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Airborne Fungal Analysis of Different Units of Hospitals Present in Bhimber, Azad Kashmir, Pakistan

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Abstract

Airborne indoor and outdoor environmental fungi were assessed from six different government and private hospitals in Bhimber, Azad Kashmir. Culture plate technique was used to determined fungal contamination. This was carried out to assess the level of airborne pathogens and to establish standards for further reference. The indoor places included laboratories, bedrooms, X-ray room, doctor rooms and emergency rooms. 28 different fungal pathogens were detected from both environments. Pathogens were generally more dominant in an indoor environment of hospitals. It was also observed that private hospitals have low concentration of pathogens as compared to government hospitals, especially indoor air of the government hospital was more contaminated than that of the private hospital in all units. These variations were observed due to better hygienic conditions of private hospitals. Maximum fungal pathogens were detected in the bed-rooms, while minimum pathogens percentages were detected in the X-ray rooms and emergency rooms. The dominant pathogens were Aspergillus niger (62.9%), *Fusarium oxysporum* (60.8%) and *Penicillium chrysogenum* (57.6%) in indoor environment of hospitals while dominant species in outdoor environment of hospitals were *Ulocladium* spp. (56.8%), *Alternaria alternata* (55.3%) and *Cladosporium herbarum* (54.9%), respectively. The time of visit showed higher microbial rates in government hospital, while the private hospital was low microbial rate. The incidence and severity of the identified fungal species, determination techniques and performance results were also summarized and discussed. We concluded that the indoor air quality of hospitals in Bhimber city, especially the government hospital, needs more care and surveillance and should be given priority in private hospitals.

Keywords: Fungal pathogens, Air fungi, Hospital pollution, Indoor and Outdoor environment

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1. Introduction

Fungi are present in different environments but its major form of distribution and dispersal is air which indicates their effects on humans and other organisms. Aero-mycologist are mainly concerned with dispersion and deposition of spores, interaction of different spores within the prevailing environment, pollution, environmental factors and their impact on different organism (Hussain *et al*., 2013). Healthcare facilities i.e., hospitals are complex places which require clean environments for comfort of patients, better care and control of harmful pathogens (Paradee *et al*., 2008). However, hospitals are the places of conjunction between staff and patients included hospital visitors. The hospital environment (air) is a source of pathogenic microorganisms to hospital staff and visitors (Obbard and Fang, 2003). In this respect, major source of airborne pathogens especially fungi inside the hospitals are the infected patients. The more dominant way of spreading airborne pathogens inner environment of hospitals are sneezing or coughing of patients. The pathogens float in the air over considerable distances and for a long time inside the hospitals as compare to outside environment (Emmerson, 1995). Hospitals aerosols have different pathogens i.e., bacteria, viruses and fungal spores (Gillette, 2000).

Some unfavourable environmental conditions i.e., UV-radiation, dryness and low temperature, which may reduce the spreading risk of growing pathogens inside the hospitals. However some fungal spores and viruses can live for longer periods of time and affects the life of visitors. In recent years, the prevalence of airborne fungal spores has increased because many new hospitals are sealed for temperature control (Matar *et al*., 2005). Various fungal spores with high concentration were isolated in the

indoor as compared to outdoor samples. Spores from the *Penicillium* and *Aspergillus* groups were the most dominant in indoors as comparison to prevalence of outdoor spores. Ascospores and basidiospores were the dominant spore type's outdoor environments. The prevalence of other spores (*Cladosporium* and *Curvularia*) was similar in both environments. Moisture-loving fungi (*Chaetomium, Stachybotrys* and *Ulocladium* species) were usually absent in indoor and outdoor air samples. Although, climatic variation, airo-spora and levels in central Florida houses are similar to those found in other geographical locations (Codina *et al*., 2008).

