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Fungal Diversity and Susceptibility Patterns of Yeast to Several Antifungals in Jakarta

Conny Riana Tjampakasari, Yeva Rosana, Mardiastuti HW

Department of Microbiology, Faculty of Medicine, Universitas Indonesia, Jakarta 10430, Indonesia
*Corresponding Author: connyrianat@yahoo.com

ABSTRACT

Fungal infections are common in the tropical country. The cause of mycoses depends on the cause and the accompanying risk factors. It is important to do culture and identification to determine the etiology and continue with antifungal sensitivity examination to determine the appropriate management. As various fungi cause infections, the antifungal used are also very diverse. The purpose of this research is determine of the diversity of fungi and the susceptibility test of yeast to several antifungals. The study was conducted during 2020 consisting of 350 samples of various specimens. Sputum is the most specimens (41.38%) and positive cultures of 116 (33.14%). Identification and susceptibility test of antifungals to yeast were carried out using Vitek 2® compact automated system while for the mold were carried out using the slide culture method. The results obtained 19 species on fungus, consisting of 99.14% yeast and 0.87% mold. The most of yeast found were *C. albicans* (26.27%) and *Aspergillus sp*; *Rhizopus* 0.86 % respectively from mold. The pattern of sensitivity of yeast to several antifungals showed that Fluconazole was effective at 96.43%, followed Voriconazole and Amphothericin B 93.24% respectively. The next are Flucytocin, Micafungin and Caspofungin 91.66%, 86.18% and 85.74% respectively. Culture, identification and susceptibility testing need to be carried out and improved to obtain an overview and information that reflects the fungal causing infection.

Keywords: yeast, mold, sensitivity patterns, antifungal

INTRODUCTION

Mycosis is an infection caused by fungi. Tropical climate and high humidity are one of the factors cusing fungal infections in Indonesia [1]. Mycosis can be experienced by anyone, but individuals immunocompromised, such as HIV/AIDS sufferers, chemotherapy patients and post-organ transplat patients, are more at risk of fungal infections [2]. Fungi are microorganisms that live naturally in soil or plants and can even live on human skin. Although normally harmless, some fungi can cause serious health problems [3].

The cause of mycoses depends on the cause and the accompanying risk factors. Candidiasis is the most common fungal infection found in the tropics, caused by the genus Candida. In normal conditions, these fungi live naturally on the surface of the skin, mucosa and gastrointestinal tract in small amounts [3]. *Candida albicans* is an opportunistic fungus that can infect humans if there are predisposing factors that cause it to becaome pathogenic. These predisposing factors include the use of broad-spectrum antibiotics, the use of topical or systemic steroids, pregnancy, a decreased immune system resulting in a moist body condition and cause candidiasis [3,4].

Installation of Medical Records RSUP Dr. Sardjito said that there were 2661 cases of local and systemic candidiasis that occurred in Yogyakarta during the period from 2014 hingga to December 2018. The Yogyakarta Ministry of Health Poltekkes reprted that in 2011 there were 7098 cases of oro-pharyngeal candidiasis in HIV/AIDS [5]. Another case was reported by the Director General of Disease Control and Preventian of the Minstry of Health of the Repuclic of Indonesia in 2017, that there were

266 cases of candidiasis, which means that it ranks second with the highest number of diseases accompanying HIV/AIDS out of a total of 10 comorbidities. While the research conducted in January – June 2016 at Hospital of Prof. Dr. Sulianti Saroso Jakarta reported opportunistic infections for candidiasis are 17,74% [6].

Besides *Candida*, there are other fungi that can infect humans, among other, *Aspergillosis* and *Rhyzous* [7]. *Aspergillus* species cause a wide spectrum of diseases in humans. Depending on the underlying immune status of the host, *Aspergillus* diseases can be roughly classified into three groups with distinct pathogenetic mechanisms, clinical manifestations, and overlapping features, which are depicted based on their relative clinical importance [7,8].

Aspergillus spp. can lead to chronic, noninvasive forms of infection with overlapping features, ranging from development of a fungus ball (aspergilloma) to a chronic inflammatory and fibrotic process currently classified as chronic pulmonary aspergillosis.[7] In particular, saprophytic colonization of a parenchymal lung cavity by *Aspergillus* is referred to as aspergilloma and consists of both dead and living mycelial elements, inflammatory cells, fibrin, mucus, and components of degenerating blood and epithelia. The mycelial mass may lie free within the cavity or be attached to the cavity wall by inflammatory/granulomatous tissue [8]. Particularly on the increase are invasive infections with Aspergillus species, resulting in high mortality rates or, if the patient survives, causing high levels of morbidity that often limit further antileukemic therapies [9].

