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Aeromycological Analysis of the Operation Theater's Indoor Environment at the Rural Healthcare Center Sindewahi Using the Petriplate Exposure Method

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Introduction

Sindewahi Rural Healthcare Centre is located in India at latitude 20.283220 and longitude 79.6667600. The three seasons of this region's climate—summer, winter, and rainy season—are determined by factors including temperature, humidity, and rainfall. From February to May, the summer season begins, with highs of 45 to 47 degrees Celsius.[1] Rainfall often falls between June and September, while winter begins in October and lasts until January, with lows of 8 to 9 degrees Celsius. Operation Theater (O.T.) air samples were taken in order to analyze the indoor aeromycoflora of the rural health care center. Regular air sampling was conducted twice a month for the two years in a row, from August 2014 to July 2016.[2] The rural health care center's operation theater is exclusively used for family planning procedures, especially during the months of December and January.[3] The O.T. was sanitized using a fumigation method prior to the procedure. Formaldehyde fumigation is the procedure used for sterilizing. Using the petriplate exposure method, air samples were routinely taken from the O.T. of the rural health care center Sindewahi.[4]

Fig. Latitude and Longitude Position of Rural Healthcare Centre Sindewahi

Results and Discussion

Petriplate exposure method

Indoor aeromycoflora in Rural Healthcare Centre Sindewahi

The Petripalte exposure method, the oldest technique for gathering and identifying airborne fungal spores, was used to collect fungal aeromycospora from O.T. at the Rural Healthcare Center Sindewahi. Agar media-

containing petriplates are incubated for 4–7 days at room temperature to determine the amount of fungus spores that fall on their surface.[5] Colonies are enumerated and identified up to genera and species after five to seven days. A total of 71 fungal species from 20 distinct fungal genera were collected using the petriplate exposure approach.[6] In addition to these white sterile mycelia, two years of research (August 2014–July 2016) also isolated black and orange sterile mycelia. Two of the 20 genera that have been identified are members of the *Phycomycotina: Rhizopus* (4 species) and *Mucor* (7 species).[7] The remaining 15 fungal genera, which include *Aspergillus* (12 fungal species), *Penicillium* (7 fungal species), *Alternaria, Cladosporium, Curvularia,* and *Trichothecium* (four fungal species each), Fusarium, Candida, *Phoma,* and *Torula* (three fungal species each), *Cercospora*, *Drechlera, Helminthosporium, Nigrospora,* and T*richoderma* (one fungal species each), represented *Deuteromycotina. Deuteromycotina* accounted for 66.39% of the 52 fungal species in the two-year study, followed by *Phycomycotina* with 15.28% and *Ascomycotina* with 7.23% and 8 fungal species, respectively. During the study period, 11.09 percent of sterile mycelium were found in the rural healthcare facility Sindewahi. In the first year of the study, *Deuteromycotina* accounted for 69.30%, *Phycomycotina* for 14.01%, and *Ascomycotina* for 7.12%. *Deuteromycotina* accounted for 64.62%, *Phycomycotina* for 16.05%, and *Ascomycotina* for 7.29% of the second-year research. Over the course of the two-year research period, from 2014 to 2016, a total of 4678 fungal colonies were identified at the rural health care center Sindewahi. A total of 1769 fungal colonies were recovered in 2014–2015, and 2909 fungal colonies were isolated in 2015–2016. *Aspergillus* dominated at 659 colonies (14.08%) across the two years of the study, followed by *Penicillium* at 570 colonies (12.18%), *Mucor* at 458 colonies (9.79%), *Alternaria* at 432 colonies (9.23%), *Rhizopus* at 257 colonies (5.49%), *Curvularia* at 179 colonies (3.82%), and *Cercospora* at 157 colonies (3.35%). *Phoma* 139 colonies (2.97%), Epicoccum 136 colonies (2.9%), and *Fusarium* 145 colonies (3.09%) 134 colonies of *Geotrichum* (2.86%) 125 colonies of *Torula* (2.62%), 123 colonies of *Cladosporium* (2.62%), 113 colonies of *Helminthosporium* (2.41%), 110 colonies of *Trichoderma* (2.35%), 105 colonies of *Nigrospora* (2.24%), and 89 colonies of *Trichothecium* (1.90%) *Chaetomium* 68 colonies (1.47%), *Drechslera* 85 colonies (1.81%), and Candida 75 colonies (1.6%). During the two-year experiment, 366 colonies of White sterile mycelia (7.82%), 145 colonies of Black sterile mycelia (3.09%), and 8 colonies of Orange sterile mycelia (0.17%) were also observed.

