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Examination of Nail Fungus in Garbage Transporters using Saboraud Dextrose Agar Culture Media

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Abstract

Onychomycosis, a fungal infection affecting both toenails and fingernails, can lead to nail discoloration, yellowing, blackening, deformation, sponginess, and brittleness. This condition is typically caused by different types of fungi, such as dermatophytes, non-dermatophyte molds, and yeasts. Onychomycosis has an estimated prevalence of 10% worldwide. The prevalence of dermatomycosis cases in Indonesia is in the low range of 3.5-4.7%. This study aims to determine the prevalence of onychomycosis (nail fungus) and explore the relationship between personal hygiene, length of work, and the use of boots in waste transporters at the UPTD cleanliness Rawalumbu area, Pengasinan Village. This type of research is descriptive. This research design is cross-sectional with ethics code 218/EC/KEPK/STIKES-PI/II/2023. The sample in this study amounted to 30 garbage transporters at the UPTD cleanliness Rawalumbu region, Pengasinan Village. The study included garbage transporters in the UPTD Rawalumbu area of Pengasinan Village who were willing to complete the Informed Consent sheet. Garbage transporters with mental disorders or difficulty participating in interviews were excluded. The sampling method used in this research is purposive sampling. The sampling place in this research was conducted at the UPTD cleanliness Rawalumbu area, Pengasinan Village. The results of this study obtained 20 samples (67%) positive for onychomycosis and ten samples (33%) negative for onychomycosis. The positive results were two nail samples detected *Trichophyton* sp. fungus type (15%) and 18 other samples found *Aspergillus* sp. fungus type (85%). This research concludes that the examination of 30 nail samples of garbage transporters found 20 samples (67%) positive for onychomycosis and 10 samples (33%) negative for onychomycosis. Of the 20 positive samples, two nail samples detected the fungus type *Trichophyton* sp. (15%), and 18 other samples found the fungus type *Aspergillus* sp. (85%). The results of the statistical test performed coefficient contingency showed a significant relationship between personal hygiene, length of work, and using boots with the incidence of onychomycosis in garbage transporters at the UPTD cleanliness Rawalumbu area, Pengasinan Village, which was assessed by statistical tests where the p-value was obtained <0.05.

Keywords: Onychomycosis, Personal Hygiene, Garbage Transporters

Introduction

Onychomycosis, a fungal infection affecting both toenails and fingernails, can lead to nails thickening and becoming uneven, cracked, and dull while the color changes to yellow, brown, or black. This condition is typically caused by different types of fungi, such as dermatophytes, non-dermatophyte molds, and yeasts. Gupta *et al.* (2020) estimated the worldwide prevalence of onychomycosis to be about 10%. Dermatomycosis has a low prevalence in Indonesia, estimated at 3.5-4.7%. The age group most susceptible to onychomycosis infections is 25-44 years, with a prevalence of 23.8%. Men have a higher incidence rate of onychomycosis (65%) compared to women (35%). The high infection rate in men may result from frequent nail injuries due to daily and sports activities (Bitew & Wolde, 2019; Gupta *et al*., 2017; Mamuaja *et al*., 2017). Nail changes resulting from onychomycosis infection can significantly decrease the quality of life and comfort for those with the condition. Onychomycosis predominantly affects individuals who work in wet and humid environments, such as garbage collection professionals. Poor personal hygiene practices may contribute to developing fungal infections (Artha & Oktasaputri, 2020; Elewski & Tosti, 2015; Hidayat, 2018). According to Lolowang *et al.* (2020), waste pickers are highly susceptible to onychomycosis because they work in humid and unhygienic environments.

A study conducted by Mulyati and Zakiyah (2020) revealed that out of the 57 scavengers at the Bantar Gebang landfill, 50 samples were positive for onychomycosis (87.72%), and seven samples were negative (12.28%). This high risk of infection is corroborated by Sari *et al.* (2016), who found that scavengers are prone to various diseases due to work conditions. This is unchanged because it already adheres to the principles and lacks context. This study aims to determine the prevalence of onychomycosis (nail fungus) and explore the relationship between personal hygiene, length of work, and the use of boots in waste transporters at the UPTD cleanliness Rawalumbu area, Pengasinan Village. Researchers chose to study waste transporters due to their increased risk of nail fungus infection from their unclean and

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humid work environment. The study's findings will serve as an informative resource for the Bekasi City government to educate garbage transporters at the UPTD cleanliness Rawalumbu area, Pengasinan Village, regarding the significance of personal hygiene during their duties.