Rhizopus is the most common fungal genus causing mucromycosis; other less common causes of infection include Mucor and Rhizomucor [10]. *Rhizopus* is ubiquitous in nature, and a number of its species can be used in food fermentation and the production of hydrolytic enzymes [10,11]. Although mucromycosis encompasses a wide range of clinical presentations caused by the family Mucoracae, the infection commonly as two clinical syndromes: sinopulmonary and rhinocerebral mucromycosis. Other reported rare forms include cutaneous, intestinal and pulmonary diseases. The infection can be acquired through inhalation or direct traumatic inoculation through the skin or mucosa.[11] Mucromycosis has been recognized as an emerging fungal infection in immunocompromised patients, especially those with hematological malignancy after stem cell transplantation and in cases of diabetic ketoacidosis. However, mucromycosis soft-tissue infection remains extremely rare in immunocompetent hosts [11, 12].

The method of treating a yeast infection depends on the type of infection, its severity and the part of the body that infected. As various fungi cause infections, the antifungal drugs used are also very diverse. There are several types of antifungals that are divided based on their chemical structure and how they work, including the Azole group, which is a broad-spectrum antifungal that works to damage fungal cell membranes; Echinocandin group, which works by destroying fungal cell walls, Amphotericin B, used to treat serious and dangerous fungal infections, this antifungal works by stopping the growth and reproduction fungi [13].

Another antifungal, Flucytosine, is widely used because of its adequate effect on systemic fungal infections. Flucytocine inhibits the uptake of purines and pyrimidines via intra cellular metabolism to 5-fluorouracil, which then binds to fungal RNA and inhibits fungal DNA and RNA synthesis [14]. Based on this study, it is necessary to identify fungi to determine species diversity and profile sensitivity to several antifungals in Jakarta. By knowing the profile of the fungus and its sensitifity test, expected that management of mycosis can be enforced even better.

METHODS

The study was conducted at the Clinical Microbiology Laboratory, 350 specimens were obtained during 2020. A variety of specimens come from patients suspected of being mycosis, patients demographic information was obtained from medical records.

Specimen Collection

Specimens were collected and placed according to the procedure and immediately processed in the laboratory. Samples from outside the laboratory were sent within one hour.

Culture, Identification and Susceptibility Test

All specimens were cultured on Sabouraud Dextrose Agar (Oxoid). Specimens derived from nail and skin scrapings were processed for KOH 10% (Merck) before culture. Another specimens such as, body fluids are inoculated on a *BacT/Alert*

(BioMerieux Inc) bottle and incubated on a BacT/Alert is an automated microbial detection system based on the colorimetric detection of CO_2 produced by growing microorganisms. Results of an evaluation of the media, sensor, detection system and algorithm indicate that the system reliably grows and detects a wide variety of fungus [15]. Specimen with a positive signal were cultured according to the standard for fungus on Sabouraud Dextrose Agar (SDA) (Merck) as well as other specimens [16].

The plates on Sabouraud Dextrose Agar (Oxoid) were incubated at 30°C and 35°C (Thermo) and observed everyday. Yeast isolates were identified by Gram staining (Becton Dickinson) and continued identification using Vitek 2® automated system with YST ID card a sensitivity examination were carried out yeast susceptibility system card (AST-YS01) (BioMerieux Inc) [17]. Meanwhile molds colonies were processed for Lactophenol Cotton Blue (LPCB) (Himedia).

If the structure of the fungus is unclear and does not point one of the genera, a slide culture method (Riddel technique) was used. This was done using Potato Dextrose Agar (PDA) (Merck) pieces measuring 1 cm x 1 cm which were inoculated on each side of the part of the fungus that had not been developed identified, covered with cover slip and placed in a strerile and damp container [18]. The slide culture were incubated at room temperature in the dark for 1o days and observed every two to three days for humidity [19]. After 10 days of incubation, the cover glass containing the sporulated fungus colud be stained directly with Lactophennol Cotton Blue (LPCB) and observed under microscope.

