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Diversity and Abundance of Aeromycobiota in Collecting Rooms, Slaughterhouses and Meat Retail Shops at Taiz City, Yemen

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Abstract: Airborne microbial contamination is a significant issue in the places of meat and meat products that have linked with adverse health effects. The incidence and distribution of mycobiota in the air of collecting room, slaughterhouse and popular markets were detected using Czapek's agar medium at 7 and 28 ± 2 ºC. The results indicated that the atmosphere of the three sites were heavily contaminated with moulds especially at 28 ºC. A total of 533 and 706 colonies/320 m³; 89 and 64 colonies/768 m³ and 34 and 208 colonies/560 m³ were collected from the air in all samples of the three sites, respectively. Fifty- four species related to 17 genera were isolated from the three sites at 7 and 28 ± 2 ºC. The atmosphere of the collecting room was highly polluted with moulds, but the popular markets having wide spectra of genera and species especially at high temperature. The ability of common fungi isolated in the current study for protease and lipase were screened. The results revealed that most isolates tested were able to produce the enzymes. Among 105 isolates tested, 87 (82.9%) and 57 (54.3%) could produce the two enzymes, respectively. From the positive isolates 4(4.6%) and 1 (1.8%) exhibited high proteolytic and lipolytic production, whereas 26 (29.9%) and 7 (12.3%) showed moderate production and 57 (65.5%) and 49 (86.0%) were weak producers. Several saprobes such as members of *Aspergillus* and *Penicillium* as well as others fungal genera are contaminated the meat products causing deterioration of meat. Also, the distribution of these fungi in such habitats which are in close association with human activities is a risk factor for the infections caused by these fungal species. So, it is important to take in consideration the different methods for prevention the fungal growth. Further knowledge of the contamination sources in the production environment would be beneficial in order to be able to control their presence in the processing plants.

Key Words: Air spora, Meat, Meat products, Slaughterhouses, Protease, Lipase, enzymes.

Introduction

Air has long been recognized as a source of microbial contamination in a range of food processing plants, including those of dairy producing plants (**Hemida and Abdel- Sater 2016, Moubasher *et al.* 2016).** Airborne molds in animal houses may also affect the health of working personal in many ways. Fungi can cause a number of different types of illness (**Singh 2004 and Vesković- Moračanin *et al.* 2009**). Fungi are ubiquitously distributed and they contaminate meat and meat products which can further lead to meat spoilage. Also, fungi contaminated meat are a health risk if they have the ability to produce mycotoxins. The main sources of moulds contaminating carcasses are air, water, walls and floors of slaughterhouses (**Refai *et al.* 1993, Rawat 2015, Anwer *et al.* 2017). Refai *et al.* (1993)** reported that *Aspergillus*, *Penicillium*, *Cladosporium* and *Mucor* were frequently isolated from the floors and walls of slaughterhouses. **Lacey and Dutkiewicz** (**1994**) noticed that microbiological composition of air, as a factor of ambient conditions in animal facilities can significantly influence performance, health and animal welfare. Also, there are numerous reports on health outcomes in occupants exposed to airborne microorganisms and their biologically active products.

Protease enzyme has the ability to break down protein. The industrial protease market for non-therapeutic use, such as food, detergents, textiles, leather, pulp and paper industry was previously recorded **(Godfrey and West 1996).** Proteases with different molecular masses, and different pH and temperature stability are produced by different fungal species (**Chou *et al.* 2001).** Proteases represent an important group of enzymes produced industrially and account for 60% of the worldwide sales value of the total industrial enzymes (**Godfrey and West 1996).** An alkaline serine protease has been identified as the major allergen from airborne *Penicillium chrysogenum (P. notatum).* It exhibits 83 and 49 % amino acid sequence identity with its counterparts from *P. citrinum* and *Aspergillus fumigatus*, respectively (**Chou *et al.* 2001).**

Lipases are produced by several microorganisms, belonging bacteria, fungi, archea, eucarya as well as by animals and plants. Commercially useful lipases are usually obtained from microorganisms that produce a wide variety of extracellular lipases (**Nwuche and Ogbonna 2011).** In 1994, Nova Nordisk introduced the first recombinant commercial lipase, which originated from the fungus *Thermomyces lanuginosus* (formerly *Humicola lanuginosa*) and was expressed in *Aspergillus oryzae.* Nova Nordisk markets a range of enzymes for various industrial purposes among which most of the enzymes are utilized mainly in food processing industries **(Padmapriya *et al.* 2011).**

The present study was planned for the first time in Yemen to assess the fungal load in the air of popular

markets, slaughterhouse and collecting animal rooms. As well as comparison between the isolated moulds from the different sources was assayed. Also, the capability of isolated moulds for their abilities to produce protease and lipase enzymes was tested.

Materials and Methods

1- Air examination

The microbial quality in the air of three areas related to fresh meat direct link (slaughterhouse, collecting room and the popular markets) has been examined where they are divided into equal region in the sedimentation method. Open Petri-dishes containing Czapek's agar medium were exposed at the measuring stations for 15 minutes (150 cm above the ground level). After that, the plates were incubated at 28 and 7 °C for 7-10 days. Results were expressed as the fungal precipitation rate on a horizontal surface and calculated as the mean log10 cfu.m-2 per minutes (Małecka-Adamowicz *et al.* 2007).

