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Fungal contamination and *Candida spp.* Identification with antifungal susceptibility in different meat and chicken luncheon brands from Asyut City markets in Egypt

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ABSTRACT:

Contamination of ready-to-eat foods sold by markets has become a global health problem. Mycological count carried out on 80 samples of luncheon, divided into 40 samples each from the meat luncheon and the chicken luncheon. The samples were collected from different markets in Asyut city, Asyut governorate, Egypt. It resulted in mould and yeast count mean (SE) in meat luncheon 11(±1), 2 x 10^4 (± 1.96 x10^4) were higher than in chicken luncheon 9 (±1), 7.5 x 10^2 (± 3x10^2), respectively. Mould detection was more in meat luncheon 27 (67.5%) than in chicken luncheon 23 (57.5%). But the opposite was found in yeast detection, where the detection was more in chicken luncheon 27 (67.5%) than in meat luncheon 25 (62.5%). All positive samples for yeast count were relieved 47 different colonies type for each group of luncheon meat and chicken. The most frequent *Candida spp.* was *C. glabrata, C. parapsilosis,* or *C. membranifaciens* 58 (61.7%) in all luncheon samples. From which 3 (3.2%) was *C. glabrata*, 6 (6.4%) were *C. parapsilosis* and others 49 (52.1%) were mixed *C. glabrata, C. parapsilosis* or *C. membranifaciens*. Followed by *C. utilis* were 17 (18.1%) then, *C. krusei* were 8 (8.5%). Only one sample (2.1%) was *C. albicans* in all luncheon samples that found in chicken luncheon. The resistance was noted only in samples against Fluconazole and Nystatin, which represented 10 (10.6%) and 7 (7.45%) for Fluconazole (25 µg) and Nystatin (100 units) in all luncheon samples, respectively. While, the most Susceptible Dose Dependent was against Nystatin was 59 (62.8%) samples in all luncheon samples. The majority of isolates were sensitive to all four antifungal drugs, except Nystatin, which was the lowest percent from all isolates, 28 (29.8%), with Amphotericin (100 units) 92 (97.9%) being the most sensitive in isolates from all luncheon samples.

KEYWORDS:

Mould, Yeast, Candida Spp., Luncheon, Antifungal Susceptibility Test

INTRODUCTION:

Ready-to-eat (RTE) meat and chicken products are very varied, and some are very easy to prepare, such as luncheon. It is a popular food in Egypt, providing a good source of protein, delicious taste, being cheap and produced in many new flavors. Thus, the manufacturing process from used raw materials passing initial processing, packaging, and storage until the product is sufficiently cooked and consumed, may provide a way for contamination (Saad *et al.* 2018). The processing of the products may lead to contamination from the hands, knives, workers' clothes, the gut, the surfaces, or from the environment, resulting in poor hygiene and an inferior quality for human consumption, and cause a public health hazard (Debabrata 2012).

Egyptian Standard (3493/2005) for Products of meat poultry treated with heat, ES (1696 – 2005) for luncheon poultry meat, and ES (1114-2005) for luncheon meat; those entire products stated that they must be free from mould and yeast contamination(EOSQ 2005).

Yeast and mould grow in a wide range of PH and Temperature. Mould causes meat deterioration and economic losses from food production by may reach 5-10%. Some toxigenic fungi produce secondary metabolites with highly toxic features recognized as mycotoxins, which are in minute amount may lead to carcinogenic, neurotoxic, nephrotoxic, immunosuppressive, and estrogenic effects (Pitt 1997). Yeast could spoil cold-preserved food like luncheon through their proteolytic and lipolytic activity, causing rancidity and off-flavor (Deak 1991). The presence of certain pathogenic yeasts in meat products renders them unfit for consumption (Ahmed and Ismail 2010).

Candida genus from all yeast have a major public health hazards especially when detected in RTE food, World health organization listed *Candida spp.* that cause 90% of infections as opportunistic pathogens; *Candida albicans, Candida glabrata, Candida tropicalis, Candida krusei,* and *Candida auris* (de Melo Pereira *et al.* 2022, Organization 2022).

Transmission candida from food to human can exhibit more danger and cause disease when *Candida spp.* acquired virulence factor as resistance against antifungals and transmitted from commensal to potential pathogenic state (Marak and Dhanashree 2018). The molecular mechanisms associated with antifungal resistance include gain of function mutations and overexpression of membrane transporters, altered cell wall and ergosterol biosynthesis. Also the resistance to antifungal drugs increased by forming biofilms (Somanon Bhattacharya 2020 , Sasani *et al.* 2021).

The study aim is to investigate the prevalence of mycological contamination in luncheon as RTE food and figure out the most common candida species found in this product and the extend of candida resistance to antifungal drug used in the study.

MATERIALS AND METHODS:

1. Sample Collection and Preparation:

1.1. Collection of Luncheon Samples:

Eighty samples of luncheon were collected from Asyut city, Asyut governorate, Egypt. The samples were divided into 40 samples of meat luncheon from eighteen different brands and 40 samples of chicken luncheon from eleven different brands, gathered from various markets across the city between January 2023 and February 2023. The samples were preserved in a cool temperature on ice until they reached the laboratory without being opened.

1.2. Preparation of Luncheon Samples (Pitt and Hocking 1995, ISO 2007)**:**

Five grams of each sample were weighed, homogenized with manual homogenizers, then added to 45 ml of sterile Nutrient broth (0.1%) (Himedia, India) in the sterile falcon tubes.

