ALCOHOL-BASED ANTISEPTIC SOLUTIONS ARE INEFFECTIVE IN INHIBITING PATHOGENIC FUNGI

Handi Suyono [1)], Laura Wihanto [2)]

ABSTRACT

Introduction: *Breaking the chain of transmission of infection using an antiseptic solution is an easy, inexpensive and effective method. The incidence of fungal infection is one of the global health problems that lead to severe complications even death, especially in patients with immunosuppressed or immunocompromised conditions and is reported to be increasing. Although alcohol-based antiseptic solutions are frequently used to prevent transmission of pathogenic organisms, these methods are rarely evaluated.* ***Aim:*** *Determine the susceptibility of three pathogenic fungi against alcohol-based antiseptic solutions with and without hydrogen peroxide addition.* ***Methods:*** *To determine the inhibition of the antiseptic solution against fungi, the Kirby & Bauer test disc diffusion method was used. After the petri dishes were incubated at 37° C for 24 hours, the diameter of the inhibition zones were measured using a caliper. The phenol coefficient test was carried out to compare the efficacy of an antimicrobial agent in this case alcohol-based antiseptic solutions against phenol. The phenol coefficient was applied as a test against Salmonella typhi, with a minimum score of 1, using the method according to SNI 1842: 2019.* ***Result:*** *The inhibition zone on Candida albicans ATCC 102231, Aspergillus niger, and Cryptococcus neoformans ATCC 14116 for solution A contains ethanol 80% v / v, glycerin 8% v / v, sterile water add 100% and solution B contains ethanol 80% v / v, H2O2 0.15% v / v, glycerin 8% v / v, add 100 sterile water % all were less than 6 mm which were classified as resistant. The result of the phenol coefficient test for solution A and B were 0.3 and 0.4 , which less than 1, indicated the antiseptic solutions were less effective than phenol.* ***Conclusion****: Antiseptic ethanol solution and a combination of ethanol + H2O2 were ineffective inhibits of pathogenic fungal growth. Further studies are needed to form a more potent antiseptic solution in order to improve management of fungal infections prevention.*

Keywords: Alcohol, *Candida albicans, Aspergillus niger, Cryptococcus neoformans*

ABSTRAK

Pendahuluan: Pemutusan rantai penularan infeksi menggunakan larutan antiseptik merupakan salah satu cara yang mudah, murah dan tergolong efektif. Peningkatan kejadian infeksi fungi menjadi salah satu masalah kesehatan global yang dapat mengakibatkan komplikasi kematian terutama pada pasien dengan kondisi imunosupresi (*immunocompromised)*. Meskipun larutan antiseptik berbahan dasar alkohol sering digunakan untuk mencegah penularan organisme patogen, metode ini tergolong jarang dievaluasi. **Tujuan:** Menentukan daya hambat tiga jenis jamur patogen terhadap larutan antiseptikberbasis alkohol dengan dan tanpa penambahan hidrogen peroksida. **Metode:** Penentuan daya hambat larutan antiseptik terhadap fungi, digunakan metode *disc diffusion* Kirby & Bauer test . Setelah cawan petri diikubasi pada suhu 37°C selama 24 jam, diameter zona hambat yang terbentuk diukur menggunakan jangka sorong. Pengujian koefisien fenol dilakukan untuk membandingkan keampuhan suatu bahan antimikroba dalam hal ini larutan antiseptik berbahan dasar alkohol terhadap fenol. Koefisien fenol yang diterapkan adalah uji terhadap *Salmonella typhi*, dengan persyaratan minimal skor 1, menggunakan metode sesuai SNI 1842:2019 **Hasil :** Zona hambat pada media mengandung *Candida albicans* ATCC 102231, *Aspergillus niger*, dan *Cryptococcus neoformans* ATCC 14116 yang diuji larutan A yang mengandung etanol 80% v / v, gliserin 8% v / v, air steril *add* 100% dan larutan B yang mengandung etanol 80% v / v , H2O2 0,15% v / v, gliserin 8% v / v, air steril *add* 100% semuanya kurang dari 6 mm yang terklasifikasi resisten. Hasil uji koefisien fenol untuk larutan A adalah 0,3 dan larutan B 0,4 (kurang dari 1) yang berarti bahwa larutan antiseptik kurang efektif dibandingkan dengan fenol**. Kesimpulan**: Larutan antiseptik etanol dan kombinasi etanol + H2O2 tidak efektif menghambat pertumbuhan fungi patogenik. Perlu dilakukan studi lebih lanjut untuk menyusun formula larutan antiseptik yang lebih poten dalam rangka memperbaiki manajemen pencegahan infeksi fungi.