Methods

The study included garbage transporters in the UPTD Rawalumbu area of Pengasinan Village who were willing to complete the Informed Consent sheet. Garbage transporters with mental disorders or difficulty participating in interviews were excluded. A total of 30 garbage transporters from the UPTD of cleanliness in the Rawalumbu area of Pengasinan Village were sampled for this study. This study was conducted under a research permit obtained from STIKes Mitra Keluarga. Additionally, an ethics letter was secured from STIKes Prima Indonesia. The research received informed consent from the garbage transporters, who participated as research subjects, after being approved by the ethics committee (registration number 218/EC/KEPK/STIKES-PI/II/2023). This research was conducted during February and March of 2023 at the UPTD Cleanliness in the Rawalumbu area of Pengasinan Village. The samples were analyzed at the Mycology Laboratory of STIKes Mitra Keluarga without deviations from standard procedures.

Sabouraud Dextrose Agar (SDA) Media Preparation Procedure : It weighed 29.25 grams (6.5%) of SDA media in a weighing cup. Transferred the weighed media into an Erlenmeyer and dissolved with 450 mL of distilled water. Then, Erlenmeyer was heated until it evaporated so that it mixed perfectly. Erlenmeyer was sterilized with an autoclave for 15 minutes at 121°C with a pressure of 2 atm. Waited until cool, added 0.225 grams (0.05%) chloramphenicol while shaking until dissolved. Then, put it into a petri dish and homogenize it. Sterile Distilled Water Making Procedure : 10 mL amount of Aquadest was added to a test tube. The test tube was then covered with cotton and sterilized using an autoclave at a temperature of 121°C for 15 minutes (Zulneti, 2020).

The nail is cleansed using an alcohol swab, followed by scraping with a scalpel. The resulting nail-scraping sample is placed into a container for examination at a laboratory. Nail samples are collected using a sterile cotton swab moistened with sterile distilled water. The swab is then used to spread the sample on the center of the surface of the SDA media. The Petri dishes are wrapped in paper and incubated at room temperature for 5-7 days. Fungal growth is observed and recorded. Observations were macroscopically conducted to identify fungal colonies that grew on SDA media by evaluating their colony shape, color, and surface, as reported in the literature. Microscopic observations were conducted to identify fungal colonies that grew on SDA media with LPCB reagents. The glass object was cleaned to remove fat residues, and LPCB was applied droplet by droplet to the glass object. Then, the fungal colonies were aseptically taken using an ose needle, and the colony was stretched and mixed with LPCB. Technical abbreviations are explained when first used. Then, the fungal colonies were aseptically taken using an ose needle, and the colony was stretched and mixed with LPCB. It was covered with a glass object and observed under a microscope at a magnification of 10x10, then continued with a magnification of 40x10 (Zulneti, 2020).

Results and Discussions

This study was conducted at the UPTD cleanliness Rawalumbu area, Pengasinan Village, Bekasi City. The study sample amounted to 30 nail scrapings from garbage transporters. The samples were then examined by two methods, namely by culturing using Sabouraud Dextrose Agar (SDA) media. Then microscopic observations were made in the mycology laboratory of STIKes Mitra Keluarga

Table 1. Percentage incidence rate of onychomycosis

Result	Amount	Percentage
Positif	20	67%
Negatif	10	33%
Total	30	100%

Table 1. illustrates that 67% of 30 nail scraping samples from garbage transporters yielded positive results for nail fungus, while 33% were negative. Confirmation tests were performed to verify whether or not the garbage transporters were infected with nail fungus. Scraping samples were taken from the toenail, planted on SDA media, and incubated at room temperature for 5-7 days. The resulting figures are depicted below.

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Figure 1. Colony growth result on SDA media

Figure 1. displays the findings of fungal colony growth in nail scraping samples on SDA media. Figure 2 displays the findings of fungal colony growth in nail scraping samples on SDA media. The samples were incubated at room temperature for 5-7 days. The following figure illustrates the results of the microscopic examination obtained in this study.'

