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Identification and analysis of major street fast food borne pathogenic fungi and its sensitivity to nystatin

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Abstract
Street foods consuming is available in Bangladesh, mainly in tea stalls and among the fast foods bread, pates, salty biscuit, cake, sweet biscuit, chatpati and pickle are popular. As these popular foods are available at street side, these are prone to have microbial attack. This study was conducted to determine the presence of pathogens responsible for food spoilage by inoculating samples into Potato-Dextrose-Agar (PDA) media. Three replications of fungi in every seven days of interval were made using the PDA media. In each replication several types of fungi were obtained. Later on evaluating the intensity of the microbes we undertook pure culture and got *Aspergillus flavus, Aspergillus niger* and *Fusarium* spp. generally which are considered as the most frequently growing fungi which were properly characterized. Then anti-fungal sensitivity was tested using an anti-fungal agent Nystat (100,000 units/ml). *Aspergillus flavus, Aspergillus niger* and *Fusarium* spp. were collected from pure culture media and it was found that *Aspergillus flavus* and *Fusarium* spp. as resistance to the aforementioned anti-fungal agent while *A. niger* remained sensitive to that agent. Isolation of major typical toxins mainly the aflatoxins and analysis of their molecular modeling using bioinformatic tools might be an epoch making event for us in ensuring food safety for all and new drug designing in future.

Keywords: food toxicity, Aflatoxins, Anti-fungal agent, culture media.

1. Introduction
In case of street fast foods health benefits, food safety etc are the buzzwords now days in case of both the industrialist and non-industrialist countries. Consumer rights and food safely issues are maintained strictly in the developed countries where, in some extent the issues aren't kept in mind on the perspective of the developing and poor countries. The matter of great regret is that, international monitoring to the developed, developing and poor countries is quite discriminatory to the aforementioned issues. In the third and fourth world countries, people are not so conscious to get the best. Bangladesh being a third world developing country, food safety regulations are not followed in most of the cases. Foods are often considered as the vector of most of the pathogens for stomach toxicity and gastrointestinal diseases [1]. Diarrheal disease is a leading killer of adults and children not only in India but also in Bangladesh. Where tracking the food borne sources of infection will be a smart way to that concern salvation [2]. To carry the consistently increased fast food demands where majority come from the bakeries when, authority add different types of emulsifiers, flavoring agents, coloring agents, imbalanced nutrients and few other food additives which reflects their intention to intensify the taste only rather than hygienic factors. Hypervitaminosis A and microbial pathogens are such serious effects that can cause serious bone pain, dizziness, headache, double vision, gastric mucosal calcinosis [3], heart valve hypercalcemis, calcification [4], liver damage [5-7]. Often both the acute and chronic toxicity suffers the people of all walks of life in Bangladesh. In the same way ataxia, lethargy and copper deficiency as the consequence of food zinc toxicity, stomach and nerve disease as the bulk of chemically synthesized colors from aniline [8], neurotoxicity for methiocrab [9] etc. are common phenomena in the perspectives of Bangladesh. In the following way, mycotoxins are secondary metabolites (or natural products) and research has established the existence of biochemical pathways solely for the purpose of producing mycotoxins and other natural products in fungi [10]. Mycotoxins like aflatoxin from *Aspergillus flavus*, lethal amatoxins in some *Amanita* mushrooms etc. are promoting new type of health concern for the people of Bangladesh because her environment is quite suitable to nurture all sorts of microbes, considered as very harmful for health [11].

The aflatoxins, which are insidious liver toxins and highly carcinogenic metabolites produced by certain *Aspergillus* species often growing in or on grains and nuts consumed by humans, ochratoxins, patulin, and trichothecenes (e.g., T-2 mycotoxin) and fumonisins, which have significant impact on human food supplies or animal livestock [12]. Not only food toxicity and stomach disorders are the concerns of the Bangladeshi people but also food spoilage and economic losses are very crucial for the people because around 13% businessmen are directly or indirectly involved in marketing bakery foods and some other local fast foods as the main street foods of Bangladesh as we have about 40% street fast food consuming people. For instance, food spoilage due to the infection of different species of *Fusarium, Stemplylium, Alternara, Aspergillus, Penicillium,* and a number of bacterial strains like *Escherichia coli, Vibrio cholera, Salmonella, Streptococci, Staphylococci etc.* This also gets an influence in our GDP. Now it is time to reveal the secrets of all sorts of food toxicity, food spoilage as well as economic losses and to establish the consumer rights to choice the best for them ensuring the food safety issues successful for all. The research is undertaken to identify different types of fungi responsible for food toxicity and to detect their sensitivity to an antifungal agent Nystatin.