Kata Kunci: Alkohol, *Candida albicans, Aspergillus niger, Cryptococcus neoformans*

1) Physiology and Biochemistry Department, Faculty of Medicine, Widya Mandala Catholic University Surabaya, Surabaya, East Java, Indonesia 2) Microbiology and Parasitology Department, Faculty of Medicine Widya Mandala Catholic University Surabaya, Surabaya, East Java, Indonesia

INTRODUCTION

Globally, the incidence of fungal infections is increasing, especially in immunosuppression or immunocompromised conditions, and supported by high population of people using medical technology such as stent implantation, increased cases of cancer and intensive care due to critical conditions, and also increased organ, tissue and stem cell transplant (1,2). Low immunity condition, late diagnosis, and resistance to antifungal drugs are supporting factors that often lead the patients to death (3).

The number of systemic pulmonary fungal infection cases in Indonesia have been increasing over the years, with mortality reaching 50-100%. Several of the risk factors are comorbid pulmonary disease (tuberculosis, malignancy, asthma, chronic obstructive pulmonary disease), prolonged use of antibiotics, long-term steroids, and chemotherapy (4).

The most nosocomial fungal pathogens are *Candida*, while *Aspergillus* is the second most. Manifestations of fungal infections can include skin infections or systemic infections. The habitat of *Candida* is in the mouth, digestive tract (intestines), vagina, and skin. Infestation of *Candida* in immunocompetent conditions is generally commensal, but in immunocompromised conditions, it becomes pathogenic. *Candida* infection occurs in mild immunocompromised patients, whereas *Aspergillus* infection occurs in moderate-severe immunocompromised patients. (1,5).

A study at Cipto Mangunkusumo Hospital, Jakarta, Indonesia, reported that the prevalence of invasive candidiasis by *Candida albicans* was 12.3% in septic patients with mortality rate of 64.8%. The clinical manifestations of invasive candidiasis include disseminated candidiasis, endocarditis, meningitis, endophthalmitis, and infection of the visceral organs. Disseminated candidiasis has reportedly increased 15-20 times over the past 2 decades

with a mortality rate of up to 40%, even up to 50% in patients receiving chemotherapy. Mortality in cases of severe sepsis can reach more than 50% in *Candida* infections, and this rate is higher than sepsis due to infection with *Pseudomonas aeruginosa* (5,6).

Nosocomial and iatrogenic events were influenced by the use of broad-spectrum antibiotics, central venous catheters, parenteral nutrition, cardiac or digestive surgery, prolonged hospitalization, intensive care in intensive care units, burns, and in premature neonates. In addition, neutropenic conditions, use of corticosteroids, comorbid human immunodeficiency virus (HIV), and comorbid diabetes mellitus also contribute to the incidence of fungal infections (1,5,6).

Candida transmitted by direct contact of the skin such as hand to hand or body, and still viable up to 45 minutes to 4 months in a hospital setting (1,5).

Aspergillosis caused by Aspergillus fungus is less common than candidiasis, but the mortality is higher, reaching 45-80%. The most common causes of invasive aspergillosis are *A. fumigatus, A. flavus, A. niger*, and *A. terreus*. *Aspergillus* can be found in soil, fruits and vegetables. Infection in humans occurs exogenously through inhaled spores. The sources of transmission are air dust and water. A dusty and damp / watery hospital environment is a potential aspect that increases the incidence of aspergillosis (1,2,7).

Aspergillus virulence is influenced by its ability to produce hydrolytic enzymes, toxins (aflatoxin), and penetrate to the host. *Aspergillus* can circulate systemically and infect various organs (3).