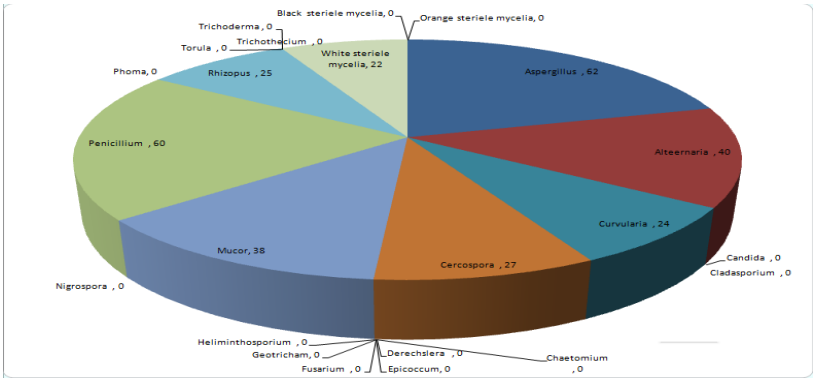
The most common species in the first year of the study were *Aspergillus* (235 colonies, 13.28%), *Penicillium* (223 colonies, 12.6%), *Mucor* (148 colonies, 8.36%), *Alternaria* (138 colonies, 7.8%), *Rhizopus* (100 colonies, 5.08%), *Curvularia* (93 colonies, 5.25%), and *Cercospora* (87 colonies, 4.91%). A two-year investigation revealed that in addition to the following: *Helminthosporium* 71 colonies (4.01%), *Geotrichum* 62 colonies (3.5%), *Fusarium* 57 colonies (3.22%), *Cladosporium* 53 colonies (2.99%), *Nigrospora* 47 colonies (2.85%), *Trichoderma* 44 colonies (2.48%), *Drechslera* 42 colonies (2.37%), *Torula, Trichothecium* 36 colonies (2.03%), *Chaetomium, Phoma* 33 colonies (1.87%), *Epicoccum, Candida* 31 colonies (1.75%), and *White sterile mycelia* 125 colonies (7%). *Aspergillus* dominated the second year of the study as well, with 424 colonies (15.19%), followed by *Penicillium* with 347 colonies (11.92%), *Mucor* with 310 colonies (10.65%), *Alternaria* with 294 colonies (10.10%), *Phoma* with 106 colonies (3.5%), *Epicoccum* with 105 colonies (3.6%), *Torula* with 89 colonies (3.05%), *Fusarium* with 88 colonies (3.02%), *Cercospora* with 70 colonies (2.4%), *Geotrichum* with 72

colonies (2.47%), *Trichoderma* with 66 colonies (2.26%), Nigrospora with 58 colonies (1.99%), Trichothecium with 53 colonies (1.82%), Candida with 44 colonies (1.51%), *Drechslera* with 43 colonies (1.47%), *Helminthosporium* with 42 colonies (1.44%), and *Chaetomium* with 35 colonies (1.2%). In addition, during the two-year study, 241 colonies of white sterile mycelia (8.28%), 109 colonies of black sterile mycelia (3.7%), and three colonies of orange sterile mycelia (0.20%) were seen.
In the first year of the study (August 2014–July 2015) Of the 1769 fungal colonies that were isolated, 308 were identified from the Operation Theater (O.T.).

