Research

Comparison sensitivity of 1% clotrimazole and 2% acetic acid to patient's otomycosis isolates

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ABSTRACT

Background: Otomycosis is a challenge in the medical field, because it requires long-term treatment and follow-up and has a high recurrence rate. In the administration of otomycosis therapy, there are various problems, such as the occurrence of resistance, difficulty in drug application, and requires a long period of time for management. Therefore, choosing the right treatment regimen is very important, so as to reduce the level of resistance caused by the fungus that causes otomycosis. **Purpose:** To compare the sensitivity of 1% clotrimazole cream and 2% acetic acid solution to fungal isolates of otomycosis patients. **Method:** This study used true experimental design in vitro with post-test only control group design. Sampling was done by total sampling method during the August-November 20215. Eleven samples were obtained and tested on 3 treatment groups with repetition 3 times, so there was a total of 33 specimens studied. **Result:** The statistical calculations with the Kruskal-Wallis test from 11 samples tested, obtained a p value < 0.05 which indicated that there was a significant difference in the sensitivity of 1% clotrimazole cream and 2% acetic acid solution. **Conclusion:** The sensitivity of 1% clotrimazole cream and 2% acetic acid solution has a significant difference on fungal isolates of otomycosis patients.

Keywords: otomycosis, sensitivity, clotrimazole, acetic acid

ABSTRAK

Latar belakang: *Otomikosis merupakan suatu tantangan dalam dunia medis, karena memerlukan pengobatan dan tindak lanjut jangka panjang, serta memiliki tingkat kekambuhan yang cukup tinggi. Dalam pemberian terapi otomikosis, terdapat berbagai macam permasalahan, seperti terjadinya resistensi, sulitnya aplikasi obat, dan membutuhkan jangka waktu yang lama dalam penatalaksanaannya. Oleh karena itu, pemilihan regimen pengobatan yang tepat sangatlah penting, sehingga dapat mengurangi tingkat resistensi yang disebabkan oleh jamur penyebab otomikosis.* ***Tujuan:*** *Membandingkan sensitivitas antara klotrimazol krim 1% dan larutan asam asetat 2% terhadap isolat jamur pasien otomikosis di RS Adam Malik dan RS Prof. Chairuddin P. Lubis.* ***Metode:*** *Penelitian ini menggunakan desain true experimental secara in vitro dengan rancangan* posttest only control group design*. Pengambilan sampel dilakukan dengan metode total sampling selama periode Agustus-November. Pada penelitian ini sampel yang diperoleh berjumlah 11 dan diuji terhadap 3 kelompok perlakuan dengan pengulangan sebanyak 3 kali, sehingga terdapat total 33 spesimen yang diteliti.* ***Hasil:*** *Hasil perhitungan statistik dengan uji Kruskal-Wallis dari 11 sampel yang diuji, didapatkan nilai $p < 0.05$ yang menandakan terdapat perbedaan signifikan terhadap sensitivitas klotrimazol krim 1% dan larutan asam asetat 2%.* ***Kesimpulan:*** *Sensitivitas klotrimazol krim 1% dan larutan asam asetat 2% memiliki perbedaan yang signifikan terhadap isolat jamur pasien otomikosis.*

Kata Kunci: *otomikosis, sensitivitas, klotrimazol, asam asetat*

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INTRODUCTION

Otomycosis is an inflammation of the external acoustic canal caused by a fungal infection. This disease is a challenge in the medical field because it requires long-term treatment and follow-up due to its relatively high recurrence rate.[1] This study compared 1% clotrimazole cream with 2% acetic acid solution. Clotrimazole is known to be one of the most effective broad-spectrum antifungals for managing otomycosis, as it does not exhibit ototoxic effects and functions by disrupting the permeability barrier of the fungal cytoplasmic membrane.[2] Meanwhile, acetic acid acts as a non-specific antifungal by lowering the pH level of the ear canal, which causes a stress response and loss of energy for fungal growth.[3]

