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PREVALENCE OF YEAST IN SOME CHICKEN MEAT PRODUCTS

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Received: 30 June 2016; **Accepted:** 31 July 2016

ABSTRACT

This study was carried out upon 60 samples of chicken luncheon, pane and nuggets (20 of each), samples were collected from different markets at Dakhlia governorate and conducted to evaluate the total yeast count and the mycological profiles. The mean values of the total yeast counts of chicken luncheon, pane and nuggets (cfu/g) were $7.1\times10^3 \pm 3.1 \times10^3$, $1.8\times10^5 \pm 8.2\times10^4$, $2.4\times10^6 \pm 1.4\times10^6$, respectively. There was a significant difference in total yeast count between chicken luncheon and nuggets. Chicken nuggets were the most contaminated samples with yeast, while chicken luncheon was the lowest contaminated samples. One hundred and five (105) yeast isolates represented 4 genera were isolated from chicken luncheon, pane and nuggets samples in the following number and percentage, Candida, 14 (58.3%), 22(59.5%), 25(56.8%) with the highest total incidence of 61(58%) followed by Rhodotorula 7(29.1%), 11(29.7), 13(29.5%) with total incidence of 31(29.5%) followed by Saccharomyces, 3 (12.5%),3 (8.1%),4 (9.1%) with total incidence 10(9.5%), and finally, Trichosporon 0(0%),1(2.7%), 2(4.5%) with total incidence 3(2.9%), respectively. Regarding yeast species isolated from chicken luncheon, pane and nuggets, C. albicans were the most predominant species which isolated from 40%, 75% and 85% of examined samples represented by the following number and percentage 8(33.3%), 15(40.5%) and 17(38.6%), respectively. The yeast isolates were identified using traditional standard method. Six samples examined using PCR, all of them were successfully amplified a products of 109 bp and interpreted as positive for Candida albicans which confirmed the results obtained by traditional method. While 2 samples amplified a products of 919 bp and proved to be positive for killer gene (KHS) of S. cerviciae and one samples amplified a product of 727 bp and give positive killer genes (PelA).

Key words: *Yeast, total count, identification, Chicken luncheon, nuggets, pane, PCR.*

INTRODUCTION

Chicken and chicken meat products are a good sources of animal protein of high biological values which contains all essential amino acids required for human nutrition, as well as they contains higher proportion of unsaturated fatty acid and less cholesterol besides, poultry meat is good source of different types of vitamins (Shedeed, 1999 and Abo Hussein, 2007).

Yeast is microscopic single organisms which are mostly saprophytic, while few species are pathogenic (Abd–El-Rhahman *et al.,* 2013). Yeast can contaminated chicken meat and their products during processing as scalding, defeathering, evisceration, cooling, packing, in addition to transportation and storage. Also through the contaminated additives which used in the manufacture of chicken meat products (Abd-El–Atti1997; Farghaly, 1998 and Solimman, 2000). Due to the ability to proliferate in a wide range of pH and temperatures, yeast are of a great importance in spoilage of poultry meat and their products, resulting in different ranging in flavor, color ranging from white, creamy to pink or brown due to pigment production by yeasts, texture (slimness) and odor which make these products unwholesome. Moreover, yeast specially genus Candida have a major public health hazards because it causes several pathogenic lesions in gastrointestinal tract such as stomatitis, diarrhea, gastric disorder, intestinal disturbance and in other organs of the body as vaginitis, pulmonary thrush, meningitis as recorded by Wilson *et al.* (1981); Bier (1994); Koneman *et al.* (1997) and Lott and Effat (2001). Saccharomyces cervisiae which is non-spore forming yeast, considered a common colonizer of human respiratory,

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gastrointestinal and urinary tract, as it causes invasive disease in immunocompromised person which could be responsible for pyelonepheritis (Pillai *et al.,* 2014). Killer yeast such as Saccharomyces cervisiae are able to secret a number of toxic protein which are lethal to susceptible yeast strains but not harmful to human or animals. The major killer strains of S. cervisiae belong to the killer class K1 and K2 which kill each other but are immune to killer toxins of their own class. Killer yeast phenotype requires presence of two different dsRNA viruses, an L-virus (helper virus) and the toxin-coding (M) (killer virus). L-A helper virus contain 2 open reading frames, the first one encodes a capsule protein (Gag) required for capsid formation and the second one encodes an RNA dependent RNA polymerase (POI) required for viral dsRNA replication (Nurzhan, 2015). There are other killer system in S.cervisiae such as KHS and KHR genes encoded in chromosomal DNA and showing weaker killer activity (Goto*etal.,* 1990 and Goto *et al.,* 1991). Killer yeasts and their toxins have many potential applications in environmental, medical and industrialbiotechnology. Killer yeast strains can be a problem in commercial processing because they can killdesirable strains. So it's necessary to detect the fungal spoilage at early stages (Dillon and Board, 1991 and Frazier and Westhoff, 1995). Killer yeast strains not being restricted to S. cervisiae, it could found in other genera including Candida, Cryptococcus, Debaromyces, Pichia. Toxin from all killer strains are protease-sensitive, heat labile and act only in acidic PH values (Maraquina *et al.,* 2002).

