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Antibiotic susceptibility of *Aspergillus* Spp. isolated from contaminated food sources

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ABSTRACT

Aspergillus niger and Aspergillus flavus are frequently reported fungal pathogens known to cause infections in food materials. Fungal growth on the food materials leads to nutritional loss and food spoilage. Consumption of these food materials can cause severe complications and diseases in humans. Antifungal agents are commonly administered to check fungal growth. Knowing the importance of food materials in daily life, we have studied the antibiotic resistance pattern of A. flavus and A. niger infecting various food materials. The fungal pathogens were isolated from various sources and phenotypically confirmed. The susceptibility of the isolates was tested using Itraconazole, Ketaconazole and Amphotericin B. The isolates showed varying degrees of antibiotic resistance resulting in moderate zone of inhibitions against the antifungal agents and MIC values were in the range from 0.008 to 0.032 for A. flavus and A. niger when tested with Itraconazole and Ketaconazole. The study predicts the importance of biodiversity and environmental issues in the developmental resistance of food-borne fungal pathogens towards standard antifungal agents and warrants prophylactic measures to be taken to eradicate fungal infections of food sources for economic benefits and health issues.

Key words: *MIC, A. flavus, A. niger, Itraconazole, Ketaconazole, amphotericin B*

INTRODUCTION

In contemporary years, yeasts and molds have emerged as chief nosocomial pathogens [1-5]. These fungi are a foremost cause of morbidity and mortality in tumor [2, 6], burn and surgical patients with neonatal intensive care unit (ICU) [7]. Rapid initiation of antifungal therapy is essential for tumbling the high mortality rate in infected patients [8, 9]. The molds also have agricultural importance as a common food spoilage organism, mainly in onion, grapes and coffee [10, 11]. Certain species contaminate food and result in the production of a secondary metabolite, mycotoxins which are highly toxic to human system. A few mycotoxins can cause autoimmune infection, include allergic properties and while others trigger teratogenic, carcinogenic and mutagenic response in hosts. In nature, *A. flavus* and *A. niger* are one of the most plentiful and broadly distributed soil-borne molds. These are the fungi of genera *Aspergilli*, which are frequently retrieved from food materials. *A. niger* (black mold) belonging to section Nigri is one of the regular food-borne fungal pathogen [12]. *A. flavus* is yet another food borne fungal pathogen classified into genus *Aspergillus,* subgenus Circumdati and Section Flavi [13]. *A. flavus* is the producers of prospective hepatotoxic and carcinogenic secondary metabolic aflatoxin. Fruits and vegetables are the prime food materials consumed throughout the world. The mycotoxin producing fungal pathogens are serious threats to human health.

Hasty detection of the fungus and defensible commencement of contaminated food resources infected with *A. niger* and *A. flavus* provides plenty time to initiate antifungal therapy. Early detection also provides sufficient time to stumble upon against ochratoxins producing *A. niger* and aflatoxin producing *A. flavus*.

In this study, we focused on the isolation and identification of the food borne pathogen, followed by determination of MIC values using standard antifungal drugs like Itraconazole, Ketaconazole and Amphotericin B.

MATERIALS AND METHODS

Isolation of fungal pathogens
Fifty food-borne fungal pathogens were isolated from various food sources in non random etiquette.

Identification of fungal isolates
Lacto Phenol Cotton Blue (LPCB) mounts be prepared by mixing a drop of LPCB stain with a small amount of fungal spores on a grease free glass microscope slide and placing a cover slip on the mixture. LPCB contains 20 g of phenol crystals, 20 ml of lactic acid, 40 ml of glycerol, 0.05 g of cotton blue stain, and 20 ml of distilled water [14].

Antifungal susceptibility test
Inoculums were prepared as illustrated in standard procedure [15]. Turbidity of the inoculums was measured by spectrophotometer and adjusted to 0.5 McFarland densities. Finally the inoculums containing 1×10^6 to 5×10^6 cells/ml. This standard suspension was used for agar diffusion methods (CLSI, 2002) Ketaconazole (10 mcg), Itraconazole (10 mcg), Amphotericin B (30 mcg) were used for antifungal testing.