In cases of otomycosis, it is also important to identify the causative agents and select the most effective antifungal based on the sensitivity of the identified fungi.[4] Despite numerous in vitro studies demonstrating the effects of various antifungal agents, clinicians still faced difficulties in identifying the most effective agent for treating otomycosis.[5] Treatment of otomycosis involves various challenges, such as resistance development, limited data regarding the safety of otic medications, irritation effects of topical antifungals on middle ear mucosa, and systemic toxicity of antifungals. Clinical or environmental exposure had led to an increase in antifungal resistance rates among various candida and aspergillus species.[6]

Furthermore, there were challenges associated with treating otomycosis, such as difficulties in drug application and prolonged treatment duration, which could lead to frustration for patients and ultimately result in non-compliance with treatment. These issues lead to urgency for this research.[6] Due to these problems, selecting an appropriate treatment regimen for specific fungal species is essential to reduce resistance rates and complications risk arising from fungal pathogens causing otomycosis.[5] The need to predict which fungal infections will or will not respond to treatment is increasing, so antifungal susceptibility testing (AFST) methods are necessary to assist in selecting the right antifungal agents for treating patients with otomycosis.[7]

METHOD

In this study, a true experimental design was utilized in vitro with a post-test only control group design. The necessary tools for this research included: inoculating loops, dropper pipette, microscope, measuring cylinder, Erlenmeyer flask, autoclave, Bunsen burner, hot plate, racks, object glasses, coverslips, biosafety cabinet, sterile cotton swabs, transport swabs, incubators, magnetic stirrers, paper discs, gloves, and caliper. Meanwhile, the required materials were: 1% clotrimazole cream, 2% acetic acid in 70% alcohol, McFarland standard turbidity 1, distilled water, sterile saline solution, Mueller Hinton Agar (MHA), and Sabouraud Dextrose Agar (SDA).

The objects of this research were fungal samples obtained from patients with otomycosis. The fungal samples were collected by sterile cotton swabs under aseptic conditions after making a clinical diagnosis. In the next step, a 10% KOH microscopic examination was carried out on all samples or specimens that had been collected.

Specimens that contained fungal elements were inoculated on the surface of Sabouraud dextrose agar (SDA) at 37°C. Then, the sensitivity of the fungus could be determined based on the formation of the diameter of the inhibitory zone around the disc.

In this study, several variables were analyzed, such as gender, age, employment status, inhibition zone diameter, and antifungal sensitivity. The data in this research were analyzed using IBM SPSS V.7. Kruskal-Wallis test was applied to analyze the sensitivity of 1% clotrimazole cream and 2% acetic acid solution. The data obtained was not normally distributed, with a degree of significance *p* value < 0.05. This indicated that there was a significant difference in the sensitivity of 1% clotrimazole cream and 2% acetic acid solution.

RESULT

Based on the results of fungal identification, it was found that 9 samples of otomycosis patients used in this study were caused by *Aspergillus sp.* (82%). Furthermore, for the other 2 patient samples, otomycosis was caused by *Candida sp.* (18%). Identification of *Aspergillus sp.* fungus was carried out using Lactophenol Cotton Blue (LPCB) staining. Based on the LPCB staining results, conidia, vesicles, conidiophores, and hyphae appear on *Aspergillus sp.* (Figure 1).

Meanwhile, to identify *Candida sp*. Gram staining could be done. In the gram staining results, it was found that there were oval or budding colonies. Furthermore, the fungal colony *Candida sp.* (Figure 2).

