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Antifungal Resistance Characterization of Food Borne Pathogenic Fungi from India

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ABSTRACT

Food borne diseases are increasing globally and disease-causing fungi are becoming resistant to antifungal agents. Errors in food processing technology, irresponsible health and hygiene measures are responsible for widespread of antifungal diseases. The health hazards of such antifungal resistant pathogens are life threatening and also puts economic burden on the patient. This study determined antifungal resistance of fungi isolated from roadside food vendors from Lucknow and Pune, India. A total of 374 food samples were collected for this study. The food samples were analysed in laboratory using prepared quantitative media and identified by conventional lactophenol cotton blue staining. Confirmed fungal isolates were further subjected to antifungal susceptibility testing by the Agar disc diffusion technique. Results showed that 110 fungal isolates showed resistance to more than three antifungal agents. The multiple antifungal resistance exhibited by the fungi in this study is an indication of possible multidrug resistant fungal strains present in roadside food items. Therefore, roadside food vendors as well as common people should be made more aware regarding food borne fungal diseases caused due to multidrug resistant pathogens.

Keywords: Antifungal resistance, Food borne diseases, WHO, Fungi, Food processing

1. Introduction

Food is essential for every living being on this planet for survival as it provides nutrition and energy. As it contains nutrients other unicellular microorganisms like bacteria, fungi also grow luxuriously. Fungi also known as moulds have fuzzy appearance and can be orange, green, black, brown, pink or purple in colour. A mould is a fungus that grows in the form of multicellular filaments called hyphae. Mould growth is encouraged by warm and humid conditions. Mould will grow in places with a lot of moisture. They can be grown on yeast and mould agar by incubating at 25°C for 48 to 72 hours in acidic conditions **(Fisheret al., 2020)**. Moulds are also used safely in the production of cheese but with failure in safety measures

while preparation, if this contaminated food is consumed, it can cause adverse effects on human health **(Benedict et al., 2016)**.

2. Significance of the Study

Naturally, fungal spores can contaminate raw as well as processed food and can cause food poisoning. This results in production of unwanted metabolites, toxins inside the human body causing clinical symptoms like nausea, vomiting, diarrhoea, abdominal pain or even death. Contamination of beverages by other fungi can cause liquor-poisoning leading to death. Onychomycosis is caused by *Trichophyton rubrum* leading to white or yellow nail discolouration and thickening of the nails. Common fungal infections on bread includes *Rhizopus mucor, Aspergillus flavus, Oxysporium, Cryptococcus neoformans*, etc. Contamination of foods by moulds can result in substantial economic losses to producer, processor and consumer. Several food-borne moulds may also be hazardous to human and animals due to their ability to produce toxins known as mycotoxins **(Helrich, 1990, Köhleret al., 2016).**

3. Review of Related Studies

Humans have researched ways to prevent cause of infection by cleaning, washing, heating and using spices while preparing food. Still, if fungal load isn't reduced, it can cause infection and person consuming becomes ill. To treat the patient, various antifungal agents are used. Fungi like *Penicillium chrysogenum* produces certain compounds, which can inhibit and kills the other fungi are known as antifungal agents. With the advancement of modern synthetic chemistry, new antifungal agents are being produced **(Krysan, 2017, Wiederhold, 2017)**. But, overuse of these drugs has developed resistance in fungi and thus new variants of antifungal resistant fungi are developed **(Perlin et al., 2015, Kontoyiannis, 2017, Caetano et al. 2018)**. Use of fungicides in agriculture to prevent and treat fungal diseases in crops can also contribute to antifungal resistance in *Aspergillus* **(Hoda et al., 2017, Georgacopoulos et al., 2021, Hagiwara et al., 2016, Averya et al., 2019Alastruey-Izquierdo et al., 2018)**. When these fungi infect the individual, the treatment with antifungal agents fails, as these fungi are already drug-resistant **(Perlin et al., 2017, Bongomin et al., 2017, Sanglard, 2016)**. Thus, new antifungals need to be developed and used which increases in complexity and cost of treatment **(Chang et al., 2019, Borman et al., 2021, Kohler et al., 2014, Jensen, 2016, Denning et al., 1997, Arendrup, 2014). Fisher et al. (2018)** have reported that the azoles are used for human and animal health care, crop protection, in antifouling coatings and timber preservation. Overuse of these azoles have resulted in increased risk in human health care from naturally occurring opportunistic drug resistant fungal pathogens. In order to control fungal infections and to avoid critical failures in medicine and food security, we must control use of drugs, promote new antifungal discovery and find alternative solutions to the existing therapy **(Vandeputte et al., 2012, Prasad et al., 2017, Davies et al., 2021, Margriet et al., 2019). Rivero-Menendez et al. (2016)** have reported about an azole-resistant *A. fumigatus* strain reported in 1997 in the USA and soon after in Europe, has now been described worldwide. The main mechanism of resistance is the modification of the azole target enzyme