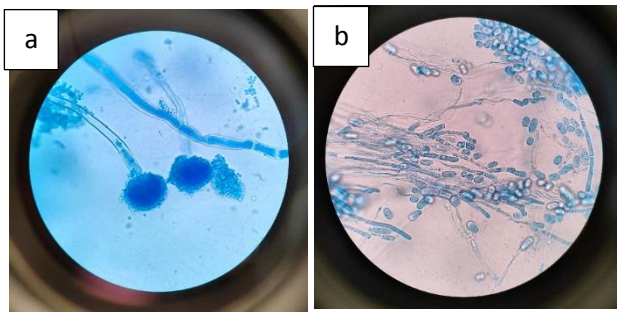

Figure 2. a. *Aspergillus* sp. b. *Trichophyton* sp. (40x10 magnification)

Figures 2. display the microscopic observations of colonies grown on SDA media for 5-7 days at room temperature. The percentage of the genus is determined based on these results and the macroscopic observations.

Table 2. Percentage of genus against positive samples

Result	Amount	Percentage
Aspergillus sp.	18	85%
Trichophyton sp.	2	15%
Total	20	100%

Table 2. displays that 18 samples (85%) were positive for *Aspergillus* sp. and 2 samples (15%) tested positive for *Trichophyton* sp. Furthermore, a survey was conducted to determine the factors that affect the incidence of onychomycosis, as shown in **Table 3.**

Table 3. Statistical Test Performed Coefficient Contingency of Factors Affecting Incidence of Onychomycosis with Sample Size of 30 Respondents

Factors affecting	Incidence of onychomycosis	p-Value < 0.05
Cutting nail frequency		
Often	8	0.038
Rarely	12	
Shower frequency		
Not shower	0	

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Shower 1x	1	0.542
Shower 2x	16	
Shower ≥2x	4	
Personal hygiene		
Hand and foot washing	23	0.033
No hand and foot washing	7	
Work duration	5	0.011
≤ 5 years	15	
≥ 5 years		
Boots wear	9	0.032
Wearing boots	11	
Not wearing boots		
Age	11	0.429
16–30 years	9	
31–45 years		

Table 3 displays the factors that impact the occurrence of onychomycosis. The statistics were then subjected to the coefficient contingency test to determine if there was an association between these factors and the incidence of onychomycosis. Of the four factors, cutting nail frequency, personal hygiene, work duration, and use of boots have a significance value <0.05, indicating a relationship with the incidence of onychomycosis. Meanwhile, the frequency of bathing and age do not show statistical significance (p>0.05), indicating no significant relationship between the frequency of daily bathing and age with the incidence rate of onychomycosis.

Based on the study results, garbage transporters took 30 nail-scraping samples. Table 1 displays the findings, with 20 samples (67%) testing positive and ten (33%) testing negative. Positive results are observed by the presence or absence of fungal growth on SDA media and the subsequent examination using LPCB under a microscope. LPCB is a dye applied to stain the cellular structure of fungal cells (Elsavira, 2021).

The macroscopic research yielded two genera, *Aspergillus* sp., and *Trichophyton* sp., as shown in **Figure 2.** To confirm the fungal genus, LPCB was used in conjunction with microscopic examination, as discussed in Figure 3. The microscopy results indicate *that Aspergillus* sp. exhibited hyphae that were difficult to discern, visible vesicles, and conidia that were not easily visible. This aligns with previous research by Ounleye and Olaiya (2015). The characteristics of *Aspergillus* sp. include skeptical hyphae, round vesicles, round conidia, and conidiophores made up of long stalks that support bulging vesicles. Conversely, *Trichophyton* is characterized by straight and smooth hyphae, with no macroconidia but abundant slightly oval round microconidia. This is in line with Kidd *et al.* (2016) study found that microconidia were present in abundant oblong, round shapes along the hyphae, while macroconidia were rare. If present, they were clavate, smooth, thin-walled, and varied in size.

According to the percentages presented in **Table 2,** the microscopic results identified *Aspergillus* sp. in as many as 18 samples (85%) and *Trichophyton* sp. in 2 samples (15%). *Aspergillus* sp. was the most frequently occurring finding in the study. This is due to *Aspergillus* sp. being one of the non-dermatophyte fungi responsible for causing onychomycosis, and its spores are present in the air as a contaminant, making it ubiquitous in the environment. This fungus can be found in soil, decaying plants, and other forms of organic waste (Ekasari & Nahlia, 2020). The high percentage of *Aspergillus* sp. fungi found in this study is in line with the results of research by Mulyati and Zakiyah (2020). The report indicated that the culture examination yielded nine fungi species, with *Aspergillus* sp. being the most prevalent, accounting for 48.72% of the total.

