

Isolation and Identification of Yeasts from Slaughterhouses in Baghdad province

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ABSTRACT: The aim of this research was isolation and identification of yeasts from slaughterhouse environment which including equipment specimens before slaughtered and after slaughtered as well as vital organs swabs in sheep carcasses were slaughtered inside of slaughterhouse, the specimens were collected in Baghdad province during winter season (2023);and two hundred (200) samples were collected from abattoirs divided in to (50) samples before slaughtered and (50) samples after slaughtered and finally another (100) samples were obtained from sheep carcasses which were distributed into twenty-five(25)swabs from each of lung,liver,heart and spleen; these samples were inoculated on Sabouraud Dextrose Agar (SDA),so the results revealed that the percentage of yeasts isolated before slaughtered was (52%) while after slaughtered it decreased to (42%),while percentage of isolation from vital organs of sheep carcasses (22%) in which most visible in abattoirs environment *C. albicans* was in percent of (10%) then *Rhodotorula mucilaginosa* was recorded (7.5%), whereas after *C. parapsilosis* appeared in percent of (4.5%) while *Cryptococcus albidus* and *C. guilliermondii* appeared in percent of(3%) ; *C. famata* appeared in percent of (2.5%), both *C. zeylanoides* and *C. sphaerica* were recorded the same percent of (1%);finally each of *C.rugose*, *C. Lusitaniae*, *C.tropicalis*, and *C. krusei* were appeared in percent of (0.5%).

Keywords: slaughterhouse, yeasts, slaughterhouse equipment, sheep carcasses



1. INTRODUCTION

Yeasts are single-cells, eukaryotic, and classified as the kingdom of the fungal; The initial yeast before hundreds of millions of years, and at minimum, 1,500 spp, it's are currently recognized. They are evaluating that represent 1% of all described fungal species. [1], Yeasts have been find out in many ecosystems and play a main role in the biodiversity of Earth [2], Candidiasis is very important a zoonotic disease caused by *Candida* spp, *Candida* genus includes nearly 150 different species [3]. *C. albicans* is the very significant pathogenic, but other yeasts can cause infection for human and animal, which is called non-candida such as *C. tropicalis*, *C. globate*, *C. krusei*, *C. parapsilosis*, and *C. kefir* [4].

The low sanitary standard of butcher shops and their premises and poor personal hygiene may have contributed to the high prevalence of fungal contamination [5]. In the abattoirs, most equipment like the bucket and knives are made of stainless-steel; If wrong to sterilize knives and tools regularly can result fungi cross-contamination of the carcass [6, 7].

Rhodotorula mucilaginosa. is saprophytic yeast that usually isolated from humid environmental, they are accounted for 4.2–6.0% among invasive non candida yeasts as recorded by [8] the yeasts such as *Rhodotorula mucilaginosa*, *C. zeylanoides*, and *Candida parapsilosis* were often have found on the border side of the splitting carcasses, as well as it was considered that yeast may have exist on the foot cutter border and side of the splitting saw after washing [9; 10; 11].

Candida spp, in high percentage (38.46%), which the percent of isolated from the lungs of the ruminants especially *C. albicans* (23.07%) and *C. parapsilosis* (4.9%) as established by [12] also [13] were recorded in percent of (43.8%) In

addition to that *Candida* spp. was the most predominant species isolated from the animal's carcasses and meat fillet as observed by [14] and [15].

C. albicans have different virulence factors that enhanced it to adhere and finally invade the tissues resulting in infections and Some of these factors are pseudohyphae formation (Germ tube) for adherence, phenotyping switching, proteinase and phospholipase production for invasion and biofilm formation and these virulence factors were associated with pathogenicity of *C. albicans* [16,17].

2. MATERIALS AND METHODS

Samples were collected from equipment and vital organs from sheep carcasses.

One hundred (100) swabs collected from equipment and building of slaughterhouse in two (2) abattoirs in Baghdad province; divided into fifty (50) samples before slaughtered and fifty (50) after slaughtered. The collection was done during three months. All samples were collected and fill in the information in the transport tube media label., While samples from sheep carcasses were one hundred (100) swabs collected from vital organs (lung, heart, liver and spleen) of sheep; that were slaughtered inside the slaughterhouse by taking twenty-five (25) swabs from each of the above organs. All samples were inoculated on sabouraud dextrose agar (SDA) with chloramphenicol and incubated in 25°C for week for yeasts isolation.