and further research is being done to control these infections **(Macedo et al, 2018, Wattal et al., 2017, Arastehfar et al., 2017).**

Depending on this review, the study was carried out with the following objective:

4. Objectives of the Study

- Isolate pathogenic fungi from street food using standard laboratory methods
- Study antifungal susceptibility test against fungal isolates using disk diffusion technique

5. Hypotheses of the Study

- There is presence of pathogenic fungi from street food
- There is antifungal resistance shown by fungal isolates

6. Population and Sample

A total of 374 samples were obtained for the study**.**

6.1. Statistical Techniques Used in the Present Study

The data recorded during the course of investigation was statistically analysed using SPSS software and conclusion was drawn accordingly.

6.2. Data Analysis and Interpretation

6.2. Materials and Methods

6.2.1 Study Area

Two metropolitan cities Lucknow and Pune were selected for the study.

6.2.1.1 Chemicals and media

Standard media and laboratory chemicals were procured from Himedia Pvt. Ltd. and were used in laboratory for microbiological analysis. Selective ready prepared media M-BCG Yeast and Mould Agar HiMedia M1504 for isolation of mould was used.

6.2.2 Sampling

A total of 374 samples were obtained for the study. Food samples were purchased from markets in the study area from food vendors and collected in separate labelled sterile polythene bags and immediately transported to the Cytogene laboratory (Lucknow) and Hydrotech laboratory (Pune) for fungal analyses.50 food samples were collected by Cytogene laboratory in Lucknow city in July and August 2020 and 324 food samples by Hydrotech laboratory in Pune city from December to February 2021.The samples were stored at 4℃ at the laboratory until processing. Demographic data was recorded using a survey form and association between fungi growth and growth factors was correlated.

6.2.3 Laboratory procedures

6.2.3.1 Isolation of fungi from food sample from Lucknow

1 g of food sample was diluted in 10 ml of sterile saline as dilution 10^{-1}.Using sterile spatula, homogenous paste was prepared. From this 10^{-1} dilution, 1 ml was taken and added to 9 ml sterile saline to prepare 10^{-2} dilution. Similarly, serial dilutions up to 10^{-9} were done. Loopful of 10^{-9} dilution was spread on nutrient agar medium. Microorganisms isolated on nutrient agar medium were streaked on selective media Potato Dextrose Agar (PDA) HiMedia M096 for identification of Fungi. The plates were kept at 25 ℃ for 5-7 days. Colonies observed on these selective media were used for further analysis.

6.2.3.2Morphological characterization of fungi isolated from food samples of Lucknow

The fungal isolates were characterized by morphological characteristics and the results were recorded as colour of mycelium, colour of spores, pigmentation.

6.2.3.3Starch hydrolysis test: Fungal isolates were inoculated in the starch agar medium HiMedia M107D. Then plates were incubated at 25℃ for 5-7 days. After that, the plates were flooded with iodine solution for 30 seconds. Observation of clear zones around the fungal growth indicated Positive result.