RESULTS

Over a period 2020, a total of 350 specimens were received in microbiology laboratory for fungus culture from patients with various infections from 47 hospitals and clinical laboratories and 12 independent medical doctors in Jakarta. Significant growth of pathogenic fungus was noted 116 patients (33.14%) from 19 hospitals and clinical laboratories and 4 independent medical doctors in the age range 19 days - 97 years, with most age range 70 - 79 years. The proportions of gender between men and woman were 60 of 116 (51.72%) and 56 of 116 (48.28%) respectively.

Our study succeeded in identifying 19 species on fungus, consisting of of 99.14% (115 of 116) yeast and 0.87% (1 of 116) mold. The most yeast found were *C. albicans 26.27%* (31 of 116) and *Aspergillus sp*; *Rhizopus* 0.86 % (1 of 116) respectively from mold (*Supplementary 1*). The culture results of the 3 most common fungal found on Sabouraud Dextrose Agar can be seen in Figure 1.

Clinical specimens came from broncho alveolar lavage 11 (9.48%), sabouraud culture 5 (4.31%), blood 7 (6.03%), abdominal wound tissue 1 (0.86%), sputum 45 (41.38%), bronchial rinse 6 (5.17%), cerebrospinal fluid 1 (0.86%), feces 6 (5.1%), tissue 3 (2.58%), nail scraping 2 (1.72%), pus wound1 (0.86%), vaginal secretions 4 (3.45%), ear swab 3 (2.58%), ulcer swab 1 (0.86%) and urine 17 (14.65%) (*Supplementary 1*).

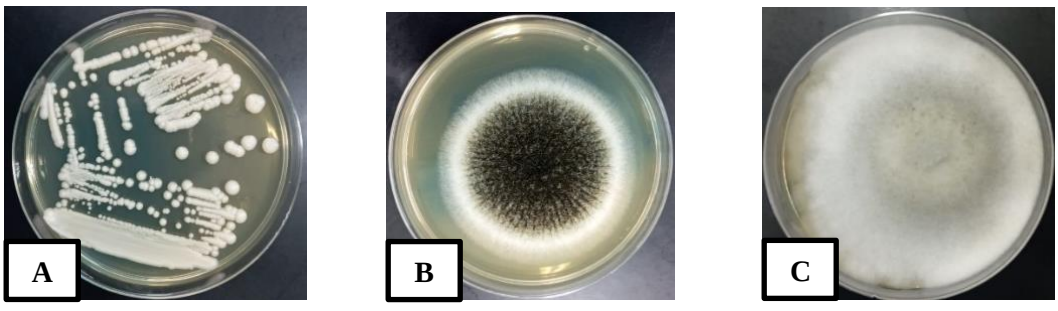

Figure 1. Fungal Culture on Sabouraud Dextrose Agar
A. *Candida* sp, B. *Aspergillus* sp, C. *Rhizopus* sp

Table 1. The Pattern of Sensitivity of Yeast to Several Antifungals

Antifungals	*C. alb* (%)	*C. dub* (%)	*C. gla* (%)	*C. kru* (%)	*C. luci* (%)	*C. par* (%)	*C. trop* (%)	*C. cif* (%)	*C. guil* (%)
Fluconazole	100			100	85.71		100		
Voriconazole	97.5	100	91.67	100	100	100	100	50	100
Caspofungin	100		28.71	100		85.71	100		100
Micafungin	97.56		100	33.33		100	100		
Amphotericin B	97.5	100	91.67	100	100	100	100	50	100
Flucitocyne	100	100	100	33.33	100	100	100		100

C. alb : *C. albican, C. dub* : *C. dublinensis, C. gla* : *C.glabrata, C. kru* : *C. kruseii, C luci* : *C. lucitaniae, C. par* :*C. parapsilosis, C. trop* : *C. tropicalis, C. cif* : *C. ciferrii, C. guil* : *C. guilliermondii*

The pattern of sensitivity of yeast to several antifungals, such as Fluconazole, Voriconazole, Caspofungin, Micafungin, Amphotericin B and Flucytocin are presented in Table 2. The result indicated that Fluconazole showed effectiveness above 80 % against the yeast tested. The antifungal sensitivity pattern showed that all antifungals were 100% sensitive to *C. tropicalis* meanwhile against *C. albicans* above 90%. The lowest yield was found in the antifungal Caspungin against *C. glabrata* (28.71%).

