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PREVALENCE OF FORBIDDEN CONTAMINANT CLONES OF AZOLES AND POLYENES RESISTANT *CANDIDA ALBICANS* IN BUTTER-CREAM ECOSYSTEM IN BAGHDAD, IRAQ

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Abstract

Special emphasis on recalcitrant electromagnetic clouds of emergent entities of biofilm-producing and azoles-polyenes multidrug-resistant forbidden clones of *Candida albicans* contaminants in our food chain, butter-cream dairy series ecosystem in Baghdad represent hazard hygienic risk in our lifestyle. The focus issue of this project was to determine the frequency and distribution pattern of denominator in scanned zones of Al-Fudhaliyah, Abu-Ghraib and Al-Sadrya within specified episodes. One hundred-eight samples of butters and creams were collected and processed according to modified-verified enrollment designed by supervisor (Al-Shammary). Butter-Cream dairy chain ecosystem unveiled recovery of twelve (12) out of 108 assembled units: 11.11 % and totally 3.174 % in which, two brand series of both (54 assembled units) from each butter ecosystem recovered frequency series of five (5: 9.26 % cascaded 4.63 % and totally 1.322 %) clones and each cream ecosystem recovered frequency series of seven (7: 12.963 % cascaded 6.481 % and totally 1.851 %) clones. Resistance profile unveiled ten (11 %) multidrug-resistant clones of *C. albicans* lineage as 4 (4.4 %): 2 (2.2 %) from Al-Fudhaliyah, one (1.1 %) from Abu-Ghraib and one (1.1 %) from Al-Sadrya. From cream ecosystem as 6 (6.6 %): 4 (4.4 %) from Abu-Ghraib, one (1.1 %) from Al-Sadrya and one (1.1 %) from Al-Fudhaliyah. In conclusion, prevalence of forbidden clones of *C. albicans* in our food chain ecosystem in Baghdad needs serious screening monitoring schedules.

Key words: *Candida albicans*, biofilm, multidrug-resistance, dairy ecosystem.

Introduction

Fungi are common contaminants of dairy products, which provide a favorable niche for their growth. They are responsible for visible or non-visible defects, such as off-odor and lead to significant food waste and losses as well as important economic losses. Control of fungal spoilage is a major concern for industrials and scientists that are looking for efficient solutions to prevent and/or limit fungal spoilage in dairy products (Garnier *et al.*, 2017). This has culminated in an increased understanding of the pathogen's genetic makeup and biology, virulence mechanisms and interaction with the host. The phenomenon of multidrug resistance in *C. albicans* and the related pathogenic species has taken toll on the clinicians because the management of fungal diseases has become extremely difficult. In order to explore alternate drug targets and develop modern age drugs and vaccines, thorough understanding of the pathogen's biology has become vital (Pal, 2014, Pal, 2016 and Prasad, 2017).

In recent years, a general shift has been observed with regard to the species, which are commonly recovered from patients suffering from candidiasis. For instance, non-albicans species such as *C. krusei* and *C. glabrata* have been found to be frequently associated with life threatening infections. Such a shift from azole susceptible to azole resistant strains is a cause of deep concern. When one considers the Southeast Asian region, *C. tropicalis* accounts for the maximum share. Interestingly, a new species *C. auris* has been in the news for outbreaks across the globe (Pal, 2014, Pal, 2016 and Pfaller and Diekema, 2007). *Candida albicans* and emerging non-albicans Candida (*NAC*) species such as *C. glabrata*, *C. parapsilosis*, *C. tropicalis* and *C. krusei* can cause superficial infections of the oral and vaginal mucosa as well as disseminated bloodstream and deep-tissue infections. Species involvement varies by infection site

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and by geography. Candida infections are most often caused by *C. albicans* as evidenced by epidemiological studies (Pal, 2014, Pal, 2016 and Whaley *et al.*, 2017). In Baghdad ecosystem, *C. albicans* recovered from human sputum samples in Al-Yarmouk educational hospital and from bovine suffered from chronic respiratory distress in Al-Karkh slaughterhouses (Al-Khalidi *et al*., 2012).

Farnesol-Tyrosol Quorum sensing behavior is the brain-like machine in forbidden clones of *C. albicans* that orchestrate and remodeling their homeostasis throughout stress-adaptation and stress-hardening cascaded phenomenon as pheromone signal that sense environment and transfer their neuron-like impulses throughout sophisticated manner across complex and hidden nano bionetwork in order to regulate their switching morphogenesis, germ tube, biofilm formation, virulence biomarkers and multidrug resistance (Rodrigues and Cernakova, 2020). Our focus of this project was to investigate the prevalence pattern of denominator *C. albicans* in butter-cream ecosystem with special emphasis on biofilm-producing and multidrug-resistant forbidden clones in Baghdad.