6.2.3.4 Cellulose hydrolysis test: Fungal isolates were inoculated in the Czapek mineral salt agar medium HiMedia M075 and incubated at 35 ℃ for 5 days. After incubation, the plates were flooded with 1% aqueous solution of hexadecyl trimethyl bromide for 3 seconds. The plates were observed for formation of clear zones around the fungal growth. Clear zone indicated cellulose hydrolysis.

6.2.3.5 Isolation of fungi from food sample from Pune

All food samples were collected, and 1 grams of each food sample were immediately processed under aseptic conditions. 1g of food sample was diluted in 10 ml of sterile saline. Homogenous paste using sterile spatula was made. Liquid part of the original dilution 10^{-1} was used for serial dilution. From this 10^{-1} dilution, 1 ml was taken and added to 9 ml sterile saline to prepare 10^{-2} dilution. Similarly, 10^{-5} dilutions were prepared. 1 ml from 10^{-5} dilution was spread on HiMedia M-BCG Yeast and Mould agar M1504 for Fungi (Mould) plate. All the plates were incubated at 25 ℃ for 7 days. After incubation, all colonies were enumerated. Control plate (without any sample addition) was also incubated.

6.2.3.6 Identification of fungal cultures isolated from Lucknow and Pune food samples by Lactophenol cotton blue staining

Identification of fungal isolates was done by staining the cultures with Lactophenol Cotton Blue Lactophenol Cotton Blue HiMedia S016.A drop of Lactophenol Cotton Blue reagent on a clean and dry slide was placed. The stain imparts a blue colouration on hyphae. By using a nichrome inoculating wire, the fungal culture into a thin preparation was carefully teased. A coverslip was placed on the preparation and after 5 minutes the slide was observed first under microscope with low power for screening in low intensity. Conidia or mycelia or hyphae were observed under microscope and identification was done by comparing with laboratory standards.

6.2.3.7Antifungal susceptibility test of Lucknow and Pune food sample isolates: The fungal isolates were tested for antifungal susceptibility test using agar-based disk diffusion method. After growth of fungi, conidia were inoculated into sterile saline, the suspension of fungi was mixed with Potato Dextrose Agar (PDA)HiMedia M096 and spread evenly in the Petri dishes and left to dry for 3 minutes in laminar flow. Antifungal discs were applied to the surfaces of inoculated plates. The plates were incubated at 30℃ for 4-7 days. Inhibition zone was measured in millimetres. Miconazole (10 µg), fluconazole (25 µg), ketoconazole (15 µg), itraconazole (8 µg) and voriconazole (1 µg) was used for antifungal susceptibility test.

6.2.4 Maintenance of isolates: The cultures were maintained on Potato Dextrose Agar (PDA) HiMedia M096 slants and stored at 4℃ until further use.

6.2.5 Statistical analysis: The data recorded during the course of investigation was statistically analysed using SPSS software and conclusion was drawn accordingly.

6.3. Results

6.3.1 Study Area

The study was carried out in Lucknow and Pune in India.

6.3.2 Sampling

Total of 374 food samples from roadside food vendors from Lucknow and Pune were collected. These food items which included samosa, wada pav, idli, batata wada, udid wada, kanda bhaji, batata bhaji, bhel, masala puri, ragada puri, ragada pattice, shev puri, dosa, uttapa, kacchi dabeli, shev pav, pohe, sev usal, kachori, sandwich, upma, bread pattice, medu wada, sabudana wada, sabudana khichadi, lassi, dhapata, appe, thalipeeth, boiled egg, egg bhurji, egg omlet, egg rice, egg pattice, gol bhaji, sheera, sambar, chatni, misal, paneer veg roll, moong bhaji, rice, flower mattur sabji, dal, french peas sabji, patti samosa, mirchi bhaji, farsan, dal wada, biryani, chhole, momos, tikki, puri. The samples were stored at 4℃ at the laboratory until processing.

