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PREVALENCE OF (*Aspergillus niger)* IN OTOMYCOSIS AT PROF. DR. MARGONO SOEKARJO HOSPITAL

PREVALENSI JAMUR (*Aspergillus niger)* PADA OTOMIKOSIS DI RSUD PROF. DR. MARGONO SOEKARJO

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ABSTRACT

Otomycosis is a chronic fungal infection with high prevalence. Environmental factors dan predisposing factors can influence a patient's vulnerability to otomycosis. According to studies conducted in several country, Aspergillus niger is the common fungus that causes otomycosis. This study aims to determine the prevalence of Aspergillus niger in patients at Prof. dr. Margono Soekarjo Hospital, who was clinically diagnosed with otomycosis. The study was done at the ENT Poly of Prof. dr. Margono Soekarjo Hospital and Microbiology Laboratory of Medicine Faculty, Jenderal Soedirman University between August 2022 to Januari 2023 as a descriptive observational study. According to the inclusion criteria, there were 42 subjects and 46 samples that ENT specialists carried out with sterile flocked swabs, the isolated the samples bedside on SDA Chloramphenicol, Czapek Dox agar, and object glass. Samples were observed macroscopically and microscopically with 10% KOH and LPCB staining. Patients with Aspergillus niger infection in otomycosis at Prof. dr. Margono Soekarjo Hospital , are represented by nine (19,56%) out of the 46 samples. The gender with the highest sex precentage is female (55,56%), folllowed by the age range of 26 – 35 years (44,45%). The prevalence of Aspergillus niger as a cause of otomycosis in Prof. dr. Margono Soekarjo Hospital is 19,56%, the most in females and the age group 26 – 35 years.

***Keywords**: Aspergillus niger; Otomycosis; Prevalence*

ABSTRAK

Otomikosis adalah infeksi jamur kronis dengan prevalensi yang tinggi. Faktor Lingkungan dan faktor predisposisi dapat mempengaruhi kerentanan seseorang terhadap otomikosis. *Aspergillus niger* merupakan jamur yang paling banyak menyebabkan otomikosis berdasarkan penelitian di berbagai negara. Penelitian ini bertujuan untuk mengetahui prevalensi *Aspergillus niger* pada pasien dengan diagnosis klinis otomikosis di RSUD Prof. dr. Margono Soekarjo. Penelitian ini merupakan penelitian deskriptif observasional yang dilaksanakan pada bulan Mei 2022 – Januari 2023 di Poli THT-KL RSUD Prof. dr. Margono Soekarjo dan Laboratorium Mikrobiologi Fakultas Kedokteran Universitas Jenderal Soedirman. Subjek pada penelitian ini adalah 42 pasien dengan 46 sampel yang diambil dengan teknik *total sampling* sesuai kriteria inklusi. Pengambilan sampel dilakukan oleh dokter spesialis THT-KL menggunakan *flocked swab steril*, kemudian diisolasi *bedside* pada SDA *Chloramphenicol*, *Czapek Dox Agar*, dan *object glass.* Dilakukan pengamatan secara makroskopis dan mikroskopis dengan pengecatan KOH 10% dan

LPCB. Dari 46 sampel, pasien yang terinfeksi *Aspergillus niger* pada otomikosis di RSUD Prof. dr. Margono Soekarjo adalah 9 sampel (19,56%). Persentase jenis kelamin terbanyak adalah perempuan (55,56%) dan kelompok usia 26 – 35 tahun terbanyak dengan 44,45%. Prevalensi *Aspergillus niger* sebagai penyebab otomikosis di RSUD Prof. dr. Margono Soekarjo adalah 19,56%, terbanyak pada perempuan dan kelompok usia 26 – 35 tahun.

