PHARMACEUTICAL JOURNAL OF INDONESIA 2022 7(2): 71 - 78

PHARMACEUTICAL JOURNAL OF INDONESIA

Available online at http://.pji.ub.ac.id

The Sensitivity of Amphotericin B, Fluconazole, and Flusitosin to Fungal Pathogens Isolated from Wounds of Diabetic Ulcer Patients

***Rafika Sari*[1]**

[1.] *Farmasi, Fakultas Kedokteran Universitas Tanjungpura, Pontianak, Indonesia*

INFO ARTIKEL

Sejarah artikel:

Penerimaan naskah: 04 Januari 2022
Penerimaan naskah revisi: 07 Mei 2022
Disetujui untuk dipublikasikan: 27 Mei 2022

Keywords : Anti-fungus, Diabetic Ulcer Wound, Sensitivity.

A B S T R A C T

A diabetic ulcer is a wound caused by infection, ulceration or destruction in people with diabetes mellitus. Mostly, diabetic ulcer found in foot. The purpose of this study was to identify pathogenic fungi in diabetic ulcers and their sensitivity to fungal drugs. The morphological determination of fungal isolates was stained with Lactophenol Cotton Blue. Determination of sensitivity using diffusion discs Kirby Bauer which are classified based on Clinical Laboratory Standard Institute (CLSI) with Amphotericin B, Fluconazole, and Flusitosin. Based on the results of the study found 8 types of pathogenic fungi namely *Candida albicans* (38.78%), *Candida guilliermondii* (4.08%), *Candida kefyr* 10.20%), *Candida parapsilosis* (10.20%), *Fusarium solani* (14.29) %), *Microsporum audouinii* (14.29%), *Penicillium sp* (2.04%), *Trichophyton mentagrophytes* (4.08%), and *Trichophyton equinum* (2.04%). The fungus was identified using Gideon software and the book Description of Medical Fungi. The results of the antifungal sensitivity test showed the results were sensitive to fluconazole. Amphotericin B is intermediate to *Candida albicans*, *Candida guilliermondii*, *Penicillium sp*. and *Trichophyton sp*. and is resistant to *Candida kefyr* and *Candida parapsilosis*. Flusitosin is only an intermediate against *Penicillium sp*. while other fungal species are immune. The conclusion of this study. The results of diabetic ulcer swabs III and IV indicate the presence of fungal colonies with species of 9 species namely *Candida albicans, Candida guilliermondii, Candida kefyr, Candida Parapsilosis, Fusarium solani, Microsporum Audouinii, Penicillium sp., Trichophyton mentagrophytes, and Trichophyton equinum*. Antifungal sensitivity tests on all pathogenic fungi showed sensitivity to fluconazole. Amphotericin Bintermediatrics against *Candida albicans, Candida guilliermondii, Penicillium sp. and Trichophyton sp*. while resistant to *Candida kefyr* and *Candida parapsilosis*. Flusitosin is only an intermediate against *Penicillium sp*. while other fungal species are resistant.

Keywords: Anti-fungus, Diabetic Ulcer Wound, Sensitivity.

* *Corresponding* author: ***Rafika Sari***, *Farmasi, Fakultas Kedokteran Universitas Tanjungpura, Pontianak, Indonesia*,. E-mail: *rafikasari@pharm.untan.ac.id*

INTRODUCTION

A diabetic ulcer is a condition of infection, ulceration or destruction of the most profound skin tissue in the feet of diabetic patients. Diabetes mellitus (DM) due to nerve abnormalities and peripheral arterial blood vessel disorders (1). Diabetic Ulcer rates in West Kalimantan, especially in Pontianak City are very high, data obtained at the Kitamura Wound Care Specialist Clinic shows that the number of patients visiting for wound care throughout 2016 amounted to 323 people (2).

Diabetes patients are prone to fungal infections because high blood glucose levels can help fungal growth (3). The growth of microorganisms in the form of fungi in Diabetic Ulcer is generally pathogenic. Fungal infections in Diabetic Ulcer patients can cause feet skin to become dry, cracked, nerve tissue death in foot bone infections (4). If fungal infections in Diabetic Ulcer patients are not treated immediately, it can cause a decrease in patient immunity and cause complications.