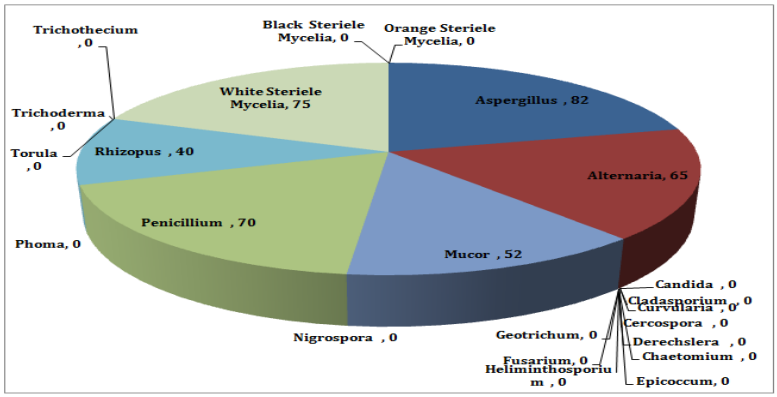
Rural Healthcare Center Sindewahi 2014-2016					
Petriplate Exposure Method /Settle Plate Method					
Total No of Fungal Colonies & Their Percentage Contribution in Operation Theatre (O.T.)					
Year August 2014- July 2016					
Sr. No	Month	Total No. of Colonies			%
		Operation Theatre		Total	
		2014-2015	2015-2016		
1	August	18	25	43	6.213872832
		19	26	45	6.502890173
2	September	17	26	43	6.213872832
		14	24	38	5.49132948
3	October	13	21	34	4.913294798
		15	20	35	5.057803468
4	November	14	19	33	4.768786127
		16	17	33	4.768786127
5	December	17	17	34	4.913294798
		13	15	28	4.046242775
6	January	11	16	27	3.901734104
		9	13	22	3.179190751
7	February	9	11	20	2.89017341
		7	9	16	2.312138728
8	March	10	9	19	2.74566474
		8	7	15	2.167630058
9	April	7	5	12	1.734104046
		7	5	12	1.734104046
10	May	8	4	12	1.734104046
		6	5	11	1.589595376
11	June	12	14	26	3.757225434

		15	20	35	5.057803468
12	July	22	27	49	7.080924855
		21	29	50	7.225433526
Total		**308**	**384**	**692**	

In 1st year study shows that, seasonal variation also exhibited by indoor aeromycoflora. In rainy season (June to September) maximum 861(48.67%) fungal colonies were isolated followed by 638(36.06%) colonies in winter season (October to January) and minimum 270(15.26%) colonies in summer (February to May)were recorded.[8] In whole study maximum 273 fungal colonies were recorded in month of July followed by Aug., Sept., Oct., Nov., Dec., Jan.,June, Feb., Mar., Apr. and minimum 44 fungal colonies were recorded in month of May.



Total No of Fungal Colonies in O.T. Sections & % Contribution to Total Aeromycoflora in 2014-2015



Total No of Fungal Colonies in O.T. Sections & % Contribution to Total Aeromycoflora for the year 2015-2016

In 2nd year investigation (Aug2015- July 2016) total 2909 fungal colonies were isolated, out of which 384 fungal colonies were recorded from Operation Theater (O.T.).[9] In 2nd year study shows that , seasonal variation changes the concentration of fungal spores in air, in rainy season (June to September) maximum 1284 (44.13%) fungal colonies were isolated after 1069 (36.74%) colonies in winter season (October to January) and minimum 556(19.11%) colonies in summer(February to May)were recorded. In whole study maximum 382 fungal colonies were recorded in month of July followed by Aug., Sept., Oct., Nov., Dec., Jan., June Feb.,

Mar., Apr. and minimum 77 fungal colonies were recorded in month of May.[10] During 1st years (Aug. 2015-July 2016) of study , in month of April and May only a 5 genera viz. *Aspergillus ,Penicillium* ,*Alternaria,Mucor* and *Rhizopus* along with White sterile mycelia were recorded from indoor air atmosphere of O.T. of Rural healthcare centre Sindewahi. While in 2nd years of study along with these 5 genera *Curvularia* was also recorded.

Indoor aeromycoflora inOperation Theater (O.T.)