2.1 YEAST DIAGNOSIS

1) Germ tube test

The primary isolation of *Candida* spp. In laboratory by distinctive characteristic of growing on SDA according to [18] then diagnosis was done in vitro and depended on Germ tube test which was rapid screening test that is used to differentiate *Candida albicans* from other yeast which applied by [19] which was done by Putting (0.5) ml of human serum into a small tube (Eppendorf tube) and use a disposable loop, touch a colony of yeast and gently emulsify it in the serum then Incubated at (37°C) for (2-3 hours) and then Transfer a (2-3) drops of the serum to a slide and covered with Coverslip and examine microscopically under (40x) objectives lens as established by [20].

2) The VITEK 2 system

All yeasts have been diagnosed by The VITEK 2 system, the VITEK ID-YST card allows the identification of clinically important yeasts and yeast-like organisms in (15 hr.), due to a sensitive fluorescence-based technology as suggested by [21] and [22].

2.2 PROCEDURE OF VITEK2 SYSTEM

A sterile swab is used to transfer a sufficient number (2-5) of colonies from a pure of yeasts culture and suspend the microorganism in (3.0) mL of sterile saline, the turbidity is adjusted accordingly and measured using a turbidity meter called the DensiChekTM; The turbidity ranges in the yeast was installed between (1.80-2.20) optical density (O.D). A test tube containing the yeast suspension is placed into a special rack (cassette) and then incubated cassette to complete biochemical reaction within (15 hr.).

Inoculated cards are passed by a mechanism, which cuts off the transfer tube and seals the card prior to loading into the carousel incubator. The carousel incubator was accommodating 60 cards. The incubation temperature was on-line at $35.5 \pm 1.0^\circ\text{C}$. Each card is removed from the carousel incubator and transported to the optical system for reaction readings. Interpretation of results were performed according to VITEK2 compact system special software to identify yeast species and strains.

3. STATISTICAL ANALYSIS

Statistical analysis of data was performed using SAS (Statistical Analysis System - version 9.1). Chi-square test was used to assess the significant differences among proportions, whereas McNemar test for paired proportions was used to compare before and after proportions $P < 0.05$ is considered statistically significant. [23].

4. RESULTS AND DISCUSSION

4.1 YEASTS ISOLATES FROM SLAUGHTERHOUSE EQUIPMENT'S AND BUILDINGS

There were many species of yeasts isolates obtained from equipment and buildings in the tow (2) Abattoirs in Baghdad city; which recorded twenty-six (26) isolates in fifty-two percent (52%) before slaughtered and twenty-one (21) isolates in percentage of forty-two (42%) after slaughtered process as in the table (1).

The *Rhodotorula mucilaginosa* recorded nine (9) isolates in percent of (18%) before slaughtered and in six (6) isolates in percentage of (12%) after slaughtered as in (Figure:1), this result was agreed with [24] who established percent of (12.5%) of *R. mucilaginosa* when isolated from the equipments used in the slaughtering line.

Candida albicans appeared in four (4) isolates in percentage (8%) before slaughtered and after slaughter process to six (6) isolates in percentage of (12%) and this disagree with [25] which were established the isolation percent in (33.30%) and (39.89%) respectively from equipment, but its compatible with [26,27] which were established the medical important of *Candida* spp as the most common opportunistic mycosis distributed worldwide and consider the common cause of nosocomial urinary tract infection (UTIs) oral candidiasis and genitourinary candidiasis, all *C. Albicans* isolates were positive when tested by germ tube which is compatible with [28] and [29] while the other yeasts species don't able to produce germ tube as showed in table (1)

Cryptococcus albidus (Figure:2) were appeared in six (6) isolates in percent of (12%) before slaughtered without any appear after slaughtered and this is agreement with [30] which were able to isolate *Cryp. albidus* in (11.11%) in slaughter environment but disagreement with [31] who were established percentage of (2.29%).

Candida parapsilosis appeared in four (4) isolates before slaughtered in percent (8%) and one (1) isolate after slaughtered in percent (2%) and this is agree with [32] who were isolated *C. parapsilosis* from plastic surfaces in percent of (3.7%) but disagree with [33] where recorded (1.6%) from the environment also agree with [34] who isolated ratio (4.5%), on the other hand *Candida guilliermondii* appeared in one (1) isolate before slaughtered in percent of (2%) and in four (4) isolates in percent (8%) after slaughtered, followed by *Candida famata* which were appeared in one (1) isolate before slaughtered in percent (2%) and two (2) isolates in percent (4%) after slaughtered and this result not consistent with [35] who achieve isolation rate of 0.5% and 1.6% for the above yeasts respectively.