2.Total Fungal (Mould and Yeast) Count of Luncheon Samples (Pitt and Hocking 1995, ISO 2007):

From the previously prepared original dilution (1:10), 0.1 ml was spread by a sterile spreader over the dry surface of previously prepared sterile Sabroud Dextrose Agar (Himedia, India) with antibiotics Chloramphenicol (0.05 g) and Gentamicin (40-80 mg/ml)/ liter plates in duplicates. The plates were then inoculated inverted at 25°C for 5 days. After the incubation period, the average of mould and yeast colonies on the plates were enumerated and recorded. The mould counts and yeast counts per each gram of the examined samples were then calculated according to the dilution and amount taken. Yeast isolates that grew on SDA were examined for colony identification. They exhibited different forms such as wrinkled, pinpointed, smooth wide flatted, smooth pasty, and concave smooth brown. Different yeast colony shapes were picked for further identification and preservation in Sabroud Dextrose broth with 15% glycerol in the freezer at -20°C.

3. Microscopic Identification for Isolated Yeast:

Afterward, different colonies were filmed on slides, stained with methylene blue, and examined under a microscope. All isolates were oval-shaped, some with buds, and all had a unicellular organism form. Only three samples were elongated in shape and included hyphae in-between cells, considered as yeast-like fungi, Figure (1).

4. HiCrome Candida Differential Agar-M1297A (Himedia, India):

All yeast isolates that exhibited more than one shape of colony for a sample on SDA were picked up by a sterile loop to be sub-cultured on HiCrome Candida Differential Agar (HiCA) medium for 48 hrs. at 37°C aerobically. The colonies were differentiated according to the color of the colony produced, as per (Table1). The three samples described as yeast-like fungi showed no growth on HiCA media.

Table (1): Differentiation of Candida with HiCrome Candida Differential Agar.

Candida spp.	**Color on HiCrome Candida Differential agar**
1-*Candida albicans (C. albicans)*	Light green
2-*Candida glabrata (C. glabrata)*	White to Cream - Cream to mauve
3-*Candida krusei (C. krusei)*	Purple, fuzzy
4-*Candida tropicalis (C. tropicalis)*	Blue to purple
5-*Candida kefyr (C. kefyr)*	Cream to White with slight Purple center or Brown
6-*Candida utilis (C. utilis)*	Pale pink to Pinkish purple
7-*Candida parapsilosis (C. parapsilosis)*	White to Cream (Cream to pale pink)
8-*Candida membranifaciens (C. membranifaciens)*	White to Cream (Occasionally blue or Blue-grey)

5. Antifungal Susceptibility Test:

Identified Candida species by HiCA and yeast-like fungi separate colonies were used for making a suspension by dissolving 1-2 colonies in 2 ml of sterile saline and comparing the suspension of turbidity to be equivalent to 0.5 McFarland standard. Using a sterile swab, the suspension was spread on Mueller-Hinton (MH) agar (Himedia, India) supplemented with 2% glucose and 0.5µg/ml methylene blue in three dimensions. After a while, the antifungal disks were placed on the medium surface and incubated at 37 °C for 24 h. Plates that did not register growth were re-incubated for an extra 24 h. The antifungal disks (Himedia, India) used were azoles (Fluconazole (25 µg), Voriconazole (1µg)) and polyene (Amphotericin (100 units) and Nystatin (100 units)). The zone diameter reading of the antifungal disk was interpreted as in table (2) (Wayne 2004).

Table (2): Zone diameter for used antifungal disc Interpretive Standards.

Antifungal agents	Zone diameter in mm		
	Resistant (R) (mm)	Susceptible Dose Dependent (SDD) (mm)	Susceptible (S) (mm)
Fluconazole FLC (25 µg)	≤14	15–18	≥19
Amphotericin AMP (100 units)	≤10mm	10–14 mm	≥15mm
Nystatin NS (100 units)	≤16	17–24	≥25
Voriconazole VRC (1 µg)	≤13	14–16	≥17

Data Analyses:

The collected data for samples were recorded manually into MS-Office Excel software Version 2010. The data was later analyzed using Statistical Package for the Social Sciences Version 27 (IBM SPSS, SPSS Inc., Chicago, IL, USA). Categorical data variables were statistically described in the form of frequencies and percentages, while continuous data variables were summarized as mean (Standard Error (SE)) and minimum and maximum (Min.- Max.).

RESULTS:

Mycological detection and counting in Luncheon samples:

From all eighty-luncheon samples, yeast was detected in 52 (65%) samples, and moulds were found in 50 (62.5%) samples. The distribution of yeast and mould in meat and chicken luncheon samples was relatively close. Yeast detection was 25 (62.5%) and 27 (67.5%) in meat and chicken luncheon samples, respectively. While mould detection was 27 (67.5%) and 23 (57.5%) in meat and chicken luncheon samples, respectively (Table 3).

Table (3): The frequency of yeast and mould detection in meat and chicken luncheon samples.

