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Fungal contamination of foods prepared in some hotels in the Kumasi metropolis

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Abstract

There is an increase of food borne diseases in Ghana and therefore a lot of street food studies have been conducted in Kumasi, the Ashanti Region of Ghana with less information on microbiological safety of hotel foods. A total of forty food samples were aseptically collected from five highly patronised hotels (three star to budget). The hotels were selected by simple random sampling. Standard methods were used for the dilution, spreading, incubation, enumeration and identification. Serial dilution of each food was prepared in buffered peptone water and inoculated onto malt extract agar (MEA). Growth were counted and later identified. The bacterial counts were expressed to log10 cfu/g. Two foods from Hotel 01 (fresh pepper sauce and chicken and vegetable sauce) and six foods from Hotel 02 (chicken with noodles and vegetables, jollof rice, fried rice, potato chips, beef in vegetable sauce and coleslaw) were above the WHO acceptable levels (< 3 Log 10 cfu/g). Again, 4 foods from Hotel 03 (boiled plain rice, fish light soup, tossed mixed vegetables and tossed salad), Hotel 04 (vegetable sauce, fried chicken, mixed salad and fried rice) and Hotel 05 (goat light soup, fried chicken, fried rice and mixed vegetable salad) were all above the WHO acceptable levels. Fungi isolated were *Eurotium herbariorum, Aureobasidium pullulans, Alternaria alternate, Botrytis cineria* and *Fusarium oxysporu*. It was observed that foods tested were above the acceptable levels and could be sources of food borne pathogens. The causes could be attributed to poor food hygiene and inadequate processing. It is recommended that hotel inspections should include microbial test on foods.

Introduction

Studies on microbiological safety of street foods have been conducted in several parts of Ghana, especially in the capital cities but not much has been done with regards to hotels. The security attached to the hotels is much stronger and makes it difficult to enter their kitchens and make enquiries about their food preparation resulting in less information about microbial food safety in hotels in Ghana. The production of safe and quality meals with no microbial growth in hotels requires effective hygienic practices in the production system especially when it is handled by humans. Hygienic measures include kitchen design and processing facilities, personal hygiene, cleaning, sanitation and pest control (FAO, 2014).

According to Nuamah (2010), the Ghana Food and Drug Authority reported that 90,692 people died in 2006 from food and personal hygiene related illnesses and that an estimated 297,104 people were recorded as having reported at the Out-Patients Departments of clinics and hospitals with food and hygiene related cases. Feglo and Sakyi (2012) also added that, in Ghana, diarrhoea has been recognised as one of the major causes of hospital attendance whilst it has been estimated that each year unsafe foods make at least two billion people, representing about one-third of the global population ill worldwide (WHO, 2007; Rheinlander *et al*., 2008; Arponutud *et al*., 2009).

In Ghana, the extrapolated incidence of food poisoning is 5.8 million annually (Salas, 2011). The high prevalence of diarrhoeal diseases in many developing countries suggests major underlying food safety problems (WHO media, 2007). Mishandling of food plays a significant role in the occurrence of food borne illnesses. For instance, improper food handling is implicated in 97% of all food borne illnesses associated with catering outlets with Africa alone contributing 90% of cholera cases globally (Addo *et al*., 2007). Ghana accounted for 27,000 of these cases with Kumasi in the Ashanti Region being the most affected. Similarly, poor food-handling practises

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were implicated to be the major cause of outbreaks of infectious intestinal diseases (IID) in England and Wales (Egan *et al*., 2007).

A growing body of data from foodborne disease outbreaks and studies of sporadic (non-outbreak-associated) gastrointestinal disease of various etiologies suggest that eating food prepared in restaurants is an important source of infection. It is also estimated that about 70% of all bacterial food poisoning is caused by caterers (Annor and Baiden, 2011). These data suggest a critical need for action that is focused on preventing disease transmission within the hotel industry. To this effect, scientists and researchers have allotted enough of attention to street foods and street food operators in Ghana concerning various aspects of food safety including the presence of fungi especially moulds in foods.