Minimal Inhibitory Concentration
The minimal inhibitory concentration (MIC) values were determined using ketaconazole, itraconazole and amphotericin B strip as per manufacturer's instructions (Himedia India). Fresh fungal conidia spores are diluted in normal saline at the concentration of 1×10^6 conidia/ml with 0.5 McFarland and used for the MIC assay. The fungal spores were lawn cultured in Mueller- Hinton agar plates. Hi comb MIC strips were placed on the plates and were incubated at 25ºC for 48 hours [16].

RESULTS AND DISCUSSION

Foodstuffs have vital nutrients for fungal growth, thus fungi can appear and ruin dissimilar foods and feeds. Fungal contamination of food resources are facing serious economic crisis throughout the world. For 20 years, analytical microbiology has been urbanized for forecasting the occurrence drug resistant food-borne fungal pathogens, even though these tools are devoted to bacteria [17]. Recently, the circumstances have altered and significant numbers of studies are available in the literature dealing with the extrapolative mock-up approach of fungi [18, 19]. *Aspergillus* species are ubiquitous fungi. Invasive aspergillosis is often lethal and the most common opportunistic mycosis following candidiasis [20]. Among the 200 *Aspergillus* species identified, *A. niger, A. flavus, A. fumigatus* are recurrently renowned to cause illness in immune compromised patients [21]. Certain other complications like, Aspergillomas is associated with the production of oxalic acid by the causative fungi, which elicit renal complications, a condition termed as otomycosis [22]. In this study isolation of fungal pathogen from various foods source and their susceptibility to commonly prescribed fungal antibiotics were tested for understanding the prevalence of emerging drug resistant fungal pathogens. The food-borne fungal pathogens were initially confirmed by LPCB. Pure cultures of *A. flavus* and *A. niger* isolates were used for antibiotic susceptibility testing. Itraconazole elicited moderate zone of inhibition (0.8 mm - 0.9 mm for *A. niger*) against the fungal pathogens tested, while ketaconazole showed zones in the range 0.8 to 1.1 mm for *A. niger* (Figure 1 & Table 1). Most of the fungal pathogens tested were resistant to amphotericin B. The MIC values were 0.016 mm (Itraconazole) and 0.032 mm (Ketaconazole) for *A. flavus* (Figures 2A & B) while the values were 0.016 mm (Itraconazole), 0.008 mm (ketaconazole) for *A. niger* (Figures 2C & D). Individual isolates showed varying degree of antibiotic resistance, a factor which may be influenced by biodiversity, climatic and environmental traits. In conclusion, the study was conducted to screen and identify the emerging drug resistant fungal pathogen known to infect diverse food sources. The evolution of drug resistant fungal pathogen in the current scenario requires frequent surveillance and management to unearth an alternative therapeutic medicine. The study concludes that the food-borne fungal

pathogens are marginally resistant to various antibiotics tested (Itraconazole, ketaconazole and amphotericin B) and requires frequent monitoring of food sources to avoid the serious consequences due to these fungal pathogens.

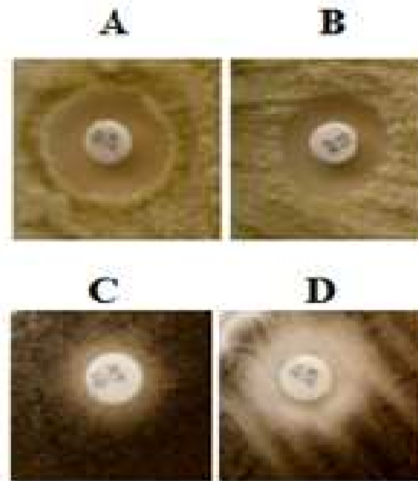
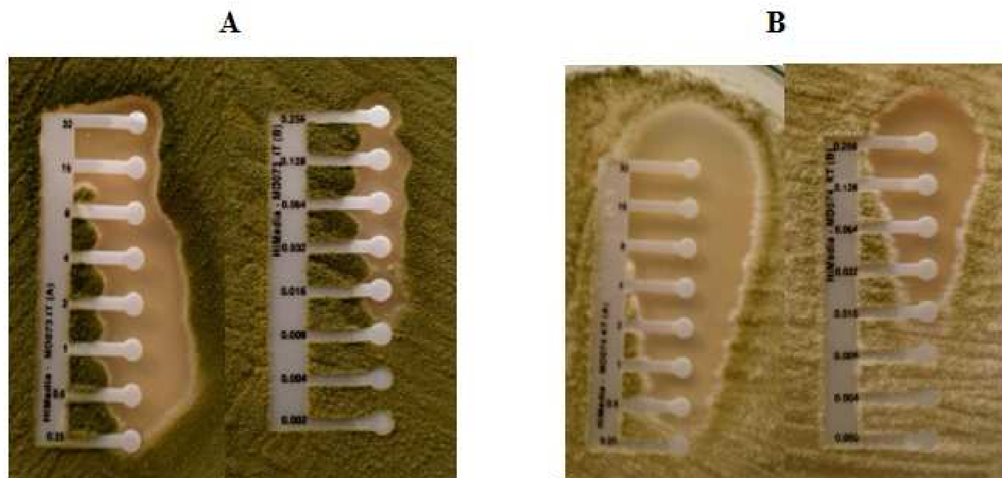


