Mycoflora of Edible- offal of Slaughtered Animals in Qena Abattoirs with The Aid of RAPD- PCR Technique.

الفطريات المتواجدة في الأحشاء الصالحة للاستهلاك الآدمي من الحيوانات المذبوحة في مجازر قنا باستخدام الوراثة الجزئية

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Abstract

Eddible- offal of slaughtered animals is considered as popular diete in Egypt, so a total of 50 samples of them were collected from large animals (cattle and buffaloes slaughtered in Qena abattoirs (10 each of heart , intestine, liver, rumen and spleen). Samples were subjected to mycological investigation for isolation and identification of various mould species with the aid of RAPD-PCR (Random Amplified Polymorphic DNA- Polymerase Chain Reaction). Rumen and intestine were heavy contaminated with moulds than other examined edible -offals of the slaughtered animals; $5.3 \times 10^4 \pm 0.3 \times 10^4$ and 4.1×10^3 / 1 g± 0.2×10^3 of the samples, respectively. Fungal contamination was not detected in all samples of heart and spleen. Ninty-eight mould strains were detected from examined samples , the majority of isolates belonging to *Aspergillus spp.* 23(23.5 %), *Trichoderma* 13(13.3 %), Fusarium spp. 12(12.3%) and Absidia corymifera 11(11.2%). The isolated species of *Aspergillus species* were further confirmed by using RAPD- PCR technique. Harmful effect on consumer health resulting from mould contamination of edible- offal and the hygienic measures adopting in abattoirs were fully discussed

Materials and Methods

A large number of different methods has been developed for examination of mycoflora in foods based on media , water activity and temperature . In order to find optimal detection and isolation media for food-borne fungi , lower water activity , lower temperature and higher carbohydrate level should be kept than bacteriological media . The following conventional standardizing methods were carried out according to *Samson and Hockstra* (1996).

1.Sampling

A total of 50 samples of edible- offal of slaughtered cattle and buffaloes were collected from different abattoirs in Qena city ; 10 each of heart , intestine , liver, rumen and spleen . The samples transported in an insulated ice bag to the laboratory without delay . Ten-fold dilutions up to 10^6 using sterile peptone water (0.1%) were prepared. Malt extract and Czapeck's-Dox agar (pH: 4.5) used for plating . The plates incubated at 25° C for 5 – 7 days and examined daily for detection of mould colonies .

2-Isolation and identification

Detected colonies in Petri-dishes were inoculated with sterile mycological needles into sterile slope agar (2 % Malt extract agar , pH : 7.0) and incubated at 25° C for 5 days . The summation of inoculated Malt extract slopes multiplied by correspondent dilutions were expressing the total mould count per one gram (TMC / g) of the sample . Identification of mould species carried out on pure cultures based on 3-point method and slide-culture technique . These methods of differentiation between mould species mainly depending on their taxonomic information and morphology of the colony , as well as , pigmentation of the reverse surface and fungus structure , according to Samson *and Hockstra*(1996).

3-Molecular technique

The strains of *Aspergillus species* were the majority of mould species isolated from edible- offal samples , therefore they subjected to further identification with the aid of RAPD-PCR analysis as Arizan et al. (1995), as follows :

a-DNA extraction :

Aspergills strains were cultures in flasks containing 25 ml (per liter : 1 g K_2HPO_4; 10 ml Czapek concentrate, 5 g yeast extract and 200 g sucrose) for one week using a rotator shaker (30°C at 150 rpm) .The mycelium was collected by filtration and ground to fine powder in a liquid N2. Fifty mg. of the powder transferred to 1.5 ml. Eppendorf tube and mixed with 700µ / 2 X CTAB buffer .The tubes incubated at 65°C for 30 min., then 700µ of chloroform were added and the mixture vortixed

briefly. The resulting mixture centrifuged at a maximum speed of 500 rpm for 30 min. and the cleared supernatant was mixed with 600µ of isopropanol chilled to -20°C . The mixture was centrifuged at the maximum speed of 500 rpm for 5 min. and the resulting pellet washed twice with 1 ml of 70% ethanol the pellet was dried under vacuum and dissolved in 100µ TE (10 mM Tris , 1 mM EDTA ,pH 7.5) buffer. The DNA concentrations were evaluated by agarose gel electrophoresis.