Cryptococcosis due to *Cryptococcus,* a facultative intracellular microbes often occurs in immunocompromised patients, especially in HIV / AIDS patients. The most common cryptococcosis is meningeal cryptococcosis, beside other body parts like skin, eyes, lungs, urinary tract, muscles, heart, gastrointestinal tract, lymph nodes, thyroid, adrenals, and neck with a mortality of up to 44%. Transmission occurs through inhalation of spores. A study in Jakarta, Indonesia, reported the incidence of meningeal cryptococcosis of 21.9% in patients with AIDS (8). *Cryptococcus* can

grow freely in the environment, within soil amoebae, and in mammals either free in body fluids or tissues, or in phagocytic cells (macrophages). (9).

The pathogenicity of *Cryptococcus* is influenced by the presence of capsules, their ability to penetrate the blood-brain barrier and produce hydrolytic enzymes, phenotypic changes, and form biofilms. Clinical manifestations vary, ranging from self-limiting cutaneous infection to systemic infection (disseminated). Cryptococcus can stay in the host latently for a long period of time, and can infect the host if immunity is weakened. (3,9).

The use of antiseptics is a way to reduce the morbidity of pathogenic fungal infections. The World Health Organization (WHO) recommends the use of antiseptics, especially for medical personnel. Prevention of infection is needed, especially in high-risk patients such as neonates (newborn), elderly people (geriatrics), and immunocompromised patients (10) .

Alcohol is an antiseptic substance recommended by WHO and is most widely used as a hand rub antiseptic. The types of alcohol used include ethanol (ethyl alcohol), isopropanol (isopropyl alcohol), and n-propanol with a content range of 60-90% (10,11). Alcohol has a broad-spectrum antimicrobial effect against bacteria, viruses and fungi. Alcohol kills fungi by damaging cell membranes and denaturing proteins. Several studies have found alcohol resistance events to certain pathogenic organisms (12).

METHODS & MATERIAL

There were two types of antiseptic solution that were tested in this in vitro experimental study, namely: 1) Solution A containing ethanol 80% v/v, glycerin 8% v/v, and sterile water add 100%. 2) Solution B containing ethanol 80% v/v, H_2O_2 0.15% v/v, glycerin 8% v/v, and sterile water add 100%. We used the pathogenic fungi, namely 1) *Candida albicans* ATCC 102231 2) *Aspergillus niger* 3) *Cryptococcus neoformans* ATCC 14116 obtained from the Central Health Laboratory, Ministry of Health of the Republic of Indonesia, Surabaya-East Java, Indonesia. Ketoconazole antifungal was used as a control substance.

The Kirby & Bauer disc diffusion method was used as a susceptibility test to determine the inhibition of the antiseptic solution against fungi (13). Pure culture of rejuvenated fungi, suspended in 10 ml of sterile saline solution, then homogenized with vortex. The suspension was compared to the absorbance value with the standard turbidity of 0.5 McFarland using a spectrophotometer with a wavelength of 625 nm to obtain an inoculum suspension according to the standard, 108 cfu / ml. Media Sabouraud Dextrose Agar + Glucose 2% that has been liquified is poured into a sterile petri dish and allowed to solidify. After solidifying, 1 mL of the fungi suspension is evenly distributed on the surface of the medium. Sterile paper discs measuring 6 mm were immersed in each antiseptic solution for 15 minutes, then placed on the surface of the media. Petri dishes were incubated at 37°C for 24 hours. The diameter of the inhibition zone is measured using a caliper

Phenol coefficient is the ratio of the potency of an antimicrobial agent compared to the phenol. The phenol coefficient is determined by dividing the highest dilution of phenol to the highest dilution of antimicrobial agents which kills microorganisms in ten minutes but not less than five minutes.

The phenol coefficient applied in this study is a test against *Salmonella typhi*, with a minimum score of 1, using the method according to SNI 1842: 2019. The exposure times used were 5, 10, and 15 minutes. Phenol coefficient testing was carried out at the Sucofindo Laboratory, Cibitung, Bekasi-West Java, Indonesia. Data were analyzed descriptively.

RESULT

The test results on agar media after incubation are shown in picture 1. The inhibition zone is declared resistance if the diameter is ≤ 6 mm and it is stated sensitive if it is > 6 mm.

The result showed that the inhibition zone on *Candida albicans* ATCC 102231, *Aspergillus niger*, and *Cryptococcus neoformans* ATCC 14116 for both solution A and B were all less than 6 mm which indicated that the antiseptic solutions did not inhibit fungal growth.