2- Medium Used for Isolation of Fungi

Modified Czapek′s Dox agar medium was used in which the 3% sucrose was substituted with 2% glucose. The composition of the medium (g/L) was: glucose 20; $NaNO_3$, 3; $KH_2PO_4.7H_2O$, 1.0; $MgSO_4.7H_2O$, 0.5; KCl, 0.5; $FeSO_4.7H_2O$, 0.01; Agar Agar, 15; Rose-bengal (1/15000) combined with Chloramphenicol (0.5 mg/ml) were used as bacteriostatic agents (**Smith and Dawson 1944 and Al-Doory 1980).**

3- Identification of Fungal Genera and Species:-

According to macro- and microscopic features, fungi isolated in the current study were identified on the bases of the following keys**: Raper and Fennell (1965), Booth (1971), Ellis (1971), Moubasher (1993), Pitt (1979), Domsch *et al.* (2007).**

4- Screening of Enzymatic Activity of Fungal Isolates

The common fungal isolates recovered in this study were tested for their abilities to produce extracellular protease and lipase on solid media as following:

a- **Screening for proteases production**

One hundred and five fungal isolates belonging to 55 species related to 18 genera, commonly isolated in the current work, were tested for their abilities to produce protease enzymes. The fungal proteolytic activity was tested using a casin hydrolytic medium as employed by **Paterson and Bridge (1994).**The composition of the medium (g/l): KH_2PO_4, 1.0; KCI, 0.5; $MgSO_4$. $7H_2O$, 0.2; $CaCl_2.2H_2O$, 0.1; 15 % skim milk, 25.0 ml; Glucose, 10; Agar, 12; 15 % skim milk was made by dissolving 3.75g skim milk in 25 ml distilled water and dissolved to a creamy texture prior to adding to the medium. The medium was sterilized by autoclaving at 121 °C for 30 minutes. Then the cooled medium was poured into 9 cm Petri-dishes (20 ml/plate). This medium was intended for presumptive protease activity and contains skim milk which gives an opaque final medium. Hydrolysis of the casein, which may also be due to acid production, result in a clear zone around the fungal colony.

b- **Screening for lipases production**

The collected 105 fungal isolates belonging to 55 species related to 18 genera, commonly isolated in the current work, were tested for their abilities to produce lipases. The fungi lipollytic activity was tested using the method of **Ullman and Blasins (1974)** with some modification. Tween 80 (poly oxy-ethylene sorbitan mono oleate) was added instead of Tween 20. The basal medium was composed of: peptone, 1 %; $MgS0_4.7H_20$, 0.2%; $CaCl_2$. $2H_20$, 0.02 %; Tween 80, 1% and Agar-agar, 1.5 %; pH 6.0. The medium was sterilized by autoclaving at 121 °C for 15 minutes. The Tween 80 was autoclaved separately and 1 ml per 100 ml of sterile tube was added to cooled basal medium. Duplicate plates from pure test tubes were inoculated on the surface of agar by single spot inoculum and incubated at 28 °C for 10 days. The formation of lipolytic enzymes by a fungal colony was seen either as a visible precipitate due to the formation of crystals of calcium salt of the oleic acid liberated by the enzyme or as opaque zone surrounding the colony consisted of calcium salts of free fatty acids.

Results and Discussion

1- Fungi Recovered from Air in the Collecting Room at 7 and 28 ± 2ºC.

The incidence of mycobiota in the air of collecting room was analyzed on Czapek's agar at 7 and 28 ± 2 ºC. The results showed that the atmosphere of the collecting room was heavily contaminated with moulds. A total of 533 and 706 colonies/320 m^3 of air in all samples represented 23 species related to 10 genera were isolated at 7 and 28 ± 2 ºC, respectively. The main sources of moulds contaminating carcasses are air, water, walls and floors of slaughterhouses **(Refai *et al.* 1993).** They reported that *Aspergillus*, *Penicillium*, *Cladosporium* and *Mucor* were frequently isolated from the floors and walls of slaughterhouses.

The highest counts of species and genera were noticed at high temperature. *Aspergillus* was the most prevalent genus which appeared in high frequency of occurrence isolated from all samples examined at both incubation temperatures. It was occurred each in 87.8% of total fungi. Of 9 species identified from the genus, *A. flavus*, *A. terreus* and *A. tubingensis* were the most common species. They were occurred in 75% of *Aspergillus* at 28 ºC and 100% of the samples on the two isolation temperatures (Table 1).

The obtained results are in line with those reported by several investigators (**Simmons *et al.* 1997, Adhikari *et al.* 2004, Menezes *et al.* 2004, Kaarakainen *et al.* 2008 and Ljaljević-grbić *et al.***

2008). They reported that mitosporic fungi showed the largest proportion of the total airborne fungal spores (83.4%) as well as culturable *Choanephora* and *Curvularia*. In the cow section of the cattle shed the range of total average monthly spore concentration was 253–2985/m^3 during the first sampling year and 308–2544/m^3 during the second sampling year. Among the recorded culturable moulds, the dominant types originated from *Aspergillus niger, Aspergillus flavus, Alternaria alternata, Cladosporium cladosporioides, Absidia blakesleeana, Penicillium citrinum, Penicillium chrysogenum, Rhizopus nigricans* and *Syncephalastrum racemosum.*

Alternaria came behind *Aspergillus* in the number of cases of isolation, isolated in high occurrence (100% at 7 ºC and 50% at 28 ºC). From the genus 3 species were encountered of which *A. chlamydospora* and *A. tenuissima* were the most dominant at 7 ºC. Both of *Cunninghamella* and *Fusarium* came behind *Aspergillus* in number of cases of isolation, isolated in high occurrence at 28ºC. From *Cunninghamella* 2 species were encountered of which *C. echinulata* was the most dominant (75% at 28 ºC) and *Fusarium* 3 species were encountered of which *F. dimerum* and *F. nivale* were the most dominant (75% at 28 ºC). **Nichita and Tirziu (2008)** investigated the airborne fungi in a poultry house and observed that high concentrations of fungal spores were detected. They isolated 11 species ascribe to 8 fungal genera of which *Aspergillus* and *Penicillium* spp. made up a vast majority of the identified fungi. Overall four species ascribed to the genus *Aspegillus* were isolated and identified, from those *A. fumigatus* and *A. flavus* prevailed and made up 34.8 and 8.7 % of all the identified isolates. The others *Aspergillus* (*A. oryizae* and *A. candidus*) species were presented in proportion of 5.7% from the total of isolated fungi. *Penicillium* was represented by *Penicililum expansum* (14.3%), *Penicillium chrysogenum* (5.8%) and *Penicillium aurantiogriseum* (4.9%), all of those species representing 24% from all isolated fungi. The other isolated fungal species were ascribe to *Alternaria, Rhizopus, Mucor, Fusarium, Cladosporium* and *Scopulariopsis*.