Previously, seven different fungal species of six genera were identified from air of Bagsar fort from Bhimber Azad Kashmir. *Cladosporium* spp. was found to be the most dominant fungal species followed by *Aspergillus niger*, *Alternaria solani*, *Alternaria alternata, Curvularia* spp., *Penicillium* spp. and *Fusarium* spp. (Hussain *et al*., 2013). Fungi are potential source of causing aeroallergens which are also creating different serious diseases to man and livestock such as aspergillosis, mycosis and irritation. *Aspergillus* infections, primarily cause pulmonary aspergillosis is observed more in hospitals (Goodley *et al*., 1994). The vicinity of ward areas can produce large aerosols of infective particles especially fungal spores due to poor hygienic conditions. It is investigated that climatic conditions did not influence spore counts of *Aspergillus fumigatus* in the air. Several mycologists have surveyed that some spores of fungi (mold spores) may enter the hospital through windows or ventilation holes (Gerson *et al*., 1994; McCarthy *et al*., 2000). In previous study, observed that the outdoor air is a potential threat to public health center because of prevalence of more pathogenic and allergic airborne fungal spores. These are the major sources of contamination of indoor environments in different places i.e., offices, homes, hospitals etc. (Masoomeh *et al*., 2014). There is no single method for sampling airborne fungi. Different methods are used for the identification and counting of microbes in air. Therefore, it is not an easy task (Dharan and Pittet, 2002; Shintani *et al*., 2004).

Control of airborne fungal pathogens in hospitals is not only important for the safety of the patient, but it is also important for hospital staff. So, different controlling procedures can reduce the risk of infection (Montz and Edward, 2000). So, there is a demand to eliminate/reduce airborne fungi and their spreading mechanism. It is very important to identify these hazardous facilities and require to maintain a clean environment in hospitals (Shintani *et al*., 2004; Li and Hou, 2003; Sudharsanam *et al*., 2008). Hospital air-mycoflora must be investigated regularly. The scientist investigated the air-fungi in hospitals for different purposes i.e., epidemiology, severity of pathogens, research purpose and management purposes (Groschel, 1980). Obbard and Fang, (2003) reported that density is a major factor affecting on prevalence of airborne fungi. Temperature and humidity is also important depending on the particular place within the hospital. This study was conducted to gain knowledge about the prevalence of airborne fungi in the indoor and outdoor environment of selected private and government hospitals in Bhimber Azad Kashmir, Pakistan. It is also suggested suitable guidelines in order to decrease spreading mechanism of fungal spores in indoor environment of hospitals.

2. Material and Methods

This study was conducted during the year 2013-2014. Culture plate technique was used for investigation of aero-mycoflora of different hospitals.

2.1. Culture Plate Technique

Plates having sterile PDA media with 0.1% streptomycin were exposed for outdoor sampling in different intervals. The petri plates used for indoor sampling having already sterilised PDA media with the addition of 0.05% Rose Bengal. The exposure time was 10 minutes. The petri plates were exposed at different places inside the hospitals and also exposed randomly outer environments. A total of 144 plates were exposed, 90 petri dishes indoors and 54 petri dishes outside. Unexposed plates at each location served as controls followed by Hameed *et al*. (2009), Hussain *et al*. (2013) and Estelle and Leon (1977).

2.2. Inoculation of Petri Plates

After exposure time, the petri dishes were covered, labeled, sealed and transported to the laboratory of MUST University Mirpur for incubation at room temperature. They were incubated for five to seven days or until fungal colonies appeared (Olugbue *et al*., 2013). The colonies that unable to sporulate on this primary PDA media were further sub-cultured onto another water agar (WA), Sabouraud dextrose agar (SDA) and Nutrient agar (NA) media for further better growth followed by Choi *et al*. (1999) and Saha *et al*. (2009).

3. Results and Discussion

Air samples from six different indoor sites and three outdoor sites of selected hospitals were taken and used for incidence, prevalence, distribution and isolation of airborne fungi on PDA plates. Two government and three private hospitals were selected

for analysis and distribution of fungal spores. A total of 27 fungal species were isolated from the selected sites of hospitals. On species is yet unidentified. The incidence of indoor and outdoor fungal species was shown in table 1. The dominant species in outdoor environment of hospitals were *Ulocladium* spp. (56.8%), *Alternaria alternata* (55.3%) and *Cladosporium herbarum* (54.9%), respectively. On the other hand the more prevalent fungal species in indoor environment of hospitals were *Aspergillus niger* (62.9%), *Fusarium oxysporum* (60.8%) and *Penicillium chrysogenum* (57.6%), respectively (Table 1). The result showed more incidences of fungi in indoor environment as compared to outdoor air sampling in the selected hospitals.