6.3.3 Laboratory procedures

6.3.3.1 Isolation of fungi from food sample from Lucknow food samples

Total of 48 fungal isolates were obtained from 50 food samples collected from different food vendors from Lucknow.

6.3.3.2 Morphological characterization of fungi isolated from Lucknow food samples

Table S1 depicts results of colour of mycelium, colour of spores and pigmentation of fungi isolated from Lucknow food samples.

6.3.3.3Starch hydrolysis test:

Table S2 shows results of starch hydrolysis test of fungal isolates from Lucknow food samples.

6.3.3.4 Cellulose hydrolysis test:

Table S3 shows results of cellulose hydrolysis test of fungal isolates from Lucknow food samples.

6.3.3.5 Isolation of fungi from Pune food samples

Total of 60 fungal isolates were obtained from 324 food samples collected from different food vendors from Pune.

6.3.3.6 Identification of fungal cultures isolated from Lucknow and Pune food samples by Lactophenol cotton blue staining

Identification on the basis of Lactophenol cotton blue staining of 48 fungal isolates from 50 Lucknow food samples and 61 fungal isolates from 324 Pune food samples was done. The isolated fungi were belonged to the genera *Aspergillus, Rhizopus, Mucor, Penicillium* and *Rhizoctonia soloni*.

6.3.3.7 Antifungal Susceptibility Testing

All the isolates were subjected to antifungal susceptibility test and response to the antifungal was recorded as susceptible, intermediate and resistant. Zone of inhibition in cm was calculated and compared with standard table given for every antifungal with specific concentration in HiMedia antifungal susceptibility systems data sheets. The results were compared with CLSI standard.

Interpretation of Table-1

For Lucknow food sample isolates, antifungal susceptibility of food borne pathogens was assessed. Total of 48fungal isolates were obtained from 50 food samples from Lucknow and assay for antifungal susceptibility was carried out. From Table 1, it can be observed that, of the total isolates, above 90% were found to be resistant to Voriconazole (1µg), Itraconazole (8µg), Miconazole (10µg), Fluconazole (25µg), Ketoconazole (15µg). Around 5% of the isolates showed sensitive while 2%isolates showed intermediate response to the antifungals tested. This data indicates that Lucknow food samples were heavily contaminated with food borne pathogens and very high number of these isolates were multi-resistant to antifungal tested.

Table 1 - Antifungal susceptibility test of Lucknow food sample isolates

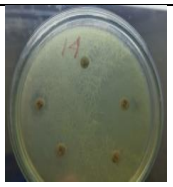
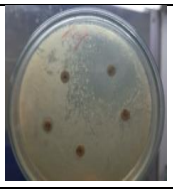
Sample code	Voriconazole (1µg)	Itraconazole (8µg)	Miconazole (10µg)	Fluconazole (25µg)	Ketoconazole (15µg)
	Zone size diameter (mm)				
AZI001B/1	6(R)	0(R)	9(R)	7(R)	5(R)
AZI001B/2	0(R)	20(I)	15(R)	0(R)	0(R)
AZI002B/1	0(R)	0(R)	0(R)	0(R)	0(R)
AZI002B/2	14(I)	0(R)	0(R)	0(R)	0(R)