2. Materials and methods

Seven food samples (patis, salty biscuit, cake, bread, sweet biscuit, pickle and chatpati) were collected from various locations where bakery foods and individually produced foods are sold by the street fast food vendors. A lab was selected and designed belonging to the Plant Pathology Laboratory of Bangladesh Agricultural Research Institute (BARI) in Jessore to perform the culture, isolation, identification, characterization and sensitivity detection of the fungi to particular anti-fungal agent, collected from the food samples.

2.1 Culture replications of the pathogens

Potato-Dextrose-Agar (PDA) media was prepared and preserved in the refrigerator for rapid growth of the fungi. The samples were taken in the *in vitro* glass chamber for 3 days at room temperature so that the pathogens can get some access to contaminate foods by the open air allowed. Afterwards, the samples were taken to the laminar air flow (LAF) to inoculate them into the growth mediums as a step of the first replication (Figure 1).

Fig 1: Seventh day of the first replication of fungi from food samples.

Single replication is not enough to get fungi from all food samples because there is possibility of adulteration of the street fast foods. Considering the possibilities of the presence of typical preservatives, the fungi from the first replication are transferred to next replication. After seven days the reading of the fungal growth were taken in the nutrient medium for second replication (Figure 2).

Fig 2: The presence of a number of fungi in the second replication.

Following the next seven days of the second replication, it was taken into LAF to undergo replication number three. This is a matter of interest that, in the third replication six samples were infected. Few samples remained unchanged which, notifies that the items chosen for consuming in the workplace are not free from risk because there may be contents of high synthetic preservative (Figure 3).

Fig 3: The third replication demonstrates the fungal inoculation in the samples remarkably.

2.2 Pure culture preparation

Few fungal species were taken into observation to undertake pure culture to get any fresh unique pathogen. Three most growing pathogens from the samples such as *Aspergillus flavus, Aspergillus niger* and *Fusarium* spp. were taken for getting them as pure cultures. Both are spore forming. *Aspergillus flavus* creates the toxin named 'Aflatoxin' for food poisoning and it covered the media plate with its deep green appearance (Figure 4) while *Fusarium* spp. is cotton shaped and white (Figure 5).

Fig 4: Pure cultured of *A. flavus*.

Fig 5: Pure cultured of *Fusarium* spp.

2.3 Anti-fungal sensitivity testing

In this experiment anti-fungal agents are applied to determine the response of the fungi to the agents. To analyze the pathogenic status of the fungus we used 0.5 ml of Nystat which contains an anti-fungal agent Nistain at 100,000 units/ ml. Three fungi *Aspergillus flavus*, *Aspergillus niger* and *Fusarium* were elected to run the process of anti-fungal sensitivity testing.

3. Results

Significant presence of *Fusarium, Aspergillus flavus* and *Aspergillus niger* was found in the culture from the sample of Chatpati and sweet biscuits. *Aspergillus candidus* and *Penicillium* spp. were present in patice and sweet biscuits. Pickles and cake samples were also found with *Aspergillus flavus* and *Aspergillus niger*, salty biscuits showed the presence of *Fusarium* only where bread was free of any fungal contamination.

