

First Mycological Investigation of Medically Important Opportunistic Fungi in Soil of Djibouti

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Abstract

Fungi are a versatile group of living eukaryotic organisms, which can live as saprobe in the environment. The present study was undertaken to determine the prevalence of medically important opportunistic fungi in 11 soil samples obtained from the livestock farm in Djibouti. Each soil sample was examined by dilution technique by inoculating on duplicate plates of "APRM" medium. All the soil samples were positive for opportunistic fungi, and yielded one or other type of fungi. The identification of the fungi was based on the macroscopic and microscopic characteristics. "Narayan" stain was used to study the detailed microscopic morphology of each fungal isolates recovered from the soil samples on "APRM" medium at 25°C. In total 128 colonies representing 11 genera of medically important fungi were isolated from 11 soil samples. *Aspergillus* species was the predominated mould (56.25%), which was isolated from all the soil samples. This was followed by *Alternaria*, *Curvularia*, *Penicillium Cladosporium*, *Fusarium*, *Mucor*, *Rhizopus*, *Rhodotorula*, *Geotrichum*, and *Candida*. All these fungi are opportunistic pathogen, which has the potential of causing the variety of infections, particularly in immunocompromised subjects. As far as could be ascertained, this seems to be the first study of isolation and identification of medically important opportunistic fungi from the environment of Djibouti.

Keywords: APRM Medium; *Aspergillus*; Djibouti; Immunocompromised Patient; Narayan Stain; Opportunistic Fungi; Soil

Introduction

Djibouti is a small economically poor tropical country, which is located between Ethiopia and Somalia. It was earlier known as French Somaliland, and then as the French Territory of Afars and Issa. Its capital is Djibouti. The country has an area of 21,783 square kilometer, and population of 5,06,221. The livestock particularly, the camels, goats, and sheep form the major wealth of the country [1].

Fungi are ubiquitously prevalent in our environment, and have been isolated from a wide variety of natural substrates including the soil throughout the world [2-9]. The natural substrates are rich in organic matters and other chemicals that may serve as an enrichment medium for fungi [10]. It has been recognized that the soil serves as the most important natural habitat for keratinophilic, opportunistic, and dimorphic fungi [9,11-17]. The fungi grow, multiply, and produce millions of spores/conidia in the environment. The inhalation of sufficient number of infectious fungal spores through respiratory tract may cause fungal infections in the susceptible humans and animals [10,18]. Several investigators from many regions of the world studied the natural occurrence of fungi in the soils [3,9,19,20]. The extensive search of literature on internet failed to provide any information on the natural occurrence of opportunistic fungi in the soil of Djibouti. Therefore, the present paper delineates the first mycological investigation of the soils from livestock farm in Djibouti. In addition, the efficacy of newly developed "APRM" medium for the isolation of fungi from environmental samples is also reported.

Materials and Methods

Eleven soil samples obtained from livestock farm in Djibouti constituted the materials for this preliminary study. Each sample contained about 10g of soil, taken from a depth of about 2 to 4 cm with the help of sterilized wooden spatula in a clean polythene bag. One gram of the soil sample was suspended in 9 ml of sterilized physiological saline in sterilized glass test tube, and was shaken on Vortex mixer. After allowing the sample to stand about 20 - 30 minutes, one ml of the supernatant fluid was transferred into a sterilized glass test tube containing 1 ml of chloramphenicol solution (0.5 mg / ml). This mixture was incubated at 37°C for 1h, and then serial dilution of 1:10 was made in sterile distilled water. From this, aliquot of 0.1ml was inoculated by surface streaking method on the newly introduced medium designated as "APRM". The name "APRM" is derived from the first letter of Anubha, Pratibha, Raj and Mahendra. This new medium contained 2.0 g of dried marigold flower, 2.0 g agar, 50 mg of chloramphenicol, and 100 ml of distilled water [9]. The inoculated plates were incubated at 25°C, and examined daily for the growth of fungi. The single colony was picked up and sub-cultured onto the slant of "APRM" for further identification. The detailed microscopic morphology was done in 'Narayan' stain, which contained 0.5 ml of methylene blue (3% aqueous solution), 4.0 ml of glycerine, and 6.0 ml of dimethyl sulfoxide [21]. All the fungal cultures recovered from the soil of livestock farm in Djibouti were identified by studying the macroscopic as well microscopic characteristics [10].

