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EVALUATION OF FUNGAL PATHOGEN AMONG VARIOUS TYPES OF FOOD MATERIALS IN RELATION TO COOKING OR PRESERVATION

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AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Many contaminated foods are one of the sources of food-borne diseases which have an incredible impact on health and survival. Fungal contamination of food may be one of the most persistent and frequently recognized causes of these diseases. In the present study, the effect of cooking and refrigeration methods were used for the food items like vegetables, eggs, and meat to detect the growth of food-borne fungal pathogens. A set of each of the food items was kept raw at room temperature as the control sample. A total of nine fungal species viz Aspergillus flavus, Aspergillus fumigates, Aspergillus niger, Aspergillus nidulance, Pencillium verrucossum, Mucor spp, Rhizopus nigricans, Syncephalastrum recemosum, and Mycelia sterile were isolated and identified according to their morphological characters. Species wise relative abundance was highest for Aspergillus flavus followed by Aspergillus niger and Aspergillus fumigates. All the isolates present in this study are potential fungal pathogens that are capable of causing serious human hazards.

Keywords: Food borne disease; fungal contamination; aspergillus sp.; fungal pathogen.

1. INTRODUCTION

Foods-borne diseases are an emerging public health problem in developed and developing countries. The term "Food Borne Diseases" encompasses a variety of clinical ad etiological conditions and described the subject of enteric diseases. Food-borne diseases are the major cause of illness and death worldwide and it has been estimated that up to 6 million (1 in 10 people) of the population suffer a food-borne illness each year [1].

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Reports validate that, bacteria and fungi are the two most common pathogens that cause food spoilage and 250 different varieties of food-borne health issues in human. in the U.S. every year 24 million to 81 million cases of food poisoning are reported, costing an estimated 5 billion to 17 billion in medical care and lost economic productivity [2]. Resilient bacteria such as Salmonella, E.coli, Listeria Monocytogenes and Cyclospora cayetaner, and fungi like Aspergillus, Pencillium inseminate themselves into fruit, vegetables, poultry, beef, and dairy product as they circulate around the globe and generating trade related infection [3].

Fungal contamination of food may be one of the more pervasive and seldom recognized causes of disease Mycotoxins are natural, secondary metabolites produced by fungi on agricultural commodities in the field and during storage under a wide range of climatic conditions. About 200 different filamentous fungi species, e.g. Aspergillus, Penicillium and Fusarium species (sp.), have been identified [4]. Mycotoxins can cause acute and chronic illness, induce cancer, and damage vital organs such as the liver, kidney, and brain. Aspergillus is a ubiquitous fungus that can fatally infect patients with reduced immunity [5]. The common fungi, which grow on food, even in the refrigerator are Pencillium, Aspergillus, and Claviceps [6]. Over 15 thermogenic mycotoxins have been isolated from these fungi. Aflatoxins, Ocharotoxins, and Patulin are some of the important mycotoxins [7].

In 2011 Patna university of India presented a project on fungal growth on boiled food items like chappathy, dal, chicken, and mixed vegetables in the International Conference on Biotechnology and Food Science. Food-borne illness is caused by consumption of food contaminated with infectious and toxigenic microbes is a major cause of death throughout the world. The past few decades have witnessed massive food-borne pathogens. The emergency food-borne pathogens are due to animal feeding practices, change in food technology and lifestyle, consumer demand, and an ever-growing number of populations. Microbial hazards have also emergency because of changes in food consumption such as eating of fresh fruit and vegetable and also recent interest of consumers in the western style of cooking, while it is not fit for tropical environment [8]. In developing countries, only poor hygienic precautions are taken during food handling. Studies on food-borne illness are very few. While information would suggest this fungal contaminant poses little risk to consumers, our results show that it is capable of causing significant infections in human beings. Hence the present study and its findings are of great importance.

2. MATERIALS AND METHODS

The study was conducted to find out the prevalent fungal pathogens associated with commonly used food items such as eggs, meat, and vegetables. The effect of cooking and refrigeration on microbes was also recorded during the study. The food samples were collected from shops in Kottayam, Kerala, India on February 10, 2014. The collected samples such as vegetables, meat, and egg were divided into three equal parts; one gram each was kept raw, refrigerated at 4°C for 24 h and other cooked for 20 minutes at 100°C Purification of cultures was done by preparing the cultures on Sabouraud Dextrose Agar (SDA). To inhibit the bacterial growth 500 µg/ ml each of penicillin and streptomycin was added to SDA plates. Initially, all three samples were inoculated into SDA broth and incubated for 7-10 days. Further, all the samples were inoculated into SDA, and incubation was done aerobically for 7-10 days at 28±2°C. All the experiments were carried out for three replications. Slides were prepared according to [9] by taking material from each colony and staining with 0.05% trypanblue in lactophenol. The slides were observed under a Digipro-labomed microscope and photographed. The fungi were identified with the help of available fungal identification keys and literature12. The samples were surfaced sterilized with 70% ethanol and rinsed with three changes of sterile distilled water. A 10 g of each sample were cut with sterile forceps, macerated aseptically in a mortar, and mixed in 10 ml of sterile peptone water. From this mixture, further tenfold dilutions were made up to 103, and 0.1 milliliters of each dilution was plated in triplicate on SDA supplemented with streptomycin to inhibit bacterial growth. Plates were incubated at 28 ± 2◦ C and examined daily for 7 days. The mean number of all fungal colonies appearing in the three plates was taken as the average number of colonies per plate for the sample. This was helped to calculate the relative frequency of fungus isolated from samples [10]. The relative abundance (%) was calculated by the following equation [11].