Result of samples	**Meat luncheon (Total No.40)**		**Chicken luncheon (Total No.40)**		**All Luncheon samples (Total No.80)**	
	Yeast	Mould	Yeast	Mould	Yeast	Mould
Negative	15 (37.5%)	13 (32.5%)	13 (32.5%)	17 (42.5%)	28 (35%)	30 (37.5%)
Positive	25 (62.5%)	27 (67.5%)	27 (67.5%)	23 (57.5%)	52 (65%)	50 (62.5%)

Mycological count in all luncheon samples, the beginning was (5 CFU/g) for both yeast and mould, and the maximum number was (4.9 x 10^5 CFU/g) for yeast and (35 CFU/g) for mould, and the mean with standard error (SE) was 1 x 10^4(± 0.94 x10^4) CFU/g for yeast and 10 (± 0.91) CFU/g for mould. The highest yeast and mould count was detected in meat luncheon samples, where yeast and mould count mean ± SE was 2 × 10^4 (± 1.96 × 10^4) CFU/g and 11(±1) CFU/g, respectively. The minimum count was (5 CFU/g) for both yeast and mould. But the maximum count was (4.9 x 10^5 CFU/g) for

yeast and (35 CFU/g) for mould. In chicken luncheon samples, yeast and mould count mean ± SE was 7.5 x 10^2 (± 3x10^2) CFU/g and 9 (±1) CFU/g, respectively. The minimum count was (5 CFU/g) for both yeast and mould. The maximum count was (6.6 x 10^3 CFU/g) for yeast and (25 CFU/g) for mould (Table 4), (Figure 2).

Table (4): Detection of Mycological counts (yeast and mould) in meat, chicken and all luncheon samples.

	Meat luncheon count (CFU/g)		**Chicken luncheon count (CFU/g)**		**All luncheon count (CFU/g)**	
	Yeast (No.25)	Mould (No.27)	Yeast (No.27)	Mould (No.23)	Yeast (No.52)	Mould (No.50)
Minimum – Maximum	(5 - 4.9 x 10^5)	(5 - 35)	(5- 6.6 x 10^3)	(5 – 25)	(5 - 4.9 x 10^5)	(5 - 35)
Mean (SE)	2 x 10^4 (± 1.96 x10^4)	11(±1)	7.5 x 10^2 (± 3x10^2)	9 (±1)	1 x 10^4(± 0.94 x10^4)	10 (±0.91)

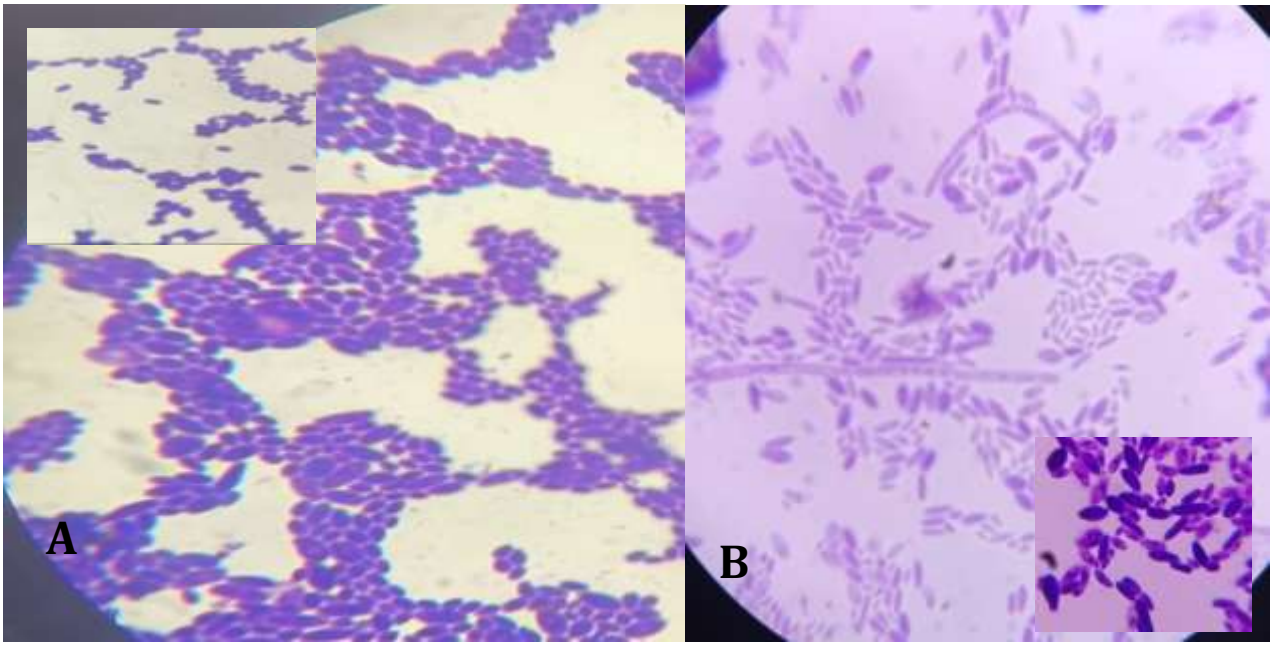

Figure (1): Film from different pure colonies stained with gram stain showing; (A): *Candida spp.*, (B): Yeast like fungi.