Treatment of fungal infections can be done using antifungal drugs. However, widespread and long-term use of antifungal agents can cause resistance. Prevalent fungal infections, inappropriate use of antifungal agents, and the lack of available therapeutic options cause resistance (5). It is crucial to test sensitivity to pathogenic fungi to determine the proper use of antifungal and prevent resistance. The sensitivity test method is done by using the disk diffusion method Kirby Bauer. This method is a simple method, inexpensive and can be used for routine testing to determine sensitivity to antifungal by looking at the inhibitory zones formed and classified according to the table of Clinical and Laboratory Standard Institute (CLSI).

METHODS

The tools used are autoclaves (All American, American), incubators (Memmert, Germany), Laminar Air Flow (Marga Cipta, Indonesia), refrigerators (Aqua, Japan), binocular microscopes (Olympus CX51, Japan) electric heating (hot plate). The ingredients used are chloramphenicol 1%, Lactophenol Cotton Blue (Himedia, USA), standard solution Mc Farland, 0.9% NaCl solution, Potato Dextrose Agar (MERCK, America), ammonium nitrate (MERCK, USA), potassium phosphate (MERCK, America) MERCK, America), magnesium sulfate (MERCK, America), iron chloride (MERCK, America), galactose (MERCK, America), glucose (MERCK, America), maltose (MERCK, America) and sucrose (MERCK, America).

The Samples

Samples used were diabetic ulcer swabs and antifungal disks, namely amphotericin B 100µg / disk (Biorad, France), fluconazole 25µg / disk (Biorad, France) and flucytosine 1µg / disk (Biorad, France).

Method of Research

Sampling

Sampling was carried out by explaining the actions to be taken to patients with ethical-clearance No.8556/UN22.9/DI/2018 from Ethical review committee Faculty of medicine Tanjungpura University. Cleaning the wound with a sterile gauze soaked with physiological NaCl carefully, then repeat the treatment three times. Swab culture is opened from the wrapper then rubbing sterile cotton swab into the ulcer without touching the edge of the wound. Then putting the cotton stick into the transport media and closing the tube tightly and naming tags and then taking it to the laboratory for examination.

Fungal Isolation

The Isolation of fungi was done by swabbing on potato dextrose jelly media, which had been added to chloramphenicol, after which it was incubated at 37 °C for 3 days (72 hours).

Equipment and material Sterilization was done by autoclaving for 15 minutes at a temperature of 120 ^{o}C and a pressure of 15 psi (per square inch) or 2 atm (6).

Fungus Morphology Tests

Fungal morphology tests were carried out by microscopic, assimilation, hair testing and urea hydrolysis, which will be explained as follows:

a. Microscopic Test
   Determination of fungal isolates is done by making fungal preparations then observed under the microscope aseptically using 1-2 drops Lactophenol Cotton Blue (LCB) for staining. Things that were observed included blastoconidia, hyphae, conidiophores, conidia, pseudohyphae, and spores. The object and the glass cover are disinfected with alcohol (7).
b. Assimilation Test
   1. Assimilation *Penicillium sp.*
      The assimilation method carried out according to Peterson's research. The assimilation media used consisted of ammonium nitrate, potassium phosphate, magnesium sulfate, and iron chloride, added with distilled water up to 100ml. Prepared sweets (galactose, glucose, maltose, and sucrose) with a concentration of 2%. Tools, materials are

developed and sterilized by autoclaving at 121 °C for 15 minutes. Assimilation media was put into the test tube as much as 5 ml, and each test tube was added with different sugars with the same volume. The mushroom colony is taken using ose, put into a test tube until it changes color from clear to turbid. They were incubated for five days (72 hours) at a temperature of 37°C — the results in the form of growing or not a colony that has different characteristics.