Kata kunci: *Aspergillus niger;* Otomikosis, Prevalensi

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INTRODUCTION

The prevalence of otomycosis or chronic infection caused by fungi on the ear canal reaches about 9 - 30 % from the externa otitis over the world (Agarwal and Devi 2017). Age group of 20 – 30 years is risky on suffering otomycosis because classified as productive age thus having firm relation to the jobs, especially outside jobs that increase the spreading of fungus spore (Aremu *et al.* 2020). Women show the higher prevalence on the otomycosis resulted by the high hygiene pattern and related to ear-scraping habit (Manjunath *et al.* 2020)

Geographic condition such heated weather, a high humidity rate and dust, on both subtropic and tropic area as Indonesia causes a higher fungus spreading rate (Merad *et al.* 2021). Otomycosis is uncomplicated to be discovered on patients with cotton buds usage history, eardrops usage, ear appliance usage, swimming habit in the places with a low quality of water, and stressing condition (Adegbiji *et al.* 2014; Marlinda dan Aprilia 2016).

Aspergillus niger has a higher prevalence compared to other fungi because it is able to intermix in the dust or air (Merza and Abdulkhaleq 2021). The research of otomycosis caused by *Aspergillus niger* is easier to conduct because the characteristics are relatively easy to be identified compared to *Candida spp.* (Manjunath *et al.* 2020). Based on the backgroind elucidation above, there are differences of fungi prevalence that predominantly cause otomycosis. The research of *Aspergillus niger* prevalence as otomycosis cause in Jawa has not been found. The researcher expects the conducting of this research can interpret *Aspergillus niger* prevalence on otomycosis in Prof. dr. Margono Soekarjo Hospital.

METHODS

This research is descriptive observational research. The targeted population on this research is ENT patients in RSUD Prof. dr. Margono Soekarjo, whereas accessible population is patients who come to ENT poly Prof. dr. Margono Soekarjo Hospital who was clinically diagnosed with otomycosis**.**

Materials

This research uses the primary data of fungi mass swab by medical ENT specialists, using flexible minitip flocked swab sterile, Saboroud's Dextrose Agar (SDA) *Chloramphenicol*, *Czapek Dox Agar*, object glass and cover glass, Lactophenol Cotton Blue (LPCB) solution, 10% KOH solution, microscope, and ose needle.

Research

This research has been approved by FK UNSOED Medical Research Ethics Commission (019/KEPK/PE/II/2022). The sampling uses total sampling technique. The inclusion criteria are

RSUD Prof. dr. Margono Soekarjo patients who are diagnosed clinical otomycosis, age > 17 years and have signed the informed consent. The exclusion criteria of this research are patients with huge degree externa otitis and uncooperative ones. Fungi mass swab sample is carried out by ENT specialists using sterile flocked swab, then isolated bed side on culture media and object glass for microscopic inspection directly using KOH 10%. Fungi mass swab outcome is inoculated on SDA Chloramphenicol culture media and Czapek Dox Agar media. Then, it is incubated on 25^{o}C and 37^{o}C temperature aerobically for four weeks and examined every 3-5 days.

Data Analysis

Data taken from this research are analyzed with univariate analysis for describing *Aspergillus niger* isolation result in the form of frequency distribution table and percentage that contain sample's characteristic data classified by age, gender, and *Aspergillus niger* prevalence on otomycosis.

RESULTS AND DISCUSSION

The subjects of this research are 42 patients who are diagnosed by clinical otomycosis with 46 samples that qualify the inclusion criteria can be seen on Table 1.

Tabel I. Characteristics of subject with otomycosis

No.	Subject Characteristics	Frequency	Persentage (%)
1.	**Gender**		
	Laki-laki	21	50,00
	Perempuan	21	50,00
	Total	42	100,00
2.	**Age**		
	17 - 25 years	6	14,28
	26 - 35 years	12	28,57
	36 – 45 years	9	21,42
	46 – 55 years	5	11,90
	56 – 65 years	8	19,04
	>65 years	2	4,76
	Total	42	100,00

Table I that shows the sample by gender obtains each man and woman result 21 people (50,00%). The highest frequency by age is in the 26-35 years old group as 12 people (28,57%).

Tabel II. Prevalence of *Aspergillus niger* in otomycosis

No.	Prevalence	Total	Persentage (%)
1.	*Aspergillus spp.*	22	47,84
2.	*Aspergillus niger*	9	19,56
3.	Other species found (*yeast*)	15	32,60
	Total	46	100,00

Table II shows 22 (47,84%) of 46 sample are otomycosis caused by *Aspergillus spp.,* 9 samples (19,56%) are otomycosis caused by *Aspergillus niger*, and 15 samples (32,60%) are yeast.