Chromagen Candida agar proved to be a useful method for identifying certain yeast species, such as C. albicans and C. krusei as early as 24 hours, while the majority of yeast isolates required 48 hours incubation before the pigmentation became distinctive enough for differentiation. Although, we could not distinguish between C.parapasilosis and. krusei by color, so required another tests for differentiation (Siriorn *et al.,* 2005). Identification of isolates by polymerase chainreaction (PCR) proved more rapid and accurate identification comparing to traditional phenotypic methods (Yamakami *et al.,* 1996).

Therefore, the present study was conducted to evaluate the total yeast count and the mycological profile of chicken meat products collected from Dakhlia governorate supermarkets.

MATERIALS AND METHODS

Collection of samples: A total of 60 random samples of chicken products represented by luncheons, pane and nuggets (20 of each) were collected from different markets at Dakhlia governorate. Samples were transferred to the laboratory in ice- box without delay.

Preparation of samples: Twenty five grams of each samples were mixed with 225ml of sterile peptone water 0.1% to give dilution of 10^{-1} from which further dilution were prepared (APHA, 2001).

Total yeast count: 1mlfrom each dilution was transfer aseptically to sterile petri dishes contain Sabaroud´s dextrose agar(SDA) (Oxoid) at 45°C supplemented with 0.05mg chloramphenicol/ml, then mixed thoroughly and allowed to solidified, the plates were incubated at 25C°-28°C for 3-4 days then the yeast colonies were enumerated according to (APHA, 1992).

Isolation and identification of yeast according to (Koneman *et al*., 1997; Isenberg and Henry 2004; David *et al.,* 2007 and Sivakumar *et al*., 2008): Yeast colonies were isolated on SDA agar and incubated at 30°C-37°C for 72hs.

Identification of suspected yeast isolates:

1-Phenotypic methods:

1.1. Macro morphological characters

A- Macro morphological characters of yeaston SDA (David *et al*., 2007; Taha, 2011 and Markey *et al*., 2013): Gross appearance of the colonies was described considering their size, consistency and surface color.

B- Cultivation on chromogenic candida agar (CCA) for identification of Candida species. Loop full from yeast colonies that identified microscopically by rice agar with 1% Tween 80 was inoculated on chromogenic candid a agar for 24- 48hs at 30°C - 35°C. The colonies color were described with reference to the color formula guides as shown in Table (1).

Table 1: Interpretation of identification of Candida species by CCA

species	Color
C. albicans	Green
C.tropicalis	metallic blue
C.krusei	Purple
C.parapasilosis	Creamy white to light pink
Trichosporium	Light blue
C.galabrata	Creamy white to light pink
Rhodotorula	Orang-pink
S. cervisae	Violet

1.2. Micro morphological characters:

- Slid mount technique: Using cotton blue stain to detect yeast cells. Candida albicans colonies produced thin walled, budding yeast cells and gram´s stain to detect gram positive large spherical yeast cells.
- Microscopical examination on rice agar contained tween 80(RAT80):

A thin layer agar were streaked out with yeast to be identified in 3 lines then covered with sterile cover slip. After incubation at 25°C for 48hrs, the plates were examined for the presences of blastospores, pseudohyphae, arthrospores and chlamydospores. The identification and differentiation into genera according to (Taha, 2011).

2- Biochemical and physiological tests:

- Sugar fermentation, Sugar assimilation, nitrateassimilation and urea's test according to (Koneman *et al.,* 1997 and David *et al.,* 2007).
- Demonstration of germ tube test (David *et al*., 2007 and Markey *et al*., 2013). Avery small inoculum of yeast colonies was suspended in 0.5ml serum of sheep or normal human in test tube. The tube was incubated at 37°C for 1-3hrs. One drop from each serum suspension was placed on a clean slid and cover with cover slip and examined microscopically under low power for detection of pseudomycelium, pseudogerm tubes appearea as small tubes projecting from some of the yeast cells, this is the characteristic of C. albicans.

3- Identification of yeast species by polymerase chain reaction (PCR):

A- Nucleic acid extraction: DNA extraction from samples was performed using QIAamp DNeasy Plant Mini kit (Qiagen, Germany, GmbH). Briefly, 200 µl of the sample was added to 400 µl Buffer AP1 and 4 µl RNase A stock solution (100 mg/ml). The mixture was incubated for 10 min at 65°C and mixed 2 or 3 times during incubation by inverting tube. Then, 130 µl Buffer P3 was added to the lysate, mixed and incubated for 5 min on ice. The lysate was centrifugated for 5 min at 14,000 rpm and then pipetted into the QIAshredder Mini spin column (lilac) placed in a 2 ml collection tube, and centrifugated for 2 min at 14,000 rpm. The flow-through fraction from was transferred into a new tube without disturbing the cell-debris pellet and then applied to silica column. The lysate was then washed and centrifuged following the manufacturer's recommendations. Nucleic acid was eluted with 50 µl of elution buffer provided in the kit.

