Fungi Load and prevalence of *Aspergillus species* in Meat Markets and Abattoirs in Ibadan, Oyo State

Ogundijo, O. A. and Adetunji, V. O.*
Department of Veterinary Public Health and Preventive Medicine,
*Fa v culty of Veterinary Medicine, University of Ibadan, Ibadan, Nigeria *Correspondence:vadetunji@gmail.com*

Abstract

Meat contamination in abattoirs and meat markets has been associated with several factors. Of major concern is contamination due to pathogenic microbes present in food processing environments. This study investigated fungal contamination in meal markets and abattoir environments. Four meat markets (Olunloyo, Oja Oba, Olorunsogo, and Olodo) and three abattoirs (Olorunsogo, Akinyele, and University[7] of Ibadan) in Ibadan, Oyo State, Nigeria, were assessed for prevalence of *Aspergillus* species. Fungi counts were determined by Pour-plate method at 10° dilution on Sucrose-Potato Dextrose Agar, supplemented with streptomycin, and incubated at 26°C for 5 days. Culture and enumeration of *Aspergillusflavus* and *Aspergillusniger* from table scrapings (n = 260) and srvabs (n=l 00) of abattoir environment were done using standard microbiological methods. The mean value for the total fungal count (TFC) was 4.46 + 0.24 log C'FUml"[1]. There were significant differences in the total fungal counts among locations, with Olunloyo market having the highest fungal load (4.73 + 0.64 log CFUml'[1]), and the lowest total fungal count was at the University of Ibadan abattoir (4.14 + 1.29 log CFUml'[1]). A. *niger* (30.18 %) w[7]as the most frequently isolated fungi, while the least w[7]as *A. fumigatus* (1.0 %). Prevalence *o(A. flavus* was 14.79 %. (Other fungi species vvere.4. *tamarii, A. terreus, Fusarium compacticum,F. oxyspomm, F. prolifemm, Penicillium chiysogenum,* and *P. oxalicum.* This study revealed a compromise in food safety in meat producing areas of Ibadan and hence, a need to enhance hygienic standards to improve food safety[7] in these locations.

Key words: *Aspergillus* spp., Contamination, Abattoir, Food safety, Ibadan.

Introduction

Meat contamination in abattoirs and meat stalls could result from contaminated water, unhygienic practices like poor handling, use of contaminated tables to display meat meant for sale and the use of contaminated knives in cutting operations. Contamination of meat and meat products occurs when raw meat is makes contact with pathogenic microbes which are ubiquitous in nature (Edema *et* al., 2005). The Food and Agricultural Organization (FAO) and World Health Organization (WHO) state that illness due to contaminated food is perhaps the most widespread health problem in the contemporary world and an important cause of reduced economic productivity (Edema *et al.*, 2005).

Serious consequences relating to public safety can arise from lack of hygiene and sanitation in abattoirs and meat stalls (Fasanmi *et al.*, 2010) which can be of adverse effect on public health. *Aspergillus* species, are ubiquitous saprophytic fungi that play a significant role in global carbon and nitrogen recycling (Pitt, 1994; Haines, 1995).They are commonly found in air, water, soil, plant debris, rotten vegetation, manure, sawdust litter, bagasse litter, and animal feed, on animals and in indoor air environments (Pattron, 2006; Bennett *et al.*, 2010). These fungi species have been found to be pathogenic in

humans, being opportunistic in nature. *A. fumigatus, A. flavus, A. terreus, A. niger,* and *A. nidulans* have all been implicated in human and animal infections (Denning, 1998; Morgan *et al.,* 2005). *A. flavus* has been found to cause upper respiratory tract infections faster than any other *Aspergillus* species (Kennedy *et al,* 1997; Panda *et al.,* 1998). They also cause a broad spectrum of diseases, ranging from hypersensitive reactions to invasive infections associated with angio- invasion (Anand and Tiwary, 2010). Studies to specifically assess the environmental impact of this organism are limited when compared to the interest of researchers in other microorganisms. This study aimed at determining the Fungi counts and the prevalence of *Aspergillus* spp. on meat tables and abattoirs in Ibadan, Oyo state.

