

Comparison of different methods for detection of *Aspergillus* infection and identification of *Aspergillus* to species level

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Introduction

Aspergillus species that cause aspergillosis are globally spread in nature without geographical exceptions. The species of these fungi were isolated from water, air, soil and food even inside and outside buildings, in highlands and lowlands, in caves and closed or open spaces. These fungi are saprobes which act as decomposers by absorbing nutrients from dead organic matters. Recently, a remarkable increase in aspergillosis is observed that could be due to increased diseased cases, which resulted from lack of immunity. The latest data indicate that aspergillosis is increasing in intensive care unit patients or even in patients with normal immunity. Additionally, it is considered as the main reason of mortality in children with Acquired Immune Deficiency Syndrome (AIDS) [1,2]. *Aspergillus fumigatus* is considered as the major cause of invasive aspergillosis, which is responsible for more than 90% of the cases. However, there would be other four species of *Aspergillus* including *A. flavus*, *A. terreus*, *A. niger*, and *A. nidulans*. [3,4].

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ABSTRACT

Objective: *Aspergillus* species are saprophytic, thermo-tolerant fungi that are ubiquitous in the air and environment. The incidences of *Aspergillus* infections is on the rise due to increased longevity, increase in lung diseases due to pollution, improved survival from other diseases like AIDS and organ transplants. The aim of the study was to compare the sensitivity of different methods for the detection of *Aspergillus* infection.

Methods: Total 200 samples were included in this study out of which positivity by KOH light microscopy was 20/180 (10%), Calcofluor white fluorescence microscopy 34/166 (17%) and by culture 39/161 (19.50%).

Results: Comparison between KOH and Culture was P value (0.007) < 0.05, Highly Significant, KOH Vs. Fluorescent P value (0.041) < 0.05, Significant and Fluorescent Vs. Culture P value > 0.5, Not Significant. Speciation showed maximum prevalence of *Aspergillus niger* followed by *Aspergillus fumigatus*, *Aspergillus flavus*, *Aspergillus brasiliensis* and *Aspergillus terreus*.

Conclusions: Comparison of these methods showed an improved detection of *Aspergillus* by Fluorescence Microscopy and culture as compared to KOH.

KEY WORDS: Calcofluor white
Fluorescence Microscope
Aspergillus
KOH

Typing of clinical and ambient isolates could help to provide hints about environmental sources of strains causing outbreaks as well as to provide guidelines for patient management. For epidemiologic studies, the subspecies characterization of isolates is as important as the species-level identification. *A. fumigatus* differentiation by phenotypic methods has a low discriminatory power and is difficult to standardize. [5,6].

It is necessary to diagnose the infection with some laboratory evidences before treating them with antifungal drugs, whose duration of treatment is long and may have some serious side effects. Currently available routine laboratory

methods are direct microscopy with potassium hydroxide mount (KOH) and mycological culture. These traditional methods give inconsistent results, and the results may be altered depending on the method of collection and preparation of specimen [7].

Materials and methods

This prospective and analytical study was carried out over a period of 12 months with effect from January 2014 to December 2014 at the Department of Microbiology and Central Clinical Laboratory, MGM Medical College and Hospital, Kamothe, Navi Mumbai.

Number of samples: 200 samples were taken for this study.

Study group: 200 samples taken from: 200 Patient attending IPD & OPD, M.G.M. Medical College and hospital, Navi Mumbai.

All chronic purulent exudates were studied for bacterial and fungal growth. Specimens which do not show any pus cells were excluded from further studies.

Ethical Clearance:

The study was cleared by institutional ethics committee of MGM Institute of Health Sciences, Navi Mumbai and written consent from the patients was taken prior to collection of samples.

Collection of samples

Various clinical samples like sputum, BAL, paranasal sinuses aspirates, eye swab, ear swab, blood and pus samples were collected in a sterile container by taking all aseptic precautions and properly labelled containers. ATCC control strain of *Aspergillus oryzae*, *Aspergillus niger* and *Aspergillus brasiliensis* was obtained from Microbiologics Inc, USA. Fluorescence Microscope (Nikon, USA, Sr.No:MBA92010)

Statistical analysis

Chi Square test, Fischer's (F) test and t test was used for testing the hypothesis.

Results

In our study, positivity of fungal elements in the KOH mount was 10% in 200 samples studied. Samples were of different types. This part of the study was carried out

(1) To compare 3 diagnostic procedures- KOH light microscopic examination, Calcofluor white fluorescent stain and culture. (2) Speciation of *Aspergillus* isolates.

Culture is universally recognised as gold standard for isolation and identification of any organism. However, it is time consuming and laborious. Hence other tests are necessary for screening purposes which will give provisional results pending culture report.

Thus KOH mount and Calcofluor White Fluorescent stain are used for screening purpose only. Considering culture as 100% sensitive and specific method, KOH and fluorescent microscopic exams were compared with these test procedures. 200 clinical samples were examined by three methods. Results are shown in Table 1 & Figure 1.

Table 1. Showing comparison of various methods for detection of Aspergillosis in patient samples.

Methods	Positive	Negative	Percentages (n=200)
KOH Light Microscopy	20	180	10%
Calcofluor White Fluorescence Microscopy	34	166	17%
Culture	39	161	19.50%

Table 2. Showing species wise distribution of *Aspergillus* in patient samples.