The fungal species recovered from air in the collecting room on Czapek's agar at 7 ± 2 ºC showed that, *A. tubingensis* was a high recorded species with 185 colonies/ 320 M^3 and 100% of the samples, *A. terreus* (183 and 100%), *A. flavus* (88 and 100%), *Alternaria. tenuissima* (17 and 100%), *A. chlamydospora* (15 and 100%), *Cladosporium macrocarpum* (15 and 75%), *A.versicolor* (7 and 25%), *A.alternata* (5 and 75%), *A. sydowii* (5 and 50%, *Cochliobolus pallescens* (5 and 50%, *F. nivale* (4 25%), *Penicillium expansum* (2 and 50%), *C. echinulata* and *F. sambucinum* (1 colony and 25% each) as shown in table (1). While, the fungal species recovered from air in the collecting room on Czapek's agar at 28 ± 2 ºC showed that, *A. tubingensis* was a high recorded species with 322 colocnies/ 320 M^3 and 100% of the samples, followed by *A. terreus* (217 and 100%), *A. flavus* (66 and 75%), *Penicillium expansum* (31 and 75%). *C. echinulata* (23 and 75%), *F. nivale* (11 and 75%), *F. dimerum* (8 and 75%), *A. tamarii* (7 and 50%**,)** *A. fischeri* (4 and 50%), *A. alternata, A. chlamydospora, A. heteromorphus, Gibberella acuminata* (3 and 25% each), *Drechslaria incurvala* (2 and 25%), *A. foetidus, C. elegans* and *Syncephalastrum racemosum* (1 and 25% each) (Table 1). These results are in agreement with those obtained by **Sørensen *et al.* (2008), Suerdem and Yildirim (2009)** and **Nichita *et al.* (2010).** They noticed that the presence of fungi in the air of two broiler houses. They found that high concentrations of fungal spores were detected in the conventional ventilated poultry house. Species of *Aspergillus fumigatus, Aspergillus flavus, Penicillium chrysogenum, Cladosporium cladosporioides* and *Scopulariopsis* spp., were prevailed in both poultry houses.

Comparison between fungi recovered from the air of collecting room at 7 and 28 ºC, It could be noticed that some species were predominant at low temperature and less common or not encountered at the other temperature. In this respect, *Aspergillus sydowii, A.versicolor, Cladosporium macrocarpum, Cochliobolus pallescens* and *F. sambucinum* were common at 7 ºC and less encountered at 28 ºC. On the other hand, *Aspergillus fischeri, A. foetidus, A. heteromorphus, A. tamarii, Cunninghamella elegans, Drechslaria incurvala, Fusariu dimerum, Gibberella acuminata* and *Syncephalastrum racemosum* were common at 28 ºC and less frequent at 7 ºC (Table 1).

2 - **Fungi Recovered from Air in the Central Slaughterhouse at 7 and 28 ± 2ºC.**

The incidence of mycobiota in the air of slaughterhouse was analyzed on Czapek's agar at 7 and 28 ± 2ºC. The results showed that the atmosphere of the slaughterhouse was heavily contaminated with moulds. Similar results were observed by **Sorensen (2009).** He made a mycological survey of Danish meat products and the processing environments and noticed that the diversity of filamentous fungi was high and especially a large number of *Penicillium* species. In the current study a total of 89 and 64 colonies/768 m^3 of air in all samples represented 14 species related to 6 genera were isolated at 7 and 28 ± 2 ºC, respectively. The highest counts of species and genera were noticed at low temperature (Table 1). In this respect, **Sorensen *et al.* (2008)** studied the filamentous fungi from air, equipment and raw materials in the processing areas of different meat products. They reported that there are high diversity of these fungi and could isolate 17 different genera. Also, the surface mycobiota of three types of Slovenian dry-cured meat products were isolated from a total of 75 items of product that were sampled periodically during the drying/ripening stage of

processing. They found that the predominant filamentous fungal genera isolated were *Penicillium* and *Eurotium* spp.

Aspergillus was the most prevalent genus which appeared in high frequency of occurrence and isolated from all samples examined at both incubation temperature. It was occurred in 33.7% and 50% of total fungi, respectively. Of 4 species identified from the genus, *A. sydowii, A. terreus* and *A. tubingensis* were the most common species. They were recovered from 75 and 38%, 75 and 63%, and 63 and 63% of the samples at the two incubation temperatures, respectively. This genus was also isolated in high occurrence in the processing areas of meat products as reported by **Sorensen *et al.* (2008)** and **Sorensen (2009).**

Cladosporium came behind *Aspergillus* in the number of cases of isolation and isolated in high occurrence (100% at 7ºC and 75% at 28ºC). From the genus 3 species were encountered of which *C. macrocarpum* was the most dominant (75% and 50%), The rest two species were less frequent. Also, three genera namely *Alternaria*, *Cochliobolus* and *Fusarium* were highly isolated from the air of slaughterhouse especially at low temperature and less encountered at high temperature. *Penicillium* (represented by one species) was isolated in rare occurrence (Table 1).