Fungi are ubiquitous, eukaryotic microorganisms which are found in many different environments wherever organic material is available. Moulds are important in food because they can grow even in conditions in which many bacteria cannot grow, such as low pH, low water activity (A_w), high osmotic pressure and low temperature (Al-Fakih, 2014). In general, moulds are able to grow at lower pH of between 1.5 and 9.0 and require water activity (Aw) of 0.80 or lower and thus can grow on partially dehydrated surfaces (including food). Moulds also tend to be less thermophillic compared to bacteria and other microbes. Moulds are important spoilage microorganisms but many strains also produce mycotoxins and have been implicated in food-borne intoxication. Some of these mycotoxins are carcinogenic or mutagenic and cause organ-specific pathology such as liver and kidney toxicities.

Food borne illness outbreak after consumption of yogurts contaminated with mold has been reported (Soo *et al.*, 2014). Again, out of 222 cooked food samples examined from 25 food service establishments serving the University of Jos community, fungi were isolated from 138 (62.0%) of the foods. The contaminated foods included okro soup, jollof rice, and pounded yam and the predominant fungi were included *Aspergillus niger, Aspergillus flavus, Mucor indicus* and *Penicillium* spp. (Ayanbimpe et al., 2007).

Data is abundant on the food hygiene knowledge attitudes and practices of food handlers and consumers, however, knowledge is scanty on the food safety and microbiological standards among food handlers in the hotel industries in Kumasi. This study focussed on the food safety standards practiced by some "three-star to budget" hotels in Kumasi, Ghana and also investigated the identity of mould contaminants on the food samples.

Materials and Methods

Study Location

The study was conducted on five hotels in the Kumasi Metropolis in the Ashanti Region of Ghana. Kumasi is the capital of the Ashanti Region of Ghana and the second largest city in Ghana with about 1.5 million inhabitants (Ghana Statistical Service, 2010). The actual locations of the hotels are withheld due to ethical reasons. The hotels selected ranged from "three star to budget" and have intense patronage throughout the year and were selected by a simple random sampling.

Ethical consideration

The data was collected after written informed consent was obtained from all the study participants (Managers of the Hotels) and the study approved by the Committee for Human Research Publication and Ethics (CHRPE) at the School of Medical Sciences, Kwame Nkrumah University of Science and Technology, Kumasi-Ghana.

Sample collection

Samples of cooked and/or uncooked foods were aseptically collected in two batches from the five hotels only during 12:00 mid-day and 1.00 pm each day with four foods in each batch. The foods were collected from the hotel restaurants at the point of serving and kept in sterile stomacher bags (Sharp and Jackson, 2000) and immediately stored inside an ice chest with ice packs while transporting them to the laboratory for analysis within two hours of collection. The foods collected were those that were available as lunch for the day. Sample collection and analysis were done between May 2013 to March 2014. A total of 40 foods were sampled from the five hotels.

Fungal counts

Ten grams of the food samples were weighed aseptically into 90 ml of peptone water (Oxoid LP0037) making a dilution ratio of 1:10. This was shaken vigorously to release adhered microbe. Further dilutions were made from the stock to obtain 10^{-1},10^{-2}, 10^{-3} and 10^{-4} . The dilutions were mixed very well and 0.1 ml of each was transferred onto sterile malt extract agar (MEA) (70145 Fluka Analytical, Sigma Aldrch Switzerland) and incubated at room temperature for 5 to 7 days. Colonies with growth between 20-200 were counted and subcultured on MEA to produce pure colonies for identification of moulds.

