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Studies Based on the Fungal Flora of Indian Currency

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Abstract:

The growth of fungal organisms colonizing the Indian currency was studied. The fungi were estimated both qualitatively and quantitatively more during February to March 2015. The currency notes of different denominations were collected from vegetable shop, personal uses and milk shop in cities. The samples collected were analyzed for microbial contamination. Totally 9 species were recorded. The results indicated the presences of fungi. The fungi isolated were Aspergillus spp., Rhizopus spp., Fusarium spp., Penicillium spp. and Alternaria spp. Most of these isolated microorganisms are pathogenic to man. The currencies used by In vegetable shop highest total viable count occur in 10 rupee notes 4.0×10^4*. In personal uses highest total viable count occur in 10 rupee notes* 2.0×10^4 *and milk shop highest total viable count occur in 10 rupee notes* 1.0×10^4*. Vegetables shops were found to be extremely contaminated with various pathogenic microorganisms followed by the currency used by personal uses and milk shop. Therefore, adequate care must be taken by those handling these currencies and public awareness of using paper currency in circulation became essential for the safety in human health.*

Key words: Indian Currency notes, fungal flora, *Aspergillus, Rhizopus, Fusarium, Penicillium* and *Alternaria*.

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Introduction:

Money is very important to human life as it facilitates the needs of common man. Paper currency is widely exchanged for goods and services in countries worldwide. It is used for every type of commerce. Infect, all types of trades depend on currency, with lower denomination notes receiving the most handling because they are exchanged many times (Sanjogita S. and Geeta S. 2014). Rupee is the name given to the official currency that is used in several countries includes India, Bhutan, Pakistan, Shrilanka, Nepal, Mauritius, Maldives and Indonesia. The name rupee comes from the Sanskrit language word rupyakam meaning silver coin –Rupee in different region is denoted with different symbols most commonly Rs and Rp. The currency in India is denoted with the Sign- Rs.

Paper money that is passed from hand to hand is likely to be contaminated with disease-causing micro-organisms especially if handling with unclean hand, or kept in dirty surroundings, walks of life, like beggars, street food vendors, shoe-shiners, school children, cashiers, salespeople, vegetable seller, milk shop and the general public to the currency notes that they handle**.** Therefore, there are chances of higher level of microbes contamination on lower denomination currency. Scientific information on the contamination of money by microbial agents is lacking in most developing countries (Badvi *et al*., 2010).

Researchers different other parts of the world have also reported fungal contamination of notes (Barolia *et al*., 2011).A survey carried out by Abirami *et al*.(2012) in Nanded city (Maharashtra) revealed heavy contamination of currency notes and coins by fungal species like *Aspergillus spp. A. flavus, A. nidulans, Penlcillum citrinum, Alternaria tenuis, Rhizopus stolonifer, Fusarium* spp. and *Trichoderma viride.* Most of the organism wereisolated from the currency counting machines while currency counting rooms had fewer isolates or none in some cases. Most commonly 4 fungal genera (*Aspergillus,*

Mucor, Rhizopus and *Penicillium)* were isolated. The identified microorganism have pathogenic potential and hence their presence on such surfaces could serve as a sources of cross-transmission of fungal infections in the banks and the general community. Money, which is responsible for solving health problems can turn out to be as a cause of creating health problems. It is very good vector for the transmission of diseases (Wamae, 2009). The possibility that currency notes might act as environmental vehicles for the transmission of potential pathogenic microorganism was suggested by Singh *et al*., (2002).

Study conducted by various researchers has reported potential threat of the contaminated notes carrying pathogenic microbes for human health. Fomites are generally the main sources of infection in a community. Communicable diseases spread through contact with fomites and transfer through paper currency is an important route (Pope *et al*., 2002).In the recent years, opportunistic fungal infections have emerged as the interesting areas of research. The moulds species isolated from paper notes namely *Penicillium, Fusarium, Rhizopus,* and*Alternaria*species cause a variety of opportunistic infections (Forbes et al., 1994). Among the various species of *Aspergillus*that are found frequently on the old paper notes and even on the intact newly dominant one. Though it is less likely to cause human diseases, yet whenever many spores are inhaled, it can cause Aspergillosis (Schuster *et al.,* 2002).

Methods and Materials:

Sources of experimental materials

Samples of currency notes of the denominations of ten and twenty were collected from vegetable shop, Personal uses and milk shop located in Haridwar, Uttrakhand during the period of February to March 2015. The collected samples were put in

polythene bags and stappled and then brought to the laboratory.

Isolation of microorganism

Stock solution was prepared by dipping currency notes in 10 ml distilled water. 7 test tube containing 9ml of sterile distilled water were placed on test tube stand. 1 ml from stock solution was transferred to the first test tube and mixed well or properly. Then 1 ml solution from the first test tube was transferred into the second test tube making dilution of 10^{-2} and repeated till the last test tube making final concentration to 10^{-7}.The 1ml from last test tube was discarded. 0.1 ml solution from 10^{-3} to 10^{-5} dilution was spread on solidified SDA medium separatly. The plates were incubated at $25^{\circ}C \pm 28^{\circ}C$ for 5-7 days.

Calculations-

$$\text{C.F.U} = \frac{\text{no.of colonies} \times \text{dilution factor}}{\text{volume of culture plate (ml)}}$$

Where, C.F.U = Colony Forming Unit
Dilution factor = 1/dilution

Pure culture preparation-

For the purification of fungus following steps were used. All plates were observed under the colony counter and the isolated colonies of fungi were picked up with the help of sterile cork borer. This fungal disc was placed in Sabouraud'd Dextrose Agar media. These slants were incubated for 5-7 days at $25^{\circ}C \pm 28^{\circ}C$. Restreaked the purified colonies of fungi on SDA slants for 5-7 days at $25^{\circ}C \pm 28^{\circ}C$.