Materials and Methods

Collection and Processing of Butter and Cream Units: A one-hundred eighty pooled samples from both brands as fifty-four assembled cuts from each butter and cream brands were enrolled in which, three pairs from each butter-cream brands from each territory per month. Assembled as two separated parts (size volume = 250 - 500 ml or g). Refrigerated and mixed well units cultured recovery behavior strategy involved. Processing proceeds by homogenization units inside collecting bags. Then taken representative sample approximately twenty-five (25) ml or g. Modified-verified enrollment were dependent (Quinn *et al*., 2004, Jay *et al*., 2005; Microbe Online, 2016 and BAM, 2020). Inoculating and incubating pooled processed sample with two-hundred fifty (250) ml doubled strengthen powered tryptone-soya yeast-extract broth at 25 and 37°C for 48 hours (modified one part processed sample: ten part enriched enrollment). Inoculated samples mixed well again for transferring by standard HiMedia loop about fiver droplets contents (each droplet equal mathematically to 0.02 micron or ml and so on, totally inoculated processed part equal to approximately one ml). Streaking by dilution technique on selective-differential HiCrome Candida agar then incubated at 25 and 37°C for (24-72) cascaded yeast, pseudo hyphal and true hyphal transformed phenotypes.

Counting Regime: Authorized systematic culturing on selective and differential HiCrome Candida agar with verified dual commands of surface viable micro droplet technique of Miles and Misra (Miles *et al*., 1938) and Pour plate (Van Soestbergen and Lee, 1969) technique, used for better estimating actual number of *C. albicans* lineage-complex in all processed brands. This modified dual decimal or logarithmic procedure get rid the aerobic and anaerobic environment for missing colonies and so counting errors. The mean log count of recovery of *C. albicans* lineage-complex was dependent on colonial phenotypes variants like structures and discoloration pattern, colonial biofilm behavior and siderophore activity of isolates. The augmented reality of *C. albicans* lineage-complex load log recovery titers calculated via mean number of colonies on cultured plate x a reciprocal of dilution factor × 50 cfu / ml (Ali Al-Shammary, 2009).

Biochemical ID: According to instructions of MacFaddin (2000), Quinn *et al*., (2004) and Microbe online (2016), a lactophenol cotton blue staining technique used for demonstration of blastoconidia, pseudo hyphae and true hyphae of *C. albicans*. This stain contains phenol, which will kill the organisms, lactic acid that preserves fungal structures, and cotton blue that stains the chitin found in the fungal cell walls. Cascade procedure enrolled by placing a drop of alcohol on a slide then taking by loop or stiff wire a yeast colony deeply from agar or some parts of filamentous hyphal growth and distributed thoroughly on a slide with immersion with LPCB dye, teased out the material very gently with mounted needles. Cover with coverslip for (10-40) X visualization or deeply scan slides with oil magnifier lens (100X). Oxoid-Remel RapID Yeast Plus System (4 hours) was dependent as a series of biochemical identification system integrated with computerized electronic certification compendium (ERIC Module) for segregation and confirmation of isolates. Detailed leaflet instruction firmware installed to verifying reactions discoloration scheme with control chart.

Biofilm: Modified-verified tissue culture technique designed by Christensen *et al*., (1985) was dependent in this enrollment. An overnight culture grown in TSB-YE at 37°C was transferred and diluted in microtiter plate as (0.1-0.5) ml approximately 7 log McFarland titer) containing approximately (5-8) ml freshly prepared TSBYE inoculated for each well. Each isolate was tested in triplicate. Wells with sterile TSB-YE alone was served as controls. The plates were incubated for 48 h at 37°C in order to creation permission of visible clear biofilm adhesive polymucoid structures, layers and dots surrounding inside periphery or rims and in the bottom of charged wells. Furthermore, the culture was removed and plates were washed three times with sensitive

phosphate-buffered saline to remove non-adherent cells and dried in an inverted position. Adherent biofilm was fixed with 2% sodium acetate for five minutes and was stained with double modified 20% biofilm-crystal violet and 20% biofilm-safranin for (15-30) minutes depending on secretory power for each clone, sensitivity, and specificity for each dye. Then, unbound stain was removed and the wells were washed three times with PBS. Plates were settled (2-3) hours for dryness then stained layers and dots of biofilm in bottom and around internal rims of wells were photographed, measured and scored according to the degree of formation, type of stain and type of isolate. Obvious result revealed after (2-3) days after complete dryness of induced biofilm.