Tabel III. *Aspergillus niger* infection on otomycosis according to age and sex

No.	Distribusi	Criteria	Total	Persentage (%)
1.	Age	17 - 25 years	0	0
		26 - 35 years	4	44,45
		36 – 45 years	2	22,22
		46 – 55 years	0	0
		56 – 65 years	3	33,33

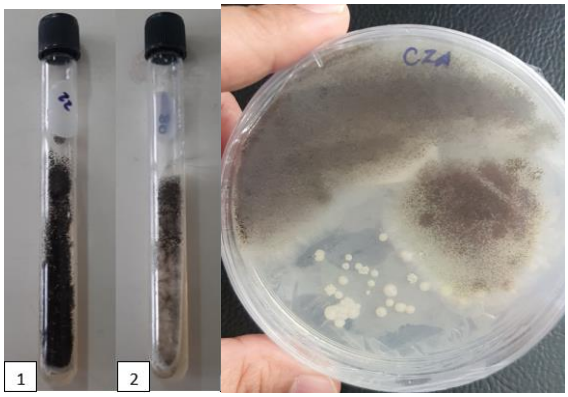
		>65 years	0	0
		Total	9	100,00
2.	Sex	Male	4	44,44
		Female	5	55,56
		Total	9	100,00

Table III shows the highest rate of *Aspergillus niger* infection as otomycosis cause is in the 26 – 35 years old group as 4 samples (44,45%). According to the sex, *Aspergillus niger* as otomycosis cause is frequently in women as 5 samples (55,56%).

Tabel IV. Predisposition factors of otomycosis sufferer

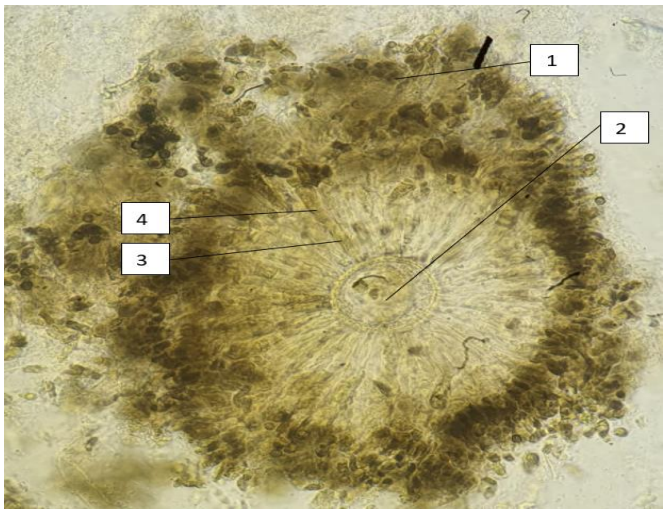
No.	**Faktor Predisposisi**	**Criteria**	**Total**	**Persentage (%)**	**Total (*A. niger*)**	**Persentage (%)**
1.	Having water entry while showering	Ya	35	76,08	7	77,78
		Tidak	11	23,91	2	22,22
2.	Ear drops usage	Ya	36	78,26	7	77,78
		Tidak	10	21,73	2	22,22
3.	Ear scraping	Ya	31	67,39	7	77,78
		Tidak	15	32,60	2	22,22

Table IV shows the predisposition sample factors. Samples which have water entry while showering as 35 samples (76,08%), and 7 samples (77,78%) of them suffer otomycosis caused by *Aspergillus niger*. As 36 samples (78,26%) have antibiotic ear drops usage history, and 7 (77,78%) of them suffer otomycosis caused by *Aspergillus niger*.



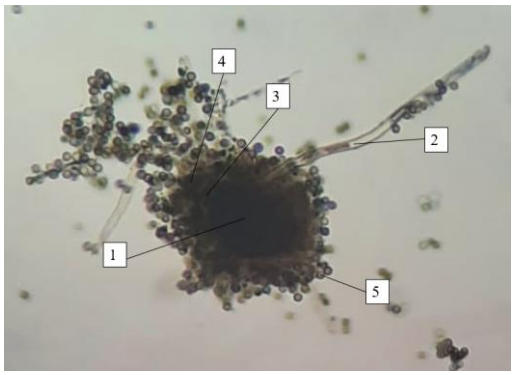
Gambar 1. Colony of *Aspergillus niger* on the SDA *Chloramphenicol* and *Czapek Dox Agar*.