2. Assimilation of *Candida sp.*
   The carbohydrate assimilation test technique used is the solid media technique, which is done by mixing yeast extract with pure agar and peptone A into distilled water, then sterilized and poured into a petri dish and allowed to stand until solidified. Subsequently, *Candida* was planted using an ose needle and etched into the media and placed a sugar disk on the surface of the media. After the whole process is complete, the petri dishes are put into an incubator at 37˚C for three days (8,9).

c. Perforation Hair Test
   The test is done by placing a small 1 cm hair cut in a vial containing aquades and sterilized using an autoclave. The hair sample come from researcher hair as preliminary studies. Then put a fungal colony using ose wire and homogenized. Work is carried out aseptically in LAF. Incubation is done within 4 weeks to get the results of hair damage (9).

d. Urea Hydrolysis Test
   The media used for the urea hydrolysis test is media Christensen's, Urea Agar. The media is made by dissolving 7.25 grams of Christensen's Urea Agar in 25 ml of distilled water and sterilized by autoclaving. The sterilized media is then cooled to a temperature of ± 50 ° C after that 0.5 grams of urea powder is added. A total of 5 ml of media was put in a tube and made to be tilted. After solidifying, the mushroom is etched on the media so that it is tilted using ose (9).

Antifungal Sensitivity Test

Making a suspension is done by taking a colony of fungi then suspended into a test tube containing 5 mL of 0.9% sterile NaCl solution. Turbidity is equivalent to standard Mcfarland 0.5. Fungus planting was carried out by spreading and then put an antifungal disk with the disk diffusion method Kirby Bauer. Incubated at 37 ˚C for five days around 72 hours. Observations were made by measuring the zone of resistance around the disk with calipers. The views were compared with the obstacle categories according to the Clinical and Laboratory Standard Institute (CLSI) (10).

Table 1. Classification of Obstacle Zones by CLSI (11)
Inhibition Zone

No	Code	Description	Diameter (mm)
1	+++	Sensitivity	≥20
2	++	Intermediate	15-19
3	+	Resistance	≤14

RESULTS AND DISCUSSION

Based on data from research, this shows that the number of patients with diabetic ulcers in men is greater than 16 of 24 people (66.67%) while in women as many as 8 of 24 people (33.33%). Following the research of Arlina et al. that most research subjects are male compared to female. This is related to the pressure on men's feet is higher than that of women. Continuous stress and friction on the feet will cause tissue damage, which initially takes the form of pre-ulcers, bleeding inside the callus, blistered skin, blisters and will become an increasingly widespread ulcer. Callus or calluses will increase foot pressure by 30%. This situation has contributed to the occurrence of Diabetes Foot Ulcers. Walking barefoot allows trauma to the foot. Also, it will result in more significant pressure when compared to walking with footwear (12).

Table 2. Results of Identification of Pathogenic Fungi for Diabetic Ulcer Swabs of Degrees III and IV Wagner

No.	Bacteria	Samples	Percentage of
1.	*Candida albicans*	12	38.78%
2.	*Fusarium solani*	5	14.29%
3.	*Microsporum Audouinii*	5	14.29%
4.	*Candida parapsilosis*	3	10.20%
5.	*Candida kefyr*	2	10.20%
6.	*Candida guilliermondii*	2	4.08%
7.	*Trichophyton mentagrophytes*	2	4.08%
8.	*Penicillium sp.*	1	2.04%
9.	*Trichophyton Equinum*	1	2.04%
Total		149	100%