B- Oligonucleotide Primers. Primers used were supplied from Metabion (Germany) and are listed in Table (2).

Table (2): Primers sequences, target genes, amplicon sizes and cycling conditions for conventional PCR.

Target gene	Primers sequences	Amplified segment (bp)	Primary Denaturation	Amplification (35 cycles)			Final extension	Reference
				Secondary denaturation	Annealing	Extension		
KHS	AAGCATCCGA AACAGTACT TCAAGGATGC TGCTAAGCTG	919 bp	94˚C 5 min.	94˚C 30 sec.	53˚C 50 sec.	72˚C 50 sec.	72˚C 10 min.	**Suga *et al*., 2002**
pelA	ATCGAATTCA TGAAGTTCAC TGCTGCTTT ACGGAATTCG CAGCTCGTGG TGGAGCCAGT	727 bp	94˚C 5 min.	94˚C 30 sec.	55˚C 45 sec.	72˚C 45 sec.	72˚C 10 min.	
C. Albicans ITS	GGTTTGCTTGA AAGACGGTAG AGTTTGAAGA TATACGTGGT AG	109 bp	94˚C 5 min.	94˚C 30 sec.	50˚C 30 sec.	72˚C 30 sec.	72˚C 7 min.	**Tarini *et al.,* 2010**

C- PCR amplification: Primers were utilized in a 25- µl reaction containing 12.5 µl of Emerald Amp Max PCR Master Mix (Takara, Japan), 1 µl of each primer of 20 pmol concentrations, 4.5 µl of water, and 6 µl of DNA template. The reaction was performed in an applied biosystem 2720 thermal cycler.

D- Analysis of the PCR Products:

The products of PCR were separated by electrophoresis on 1.5% agarose gel (Applichem, Germany, GmbH) in 1x TBE buffer at room temperature using gradients of 5V/cm. For gel analysis, 15 µl of the products was loaded in each gel slot. A gene ruler 100 bp DNA Ladder (Fermentas) and a gelpilot 100 bp ladder (Qiagen, Germany, GmbH) were used to determine the fragment sizes. The gel was photographed by a gel documentation system (Alpha Innotech, Biometra) and the data was analyzed through computer software.

- Statistical analysis: The obtained data were analyzed using Analysis of Variance (ONE WAY ANOVA) SPSS according to Sabine and Brian (2014).

RESULTS

Table 3: Statistical analytic results of total yeast count (cfu/gm) of examined chicken product samples (n=20 of each).

Product /Count	Min	Max	Mean± S.E.
Luncheon	1×10^2	$3 \times 1o^4$	$7.1 \times 1o^3 \pm 3.1 \times 1o^3$
Pane	2×10^2	$7.4 \times 1o^5$	$1.8 \times 1o^5 \pm 8.2 \times 1o^4$
Nuggets	1×10^3	$1.4 \times 1o^7$	$2.4 \times 1o^6 \pm 1.4 \times 1o^6$

Significant difference between luncheon and nugggets ($P \leq 0.05$)

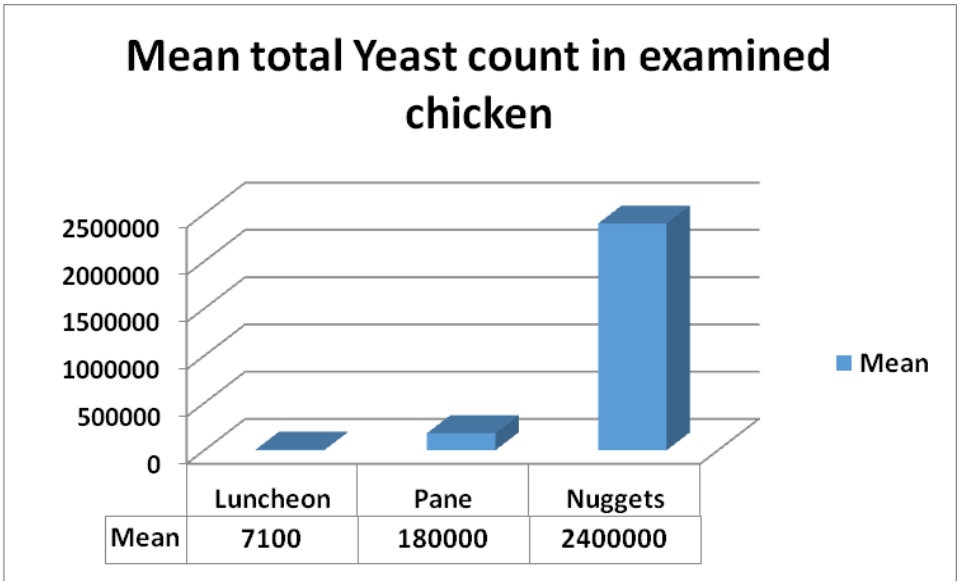


Fig. 1: Mean Yeast count in examined chicken products.

