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Research Article

Evaluating drug susceptibility of fungal species isolated from caught ants in Babol teaching Hospitals

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ABSTRACT

Considering the role of ants as potential carriers of a wide range of fungal agents, including *Aspergillus* spp. and *Candida* spp. as well as the increasing incidence of treatment-resistant hospital fungal infections, the antifungal susceptibility of fungal isolated from the surfaces of ants caught from teaching hospitals of Babol city were evaluated. In the present experimental study, 46 fungal isolates from caught ants in Babol teaching Hospitals including 15 strains of *Aspergillus niger*, 15 strains of *Aspergillus flavus*, 11 strains of *Aspergillus fumigatus* and 5 strains of *Penicillium* spp. were cultured on PDA medium and drug susceptibility to posaconazole, Isavuconazole, itraconazole, luliconazole and voriconazole was determined according to CLSI (M238-A2) guidelines. The best antifungal effect was related to loliconazole, itraconazole and Isavuconazoleagainst to *Aspergillus niger*, *Aspergillus fumigatus* and *Aspergillus flavus*, respectively and posaconazole showed the weakest antifungal effect against to the studied isolates. Our knowledge regarding the level of sensitivity of fungal species with the source of hospital ants can highlight the role of these insects as carriers of hospital infections and also demonstrate the need for a regular preventive programmer to control these insects in different hospitalized areas.

1. Introduction

Ants, social insects belonging to the order Hymenoptera, exhibit remarkable adaptability to their environments, characterized by their adeptness in locating food, moisture, and suitable habitats (Rodríguez, Flórez et al. 2016). Originating in the middle of the Cretaceous period approximately 110 to 130 million years ago (Soares, Almeida et al. 2006), ants inhabit diverse regions across the globe, with the exception of the Arctic and Antarctic regions, as well as remote and inaccessible islands (Czaczkes, Franz et al. 2015).

These organisms constitute 15-20% of all living organisms on Earth. Their success can be

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attributed to their social structure, high adaptability, and capacity to modify their habitats, resource exploitation, and mutual defense. In Iran, 110 species belonging to 26 genera from six subfamilies of Formicidae (Formicinae, Myrmicinae, Ponerinae, Dolichoderinae, Dorylinae, and Aenictinae) have been identified (Paknia, Radchenko et al. 2008).

Ants are among the organisms that can impact human health by influencing environmental quality and serving as vectors for disease transmission (Pinter-Wollman, Jelić et al. 2018). Their presence in the outdoor areas surrounding hospitals, as well as their infiltration into indoor environments, poses significant health risks. Certain ant species have been identified as carriers of pathogenic microorganisms, including *Staphylococcus*, *Klebsiella*, *Acinetobacter*, *Streptococcus*, *Enterobacteriaceae*, and *Enterococcus*, amplifying the potential threat to human health (Rodovalho, Santos et al. 2007).

In addition to transmitting eggs of intestinal worms, viruses, and protozoa, ants can also transmit fungal spores (Kistner, Saums et al. 2015). The high prevalence of fungal carriage is significant due to its implications for the transmission of fungal pathogens associated with nosocomial infections. Pathogenic fungal species such as *Aspergillus*, *Penicillium*, *Acromonium*, *Rhizopus*, and dermatophytes have been found in the soil of potted plants in hospitals, potentially serving as a source of hospital-acquired infections (Hedayati, Mohseni-Bandpi et al. 2004).

There have also been reports of the isolation of *Penicillium*, *Rhizopus*, *Aspergillus*, and *Alternaria* fungi in the air of hospitals, as well as the presence of *Candida*, *Penicillium*, and *Cladosporium* species on operating room equipment and surfaces. This highlights the contamination of hospital environments with potentially pathogenic fungi in the air, on surfaces, and in the soil (Arab, Ghaemi et al. 2006) .

In recent years, the occurrence of diseases related to the immune system, the increased use of immunosuppressive drugs, coupled with advancements in medical science and the rise in organ transplant procedures necessitating broader and longer treatment regimens, have led to an increased risk of invasive fungal infections among patients in hospital environments (Perlroth, Choi et al. 2007, Alangaden, 2011).

The global rise in the incidence of invasive fungal infections, coupled with the emergence and spread of fungal pathogens resistant to antifungal drugs, poses a growing threat to human health, resulting in increased mortality rates(Hadrich, Makni et al. 2012). Moreover, the transmission of these drug-resistant fungi by mechanical carriers, including insects such as ants, is of significant concern (Pantoja, Filho et al. 2009).

Additionally, the hot and humid weather, along with the forest ecosystem in Mazandaran province, creates favorable conditions for ant activity year-round, potentially increasing the risk of fungal pathogens being transmitted by ants. Given the presence of ants in hospital soil and their interaction with ambient air and surfaces, their role as carriers of pathogenic agents, and the significance of drug-resistant fungal species, there is a need to investigate the drug sensitivity profiles of fungal isolates obtained from the external surfaces of ants captured in teaching hospitals in Babol City.