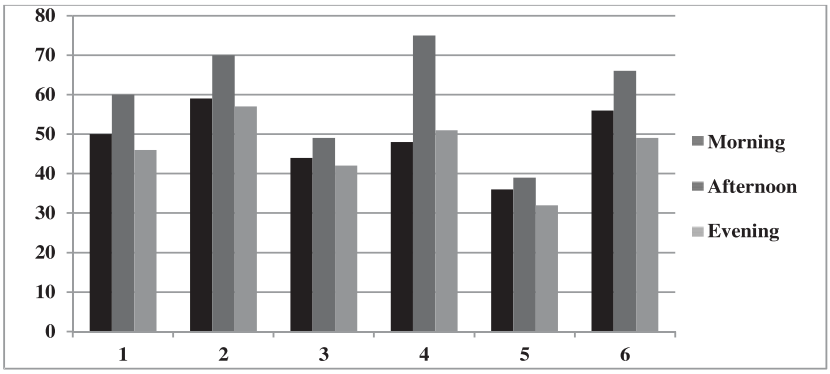

Fig. 1. Type of hospital and the time of sampling on fungi (CFU/m3) in indoor air of DHQ hospital Bhimber, Azad Kashmir

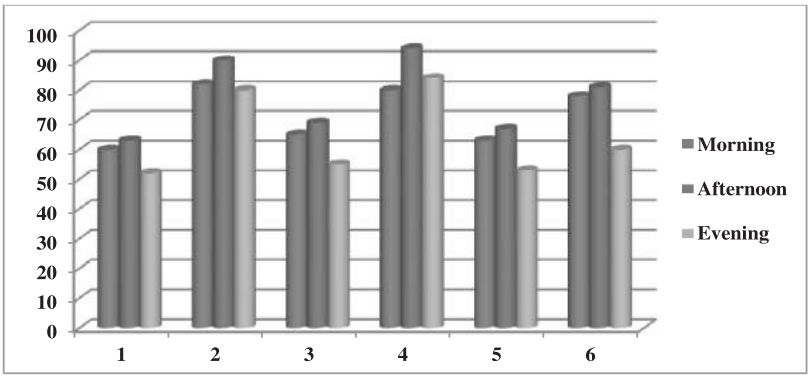

Fig. 2. Comparison between the time of sampling and fungal distribution (CFU/m3 air) of six indoor sites of THQ hospital Samahni, Azad Kashmir

The prevalence of airborne fungi in six locations of governmental hospitals was discussed in table 2. More fungal spores were observed in bed-room (BR) where patients admitted. In bed-rooms, *Aspergillus niger* was prevalent with 62.0%, *Fusarium oxysporum* with 57.5% and *Penicillium chrysogenum* was prevalent with 53.2%. Second prevalent site was surgical wards (SW). In this site *Aspergillus niger* (49.4%) was more prevalent. Third prevalent site was doctor sitting room (DR), where more prevalent species was *Penicillium chrysogenum* (45.2%). Fourth site was selected laborities (L) of hospitals. The more dominant species in this room was *Fusarium oxysporum* (40.0%). The fifth observed site was X-ray room (X-Rr), where more prevalent fungi was *Alternaria alternata* (36.2%). The sixth location inside government hospitals was Emergency rooms (ER). These rooms were also having *Alternaria alternata* (35.0%). Over all prevalence of the fungal spores in the selected locations were appeared as; BR (922.3%), SW (814.4%), DR (749.6%), L (600.6%), X-Rr (581.1%) and ER (578.6%) respectively (Table 2).

Table 1. Incidence of aero-fungi in indoor and outdoor environments of selected hospitals of district Bhimber Azad Kashmir

S/No	Isolated Species	Outdoor Environment *Incidence (%)*	Indoor Environment *Incidence (%)*
01	*Acremonium spp*	20.0	36.0
02	*Aspergillus niger*	40.4	62.9