The obtained results in Table (3) and Fig. (1) revealed that the mean yeast count (cfu /gm) were $7.1 \times 1o^3 \pm 3.1 \times 1o^3$, $1.8 \times 1o^5 \pm 8.2 \times 1o^4$ and $2.4 \times 1o^6 \pm 1.4 \times 1o^6$ for chicken luncheon, pane and nuggets, respectively. There was a significant difference between total yeast count of chicken luncheon and nuggets.

Table 4: Incidence of isolated yeast genera in chicken meat products (n=20 of each)

Sample / Yeast Species	Luncheon		Pane		Nuggets		Total	
	N	%	N	%	N	%	N	%
Candida	11	55	17	85	19	95	47	78.3
Rhodotorula	5	25	7	35	8	40	20	33.3
Saccharomyces	3	15	4	20	4	20	11	18.3
Trichosporon	-	-	1	5	2	10	3	5

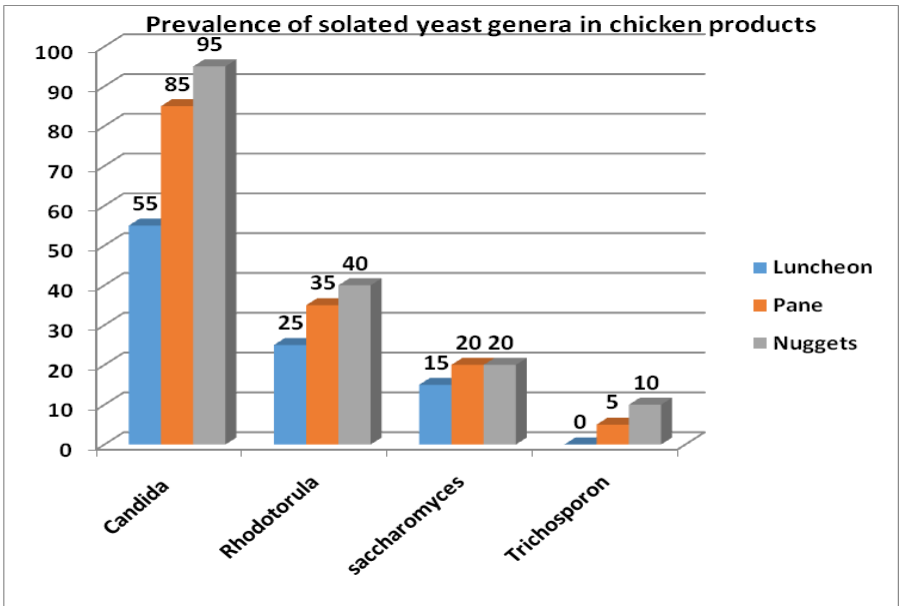


Fig. 2: Prevalence of isolated yeast genera from examined chicken products

The results in Table (4) and Fig. (2) revealed that the predominant yeast genera isolated from examined luncheon, pane and nuggets were Candida 11(55%), 17 (85%), 19 (95%) followed by Rhodotorula 5(25%), 7(35%), 8(40%), Saccharomyces 3(15%), 4(20%), 4(40%) and lastly, Trichosporon with incidence of 0(0%), 1(5%), 2(10%), respectively. Concerning the proportion of yeast genera individually compared to other isolates from chicken luncheon, pane and nuggets as declared in Table (5), Candida species represented by 14(58.3%), 22(59.4%), 25(56.8%) with the highest total incidence 61 (58%) followed by Rhodotorula 7(29.1%),11(29.7%), 13(29.5%) with total incidence 31(29.5%) then Saccharomyces 3(12.5%), 3(8.1%), 4(9.1%) with total incidence 10 (9.5%), finally Trichosporon 0(0%), 1(2.7%), 2(4.5%) with the lowest total incidence 3(2.9%), respectively. Also Table (5) showed that chicken nuggets have the highest number of isolated yeast genera (44) followed by chicken pane (37) then chicken luncheon (24).

Table 5: Number and percentage of isolated genera in examined chicken meat products in relation to the total isolates (n=20 of each).