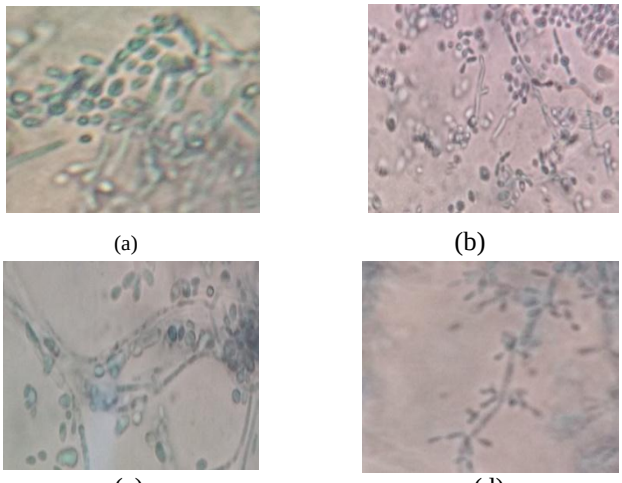
The most significant percentage of patients with diabetic ulcers are patients aged ≥60 years as many as 12 out of 24 people (50%), then the age group of 50-59 years as many as 7 of 24 people (29.17%) and the last age group less than 50 years was 5 of 24 people (20.83%). This is consistent with Waspadji's research, which states that as age increases, glucose intolerance also increases. In the elderly degenerative decline is due to the sensitivity of peripheral nerves and decreased tissue flexibility so that it will spur the onset of diabetic ulcers (13)

The purpose of isolating fungal pathogens is to take pathogenic fungi from their native environment and grow them on artificial media so that pure culture is obtained and the type of pathogenic fungi that can infect a sample can be identified. After the identification of fungal isolates, eight types of fungi were obtained, namely *Candida albicans, Candida guilliermondii, Candida kefyr, Candida parapsilosis, Penicillium lanosum, Penicillium resticulosum, Trichophyton mentagrophytes,* and *Trichophyton equinum.*

Microscopic Identification

Observations of fungi are carried out using a microscope to see the shape of the fungus by observing characteristics such as conidiophores, hyphae, conidia, types, and forms of spores. Observations were made by staining lactophenol cotton blue using a sterile technique, then viewed under a microscope using the same magnification of 100 times. The fungi identified in this study were *Candida albicans, Candida guilliermondii, Candida kefyr, Candida parapsilosis, Penicillium lanosum, Penicillium resticulosum, Trichophyton mentagrophytes,* and *Trichophyton equinum.*

Observation of the test results showed the microscopic characteristics of the fungus *Candida sp*., *Penicillium sp*., and *Trichophyton sp*.



(a) (b) (c) (d)

Figure 1. Microscopic identification of Fungus

Figure 1 descriptions: *Candida albicans* has branched pseudohyphae with many blastoconidia. The formation of klamidospora forms spherical and terminal. *Candida guilliermondii* has the characteristic of branching pseudohyphae with many blastoconidia. The structure of blastoconidia forms spherically. Candida parapsilosis has the attributes of many pseudohyphae branched. Blastoconidia has subglobose, which functions to form buds. The characteristics of Candida kefyr microscopically are pseudohyphae branching, long, wavy. Blastoconidia are shoots or form chains. In *Candida kefyr*, pseudohyphae are rarely found or almost absent. (a). *Candida albicans* (b). *Candida guilliermondii* (c). *Candida kefyr* (d). *Candida parapsilosis.*

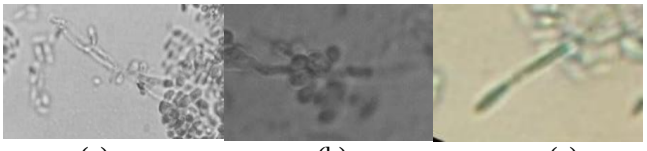
Figure 2. Microscopic of *Penicillium lanosum*

Figure 2 descriptions: Characteristics of *Penicillium lanosum* microscopic have erect, branched conidiophores; there are 2-3 metulas at the top. The vertical phialides in each metula are adjacent, dense, conidial heads consisting of conidia that are chained in each phialide. Conidia in the form of penicillin (brush). (a) Conidiophores; (b) Conidia; (c) Fialid.

(a) (b) (c)

Figure 3. Microscopic of *Penicillium resticulosum*

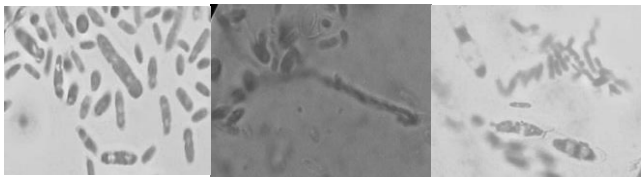
Figure 3 descriptions: *Penicillium resticulosum* microscopically that is having conidiophores that are not upright and unbranched, there are 2-4 metula at the peak and 3-4 phialides. The shape of the conical is oval. (a) Conidiophores; (b) Conidia; (c) Fialid.