03	*Aspergillus oryzae*	32.6	0.0
04	*Alternaria alternata*	55.3	44.0
05	*Alternaria solani*	28.6	32.4
06	*Botrytis spp.*	0.0	18.0
07	*Curvularia lunata*	22.2	35.5
08	*Cladosporium cladosporioides*	42.8	44.0
09	*Cladosporium herbarum*	54.9	50.5
10	*Drechslera spp.*	0.0	13.4
11	*Fusarium moniliforme*	12.6	26.2
12	*Nigrospora oryzai*	32.4	38.0
13	*Rhizopus stolonifer*	0.0	10.5
14	*Fusarium avenaceum*	33.5	0.0
15	*Fusarium oxysporum*	46.2	60.8
16	*Penicillium nigricans*	11.9	0.0
17	*Penicillium chrysogenum*	49.6	57.6
18	*Penicillium citrinum*	16.9	18.0
19	*Helminthosporium spp.*	14.5	0.0
20	*Puccinia spp.*	32.4	30.0
21	*Stemphylium spp.*	16.2	20.5
22	*Trichoderma virens*	40.0	46.8
23	*Trichoderma spp.*	0.0	18.2
24	*Neurospora* sp.	18.4	24.5
25	*Ulocladium spp.*	56.8	49.0
26	*Ustilago spp.*	16.2	0.0
27	*Unidentified*	40.5	52.0
28	*Verticillium spp.*	36.5	38.4

The distribution of fungi (CFU/ m3 air) was detected in five selected hospitals of government and private sector according to type of locations and sampling time (Table 3). There was commonly observed that more fungal distribution (CFU/m3 air) appeared at afternoon as compared to morning and evening (Figure 1-5). It was also observed that government hospitals showed more fungal pollution (CFU/m3 air) than that of private hospitals in all units. In the governmental hospital, there were no significant differences between the different units. On the other hand, the private hospitals were showed significant difference from that of government hospitals (Table 3).

The microfungi of indoor air in hospitals are much more as compared to outdoor environment because of the open holes in the buildings and entrance of more peoples included patients (Table 1). The fungal spores were attached with the hands and clothes of visitors entrapped in indoor environment also another factor of increasing indoor fungal pathogens. Many patients are actually at increased risk of infection in the older hospital because of poor hygienic condition in those hospitals. Generally, older government hospitals which may have large wards and poor mechanical ventilation system have more fungal spore inside environment (Obbard and Fang, 2003; Pastuszka *et al*., 2005).

In Table 3, three factors were investigated, namely the kind of hospital, the type of room and the time of sampling, individually. They showed the distribution of fungi (CFU/m3 air) in indoor air of hospitals. The results from this investigation revealed that the governmental hospitals had a higher degree of contamination with airborne fungi in indoor air rather than the private hospitals. These high rates in the governmental hospitals might be the age of the building and poor hygienic conditions

Table 2. Prevalence (%) of airborne fungi isolated from indoor five sites of governmental hospitals of Bhimber Azad Kashmir

S.No	Fungal Species Names	Labora-tories	Bed-room	X-ray rooms	Doctor rooms	Emergency rooms	Surgical Wards
1	*Acremonium spp*	20.5	36.0	18.0	32.4	22.0	34.5
2	*Aspergillus niger*	26.2	62.0	24.6	38.3	32.3	49.4
3	*Aspergillus oryzae*	0.0	0.0	0.0	0.0	0.0	0.0
4	*Alternaria alternata*	29.8	47.8	36.2	43.6	35.0	45.0
5	*Alternaria solani*	36.5	45.0	32.2	40.5	33.6	41.8
6	*Botrytis spp.*	32.7	47.4	30.6	40.5	30.7	43.0
7	*Curvularia lunata*	28.3	36.8	20.3	32.4	22.0	34.0

