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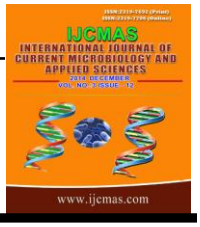

Original Research Article

Pilot study on identification of *Aspergillus* species and its antifungal drug sensitivity testing by disc diffusion method

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Keywords

Aspergillus species, disc diffusion method, antifungal drug sensitivity test,

A B S T R A C T

The aim of this study was identification of *Aspergillus* species and standardization of antifungal drug susceptibility test by disc diffusion method. 11 isolates were included in this study out of which *Aspergillus niger* (55%), *Aspergillus fumigatus* (27%) and *Aspergillus flavous* (18%). This study supports the use of Miconazole and Nystatin as a choice of drugs for treatment of Aspergillosis.

Introduction

Aspergillosis is the most common fungal infection of the respiratory tract in birds. The illness affects all species of poultry, particularly geese and ducks, as well as ornamental birds and wild birds kept in aviaries. The highest sensitivities are found in embryos and chicks, in which the course of the disease is acute. Infection in adults occurs predominantly in its chronic form. [1]

Aspergillosis is usually caused by *Aspergillus* fumigatus and less commonly by A. flavous or A. niger. [2-4]

Aspergillus species are frequent causes of invasive fungal infections in immunocompromised patients; they are also associated with allergic bronchopulmonary diseases, mycotic keratitis, otomycosis and nasal sinusitis. At least 30 *Aspergillus* species have been associated with human diseases, and A. fumigatus remains the most frequent cause of invasive Aspergillosis (IA) but recently in some institutions, *A. tereus* is becoming more common and it is less susceptible to Amphotericine B. *A. nidulans* has also been reported to be less susceptible to this drug in comparison with *A. fumigatus.* *A. ustus* as a rare cause of invasive disease has been, reported to be resistant to Amphotericine-B while remaining susceptible to itraconazole. A. ustus has also been reported to be less susceptible to voriconazole. Therefore accurate identification of species is

important for the management of IA as well as for surveillance and other epidemiological purposes. Generally identification of the *Aspergillus* species is based on the morphological characteristics of the colony and microscopic examinations.1 Although molecular methods continue to improve and become more rapidly available, microscopy and culture remain commonly used and essential tools for identification of *Aspergillus* species. [5]

Materials and Methods

This prospective study was carried out at Department of Microbiology, MGM Medical College and Hospital, Navi Mumbai, India, over a period of six months from May 2014 to October 2014. The ATCC control strain of *Aspergillus niger* (6275) was obtained from HI-Media, Lot No: 500-19-13, Ref: 0500P, Expiry date: 11-2014.

Sampling strategy

The patient's name, age, sex, details of history and clinical examination findings, antifungal treatment if any were recorded in requisition form. Samples were collected after obtaining informed consent from all patients.

KOH Microscopic examination

The samples like sputum and purulent samples were mixed in 2-3 drops of potassium hydroxide and kept digestion of purulent materials which then help to visualize the fungal elements.

Culture: The specimen were cultured on Sabouraud's dextrose agar **(**SDA), Potato dextrose agar (PDA), Czapek Dox medium by inoculation with sterile straight wire and incubated at 25 ±5°C in Biological Oxygen Demand incubator for 48 to 72 hours.

LPCB Mount preparation: Growth of Fungus were taken from culture medium (SDA, PDA, Czapek Dox medium) on clean grease free sterile microscopic slide and mixed with 1-2 drops of LPCB stain. Focused the slide first under 10x to find the area and then under 40x for identification of fungus.

Slide Culture: 10 mm square block from Potato dextrose agar (PDA) was cut using sterile coverslip or scalpel. The growth of Fungus were taken from culture medium i.e. Sabouraud's dextrose agar (SDA) and Czapek Dox medium with the help of L-shaped wire and inoculated into four corner of the block and then a sterile coverslip was kept over the block. The inoculated side culture was incubated in a wet Petri dish at 25 ±5°C in Biological Oxygen Demand incubator for 48 to 72 hours. Then the coverslip was removed and placed over a drop of Lactophenol Cotton Blue **(**LPCB) stain in clean sterile glass slide and then focused under 10x to find the area and then under 40x for identification of fungus.

Antifungal drug susceptibility testing:

Antifungal drug susceptibility testing was done according to Clinical and Laboratory Standards Institute (CLSI) guidelines. [8] Growth of all the samples was inoculated in Sabouraud's dextrose broth from solid culture media and incubated for 4-6 hours. The suspension was matched with 0.5 McFarland standards before performing antifungal drug sensitivity testing.

Result and Discussion

This prospective study was carried out at Mycology Laboratory, Department of Microbiology, MGM Medical College and Hospital Navi Mumbai. Total numbers of 11 isolates were included in the study which

was collected from environmental and patient samples.