2. Materials and Methods

2.1. Study Design and Sample Collection

This experimental study, conducted in 2022, utilized 46 fungal isolates obtained from ants in educational and therapeutic hospitals in Babol County (*Ayatollah Rouhani*, *Shahid Yahya Nejad*, and *Shahid Beheshti* hospitals). These isolates had previously been identified to the species level using polymerase chain reaction (PCR).

2.2. Fungal Isolates and Culturing Conditions

The study included 15 isolates of *Aspergillus niger*, 15 isolates of *Aspergillus flavus*, 11 isolates of *Aspergillus fumigatus*, and 5 isolates of *Penicillium* species. The selected isolates were cultured under sterile conditions on Sabouraud dextrose agar (SDA) medium supplemented with chloramphenicol (SC) and potato dextrose agar (PDA). The cultures were incubated at 35°C for 24 to 48 hours.

2.3. Antifungal Drug Sensitivity Testing

The sensitivity of the fungal isolates to five antifungal drugs—posaconazole, isavuconazole, itraconazole, luliconazole, and voriconazole—was evaluated using the broth microdilution method, following the guidelines of the Clinical and Laboratory Standards Institute (CLSI, M38-A2)(HR 2008).

2.4. Preparation of Drug Solutions

All antifungal drugs were sourced from Sigma-Aldrich (USA). To prepare a final dilution of 16 µg/ml, 3.2 mg of pure drug powder was dissolved in one milliliter of DMSO to create a stock solution with a concentration of 3200 µg/ml. From this stock, 200 microliters of each drug solution were added to the first column of a 96-well plate. Serial dilutions were then performed by adding 100 microliters of RPMI 1640 medium to the wells in columns two through ten. Subsequently, 100 microliters of a fungal suspension, adjusted to a light transmission of 83-85% at a wavelength of 530 nm and a final concentration of 10^4 CFU/ml, were added to all wells except for the negative control well. Each plate included a positive control (fungal suspension without the drug) and a negative control (drug without fungal suspension) to validate the experiment's accuracy. The plates were incubated at 35°C for 48 hours, after which the results were visually assessed. The minimum inhibitory concentration (MIC) was determined as the lowest concentration at which complete inhibition of fungal growth was observed.

3. Results

The antifungal susceptibility of fungal isolates to five azole drugs was investigated, and the results are presented below in Tables 1 to 3.

Based on the geometric mean (G-Mean) values of the drugs studied, luliconazole, itraconazole, and isavuconazole exhibited the most potent antifungal effects on *Aspergillus niger*, *Aspergillus fumigatus,* and *Aspergillus flavus* isolates, respectively. Posaconazole demonstrated the weakest antifungal activity against the tested isolates.

Due to the limited number of *Penicillium* isolates (less than 10), MIC 50, MIC 90, and G-Mean could not be calculated for them. However, posaconazole (0.5-4 µg/ml) and voriconazole (0.25-2 µg/ml) showed the least antifungal effects against *Penicillium* isolates. Conversely, luliconazole (0.016-32 µg/ml) and isavuconazole (0.125-0.25 µg/ml) displayed the most potent antifungal effects.

Table 1. Antifungal Susceptibility Results of *Aspergillus fumigatus* Isolates from Ant Surfaces

Fungal Species	**MIC (µg/ml)**	**Posaconazole**	**Itraconazole**	**Voriconazole**	**Luliconazole**	**Isavuconazole**
Aspergillus fumigatus (11 isolates)	MIC 50	1	0.25	0.25	0.016	**0.25**
	MIC 90	2	1	1	0.032	**0.5**
	Minimum	0. 25	0.063	0.125	0.016	**0.032**
	Maximum	4	2	1	0.032	**0.5**
	G-Mean	0.890	0.223	0.314	0.190	**0.264**

Table 2. Antifungal Susceptibility Results of *Aspergillus niger* Isolates from Ant Surfaces

Fungal Species	**MIC (µg/ml)**	**Posaconazole**	**Itraconazole**	**Voriconazole**	**Luliconazole**	**Isavuconazole**
Aspergillus niger (15 isolates)	MIC 50	1	0.25	0.5	0.016	**0.25**
	MIC 90	2	0.5	1	0.032	**0.5**
	Minimum	0.25	0.063	0.125	0.016	**0.125**
	Maximum	2	0.5	1	0.032	**0.5**
	G-Mean	0.890	0.223	0.314	0.019	**0.265**

Table 3. Antifungal Susceptibility Results of *Aspergillus flavus* Isolates from Ant Surfaces