Candida rugosa appeared only in one (1) isolate before slaughtered in percent of (2%) and this is disagreeing [36] who was fixed the ratio of (7%) in cow slaughterhouse environment.

finally, both *Candida Lusitaniae* and *Candida zeylanoides* were appeared in one (1) isolate after slaughtered only, which was compatible with [37] who were able to isolates *C. Lusitaniae* from environment and soil of abattoirs in percent of (1%) but disagree with [38] which found (30%) isolates from slaughter line, and equipments were from *C. zeylanoides*, So the ratio of yeasts isolation in this research recorded higher isolation percent than established by [39] which was (23.3%) in abattoirs environment.

According to p-value which were $0.05 < 0.06$ so there is no significant differences among isolation proportions of yeasts appeared before and after slaughter process.

Table 1. - comparison between yeast isolates from the equipment and building of slaughterhouse before and after slaughtering

Yeast spp	Before slaughter process		After slaughter process		P-value				
	NO	%	G. tube	%		NO	%	G. tube	%
<i>Rhodotorula mucilaginosa</i>	9	18%	0		6	12%	0		0
<i>candida albicans</i>	4	8%	4	8%	6	12%	6	12%	
<i>Cryptococcus albidus</i>	6	12%	0		0	0			0
<i>Candida parapsilosis</i>	4	8%	0		1	2%			0
<i>Candida guilliermondii</i>	1	2%	0		4	8%			0
<i>Candida famata</i>	1	2%	0		2	4%			0
<i>Candida rugosa</i>	1	2%	0		0	0			0
<i>Candida Lusitaniae</i>	0	0%	0		1	2%			0
<i>Candida zeylanoides</i>	0	0	0		1	2%			0
Total	26	52%			21	42%			0.06

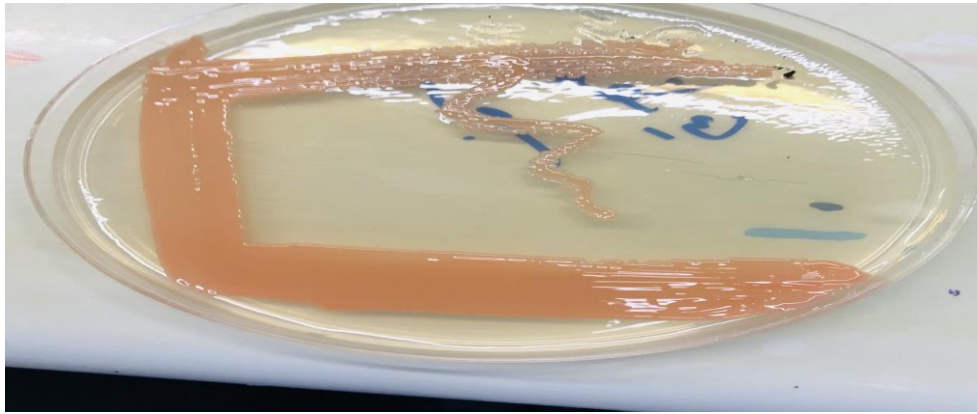


FIGURE 1. - Macroscopical appearance of Rhodotorula spp. on SDA incubated in 25°C for (2-3) days

bioMérieux Customer:		Microbiology Chart Report		Printed Feb 15, 2019 22:03 CST													
Patient Name:				Patient ID:													
Location:				Physician:													
Lab ID: h1				Isolate Number: 1													
Organism Quantity:																	
Selected Organism : Cryptococcus albidus																	
Source:				Collected:													
Comments:																	
Identification Information		Analysis Time: 18.00 hours		Status: Final													
Selected Organism		96% Probability		Cryptococcus albidus													
ID Analysis Messages		Bionumber:		4114145263673530													
Biochemical Details																	
3	LysA	-	4	IMLTa	-	5	LeuA	+	7	ARG	+	10	ERYa	-	12	GLYLa	-
13	TyrA	+	14	BNAG	-	15	ARBa	-	18	AMYa	-	19	dGALa	-	20	GENa	+
21	dGLUa	+	23	LACa	-	24	MAdGa	-	26	dCELa	-	27	GGT	-	28	dMALa	+
29	dRAFa	+	30	NAGA1	-	32	dMNEa	+	33	dMELa	-	34	dMLZa	+	38	ISBEa	-
39	IRHAa	-	40	XLTa	+	42	dSORa	(+)	44	SACa	(+)	45	URE	+	46	AGLU	-
47	dTURa	-	48	dTREa	(+)	49	NO3a	+	51	IARaA	+	52	dGATa	+	53	ESC	+
54	IGLTa	+	55	dXYLa	+	56	LATa	-	58	ACEa	+	59	CITa	-	60	GRTas	+
61	IPROa	+	62	2KGa	+	63	NAGa	-	64	dGNTa	-						