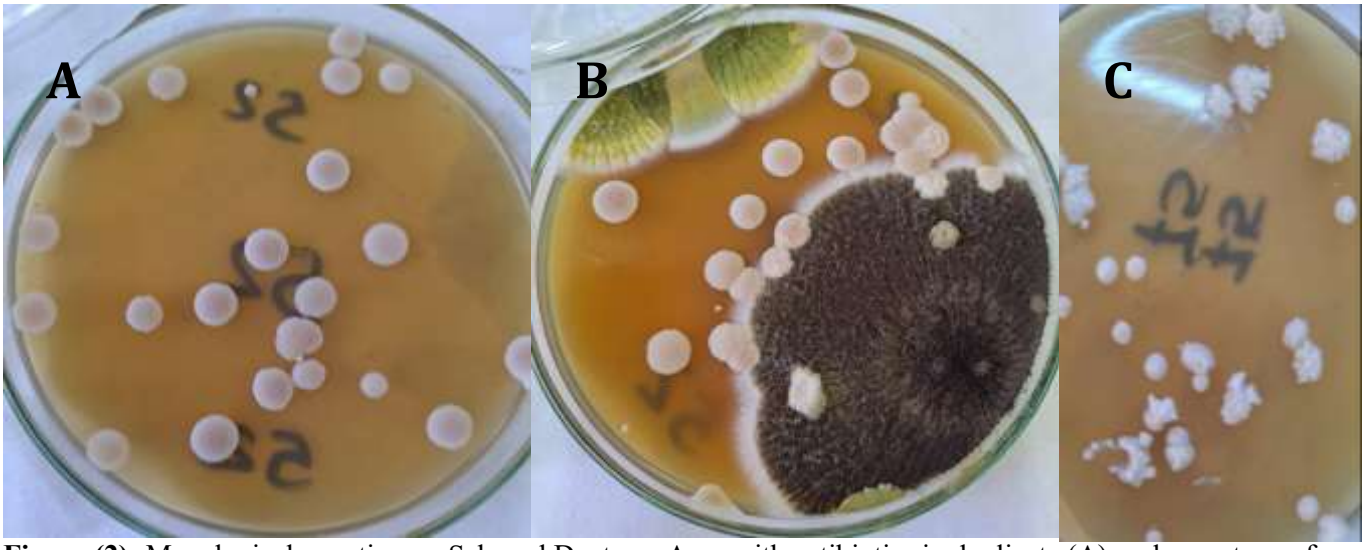

Figure (2): Mycological counting on Sabroud Dextrose Agar with antibiotics in duplicate (A): only one type of yeast growth (B): yeast growth plus mould growth, but (C): demonstrate different colonies types of yeast growth.

Yeast differentiation by microscopic examination and HiCA:

Mycological count showed on SDA different shapes of colonies. Microscopic examination and culturing of 25 samples of meat luncheon yeast and 27 samples of chicken luncheon yeast on HiCA revealed 47 types of *Candida spp.* in meat luncheon samples, and 44 (93.6%) types of *Candida spp.* and 3 (6.4%) yeast-like fungi isolates in chicken luncheon samples. The most frequent *Candida spp.* were *C. glabrata, C. parapsilosis,* or *C. membranifaciens* 58 (61.7%) in all luncheon samples, 31 (65.96%), and 27 (57.4%) in meat luncheon and chicken luncheon samples, respectively. From culturing on HiCA 3 (3.2%), colored *C. glabrata* (Cream to mauve), 6 (6.4%) were *C. parapsilosis* (Cream to pale pink), and the remaining 49 (52.1%) were mixed *C. glabrata, C. parapsilosis,* or *C. membranifaciens* (white to cream) in all luncheon samples. In meat luncheon samples, 1 sample (2.1%) was *C. glabrata*, 4 (8.5%) were *C. parapsilosis*, and the remaining 26 (55.3%) were mixed *C. glabrata, C. parapsilosis,* or *C. membranifaciens*. In chicken luncheon samples, 2 (4.3%) were *C. glabrata*, 2 (4.3%) were *C. parapsilosis*, and the remaining 23 (48.9%) were mixed *C. glabrata, C. parapsilosis,* or *C. membranifaciens*. Followed by *C. utilis* were 17 (18.1%) in all luncheon samples divided to; 8 (17.0%) and 9 (19.1%), then, *C. krusei* were 8 (8.5%) in all luncheon samples divided to; 5 (10.6%) and 3 (6.4%), finally *C. kefyr were* 7 (7.45%) in all luncheon samples divided to; 3 (6.4%) and 4 (8.5%), in meat luncheon and chicken luncheon samples, respectively. Only one sample (2.1%) was *C. albicans* in chicken luncheon samples (Table 5).

Table (5): Frequency of detected candida spp. on HiCrome candida differential agar and yeast like after microscopic examination in meat, chicken and all luncheon samples.

Types / samples	Meat Luncheon (No.25)	Chicken Luncheon (No.27)	All Luncheon samples (No.52)
***Candida spp.* Isolates on HiCrome**	47 (100%)	44(93.6%)	91 (96.8%)
1-*C. albicans*	0 (0%)	1 (2.1%)	1 (1.1%)
2-*C. glabrata*	1 (2.1%)	2 (4.3%)	3 (3.2%)
3-*C. krusei*	5 (10.6%)	3 (6.4%)	8 (8.5%)
4-*C. kefyr*	3 (6.4%)	4 (8.5%)	7 (7.45%)
5-*C. utilis*	8 (17.0%)	9 (19.1%)	17(18.1%)
6-*C. parapsilosis*	4 (8.5%)	2 (4.3%)	6 (6.4%)
			49 (52.1%)

7-*C. glabrata, C. parapsilosis or C. membranifaciens*	26 (55.3%)	23 (48.9%)	
Yeast like fungi isolates (Non candida)	0 (0%)	3 (6.4%)	3 (3.2%)
Total	47 (100%)	47 (100%)	94 (100%)

(%): Related to total Candida spp. number =47

Antifungal Susceptibility Test Outcome:

Different *Candida spp.* and yeast-like fungi were counted and scaled from one colony per sample to four colonies per sample. The mean (SE) of different colonies was (1.9 ± 0.16), (1.7 ± 0.16), and (1.8 ± 0.11) for meat, chicken, and all luncheon samples, respectively. The resistance was noted only in samples against Fluconazole and Nystatin, which represented 10 (10.6%) samples for Fluconazole and 7 (7.45%) samples for Nystatin in all luncheon samples. The most Fluconazole resistance was in chicken luncheon samples 8 (17%). The highest Nystatin resistance was in meat luncheon samples 4 (8.5%). The majority of isolates were sensitive to all antifungal drugs except Nystatin, with Amphotericin 92 (97.9%) being the most sensitive in isolates from all luncheon samples, where all chicken luncheon samples were sensitive to Amphotericin 47(100%), and 45 (95.7%) samples were sensitive in meat luncheon samples. Followed by Voriconazole, sensitivity against it was 91(96.8%) in all luncheon samples, then sensitivity against Fluconazole was 77(81.9%), and finally sensitivity against Nystatin was 28 (29.8%) in all luncheon samples. The most susceptible dose-dependent was against Nystatin 59 (62.8%) samples in all luncheon samples, followed by 7 (7.45%), 3 (3.2%), and then 2 (2.13%) samples from all luncheon samples in Fluconazole, Voriconazole, and Amphotericin, respectively. Only one Candida isolate was resistant to two types of antifungal drugs from meat luncheon samples and identified as *C.utilis* (Tables 6, 7 &8)(Figure 3).

Table (6): Frequency and description of all different colonies types per sample and zone diameter in mm of different antifungal disc for total yeast samples to meat, chicken and all luncheons.

	Yeast types of colonies N. / samples Mean (SE) (Min.-Max.)	**FLC (25 mcg) Zone in mm Mean (SE) (Min.- Max.)**	**AMP (100 Unit) Zone in mm Mean (SE) (Min.- Max.)**	**NS (100 Unit) Zone in mm Mean (SE) (Min.- Max.)**	**VRC I mg Zone in mm Mean (SE) (Min. - Max.)**
Meat Luncheon all yeast +ve isolates (No.25)	Colonies N. = 47 (1.9 ± 0.16) (1 - 4)	33.38 (±1.7) (13 - 80)	21.02 (± 0.7) (13 - 37)	21.96 (± 0.7) (12 - 35)	39.66 (±1.98) (15 - 80)
Chicken Luncheon all yeast +ve isolates (No.27)	Colonies N. = 47 (1.7 ± 0.16) (1– 4)	33.89 (±2.9) (0 - 67)	24.00 (± 1.15) (15 - 46)	25.40 (± 1.11) (13 - 46)	43.94 (±2.41) (15 - 76)
All Luncheon all yeast +ve isolates (No.52)	Colonies N. = 94 (1.8 ± 0.11) (1– 4)	33.64 (±1.7) (0 - 80)	22.5 (± 0.68) (13 - 46)	23.7 (± 0.69) (12 - 46)	41.80 (±1.57) (15 - 80)

Figure (3): Antifungal Susceptibility Test to the four antifungal drugs used in the study.

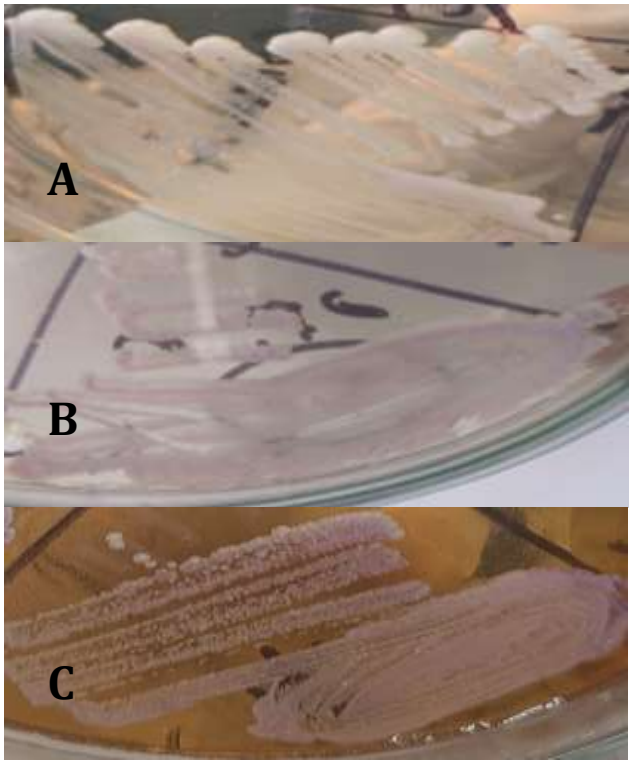

Figure 4: HiCrome Candida Differential Agar inoculated with yeast, (A) White creamy color (*C. glabrata, C. parapsilosis or C.membrani-faciens)*, (B) White with slight Purple center *(C. kefyr)* (C) Cream to mauve *(C. glabrata)*