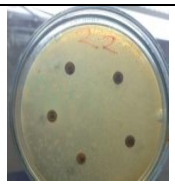
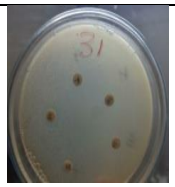
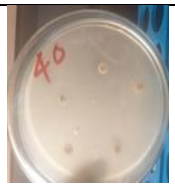
AZI006B/1	7(R)	6(R)	0(R)	6(R)	6(R)
AZI006B/2	8(R)	0(R)	0(R)	0(R)	10(R)
AZI008B/1	6(R)	8(R)	27(S)	6(R)	8(R)
AZI013B/1	0(R)	0(R)	0(R)	0(R)	0(R)
AZI015B/1	0(R)	20(I)	16(R)	20(S)	18(R)
AZI015B/3	0(R)	11(R)	13(R)	0(R)	0(R)
AZI018B/1	0(R)	0(R)	0(R)	0(R)	10(R)
AZI019B/1	0(R)	0(R)	17(R)	0(R)	0(R)
AZI021B/1	0(R)	0(R)	10(R)	0(R)	0(R)
AZI022B/1	6(R)	12(R)	0(R)	13(R)	0(R)
AZI023B/1	0(R)	14(R)	15(R)	13(R)	15(R)
AZI023B/2	0(R)	0(R)	0(R)	0(R)	0(R)
AZI026B/1	0(R)	0(R)	0(R)	8(R)	0(R)
AZI027B/1	0(R)	0(R)	25 (I)	25(S)	0(R)
AZI028B/1	0(R)	19(I)	0(R)	25(S)	22(S)
AZI029B/1	0(R)	0(R)	6(R)	8(R)	8(R)
AZI030B/1	0(R)	0(R)	0(R)	0(R)	0(R)
AZI031B/1	0(R)	8(R)	10(R)	0(R)	10(R)
AZI031B/3	0(R)	0(R)	0(R)	0(R)	0(R)
AZI032B/1	0(R)	0(R)	0(R)	0(R)	0(R)
AZI033B/2	0(R)	0(R)	0(R)	0(R)	0(R)
AZI034B/1	0(R)	0(R)	0(R)	0(R)	6(R)
AZI035B/1	10(R)	10(R)	7(R)	10(R)	7(R)
AZI036B/1	0(R)	0(R)	12(R)	19(S)	6(R)
AZI036B/2	0(R)	0(R)	0(R)	6(R)	7(R)
AZI036B/3	0(R)	0(R)	0(R)	0(R)	0(R)
AZI037B/1	6(R)	0(R)	8(R)	8(R)	7(R)
AZI038B/1	0(R)	6(R)	6(R)	0(R)	0(R)
AZI039B/1	7(R)	0(R)	0(R)	0(R)	10(R)
AZI039B/2	0(R)	0(R)	0(R)	0(R)	0(R)
AZI041B/1	6(R)	6(R)	6(R)	0(R)	0(R)
AZI041B/4	6(R)	0(R)	0(R)	0(R)	0(R)
AZI042B/1	6(R)	7(R)	0(R)	0(R)	7(R)
AZI042B/2	0(R)	15(R)	0(R)	13(R)	0(R)
AZI042B/3	0(R)	0(R)	0(R)	0(R)	0(R)
AZI043B/1	9(R)	9(R)	0(R)	0(R)	15(R)
AZI044B/1	0(R)	20(I)	15(R)	19(S)	13(R)
AZI044B/2	0(R)	10(R)	0(R)	0(R)	0(R)
AZI045B/1	0(R)	22(S)	20(R)	0(R)	25(S)
AZI047B/1	0(R)	0(R)	7(R)	7(R)	8(R)
AZI048B/1	0(R)	6(R)	6(R)	6(R)	7(R)
AZI049B/1	0(R)	23(S)	0(R)	26(S)	22(S)

AZI050B/1	6(R)	0(R)	6(R)	0(R)	0(R)
AZI050B/2	0(R)	0(R)	0(R)	0(R)	0(R)

All the fungal isolates were tested for five antifungal agents. The zone of diameter was measured and comparative table from Himedia datasheets were used. Above 80% of the isolates were found to be resistant to Miconazole MIC (10 µg), Fluconazole FLU (25 µg), Ketoconazole KET (15 µg), Itraconazole ITR (8 µg) and Voriconazole VOR (1 µg) (Table 2).