FIGURE 2. - Result sheet of Vitek 2 system, Cryptococcus albidus

4.2 YEASTS ISOLATION FROM VITAL ORGANS OF SHEEP CARCASSES

The yeasts isolation from (heart, lung, liver and spleen) of sheep carcasses, were appeared in twenty-two (22) isolates in percent of (22%) as revealed in the table (2).

Candida albicans was the most visible yeast which appeared in ten (10) isolates in percent of (10%), so this result was close to what was proven by [40] which were established the occurrence of C. albicans in lower respiratory tract, in sheep with (12%) and goat with (13%) but disagree with [41] which were found a percent of (6.7%) of C. albicans from cattle nasal swabs, as well as result match with [42] who were found the C. albicans in percent of (9,9%) in the intenal organs of sheep, similar to series of diagnosis has been established in this research the researchers were also used the G. tube test in diagnosis C. albicans (Figure:3).

Candida parapsilosis (Figure:4) were appeared in four (4) isolates in percent of (4%) and this is disagreed with [43] who found the percent of (20%) of C. parapsilosis was isolated from cut meat of internal organs for sheep and cattle, while compatible with [44] who established C. parapsilosis in percent of (4%) from animals.

Candida Famata and Candida sphaerica appeared in two (2) isolates for both of them in percentage of (2%); this is agreeing with [45] who affirm percent of (2.5%) in isolation of C. sphaerica which was grown in a low cost medium in environment of abattoirs but disagree with [46] who were able to confirm C. Famata in percent (12%) in vital organs of lamb.

Finally Candida tropicalis, Candida gullermondii, Candida zeylanoides and Candida krusei were appeared in one (1) isolate in percent (1%) for each of them; this doesn't match with [47] who were isolated C. tropicalis in percent of (6.6%) from carcasses in summer while in winter the percent was (13.3%) as well as isolated percent was (5%) from C. gullermondii, The isolation percent of C. zeylanoides in this research disagree with [48] who were isolated C. zeylanoides in percentage (4%) from slaughterhouse as well as, [49,50] were able to isolate C. krusei in percent (11%) in poultry meat which is not consistent with this research.

According to p-value which were $0.05 < 0.64$ so there are no significant differences among isolation proportions of yeasts appeared in vital organs of sheep carcasses in abattoirs.

Table 2. - Numbers and percentage of yeasts isolated from vital organs of sheep slaughtered in abattoirs

Yeasts spp					
	No	%	G.tube	%	p-value
Candida albicans	10	10%	10	10%	
Candida parapsilosis	4	4%	0		
Candida sphaerica	2	2%	0		
Candida Famata candida gullermondii	2	2%	0		
Candida tropicalis	1	1%	0		
Candida zeylanoides	1	1%	0		
Candida krusei	1	1%	0		
	1	1%	0		
Total	22	22%			0,64