Table 1. Growth inhibition zone from disc diffusion assay

Antiseptic solutions	***C. albicans* ATCC 102231**	***A. niger***	***C. neoformans* ATCC 14116**	**Phenol coefficient**
A (Ethanol 80%)	Resistance (≤ 6 mm)	Resistance (≤ 6 mm)	Resistance (≤ 6 mm)	0.30
B (Ethanol 80% + H_2O_2 0,15%)	Resistance (≤ 6 mm)	Resistance (≤ 6 mm)	Resistance (≤ 6 mm)	0.40
Controls (Ketoconazole)	Sensitive (46 mm)	Sensitive (25 mm)	Sensitive (35 mm)	NA

The phenol coefficient test results for solution A and were 0.3 and 0.4. The phenol coefficient is a test to show the effectiveness of a biocide material, which was established by Joseph Lister, then further developed by Samuel Rideal and J. T. Ainslie Walker, which is known as the Rideal-Walker coefficient. Phenol is used as a comparison because phenol is often used to kill microorganisms. The phenol coefficient of less than 1, as in both A-B solutions, indicates that the antimicrobial agent is less effective than phenol. Conversely, if the phenol coefficient is more than 1, it means that the microbial material is more potent than phenol.

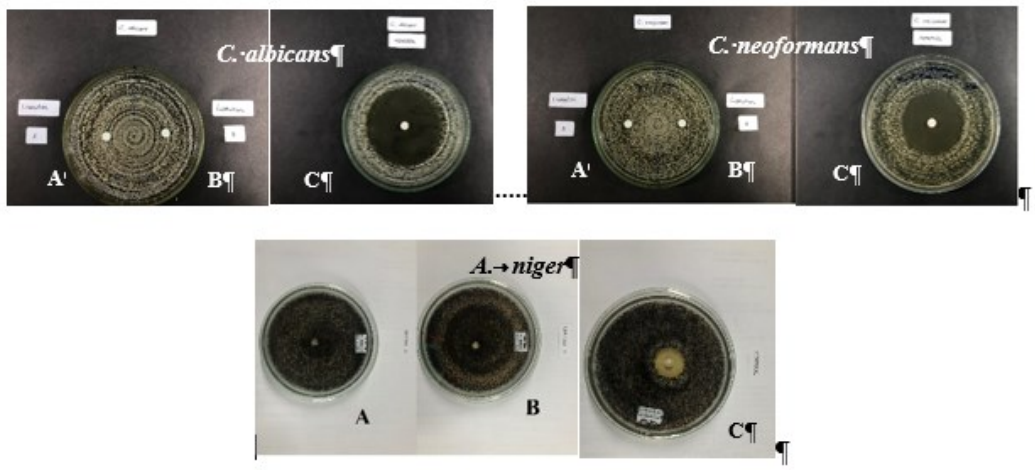

Figure 1. Disc diffusion assay on pathogenic fungi

Note: A. Ethanol 80%, B. Ethanol 80% + H_2O_2 0,15%, C. Ketoconazole

DISCUSSION

Ethanol is a type of alcohol that is often applied as a cleaner, antiseptic, and disinfectant. Alcohol consider as a broad-spectrum antiseptic that is environmentally friendly. It has moderate strength against fungi but is ineffective at killing spores (12).

Our research shows ethanol 80% is ineffective at inhibiting *Candida, Aspergillus,* and *Cryptococcus* fungi. Higher levels of alcohol have the potential to irritate the skin. Glycerin is added as skin conditioning agent to prevent dryness of the skin. Fungal resistance to ethanol is mainly associated with phenotype adaptation and biofilm formation (10,12).

Candida is able to form biofilms and adapt into various morphologies / phenotypes including yeast, khamir, hyphae and pseudohyphae, also forming colonies which increases the virulence of *Candida* (3). Biofilms are communities of microorganisms attached to a solid surface covered by an exopolymeric matrix. Biofilms can form on medical equipment and are proposed to decrease the antifungal drug sensitivity (12).