(a) (b) (c)

Figure 4. Microscopic *Trichophyton Magnification* of equinum

Figure 4 descriptions: Microscopic *Trichophyton Magnification* of equinum, there are a lot of microconidia, spherical, and along the hyphae and Macroconidia are rarely produced (a) Microconidia; (b) Nodular organs; (c) Macroconidia



(a) (b) (c)

Figure 5. Microscopic *Trichophyton mentagrophytes*

Figure 5 descriptions: *Trichophyton mentagrophytes* has the characteristics of single-celled microconidia. Microconidia are mostly spherical. Hyphae are spiral and (a) Microconidia; (b) Macroconidia; (c) Hifa Spiral

Assimilation Test

Assimilation test of *Candida sp*. and *Penicillium sp*., which has been identified microscopically, further testing is done to determine the properties of fungi on various substances by looking at the different fungal properties of each species. Tests carried out are assimilation tests of sugars (galactose, glucose, maltose, glucose, maltose, sucrose, lactose, raffinose, and trehalose). This assimilation determines whether microorganisms metabolize each of these sugars and form a fungi body. Following are the assimilation test results of *Penicillium sp*. *and Candida sp.*

Table 3. Assimilation test *Penicillium sp.*

Assimilation Test Type	Fungus type			
	lanosum Penicillium		Penicilliumresticulosum	
	Observations	According to Peterson	Observations	According to Peterson
Maltose	+	+	+	+
galactose	-	-	-	-
Glucose	+	+	-	-
Sucrose	+	+	-	-

Table 4. Test Assimilation of *Candida sp.*

Mushroom	**Sugar**					
	Glucose	**Maltose**	**Lactose**	**Sucrose**	**Trehalose**	**Raffinose**
CA	+	+	-	+	+	-
CG	+	+	-	+	+	+
CK	+	-	+	+	-	+
CP	+	+	-	+	+	-

Perforation Hair Test

Trichophyton sp. is a type of dermatophyte fungus that can digest keratin, for example, the stratum corneum of the skin (epidermis), hair, nails. Isolates *Trichophyton Mentagrophytes* produce damage to keratin tissue in the hair, which will cause the cuticles of the hair to become thin and damaged. *Trichophyton mentagrophytes* show positive results because they cause severe damage to the hair with erosion in the hair walls so that the hair walls become weak, and there are holes in the hair tissue. Trichophyton equinum does not show any damage to the hair and the hair wall does not thin.

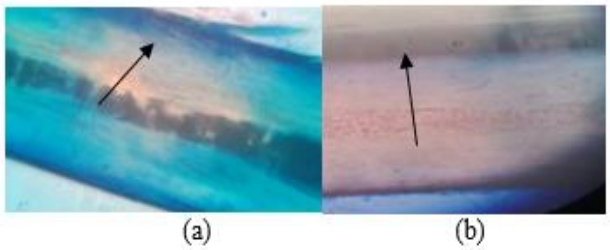


Figure 6. Microscopic Observation of Hair Test Results of Trichophyton mentagrophytes and Trichophyton equinum
Description: (a) Hair damage occurs; (b) No hair damage occurs

Urea Hydrolysis Test

The breakdown of urea causes the formation of a product in the form of ammonia which will create an alkaline environment that causes the red phenol indicator to turn pink. Color changes that occur in the media indicate the presence of urease enzymes produced by fungi, whereas if there is no color change in the media indicates that there are no urease enzymes produced by fungi. Tests carried out on Trichophyton mentagrophytes and Trichophyton equinum show positive results due to changes in color from orange to pink. Test results carried out on Trichophyton mentagrophytes showed a faster color change on the third day compared to Trichophyton equinum which showed the results of the color change on the fifth day.