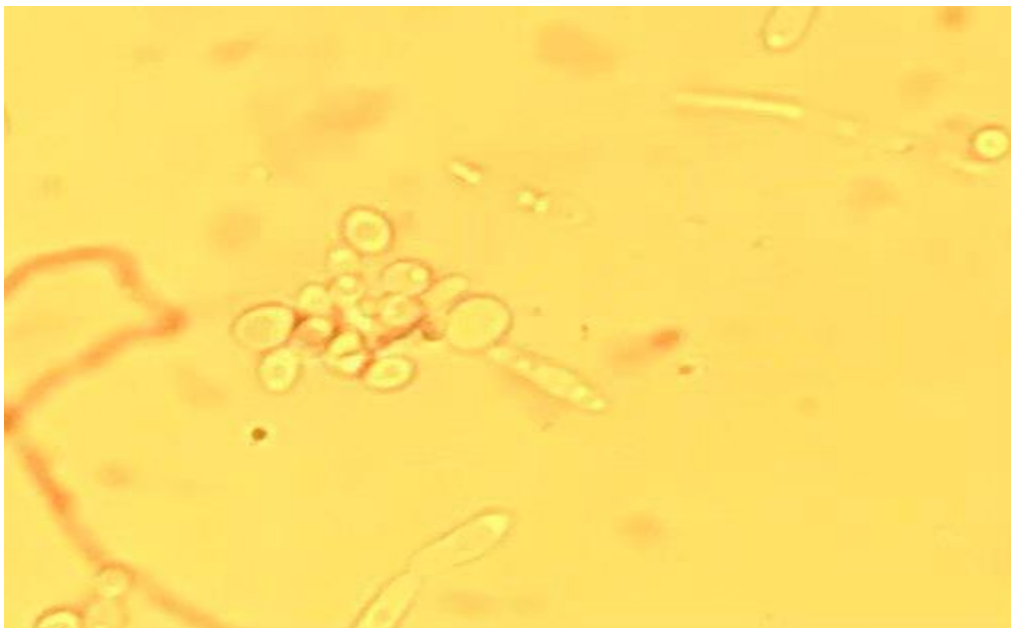


FIGURE 3. - Microscopical appearance of candida albicans under (40x) by use germ tube test after inoculation of human serum and incubated at 37°C for 3 hr. (blue arrow)

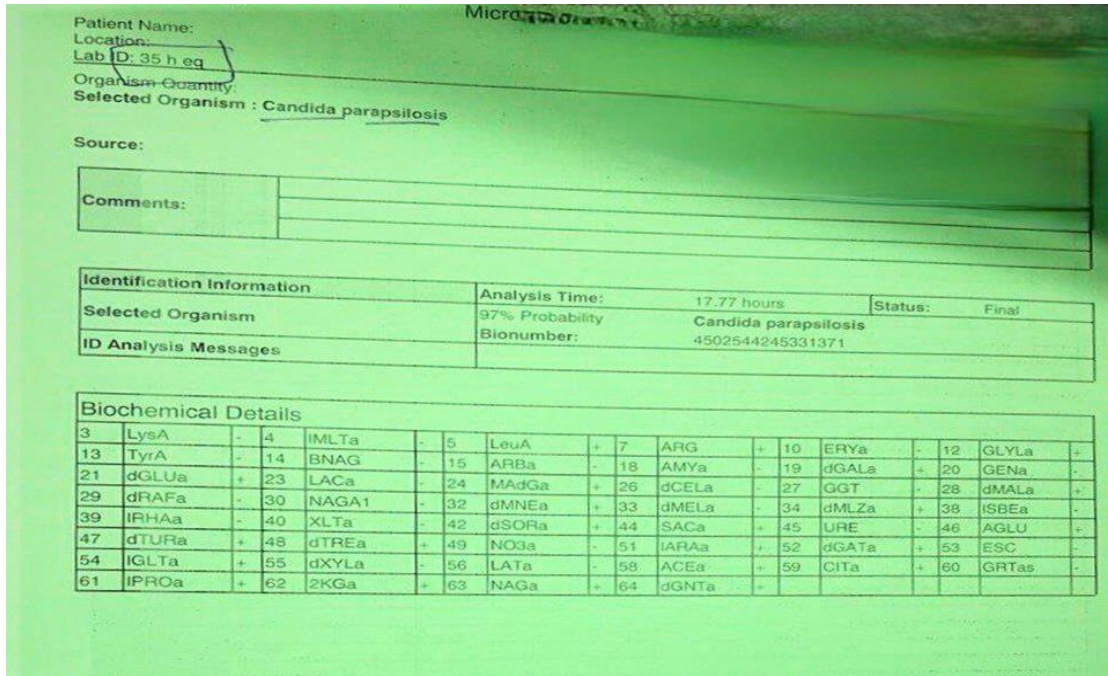


FIGURE 4. - Result sheet of Vitek 2 system, Candida parapsilosis

5. CONCLUSION

There will be higher isolation percent from equipments and building before slaughtered in compare with that appeared after slaughter process which indicate appearance of slaughterhouse environment and this is related with management hygiene protocol, as well as indicate the ability of these species of yeasts to transmitted to human via consumption of meat polluted with such yeasts appeared in immunocompromised like *C.albicans* and *R. mucilaginosa* also recorded pathological types like *Cryptococcus albidus* ;and consider *C.albicans* and *C.parapsilosis* which were isolated from sheep carcasses in high percent on of most important yeasts that cause invasive infection in animals and can be transmitted to human.

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CONFLICTS OF INTEREST

The author declares no conflict of interest

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