Materials and Methods Study location and sample size

This study was carried out in six locations of five different Local Government areas (LGA) in Ibadan, located at 7° 23' N, 3° 55' E, with an average temperature of 23.94 ^{o}C and relative humidity of 74.55 %., Oyo State, Nigeria. The selected study areas were; Olunloyo market (Ona Ara LGA), Oja Oba market (Ibadan South-West LGA), Olorunsogo market (Ona Ara LGA), Olodo market (Lagelu LGA), Akinyele slaughter house (Akinyele LGA) and University of Ibadan abattoir (Ibadan North LGA). Total number of samples collected was 360 samples consisting of meat table scrapings (*n* = 260) and swabs from slaughter slabs and walls of abattoirs (n = 100).The studied areas and samples were selected using the random sampling technique.

Sample collection and processing

Samples of table scrapings were collected from Olunloyo (n = 70), Oja Oba (n = 60), Olorunsogo (n = 80), and Olodo (n = 50), while swabs from slaughter slabs and walls were taken from Olorunsogo (*n* = 20), Akinyele (n = 40), and University of Ibadan (n = 40). Samples of table scrapings were collected using sterile universal bottles and swabs were taken with sterile swab sticks. Collected samples were placed in 9 mls of peptone water, placed in ice packs and transported to the laboratory for processing within 12 hours of collection. The samples were analysed at the Microbiology laboratory of the Institute for Agricultural and Research Training, Moor, Apata, Ibadan.

A ten-fold serial dilution to a factor of five (10^{-5}) of 1 ml of the collected samples were prepared using 9 mls of physiological saline solution in test tubes. Using the Pour- plate method, 1 ml of the serially diluted samples were cultured in 10 mls of Sucrose-Potato Dextrose Agar supplemented with streptomycin to prevent other microbial growth, and incubated at 26 °C for five days. Fungal counts for each of the plates were done using a colony counter and pure colonies were sub-cultured in Sabouraud dextrose agar for identification.

Fungal isolation and identification

The identification of fungal specieswas based on gross colony morphology, colour and on microscopic features.Distinct colonies were stained on glass slides using Lactophenol cotton blue for proper examination with the aid of a photomicroscope as described by Singh *et al.* (1991). This was carried out in the Veterinary Pathology Laboratory, University of Ibadan, Ibadan.

Statistical analysis

One-way analysis of variance (ANOVA) was performed on the data and standard deviations for the locations and organisms were calculated. Significantly different means were separated using Duncan Multiple Range Test ($p<0.05\%$) (Obi, 1990).

Results

The identification of all cultured organisms was based on their macroscopic and microscopic features on Sabouraud dextrose agar (Plates 1 and 2) and

photomicroscope (Plate 3 and 4). *A. niger* was identified by its blackish-brown colour, which was cream-yellow to yellow on the reverse side of the plate on Sabouraud dextrose agar (Plate 2), while *A. flavus* was identified with its characteristic yellowish- green to green colour which was cream- yellow on the reverse side of the plate (Plate 2). At *400 magnification, the conidial head of *A. niger* was globose, with globose to elliptical, rough, and dark brown to black conidia (Plate 3a). For A. flavus, the conidial head was radiating, which became loosely columnar with time (Plates 3b and 4) (Singh *et al.*, 1991).

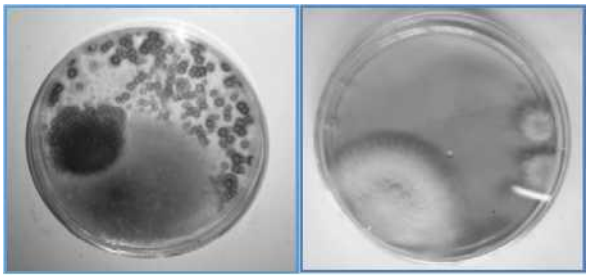

Plate 1. Mixed cultures showing *Penicillium oxalicum* (left) and *Fusarium oxysporum* (right)on Sucrose-Potato dextrose agar (Aspect Ratio: 4: 3).

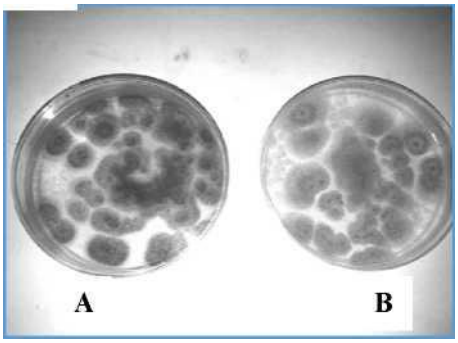

Plate 2. Pure culture plates showing *A. niger* (A)and *A. flavus* (B)on Sabouraud Dextrose agar (Aspect Ratio: 4: 3).