Table 1: Presence of different mycoflora in street food items from different locations of Bangladesh

Name of the samples	*Fusarium*			*Aspergillus* Spp.												*Penicillium* spp.			*Stemphylium* spp.			*Alternaria* spp.			Bacteria Strains		
				Aspergillus flavus			*Aspergillus candidus*			*Aspergillus niger*			*Aspergillus parasiticus*														
	R1	R2	R3	R1	R2	R3	R1	R2	R3	R1	R2	R3	R1	R2	R3	R1	R2	R3	R1	R2	R3	R1	R2	R3	R1	R2	R3
Chatpati	+	+	+	-	+	+	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+
Patice	+	-	-	-	-	-	+	+	+	-	-	-	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-
Sweet Biscuits	+	+	-	+	+	+	-	-	+	-	+	+	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-
Salty Biscuits	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Pickles	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	_
Cake	-	-	+	-	-	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+	_
Brade	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	_

Aspergillus flavus and Fusarium gave negative result in food safety issues with the properties of having no sensitivity to Nystatin (Figure 6). Aflatoxins are the means of most of our food toxicity. *Aspergillus flavus* is responsible for synthesizing these mycotoxins.

Fig 6: *Aspergillus flavus* and *Fusarium* showing no sensitivity to Nystatin in a mixed culture.

Aspergillus niger could not survive due to its sensitivity to Nystatin (Figure 7).

Fig 7: Positive sensitivity of *Aspergillus niger* to the anti-fungal agent Nystatin.

4. Discussion

We carried our research out in search of microbes in the popular and available street foods of Bangladesh. After collecting the samples we made three times replication of the fungi in PDA media inside the Laminar Air Flow (LAF) facility and other controlled aseptic conditions. On each replication with 7 days of interval we took data of microbial species. After the completion of a proper data analysis we inoculated fungi for pure culture and finally we observed the

antimicrobial status of few of the fungi collected and cultured from the replications. In resultant, we got *Aspergillus flavus* and *Fusarium spp.* were resistance to the anti-fungal agent called Nystatin while *Aspergillus niger* was not. Spoilage of fresh foods is limited to particular species. Such specific relationships between fresh food and fungi are mentioned in research purposes because susceptibility of foods varies to the fungal infection. There are number of fungi that are substrate specific [13]. In most of the cases fungal infection is more identical than others [14].

We got few major groups of pathogens such as *Aspergillus flavus* and *Fusarium spp.* Mycotoins are the main concerns of their presence. The mycotoins as diversified secondary metabolites synthesized by the pathogens like the *Aspergillus spp*. and few others.

The presence of fungi is really harmful for health due to having toxigenic effect on health. The presence of *Fusarium spp.* reflects the probable damage of foods mainly the street fast foods. Four types of toxigenic fungi have elected for crop's toxicity- *Fusarium graminearum* (as plant pathogen), *Fusarium moniliforme* and *Aspergillus flavous* (as mycotoxin producers in senescent or stressed plants) [15], where *Aspergillus flavus* and *Fusarium spp.* both are present in street fast food samples we derived.

In our research the extensive presence of *A. flavus* can be alarming to secret aflatoxins, might be very horrible for the agro-economy of a country. Studies conducted regarding the losses of agro-products not only from the street fast foods but also the ingredients of street fast foods like groundnut and ground products due to fungal infection [16-19]. The risk is always strong when fungal pathogens are available in our fast foods as we got in our research because the toxic effect of mycotoxins can be significant and varied depending on the toxin, dose, host and food matrix involved. The effects include- carcinogenicity (cancer causing) especially in the liver, hepatotoxocoty (liver damage), mutagenicity (changes to DNA), toxic effects including kidney disease [20], immumo-suppression and disturbance to the nervous and hormonal systems. These could be of great hazard for all.

We experienced huge fungal mycelium on the surface of the food samples. We then compared that with the research [21] stated that fungi tend to grow on the surface of objects in the shape of a visible mycelium made up of a number of cells. Ochratoxin A (OTA) producing species *Aspergillus spp.* are known to be a potential source of OTA contamination of cereals and certain common foods and beverages such as bread, beer, coffee, dried fruits, grape juice and wine [22].

At the same way we wanted to analyze fungal presence with the assistance of other research [23]. They establish the idea that very limited fungal strains iare responsible for the spoilage of each kind of food. We re-establish the concept that the presence of any type of fungal pathogen is alarming for human food safety especially for the street fast food consumer. Mycotoxins may cause mycotoxicosis which can result in an acute or chronic disease episode.

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