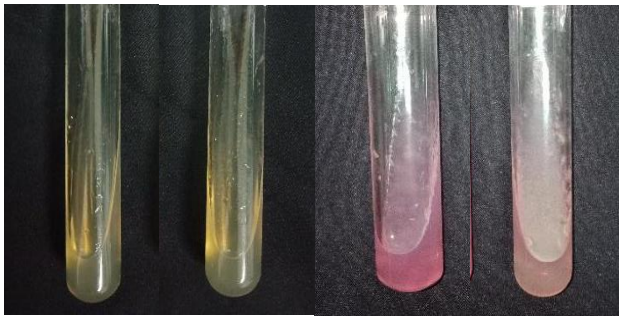


Figure 7. Urea Hydrolysis Test

Description:
(a) Day 0 Trichophyton mentagrophytes; (b) Day 0 of Trichophyton equinum; (c) Day 3 Trichophyton mentagrophytes; Day 3 Trichophyton equinum Antifungal

Sensitivity Test Antifungal

Sensitivity test uses three types of antifungal discs from different groups, namely the triazole group (fluconazole 25 µg), the pollen group (amphotericin B 100 µg), pyrimidine group (flucytosine 1 µg). Anti-fungal sensitivity of amphotericin B, fluconazole and flucytosine can be observed through the diameter of the inhibitory zone formed on the test media Potato Dextrose Agar (PDA) that has been placed with an antifungal disk. The results of the inhibition zone diameter formed can be seen in **Figure 8.**

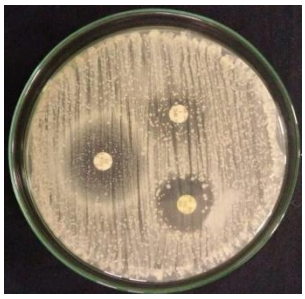


Figure 8. Antifungal Sensitivity Test Results

Measurement of the diameter of the inhibition zone was carried out three times using a caliper. The width of the inhibition zone that is formed is then calculated the average value which can be seen in the following table: width of the inhibitory zone, Interpretation of the results of the inhibition zone diameter can be seen from the following table 5**.**

Table 5. Anti-Fungal

Fungal Species	Average Diameter of the Inhibition Zone (mm) ± SD		
	Amphotericin B	Fluconazole	Flucytosine
Candida albicans	14.8 ± 1.0	35.1 ± 0.9	6.3 ± 0.2
Candida guilliermondii	16.1 ± 0.7	38.7 ± 0.8	8.3 ± 0.3
Candida Kefyr	11.8 ± 4.0	26.1 ± 7.7	0.0 ± 0.0
Candida parapsilosis	13.2 ± 0.8	17.5 ± 0.9	7.3 ± 0.2
Penicillium lanosum	14.7 ± 0.2	24.9 ± 1.0	14.4 ± 0.6
Penicillium resticulosum	18.9 ± 0.4	24.8 ± 4.2	17.9 ± 0.5
Trichophyton equinum	18.1 ± 0.3	23.5 ± 0.3	0.0 ± 0.0
Trichophyton mentagrophytes	15.6 ± 0.5	21.6 ± 0.4	0.0 ± 0.0

Based on table 5, antifungal fluconazole has the sensitivity to the largest of all fungal species; this can be seen from the diagram showing that the diameter of inhibitory zones formed from fluconazole is greater than the antifungal amphotericin B and flucytosine. Antifungal flucytosine against *Candida sp*. and *Trichophyton sp*. Has properties resistant whereas in *Penicillium sp*. Antifungal properties are intermediate.