Samples / yeast species	Luncheon		Pane		Nuggets		Total	
	N	%	N	%	N	%	N	%
Candida	14	58.3	22	59.5	25	56.8	61	58
Rhodotorula	7	29.1	11	29.7	13	29.5	31	29.5
Saccharomyces	3	12.5	3	8.1	4	9.1	10	9.5
Trichosporon	-	-	1	2.7	2	4.5	3	2.9
Total	24	100	37	100	44	100	105	100

Table 6: Incidence of identified yeast species in chicken meat products (n=20 of each)

Sample / Yeast Speacies	Luncheon		Pane		Nuggets	
	N	**%**	**N**	**%**	**N**	**%**
C. albicans	8	40	15	75	17	85
C.tropicalis	3	15	2	10	4	20
C. parapasilosis	2	10	3	15	1	5
C.krusi	-	-	1	5	1	5
C.galabrata	1	5	1	5	2	10
Rh. Rubra	4	20	7	35	7	35
Rh.glutini	3	15	4	20	6	30
S. cervisiae	3	15	3	15	4	20
Trichosporon	-	-	1	5	2	10

Table 7: Total number and percentage of identified yeast species in chicken meat products in relation to the total isolates (20 of each).

Sample / Yeast Speacies	Luncheon		Pane		Nuggets	
	N	%	N	%	N	%
C. albicans	8	33.3	15	40.5	17	38.6
C.tropicalis	3	12.5	2	5.4	4	9.1
C. parapasilosis	2	8.3	3	8.1	1	2.3
C.krusi	-	-	1	2.7	1	2.3
C.galabrata	1	4.2	1	2.7	2	4.5
Rh. Rubra	4	16.7	7	18.9	7	15.9
Rh.glutini	3	12.5	4	10.8	6	13.6
S. cervisiae	3	12.5	3	8.1	4	9.1
Trichosporon	-	-	1	2.7	2	4.5
Total	24	100	37	100	44	100

Concerning yeast species identified from Chicken Luncheon, pane and nuggets Table (6) showed that Candida albicans were the most predominant species which isolated from (40%, 75%, and 85%) of examined luncheon, pane and nuggets samples, respectively. While Trichosporon was the lowest species which isolated from (0%, 5% and 10%), respectively. The results illustrated in Table (7) showed that concerning to chicken luncheon, Candida isolates identified as C.albicans 8 (33.3%), C.parapasilosis 2(8.3%), C.tropicalis 3(12.5%), C.galabrata 1(4.2%), while Rhodotorula identified as Rh. rubra 4 (16.7%), Rh.glutini 3(12.5%) wherease Saccharomyces identified as S. cervisiae 3(12.5%). Dealing with chicken pane, the isolates identified as C. albicans 15(40.5%), C. tropicalis 2(5.4%), C.parapasilosis 3(8.1 %), C. galabrata 1(2.7%), C.krusi 1(2.7 %), Rh. rubra 7(18.9%), Rh. Glutini 4(10.8 %), S. cervisiae 3(8.1 %) and Trichosporon sp. 1(2.7%). Regarding to chicken nuggets the isolates identified as C.albicans 17(38.6%), C.tropicalis 4(9.1%), C. parapasilosis 1 (2.3 %), C.galabrata 2(4.5%) C.krusi 1(2.3%), Rh. Rubra 7(15.9%), Rh. glutini 6(13.6 %), S.cerivisae 4 (9.1%) and Trichosporon species 2(4.5%).

In concerning with PCR examination, the primer used designed for amplification of the ITS region (Tarini *et al.*, 2010). Table (8) showed, all examined 6 samples were successfully amplified a product of 109 pb and give +ve C.albicans with PCR examination (figure10). While 2 samples amplified a products of 919 bp and interpreted as positive killer gene (KHS) of S.cervisiae. Wherease, one sample amplified a product of 727 bp and give positive of killer gene (pelA) (figure11).

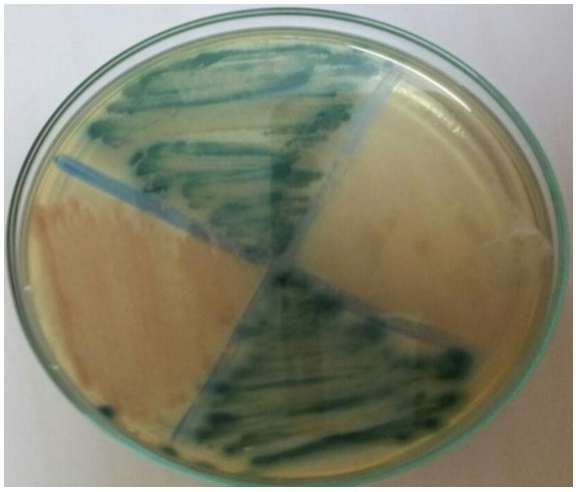


Fig. 3: Candida albicans green, C.parapasilosis white creamy to light pink on CCA