DISCUSSION

In our study, we found the majority of infections in the age range of 19 days – 97 years, with most age 70-79 years (5.17%) and male gender predominance was noted (51. 72%). Insidence by gender varies quite a bit in several countirs but dermatophyte infecions the prevalence of menis 5 times more than women. Akihiko *et al* (2021) also showed the prevalence of men suffering from fungal infections was more dominant than women with ratio 1.67: 1 [20,21].

The highest distribution of age groups is 70-79 years, followed by the age froup 60-69 years. These groups are elderly. Geriatrics experience an immunosence condition, which is a decreasr in a person's immunity to exposyre to external antigens due to age. So that the body's immune response to infection defense decrease. As a result, geriatrics are susceptible to infection and are often accompanied by severa complications. If already infected, controlling the disease will be more difficult, thus increasing the mortality rate in the elderly. In this age group the presence pf predisposing factors as a lot of sweat, wet or damp and trauma increases the risk for suffering from fungal infections is greater than other age groups [21,22].

The most specimens from our study were sputum samples which indicated that these patients had respiratory tract infections, as it is known that respiratory are third highest infection in the world. One of the microbes that cause these infections is a fungus [23]. Generally respiratory infection patients are immunosuppressed patients with the most common isolate being *Aspergillus sp*, *C. albicans*, *Blastomyces dermatitidis*, *Histoplasma capsulatum*, *Coccidioides immitis* and *Cryptococcus neoformans* [24]. Consistent with our study where *Aspergillus sp* and *Rizopus* is the common fungi found in sputum apart from *C. albicans.*

Reaserch by Meersseman *et al* (2009) showed *C. albicans* is the most frequent species isolated from respiratory samples (approx. 50%) followed by *C. parapsilosis*, *C. tropicalis* and *C. glabrata.* Despite the frequent isolation of *Candida* spp. from respiratory samples, isolation in non-neu tropenic patients is not considered diagnosis of pneumonia regardless the species isolated [25].

Research by Azoulay et al (2006), revealed in contrast to *Candida*, the genus *Aspergillus* acquires relevance in the respiratory infection of the critically ill patient. *Aspergillus fumigatus* is the most frequent species (80-90% cases) causing invasive pulmonary aspergillosis (IPA), although its frequency seems to be declining in last years with an increase in cases by other no-fumigatus species, especially *Aspergillus flavus* or *Aspergillus terreus* [26]. Conidia of *Aspergillus* are easily aerosolized, being transmission by air nearly universal. *A fumigatus* presents rapid replication and small size conidia, thus favoring its frequency as etiological agent of IPA. Humans continuously inhale *Aspergillus* conidia but, in general, they are efficiently eliminated by the immune system [27]. In accordance with our study, Soontrapa et al (2010) found Rizopus as one of the etiologies of 37 samples with positive fungal cultures in patients with invasive and non-invasive rhinosinusitis [28].

In our study, the sensitivity examination was carried out only on yeast isolates because Vitek

2® automated system did not provide a sensitivity examination against the mold. This is because so far the mold still has good sensitivity to fungals [29]. Based on the results, it was that not all antifungals were examinationed against yeast isolates. This depends on the suitability and availability on the Vitek card for certain types of yeast on the automatic machine. In this study, antifungal sensitivity test were carried out automatically with Biomerieux Vitek 2® System using an AST card for yeast, which was able to perform sensitivity test to several types of antifungals, such as fluconazole, voriconazole, caspofungin, micafungin, amphotericin B and flucytosine.

Our study shows Fluconazole is effective against yeast with an average 96.43%, followed by Voriconazole and Amphotericin B at 93.24% respectively. The next are nilai Flucitocyne, Micafungin and Caspofungin with 91.67%, 86.12% and 85.74% respectively. This is accordance with Berkow *et al* (2017) research which revealed that the fluconazole against *C. albicans* achieved a sensitivity 100% in patients with oropharyngeal Candidiasis and HIV/AIDS. It is also similar to Salari et al (2016) about mechanisms of resistance to fluconazole in *Candida albicans* clinical isolates trevealed [30, 31].