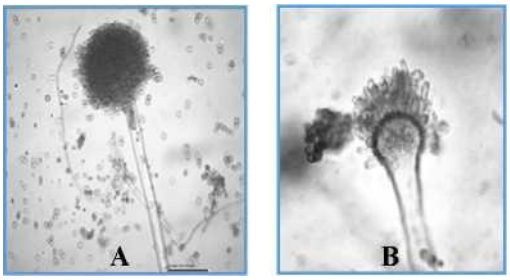

Plate 3. The globose conidial head of *A. niger* (A) (*40 magnification) and radiating conidial head of *A. flavus* (B) (*400 magnification), using Lactophenol cotton blue staining technique.

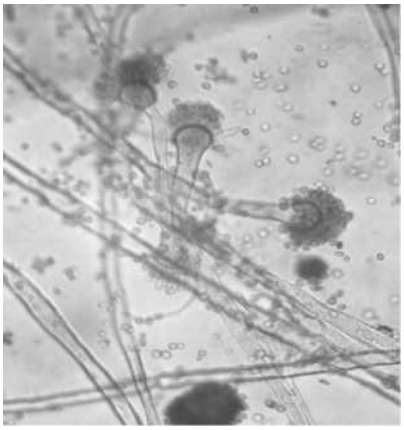

Plate 4. The radiating conidial heads of *A. flavus,* with sub-globose, rough and yellowish- green conidia (*400 magnification).

A total of ten (10) fungal organisms were isolated and identified. The isolates include;
Aspergillus flavus, A. fumigatus, A. niger, A. tamarii,A. terreus, Fusarium compacticum, F. oxysporum,F. proliferum, Penicillium chrysogenum,,and *P. oxalicum* (Table 1). The most frequently encountered Fungi was *A. niger* (30 %), followed by *F. oxisporum*while the least frequently encountered in this study was *A. fumigatus* (1 %) (Table 1).

Olodo market (18.9 %) had the highest fungal contamination,followed by Oja oba (18%) and the least was found in UI abattoir (13.6 %) (Table 2). *A. niger, A. flavus* and *F. oxisporum* where the highest contaminating fungi. The mean total fungal count (TFC) was 4.46 + 0.24 (Table 3). The total fungal load for Olunloyo market (4.73 + 0.64) was found to be the highest, and the lowest total fungal count was at the University of Ibadan (UI) abattoir (4.14 + 1.29).

Table 1. Percentage Occurrence of different fungal species

Fungus	Frequency	Percentage Occurrence (%)
Aspergillus niger	198	30.18
Aspergillus flavus	97	14.79
Aspergillus fumigatus	5	0.76
Aspergillus terreus	28	4.30
Fusarium oxysporum	162	24.70
Fusarium compacticum	26	3.96
Penicillium oxalicum	117	17.80
Penicillium chrysogenum	8	1.22
Fusarium proliferum	8	1.22
Aspergillus tamari	7	1.07
Total	656	100

Table 2.Occurence of each fungal organism in the different study locations

Locations	*proliferum* K	5 a K su s? -a o	*niger*	â 5 s	*A. flavus*	*A. fumigat*	*A. terreus*	*F. oxyspor*	*F. compac*	*P. oxalicu*	% fungal contamina
UI	0	0	26	0	9	0	4	32	0	18	13.6
Olunloyo	7	0	36	0	13	2	0	23	14	17	17.1
Oja Oba	0	0	35	0	12	0	9	33	0	29	18.0
Olorunsogo	0	0	36	6	26	0	8	29	2	7	17.4
Akinyele	1	0	29	1	11	1	4	31	6	15	1 5.1
Olodo	0	8	36	0	26	2	3	14	4	31	18.9
Total	8	8	198	7	97	5	28	162	26	117	

Table 3. Fungal loads for sampled locations.