Identification and characterization of the organism

A clean dried glass slide was taken. A drop of Lectophenol cotton blue stain was placed in the centre of the slide. With the help of inoculation loop a small portion of colony was scratched

and placed in the drop of stain. Then it was teased into small bits with the help of needle. A cover slip was placed on top and gentle pressure was applied for even spreading. It was observed under the microscope.

Results:

Enumeration of fungus isolated from currency notes -

The total 9 colonies of fungus were observed on SDA media plates. From vegetable shop in 10 rupee notes 4 colonies and in 20 rupee notes 2 colonies of fungi were observed. Personal uses in 10 rupee notes 2 colonies and in 20 rupee notes no colonies of fungi were observed and from milk shop in 10 rupee notes 1 colonies and in 20 rupee note No colonies of fungi were observed.

In vegetable shop highest total viable count occur in 10 rupee notes 4.0×10^4. In personal uses highest total viable count occur in 10 rupee notes 2.0×10^4 and milk shop highest total viable count occur in 10 rupee notes 1.0×10^4. As shown in table no 1 and 2.

The antifungal activity of three types commercial antifungal (Fluconazole, Terbinafine and Levocentirizes) were assayed using Agar Well diffusion method. Approximately 80% of the recovered isolate have shown the sensitive activity against Fluconazole, 60% of the recovered isolate have shown the sensitive activity against Terbinafine and 40% of the recovered isolate have shown the sensitive activity against Levocentirizes. The diameter of inhibition zone observed in different types of antifungal used by different concentration. Out of zone domination against Fluconazole (Table 3).

Discussion:

In this study different fungal species were isolated from the currency notes, this confirmed that currency notes play a role

vector in transmission of microorganism. Paper currency provides large surface area as a breeding ground for pathogens. The use of saliva and water pads for counting notes moistens the notes and encourages the growth and establishment of a micro habitat for microbes.

Among the different isolated fungal species five were found to be dominant in occurrence and these were identified as *Aspergillus* spp,*Rhizopus* spp*, Fusarium* spp, *Penicillium* spp and *Alternaria* spp. Fungal species are known to be distributed in every environment. Survey carried out by **Abirami *et al.*, 2012** in Nanded city (Maharashtra) also revealed heavy contamination currency notes and coins by important fungal species like *Aspergillus* spp*., Penicillium* spp*., Alternaria* spp*., Carvularia* spp*., Cladosporium* spp*., Fusarium* spp.and *Trichoderma* spp. Some of these isolated fungal species are potential pathogens of human beings. The mold species isolated from paper notes and coins namely *Penicillium* spp, *Fusarium* spp, *Rhizopus* spp and *Alternaria* spp.cause a variety of opportunistic infection **(Forbes *et al.*, 1994).**

The degree of contamination and types of microbes present on the currency notes is dependent on sanitary conditions of the area, microbes endemism and texture of paper notes **(Dehgani *et al.*, 2011).** A total Cfu/ml for fungal isolates were also observed from each samples collected from different locations such as vegetable shop, personal uses and milk shop. Maximum Cfu (4.0×104) in 10 rupee and (2.0×10^4) in 20 rupee notes was observed in vegetables shop. It was also observed that fungal flora isolated from 10 rupee notes have more Cfu than that of 20 rupee notes. Earlier studies have also shown that the lower denomination notes harbor the greatest bulk of infectious agents since they are exchanged more than higher denomination notes **(Gadsby, 1998, Uneke and Ogbu, 2007).**

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Table 1→ Enumeration and total viable counts of currency notes.

S.N.	Samples	Date of sample	Rupee	No. of Colonies	Cfu/ml
1	Vegetable shop	14/02/2015	10	4	4.0×10^4
			20	2	2.0×10^4
2	Personal Uses	18/02/2015	10	2	2.0×10^4
			20	—	—
3	Milk Shop	23/02/2015	10	1	1.0×10^4
			20	—	—

(-) = No colony

Table 2→Showing characteristics & identify of fungi isolated from currency Notes.

Morphological characteristics					
S/NO.	**Colour**	**Elevation of colony**	**Shape and arrangement**	**Mycelium**	**Fungi**
1	Greenish-blue, black or green colonies	Flat	Conidiophores arising from a foot-cells, catenate (basipetal) conidia on phialides on vesicles	Septate	*Aspergillus*spp.
2	White to dark gray, black columellates	Raised	Sporangiophores in cluster	Non septate mycellium with root like rhizoids	*Rhizopus*spp.
3	Woolly, white to pink	Flat	Sickle-shaped	Septatemacroconidia produce in sporodochia	*Fusarium*spp.
4	Greenish or blue green colonies	Flat	Conidia in long chains on repeatedly branched	Septate	*Penicillium*spp.

			conidiophores resembling a brushlike heads (penicillus)		
5	Grayish green or blacks	Flat	Beaked conidia, in acropetal manner	Transversely and longitudinally septate (muriform)	*Alternaria*spp.

Table 3 → Antifungal sensitivity of fungal isolates.

S.N.	Antifungal drug	Concentration (mg/ml)	Zone of inhibition (mm)				
			*Aspergillus*spp.	*Rhizopus*spp.	*Fusarium*spp.	*Penicillium*spp.	*Alternaria*spp.
1	Fluconazole	5mg/ml	21	18	21	23	—
2	Terbinafine	5mg/ml	40	—	38	—	21
3	Levocentirizes	5mg/ml	38	29	—	—	—

(—) = No zone of inhibition