Table 6. Result Classification Antifungal against Pathogenic Fungus

Antifungal	CLSI (mm)			*Candida albicans*	*Candida guilliermondii*	*Candida kefyr*	*Candida parapsilosis*	*Penicillium lanosum*	*Penicillium resticulosum*	*Trichophyton equinum*	*Trichophyton mentagrophytes*
	T	I O	R								
Amphotericin B	≥20	15-19	≤14	I	S	R	R	I	I	I	I
Fluconazole	≥20	15-19	≤14	S	S	S	S	S	S	S	S
Flucytosine	≥20	15-19	≤14	R	R	R	R	I	I	R	R

According to **table 6,** diameter Inhibited zones formed are classified descriptively based on the Clinical and

Laboratory Standard Institute (CLSI) which are resistant (≤ 14 mm), intermediates 15-19(mm), and sensitive (≥ 20 mm). The results of the classification that all fungal species (100%) are sensitive to fluconazole so that fluconazole can be used in the treatment of fungal infections in diabetic ulcers. Fluconazole can give sensitive results to all fungal species by inhibiting the enzyme 14-α-demethylase in fungal cell membranes. The protein 14-α-demethylase is needed to convert lanosterol to ergosterol. Inhibition of the enzyme 14-α-demethylase causes metabolite imbalance and disruption of fungal membrane permeability so that there is no ergosterol in the fungal cell membrane and then causes death in fungi (14)**.** Enzyme activity that is bound to the membrane stops so that the growth of fungal cells will halt and the inhibition zone diameter will be formed ≥20mm.

Amphotericin B has different activities against fungal species in which 62.5% are intermediates, namely *Penicillium sp*., *Trichophyton sp*., and *Candida albicans*, 25% are resistant to *Candida kefyr* and *Candida parapsilosis*, and 12.5% are sensitive to *Candida guilliermondii*. Amphotericin B gives intermediate results against *Penicillium Lanosum, Penicillium resticulosum, Trichophyton mentagrophytes*, and *Trichophyton equinum* where fungi can be inhibited but with a weaker inhibition compared to sensitive. Results intermediates can be due to the concentration on the disc being too large amphotericin B is 100 mg. The results of a study conducted by Espinel-Ingroff et al., 2007 showed that amphotericin B could provide results that are sensitive to *Trichophyton sp*. with MIC values ≤ 1 µg / ml. Amphotericin B is only sensitive to *Candida guilliermondii*, where amphotericin B has a mechanism that is bound to ergosterol in the fungal cell membrane to form pores along with the layer. This can cause leakage in the fungal cell membrane and the ions contained in the cell exit, resulting in death in the fungus. Amphotericin B results are resistant to *Candida kefyr* and *Candida parapsilosis*, where this has similarities in previous studies where the antifungal amphotericin B 0.002-32 µg stated non-Albicans resistant of 22.2-66.6% (15).

The results of tests on flucytosine showed that 75% of fungi are resistant and 25% are intermediates. Antifungal flucytosine inhibits the growth of fungal cells *Penicillium lanosum*, and *Penicillium resticulosum* will enter the fungal cells with the help of cytosine deamination and in the cytoplasm will join the RNA after deamination to become 5-fluorouracil, inhibiting the synthesis of fungal cell proteins so that the diameter of the inhibitory zone formed 14-19mm (16). The occurrence of syplusitosin resistance in *Trichophyton sp*. and *Candida sp*. can be caused by the presence of mutations in the FCY 2 gene that encodes the cytosine permease enzyme, FCY 1 which codes for the cytosine deaminase enzyme to convert flucitosin to fluorouracil, and FUR 1 which encrypts for the enzyme uracil phosphoribosyltransferase. The existence of mutations that occur in FCY 2 can cause flucitosina not to enter fungal cells, FCY 1 causes cytosine deaminase not to form so that no change in flucitosina becomes fluorouracil, and FUR 1 that causes uracil phosphoribosyltransferase does not convert fluorouracil into fluorine uridine monophosphate used so to inhibit DNA and RNA synthesis (17). Based on the above explanation, it can be seen that the use of antifungal is appropriate in the treatment of fungal infections in diabetic ulcer degrees III and IV Wagner, namely by using antifungal in the form of fluconazole. This can be seen from the test results that showed fluconazole (25 µg) has a value that is sensitive to all fungal species.