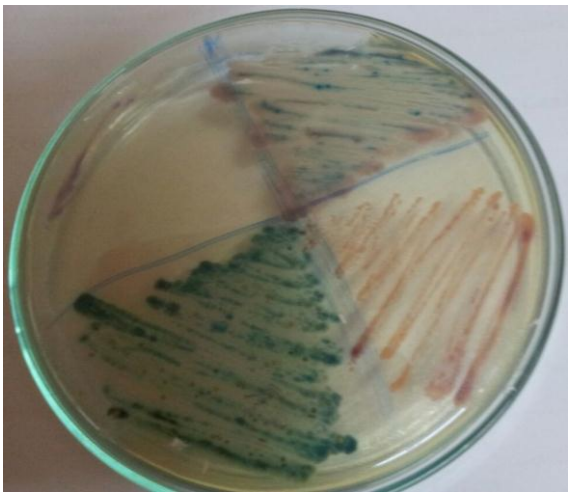


Fig. 4: Candida albicans green, *C.tropicalis blue, C.krusi pink, C.galabrata and C. Parapassilosis* white creamy to light pink on CCA

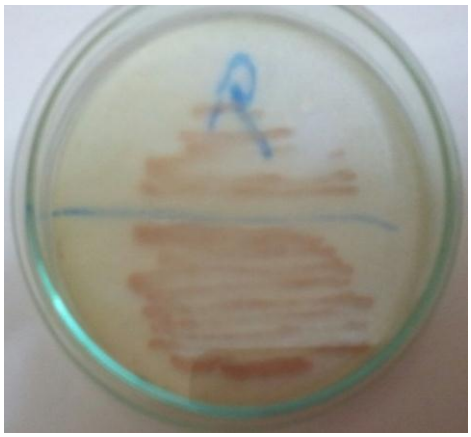


Fig. 5: Rhodotorula orange -pink on CCA

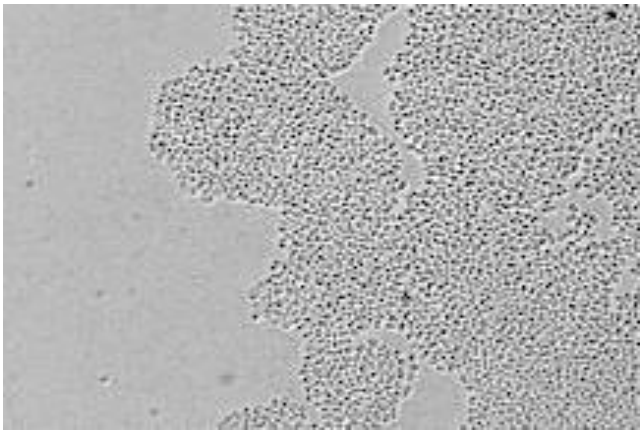


Fig. 6: *C. galabrata* on RAT showing oval budding yeast cells without pseudohyphae

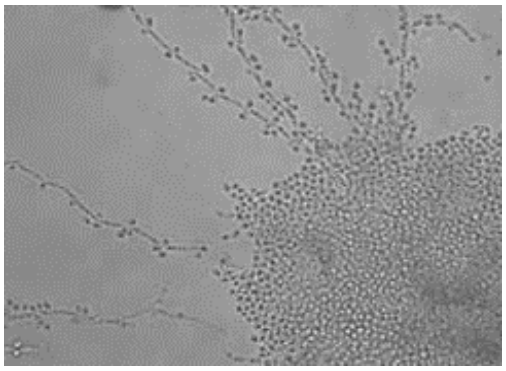


Fig. 7: *C. parapasilosis* on ART showed giant pseudohyphae with few blastospores

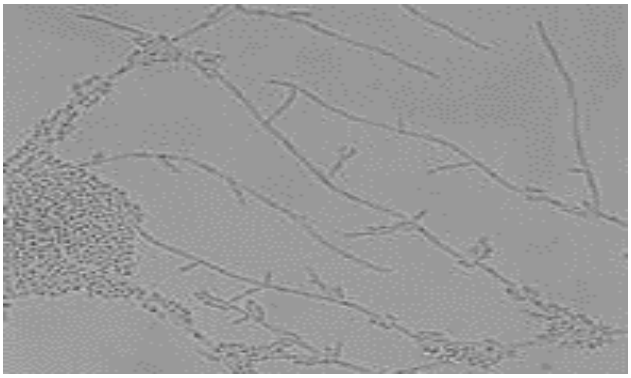


Fig. 8: *C.krusei* on RAT showed primitive pseudohyphae (tree like branching) with eleptical rice like blastospores

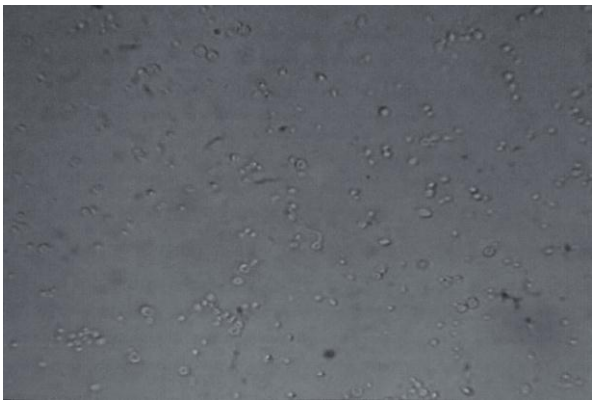


Fig. 9: Microscopic germ tube test, pseudogerm tube appears from some yeast cells which is characteristic of C.albicans