Figure 1. Antibiotic susceptibility pattern of *A. flavus* and *A. niger*. A) Sensitivity of *A. flavus* isolate 1 to ketaconazole (10 mcg), B) Sensitivity of *A. flavus* isolate 2 to ketaconazole (10 mcg). C) Sensitivity of *A. niger isolate* 1 to ketaconazole (10 mcg), D) Sensitivity of *A. niger* isolate 2 to ketaconazole (10 mcg).



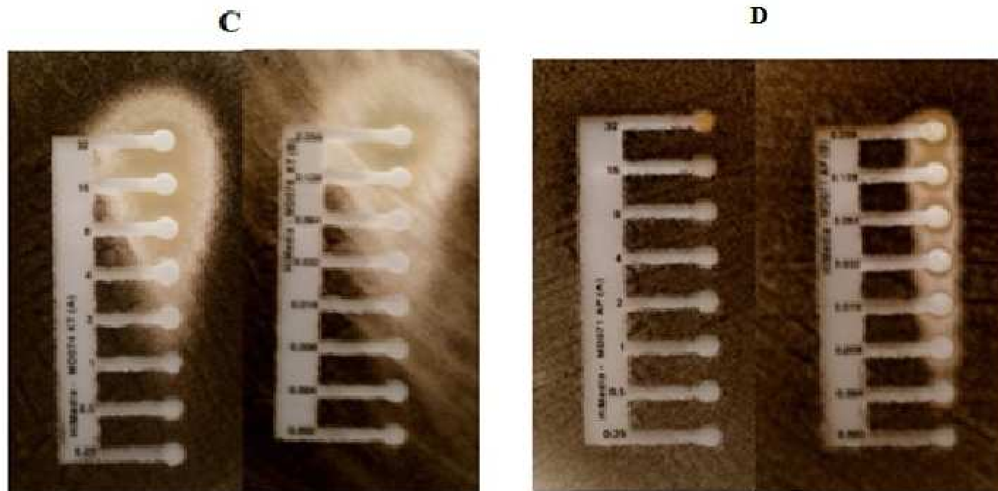


Figure 2. MIC values of Itraconazole, ketaconazole and amphotericin B. A) Hicomb Strip A & B showing the MIC value of Itraconazole against *A. flavus*. B) Hicomb Strip A & B showing the MIC value of ketaconazole against *A. flavus*. C) Hicomb Strip A &B showing the MIC value of ketaconazole against *A. niger*. D) Hicomb Strip A & B showing the MIC value of amphotericin B against *A. niger*.

Table 1. Antibiotic susceptibility pattern of *A. niger* and *A. flavus* isolated from various food sources

Food Sources	Species/Isolates	Itraconazole (10 mcg)	Ketaconazole (10 mcg)
	A.niger		
Onion	32	0.8 ± 0.00	1.1 ± 0.21
Pomegranate	4	0.9 ± 0.00	1 ± 0.00
	A. flavus		
Cake slice	8	0.83 ± 0.04	1.9 ± 0.00
Green chilly	2	0.86 ± 0.05	1.7 ± 0.00
Orange	1	0.9 ± 0.00	1.7 ± 0.00
Total	50		

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