Frequency = Number of isolates of a genus or species / Total number of fungi isolated

3. RESULTS

The microbial evaluation of prevalent fungal pathogens among various types of food materials about cooking or preservation has been conducted. In general, a total of 9 species of fungal including potential pathogens were isolated (Table 1). Among the isolates, Aspergillus shows the highest frequency of occurrence (Table 1) and the entire tested sample fetches at least one fungal colony. Genus-wise

isolation of fungal pathogen showed that Aspergillus has the highest share followed by Pencillium, Mucor, Rhizopus, and Synchephalastrum in almost same (Table 1). Both frequencies of occurrence and species-wise relative abundance were recorded highest for Aspergillus flavus followed by Aspergillus niger and Aspergillus fumigates. The comparison on the pathogenic diversity in different tested food showed that the highest diversity in beef followed by egg and the least diversity observed in lady's finger (Table 2). Aspergillus showed the highest frequency of occurrence in all the three tested food substances but from egg and beef, few others have been isolated (Table 3).

The sterilization effect of cooking and preservation by freezing were also evaluated. In the case of all food processing methods, it was found that cooking is much effective to reduce the pathogenic diversity to a considerably low level (Table 3). However, it is interesting to note that freezing is not effective to reduce the pathogenic invasion but ironically in the case of beef and lady's finger the pathogenic diversity increased after freezing (Table 3). For fungal pathogens cooking is effective to remove around all pathogenic fungi compared to other methods such as freezing and keeping at normal room temperature (raw). But in the case of egg and beef cooking promotes the growth of several fungal pathogens.

4. DISCUSSION

Food is one of the most important transmission routes of diseases globally due to microbial contaminations [12]. They are burgeoning on an alarming scale throughout the world; currently, more than 2 million deaths occur every year in developing countries due to foodborne diseases [13]. Trends in global food production, processing, distribution, and preparation present new challenges to food safety. The new condition that is borne pathogen is challenges in animal husbandry, changes in international trade and lifestyle, and consumer changes. Tackling the issues of food is without doubt one of the major challenge with regard to the protection of human health. There is an urgent need for more systemic and aggressive steps to be taken to reduce the risk of food-borne disease. The present study was conducted for the analysis of fungal pathogen in egg, meat, and vegetable in three conditions and revealed that the food contains relatively less number of pathogenic fungi. Out of which Aspergillus flavus showed the highest rate of growth followed by Aspergillus fumigates and Aspergillus niger. Although the A. flavus was isolated from all the three food substances and is thermotolerant which can thrive below 5°C. [14,15], reported that A. flavus was isolated from foodstuff and poultry feed cause hepatocellular carcinoma in humans. It is the 2nd abundant fungus that causes aspergillosis [16].

Aspergillus fumigates showed the most important one which causes aspergillosis [17,18]. The highest occurrence of A. fumigates was reported in beef. [19] reported that A. fumigates isolated from polluted meat and seedling of vegetables. It can cause human and animal aspergillosis infecting pulmonary, bone, ocular, cardiovascular, nasal, and deep organ disease; the organism is particularly angioinvasive in the immunocompromised host [20,21]. A. niger isolated from vegetable and egg samples having an isolation rate of about 48%. Aspergillus flavus, A. parasiticus, A. fumigatus, A. niger, and Penicillium species were the predominant moulds isolated from both processed meat products and spices [22]. Incidence of aflatoxins in other food products such as smoked-dried fish, dried meats, dried yam chips, has been reported as well [23]. The mycological examination carried out in the current work revealed the isolation of named six genera, Aspergillus spp., Penicillum spp., Fusarium, Rhizopus spp., and Mucor spp. which agree with that obtained by [24] and [25]. For a very less percentage Aspergilus nidulance were isolated from the meat sample. Pencillium verucossum was isolated from the egg is also a pathogenic fungus and the fungal species can live in refrigerated conditions. [26] reported that P. verucossum was also isolated from foodstuff. P. verucossum produce ocharotoxin frequently found in pork and beef intended that in Africa and Somalia nations the consumption of cereals contaminated by P. verocossum cause neurotoxic, immunotoxic, and nephrotoxic effect on human and which cause kidney disorder called Balkan Endemic Nephropathy [27]. [28] reported that A. flavus, A. niger, A. candidus, A. fumigatus, A. nidulans, A. glaucus, Alternaria spp., Penicillium spp. And Rhizopus could be isolated from fresh meat, cold-stored meat, and even in undercooked meat [29, 30] also reported the presence of Aspergillus, Penicillium, Cladosporium, Rhizopus, Mucor, and Fusarium species in frozen meat samples in the following frequencies ((51.1%), (44.6%), 26.1%), (22.9%), (18.5%).