8	*Cladosporium cladosporioides*	38.2	48.6	34.8	37.2	30.8	39.9
9	*Cladosporium herbarum*	23.6	35.2	29.2	32.6	27.5	34.0
10	*Drechslera spp.*	10.6	26.0	15.2	20.5	17.4	22.0
11	*Fusarium moniliforme*	32.0	48.6	26.1	36.4	22.5	42.6
12	*Nigrospora oryzai*	18.4	40.5	23.6	34.2	24.3	36.4
13	*Rhizopus stolonifer*	22.5	38.2	20.4	32.4	22.0	34.0
14	*Fusarium avenaceum*	0.0	0.0	0.0	0.0	0.0	0.0
15	*Fusarium oxysporum*	40.0	57.5	30.2	35.1	31.0	37.0
16	*Penicillium nigricans*	0.0	0.0	0.0	0.0	0.0	0.0
17	*Penicillium chrysogenum*	38.2	53.2	36.2	45.2	33.6	47.2
18	*Penicillium citrinum*	24.0	36.2	26.0	31.0	25.0	33.4
19	*Helminthosporium spp.*	0.0	0.0	0.0	0.0	0.0	0.0
20	*Puccinia spp.*	12.0	19.2	14.0	16.2	14.6	17.9
21	*Stemphylium spp.*	16.0	28.4	14.8	24.0	15.0	26.9
22	*Trichoderma virens*	26.4	40.3	26.2	32.1	22.0	38.0
23	*Trichoderma spp.*	32.7	42.0	28.6	37.2	27.0	40.2
24	*Neurospora* sp.	33.0	40.5	26.5	33.0	25.4	35.8
25	*Ulocladium spp.*	10.4	19.3	12.4	15.4	14.3	17.0
26	*Ustilago spp.*	0.0	0.0	0.0	0.0	0.0	0.0
27	*Unidentified*	22.6	33.8	26.0	27.0	20.6	30.2
28	*Verticillium spp.*	26.0	39.8	29.0	32.0	28.0	34.2
Total Percentage of Species		**600.6**	**922.3**	**581.1**	**749.6**	**578.6**	**814.4**

(Olugbue *et al*., 2013). Another factor which might be involved in more contamination is the number of beds in these hospitals. This more bed numbers in governmental hospital means a high number of patients and visitors that enter the patient rooms everyday with materials brought from outside such as food, fruits, and flowers, were more common in patients rooms. These are major sources of high contamination and high density of fungal spores in indoor hospitals (Jaffal *et al*., 1997; Qudiesat *et al*., 2009).

These results (Table 3) also indicate that the type of hospital has a significant effect on the rate of aero-fungi in indoor environment. Each hospital consists of different rooms with different levels of services. Some units are sealed and minimum interruption of visitors indicates the low prevalence of spores. Similar findings were obtained by the some mycologists (Chuaybamroong *et al*., 2008; Augustowska and Dutkiewicz, 2006; Krogulski, 2008). They observed the type of room as a factor affecting the indoor rate of fungal spores, there was a significant effect of different level of rooms.

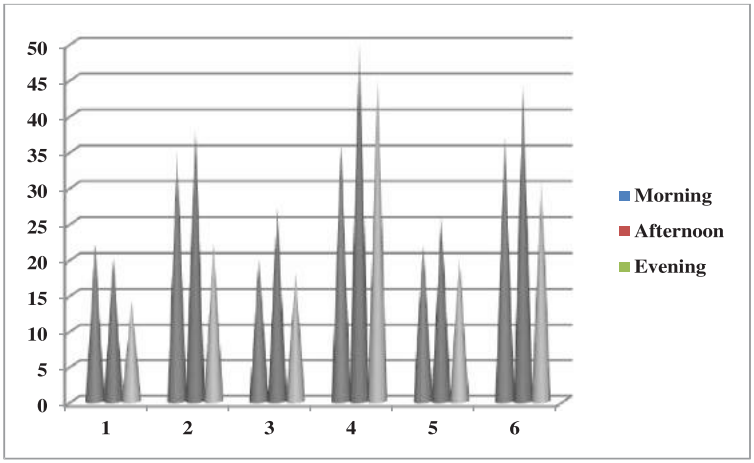

Fig. 3. Comparison between the time of sampling and fungal distribution (CFU/m3 air) of six indoor sites of Rehman Children hospital Bhimber, Azad Kashmir