The fungal species recovered from air in the slaughterhouse on Czapek's agar at 7 ± 2 ºC showed, that *Cochliobolus pallescens* was a high recorded species with 25 colonies/768M^3 and 100% of the samples, *Cladosporium macrocarpum* (17 and 75%), *A. sydowii* (12 and 75%), *A. terreus* (9 and 75%), *A. tubingensis* (8 and 63%), *A. alternata* (4 and 25%), *F. nivale* (4 and 50%), *A. tenuissima* (3 and 38%), *C. sphaerospermum* (2 and 25%), *A. tamarii, C. cladosporioides, C. ovoidea, F. dimerum* and *Penicillium expansum* (one colony and 13%) at 7± 2 ºC. Also showed that the fungal species recovered from air in the slaughterhouse on Czapek's agar at 28 ± 2 ºC *A. terreus* was a high recorded species with 14 colonies/ 768 M^3 and 63% of the samples followed by *A. tubingensis* (9 and 63%), *A. sydowii* (7 and 38%), *C. sphaerospermum* (6 and 25%), *F. dimerum* (6 and 13%), *C. macrocarpum* (5 and 50%), *F. nivale* (4 and 25%), *A. alternata* (3 and 13%), *A. tenuissima* (3 and 38%), *C. pallescens* (3 and 25%) and *A. tamari* (2 and 13%).

Comparison between fungi recovered from the air of slaughterhouse at 7 and 28 ºC, it could be noticed that some species were predominant at low temperature and less common or not encountered at the other temperature. In this respect, *Cladosporium macrocarpum* and *Cochliobolus pallescens* were common at 7 ºC and less encountered at 28 ºC. Whereas, *Fusarium dimerum* was common at 28 ºC and less frequent at 7 ºC. *Cladosporium cladosporioides, C. ovoidea* were isolated at low temperature only (Table 1). Few studies have focused on the mycobiota in the processing areas of meat processing plants. **Ismail *et al.* (1995)** examined the surroundings in Egyptian abattoirs and **Spotti *et al.* (1989). Andersen (1995) and Battilani *et al.* (2007)** examined the air conidia of north Italian ham and fermented sausage production plants. The level of fungal contamination varied between the individual meat processing plants, probably influenced by the general hygiene, the buildings, the airflow, the outdoor environments and time of the year. Also, **Scholte *et al.* (2002)** noticed that the diversity of isolated fungi was relatively high, especially in the plants examined in the autumn. This probably reflects that fungal conidia are air-borne and are therefore easily spread. Important reservoirs can be humans, soil, dust, raw materials, drains, equipment surfaces and ventilation ducts.

3- Fungi Recovered from Air in the Popular Markets at 7 and 28 ±2ºC.

The incidence of mycobiota in the air of Popular Markets was analyzed on Czapek's agar at 7 and 28 ± 2 ºC. The results showed that the atmosphere of the Popular Markets was heavily contaminated with mould especially at 28 ºC. A total of 34 and 208 colonies/560 m³ of air in all examined area represented by 31 species related to 12 genera were isolated at 7 and 28 ± 2ºC, respectively. The highest counts of species and genera were noticed at high temperature (Table 1). Presence of filamentous fungi on meat products as fermented sausages and dry-cured hams has been the subject of several studies of various geographical origins and with an increasing attention within the last 50 years (**Sorensen 2009). Zohri *et al.* (2013)** made a survey on mycobiota contaminating beef burger and sausage and reported that the total number of fungal species on DRBC was higher than that on DG18 (46 versus 31 species in case of beef burger and 41 versus 33 in sausage). They noticed that *Aspergillus*, *Penicillium* and yeasts were the most prevalent fungi contaminating about 70- 100% of the samples.

Alternaria was the most prevalent genus which appeared in high frequency of occurrence isolated from all samples examined on both incubation temperatures. From 2 species encountered *A. alternata* was the most dominant (86% each) and the other was 57% and 43%. These results are in agreement with those obtained by **Sorensen (2009)** who reported that the genera *Alternaria*, *Aspergillus*, *Cladosporium*, *Eurotium*, *Mucor, Penicillium, Rhizopus* and *Scopulariopsis* were the most dominant fungi in meat products and processing areas.

Aspergillus came behind *Alternaria* in the number of cases which appeared in high occurrence isolated from all samples examined at 28 ºC and 71% of the samples at 7 ºC. It was recovered from 20.6% and 29.3% of total moulds at the two incubation

temperature, respectively. Of 10 species identified from the genus, *A. aculeatus, A. caespitosus* and *A. niger* were the most common species. They were occurred in 21% - 29% of total fungi. Also, *Cladosporium herbarum* (86% each), *Fusarium dimerum* (14% and 57%) and *Penicillium caseicolum* (43% only at 28 ºC), *P. funiculosum* (14% and 57%) and *P. rubrum* (29% and 43%) were highly isolated (Table 1**).** In this instance, **Sorensen *et al.* (2008)** noticed that the high prevalence of *Penicillium* species in the processing areas and their presence on meat products does together with their toxinogenic properties make *Penicillium* species the most important filamentous fungi to be aware of in the production environment.

Lichtheimia corymbifera, Cochliobolus pallescens, Geotrichum candidum, Paecilomyces farinosus, Scopulariopsis candida and *Trichoderma longibrachiatum* were isolated in fewer occurrences (Table 1)**.**

The fungal species recovered from air in the Popular Markets on Czapek's agar at 7 ± 2 ºC showed that, *A. alternata* was a high recorded species with 6 colonies/560 M^3 and 86% of the samples *Cladosporium herbarum* (6 and 86%), and *A. chlamydospora* (4 and 57%).