Table 2 - Antifungal susceptibility test of Pune food sample isolates

Sample No.	**MIC (10 µg)**	**FLU (25 µg)**	**KET (15 µg)**	**ITR (8 µg)**	**VOR (1 µg)**	**Photo**
	Zone size diameter (mm)					
1	7 (R)	0(R)	0(R)	19 (I)	0(R)	
2	8 (R)	0(R)	0(R)	25(S)	0(R)	
3	16(R)	0(R)	0(R)	45(S)	0(R)	
5	15(R)	7(R)	0(R)	50(S)	0(R)	
6	15(R)	0(R)	0(R)	40(S)	0(R)	
7	11(R)	0(R)	0(R)	21(S)	0(R)	
8	8(R)	0(R)	0(R)	39(S)	0(R)	

9	11(R)	0(R)	0(R)	25(S)	0(R)	
10	7(R)	0(R)	0(R)	48(S)	0(R)	
11	0(R)	0(R)	0(R)	0(R)	0(R)	
13	0(R)	0(R)	0(R)	0(R)	0(R)	
14	0(R)	0(R)	0(R)	0(R)	0(R)	
15	12(R)	0(R)	0(R)	40(S)	0(R)	
16	0(R)	0(R)	0(R)	0(R)	0(R)	
19	0(R)	0(R)	0(R)	14(R)	0(R)	
24	12(R)	0(R)	0(R)	10(R)	0(R)	
27	0(R)	0(R)	0(R)	7(R)	0(R)	
28	8(R)	10(R)	0(R)	40(S)	0(R)	
29	0(R)	0(R)	0(R)	10(R)	0(R)	
30	11(R)	0(R)	0(R)	45(S)	0(R)	

31	9(R)	0(R)	0(R)	45(S)	0(R)	
32	0(R)	0(R)	0(R)	0(R)	0(R)	
33	0(R)	0(R)	0(R)	0(R)	0(R)	
35	0(R)	0(R)	0(R)	14(R)	0(R)	
40	0(R)	0(R)	0(R)	35(S)	0(R)	
42	0(R)	23(S)	0(R)	0(R)	0(R)	
45	0(R)	0(R)	0(R)	0(R)	0(R)	
46	0(R)	0(R)	0(R)	0(R)	0(R)	
48	0(R)	0(R)	0(R)	0(R)	0(R)	
50	0(R)	0(R)	0(R)	0(R)	0(R)	
79	7(R)	9(R)	0(R)	9(R)	7(R)	
81	0(R)	0(R)	0(R)	0(R)	0(R)	
82	0(R)	0(R)	0(R)	0(R)	0(R)	
84	0(R)	0(R)	0(R)	0(R)	0(R)	
100	0(R)	0(R)	0(R)	0(R)	0(R)	
105	0(R)	0(R)	0(R)	23(S)	0(R)	
107	0(R)	0(R)	0(R)	0(R)	0(R)	
109	0(R)	0(R)	0(R)	0(R)	0(R)	
110	0(R)	0(R)	0(R)	0(R)	0(R)	
113	0(R)	27(S)	0(R)	0(R)	0(R)	

116	0(R)	0(R)	0(R)	29(S)	0(R)	
136	0(R)	0(R)	0(R)	0(R)	0(R)	
143	0(R)	0(R)	0(R)	0(R)	0(R)	
167	0(R)	0(R)	0(R)	0(R)	0(R)	
192	10(R)	12(R)	7(R)	27(S)	13(R)	
196	10(R)	20(S)	0(R)	24(S)	22(S)	
210	10(R)	25(S)	8(R)	26(S)	25(S)	
235	12(R)	10(R)	0(R)	37(S)	12(R)	
238	10(R)	10(R)	8(R)	34(S)	7(R)	
263	11(R)	20(S)	7(R)	21(S)	21(S)	
266	11(R)	28(S)	8(R)	14(R)	28(S)	