N. (%*) / Samples	Flc(25 mcg)			AMP (100 Unit)		NS (100 Unit)			VRC (I mg)	
	S	SDD	R	S	SDD	S	SDD	R	S	SDD

Meat Luncheon										
• *Candida spp.* Isolates	43(91.5%)	2(4.3%)	2(4.3%)	45(95.7%)	2(4.3%)	8(17 %)	35(74.5%)	4(8.5%)	46(97.9%)	1(2.1%)
2- *C. glabrata*	1 (2.1%)	0(0%)	0(0%)	1(2.1%)	0(0%)	0(0%)	1(2.1%)	0(0%)	1(2.1%)	0(0%)
3- *C. krusei*	3 (6.4%)	2(4.3%)	0(0%)	4(8.5%)	1(2.1%)	1(2.1%)	3(6.4%)	1(2.1%)	5(10.6%)	0(0%)
4- *C. kefyr*	3 (6.4%)	0(0%)	0(0%)	3(6.4%)	0(0%)	1(2.1%)	2(4.3%)	0(0%)	3(6.4%)	0(0%)
5- *C. utilis*	7(14.9%)	0(0%)	1(2.1%)	7(14.9%)	1(2.1%)	1(2.1%)	6(12.8%)	1(2.1%)	8(17.0%)	0(0%)
6- *C. parapsilosis*	3(6.4%)	0(0%)	1(2.1%)	4(8.5%)	0(0%)	3(6.4%)	1(2.1%)	0(0%)	4(8.5%)	0(0%)
7- *C. glabrata, C. parapsilosis or C. membranifaciens*	26 (55.3%)	0(0%)	0(0%)	26(55.3%)	0(0%)	2(4.3%)	22(46.8%)	2(4.3%)	25 (53.2%)	1(2.1%)
• Yeast like fungi (non candida)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Total (N.47)	**43 (91.5%)**	**2 (4.3%)**	**2 (4.3%)**	**45 (95.7%)**	**2 (4.3%)**	**8 (17 %)**	**35 (74.5%)**	**4 (8.5%)**	**46 (97.9%)**	**1 (2.1%)**
Chicken Luncheon										
• *Candida spp.* Isolates	31(65.9%)	5(10.6%)	8(17%)	44(93.6%)	-	19(40.4%)	22(46.8%)	3(6.4%)	42(89.4%)	2(4.3%)
1- *C. albicans*	1(2.1%)	0(0%)	0(0%)	1(2.1%)	-	1(2.1%)	0(0%)	0(0%)	1(2.1%)	0(0%)
2- *C. glabrata*	0(0%)	1(2.1%)	1(2.1%)	2(4.3%)	-	0(0%)	2(4.3%)	0(0%)	2(4.3%)	0(0%)
3- *C. krusei*	1(2.1%)	0(0%)	2(4.3%)	3(6.4%)	-	2(4.3%)	1(2.1%)	0(0%)	3(6.4%)	0(0%)
4- *C. kefyr*	3(6.4%)	0(0%)	1(2.1%)	4(8.5%)	-	1(2.1%)	2(4.3%)	1(2.1%)	4(8.5%)	0(0%)
5- *C. utilis*	5(10.6%)	3(6.4%)	1(2.1%)	9(19.1%)	-	6(12.8%)	3(6.4%)	0(0%)	8(17.0%)	1(2.1%)
6- *C. parapsilosis*	1(2.1%)	0(0%)	1(2.1%)	2(4.3%)	-	1(2.1%)	1(2.1%)	0(0%)	2(4.3%)	0(0%)
7- *C. glabrata, C. parapsilosis or C.membrani-faciens*	20(42.6%)	1(2.1%)	2(4.3%)	23(48.9%)	-	8(17.0%)	13(17.7%)	2(4.3%)	22(46.8%)	1(2.1%)
• Yeast like fungi (non candida)	3(6.4%)	0(0%)	0(0%)	3(6.4%)	-	1(2.1%)	2(4.3%)	0(0%)	3(6.4%)	0(0%)
Total (N.47)	**34 (72.3%)**	**5 (10.6%)**	**8 (17 %)**	**47 (100%)**	**-**	**20 (42.6%)**	**24 (51.1%)**	**3 (6.4%)**	**45 (95.7%)**	**2 (4.3%)**

Table (7): In vitro Susceptibility patterns of different *Candida spp.* and non- Candida yeast like fungi to Fluconazole (25 µg), Amphotericin (100 units), Nystatin (100 units) and Voriconazole (1 µg) for meat or chicken luncheon (N. = 47).

(%*): Related to total Candida spp. number = 47/ type (meat or chicken) or total = 94.

Table (8): In vitro Susceptibility patterns of different *Candida spp.* and non- Candida yeast like fungi to different antifungal drugs for all luncheon samples (N. =94).