This prospective and analytical study was done to find prevalence of Aspergillosis in a tertiary care hospital, Navi Mumbai and also to find antifungal drug resistance pattern.

In our study, total 11 isolates were included for species characterization in which *Aspergillus niger* was found 6 (55%), *Aspergillus fumigatus* was 3 (27%) and *Aspergillus flavous* was 2 (18%).

However a study was conducted by K Diba et al. [5] from Tehran, Iran, they found *A. flavus* (55%), *A. niger* (31.7%) and *A. fumigatus* (8.7%).

However another study was conducted by Immaculata Xess et al.[6] *A. flavus* (46.93%) was the most common isolate, followed by *A. fumigatus* (37.72%) and *A. niger* (15.35%).

In our study antifungal drugs sensitivity *Aspergillus niger* showed maximum sensitive to Miconazole (MIC) i.e. 100%, Nystatin (NS) i.e. 100%, Amphotericin-B (AP) i.e. 50%, and minimal sensitive to Ketoconazole (KT) i.e. 33%, Itrconazole (IT) i.e. 17%, Fluconazole (FLU) i.e. 0%.

Tokarzewski S et al. [1] studied that *A. niger* exhibited high susceptibility to enilconazole, terbinafine, voriconazole, tioconazole and ketoconazole, low susceptibility to clotrimazole, miconazole and nystatin, and resistance to amphotericin B, itraconazole, pimaricin, fluconazole and 5-fluorocytosine.

In our study antifungal drugs sensitivity *Aspergillus fumigatus* showed maximum sensitive to Miconazole (MIC) i.e. 100%, Nystatin (NS) i.e. 100% and minimal sensitive to Ketoconazole (KT) 33%, Itrconazole (IT) i.e. 0%, Fluconazole (FLU) i.e. 0% and Amphotericin-B (AP) i.e. 0%.