In Rural healthcare centre sindewahi the air sample were collected from O.T.By using petriplate exposure method total 39 fungal species belongs to 7 different fungal genera were isolated.[11] Beside these white sterile mycelia, was also isolated in two years (Aug2014- July 2016) of research work. In 1st year (Aug 2014- July 2015) of study out of 7 fungal genera, 2 fungal genera i.e. *Mucor* (7fungal species), and *Rhizopus* (4fungal species) represent to *Phycomycotina.* and 5 fungal genera i.e. *Aspergillus* (12 fungal species), *Penicillium*(7 fungal species) ,*Alternaria, Curvularia* (each 4 fungal species) ,*Cercospora* (1 fungal species) represent to *Deuteromycotina.* Total 692 fungal colonies were recorded in O.T. of rural health centre Sindewahi during the two year research period i.e. Aug. 2014- July 2016. In 1st year investigation (Aug2014- July 2015) total 308 fungal colonies were isolated.[12] Seasonal variation also shown by fungal spores in indoor air but in O.T. fumigation was done before and after the operation therefore at that time fungal spores were not recorded.[13] In rainy season (June to September) maximum 138(44.80%) fungal colonies were isolated followed by 108(35.06%) colonies in winter season (October to January) and minimum 62(20.2%) colonies in summer (February to May)were recorded. In whole study maximum 43 fungal colonies were recorded in month of July followed by Aug.,Sept., Nov Dec., Nov.,Oct., June, Jan. Mar., Feb., April, May and minimum 14 fungal colonies were recorded in month of May. In 2nd year investigation (Aug2015- July 2016) total 99fungal colonies were isolated.[14] In rainy season (June to September) maximum 191 (49.73%) fungal colonies were isolated ,138 (35.93%) colonies in winter season (October to January) and 55 (14.32%) colonies in summer(February to May)were recorded. In whole study maximum 56 fungal colonies were recorded in month of July followed by in Aug., Sept., Oct., Nov., Dec. June, Jan., Feb., March, Apr. and minimum 12 fungal colonies were recorded in month of May. In two years study *Deuteromycotina*(53.25 %) were dominant with 28 fungal species, followed by *Phycomycotina* with 22.83% (11fungal species).[15] Besides these White sterile mycelia was 15.46 % were recorded in rural healthcare centre Sindewahi during a research period. No black and orange sterile mycelia were observed.[8-14] In 1st year study *Deuteromycotina* were noted as 50%, and *Phycomycotina* were noted as 21.42%. In 2nd year study *Deutoromycotina* were 56.51 % and *Phycomycotina* were 23.95%. Throughout the two years study *Aspergillus* were dominant having 144 colonies (20.8%) followed by *Penicillium* 130 colonies (18.78%), Mucor93 colonies, (13.43%) ,*Alternaria* 105 colonies (15.17 %),*Rhizopus* 11 colonies (6.04 %), *Curvularia* , *Cercospora* 24 colonies (3.46%).Other than these White sterile mycelia 107colonies (15.46%) were recorded in two years of investigation In 1st year investigation (Aug2014- July 2015) ,*Aspergillus* were dominant having 62 colonies (20.12 %) followed by *Penicillium* 60 colonies (19.48 %), Mucor41 colonies (13.33 %), *Alternaria* 40 colonies (12.98%),*Rhizopus* 25 colonies (8.1%),*Curvularia* , *Cercospora*(7.79%).Other than these White sterile mycelia32 colonies (10.38%) were noted. In 2nd year investigation (Aug2015- July 2016), *Aspergillus* were dominant having 35 colonies (35.35%) followed by *Penicillium* 21 colonies (21.21%), *Mucor*12 colonies (12.12%) ,*Alternaria* 11 colonies (11.11%),*Curvularia,* 9

colonies (9.09 %),*Rhizopus* 7 colonies (7.07%). Other than these White sterile mycelia9 colonies (9.09%) was noted.

Discussion

Using the petriplate exposure method with a Hi Media Air Sampler Mark II, an aeromycological survey was carried out every two weeks for two years (Aug 2014-July 2016) from the indoor environment of the rural health care center Sindewahi, Operation Theater Chandrapur District. Tilak (1982) was recommended.[16]According to a two-year study conducted at the rural health care center Sindewahi between August 2014 and July 2016, 71 fungal species from 20 different fungal genera were recovered. In addition, sterile mycelia that were white, black, and orange were isolated.[17] where *Deuteromycotina* accounted for 66.39% of the fungal species, followed by *Phycomycotina* with 15.28% and *Ascomycotina* with 7.23% and 8 fungal species, respectively. 11.09 percent sterile mycelium was found (table No. 4.1 & 4.2).According to the findings of Kotwal S.G. and Gosavi S.V. (2010), *Deuteromycotina* predominated in the indoor environment of the healthcare facility, followed by *Phycomycotina* and *Ascomycotina./*18]

In terms of colonies, *Aspergillus* dominated with 659 (14.08%), followed by *Penicillium* with 570 (12.18%), *Mucor* with 458 (9.79%), *Alternaria* with 432 (9.23%), *Rhizopus* with 257 (5.49%), *Curvularia* with 179 (3.82%), and *Cercospora* with 157 (3.35%). *Phoma* 139 colonies (2.97%), *Epicoccum* 136 colonies (2.9%), and *Fusa*rium 145 colonies (3.09%) 134 colonies of *Geotrichum* (2.86%) 125 colonies of *Torula* (2.62%), 123 colonies of *Cladosporium* (2.62%), 113 colonies of *Helminthosporium* (2.41%), 110 colonies of *Trichoderma* (2.35%), 105 colonies of *Nigrospora* (2.24%), and 89 colonies of *Trichothecium* (1.90%) *Chaetomium* 68 colonies (1.47%), *Drechslera* 85 colonies (1.81%), and Candida 75 colonies (1.6%). In addition, during the two-year examination, 366 colonies of white sterile mycelia (7.82%), 145 colonies of black sterile mycelia (3.09%), and 8 colonies of orange sterile mycelia (0.17%) were observed. The most prevalent aeroallergens in indoor air are *Aspergillus* and Penicillium spores.The fundamental element of the air mycoflora was *Aspergillus.* Harzara and Majumdar (2005).