298	11(R)	25(S)	10(R)	17(I)	26(S)	
300	8(R)	22(S)	7(R)	25(S)	20(S)	
302	0(R)	12(R)	0(R)	25(S)	20(S)	
305	8(R)	15(I)	0(R)	23(S)	18(S)	
306	7(R)	8(R)	7(R)	25(S)	10(R)	
317	10(R)	20(S)	7(R)	13(R)	28(S)	
319	10(R)	20(S)	7(R)	13(R)	28(S)	
321	10(R)	20 (S)	8(R)	17(I)	25(S)	
324	10(R)	18 (I)	10(R)	16(I)	18(S)	

Interpretation of Table-2

These results indicate that heavy contamination of food borne pathogen in food samples and these pathogens have shown multi-drug resistant. Thus, this report becomes a novel study

representing huge amount of statistically analysed data giving critical information regarding the antifungal resistance shown by food borne pathogens in India.

As we can see in the above table, most of the isolates have showed resistance to antibiotics and antifungal agents. This is serious in the sense that if the food poisoning happens through these food items supplied by food vendors, these harmful pathogens are not likely treatable using antibiotics or antifungal agents. This can lead to increase in mortality rate due to food poisoning.

6.3.5 Statistical analysis

Interpretation of Table-3

From Table 3, it can be seen that the antifungal susceptibility test showed maximum resistance in Lucknow food pathogenic fungi was found for Voriconazole (97.9%), Miconazole (95.8%), Ketoconazole(93.8) and (87.5%) for both Itraconazole and Fluconazole. For Pune pathogenic fungi, maximum resistance was seen 100% for both Miconazole and Ketoconazole while Voriconazole (80.3%), Fluconazole (78.3%), Itraconazole (52.5%) was observed. These statistical data confirm that most of the isolated pathogens were resistant to more than 50% of the antifungal agents tested.

Table – 3: Statistical analysis of antifungal susceptibility test of Lucknow and Pune food fungal isolates

Place	Result	VOR (1 µg)		ITR (8 µg)		MIC (10 µg)		FLU (25 µg)		KET (15 µg)	
		N	%	N	%	N	%	N	%	N	%
Lucknow (N=48)	Resistance	47	97.9	42	87.5	46	95.8	42	87.5	45	93.8
	Sensitive	1	2.1	4	8.3	1	2.1	0	0.0	0	0.0
	Intermediate	0	0.0	2	4.2	1	2.1	6	12.5	3	6.3
Pune (N=61)	Resistance	49	80.3	32	52.5	61	100.0	48	78.7	61	100.0
	Sensitive	0	0.0	4	6.6	0	0.0	2	3.3	0	0.0
	Intermediate	12	19.7	25	41.0	0	0.0	11	18.0	0	0.0

The Logistic Regression Analysis to find relationship of Fungus growth with significant factors revealed there was high risk of growth with those who had higher income, vending site nearby market area, site with heavy traffic, higher no. of workers, served before 1 hr prepared food, food served on paper plate, water not changed, food handling is wearing gloves, cloth is the same for everything, frying in oil once, not heard about food poisoning, eating food carry from home and touching nose during handling food as the corresponding beta coefficient for these categories were either positive or negative for their conjugate.

7. Recommendations

It is recommended that local government authorities should take strict actions if food vendors do not follow food regulations, food borne awareness campaigns and improvement of food borne surveillance to prevent and stop food borne disease. New rapid techniques should be developed to identify and treat antifungal resistance so that spread of fungal infections is restricted.

8. Conclusion

In this study, 374 food samples from food vendors from Lucknow and Pune were analysed. It was observed that food samples were contaminated with 109 pathogenic fungi in total and they were belonging to genera *Aspegillus, Mucor, Rhizopus, Rhizoctonia soloni* and *Penicillium*. It was observed that majority of the isolated pathogens were resistant to more than 50% of the antifungal agents tested. These results suggest that these pathogens are harmful in nature and can cause food poisoning in mass public.

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