The remaining fungi were represented by 1 or 2 colonies and 14 or 29%. **Sorensen (2009)** isolated *Alternaria, Aspergillus, Cladosporium، Eurotium, Mucor, Penicillium, Rhizopus* and *Scopulariopsis. The Penicillium* was isolated within all examined studies.

The fungal species recovered from air in the Popular Markets on Czapek's agar at 28 ± 2 ºC revealed that, *Cladosporium herbarum* was a high recorded species with 45 colocnies/560 M^3 and 86% of the samples, *A. alternata* (26 and 86%), *A. niger* (20 and 100%), *A. caespitosus* (17 and 57%), *P. caseicolum* (16 and 43%), *A. aculeatus* (12 and 71%), *P. funiculosum* (11 and 57%), *Scopulariopsis candida* (7 and 29%), *A. chlamydospora* (7 and 43%), *A. melleus* 95 and 29%), *F. dimerum* (4 and 57%), *P. brevicompactum* (4 and 29%), *P. rubrum* (4 and 43%), *Lichtheimia corymbifera* (3 and 29%), Setrile mycelium (3 and 14%), *A. flavipes* (2 and 29%), *F. chlamydospoarum, F. oxysporum, P. glabrum, P. implicatum P. oxalicum* (2 and 14% each), The rest species were present each by one colony and 14% of the samples.

Sorensen *et al.* (2008) noticed that the diversity of isolated fungi was relatively high, especially in the plants examined in the autumn. This probably reflects that fungal conidia are air-borne and are therefore easily spread. Important reservoirs can be humans, soil, dust, raw materials, drains, equipment surfaces and ventilation ducts (**Scholte *et al.* 2002)**. From this criterion, the important genera were *Aspergillus, Botrytis, Cladosporium, Epicoccum, Eurotium, Penicillium, Phaeoacremonium* and *Phoma*. **Zohri *et al.* (2013)** reported that *Aspergillus, Penicillium* and yeasts were the most prevalent fungi contaminating 70-100 % of the samples. *Aspergillus niger, A. terreus, A. flavus, Penicillium chrysogenum* and *P. citrinum* were the most common mould species on both beef burger and sausage samples.

Comparison between fungi recovered from the air of Popular Markets at 7 and 28 ºC, It could be noticed that some species were predominant at low temperature and less common or not encountered at the other temperature. In this respect, no fungi were common at 7 ºC and less encountered at 28 ºC. whereas, *Lichtheimia corymbifera, Aspergillus awamori, A. caespitosus, A. candidus, A. flavus, A. janus, A. melleus, Cochliobolus pallescens, Emericella nidulans, Penicillium brevicompactum, P. caseicolum, P. implicatum, P. oxalicum, Trichoderma longibrachiatum* and *Trimmatostroma betulinum* were common at 28 ºC and less frequent at 7 ºC.

Comparing the results obtained from the different habitats it was observed that, 35 species of fungi were recorded from fresh meat at 7 ºC (5 species were appeared in the air of central slaughterhouse, 6 species in collecting room and 12 species in popular market, whereas 20 species were appeared from other contamination sources) 50 species of fungi were recorded from fresh meat at 28ºC (4 species were appeared in the air of central slaughterhouse, 8 species in collecting room and 15 species in popular market, whereas 34 species were appeared from other contamination sources). Some Species were appeared only in one or two sites and disappeared from the other site and vise versa. Also, several species were dominance in the air of one site and low frequency of occurrence in other sites (Table 1).

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Table (1): Total counts (TC, /320, 768 and 560 M³ of air in the three sites, respectively) and frequency percent (F%) of fungal genera and species recovered from air in the collecting room, central slaughterhouse and meat popular markets on Czapek's agar at 7 and 28ºC and 28 ± 2 ºC.

Genera & Species	Collecting room				Slaughterhouse				Popular markets			
	7 ± 2 ºC		28 ± 2 ºC		7 ± 2 ºC		28 ± 2 ºC		7 ± 2 ºC		28 ± 2 ºC	
	TC	F%	TC	F%	TC	F%	TC	F%	TC	F%	TC	F%
Alternaria	37	100	6	50	7	63	6	51	10	100	33	100
A. alternata	5	75	3	25	4	25	3	13	6	86	26	86
A. chlamydospora	15	100	3	25	0	0	0	0	4	57	7	43
A. tenuissima	17	100	0	0	3	38	3	38	0	0	0	0
Aspergillus	468	100	620	100	30	100	32	100	7	71	61	100
A. aculeatus	0	0	0	0	0	0	0	0	2	29	12	71
A. awamori	0	0	0	0	0	0	0	0	0	0	1	14
A. caespitosus	0	0	0	0	0	0	0	0	0	0	17	57
A. candidus	0	0	0	0	0	0	0	0	0	0	1	14
A. cervinus	0	0	0	0	0	0	0	0	1	14	1	14
A. fischeri	0	0	*4*	50	0	0	0	0	0	0	0	0
A. flavipes	0	0	0	0	0	0	0	0	2	29	2	29
A. flavus	88	100	66	75	0	0	0	0	0	0	1	14
A. foetidus	0	0	1	25	0	0	0	0	0	0	0	0
A. heteromorphus	0	0	3	25	0	0	0	0	0	0	0	0
A. janus	0	0	0	0	0	0	0	0	0	0	1	14
A. melleus	0	0	0	0	0	0	0	0	0	0	5	29
A. nidulans	0	0	0	0	0	0	0	0	0	0	1	14
A. niger	0	0	0	0	0	0	0	0	2	29	20	100
A. sydowii	5	50	0	0	12	75	7	38	0	0	0	0
A. tamarii	0	0	7	50	1	13	2	13	0	0	0	0
A. terreus	183	100	217	100	9	75	14	63	0	0	0	0
A. tubingensis	185	100	322	100	8	63	9	63	0	0	0	0
A. versicolor	7	25	0	0	0	0	0	0	0	0	0	0
Cladosporium	15	75	0	0	20	100	11	75	6	86	45	86
C. cladosporioides	0	0	0	0	1	13	0	0	0	0	0	0
C. herbarum	0	0	0	0	0	0	0	0	6	86	45	86
C. macrocarpum	15	75	0	0	17	75	5	50	0	0	0	0
C. sphaerospermum	0	0	0	0	2	25	6	25	0	0	0	0
Cochlioblus	5	50	0	0	26	100	3	25	0	0	1	14
C. ovoidea	0	0	0	0	1	13	0	0	0	0	0	0