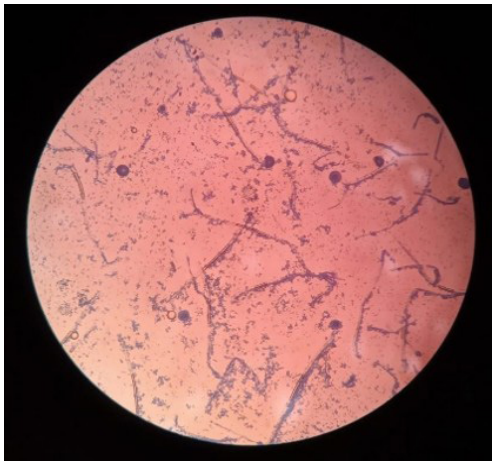

Figure 1. LPCB results of fungal isolates from otomycosis patients

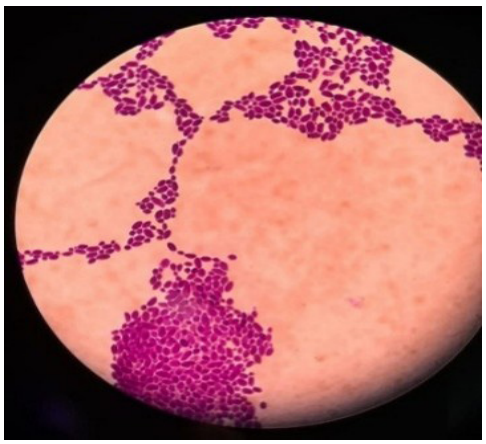

Figure 2. Results of gram staining of fungal isolates from otomycosis patients

A total of 11 patients with a diagnosis of otomycosis were included in the analysis as a sample in the study. In this study, it was recorded that there were 7 female patients (63.6%), while there were 4 male patients (36.4%). The ratio of the ages of the patients used as research samples was divided into 5 groups. The majority of the age group of research subjects were in the 26-45 years age group, totaling 6 people (54.5%), followed by the 46-65 years age group, totaling 3 people (27.3%). In the research subjects, the youngest age was 22 years and the oldest was 72 years. Then, the majority of research subjects were working. It was recorded in this study that the research subjects who worked were 7 people (63.6%) and those who did not work were 4 people (36.4%) (Table 1).

In this study, it was found that all samples had an average diameter of the inhibition zone against 1% clotrimazole cream. This indicates that all samples are sensitive to 1% clotrimazole cream. Meanwhile, only 5 samples had an inhibition zone diameter and 6 samples did not have an inhibition zone against the 2% acetic acid solution. This indicates that 5 samples are sensitive to 2% acetic acid solution and 6 other samples are resistant (Figure 3).

Table 1. Characteristic of research subject

Variable	Frequency	Percentage (%)
Gender		
Male	4	36.4
Female	7	63.6
Age		
0-11 years	0	0
12-25 years	1	9.1
26-45 years	6	54.5
46-65 years	3	27.3
>65 years	1	9.1
Occupation status		
Work	7	63.6
Not work	4	36.4

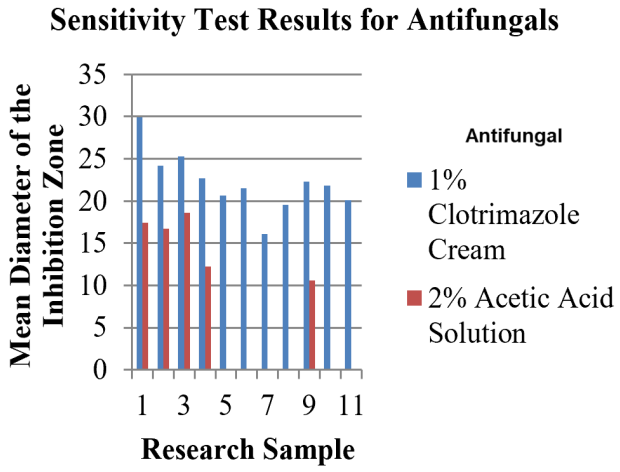

Figure 3. Diagram of sensitivity test results for clotrimazole cream 1% and acetic acid solution 2%

After incubation, the zone sizes are measured and interpreted. In the interpretation of the diameter of the inhibitory zone of 1% clotrimazole cream, it was found that 82% of samples were sensitive to clotrimazole, 18% were intermediate, and none were resistant (Figure 4).