The present study revealed that cooking and freezing are not only effective to make food substances sterile. It should be noted that freezing contributed more pathogenic invasion in the entire three samples tested. The fungi showed an increased rate of isolation in a frozen state. In the case of beef, egg, and vegetables the pathogenic diversity increased after freezing, the low temperature favors the growth of the same fungal groups. [31] reported the ability of reproduction of Cladoporium sp. in frozen meat and produce St.Anthony's fire in humans. In this experiment,

freezing promotes the growth of fungi in all three food samples. P. verrucosum, Mucor, Rhizopus, etc are isolated after freezing shows a high risk on food. The production of mycotoxins contaminates food and thereby causes serious diseases in humans. The sterilization effect of cooking was also evaluated and shows that in the case of all food substance tested cooking is much effective, that reduce the pathogenic diversity to a considerably low level for fungal pathogens. Cooking almost removes all potential fungal pathogens from food items and at high temperatures spores are killed so that they could not survive at all. The cooking provides different levels of temperature at various regions of the same food which may cause the killing of microorganisms [32] reported that improper cooking leads to fungal contamination on food items. The present study revealed that most of the food substances available in the market are highly contaminated with microorganisms. Moreover, the usual preservation practices are insufficient, which in turn enhances the pathogen diversity. But at the same time, normal cooking techniques are also not totally safe. Hence more scientific preservation and cooking practices should be implemented and due care should be given in hygienic status regarding food preservation.

Table 1. List of fungal pathogens isolated from various food items (n=36)

Sl no.	Fungi	Frequency of occurrence (%)
1	*Aspergillus flavus*	60.00
2	*Aspergillus fumigates*	31.00
3	*Aspergillus niger*	48.00
4	*Aspergillus nidulance*	8.00
5	*Pencillium verrucossum*	41.00
6	*Mucor spp*	25.60
7	*Rhizopus nigrican*	11.13
8	*Syncephalastrum recemosum*	8.00
9	*Mycelia sterila*	8.00

Table 2. Source wise analysis of fungal pathogens (n=36)

Sl no.	Food items	List of fungi	Frequency of occurrence
1	Beef	*A. flavus*	25.00
		A.fumigates	41.66
		A. nidulance	8.33
		R. nigrican	13.88
		Mucor spp	16.11
		M.sterila	8.33
2	Egg	*A.flavus*	30.50
		A.niger	22.50
		P.verrucossum	16.11
		Mucor spp	8.33
3	Vegetables	*A. flavus*	13.88
		A. niger	8.33
		S. recemosum	8.33

Table 3. List of fungal pathogens isolated with respect to cooking and preservation

Sl no.	Food items	Fungi isolated		
		Raw	Freezed	Cooked
1	Beef (n=4)	*A. flavus* *A. fumigates* *A. nidulance* *M. sterila*	*R. nigrican Mucor sp* *A. fumigates*	*R.nigrican Mucor sp*
2	Egg (n=4)	*A. flavus Mucor sp* *A. niger*	*A. flavus* *A. niger* *P.verrucossum*	
3	Lady's finger (n=4)	*A. flavus* *A. niger* *S. recemosum*	*A.flavus A.niger*	

5. CONCLUSION

Food-borne transmission is considered as emerging worldwide, more especially in developing countries where poor sanitation and hygienic environment is existing. All the tested food items showed a high level of fungal contamination including vegetables and non-vegetable items. Out of the three tested food samples, meat showed the highest rate of fungal isolates followed by egg and the Lady's finger. The preservation through freezing and normal cooking practices is found insufficient to make the food items sterile too. Freezing promotes the growth of mycoflora on food items due to the moisture content. Proper cooking kills most of the fungal pathogens successfully. Hence the present finding is very significant both from the academic and public point of view. However, more detailed studies are essential in order to find out the contamination status of different food items and related diseases among the human population. Hence considering the public health significance more elaborate studies are recommended.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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