Species	No. of isolates	Percentages (n=39)
<i>A. niger</i>	18	46.15%
<i>A. fumigatus</i>	9	23.08%
<i>A. flavus</i>	7	17.95%
<i>A. brasiliensis</i>	3	7.69%
<i>A. terreus</i>	2	5.13%
Total	39	100%

Chi-Square = 16.2, df=2, P value <0.005, Significant.

Positivity for these 3 methods was as follows KOH examination 20/200 (11.11%), Calcofluor White Fluorescent microscopy 34/200 (17%) and culture 39/200 (19.5%).

1. KOH Vs. Culture: Chi-Square = 7.18, df=1, P value (0.007) <0.05, Highly Significant.

2. KOH Vs. Fluorescent: Chi-Square = 4.20, df=1, P value (0.041) < 0.05, Significant.

3. Fluorescent Vs. Culture: Chi-Square = 0.419, df=1, P value >0.5, Not Significant.

Comparison of statistical analysis shows that differences in positivity between KOH and culture is highly significant [Chi-Square = 7.18, df=1, P value (0.007) <0.05].

Figure 1. Showing sensitivity of different methods.

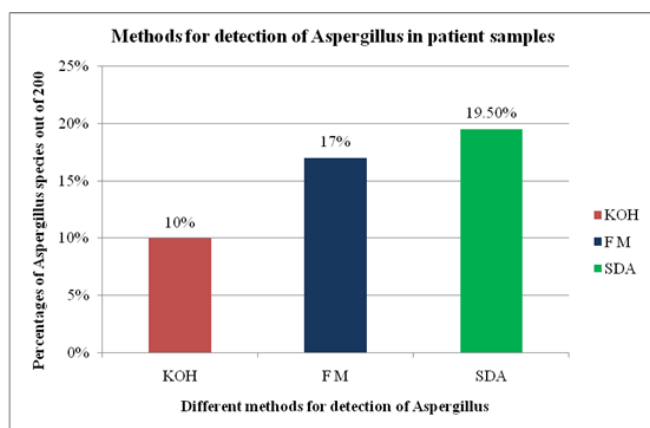
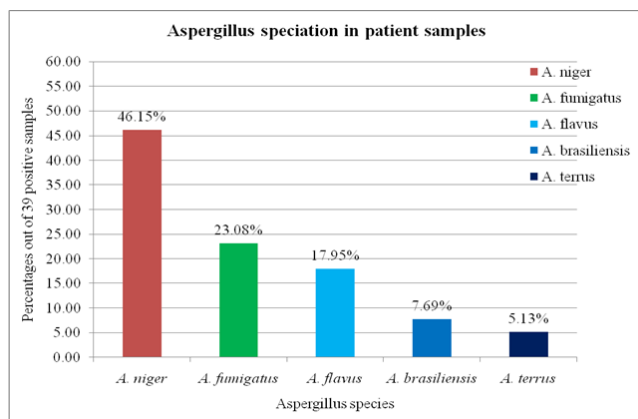


Figure 2. Showing Aspergillus speciation in patients samples



Discussion

Differences in positivity between KOH and fluorescent microscopy is also statistically significant [Chi-Square = 4.20, df=1, P value (0.041) < 0.05]. However the difference in positivity between fluorescent microscopy and culture is not statistically significant which means sensitivity and specificity of fluorescent microscopy is nearing gold standard of cultures. Comparative study of KOH, Fluorescent microscopy and culture was done by following workers and they have found greater positivity with fluorescent staining than KOH mount. Anusuya devi D et

al., Sahay R et al., Bonifaz A et al., Omaima A. El-Sayed et al [8-11].

As regards comparison of KOH examination and culture positivity Jacob JM et al., and Omaima A. El-Sayed et al. have reported higher positivity in culture than KOH examination which is similar to our finding [11,12].

However, Shenoy MM et al., Sathyanarayan MS et al., Anusuya devi D et al., Sahay R et al., Pratibha et al., Bonifaz A et al., have reported less positivity in culture than KOH examination. Here also, differences could be due to over reporting of fungal elements in KOH, as culture is gold standard for positivity [8,9,13-15]. However, prior treatment can inhibit growth in culture. Most of these studies have been done on fungal infections of eyes.

Our results: Table 2 & Figure 2.

1) *Aspergillus niger* 46.15% similar results are Sathyanarayan MS et al., Diba K et al., less prevalence reported by Anusuya devi D et al., Chander J et al. [8,14,1617].

2) *Aspergillus fumigatus* 23.08% closer results are Sathyanarayan MS et al., Anusuya devi D et al., less prevalence reported by Diba K et al., Omaima A. El-Sayed et al., more Chander J et al., [8,14,1617].

3) *Aspergillus flavus* 17.95% closer results are Sathyanarayan MS et al., Chander J et al., less prevalence reported by Anusuya devi D et al., and higher Diba K et al., [8,14,1617].

4) *Aspergillus brasiliensis* 7.69%, nil reported by others.

5) *Aspergillus terreus* 5.13% similar to Sathyanarayan MS et al. [14]

200 patients were examined for Aspergilli by KOH, Fluorescence Microscopy and culture. Comparison of these methods showed improved detection of aspergilla by Fluorescence Microscopy and culture as compared to KOH.

Speciation showed maximum prevalence of *Aspergillus niger* followed by *Aspergillus fumigatus*, *Aspergillus flavus*, *Aspergillus brasiliensis* and *Aspergillus terreus*.

Conflict of Interest

We declare that we have no conflict of interest.

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