Figure.1 Showing *Aspergillus niger* in LPCB mount

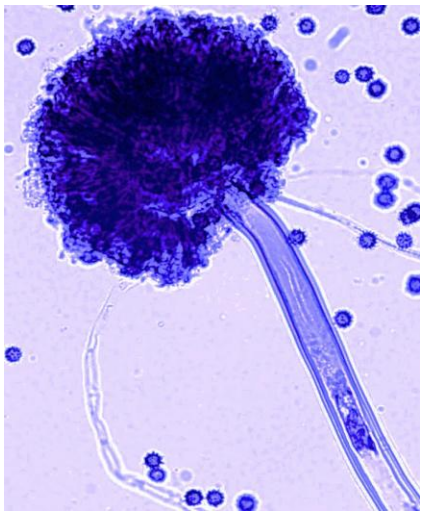


Figure.2 Showing *Aspergillus fumigatus* in LPCB mount

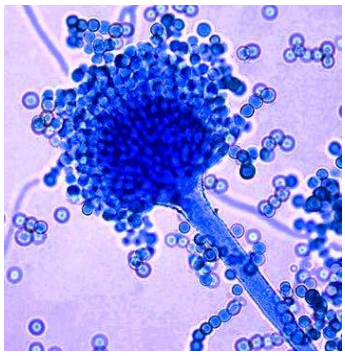


Figure.3 Showing *Aspergillus flavous* in LPCB mount

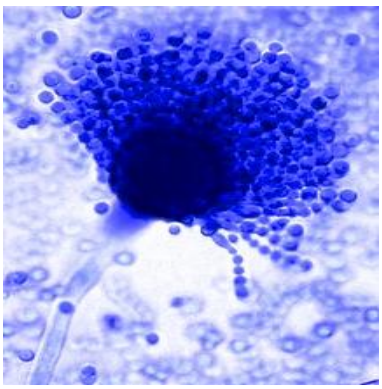


Figure.4 Showing *Aspergillus niger* growth on Czapek Dox agar plate

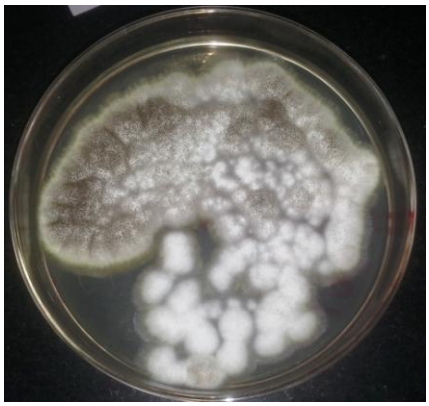
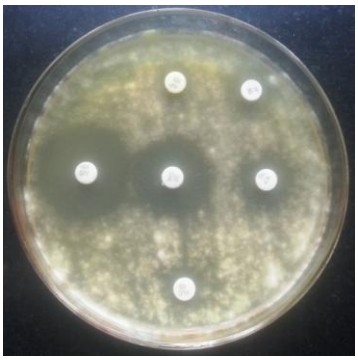
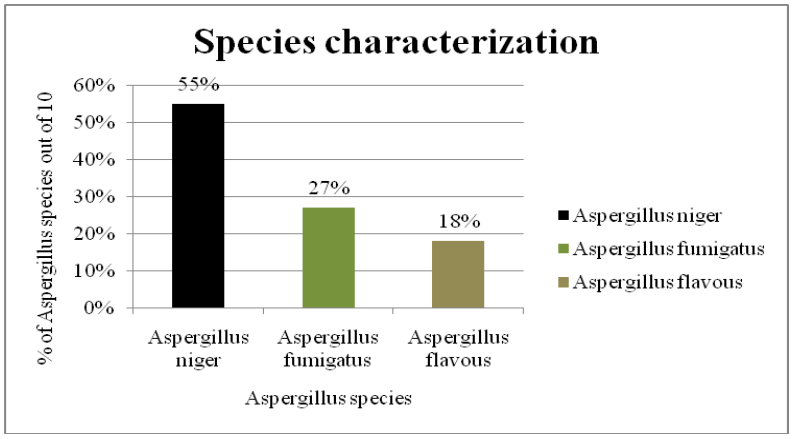
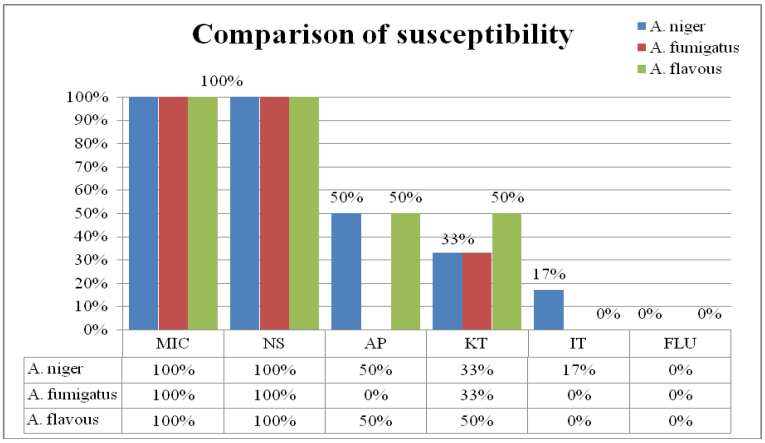