In our study, Voriconazole as one of the azole groups showed a high average sensitivity along with Amphotericin B. This is accordance with Raoul et al (2004) study which stated that the new triazole antifungal, voriconazole was developed for the treatment of life-threatening fungal infections in immunocompromised patients. The drug, which is available for both oral and intravenous administration, has broad-spectrum activity against pathogenic yeasts, dimorphic fungi and opportunistic moulds [30,31].

Whereas fluconazole acts on cytochrome P-450 *lanosterol 14-alpha-demethylase* enzyme which functions in the biosynthesis of ergosterol. The spesifci mechanism is the formation of a free nitrogen atom from the azole with the iron atom in the heme of the enzyme. This condition will prevent the activacation of oxygen and demethylation of lanostrol, thereby inhibiting the biosynthesis of ergosterol which is an important component of fungal cell membranes. The loss of normal sterols is in line with the accumulation of *14 alpha-methyl* sterols which are toxic to fungal cells membranes and can stop cell growth [32,33].

In our study, Amphotericine B was effective against all isolates except for *C. ciferrii* (50%). The main indication for Amphotericine B is to treat systemic fungal infection, because of its many side effect, this antifungal ism only used in patient with severe infections or immunodeficient patients. It is used as first-line therapy for in vasive mucormycosis infections, cryptococcal meningitis, aspergillus and certain candida infections [34,35].

Flucitocyne shown effective was 91.66% against *Candida* except *C. krusei*. Research by Cana (2019), showed the similar research that is 89.33% effective against *Candida* except for 2 isolates *Candida krusei* dan 1 *Candida albicans* (10,7%). Resistance to flucitocine is rare because occurs due to the inactivation of different enzymes in the pyrimidine pyrimidine pathway [36].

Against Micafungin, our study showed the effectiveness of 86.18%. This is similar to the syudy of Pola et al (2014), patients invasive candida in the micafungin arm had a higher overall treatment success rate at 80.0% [37]. Micafungin is an echinocandin antifungal to treat fungal infectios by inhibiting the production of beta 1,3-glucan which is an important component of fungal cell walls [38].

Caspofungin in our study showed an effectiveness of 85.74%. This result is use in patients with invasive Candidiasis showed the effectiveness of this antifungal although the sample sizes were limited. Caspofungin demonstrated favorable efficacy and safety profiles in the treatment of invasive candidiasis with a range between 71-78% [38]. Mechanism of Caspofungin inhibits the synthesis of β-1,3-D-glucan which is a component of fungal cell walls. Spectrum activity has a limited. Caspofungin is effective dermatophytes. Caspofungin is also effective against most Candida species with a high fungicidal effect but against *Candida parapsilosis* and *Candida krusei* is less effective and resistant to *Cryptococcus neoformans* but in our study it was resistant to *Candida glabrata* [38].

CONCLUSION

One hundred sixteen (33.14%) positive culture from a 350 samples of various specimens were obtained. Nineteen species on fungus, consisting of 99.14% yeast and 0.87% mold were identified. *C. albicans* (26.27%) is the most common yeast found, while *Aspergillus sp* and *Rhizopus* (0.86 %) respectively most common from mold. The sensitivity examination to several antifungal demonstrated Fluconazole as 96.43% effective, followed Voriconazole and Amphothericin B 93.24% respectively. The next are Flucytocin, Micafungin and Caspofungin 91.66%, 86.18% and 85.74% respectively.

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REFERENCES

[1] Apriliana P, Arthur PK, Evy E, Abu R. Periodical of dermatology and venereology. Profile of New Patients of Candidiasis 2019; 31(1):24-34.

[2] Wahyuningsih R, Tsuboi R, Burhan E, Rusyati LMM, Ariwatin NL, Miranda E. Medical Mycology in Asia Pacific: towards one health. Programme and Abstract Book The 6th Asia Pasific Society for Medical Mycology (APSMM) Congress Indonesia Society for Human and Animal Micology Indonesia. Bali 2016 Oct: 20-22.

[3] Rahman MH, Hadiuzzaman MD, Jaman MK, Bhuiya, Islam N, Ansari NP. Prevalence of superficial fungal infections in the rural areas of Bangladesh. Iran J Dermatol 2011; 14:86- 91.

[4] Dabas PS. An approach to etiology, diagnosis and management of different types of candidiasis. Academic J 2013; 4: 63-74.