Hydrogen peroxide (H_2O_2) is an oxidizing agent that is often used as an antiseptic or disinfectant. H_2O_2 is a strong oxidant that works through the oxidation process and the presence of breakdown products such as the superoxide and hydroxyl radicals will damage the pathogen cells. H_2O_2 is environmentally friendly because it can be broken down into water and oxygen when exposed to high temperatures or catalysts such as certain enzymes and metals. Levels of 3% -3.5% are used as a disinfectant in liquid or cream / gel form for wound care. WHO recommends hydrogen peroksida (H_2O_2) concentration at 0.125% v / v for hand sanitizer formula, and in combination with alcohol (10).

H_2O_2 resistance can arise mainly caused by catalase enzyme properties that is able to degrade H_2O_2 into water and oxygen rendering them lost its oxidant activity (12).

CONCLUSION

Ethanol antiseptic solution and the combination of ethanol + H_2O_2 are ineffective in inhibiting pathogenic fungal growth. The increasing prevalence of nosocomial fungal infections stimulates regular assessment on the effectiveness of alcohol-based antiseptic solutions

against other pathogenic fungi. It is necessary to consider the addition of other active ingredients to form a more potent antiseptic solution in order to improve prevention management of fungal infections.

ACKNOWLEDGMENT

Researchers would like to thank the Faculty of Medicine and Institute for Research and Community Service (LPPM), Widya Mandala Catholic University, Surabaya for the support of research funds.

REFERENCES

1. Perlroth J, Choi B, Spellberg B. Nosocomial fungal infections: Epidemiology, diagnosis, and treatment. Med Mycol. 2007;45(4):321–46.
2. Alangaden GJ. Nosocomial Fungal Infections: Epidemiology, Infection Control, and Prevention. Infect Dis Clin North Am [Internet]. 2011;25(1):201–25. Available from: http://dx.doi.org/10.1016/j.idc.2010.11.003
3. Karkowska-Kuleta J, Rapala-Kozik M, Kozik A. Fungi pathogenic to humans: Molecular bases of virulence of Candida albicans, Cryptococcus neoformans and Aspergillus fumigatus. Acta Biochim Pol. 2009;56(2):211–24.
4. Rozaliyani A, Jusuf A, ZS P, Burhan E, Handayani D, Widowati H, et al. Infeksi Jamur Paru di Indonesia: Situasi Saat Ini dan Tantangan Di Masa Depan. J Respirologi Indones. 2019;39(3):210–4.
5. Jahagirdar VL, Davane MS, Aradhye SC, Nagoba BS. Candida species as potential nosocomial pathogens – A review. Electron J Gen Med. 2018;15(2):0–4.
6. Kalista KF, Chen LK, Wahyuningsih R, Rumende CM. Karakteristik Klinis dan Prevalensi Pasien Kandidiasis Invasif di Rumah Sakit Cipto Mangunkusumo. J Penyakit Dalam Indones. 2017;4(2):56.
7. Mattei AS, Madrid IM, Santin R, Schuch LFD, Meireles MCA. In vitro activity of disinfectants against aspergillus spp. Brazilian J Microbiol. 2013;44(2):481–4.

8. Adawiyah R, Wahyuningsih R. Kriptokokosis : Epidemiologi , Manifestasi Klinis dan Diagnosis. Maj Kedokt FK UKI. 2012;XXVIII(3):133–43.
9. Srikanta D, Santiago-Tirado FH, Doering TL. Cryptococcus neoformans: Historical curiosity to modern pathogen. Yeast. 2014;31(2):47–60.
10. WHO. WHO Guidelines on Hand Hygiene in Health Care. Geneva, Switzerland: WHO Press; 2009. 1–64 p.
11. RI DJBK dan AKKK. Pedoman bahan berbahaya alkes dan pada produk perbekalan kesehatan rumah tangga. Kementrian Kesehatan RI; 2012.
12. McDonnell GE. Antisepsis, disinfection, and sterilization /. Second. Washington DC: ASM Press; 2017.
13. Hudzicki J. Kirby-Bauer Disk Diffusion Susceptibility Test Protocol Author Information. Am Soc Microbiol [Internet]. 2009;(December 2009):23. Available from: https://www.asm.org/Protocols/Kirby-Bauer-Disk-Diffusion-Susceptibility-Test-Pro