Germ Tube: Screening test, which is used to differentiate *C. albicans* from other yeast (Sheppard *et al*., 2008; Matare *et al.*, 2017; Aryal, 2018; Jan *et al*., 2018). Reynolds and Braude first reported germ tube (GT) formation in 1956. When Candida is grown in human or sheep serum at 37°C for at least 30 minutes or (2-4) hours, they forms a germ tubes, which can be detected with a wet KOH films as filamentous outgrowth extending from yeast cells.

Composition Each ring contains	
Antibiotic	Concentration
Amphotericin-B (AP)	100Unit
Clotrimazole (CC)	10ìg
Fluconazole (FLC)	25ìg
Itraconazole (IT)	10ìg
Ketoconazole (KT)	10ìg.
Nystatin (NS)	100Unit
Zone of diameter (mm)	
Amphotericin -B AP 100 units	10 -18 mm
Clotrimazole CC 10 mcg	12 -18 mm
Fluconazole FLC 25 mcg	25 -30 mm
Itraconazole IT 10 mcg	18 -22 mm
Ketoconazole KT 10 mcg	18 -22 mm
Nystatin NS 100 units	15 -23 mm

Fig. 1: Radial Sensititer Hexa Antimyco-01 (HX104) (HiMedia, 2019) with reference zones of inhibition.

Radial Sensititer Hexa Antimyco-01 (HX104): A sunflower like filter paper containing six protrusions, each one ended with circular disc containing standardized concentration of antifungal agent. Hexa Antimyco-01 is an inert flat circular ring having six discs of 6 mm diameter on its projections. These discs are coated with antibiotics that aid antibiotic susceptibility testing of fungal cultures as in Fig. 1.

Fluconazole Ezy MICTM Sensititer Strip (FLC Epsilometer (E test) (EM072) (0.016-256 mcg/ml): Fluconazole HiComb™ MIC Strip is a rapid and reliable method for determining the antimicrobial susceptibility of different microorganisms against Fluconazole. This system provides a set of 16 different concentrations in gradient that can be easily used to deduce a functionally accurate the Minimum Inhibitory Concentration (MIC) in microgram levels. It is a unique MIC determination paper strip, which is coated with Fluconazole on a single paper strip in a concentration gradient manner, capable of showing MICs in the range of 0.016mcg/ml to 256 mcg/ml, on testing against the test organism Fig. 2.

Statistical analysis: Clinically and statistically dependent software of Statistical Package for the Social Sciences (SPSS, version 25, 2019), including t-test and Chi-square for significance variations among data enrolled.

Results and Discussion

Frequency and distribution pattern of cross-linked series in Baghdad food-chain dairy ecosystem unveiled variable interconnected recovery results in phenotypically diverse clones of *C. albicans* lineage especially biofilm-producing and Polyenes-Azoles multidrug-resistant

Interpretation :

Use following interpretive criteria for susceptibility categorization.

When testing	Incubation	Interpretative Criteria		
		≤ S	S-DD*	≥ R
Candida spps. #	35-37°C for 24 hrs.	8	16-32	64

* S-DD - Susceptible - Dose Dependent.
#: Isolates of *C.krusei* are assumed to be intrinsically resistant to Fluconazole. The results of Fluconazole susceptibility testing should not be interpreted using this criterion for this species.

Fig. 2: E-test strips (HiMedia, 2019) with reference chart.

forbidden infectious foci in epidemiologically scanned territories of Abu-Ghraib, Al-Fudhaliyah and Al-Sadrya. Virulence biomarkers from these recovered entities unveiled their genetic elasticity phenotypically as transformation from yeast to pseudo hyphae to true hyphae with white-opaque switching yeast phenomenon cascaded by biofilm, germ tube formation and multidrug resistance profile.

Butter-Cream dairy chain ecosystem unveiled recovery of twelve (12) out of 108 assembled units: 11.11% and totally 3.174% in which, two brand series of both (54 assembled units) from each butter ecosystem recovered frequency series of five (5:9.26% cascaded 4.63% and totally 1.322%) clones and each cream ecosystem recovered frequency series of seven (7:12.963% cascaded 6.481% and totally 1.851%) clones. Butter brand distribution chain unveiled recovery of three (3) clones from Al-Fudhaliyah out of assembled 18 (16.67% cascaded 5.56% cascaded 2.78% and totally 0.793%) followed by one (1) clone from Abu-Ghraib out of assembled 18 (5.56% cascaded 1.851% cascaded 0.926% and totally 0.264%) and one (1) clone from Al-Sadrya out of assembled 18 (5.56% cascaded 1.851% cascaded 0.926% and totally 0.264%). Cream brand distribution chain unveiled recovery of five (5) clones from Abu-Ghraib out of assembled 18 (27.78% cascaded 9.26% cascaded 4.63 % and totally 1.322%) followed by one (1) clone from Al-Sadrya out of assembled 18 (5.56% cascaded 1.851% cascaded 0.926% and totally 0.264%) and one (1) clone from Al-Fudhaliyah out of assembled 18 (5.56% cascaded 1.851% cascaded 0.926% and totally 0.264%). Resistance profile unveiled ten (11%) multidrug-resistant clones of *C. albicans* lineage as 4 (4.4%): 2 (2.2%) from Al-Fudhaliyah, one (1.1%) from Abu-Ghraib and one (1.1%) from Al-Sadrya. From cream ecosystem as 6 (6.6%): 4 (4.4%) from Abu-Ghraib, one (1.1%) from Al-Sadrya and one (1.1%) from Al-Fudhaliyah. Tables (1-4) and Fig. 3 unveiled these events.