CONCLUSIONS

The results of diabetic ulcer swabs III and IV indicate the presence of fungal colonies with species of 9 species namely *Candida albicans, Candida guilliermondii, Candida kefyr, Candida Parapsilosis, Fusarium solani, Microsporum Audouinii, Penicillium sp., Trichophyton mentagrophytes*, and *Trichophyton equinum.* Antifungal sensitivity tests on all pathogenic fungi showed sensitivity to fluconazole. Amphotericin Bintermediatrics against *Candida albicans, Candida guilliermondii, Penicillium sp*. and *Trichophyton sp*. while resistant to *Candida kefyr* and *Candida parapsilosis*. Flusitosin is only an intermediate against *Penicillium sp*. while other fungal species are resistant.

REFERENCES

1. Roza RL, Rudy A, Zulkarnain E. Faktor Risiko Terjadinya Ulkus Diabetikum pada Pasien Diabetes Mellitus yang Dirawat Jalan dan Inap di RSUP Dr. M. Djamil dan RSI Ibnu Sina Padang. *J Kesehat Andalas* 2015;4(1):243.
2. Nurmansyah AS, Rochmawati E, Primanda Y. *Dukungan* Keluarga *pada* Pasien *Luka Kaki Diabetes di Klinik Kitamura Pontianak: Studi Fenomenologi*. 2018;
3. Santosh Y, Ramanath K, Naveen M. Fungal Infections In Diabetes Mellitus: An Overview. *Int J Pharm Sci Rev Res* 2011;7(2):222.
4. Suarez MG. Identification and Antifungal Susceptibility of Penicillium-Like Fungi From Clinical Samples in The United States. *J Clin Microbiol Am Soc Microbiol* 2016;54(8).
5. Siswandono, Soekardjo B. *Kimia Medisinal*. 2nd ed. Surabaya: Airlangga University Press; 2000.

6. Hendrawati TY, Utomo S. Optimasi Suhu dan Waktu Sterilisasi pada Kualitas Susu Segar di Kabupaten Boyolali. *J Teknol Univ Muhammadiyah Jakarta* 2017;9(2).
7. Fauziyyah NA, Putri DAS. Isolasi Jamur dari Batuan Penutup Drainase Pada Sisi Selatan Lantai II Bidang H Candi Borobudur. *J Konserv Cagar Budaya Borobudur* 2016;10(2):40–4.
8. Deorukhkar SC, Shahriar R. Identification of Candida Species: Conventional Methods in the Era of Molecular Diagnosis. *Ann Microbiol Immunol* (Review Artic 2018;1(1):1–4.
9. Kidd S, Catriona H, Helen A, David E. *Descriptions Of Medical Fungi*. Third Edit. Australia: National Mycology Reference Centre; 2016.
10. Wayne P. *Method for Antifungal Disk Diffusion Susceptibility Testing of Yeast Approved Guideline CLSI document M44-A2*. Second Edi. Clinical and Laboratory Standards Institute; 2009.
11. Cockerill FR, Matthew AW, Jeff A, Michael ND, George ME, Mary JF. *Performance Standards for Antimicrobial Disk Susceptibility Tests; Approved Standard. CLSI document M02-A11*. Eleventh E. Clinical and Laboratory Standards Institute; 2012.
12. Dewi A. Hubungan Aspek-aspek Perawatan Kaki Diabetes dengan Kejadian Ulkus Kaki Diabetes pada Pasien Diabetes Mellitus. *Mutiara Med* 2007;7(1):13–21.
13. Waspadji S. *Penatalaksanaan Diabetes Melitus Terpadu*. Jakarta: Balai Penerbit Fakultas Kedokteran, Universitas Indonesia; 2005.
14. Jawetz E, JL M, EA A. *Mikrobiologi Kedokteran*. Jakarta: EGC; 1996.
15. Sarenje K. Distribution and Antifungal Susceptibility of Candida Species Isolated from Clinical Specimens at the University Teaching Hospital, Lusaka, Zambia. *IOSR J Pharm* 2017;7(2).
16. Pelczar J, Chan ECS. *Dasar-Dasar Mikrobiologi*. Jilid 2. Jakarta: UI Press; 2005.
17. Verma S, Heffernan M. *Superficial fungal infection:* dermatophytosis, *onychomycosis, tinea nigra, piedra*. Seventh Ed. New York: McGraw Hill; 2010.