Meanwhile, in interpreting the diameter of the inhibition zone of the 2% acetic acid solution, it was found that no samples were sensitive, 73% of the samples were intermediate to the acetic acid solution, and 27% of the other samples were resistant (Figure 5).

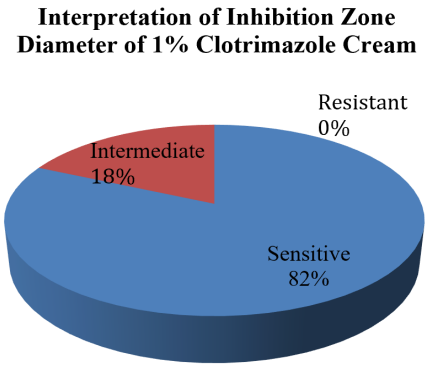

Figure 4. Interpretation of inhibition zone diameter of 1% clotrimazol cream

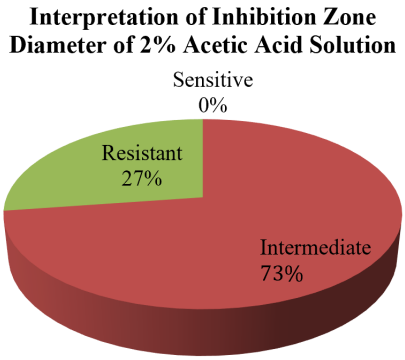

Figure 5. Interpretation of inhibition zone diameter of 2% acetic acid solution

DISCUSSION

In this study, it was found that otomycosis occurred more frequently in females, accounting for 7 cases (63.6%), compared to males with 4 cases (36.4%). This finding aligns with the study by Sangaré et al.[8] which reported that otomycosis is more common in females because they are generally more attentive and concerned about their health. Additionally, this can be associated with the habit of wearing head coverings, such as hijabs, which can increase the moisture in the external ear canal when worn for extended periods.[9] Meanwhile, another study by Ahmed et al.[10] found a higher incidence in males, attributing this to their greater involvement in outdoor activities, which increases the risk of exposure to fungal spores. However, Mistry et al.[11] noted in their research that fungal infections are not gender-specific, and other studies on otomycosis have shown varying results regarding gender distribution.

The distribution of patients was based on age group (54.5%), followed by the 46–65 years age group (27.3%). This result is consistent with the study conducted by Zulhaidah et al.[12] which stated that the highest incidence is observed in the 26–45 years age

group, categorized as adults, followed by the 46–65 years age group. Meanwhile, the lowest incidence was found in the <11 years and >65 years age groups. Kalpana et al.[13] also reported that the majority of otomycosis cases occurred in the 21–30 years age group, with fewer cases in those over 50 years. According to this study, the high incidence of otomycosis in the adult age group may be due to their frequent engagement in outdoor activities. Exposure to water and dust in outdoor environments increases the risk of fungal ear infections too.[14]

The employment status of patients showed that 7 individuals (63.6%) were employed, while 4 individuals (36.4%) were unemployed, out of a total of 11 patients. This finding aligned with the study conducted by Zulhaidah et al.[12] which mentioned that workers are more susceptible to otomycosis due to their exposure to hot and dusty environments that favor fungal growth. Kiakojuri et al.[15] also emphasized that workers were the most affected by otomycosis, as opportunistic and pathogenic fungi in their surroundings could be transmitted to the human ear through dust and contaminated tools.

In this study, *Aspergillus sp.* was the most common fungal species causing otomycosis. This finding is consistent with previous studies by Haq et al.[2] which identified *Aspergillus sp.* as the predominant causative agent of otomycosis. This might be attributed to the spores of *Aspergillus sp.*, which were carried by airborne dust or water particles and spread widely, as observed in the study by Victoria et al.[16] which investigated the prevalence of aspergillus in otomycosis patients. Bojanović et al.[4] also noted that the dissemination of aspergillus spores can be influenced by environmental factors such as warm weather, air humidity, and the tropical and subtropical climates. Furthermore, the identification of fungal species in this study was limited to the genus level due to time and equipment constraints.