Table 8: Identification of yeast isolates by polymerase chain reaction (PCR)

Sample	Results		
	C. albicans ITS	***KHS***	***pelA***
1	+	-	-
2	+	+	-
3	+	-	+
4	+	+	-
5	+	-	-
6	+	-	-

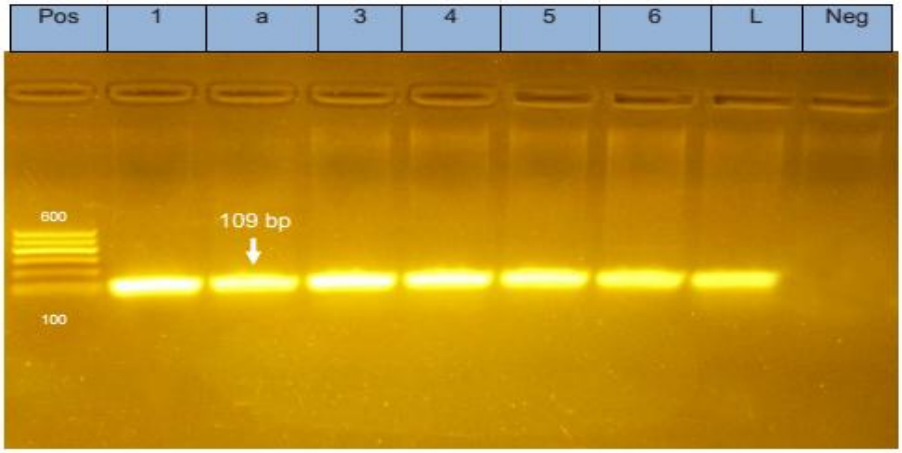
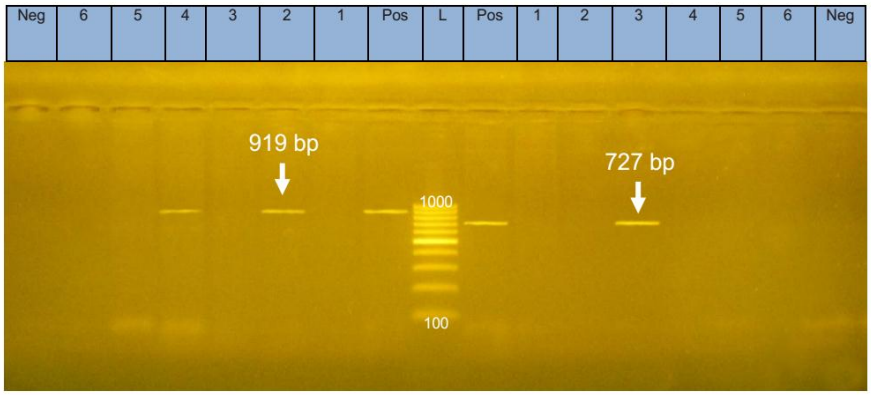


Figure (10): C.albicans ITS
Lane L.: 100-600bp DNA ladder
Lane pos.: positive control at 109 bp, **Lane N**.: negative control
Lane1-6: positive C.albicans



Figuer (11): Killer genes of yeast cells ITS1
Lane L.: 100-1000 bp DNA ladder, **LaneN: negative control**
Lane pos.: Positive control at 919bp, 727bp, **Lane 2,4**: positive KHS, Lane3: positive **Lane 2,4**: positive KHS, **Lane3**: positive pelA

DISSCUSION

The mean yeast count (cfu /gm) were $7.1\times1o^{3}\pm3.1\times1o^{3}$, $1.8\times1o^{5}\pm8.2\times1o^{4}$ and $2.4\times1o^{6}\pm 1.4\times1o^{6}$ for chicken luncheon, pane and nuggets, respectively. The results obtained from total yeast count of chicken pane and nuggets seem to be in agreement with that recorded by Abd El-Azizand Mahmud (2005) and Ibrahim *et al.* (2014). Concerning chicken luncheon samples, the results achieved seems to be in agreement with that recorded by Ismail (1995) and Abd –El Rhman *et al.* (2013) while higher results recorded by Samaha and Abd El-Hafeiz (1997), Abd-El Aziz and Mahmoud (2005), El-Tabiy (2006), Saleh and Salah El Dien (2006), Asefa *et al.* (2010), Ouf *et al*. (2010) and Sharaf and Sabra (2012). These variationscould be attributed to the applied hygienic measures, type and amount of additives used in manufacturing of chicken luncheon, and the time and temperature of exposure of the products. Our results pointed out that chicken nuggets was the most contaminated products with yeast followed by chicken pane, while chicken luncheon was the lowest contaminated product with yeast. The highest yeast count in chicken nuggets may be attributed to the use of raw chicken meat of bad quality as well as unhygienic conditions during slaughtering, handling, processing, storage and transport of these chicken products. Regardless the type of chicken products the minimum yeast count was 1×10^{2}, while the maximum yeast count was$1.4\times1o^{7}$. Nearlysimilar results were obtained by (Jay, 1978, Edris et al., 1992, Ismail, 1995 and Mahmoud and El-Taher, 2001).