Variable and diverse colonial types and morphology displayed at 25 and 37°C episodes. Yeast-Mold pattern displayed at 25°C more than at 37°C due to versatile genetic makeup of target deciphered by growth curve pattern, temperature, time, water activity (a_w), brand type, zone, etc. Yeast phenotypes with dimorphic phenomenon of white-opaque transformers plus variable textured entities with hyphal architectures and different discoloration behavior from green to blue to mixed to white to purple, *i.e.* rainbow cottony to velvet, small to large size, regular to irregular and so on noticed on HiCrome agar enrolled with serum or glucose potentiated TSA-YE agar at 25°C after (2-3) days incubation (finest quest verified temperature). Culturing at 37°C displayed gradual time-temperature growing transformation from yeast phenotypes within (18-24) hours to hyphal swarming

Table 1: Recovery profile index of *C. albicans* from butter (scanned zones).

Scanned Zones	Brands Units	*C. albicans*	Recovery Percentages		
			18 %	54 %	378 %
Al-Fudhaliyah	18	3 ^{A*}	16.67	5.56	0.793
Al-Sadrya	18	1 ^{B}	5.56	1.851	0.264
Abu-Ghraib	18	1 ^{B}	5.56	1.851	0.264
Total	54	5	27.78	9.26	1.322

A, B: Vertically post bio statistically significant differences among zones at level ($p \leq 0.05$).

Table 2: Recovery profile index of *C. albicans* from butter (Episodes).

Episodes	Brands Units	*C. albicans*	Recovery Percentages		
			9 %	54%	378%
January	9	0 ^{C}	0	0	0
February	9	0 ^{C}	0	0	0
March	9	1 ^{B}	11.11	1.851	0.264
April	9	4 ^{A*}	44.44	7.407	1.058
May	9	0 ^{C}	0	0	0
June	9	0 ^{C}	0	0	0
Total	54	5	55.56	9.26	1.322

A,B,C: Vertically post bio statistically significant differences among episodes at level ($p \leq 0.05$).

Table 3: Recovery profile index of *C. albicans* from cream (scanned zones).

Scanned Zones	Brands Units	*C. albicans*	Recovery Percentages		
			18 %	54 %	378 %
Abu-Ghraib	18	5 ^{A*}	27.78	9.26	1.322
Al-Sadrya	18	1 ^{B}	5.56	1.851	0.264
Al-Fudhaliyah	18	1 ^{B}	5.56	1.851	0.264
Total	54	7	38.89	12.963	1.851

A, B: Vertically post bio statistically significant differences among zones at level ($p \leq 0.05$).

Table 4: Recovery profile index of *C. albicans* from cream (Episodes).

Episodes	Brands Units	*C. albicans*	Recovery Percentages		
			9 %	54%	378%
January	9	0^{C}	0	0	0
February	9	0^{C}	0	0	0
March	9	2^{B}	22.22	3.703	0.53
April	9	5^{A*}	55.56	9.26	1.322
May	9	0^{C}	0	0	0
June	9	0^{C}	0	0	0
Total	54	7	77.78	12.963	1.851

A, B, C: Vertically post bio statistically significant differences among episodes at level ($p \leq 0.05$).