Genera & Species	Collecting room				Slaughterhouse				Popular markets			
	7 ± 2 ºC		28 ± 2 ºC		7 ± 2 ºC		28 ± 2 ºC		7 ± 2 ºC		28 ± 2 ºC	
	TC	F%	TC	F%	TC	F%	TC	F%	TC	F%	TC	F%
C. pallescens	5	50	0	0	25	100	3	25	0	0	1	14
Cunninghamella	1	25	24	100	0	0	0	0	0	0	0	0
C. echinulata	1	25	23	75	0	0	0	0	0	0	0	0
C. elegans	0	0	1	25	0	0	0	0	0	0	0	0
Drechslera incurvata	0	0	2	25	0	0	0	0	0	0	0	0
Fusarium	5	50	19	100	5	63	10	38	3	43	8	86
F. chlamydosporum	0	0	0	0	0	0	0	0	0	0	2	14
F. dimerum	0	0	8	75	1	13	6	13	1	14	4	57
F. nivale	4	25	11	75	4	50	4	25	0	0	0	0
F. oxysporum	0	0	0	0	0	0	0	0	2	29	2	14
F. sambucinum	1	25	0	0	0	0	0	0	0	0	0	0
Geotrichum candidum	0	0	0	0	0	0	0	0	0	0	1	14
Gibberella acuminata	0	0	3	25	0	0	0	0	0	0	0	0
Paecilomyces farinosus	0	0	0	0	0	0	0	0	0	0	1	14
Penicillium	2	50	31	75	1	13	2	13	6	57	42	100
P. brevicompactum	0	0	0	0	0	0	0	0	0	0	4	29
P. caseicolum	0	0	0	0	0	0	0	0	0	0	16	43
P. expansum	2	50	31	75	1	13	2	13	1	14	1	14
P. glabrum	0	0	0	0	0	0	0	0	2	29	2	29
P. implicatum	0	0	0	0	0	0	0	0	0	0	2	29
P. oxalicum	0	0	0	0	0	0	0	0	0	0	2	29
P. rubrum	0	0	0	0	0	0	0	0	2	29	4	43
Scopulariopsis candida	0	0	0	0	0	0	0	0	2	29	7	29
Syncephalastrum racemosum	0	0	1	25	0	0	0	0	0	0	0	0
Talaromyces funiculosus	0	0	0	0	0	0	0	0	1	14	11	57
T. ruber	0	0	0	0	0	0	0	0	2	29	4	43
Trichoderma longibrachiatum	0	0	0	0	0	0	0	0	0	0	1	14
Trimmatostroma betulinum	0	0	0	0	0	0	0	0	0	0	1	14
Sterile mycelium	0	0	0	0	0	0	0	0	0	0	3	14
Yeast	0	0	0	0	0	0	0	0	56	29	43	71
Totel count	533		706		89		64		34		208	
No. of genera = 17	7		8		6		6		6		14	
No. of species = 54	14		15		14		12		14		33	

4- Protease

The ability of common fungal isolates, recovered in the current study for protease enzyme was screened using a casin hydrolysis medium and measuring the clear zones (cm). The results revealed that most tested isolates were able to produce the enzyme. Among 105 isolates tested, 87 (82.9%) could produce the enzymes. From the positive isolates (4.6%) exhibited high proteolytic activity (2.1 -3 cm), whereas 26 (29.9%) showed moderate production (1.1-2 cm) and 57 (65.5%) were weak producers (0.1 - 1 cm) as show in Table (2).

The results indicated that the high proteolytic producers were related to *Aspergillus melleus, A. sydowii, A. terreus* and *Cladosporium sphaerospermum*, whereas the moderate related to *Alternaria chlamydospora, A. tenuissima, Penicillium brevicompactum* and *P.caseicolum.* The remaining isolates were weak producers. These results are great similar to those obtained by **Ahmed and Abdel-Sater (2003)**. They reported that, among 73 fungal isolates tested for proteolytic activity about 84.9 % of the isolates (62 isolates) could produce protease with variable degrees. From the positive producers 30 isolates (48.4 %) exhibited the highest protease production and these isolates related to *Aspergillus niger, A. flavus, A. terreus*, and *A. sydowii.* Nineteen (30.6 %) of the positive isolates could produce enzyme with moderate degree including *Fusarium oxysporum, A. niger, Cladosporium* and *Penicillium* species and thirteen (21 %) isolates were weakly producers. **El- Diasty and Salem (2007)** studied lipolytic and proteolytic activities of fungi isolated from some milk products and their public health significance.