Picture 1 shows *Aspergillus niger* colony on SDA *Chloramphenicol* media with black surface, grayish cream reverse, and soft texture. The right picture shows *Aspergillus niger* colony on *Czapek Dox Agar* media with blackish pigment on the surface that is the color of fungus spore colored dark black.



Picture 3. Cell Morphology of *Aspergillus niger* in KOH 10%.
Keterangan: 1) Conidia, 2) Vesicle, 3) Metula, 4) Phialid

Picture 3 shows *Aspergillus niger* cell morphology appearance in KOH 10% test showing *Aspergillus niger* fungi, there is vesicle in the center with the whole surface surrounded by metula and phialid. In the fialid edge, there is round blackish dark conidia.



Picture 4. Cell Morphology of *Aspergillus niger* in LPCB staining.
Explanation: 1) Vesicle, 2) Conidiophore, 3) Metula, 4) Phialid, 5) Conidia

Picture 4 shows *Aspergillus niger* cell morphology with vesicle, biseriate phialid and blackish conidia.

The highest rate of otomycosis sufferer is in 26 – 35 years group, as 12 patients (28,57%) in the Table I, and 4 samples (44,45%) in the Table III are samples who suffer otomycosis caused by *Aspergillus niger*. As a productive age group, the occupation factor has a big impact on the spreading of fungus spores (Aremu *et al.* 2020). Women are the most frequent sex who suffer otomycosis caused by *Aspergillus niger* as 5 samples (55,56%) in the Table 3.

Women have a high hygiene factor as one risk factors that has a big impact on otomycosis. The excessive amount of cerumen will result in distressing feelings and hearing loss. Therefore, women tend to scrap their ears to reduce the complaint. People tend to alleviate the complaint by carrying the cerumen out using cotton bud, ear scrapping tools, and othe tools that are not hygiene guaranteed. The tools are vulnerable to contamination by *Aspergillus niger* spores beacuse these spores actively spread in the air (Adegbiji *et al.* 2014). Moreover, ear scrapping can scratch the

crust on the external acoustic canal, easing the infection experience resulting from the fat layer loss that has a role on the bacterial and fungi groeth suppresion (Humaira 2012; Khan *et al.* 2017).

Aspergillus niger prevalence as the cause of otomycosis in this research is 19,56% or 9 out of 46 samples in the Table II. The difference of result study to other research is influenced by various factors such as environmental factors and predisposing factors. *Aspergillus niger* spores that are carried out by water particles and intermixed in the dust or air can widely spread and be affected by environmental factors, such hot weather, humidity, also both tropic and subtropic areas.

The environmental condition in Indonesia, especially in Purwokerto is definitely different from the environment in Egypt, Bahrain, and India. It is the reason why the fungi spreading in Purwokerto is different with the fungi spreading out of Purwokerto. Beside the environmental factor, *Aspergillus niger* fungi growth depends on the external acoustic canal that is influenced by pH, humidity, and optimal temperature as it can grow well on the external acoustic canal (Merad *et al.* 2021; Merza dan Abdulkhaleq 2021). The condition in the external acoustic canal is affected by the cerumen that is ear physiologic substance. Cerumen has 4-5 pH level that can inhibit bacterial and fungi growth.

Aspergillus niger has virulence factor as phospholipase activity that impacts on fungi invasion to the external acoustic canal. The phospholipase activity can hydrolyze the glycerophospholipid ester bonds in the cell membrane and ease the fungi to infect tissue. Moreover, biofilm production in fungi can inhibit fungal phagocytosis (Raksha *et al.* 2017).

CONCLUSION

According to this study, prevalence of *Aspergillus niger* infection in otomycosis at Prof. dr. Margono Soekarjo Hospital between May 2022 to Januari 2023 is 19,56%. The gender with highest sex percentage is female (55,56%), followed by the age range of 26 to 35 years (44,45%). It is expected to conduct further research by further discussing *Aspergillus niger* and otomycosis related to virulence factors such as biofilm production, phospholipase activity and protease fermentation,

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