The sensitivity testing of 11 samples from otomycosis patients revealed significant differences in sensitivity between clotrimazole cream and acetic acid solution tested in vitro. The results support the hypothesis that there is a specific antifungal sensitivity difference between 1% clotrimazole cream and 2% acetic acid solution in fungal isolates from otomycosis patients at Adam Malik Hospital and Prof. Chairuddin P. Lubis Hospital. Statistical analysis showed significant sensitivity differences, where clotrimazole cream was found to be sensitive to 9 samples and intermediate to 2 samples, whereas acetic acid solution showed intermediate sensitivity to 3 samples and resistance to 8 samples.

These findings indicated that clotrimazole cream was more effective than acetic acid solution in treating otomycosis patients. The results of this study aligned with previous research conducted by Lee et al.[17] which stated that clotrimazole was the most commonly used topical azole antifungal agent. Clotrimazole effectively disrupts the fungal cell wall by inhibiting ergosterol biosynthesis, increasing membrane permeability, causing fungal cell lysis, and leading to cellular death. Clotrimazole also has broad-spectrum antifungal activity against aspergillus and candida species. Meanwhile, acetic acid works by inhibiting fungal growth through disruption of acid-base balance within fungal cells by lowering the pH in the ear canal, as described by Sudrajad et al.[1] Furthermore, Chaitanya et al.[19] found that clotrimazole and acetic acid have similar effectiveness in treating otomycosis.

The resistance of some samples to acetic acid solution could be attributed to factors such as inappropriate antifungal dosage. This might occur when treatment is discontinued prematurely or prescribed in insufficient doses, enabling fungi to develop resistance.

Additionally, antifungal resistance can arise from frequent fungicide use in agriculture, exposing fungi to higher levels of fungicides, thereby increasing the risk of resistance in individuals working in such environments. Another factor was spontaneous resistance, where fungi became unresponsive to previously effective treatments without a clear cause.[20]

In this study, the fungal isolates resistant to acetic acid belonged to the *Aspergillus sp.* Ali et al.[21] revealed that filamentous fungi producing urease enzymes played a crucial role in fungal pathogenicity by impairing macrophage ability to destroy fungal spores. This enzyme played an important role for fungal pathogens because it could reduce the ability of macrophages to destroy spores.[22] Meanwhile, *Candida sp.* isolates, which lacked or were negative for urease, were more susceptible to macrophage-mediated destruction. The urease-positive enzyme of aspergillus enhanced its virulence and resistance levels. Furthermore, the high enzymatic capability of filamentous fungi contributed to their increased virulence and more severe infection outcomes.[21]

In conclusion, the 2% acetic acid solution was not better than 1% clotrimazole cream because it did not directly kill the fungus, but was effective in making the pH of the external acoustic canal environment more acidic, so that it was not suitable for the fungal growth environment.