The obtained results showed that most isolates of *A. flavus, A. niger*, *Cladosporium* spp. *Mucor* spp. and *Penicillium* spp. having a proteolytic activity with different strength. *Geotrichium* spp. also having a lipolytic activity. The obtained results are in line with those obtained by **Saleem and El-Said (2009)** and **Djmel *et al.* (2009).**

Data showed that there were some species recored as high, moderate and weak abilities for production of protease, *Alternaria.alternata* (1 isolate high, 1 isolate moderate and 5 isolates weak), *A. chlamydospora* (3 moderate and 4 weak), *Aspergillus caespitosus* (1 moderate and 1 weak), *A. melleus* (1 high and 1 weak), *A. sydowii* (1 high and 1 weak), *A. terreus* (1 high, 1 moderate and 1 weak), *Cladosporium sphaerospermum* (1 high and 2 weak), *Cochliobolus pallescens* (1 moderate and 1 weak), *Fusarium acuminatum* (1 moderate and 1 weak), *Penicillium chrysogenum* (3 moderate and 1 weak), *P. corylophilum* (1 moderate and 1 weak), *P. expansum* (5 moderate and 3 weak), *P . jenseni* (3 moderate and 2 weak), *P. steckii* (1 moderate and 3 weak), *P. herbarum* (1 moderate and 3 weak) and *Scopulariopsis candida* (1 moderate and 1 weak) (Table 2).

5- **Lipase**

The ability of common fungal isolates, recovered in the current study for lipase production was screened, using tween 80 medium and measuring the clear zones (cm). The results revealed that most of the isolates tested were able to produce lipase. Among 105 isolates tested, 57 (54.3%) could produce the enzyme. From the positive isolates, 1 (1.8%) exhibited high enzyme production (1.7 - 2.4 cm), whereas 7 (12.3%) showed moderate production (0.8-1.6 cm) and 49 (86.0%) were weak producers (0.1- 0.7 cm) (Table 2).

The results indicated that the high lipolytic producer isolates were related to *Cladosporium sphaerospermum*, whereas the moderate were related to *Aspergillus caespitosus, A. flavipes, A. tamarii, Cladosporium macrocarpum, C. sphaerospermum, Penicillium brevicompactum* and *Phoma herbarum.* There were some species recorded as high, moderate and weak producers of lipase. **Ghosh *et al.* (1996)** studied microbial lipases production and discovered that lipase production using *Pseudomonas fragi, P. aeruginosa* and *Rhizopus oligosporus* was reduced by vigorous aeration. Growth and lipase production increased and followed by a rapid decrease of lipase activity was observed in *P. fragi* with continuous shaking. **Laila *et al.* (1998)** made an extensive study on lipase production by 90 fungal isolates identified from keratinaceous materials. They observed that 38 % of the isolates have the ability to produce this enzyme. Among the positive isolates 14 (13 % of total isolates) exhibited the highest lipase production and these fungi related to *Aspergillus versicolor, A. wentii, Geotrichum candidum, Penicillium camemberti, P. chrysogenum, P. jensenii, P. roqueforti, P. verrucosum* and *Scopulariopsis brevicaulis.* Twenty-four (23 % of total isolates) could produce enzyme with moderate degree and 31 (30 % of the positive isolates) are weakly producers.

Barakat and Abdel-Sater (1999) examined one hundred and five fungal isolates collected from raw butter (milk products) and noticed that, 69 isolates showed lipolytic activity with variable degrees. **Maia *et al.* (1999)** studied extracellular lipase by the phytopathogenic fungus *Fusarium solani*. They found that the optimum lipase

activity was achieved at pH 8.6 and 30ºC and a good enzyme stability (80% activity retention) was observed at pH ranging from 7.6 to 8.6, and the activity rapidly dropped at temperatures above 50ºC. **Wahba (2003)** tested 31 isolates representing 7 species for lipase production. He found that, the most active isolates for enzyme production were related to *Aspergillus niger* and *Alternaria alternata*. On the other hand moderate activity was achieved by *Alternaria tenuissima, F. moniliforme, M. racemosus, Penicillium aurantigriseum, P. chrysogenum* and *P. corylophiluum.* No activity was observed in culture of *A. terreus, Epicoccum purpurascens* and one isolate of *P. corylophilum*.

Nagy *et al.* (2006) screened thirty-eight filamentous fungi cultivated under solid state fermentation conditions for lipase production. They observed that many of these fungi yielded good activities of enzyme. *Gliocladium roseum* and *Trichoderma harzianum* produced the highest amount of lipase. **Aravindan *et al.* (2007)** found that lipase producers are widespread in the fungal kingdom and are of much biotechnological interest in both research and applications. The main fungal producers of commercial lipases were *A. niger, A. terreus, A. carneus, C. cylindracea, H. lanuginose* (*T. lanuginosus*), *Mucor miehei, Rhizophus arrhizus, R. delemar, R. japonicus, R. niveus* and *R. oryzae.* Media supplemented with glucose stimulated lipase production in case of all these fungi. **Saleem (2008)** isolated thirty one fungal species and 3 species varieties from 30 samples of beef luncheon meat collected from different supermarkets in Qena.

Screening of fungi for their abilities to produce lipase enzyme showed that, ten isolates represented 32.26% of total isolates appeared high lipase production, while sixteen isolates (51.61%) were moderate and 5 isolates(16.13%) were low producers. *Aspergillus niger*, *Fusarium oxysporum* and *Nectria haematococca* produced the highest amount of lipase. **Griebeler *et al.* (2011)** screened lipase-producing fungi with hydrolytic activity. They noticed that among 24 fungi, five were good lipase producers using tributyrin on agar plates and solid state fermentation of soybean bran and these fungi were belonging to *Penicillium* and *Aspergillus* genera.