Table 1. Fungal counts of food and organisms identified from Hotel-01

Food	LOAD cfu/g	Log cfu/g	Organisms identified
Fufu	TFTC	-	*Eurotium herbariorum*
Boiled plain rice	5.0×10^1	1.7	
Beef sauce	1.3×10^2	2.0	
Goat light soup	2.3×10^2	2.4	
Tosse mixed vegetable	2.7×10^2	2.4	
Fresh pepper sauce	1.7×10^3	3.2	*Eurotium chevalien*
Chicken and vegetable sauce	1.0×10^4	4.0	*Cladosporium herbarium, Eurotium herbarium*

Table 2. Fungal counts of food and organisms identified from Hotel-02

Food	LOAD cfu/g	Log cfu/g	Organisms identified
Braised rice	1.7×10^2	2.2	-
Grilled steak	2.3×10^2	2.4	-
Chicken with noodles and vegetables	1.0×10^3	3.0	*Cladosporium herbarium, Penicillum comune*
Jollof rice	1.0×10^3	3.0	*Eurotium amsteloclami, Aureobasidium pullulans*
Fried rice	5.3×10^3	3.7	*Eurotium amsteloclami, Aureobasidium pullulans*
Potato chips	5.7×10^3	3.8	*Eurotium herbariorum*
Beef in vegetable sauce	6.7×10^3	3.8	*Cladosporium herbarum, Alternaria alternate, Aspergillus tamaric, Aureobasidium pullulans*
Coleslaw	2.3×10^4	4.4	*Cladosporium herbarum, Eurotium amsteloclami*

Fungal identification

Slides of fungal cultures were prepared by gently lifting the mycelial mat with a sterile inoculation pin into a drop of lactophenol blue on a slide, teased, covered with a slip and observed under microscope. Different characteristic features of the isolated fungi were observed and used in their identification using the method by Moss (1998).

Results

Counts of fungal colonies on foods from Hotel 01

Foods from Hotel-01, exhibited fungal counts ranged from 1.0×10^4 (4 Log_{10}) cfu/g to 5.0×10^1 (1.7 Log_{10}) cfu/g. The food with the highest colony count was chicken and vegetable sauce whilst fufu had too few cells to count (TFTC). Fresh pepper sauce and chicken and vegetable sauce were above the acceptable limits (Table 1).

Counts of fungal colonies on foods from Hotel 02

Foods from Hotel-02, colony counts ranged from 1.7×10^2 (2.2 Log_{10}) cfu/g to $2.3 \times 10_4$ (4.4 Log_{10}) cfu/g. The food with the highest fungal count was coleslaw with braised rice. All the foods in this hotel were above the acceptable limits of < 3.0 Log_{10} cfu/g except braised rice and grilled steak (Table 2).

Counts of fungal colonies on foods from Hotel 03

From Hotel-03, the counts ranged from 1.0×10^2 (2.0 Log_{10}) cfu/g to 2.3×10^5 (5.4 Log_{10}) cfu/g. Tossed salad recorded the highest growth with Beef sauce recording few cells to count (TFTC). Boiled plain rice, fish light soup, tossed mixed vegetables and tossed salad were above the acceptable limits (Table 3).

Counts of fungal colonies on foods from Hotel 04

As shown in Table 4, microbial count ranged from 1.7×10^2 (2.2 Log_{10}) cfu/g to 1.1×10^4 (4.0 Log_{10}) cfu/g for Hotel-04. The food with the highest colony count was fried rice with few cells to count (TFTC) from jollof rice. The foods above the acceptable limits were boiled plain rice, vegetable sauce, mixed salad and fried rice.

Table 3. Fungal counts of food and organisms identified from Hotel-03

Food	LOAD cfu/g	Log cfu/g	Organisms identified
Beef sauce	TFTC	-	-
Tomato sauce	$1.0x10^2$	2.0	-
Fried rice	$1.0x10^2$	2.0	-
Fried fish	$1.7x10^2$	2.2	-
Boiled plain rice	$1.0x10^3$	3.0	-
Fish light soup	$1.7x10^3$	3.2	*Aureobasidium pullulans, Penicilium poloticum*
Tossed mixed vegetable	$2.0x10^3$	3.3	
Tossed salad	$2.3x10^5$	5.4	*Cladosporum herbarum, Eurotium amsteloclami, Fusarium oxysporum*