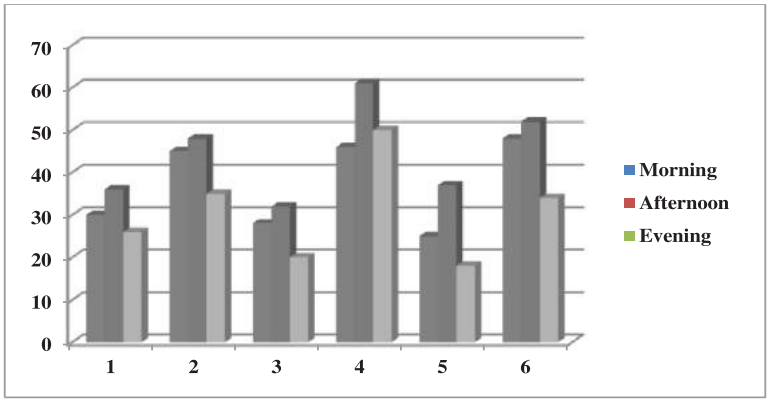

Fig. 4. Comparison between the time of sampling and fungal distribution (CFU/m3 air) of six indoor sites of Akbar Khanum hospital Bhimber, Azad Kashmir

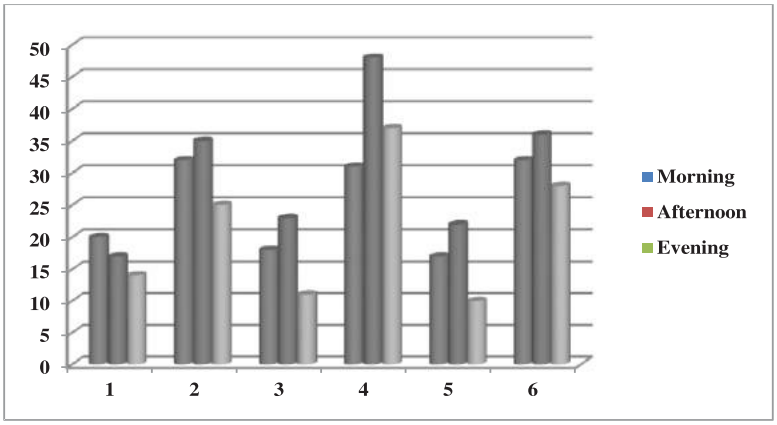

Fig. 5. Comparison between the time of sampling and fungal distribution (CFU/m3 air) of six indoor sites of Nazeer hospital Bhimber, Azad Kashmir

The prevalence(%age) of fungi in the hospital environment increased the some fungi related health problems to hospital staffs of various forms like the allergic reactions, superficial mycoses, cutaneous mycoses and sub-cutaneous mycoses. These were produced due to air fungal infection (Olugbue *et al*., 2013; Ayanbimpe *et al*., 2010; Chadeganipour *et al*., 2010).

Table 3. Distribution of fungi (CFU/m^3 in air) in private and government hospitals according to type of room and time of sampling

Hospital Name	Type of Hospitals	Indoor Locations	Fungal CFU/m^3 in air		
			Morning (9am)	Afternoon (3pm)	Evening (7pm)
1- District Head Quarter (DHQ) Hospital Bhimber	Government	laboratory	50	60	46
		Bed-room	59	70	57
		X-ray room	44	49	42
		Doctor room	48	75	51
		Emergency room	36	39	32
		Surgical Ward	56	66	49
2- Tehsil Head Quarter(THQ) Hospital, Samahni	Government	laboratory	60	63	52
		Bed-room	82	90	80
		X-ray room	65	69	55
		Doctor room	80	94	84
		Emergency room	63	67	53
		Surgical Ward	78	81	60
3- Akbar Khanum Hospital Bhimber	Private	laboratory	30	36	26
		Bed-room	45	48	35

		X-ray room	28	32	20
		Doctor room	46	61	50
		Emergency room	25	37	18
		Surgical Ward	48	52	34
4- Rehman Children Hospital Bhimber	Private	laboratory	22	20	14
		Bed-room	35	38	22
		X-ray room	20	27	18
		Doctor room	36	50	45
		Emergency room	22	26	20
		Surgical Ward	37	44	31
5-Nazeer Hospital Bhimber	Private	laboratory	20	17	14
		Bed-room	32	35	25
		X-ray room	18	23	11
		Doctor room	31	48	37
		Emergency room	17	22	10
		Surgical Ward	32	36	28

Airborne fungal spreading cannot be completely wiped-off but can be reduced to some extent due to awareness of their fatal effects. We should try to clean the hospital units daily. The visitor entrance in hospitals should be minimized. Therefore, there is the need for regular surveillance of the air to minimize the level of contamination and their risk of exposure in hospital for better workers health.

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Conflict Of Interest

Authors have no conflict of interest

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