Nwuche and Ogbonna (2011) isolated lipase producing fungi from palm oil mill effluent (pome) dump sites at Nsukka and they showed that the highest lipase producing strains belong to the *Trichoderma* while the lowest was *Mucor* sp. The lipase activity of the *Aspergillus* species was high but varied significantly among the isolates which probably were different species of *Aspergillus.* **Rajendra (2011)** tested the extracellular lipase enzyme production by seed-borne fungi under the influence of physical factors. He found that at 20 ºC *Penicillium notatum* showed significant maximum lipase activity which was followed by *Fusarium equiseti*. *Penicillium chrysogenum* showed maximum lipase production at 30 ºC as compared to other fungi. On the other hand *Curvularia lunata* and *Curvularia pallescens* did not show lipase activity at 10 ºC while, at 30 ºC lipase activity of *Fusarium oxysporum* was found to be increased.

Table (2): Protease and lipase production by fungal isolates recovered from air in the collecting room, central slaughterhouse and popular markets.

Genera & Species	NIT	Protease production				lipase production			
		NIP	High	Moderate	Weak	NIP	High	Moderate	Weak
Alternaria alternata	6	6	–	1	5	4	–	–	4
A. chalamydospora	7	7	–	3	4	5	–	–	5
A. tenuissima	2	2	–	2	–	1	–	–	1
Aspergillus aculeatus	3	3	–	–	3	3	–	–	3
A. awamori	2	2	–	–	2	–	–	–	–
A. caespitosus	3	2	–	1	1	2	–	1	1
A. candidus	1	1	–	–	1	1	–	–	1
A. cervinus	1	1	–	1	–	1	–	–	1
A. fischeri	1	1	–	–	1	–	–	–	–
A. flavipes	2	2	–	1	1	2	–	1	1
A. flavus	2	2	–	–	2	1	–	–	1
A. foetidus	1	1	–	–	1	–	–	–	–
A. heteromorphus	1	1	–	–	1	1	–	–	1
A. janus	1	–	–	–	–	–	–	–	–
A. melleus	2	2	1	–	1	2	–	–	2
A. nidulans	1	–	–	–	–	–	–	–	–
A. niger	1	–	–	–	–	–	–	–	–
A. sydowii	2	2	1	–	1	2	–	–	2
A. tamarii	1	1	–	–	1	1	–	1	–
A. terreus	5	5	2	1	3	1	–	–	1
A. tubingensis	1	1	–	–	1	–	–	–	–
A. versicolor	1	1	–	–	1	1	–	–	1
A. violaceus	1	1	–	–	1	1	–	–	1
Cldosporium herbarum	1	–	–	–	–	–	–	–	–
C. macrocarpum	1	1	–	1	–	1	–	1	–
C. sphaerospermum	3	3	1	–	2	3	1	1	1

Table (2): Continued.

Genera & Species	NIT	Protease production				lipase production			
		NIP	High	Moderate	Weak	NIP	High	Moderate	Weak
Cochliobolus ovoidea	2	2	–	–	2	1	–	–	1
C. pallescens	**2**	**2**	–	**1**	**1**	**1**	–	–	**1**
Cunninghamella echinulata	**3**	**3**	–	–	**3**	**1**	–	–	**1**
C. elegans	**1**	**1**	–	–	**1**	–	–	–	–
Drechslera incurvata	**1**	**1**	–	–	**1**	**1**	–	–	**1**
Fusarium acuminatum	**2**	**2**	–	**1**	**1**	**1**	–	–	**1**
F. chlamydospoarum	**1**	–	–	–	–	–	–	–	–
F. dimerum	**5**	**5**	–	–	**5**	**2**	–	–	**2**
F. nivale	**2**	**2**	–	–	**2**	**2**	–	–	**2**
F. oxysporum	**2**	**2**	–	–	**2**	–	–	–	–
F. sambucinum	**2**	**2**	–	–	**2**	–	–	–	–
Lichtheimia corymbifera	**1**	–	–	–	–	–	–	–	–
Geotrichum candidum	**1**	**1**	–	–	**1**	**1**	–	–	**1**
Paecilomyces farinosus	**1**	–	–	–	–	–	–	–	–
Penicillium brevicompactum	**2**	**2**		**2**		**1**	–	**1**	–
P. caseicolum	**4**	**4**	–	**3**	**1**	**3**	–	–	**3**
P. expansum	**5**	**5**	–	**3**	**2**	**4**	–	–	**4**
P. glabrum	**1**	**1**	–	**1**	–	–	–	–	–
P. implicatum	**1**	**1**	–	–	**1**	–	–	–	–
P. jenseni	**1**	–	–	–	–	**1**	–	–	**1**
P. megasporum	**2**	**1**	–	**1**	–	**2**	–	–	**2**
P. oxalicum	**1**	**1**	–	**1**	–	**1**	–	–	**1**
Phoma herbarum	**1**	**1**	–	–	**1**	**1**	–	**1**	–
Talaromyces funiculosus	**2**	**2**	–	**1**	**1**	–	–	–	–
T. ruber	**1**	**1**	-	**1**	-	–	–	–	–

Table (2): Continued.

Genera & Species	NIT	Protease production				lipase production			
		NIP	High	Moderate	Weak	NIP	High	Moderate	Weak
Rhizopus stolonifer	1	–	–	–	–	–	–	–	–
Scopulariopsis candida	2	2	–	1	1	–	–	–	–
Trichoderma lengibrachiatum	1	–	–	–	–	–	–	–	–
Trimmatostroma betulinum	1	1	-	-	1	1	-	-	1
Sterile mycelia	1	1	-	-	1	-	-	-	-
Total isolates	105	87	4	26	57	55	1	6	48

NIT= Number of isolates tested.
NIP= Number of isolates positive.
H =High activity, 3 - 2.1 cm for protolytic, 2.4 - 1.7 cm for lipolytic.
M = Moderate activity, 2 - 1.1cm, for protolytic, 1.6 - 0.8 cm for lipolytic.
W = Weak activity, 1 - 0.1 cm, for protolytic, 0.7 - 0.1 cm for lipolytic

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