Table 4. Fungal counts of food and organisms identified from Hotel-04

Food	LOAD cfu/g	Log cfu/g	Organisms identified
Jollof rice	TFTC	-	-
Beef sauce	$1.7x10^2$	2.2	-
Boiled plain rice	$3.3x10^2$	2.5	*Aureobasidium pullulans, Cladosporium herbarum, Fusarium oxysporum, Penicillium verrucosum*
Vegetable sauce	$1.1x10^3$	3.0	*Aspergillus niger, Cladosporium herbariorum*
Fried chicken	$1.3x10^3$	3.1	
Mixed salad	$2.3x10^3$	3.4	*Fusarium chevalien*
Fried rice	$1.1x10^4$	4.0	*Eurotium amsteloclami, Cladosporium herbarum*

Counts of fungal colonies on foods from Hotel 05

Microbial count ranged from 1.3×10^2 (2.1 Log_{10}) cfu/g to 1.3×10^6 (6.1 Log_{10}) cfu/g as shown in Table 5 for Hotel-05. The food with the highest count was mixed vegetable salad while boiled fish had the least colony count. The foods that did not fall within the acceptable limits (<3.0 Log_{10} cfu/g) were goat light soup, fried chicken, fried rice and mixed vegetable salad.

Discussion

The hotel industry in Ghana has a high reputation and it is perceived that the services they provide are the best, including the foods they serve. It is also believed that the higher the star rating, the better the food. In this study, the foods observed in the various hotels showed some levels of contamination with most of the foods regardless of their rating having contaminants above the acceptable limit of < 3.0 cfu/g Log10. For instance, Fresh pepper sauce (Table 1) and the organisms identified were: *Eurotium chevalien,* coleslaw (Table 2) fungi isolated were *Cladosporium herbarum* and *Eurotium amsteloclami*, tossed salad (Table 3) the fungi identified were:, *Cladosporium herbarum, Eurotium amsteloclami, Fusarium oxysporum,* mixed salad (Table 4) fungi isolated was Eurotium chevalien and mixed vegetable salad (Table 5) and the isolated fungi were Cladosporium herbarum and Eurotium amsteloclami all were above the acceptable limits. Raw vegetables were used in the preparation of fresh pepper sauce as well as all the salads and these are always eaten without further cooking. Perhaps the holding temperatures were not adhered to because these foods must be kept cool at holding temperatures of 1-5^{0}C before and during service (Ceserani and Foskett, 2007). The fungal contamination on these foods may also be due to the fact that the vegetables might have come from the farm where polluted water was used for irrigation (Gosh *et al*., 2007; Donkoh *et al.*, 2008). Studies in Kumasi-Ghana by Feglo and Sakyi (2012) revealed high levels of contamination in salads. These salads contained raw onion which are normally associated with toxigenic species such as *Penicillium* spp. and *Aspergillus* spp. (El-Nayerabi and Abdallah, 2004). Again, these fungi adhere to plant surfaces as black moulds, therefore, improper washing by food preparers can cause contamination in the food. Samson *et al*. (2001) reported that thousands of fungal species such as *Aspergillus niger* are commonly found in indoor environment

Table 5. Fungal counts of food and organisms identified from Hotel-05

Food	LOAD cfu/g	Log cfu/g	Organisms identified
Boiled fish	$1.3x10^2$	2.1	-
Jollof rice	$1.7x10^2$	2.2	-
Boiled plain rice	$2.0x10^2$	2.3	-
Vegetable sauce	$6.7x10^2$	2.8	-
Goat light soup	$1.0x10^3$	3.0	*Aureobasidium pullulans, Botrytis cineria, Cladosporium herbarum*
Fried chicken	$1.0x10^4$	4.0	
Fried rice	$1.7x10^5$	5.2	*Cladosporium herbariorum*
Mixed vegetable salad	$1.3x10^6$	6.1	*Cladosporium herbarium, Eurotium amsteloclami.*

and can easily contaminate the environment in food processing areas which can spread onto food and cause contamination. Recent evidence suggests that some true *A. niger* strains do produce ochratoxin (Samson *et al*., 2004) hence, this calls for regular and proper sanitation in the kitchen and its environs.