Results

In all, 11 soil samples were investigated for the prevalence of medically important opportunistic fungi by dilution technique. All the plates of "APRM" medium were positive for various types of opportunistic fungi. Mixed growths of fungi were observed in all the soil samples. A total of 128 colonies, which comprised of 11 different genera of fungi were recovered from 11 soil samples. *Aspergillus* was the predominant fungus, which was represented by 72 colonies (56.25%), followed by *Alternaria*, *Curvularia*, *Penicillium*, *Cladosporium*, *Fusarium*, *Mucor*, *Rhizopus*, *Rhodotorula*, *Geotrichum*, and *Candida* (Table 1). The gross cultural and microscopic examination helped in the identification of these fungal isolates [10].

Fungal species	Number of colonies	Percentage
<i>Aspergillus niger</i>	21	16.40
<i>A.flavus</i>	19	14.84
<i>A.fumigatus</i>	17	13.28
<i>A.terreus</i>	15	11.71
<i>Alternaria</i>	11	08.59
<i>Cuvularia</i>	9	07.03
<i>Penicillium</i>	8	06.25
<i>Cladosporium</i>	7	05.46
<i>Fusarium</i>	6	04.68
<i>Mucor</i>	5	03.90
<i>Rhizopus</i>	4	03.12
<i>Rhodotorula</i>	3	02.36
<i>Geotrichum</i>	2	01.56
<i>Candida</i>	1	00.78
Total	128	100

Table 1: Fungi isolated from 11 soil samples in Djibouti by dilution technique on "APRM" medium.

Discussion

The findings of the current study revealed eleven genera of medically important opportunistic fungi from the soil samples collected from the livestock farm in Djibouti. The other researchers also reported the similar types of fungi from the soils [6,9,14,20]. Very recently,

Pal [8] isolated several species of *Aspergillus* from the soil of plotted plants in Debre Zeit, Ethiopia. The isolation of many opportunistic fungi on "APRM" medium from the soils of livestock farm for the first time in Djibouti is an interesting observation. All this soil borne fungi recovered in the present study are opportunistic, which can cause a variety of infections, particularly in the immunocompromised patients [10]. Therefore, the etiologic role of these fungi in various clinical disorders of animals as well as in humans in Djibouti needs to be established. Since most of the fungi are air borne, and the respiratory tract is considered as prime portal of entry [10,18], the emphasis is given on the use of face mask, when working in the highly dusty environment. It is interesting to note that all the medically important opportunistic fungi were easily isolated on "APRM" medium. This observation is in accordance with the findings of Dave and Pal [9] who recovered many opportunistic fungi from clinical and environmental samples on "APRM" medium. Therefore, it is advised that "APRM" medium, which is very cheap and easy to prepare, should be routinely used for primary isolation of fungi from the clinical as well as environmental materials in all the microbiology and public health laboratories, particularly in poor resource countries.

Conclusion

The mycological investigation of 11 soil samples obtained from the livestock farm in Djibouti revealed the presence of 11 genera of opportunistic fungi on "APRM medium". Among the opportunistic fungi, *Aspergillus* showed the highest prevalence (56.25%). As "APRM" medium is comparatively cheaper with Sabouraud dextrose medium and other nutrient media, its wider application for the primary isolation of opportunistic fungi is emphasized. This is believed to be the first report of isolation of opportunistic fungi from the soil of the livestock farm in Djibouti.

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