Using the petriplate exposure method, a total of 1769 fungal colonies were isolated during the first year of the study (August 2014–July 2015), with 308 of those colonies being identified from the Operation Theater (O.T.).

A total of 2909 fungal colonies were isolated during the second year of the study (August 2015–July 2016), with 384 of those colonies being identified from the Operation Theater (O.T.).[19] The results of a study carried out at a university hospital in Rotterdam, Netherlands, were connected with the current findings. In contrast to wards and operating rooms, the concentration of fungal spores was higher in indoor open area sections.by Leenders, A.C., and others (1999) [20]

Seasonal Variation

The table shows the monthly contribution of all the colonies counted in three distinct areas of the rural health care center Sindewahi for the years 2014–2015 and 2015–2016, respectively.[21] During the first year of the study, the number of colonies in each month ranged from 44 to 273, and during the second year, it ranged from 77 to 382. In 2014-2015, the months of July (273) and August (242) had the highest colony counts.[22] During the following year of the study, the highest number of colonies was recorded in July (382) and August (350).[21]

Between 2.64% and 13.13% in both years, and between 2.48% and 15.43% on average, the monthly colony counts' percentage contribution to the total colony counts fluctuated. July (15.43%) had the highest colony counts in the first year, followed by August (13.68%), September (12.88%), October (11.02%), November (99.83%), December (8.3%), January (6.89%), June (6.67%), February (5.25%), March (4.4%), and April (3.1%), with May (2.48%) having the lowest colony counts.[23]The months with the highest colony counts for the second year of the study (2015–2016) were July (13.13%), August (12.03%), and September (11.44%). November (9.62%), December (8.62%), January (7.8%), and October (10.69%) The lowest percentage was in May (2.64%), followed by June (7.52%), February (6.66%), March (5.77%), and April (4.02%).

When the study's two years were compared season-wise using the exposure petriplate method—that is, summer, rainy, and winter—the highest number of colonies was found during the rainy season as opposed to the winter and summer seasons.[24] Rainy season prevailed in 2014–2015, contributing 48.67 percent with 861 colony counts, whilst in 2015–2016, it contributed 44.13% with 1284 colony counts.[25] The winter (October to January) followed the dominance of the rainy season. In 2014–2015, 638 colonies were found, with a frequency of 36.05%.[26] With a colony count of 1069 in the second year of the study, or 2015–2016, that was 36.74%. Summertime makes up a smaller portion. It was 270 (15.26%) in 2014–2015 and 556 (19.11%) in 2015–2016.

Because of the ideal climate, which includes considerable rainfall with rising humidity and moderate temperatures that are conducive to fungal growth and development, the highest number of fungal colonies were seen during the rainy season.[27] Additionally, fungi's growth and development are stopped by high temperatures and decreasing humidity (dry conditions).[28] Fungal spore concentration and metrological parameter were found to be correlated by Sudharsanam S. and Srikanth P. (2008), Pandey (1992), and Tilak and Vishwe (1975).[29-30]

Conclusion

The Rural Healthcare Centre Sindewahi in India, a facility for family planning operations, has been studying its indoor aeromycoflora using the Petripalte exposure method. The study found that 71 fungal species, including white, black, and orange sterile mycelia, were recovered from the indoor environment. *Deuteromycotina* was the dominant group, with 66.39% of the 52 fungal species. The study also recorded white, black, and orange sterile mycelia. In the second year, Aspergillus was the dominant fungal species, with 424 colonies (15.19%). Seasonal variation in indoor aeromycoflora was observed, with maximum colonies in the rainy season and minimum in the summer. The study found that *Deuteromycotina* was the most abundant aeroallergen in the indoor air, with 659 colonies (14.08%). The maximum colony counts were observed in the rainy season, with the rainy season dominated with 861 colony counts in 2014-2015 and 638 in 2015-2016.

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