Fufu (Table 1) had contaminants that were too few to count (TFTC) which is surprising, however *Eurotium herbariorum* was isolated from the fufu. Fufu is handled excessively during its preparation with the bare hands which is a possible source of contamination. Mensah *et al*. (2002) also reported in their study that enteropathogens can survive on the hands for three hours or longer. Majority of food worker associated outbreaks reviewed by Todd *et al.* (2007) involved transmission of the pathogen to food by the food workers' hands. Probably extensive hand washing was not done before handling the fufu and hence the contamination. Fufu pounding also generate sweat because of the excessive energy used in the pounding process and the sweat may find its way into the fufu unknowingly. The cassava and plantain which were used in preparing the fufu may have been stored for a period of time before being used to prepare the fufu and hence the isolation of the fungus. Cassava is a highly perishable commodity and is easily contaminated by fungi (Bankole and Adebanjo, 2003). Maybe thorough cleaning of the cassava was not done by the cooks in the kitchen. In Ghana post- harvest handling of vegetables in the market is very poor and according to Udoh *et al.* (2015) different fungal species have been reported to be associated with the post-harvest deterioration of fruits and vegetables. Again, fufu is pounded in the open and could have been contaminated with spores and mycelium fragments from the environment.

Contaminants found in the boiled plain rice (Table 4) were above the acceptable limits of < 3 Log10 cfu/g and the organism identified were *Aureobasidium pullulans, Cladosporium herbarum, Eurotium herbariorum Fusarium oxysporum* and *Penicillium verrucosum*. This compares with the study by Annan-Prah *et al.* (2011), who also found fungi including *Fusarium* spp. in cooked rice. From personal observations at the hotels, imported rice especially the parboiled and perfumed ones were the types used in all the rice dishes and were stored in large quantities, however, stored rice from Argentina and Paraguy have been found to be dominated especially by *Penicillium citrinum, Aspergillus niger, Aspergillus flavous* and *Alternaris* spp. (Anderson and Thraine, 2006). Anderson and Thraine (2006) again added that the mycobiota of rice may establish itself on parboiled rice even though this mycobiota is expected to have been eliminated by boiling. This, thus, calls for proper washing of the rice, whether perfumed or parboiled, before cooking. The cooks can also introduce the fungi onto food through talking and coughing without covering their mouth in the kitchen and this may occur if supervision is poor.

The counts on fried rice were 3.7 Log_{10} cfu/g (Table 2) and the fungi identified were *Eurotium amsteloclami* and *Aureobasidium pullulans*, Colony count on fried rice again in Table 4 was 4.0 Log_{10} cfu/g the fungi isolated were *Eurotium amsteloclami* and *Cladosporium herbariorum* and 5.2 Log_{10} cfu/g in (Table 5), the isolates were *Cladosporiuo herbariorum* and were all above the acceptable limit of < 3.0 Log_{10} cfu/g . Fried rice may be susceptible to microorganism because of its composition. For instance, fried rice is prepared by first cooking the rice and then mixing with chopped fried vegetables and soy sauce which may create a favourable condition for the growth of fungi. This result is compared with work done by Wogu *et al.* (2011) on microbial load in ready-to-eat rice sold in Benin City where high levels of fungi, thus *Saccharomyces cerevisae* and *Aspergillus niger* were detected in ready-to-eat fried rice in Benin City, Nigeria.

Fungi in Jollof rice in Hotel-02 (Table 2) did not

meet the acceptable levels of fungi, the isolated fungi were Eurotium amsteloclami and Aureobasidium pullulans. There were too few cells to count (TFTC) in the jollof rice sampled from Hotel-04 (Table 4) and this result agrees with work by Addo *et al.* (2007) who did not find any contamination in jollof rice in their study of foods from hotels in Accra. Hotel-04 is a one-star hotel but served less contaminated foods than Hotel-02 which a three-star hotel. Complacency might have accounted for the high fungal levels in Hotel-02. The contaminations could be due to the presence of spores and mycelium fragments from the environment. Additionally, the ingredients used in the preparation of the jollof rice could have been contaminated by spores that were not visible to the naked eye, thus, if the washing was not done properly the spores will grow into the vegetative form and contaminate the food when ready for service. Contamination could have also occurred during blending of the vegetables from the blender which may be handled unhygienically since blending of ingredients is part of the jollof preparation.