b-RAPD-amplification

PCR conditioned and separation of RAPD-PCR fragments were carried out according to Messner *et al*. (1994) .Using the primers M13 (5' dGAGGGTGGCGGTTCT O'Donnell., 1999).Synthesis of primers performed by (Codon Genetical Systems, Vienna, Austria), using a model 392 DNA synthesiser (Applied Biosystems, Foster city, CA, USA) . The temperature profile of primers was subjected for denaturation at 98°C for 15 sec.; annealing at 40°C for 90 sec. and extension at 72°C for 100 sec. to a total of 40 cycles .

Table (1) Total moulds count / 1 g from 50 samples of edible- offal of slaughtered animals in Qena abattoirs.

Source			
Count	Intestine	Liver	Rumen
Minimum	3.1×10^3	7.2 X 10	4.2×10^4
Maximum	5.1×10^3	2.7×10^2	6.8×10^4
Mean	4.1×10^3	5.3×10^2	5.3×10^4
Stand. Error	**0.2×10^3**	0.4 X10	0.3×10^4
Stand. deviation	0.8×10^3	0.9 X 10	3.7×10^4

Table (2) Frequencies of isolated mould species from 50 samples of edible offals of slaughtered animals in Qena abattoirs.

Mould spp.	Intestine		Liver		Rumen		Total*	
	No.	F%	No.	F%	No.	F%	No.	F%
Absidia corymbifera	4	4.1	1	1.0	6	6.1	11	11.2
Alternaria alternata	0	0.0	3	3.1	5	5.1	8	8.2
Aspergillus								
A. amstelodami	0	0.0	2	2.0	4	4.1	6	6.1
A. flavus	3	3.1	1	1.0	0	0.0	4	4.1
A. fumigatus	0	0.0	0	0.0	3	3.1	3	3.1
A.nidulans	1	1.0	0	0.0	2	2.0	3	3.1
A. niger	3	3.1	0	0.0	4	4.1	7	7.1
Fusarium								
F. poae	3	3.1	3	3.1	2	2.0	8	8.2
F. oxysporum	4	4.1	0	0.0	0	0.0	4	4.1
Mucor racemosus	5	5.1	1	1.0	4	4.1	10	10.2
Penicillium expansum	4	4.1	0	0.0	5	5.1	9	9.2
Rhizopus stolonifer	0	0.0	0	0.0	3	3.1	3	3.1
Thamnidium elegans	2	2.0	2	2.0	2	2.0	6	6.1
Trichoderma								
T. harzianum	0	0.0	0	0.0	7	7.1	7	7.1
T. viride	6	6.1	0	0.0	0	0.0	6	6.1
Total	36	36.7	13	13.3	49	50.0	98	100.0

* Moulds not detected at all samples of heart and spleen.