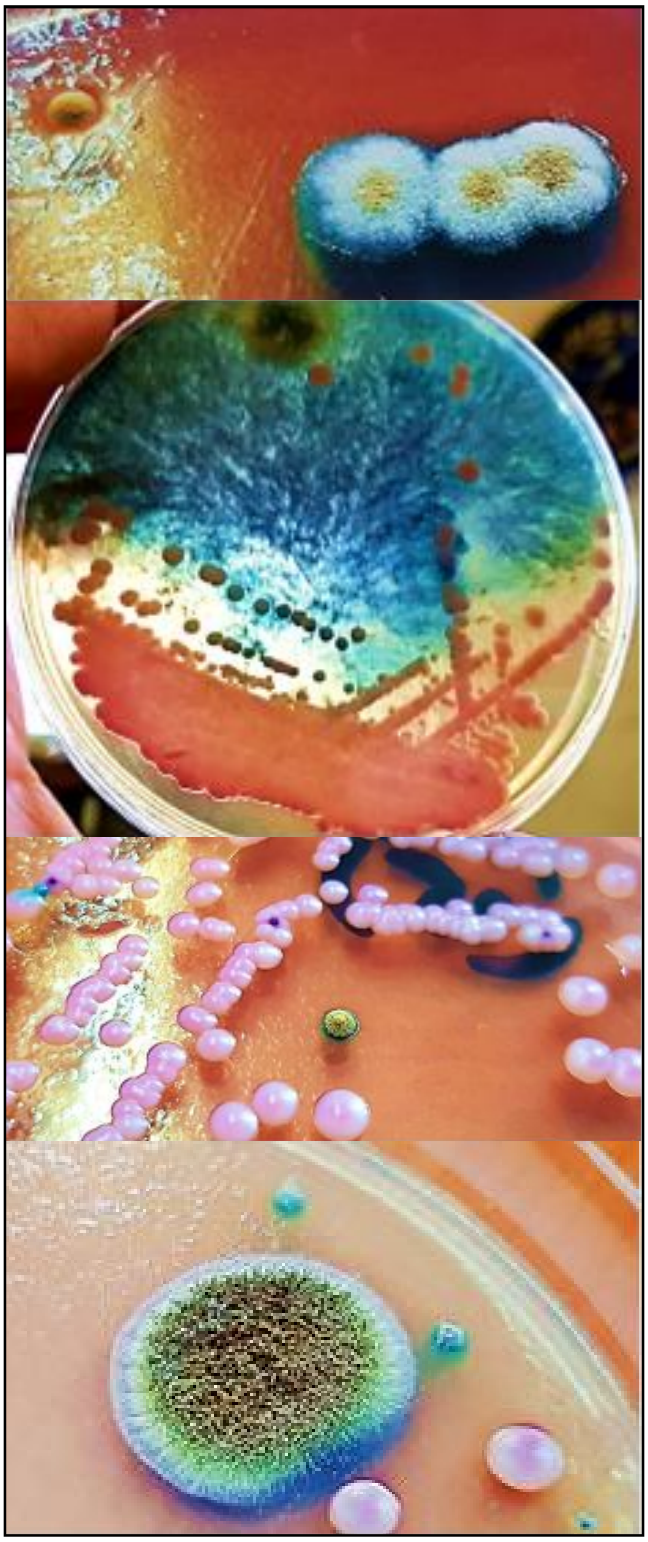

Fig. 3: Yeast-Mold Pattern of Candida species with green colonies of *C. albicans* on HiCrome Candida agar at 25 and 37°C recovered from locally dairy products in Baghdad.

behavior throughout (2-3) days with scattered seeds or flour and diffuse capillary threads or strings. Transformed time-temperature phenotypes not displayed in all recovered clone and not from all samples or from all scanned zones and episodes. From tables upstairs the predominant butter predominant clones were from Al-Fudhaliyah versus cream clones from Abu-Ghraib.

Predominant episodes for isolation profile occurred within coherent prevalence of March-April time frequency. Overall mean log counts unveiled versatile frequency and distribution pattern in which, yeast pattern at 25°C was predominant in Abu-Ghraib while mold pattern at 37°C was predominant in Al-Sadrya. Butter enrolled yeast pattern from Al-Fudhaliyah versus mold pattern from Abu-Ghraib. Cream enrolled yeast pattern from Abu-Ghraib versus mold pattern from Al-Sadrya. Tables 5 and 6 with Fig. 3 illustrate these achievements.

The heterogeneous genus *Candida* currently belongs to the order *Saccharomycetales* within the *ascomycetes*. Deciphered illustrated pleomorphic mucoid encapsulated green growth patterns of *C. albicans* on HiCrome Candida agar with white-opaque phenotypic transition phases on serum or glucose enriched TSA-YE agar revised by versatile and diverse morphological features of yeast, pseudo non-septate and true-septate hyphae

Table 5: Combo mean log count of *C. albicans* from butter at 25 & 37°C.