[5] Kementerian Kesehatan Republik Indonesia. Profil Kesehatan Indomesia 2019. Kementerian Kesehatan Republik Indonesia.

[6] Kementerian Kesehatan Republik Indonesia. Laporan Kinerja Tahun 2017. Dirtektorat Jenderal Pencegahan dan Pengemdalian Penyakit 2018.

[7] Peláez T, Muñoz P, Guinea J, Valerio M, Giannella M, Klaassen CH. Outbreak of invasive aspergillosis after major heart surgery caused by spores in the air of the intensive care unit. Clin Infect Dis 2012; 54:e24-3.

[8] Khalife S, Aliouat EM, Aliouat CM, Gantois N, Devos P, Mallat H, Del-Cas E, Dobbousi F, Hamze M, Realle E. First data on *Pneumocystis jirovecii* colonization in patients with respiratory diseases in north Lebanon. N Microb N Infect. July 2015;6:11-4.

[9] Deepa, Srikanta, Felipe H, Santiago T, Tamara LD. *Cryptococcus neoformans*: Historical curiosity to modern pathogen. Yeast. 2014 Feb; 31(2): 47–60.

[10] Natalia Rakislova, Juan Carlos Hurtado, Antonio E. M. Palhares, Luiz Ferreira, Monique Freire, Marcus Lacerda, Wuelton Monteiro, Mireia Navarro, Isaac Casas, Marcus de Melo Teixeira, Paola Castillo, Maria Teresa Rodrigo-Calvo, Lorena Marimon and Miguel J. Martínez. High prevalence and mortality due to *Histoplasma capsulatum* in the Brazilian Amazon: An autopsy study. Plos Negleted Tropical Diseases April 2021;5:15(4):E0009286.

[11] Hariprasath P, Arunaloke C. Global epidemiology of Mucormycosis**.** *J Fungi 2019;5(1):26.*

[12] Héctor M, Mora M. Special isssue *Sporothrix* and Sporotrichosis. J Fungi 2018;4(4):116.

[13] Ben-Ami R, Giladi M. Fluconazole-resistant Candida: collateral damage associated with prior antibacterial exposure? Future Microbiol 2012;7(9): 1029-31

[14] Ullmann AJ, Sanz MA, Tramarin A, Barnes RA, Wu W, Gerlach BA, Krobot KJ, Gerth WC. Longitudinal evaluation of Antifungal Drugs (LEAD I) envestigators. Prospective study of amphotericin B formulations in immunocompromised patients in 4 European countries. Clin Infect Dis. 2006 Aug 15;43(4):e29-38.

[15] Cécile E, Seher Y, Nicolas F, Gaëlle C, Laurent D. Quality indicators for blood culture: 1 year of monitoring with BacT/Alert Virtuo at a French hospita, Journal of Medical Microbiology 2021;70:001300.

[16] Viroj W. Sabouraud dextrose agar for the diagnosis of fungal keratitis. Indian Journal of Ophthalmology 2010 Nov-Dec; 58(6): 549–550.

[17] Cara W. Introduction of the VITEK 2 Compact and Implementation of EUCAST Guidelines in a Microbiology Department. Royal College of Surgeons in Ireland. Royal College of Surgeons in Ireland e publications@RCSI. Theses and Dissertations. http://epubs.rcsi.ie/msctttheses/80

[18] Frederik C, Cátia A, Sousa, Manuela DM, Eduardo VS. Improvement of the slide culture technique for the assessment of yeast viability. J. Inst. Brew.2017; 123: 39–44.

[19] Arunava K, Sreenivasan S, MV Pravin Cs. A modified fungal slide culture technique. Indian J Pathol Microbiol (serial online) 2014 (cited 2021 Dec 21); 57:356-7.

[20] Akihiko S, Yoshifumi N, Shota Y, Hiroaki Y, Hiroko K, Shintaro H, Koji A, Yoko I, Hiroshi M, Daisuke K, Shin K. Clinical Surveillance of Candidemia at our hospital. Medicol Mycol Journal 2021. Vol 62. 29- 34. ISSN 2185-6486.

[21] Irma TL, Sunarso S, Rahmadewi. Profile of fungal and bacterial infections in inguinal erythrosquamous dermatoses. Berkala Ilmu Kesehatan Kulit dan Kelamin – Periodical of Dermatology and Venereology 2017;29(3):204-11

[22] Yosa TM , Roza M. Infeksi jamur pada geriatri (Fungal infection in geriatics). Human Care Journal 2020;5(1):328-33 e-ISSN:2528-66510.