Figure.5 Showing *Aspergillus niger* growth on Potato dextrose agar plate

Figure.6 Showing antifungal drug sensitivity test

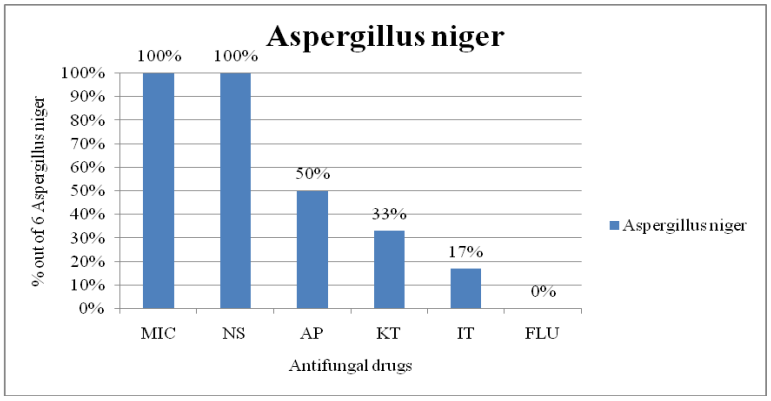


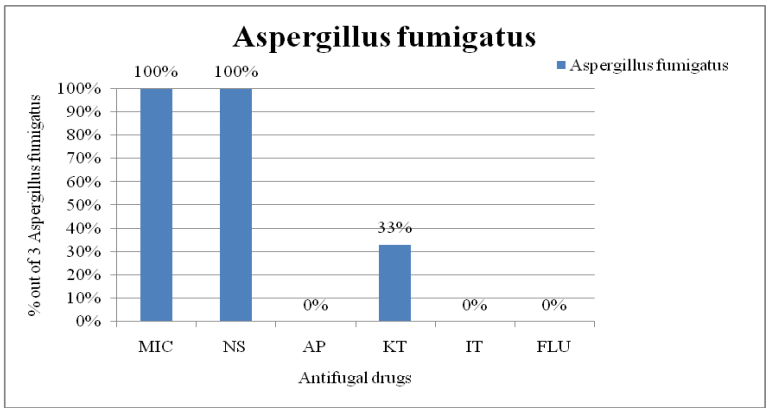


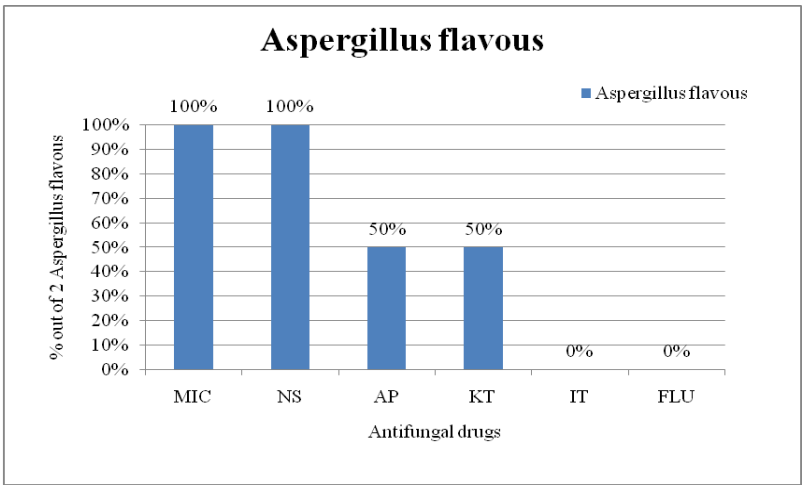


	MIC	NS	AP	KT	IT	FLU
A. niger	100%	100%	50%	33%	17%	0%
A. fumigatus	100%	100%	0%	33%	0%	0%
A. flavous	100%	100%	50%	50%	0%	0%

Abbreviations: MIC: Miconazole, NS: Nystatine, AP: Amphoterecin-B, KT: Ketoconazole, IT: Itraconazole, FLU: Fluconazole.







In our study antifungal drugs sensitivity *Aspergillus flavous* showed maximum sensitive to Miconazole (MIC) i.e. 100%, Nystatin (NS) i.e. 100%, Amphotericin-B (AP) i.e. 50%, and minimal sensitive to Ketoconazole (KT) i.e. 5%, Itrconazole (IT) i.e. 0%,Fluconazole (FLU) i.e. 0%. Ravi Kumar et al.[7] studied that the maximum sensitivity was to 5 flucytosine i.e. 100%, Ketoconazole i.e. 100%, Itraconazole i.e. 100% and Amphotericin B i.e. 96% whereas resistant to Fluconazole i.e. 0%.

We concluded that our study showed that the *Aspergillus niger* was the major fungal isolates, followed by *Aspergillus fumigatus* and *Aspergillus flavous*. The anifungal drug susceptibility test showed maximum sensitive to Miconazole and Nystatin and minimal sensitive to Amphoterecin-B where as the resistant to Itraconazole, Fluconazole and Ketoconazole. This study supports the use of Miconazole and Nystatin as a choice of drugs for treatment of Aspergillosis.

References

1. Tokarzewski S, Ziółkowska G, Nowakiewicz A. Susceptibility testing of *Aspergillus niger* strains isolated from poultry to antifungal drugs--a comparative study of the disk diffusion, broth microdilution (M 38-A) and Etest methods. *Pol J Vet Sci.* 2012;15(1):125-33.
2. Akan M, Haziroglu R, Ilhan Z, Sareyyupoglu B, Tunca R (2002) A case of aspergillosis in a broiler breeder flock. *Avian Dis* 46: 497-501.
3. Martin MP, Bouck KP, Helm J, Dykstra MJ, Wages DP, Barnes HJ (2007) Disseminated *Aspergillus flavus* infection in broiler breeder pullets. *Avian Dis* 51: 626-631.
4. Khosravi AR, Shokri H, Ziglari T, Naeini AR, Mousavi Z, Hashemi H (2008) Outbreak of severe disseminated aspergillosis in a flock of ostrich (Struthio camelus). *Mycoses* 51: 557-559.
5. K Diba, Kordbacheh P, Mirhendi SH, S Rezaie, M Mahmoudi. Identification of *Aspergillus* species using morphological characteristics. *Pak J Med Sci* 2007; 23(6): 867-872.
6. Immaculata Xess, Srujana Mohanty, Neena Jain, Uma Banerjee. Prevalence of *Aspergillus* species in clinical samples isolated in an Indian tertiary care hospital. *Indian J Med Sci* 2004; 58(12): 513-520.

7. Ravi Kumar, Sandeep Kumar Shrivastava, Arunaaloke Chakraborti. Comparison of Broth Dilution and Disc Diffusion Method for the Antifungal Susceptibility Testing of *Aspergillus flavus. Am. J. Biomed. Sci.* 2010, 2(3), 202-208; doi: 10.5099/aj100300202
8. Clinical and Laboratory Standards Institute. 2004. Method for antifungal disk diffusion susceptibility testing of yeasts. Approved guideline. Document M44-A. Clinical and Laboratory Standards Institute, Wayne, PA.