Zones	Brands Units	Mean log count of C. albicans CFU.ml^{-1}		Total
		Yeast at 25°C	Mold at 37°C	
Al-Fudhaliyah	18	4.342^{Aa}	3.903^{Aa}	4.122^{A}
Al-Sadrya	18	3.301^{Ba}	$None^{Bb}$	1.65^{c}
Abu-Ghraib	18	$None^{Cb}$	4.255^{Aa}	2.127
Total	54	2.547^{a}	2.719^{a}	2.633

A, B, C: Vertically post bio statistically significant differences among zones at level ($p \leq 0.05$).

a, b: Horizontally post bio statistically clinical differences within colonial variants at level (0.5> count >0.5 log).

Table 6: Combo mean log count of *C. albicans* from cream at 25 & 37°C.

Zones	Brands Units	Mean log count of C. albicans CFU.ml^{-1}		Total
		Yeast at 25°C	Mold at 37°C	
Abu-Ghraib	18	3.778^{Ab}	4.602^{Ba}	4.19^{A}
Al-Sadrya	18	$None^{Bb}$	5.26^{Aa}	2.63^{B}
Al-Fudhaliyah	18	$None^{Bb}$	5.255^{Aa}	2.627^{B}
Total	54	1.26^{b}	5.04^{a}	3.149

A, B: Vertically post bio statistically significant differences among zones at level ($p \leq 0.05$).

a, b: Horizontally post bio statistically clinical differences within colonial variants at level (0.5> count >0.5 log).

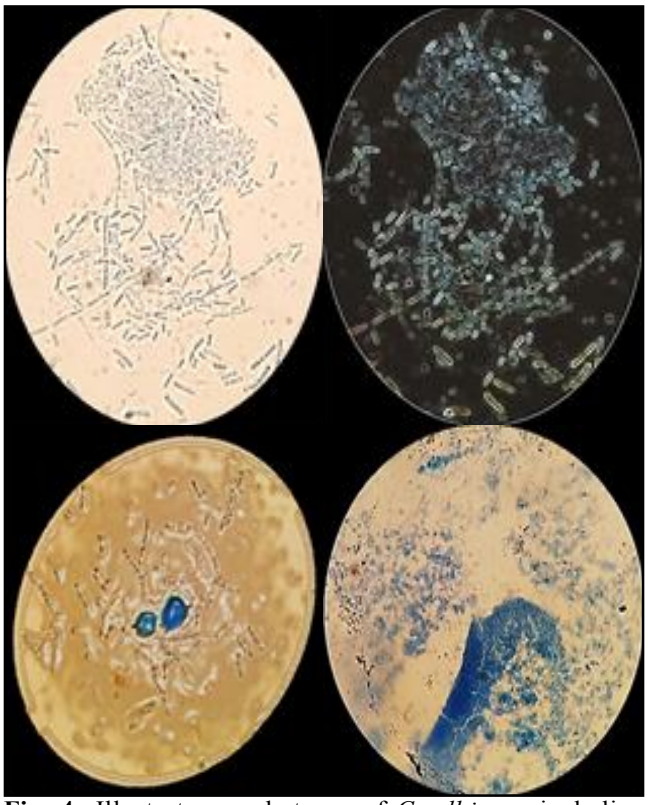

Fig. 4: Illustrate morphotypes of *C. albicans* including blastoconidia, chlamydo or ascospores, pseudo non-septate and true septate hyphae or mycelium under 100X zoom.

unveiled during staining with LPCB. According to the online microbiological notes on *Candida albicans* lifestyle (Howell *et al*., 2015 and Aryal, 2020), similar findings sensed in which, branched tree-like blue segments with small oval or irregular budding blue blastoconidia and chlamydospores or ascospores with polymorphic mold-like or sugar cane-like blue mycelium or hyphae. Blastoconidia looks like a bunch or clusters of diffused or scattered grapes surroundings the hyphae or mycelium while, chlamydospores looks like a pyriform large enclosed dark blue bodies nearby the stained entities. Fig. 4 illustrate these morphogenesis features.

Visualized discoloration biochemical ID throughout Oxoid-Remel RapID Yeast Plus System (4 hours) was dependent as a series of biochemical identification system integrated with computerized electronic certification compendium (ERIC Module) for segregation and confirmation of isolates. Detailed leaflet instruction firmware installed to verifying reactions discoloration scheme with control chart. Plus Panel has several reaction cavities molded into the periphery of a plastic disposable tray. Reaction cavities contain dehydrated reactants and the tray allows the simultaneous inoculation of each cavity with a predetermined amount of inoculum. A suspension of the test organism in RapID Inoculation Fluid is used as the inoculum, which rehydrates and initiates test reactions. After incubation of the panel, each test cavity is examined for reactivity by noting the development of color. In some cases, reagents must be added to the test cavities to provide a color change. The resulting pattern of positive and negative test scores is used as the basis for identification of the test isolate by comparison of test results to reactivity patterns stored in the Electronic RapID Compendium (ERIC™) database or by use of the RapID Yeast Plus Differential Chart. The RapID Yeast Plus Differential Chart illustrates the expected results for RapID Yeast Plus System.