N. (%*)	Flc (25 mcg)	AMP (100 Unit)	NS 100 Unit	VRC I mg

Samples	S	SDD	R	S	SDD	S	SDD	R	S	SDD
All Luncheon										
• *Candida spp.* Isolates	74(78.7%)	7(7.4%)	10(10.6%)	89(94.7%)	2(2.1%)	27(28.7%)	57(60.6%)	7(7.4%)	88(93.6%)	3(3.2%)
1- *C. albicans*	1 (1.1%)	0(0%)	0(0%)	1(1.1%)	0(0%)	1(1.1%)	0(0%)	0 (0%)	1(1.1%)	0(0%)
2- *C. glabrata*	1 (1.1%)	1(1.1%)	1(1.1%)	3(3.2%)	0(0%)	0 (0%)	3(3.2%)	0 (0%)	3(3.2%)	0(0%)
3- *C. krusei*	4 (4.25%)	2(2.1%)	2(2.1%)	7(7.4%)	1(1.1%)	3(3.2%)	4 (4.25%)	1(1.1%)	8(8.5%)	0(0%)
4- *C. kefyr*	6(6.4%)	0(0%)	1(1.1%)	7(7.4%)	0(0%)	2(2.1%)	2(2.1%)	1(1.1%)	7(7.4%)	0(0%)
5- *C. utilis*	12(12.8%)	3(3.2%)	2(2.1%)	16(17%)	1(1.1%)	7(7.4%)	9(9.6%)	1(1.1%)	16(17%)	1(1.1%)
6- *C. parapsilosis*	4(4.25%)	0(0%)	2(2.1%)	6(6.4%)	0(0%)	4 (4.25%)	2(2.1%)	0 (0%)	6(6.4%)	2(2.1%)
7- *C. glabrata, C. parapsilosis or C. membranifaciens*	46(48.9%)	1(1.1%)	2(2.1%)	49(52.1%)	0(0%)	10(10.6%)	35(37.2%)	4(4.25%)	47(50%)	0(0%)
• Yeast like fungi (non candida)	3(3.2%)	0 (0%)	0 (0%)	3(3.2%)	0 (0%)	1(1.1%)	2 (2.1%)	0 (0%)	3(3.2%)	0 (0%)
Total Luncheon samples (N.94)	**77 (81.9%)**	**7 (7.45%)**	**10 (10.6%)**	**92 (97.9%)**	**2 (2.13%)**	**28 (29.8%)**	**59 (62.8%)**	**7 (7.45%)**	**91 (96.8%)**	**3 (3.2%)**

DISCUSSION:

Quality control of RTE meat and chicken luncheon could be detected from mycological counting and its presence in food spoil it by putrefactive processes. In table 3 and 4, yeast and mould positive samples were more than the half. Yeast detection was 62.5% and 67.5% in meat and chicken luncheon samples, respectively. While mould detection was 67.5% and 57.5% in meat and chicken luncheon samples, respectively. Ali *et al.,* (2005) found lower percent than ours, mould isolation from meat and chicken luncheon samples was 45% and 20%, respectively. Sabra, (2012) and Shaltout, (2014) detected 50% and 64% mould, but yeast was 65% and 80% from chicken luncheon samples respectively, which is close to our finding. Mousa *et al.,* (2014) found that total yeast and mold count of examined meat luncheon samples was 100%. A *et al.,* (2016), in his study 70% from isolates contain mould in meat luncheon samples and this percent consider close to this study.

The highest yeast and mould count was detected in meat luncheon samples against chicken luncheon, where yeast and mould count mean ± SE was $2 \times 10^4 \pm 1.96 \times 10^4$ and 11±1 CFU/g, respectively. While, the maximum count was 4.9×10^5 for yeast and 35 CFU/g for mould. Mould count in our study was low amount in comparing with other previous studies but yeast count was similar to some studies. A *et al.,* (2016), Kasem ,(2016), Elsayed *et al.,* (2018) and Akwieten *et al.,* (2022) Found that mould count mean value was 6.4×10^3, ranged from 2x10 to $4.2x10^3$ with a mean value of $4.7x10^2 \pm 2.1x10^2$,the mean mould count was $1.3x10^2 \pm 2.1x10$, and ranged from 1×10^2 to 1.1×10^3 with a mean value of $6.1 \times 10^2 \pm 1.1x10^2$ CFU/g respectively, while yeast count mean value was 1.5×10^4 and ranged from 6×10^2 to $3.2x10^3$ with a mean value of $1.1 \times 10^3 \pm 4.1 \times 10^2$ CFU/g according to Kasem and Hana respectively, in their studies on meat luncheon.

In chicken luncheon samples, yeast and mould count mean ± SE was $7.5 \times 10^2 \pm 3x10^2$ and 9 ±1 CFU/g, respectively. The maximum count was 6.6×10^3 for yeast and 25 CFU/g for mould. The beginning number both yeast and mould in all counts was 5 CFU/g. Ali *et al.,* (2005) found that the mean value of mould count respectively was $3.1 \times 10^3 \pm 0.3x10^3$ and 4.0×10^2 /g $\pm 0.2x\ 10^2$ CFU/g in meat luncheon and chicken luncheon. Sabra, (2012) and Shaltout, (2014) studies on chicken luncheon detected that; mould count was 8×10^2 and $3.3 \times 10^2 \pm 2.0 \times 10^2$ but yeast counts were 4.3 x

10^3 and 3.3 x10^2± 2.0 x 10^2 CFU/g. El-Matary and ZAKI, (2016) studied the mean value of the total yeast counts of chicken luncheon in her research and was 7.1×10^3 ±3.1 $\times10^3$ CFU/g.

Microscopic examination and culturing of 25 samples of meat luncheon yeast and 27 samples of chicken luncheon yeast on HiCA revealed 100% different *Candida spp.* in meat luncheon samples, and 93.6% *Candida spp.* and 6.4% yeast-like fungi isolates in chicken luncheon samples. The sensitivity and specificity of HiCrome agar for identificationnd detection of *C. albicans*, *Candida* - parapsilosis and *Candida glabrata* were above 90%, whereas sensitivity and specificity of carbohydrate fermentation test were 86.67% and 74.07%, respectively according to Charles *et al.,* (2015). In a study by Shaltout, (2014) stated that, the predominant species of yeasts isolated from different chicken product including chicken luncheon were *Candida spp.* represented 32.5%. Rahal *et al.,* (2013) and El-Matary and ZAKI, (2016) found 54.5% and 58.3% from isolated yeast *Candida spp*. in chicken luncheon, respectively.

