk	≥ 15	10-14	Not formed zone
Fluconazole	25 μg/disk	≥ 19	15-18	≤ 15

Table 6: Interpretation of inhibition zone diameter disk diffusion test (CLSI) against Candida sp. (nystatin, ketokonazole, fluconazole).

In this study, it was found that there was a spectrum of changes in the causes of oral candidiasis eventhough *Candida albicans* was still more common than the raising in number *non-albicans Candida* species. *Candida* species isolates that are resistant to fluconazole were found in *non-albicans Candida* 13 species (76.4%) which this was due to the presence of *Candida krusei* which was intrinsically resistant to fluconazole. Identification and sensitivity test of *Candida* causing oral candidiasis in patients with HIV/AIDS should be conducted periodically to see changes in the spectrum of *Candida* species that cause oral candidiasis and to reevaluate oral candidiasis treatment guidelines.

Conclusion

Resistancy rate of candida species to fluconazole is increasing so that clinician should put more attencio and insight in the management of oral candidiasis with HIV/AIDS patient.

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