Differential Chart results are expressed as a series of positive percentages for each system test. This information statistically supports the use of each test and provides the basis, through numerical coding of digital test results, for a probabilistic approach to the identification of the test isolate. Identifications are made using individual test scores from RapID Yeast Plus panels in conjunction with other laboratory information to produce a pattern that statistically resembles known reactivity for taxa recorded in the RapID System database. These patterns are compared using the RapID Yeast Plus Differential Chart, or by derivation of a microcode and the use of ERIC. All lot numbers of RapID Yeast Plus System have been tested using the following quality control organisms and have been found to be acceptable. Testing of control organisms should be performed in accordance with established laboratory quality control procedures. If aberrant quality control results are noted, patient results should not be reported. The sensitivity and specificity of this test color kit agreed with that of API 20C kit series of yeasts as more than 95% significance levels ($p \leq 0.05$). Six-digit series comb biochemical ID numbers calculated by visual color verification with standard eighteen to twenty interconnected test series. Certification online throughout Thermo-Remel ERIC software, resulting in 99% specificity and sensitivity identity or matching of *C. albicans* with comparison to API 20 yeast panel but not Vitek 2 cascaded by molecular PCR enrollment, including more than one microcodes certificates of 303011 (supreme), 303001, 303003, 303007, 303010, 303013, 303017, 303031, 303033 and 303037. Online Microcodes certification reports forms plus manually-visually filled and signed Microcodes (digitally calculated numbers series comb) Worksheets reports forms guided by differential color chart illustrated in Fig. 5.

For many years, the quorum sensing and biofilm growth-pattern strategy was a predominant behavior for most if not all microbiota as an organized symposium lifestyle. *C. albicans* forming enclosed and recalcitrant

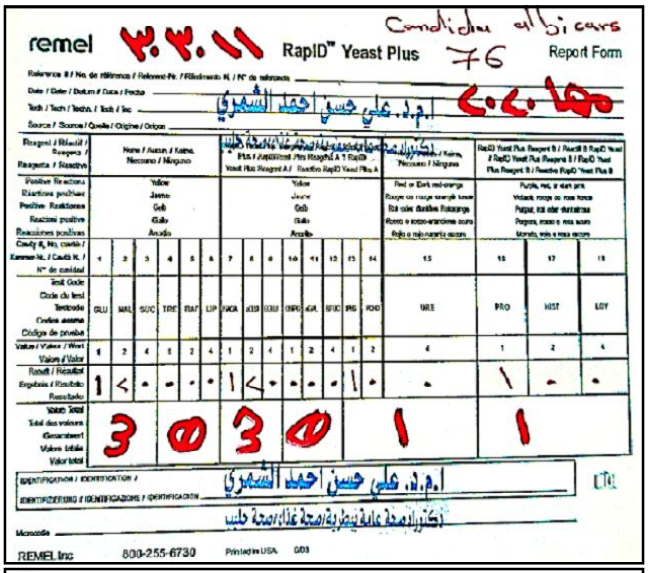

Fig. 5: ERIC Yeast Plus Compendium (Oxoid-Remel, 2019).

multilayers architecture of biofilm with an upper region of hyphae encapsulated in extracellular matrix with other microbiota throughout bridges of quorum-plasmids mediators for genes sharing strategy. Therefore, multidrug resistance sensing behavior provoked and tolerance-persister behavior initiated even without exposure to that drug. Stress-adaptation or stress-hardening phenomenon with mixed biofilm entities could transfer this tolerance behavior or induced signal stimuli to other controlling strategies in food processing like thermal timeout pasteurization. Biofilm formation displayed in all recovered clones and their phenotypes as blue and red mucoid layers and dots in the bottom of microtiter plates and surrounding their interior rims, as well as floating clouds in TSB-YE broth and mucoid translucent glues around their colonies on agars surfaces. Fig. 6 illustrate biofilm architecture.

Fig. 6: Biofilm architecture.