From table 5, The most frequent isolated *Candida spp.* were mixture of *C. glabrata, C. parapsilosis,* or *C. membranifaciens* 52.1% in all luncheon samples, 55.3% and 48.9% in meat luncheon and chicken luncheon samples, respectively. In a study to Brr and Mahmoud, (2005) found that isolated *C. glabrata* and *C. parapsilosis* represented 35% of isolated *Candida spp.* from chicken luncheon.
In meat luncheon samples; 2.1% were *C. glabrata*, 8.5% were *C. parapsilosis* and in chicken luncheon samples; both *C. glabrata* and *C. parapsilosis* were 4.3%. Shaltout, (2014) found 1.3% *C. parapsilosis* from yeast in chicken luncheon samples. El-Matary and ZAKI, (2016) found that 8.3% *C.parapasilosis* and 4.2% *C.galabrata* were from isolated yeast from chicken luncheon. Mohamed *et al.,* (2023) detected 33.3% *C.parapasilosis* from yeast in frozen meat samples.

Followed by *C. utilis* were 17.0% and 19.1%, then, *C. krusei* were 10.6% and 6.4%, finally *C. kefyr* were 6.4% and 8.5%, in meat luncheon and chicken luncheon samples, respectively. *C. kefyr* is recognized for its presence in dairy products and had been identified as an emerging pathogen, particularly in immunocompromised individuals (Karstrup *et al.* 2017, Ahmad 2020). *C. utilis* is edible yeast, which as a old food or feed additive and in the food industry categorized as BGRAS (generally recognized as safe) by the US Food and Drug Administration, for medical applications can be used as probiotic agents and induces tolerance to myelin antigen on its surface (Boze *et al.* 1992, Buerth *et al.* 2016). While its role as a spoilage organism in meat products is less frequently highlighted compared to other yeasts, its presence warrants investigation to understand its potential impact on food quality and safety. *C. krusei* was 9 % of *Candida spp.* in chicken luncheon and couldn't be detect in meat luncheon samples according to Rahal *et al.,* (2013) and Mousa *et al.,* (2014). Only 2.1% was *C. albicans* in chicken luncheon samples. Which is a small percent when compare to a study on chicken luncheon, that indicated that *C. albicans* represent 33.3% from isolated yeast and couldn't find any *C. krusei* from isolates. While this study identified Candida as prevalent, it did not specifically detail the prevalence of C. kefyr or C. utilis in chicken luncheon (El-Matary and ZAKI 2016). On the opposite side Shaltout, (2014) couldn't find any *C. albicans* from chicken luncheon in his study but detected 1.3% *C. krusei* .

Tables 6,7 & 8, showing the antifungal pattern of candida isolates, the majority of isolates were sensitive to Amphotericin 97.9%, 100%, and 95.7% in all luncheon samples, all chicken luncheon and meat luncheon samples respectively. Followed by Voriconazole, Fluconazole and Nystatin sensitivity against them were 96.8%, 81.9%, and 29.8% in all luncheon samples, respectively. The most susceptible dose-dependent was against Nystatin 62.8% followed by 7.45%, 3.2%, and then 2.13% in all luncheon samples to Fluconazole, Voriconazole, and Amphotericin, respectively. The resistance was noted only in samples against Fluconazole and Nystatin, which represented 10.6% for Fluconazole and 7.45% for Nystatin in all luncheon samples. The most Fluconazole resistance was in

chicken luncheon samples 17%. The highest Nystatin resistance was in meat luncheon samples 8.5%. This is close to Marak and Dhanashree, (2018) detected antifungal pattern, which found that all 90 Candida isolates were susceptible to amphotericin B, 75.5% strains were sensitive to Fluconazole, and 24.4% were resistant. 81.1% of the Candida spp. was sensitive to Voriconazole. Most studies report high sensitivity rates for Amphotericin. *C.albicans* isolates in Egypt showed sensitivity rates exceeding 90% (Aboueldahab *et al.* 2023). In a study, isolated *candida spp.* showed high susceptibility levels to Amphotericin 100% and Nystatin 94.9%. However, Fluconazole showed reduced activity sensitivity, SDD and resistance were 61.5%, 10.2% and 28.2% respectively (Khalil 2022). A study on frozen beef isolates in Egypt found that all yeast isolates, including *Candida spp.*, showed notable resistance to fluconazole 57% (Mohamed *et al.* 2023). In a long-lasting study from 90th to 20th, they found that 90.1% of all Candida isolates tested were susceptible to Fluconazol e, 94.8% isolates of Candida spp. tested against Voriconazole were sensitive, and 3.1% were resistant. From fluconazole-resistant isolates, less than 30% of remained susceptible to Voriconazole (Pfaller *et al.* 2007).

CONCLUSIONS:

The introduced RTE meat and chicken luncheon contains mycological contamination in more than half of the isolates from different brands in this study. Yeast isolates in the beginning stage of acquiring resistance, only one sample showed resistance to two different antifungal drugs. Accordingly, consideration and strict hygienic control methods should be applied in the whole chain of processing till delivery to the consumer. To improve the hygienic quality of meat and chicken products and ensure they are safe for human consumption, they must follow planned measures. This could be achieved by implementing satisfactory manufacturing practices and effectively training plant workers in hygiene safety assurance, and following up continuously. Also, the application of strict hygiene measures during the handling, preparation, using treated food additives and serving of the products is necessary.

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