Fungal contaminants in the braised rice samples were within the acceptable limits (Table 2). This food is prepared with fried chopped onions with the rice and water added and then allowed to boil till cooked (Personal observation). This long cooking which could be a special method used in Hotel-02 might have contributed to the acceptable levels of the fungi in the foods served.

Vegetable source (Table 4) had fungal counts of 3 Log_{10} cfu/g.and the fungal present were: *Aspergillus niger* and *Cladosporium herbariorum* Again, chicken and vegetable sauce had fungal count of 4 Log_{10} cfu/g in Table 1 and the fungal present were: *Cladosporium herbarium* and *Eurotium herbariorum,* Beef in vegetable sauce 3 Log_{10} as shown in Table 2 and did not meet the WHO acceptable levels (< 3.0 cfu/g Log_{10}). The fungi isolated were: *Cladosporium herbarium, Alternaria alternate, Aspergillus tamaric* and *Aureobasidium pullulans*. This is in agreement with Mensah *et al*. (2002) who also found sauces in their study to be above the acceptable levels. *Cladosporium herbarum* dominated the organisms identified in the sauces. *Cladosporium* spp. includes some of the most common indoor and outdoor moulds and grow indoors where moisture is present. There are no major mycotoxins produced from *Cladosporium* spp. and they are rarely pathogenic but can cause infection of the skin and toenails. In the process of sauce preparation, a lot of steam is generated which may allow the fungal contaminants in the sauce. Steam generation is common in food preparation and it is important to have extractor fans installed in the kitchen walls to remove the steam to avoid contamination especially by moulds. In this regard regular cleaning and sanitation of the kitchen is very important. For instance, equipment for cooking should be sanitized regularly to remove organisms that adhere to the surfaces.

Fish light soup 3 Log_{10} (Table 3) and goat light soup 3 Log_{10} (Table 5) had growth that were above the acceptable limits.The fungi identified were: *Aureobasidium pullulans, Penicillium poloticum* for the fish light soup and *Aureobasidium pullulans, Botrytis cinerea* and *Cladosporium herbarium* for the goat ligjt soup. Probably left-over soup was mixed with freshly prepared one during the preparation as this practice is not uncommon with commercial food preparers to prevent waste and loss of profit. The possible causes of the contamination could be due to poor personal hygiene by the food handlers and can be corrected by proper kitchen supervision. Potato chips (3.8 Log_{10} cfu/g) and chicken with noodles (3 Log_{10} cfu/g) as shown in Table 2 did not meet the acceptable limits. The isolates for the chicken with noodles were: *Cladosporium herbarium* and *Penicillium commune* whiles *Eurotium herbariorum* was isolated from potato chips The potatoes used for the chips may have been stored for too long and in large quantities and also may not have been washed thoroughly to remove adhered fungus before frying hence the fungal contamination Again, the oil for frying may have been used to fry several batches and could lead to aldehydes and acrylamide formation Other possible cause may be due to the use of defective and unsanitized equipment which may habour organisms and result in contamination of the food.

Conclusions

Most of the foods from the hotels regardless of their rating were above the acceptable limit (< 3.0 cfu/g Log_{10}) and also had mould contamination in them. Some of the foods in the budget and one-star hotels were better than those in the two-star and three-star hotels. This may mean that, the quality of the food sampled did not correlate to the star-rating of the hotels studied. The salads and vegetable dishes were the most contaminated food which may show lack of strict supervision in the kitchens. Poor kitchen sanitation in the low level and in some of the high level hotels accounted for the contamination of the foods. Also some of the fungi identified may have come from the ingredients with which the foods were prepared. Personal observations and laboratory analysis made in this study have shown

that supervision at the various hotels studied was very poor.

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