Fungal Species	MIC (µg/ml)	Posaconazole	Itraconazole	Voriconazole	Luliconazole	Isavuconazole
Aspergillus flavus (15 isolates)	MIC 50	1	0.25	0.5	0.016	**0.25**
	MIC 90	4	1	0.5	0.032	**0.5**
	Minimum	0.125	0.032	0.25	0.016	**0.125**
	Maximum	4	2	0.5	0.032	**0.5**
	G-Mean	1.096	0.274	0.396	0.018	**0.238**

4. Discussion

In recent years, the prevalence of hospital-acquired fungal infections has surged alongside the rising utilization of prolonged and extensive treatments across various hospital departments(Perlroth, Choi et al. 2007, Suleyman and Alangaden 2016). Concurrently, there has been an alarming increase in reports of pathogenic fungal agents resistant to antifungal drugs, posing significant threats to patient well-being and contributing to mortality rates (Orasch, Marchetti et al. 2014). Furthermore, the global surge in invasive fungal infections, coupled with the emergence and dissemination of fungal strains resistant to all known classes of antifungal agents, underscores the acute threat these organisms pose to human health (Benedict, Jackson et al. 2019).

Moreover, the transmission of these treatment-resistant fungi by mechanical carriers, including insects such as ants, is significant. In our current investigation, we assessed the drug sensitivity profiles of opportunistic fungal isolates obtained from the external surfaces of ants captured within teaching hospitals in Babol city. Specifically, we evaluated the susceptibility of *Aspergillus fumigatus*, *Aspergillus niger*, *Aspergillus flavus*, and *Penicillium* isolates to five antifungal drugs: posaconazole, isavuconazole, itraconazole, luliconazole, and voriconazole. This analysis aims to shed light on the potential role of ants as vectors for drug-resistant fungal pathogens within healthcare settings.

In the current study, all isolates of *Aspergillus* and *Penicillium* were sensitive to the azoles tested. In line with the study of Akbari et al., who evaluated the drug sensitivity of 34 isolates of *Aspergillus flavus* isolated from clinical samples, sensitivity to itraconazole (MIC range 0.5-0.125 µg/ml) and voriconazole (MIC range 0.5-1 µg/ml) were found in all samples and 14.7% of *Aspergillus* were considered as clinical isolates resistant to amphotericin B with MIC > 2 µg/ml (Akbari, Naseri et al. 2020).

In contrast, Rezaei et al. reported a case *of Aspergillus niger* resistant to itraconazole in their study on drug sensitivity of the same species isolated from the air in the special wards of Arak Hospital (Rezaei, Didehdar et al. 2021). Additionally, Nasrolahi Omran and colleagues evaluated the drug sensitivity of 11 *Aspergillus* species isolated from the ICU department of a hospital in Tonekabon (Nasrolahiomran 2018). Although the sources of fungal isolates in these previous studies differ from those in the present investigation, there remains a possibility that these isolates could be found on the body surfaces of ants.

In the study by Nasrolahi Omran et al., isolates of *Aspergillus niger* and *Aspergillus fumigatus* resistant to itraconazole were found, which contrasts with the findings of the present study where no fungal isolate was resistant to itraconazole. However, in agreement with their study, all isolates of *Aspergillus flavus* in our research were sensitive to the antifungal drugs tested. Duarte et al. investigated the activities of 5-flucytosine, amphotericin B, itraconazole, voriconazole, and terbinafine on environmental isolates of *Exophiala* strains, including four isolates from ant cuticles. They identified terbinafine as the most effective drug in inhibiting the growth of environmental *Exophiala*. Black fungi, such as *Exophiala*, are dangerous opportunists found in various environments and can cause mild to severe fungal complications in immunocompromised individuals. Therefore, the presence of these fungi on the surfaces of ants poses a risk of spore transmission in high-risk hospital environments. Controlling these mechanical carriers is crucial for preventing the spread of fungal infections(Duarte, Pagnocca et al. 2013).

In addition to the fact that ants can transmit fungal spores, they can also be a rich source of natural antifungal products due to actinobacteria in their microbiomes. Studies have shown that compounds with unique structures extracted from ant microbiomes possess antifungal properties (Liu, Xu et al. 2016, Cao, Mu et al. 2017). Notably, amphotericin B and kanamycin have been identified as effective antifungal agents derived from ants (Fiedler, Nega et al. 1996, Jiang, Piao et al. 2018). However, the role of ants as reservoirs and mechanical carriers of opportunistic hyaline and black fungi cannot be overlooked. There is a potential risk of treatment-resistant pathogens being present on ants, posing a danger to patients.

Conclusion

This highlights the importance of understanding the role of ants in the spread of fungal diseases and underscores the necessity for managing health risks associated with vectors. Careful planning and implementation of vector control measures, especially within hospital environments, are essential to mitigate these risks.

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