[23] José GM, Pedro O, Francisco AL, Luis AR, José B, José MA, José M, Amparo S, José B. Epidemiology, diagnosis and treatment of fungal respiratory infections in the critically ill patient. Rev Esp Quimioter 2013;26(2):173-88.

[24] Zhirong Y, Wanqing L. Fungal respiratory disease. Curr Opin Pulm Med. 2006 May;12(3);222-7. Affiliations expand PPMID.26582678.

[25] Meersseman W, Lagrou K, Spriet I, Maertens J, Verbeken E, Peetermans WE. Significance of the isolation of *Candida* species from airway samples in critically ill patients: a prospective, autopsy study. Intensive Care Med 2009; 35:1526-31.

[26] Azoulay E, Timsit JF, Tafflet M, de Lassence A, Darmon M, Zahar JR, *Candida* colonization of the respiratory tract and subsequent pseudomonas ventilator-associated pneumonia. Chest 2006;129:110-7.

[27] Peláez T, Muñoz P, Guinea J, Valerio M, Giannella M, Klaassen CH. Outbreak of invasive aspergillosis after major heart surgery caused by spores in the air of the intensive care unit. Clin Infect Dis 2012; 54:e24-31.

[28] Soontrapa, P., Larbcharoensub, N., Luxameechanporn, T., Cheewaruangroj, W., Prakunhungsit, S.W., Sathapatayavong, B., Chongtrakool, P., and Leopairut, J., 2010. Fungal rhisnosinusitis: a restrospective analysis of clinicopathologic features and treatment outcomes at ramathibodi hospital. Departement of Pathology, Ramathibody Hospital, Mahidol University,Thailand, 41 (2), pp. 442-49. PMID: 20578529

[29] Roya VS, Cornelia LF. Novel Antifungal agents and their activity against *Aspergillus* Species. *J Fungi* 2020, *6*(4), 213.

[30] Raoul H. Drug Evaluation. Voriconazole : therapeutic review of a new azole antifungal. Expert Review of Anti-infective Therapy. 2004. Vol 2. Issue 4.

[31] Berkow EL, Lockhart SR. Fluconazole resistance in *Candida* species: a current perspective. Infect Drug Resist. 2017(31)237-45.

[32] Salari S, Khosravi AR, Mousavi SA, NikbakhtBrojeni GH. Mechanisms of resistance to fluconazole in Candida albicans clinical isolates from Iranian HIV-infected patients with oropharyngeal candidiasis. JMM 2016; 26(1): 35-41.

[33] Zeichner LO, Harrington R, Azie N, Yang H, Li N, Zhao J. A Risk Score for Fluconazole Failure among Patients with Candidemia. Antimicrob Agents Chemother 2017(61)1-16.

[34] Jansook P, Fülöp Z, Ritthidej GC. Amphotericin B loaded solid lipid nanoparticles (SLNs) and nanostructured lipid carrier (NLCs): physicochemical and solid-solution state characterizations. Drug Dev Ind Pharm. 2019 Apr;45(4):560-67.

[35] Hamill RJ. Amphotericin B formulations: a comparative review of efficacy and toxicity. Drugs 2013 Jun;73(9):919-34.

[36] Cana RRS. Identifikasi spesies *Candida* dan uji sensitifitas antijanur pada dermatitis popok bayi (Identification of *Candida* pecies and antifungal sensitivity in infant diaper dermatitis). Thesis. Program Studi Magister Ilmu Kedokteran Universitas Sumatera Utara Medan 2019.

[37] Pola de la Torre, Annette C Reboli. Micafungin: an evidence-based review of its place in therapy. Core Evidence 2014:9. 23 Feb 2014, p.27-39.

[38] Arnaldo L. Colombo,Angela L. Ngai, Michael Bourque, Susan K. Bradshaw, Kim M. Strohmaier, Arlene F. Taylor, Robert J. Lupinacci, Nicholas Kartonis. Caspofungin use in patients with invasive candidiasis caused by common non-albicans *Candida* species:mReview of the Caspofungin Database. Antimicrobial agents and chemotherapy 2010;54(5 0066-4804/10/$12.00):1864-71