Study locations	Location Type	Sample size (n)	Total fungal count +SD (Log cfuml"[1])
UI slaughter house	Abattoir	40	4.14 ± 1.29
Olunloyo market	Meat Market	70	4.73 ± 0.64
Oja Oba market	Meat Market	60	4.38 ± 1.20
Olorunsogo market	Abattoir/Meat Market	100	4.55 ± 0.88
Akinyele slaughter house	Abattoir	40	4.26 ± 1.18
Olodo market	Meat Market	50	4.68 ± 0.18
Mean (Log cfuml"[1])			4.46 ± 0.24

n = 360

Discussion

The mean total fungal counts for the study locations of 4.46 log $CFUml^{-1}$ as reported in this study exceeded the FAO/WHO standard limit of 2.0 $logCFUml^{-1}$ for total microbial count offood products and water (FAO/WHO, 2000). *Penicillium oxalicum, A. flavus, A. niger, A. terreus,* and *A. tamari* among other isolates were alsoreported by Ehigiator *et al.* (2014), which were isolated from shrimps in local meat shops in Benin, except for *A. fumigatus.* This may be due to the difference in the nature of samples collected. *Penicillium oxalicum* was similarly isolated in the work of Fasanmi *et al.* (2010) and Ehigiator *et al.* (2014). Fasanmi *et al.* (2010) also reported the identification of *Saccharromyces* spp. which was absent for this study due to the initial precaution taken to prevent yeast contamination of the cultured plates. *A. flavus, A. fumigatus* and *A. terreus* as reported in this study were not observed in the study of Fasanmi *et al.* (2010). This could be due to the difference in the studied locationsandsample size (50 samples as

compared to 360 samples for this study). According to Anand and Tiwary (2010), *Aspergillus flavus* is the second leading cause of invasive and non-invasive aspergillosis. The isolation of *A. fumigatus* and *A. flavus* in food processing areas of Olunloyo meat market, Olodo meat market and Akinyele slaughter house poses public health risk to the consumers and meat sellers if proactive measures are not put into place as appropriate.

Penicillium spp. and *Aspergillus* spp. as isolated from this study also confirmed the ubiquitousness of fungi species through similar isolation of these fungal organisms together with *Cladosporium* spp. and yeasts from both kitchen and other facilities in school environment as carried out by Shelton *et al*. (2002) and Lignell, (2008). Bryden, (2007) identified *Aspergillus*, *Fusarium* and *Penicillium* species as mycotoxin producers, while Hymery *et al.,* (2014) further defined them as food spoilage fungi. Various species of *Fusarium* have been known to produce mycotoxins, which have osteogenous action and significantly toxic to the reproductive system of animals and humans, according to Milicevic *et al,* (2010). The most important of these mycotoxin-producers are *Aspergillus* species as they are responsible for the production of aflatoxins (Bennet and Klich, 2003). *A. flavus* has been identified as the most potent producer of aflatoxins with mutagenic, hepatotoxic, and carcinogenic properties (Zhang *et al.,* 2012) which have been confirmed to have detrimental effects on both humans and animals (Barret, 2000). Mohamed (2010) also described the economic importance of mycotoxins in animal production. Therefore, the presence of *A. flavus* and *Fusarium* species from meat-processing areas in this study poses a threat to public health, as supported by Pitt, (2000) and Hymery *et al.* (2014), in meat markets and slaughter houses in Ibadan.

According to Abdullahi *et al*. (2006), unhygienic practices in abattoirs around the meat markets are associated with potential health risk to consumers due to the presence of pathogens in meat and environmental contamination. Also, lack or inadequate veterinary inspection as observed in most of the abattoirs and meat markets visited, supplemented with lack of potable water, proper waste disposal facilities, and sanitary inspectors, is now becoming a normal trend in many slaughterhouses in Nigeria (Okoli *et al.,* 2006). These factors may contribute to the high fungal contamination observed in this study. Increased level of fungal contamination in food processing environment is expected in time as a result of the biofilm forming abilities of *Aspergillus* organisms as reported by Ogundijo and Adetunji (2017).

Conclusion and Recommendation

There is strong compromise in food safety with fungal contamination in the meat processing areas covered in this study. More research focus should be driven towards preventing an outbreak or emergence of fungal zoonoses in the studied locations and other related areas. Thisis attainable by ensuring that strict hygiene and sanitation measures be put in place in meat markets and abattoirs, in order to ensure cleanliness and safety of meat tables and slaughter slabs respectively, to ensure meats and meat products are safe and wholesome for consumption.

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