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REFERENCE

1. Sudrajad H, Hendradewi S, Sinaga Y. Efektivitas asam asetat 2% dalam alkohol 70% dibanding ketokonazol 2% topikal pada terapi otomikosis. ORLI. 2018;48(1):26-33.
2. Haq M, Deshmukh P. Review of recurrent otomycosis and clotrimazole in its treatment. Cureus. 2022;14(10):e29975.
3. Elhage KG, St Claire K, Daveluy S. Acetic acid and the skin: a review of vinegar in dermatology. Int J Dermatol. 2021;60(7):804-11.
4. Bojanović M, Stalević M, Arsić-Arsenijević V, Ignjatović A, Ranđelović M, Golubović M, et al. Etiology, predisposing factors, clinical features and diagnostic procedure of otomycosis: a literature review. J Fungi (Basel). 2023;9(6):629.
5. Mofatteh MR, Fersheh MA, Nikoomanesh F, Namaei MH. Comparing the therapy of otomycosis using clotrimazole with iodine tincture: a clinical trial. Iran J Otorhinolaryngol. 2021;33(4):229-35.
6. Sulaiman E, Purwanto B, Lasminingrum L, Dewi YA, Mahdiani S. Potency of vinegar therapy in otomycosis patients. J Med Health. 2015;1(2):143-55.
7. Berkow EL, Lockhart SR, Ostrosky-Zeichner L. Antifungal susceptibility testing: current approaches. Clin Microbiol Rev. 2020;33(3):e00069-19.
8. Sangaré I, Amona FM, Ouedraogo RW, Zida A, Ouedraogo MS. Otomycosis in Africa: epidemiology, diagnosis and treatment. J Mycol Med. 2021;31(2):101116.
9. Wijaya HA, Darmawan AB, Nur Hestiyani RA, Krisniawati N, Rujito L. Prevalensi Candida albicans pada pasien otomikosis di RSUD Margono Soekarjo. Med Health J. 2023;3(1):40-49.
10. Ahmed MR, Abou-Halawa AS, Hessam WF, Abdelkader DSA. A search for new otomycotic species and their sensitivity to different antifungals. Interv Med Appl Sci. 2018;10(3):146-9.
11. Mistry M, Pathak A. To study the prevalence and clino-mycological profile of otomycosis in Saurashtra and Kutch region of Gujarat. Int J Med Microbiol Trop Dis. 2020;6(4):213-6.

12. Zulhaidah F, Budiman P, Bakri ER, Purnamasari F, Ruhyana N, Zaidah N. Profil klinis dan gabungan terapi pasien terdiagnosis otomikosis di Poliklinik THT-KL RSUD Waled periode tahun 2018-2022. Tunas Med J Kedokt Kesehat. 2025;11(1).

13. Kalpana S, Ansari M, Sameena ARB. Clinico-mycological analysis of otomycosis in a tertiary care hospital. Int J Pharm Clin Res. 2023;15(6):913-8.

14. Susana EN. Hubungan kebiasaan mengorek telinga dengan kejadian otomikosis pada pasien di Poliklinik THT Rumah Sakit Budi Kemuliaan Kota Batam. J Kesehat Kedokt Vitalitas Medis. 2024;1(2):68-80.

15. Kiakojuri K, Rajabnia R, Mahdavi Omran S, Pournajaf A, Karami M, Taghizadeh Armaki M. Role of clotrimazole in prevention of recurrent otomycosis. Biomed Res Int. 2019;2019:1-6.

16. Victoria D, Widhi APKN, Darmawan AB. Prevalence of Aspergillus niger in otomycosis at Prof. Dr. Margono Soekarjo Hospital. Mandala Health. 2023;16(1):1-7.

17. Lee A, T S Jr, T S Sr. Topical azole treatments for otomycosis. Cochrane Database Syst Rev. 2021;(5):CD009289. doi: 10.1002/14651858.CD009289.pub2.

18. Maha HL, Sinaga KR, Masfria. Formulation and evaluation of miconazole nitrate nanoemulsion and cream. Asian J Pharm Clin Res. 2018;11(3):319-21.

19. Chaitanya MN, Ambika N, Goud PS, Vantaram D, Singh S. A comparative study between clotrimazole drops and acetic acid wash as a treatment for otomycosis. Int J Curr Pharm Rev Res. 2023;15(11):352-6.

20. Hossain CM, Ryan L, Gera M, Choudhuri S, Lyle N, Ali K, et al. Antifungals and drug resistance. Encyclopedia. 2022;2:1722-37.

21. Ali K, Hamed MA, Hassan H, Esmail A, Sheneef A. Identification of fungal pathogens in otomycosis and their drug sensitivity: our experience. Int Arch Otorhinolaryngol. 2018;22(4):400-3.

22. Xiong Z, Zhang N, Xu L, Deng Z, Limwachiranon J, Guo Y, et al. Urease of Aspergillus fumigatus is required for survival in macrophages and virulence. Microbiol Spectr. 2023;11(2):e04535-22.