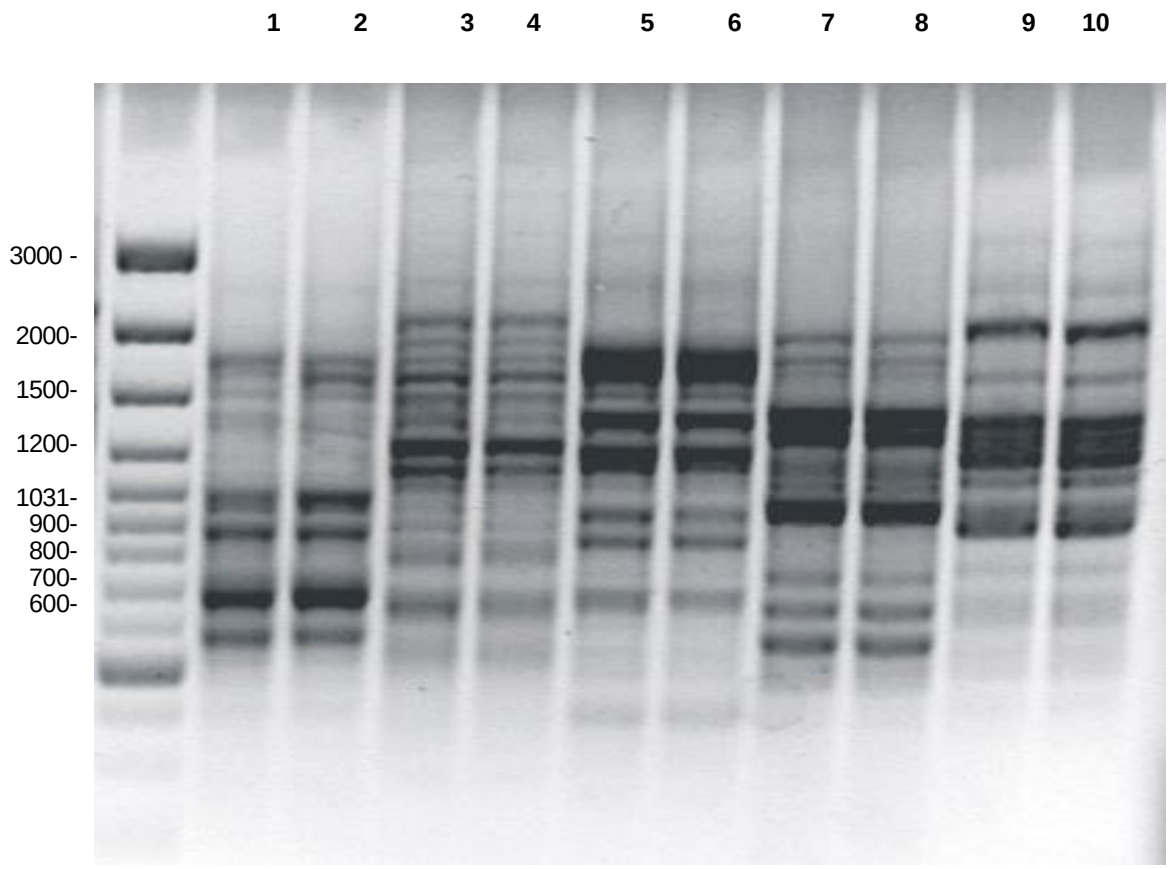

Fig .4 Pattern of fragments from RAPD analysis of different *Aspergillus* species , printed by M13 oligonucleotide (GAGGGTGGCGGTTCT K.O'Donnell *et al*., 1999).

Lane1 *Aspergillus flavus* from our study

Lane2 *Aspergillus flavus* MA 86 from IAM
Lane3 *Aspergillus fumigatus* from our study
Lane4 *Aspergillus fumigatus* MA 148 from IAM
Lane5 *Aspergillus niger* From our study
Lane6 *Aspergillus niger* MA 1922 from IAM
Lane7 *Aspergillus nidulans* from our study
Lane8 *Aspergillus nidu*lans MA 337 from IAM
Lane9 *Aspergillus amstelodami* from our study
Lane10 *Aspergillus amstelodami* MA 1068 from IAM

Conclusion

The obtained results in this study declared that fungal contamination of the edible offal, particularly intestine and rumen , frequently occurred in abattoirs. Such contamination may be attributed to their fecal contents or poor hygienic measures adopting during slaughtering , evisceration and handling of the carcasses .

The direct hazard to human health resulting from consumption of contaminated offal with moulds or their mycotoxins needs to be carefully controlled .Therefore, rigid attention for hygienic design , cleaning of equipment and sanitation procedures for eliminating sources of contamination. Offal including liver, intestine, rumen, heart and spleen should be trimmed rapidly and then washed using a continuous flow of clean water before placed in chilling room at a temperature not exceeding 3º C or freezing room for long storage (more than 72 h) and the temperature must be maintained below -10º C. These methods are usually sufficient to avoid a significant build-up of fungi during dressing of the slaughtered animals in different abattoirs.