Garbage transporters who frequently trim their nails had an onychomycosis incidence of 8 people, whereas those who rarely trim their nails had an incidence of 12. The entry of dirt into the nails is a possibility for a person not trimming their nails regularly. The statistical test in Table 3 shows a significance value of 0.038 <0.05, indicating a relationship between nail trimming and onychomycosis incidence. This study aligns with Khotimah's (2020) research, which states that individuals who do not maintain proper hand and foot hygiene, including failure to cut and clean nails with soap, may be more susceptible to developing onychomycosis fungal infection.

The study examined 30 garbage transporters and found that one person who showered once a day, 16 people who showered twice a day, and four people who showered two or more times a day had onychomycosis. According to the statistical analysis in Table 3, the significance value was 0.542, greater than the threshold of 0.05, indicating no

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significant correlation between the frequency of daily showers and the incidence of onychomycosis. However, even if a person showers regularly, neglecting proper nail hygiene can still result in uncomplicated onychomycosis infection. According to Kamil *et al.* (2021), proper personal hygiene, including showering, can affect the occurrence of onychomycosis. It is essential to maintain good personal hygiene by keeping nails short and tidy.

Personal hygiene can be observed when garbage transporters clean their hands and feet after picking up and transporting garbage. Of the workers, 23 tested positive for onychomycosis and promptly washed their hands and feet, while seven did not. According to Andarmoyo (2012), proper hand and foot care involves maintaining cleanliness by rinsing with clean water, using soap to clean, and properly drying. According to statistical tests in Table 3, the significance value of 0.033 < 0.05 indicates a relationship between personal hygiene and the incidence of onychomycosis.

The most prevalent onychomycosis infection was observed in individuals who had been working for ≥ 5 years, with 15 people affected, compared to 5 people who had worked for ≤ 5 years. This study is by the research of Amalia *et al.* (2018). The duration of work reflects the length of time an individual is exposed to dermatophyte infection agents. The longer the duration of exposure to fungal infection risk factors, the higher the likelihood of contracting an infection. **Table 3** suggests a significant relationship between the duration of work and the incidence of onychomycosis, with a statistical value of 0.011 <0.05.

Onychomycosis infection was found in 10 individuals who wore boots and ten who did not wear boots. The risk of contracting onychomycosis is equal for those who wear boots and those who do not wear boots while working. This study is in line with the research of Laksono *et al.* (2020). Wearing closed-toe shoes, specifically boots, for more than six hours daily and poor foot hygiene can lead to increased foot moisture and fungal colonization. Statistically, the results show a significant relationship between wearing boots and the occurrence of onychomycosis with a value of 0.032 <0.05.

Age is considered among various risk factors that contribute to fungal infections as the immune system typically weakens with age. The age group between 16-30 years exhibited the highest prevalence of nail fungal infections in this study. Because the majority of participants in this study fell within this age bracket, the data could have been more varied. The statistical tests yielded a significance value of 0.429, indicating no clear association between age and the prevalence of onychomycosis. The results demonstrate that onychomycosis can affect individuals of all ages, indicating that age plays a relatively minor role in the risk of developing onychomycosis.

The study's limitations include the absence of 20-40% KOH reagent examination as an initial screening for nail fungus detection and the lack of identification at the species level. Future research should consider performing examinations using 20-40% KOH reagents as an initial screening for nail fungus detection while also analyzing data results to the species level.

Conclusions

This study concludes that among 30 nail samples from garbage transporters, 20 samples (67%) were positive for onychomycosis, while ten samples (33%) were negative. Of the 20 positive samples, two (15%) detected the fungus type *Trichophyton* sp. and 18 (85%) identified *Aspergillus* sp. The statistical tests revealed a significant correlation between onychomycosis incidence and personal hygiene habits, duration of work, and the use of boots among garbage transporters at the UPTD cleanliness Rawalumbu area, Pengasinan Village, with a p-value less than 0.05. It is suggested to maintain good personal hygiene practices, limit work duration, and wear appropriate boots to prevent onychomycosis.

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