The hight proportion of candida yeast genera in all chicken product samples as mentioned in table (4) were inagreement with those observed by (Ismail, 1995; Vilioen *et al.*, 1998; Mahmoud and El-Taher, 2001; Basyoni; 2003 and Abd El-Rhman *et al.*, 2013). Table (5) revealed that Candida have the highest total incidence 61(58%), followed by Rhodotorula with total incidence 31(29.5%) then Saccharomyces 3 (12.5%) finally Trichosporon with lowest total

incidence 3 (2.9%). These results nearly similar to that obtained by Abd El-Rhmanand Ekhateib (1993); Ismail (1995); Pitt and Hocking (2000); Mohamoud and El-Taher (2001); Abd El- Aziz and Mahmoud (2005), El-Tabiy (2006); Hammad *et al.* (2006); Asefa *et al*. (2010); Ouf *et al*. (2010) and AbdEl-Rhman *et al.* (2013). The results of mycological identification of yeast genera revealed that Candida albicans was the most predominant species isolated from chicken luncheon, pane and nuggets. These results nearly similar to that obtained by Mohmoud and El-Taher (2001); Abd El-AzizMahmoud (2005); El–Tabiy (2006); Ouf *et al.* (2010) and Abd El-Rhman *et al.* (2013). All examined samples which identified as Candida albicans by traditional standard methods were give positive Candida albicans by PCR examination and 2 sampls interpreted as positive killer gene (KHS) of S.cerviciae while one samples give positive of killer gene (pelA). These results came in accordance with those recorded by Lim and Lee (2000) and Tarini *et al.* (2010).

CONCLUSION AND RECOMMENDATIONS

From our results we can concluded that Chicken meat products were subjected to the contamination by various type of yeast species which have public health hazard. Therefore, this study was designed to throw spotlight upon yeast isolates from chicken luncheon, pane and Nuggets, studying their culture, macroscopic, microscopic and biochemical characters with special references to identification and genotypic characters of them using PCR which give rapid and accurate results. Accordingly the hygienic quality of chicken meat products must be improved to be safe for human consumption. The contamination must be reduced by implementing satisfactory manufacturing practices and effectively training plant workers in hygienesafety assurance, application of strict hygienic measures during handling, preparation and serving the products.

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مدى تواجد الخمائر فى بعض منتجات لحوم الدواجن

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تم اجراء هذه الدراسة على ٦٠ عينة من منتجات الدجاج المطهية والنصف مطهية مثل الانشون والبانية والناجتس تم تجميعها من الأسواق المختلفة بمحافظة الدقهلية وتم فحصها من حيث مدى تلوثها بالخمائر وكانت القيم المتوسطة لاجمالى عدد الخمائر فى اللانشون والبانية والناجيتس $٣.١\times١٠^{٣}\pm٧.١\times١٠^{٣}$ ، $٨.٢\times١٠^{٤}\pm١.٨\times١٠^{٥}$ ، $١.٤\times١٠^{٦}\pm٢.٤\times١٠^{٦}$ علي التوالى وقد تبين من النتائج أن عينات الناجيتس أكثر تلوثا بالخمائر حيث سجلت أعلى قيمة للعد الكلى للخمائر يليها البانية وأقلهم اللانشون ، وقد تم عزل 105 عينة من الخمائر وصنفت كالأتي : كانديدا بنسبة (58.3 % ، 59.5% و 56.8%) ، والرودوتوريولا بنسبة (29.1% ،29.7% ،)29.5%، والسكارواميسيس بنسبة (12.5% ، 8.1%، 9.1%) لكل من عينات اللانشون والبانية والناجيتس علىالتوالى. وقد كانت الكانديدا البيكانز أكثر العترات التى تم عزلها ولقد تم عمل تفاعل البلمرة المتسلسل للتعرف على الصفات الجينية لفطر الكانددا البيكانز وتم الحصول على ناتج من عملية البلمرة المتسلسل وزنه الجزيئى ٦٠٠ من كل العينات التى تم فحصها أما بالنسبة لخمائر الكانديدا البيكانيز فكان الوزن الجزيئى ١٠٩ وتم الحصول على ناتج من عملية البلمرة المتسلسل وزنه الجزيئى ١٠٠٠ من كل العينات التى تم فحصها أما الجين القاتل لفطر السكارواميسيس فكان وزنه الجزيئى ٩١٩ تم الحصول علية من عدد ٢ عينة من العينات التى تم فحصها.