The clinical pathogen *Candida albicans* is a budding yeast that is capable of forming a range of polarized and expanded cell shapes from pseudohyphae to true nonconstricted hyphae. Filamentous forms consist of contiguous uninucleated compartments that are partitioned by septa. It has long been held that the so-called "dimorphic transition" from a budding to a filamentous form may aid the fungus to penetrate epithelia and may therefore be a virulence factor. New evidence has demonstrated that hyphae of *C. albicans* have a sense of touch so that they grow along grooves and through pores (thigmotropism (Gow, 1997). Selective and differential, characteristic and fingerprint feature of *C. albicans* that can be distinguished it from other genus members or other yeasts, their ability to outgrowth in the serum of human and sheep with the formation of characteristic cross-linked microtubules from budding yeast. The number and thickness of germinated tubes with the degree of branching reflect their virulence score, as well as the time or duration of growth (short) and serum species preferred for chainsaw puzzle of their epidemiological ancestor. Temperature dependent serum could encourage induction of elongated filamentous grooves or hyphae from *C. albicans*. Recently reports on the localization of septin rings in *C. albicans* during the development of pseudo hyphae and true hyphae from round, non- budded yeast mother cells (Sudbery, 2001). Previous observations are extended by showing that the pattern and location of the septin ring are different in hyphae and pseudohyphae. Endotrophic germ tube formation is the endogenous germination of *C. albicans* yeast cells. The germ tube has parallel walls and no constriction at the point of origin at the blastospore mother

cell (Matare *et al*., 2017). All recovered clones displayed these diagnostic features as appeared in Fig. 7.

Fig. 7: Germ Tube formation by *C. albicans*.

Circular sunflower-like strip deciphered Radial Sensititer Hexa Antimyco-01 (HX104). Each ring contains Polyenes-Azoles components: Amphotericin-B (AP) 100Unit, Clotrimazole (CC) 10µg, Fluconazole (FLC) 25µg, Itraconazole (IT) 10µg, Ketoconazole (KT) 10µg and Nystatin (NS) 100Unit. Each finger-like protrusion impregnated with standard reference antifungal agent cascaded hexa susceptibility module for checkerboard resistance pattern of recovered *C. albicans* clones. Most recovered clones were multidrug resistant to all antifungal agents except Itraconazole (IT) while butter clones were resistant to all and intermediate to resistant for IT versus cream clones were completely resistant to all even IT. These sensing behaviors reflect the development of resistance to azoles even not exposed to it in food chain or developed during subclinical mixed infection or active carriers or else. Table 7 and Fig. 8 illustrate these cross-sensed resistance profiles.

Fluconazole Ezy MICTM Sensititer Strip (FLC Epsilometer (E test) (EM072) (0.016-256 mcg/ml). Fluconazole HiComb™ is a unique Minimum Inhibitory Concentration (MIC) determination paper strip, which is

Fig. 8: Resistance Profile of *C. albicans* clones to Polyenes-Azoles circular antifungal strip.

coated with Fluconazole on a single paper strip in a sixteen concentration gradient manner, capable of showing MICs in the range of 0.016 to 256 mcg/ml, on testing against the targeted species. Titer index throughout E test determination profile for each clone. This gradient concentration strip with cascaded amounts of fluconazole replace time and materials consuming traditional titration techniques for estimation of lower concentration of tested antifungal *in vitro* that inhibit (MIC or MIFC) or kill (MBC or MFC) the targeted isolate of *C. albicans*. This test is complementary to upstairs Radial Sensititer Hexa Antimyco-01 (HX104) test in which, the latter test the sensitivity of isolate to that antifungal agent and the former determine the lowest concentration or titer of that tested antifungal to inhibit or kill the recovered target. According to Manufacturer Company, reference gradient concentrations that inhibit or kill *C. albicans* vary from (0.25-8) $\mu.ml^{-1}$ due to genetic diversity of recovered clones to susceptible dose-dependent clones range from (16-32) $\mu.ml^{-1}$ due to intermediate tolerance behavior and finally resistant clones that can tolerate more than 64 $\mu.ml^{-1}$ from that antifungal. Firstly must observe that guidelines hints in which, isolated colonies, pinpoint micro colonies and hazes may appear within the zone of inhibition frequently and they should be ignored. In such cases, consider reading for MIC determination at a point on the

Table 7: Multidrug Resistance Pattern Index in *C. albicans* clones-lineage from all zones.

Dairy Brand	Radial Sensititer Hexa Antimyco-01 (HX104) profile					
	Amphotericin-B (AP) 100Unit	Clotrimazole (CC) 10µg	Fluconazole (FLC) 25µg	Itraconazole (IT) 10µg	Ketoconazole (KT) 10µg	Nystatin (NS) 100Unit
Butter	None Resistant	None Resistant	None Resistant	50%Sensitive	None Resistant	None Resistant
Cream	None Resistant	None Resistant	None Resistant	NoneResistant	None Resistant	None Resistant
Total %	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant

Table 8: E-test limits for recovered clones of *C. albicans*.

Dairy Brand	Fluconazole Epsilometer Index					
	Al-Fudhaliyah		Abu-Ghraib		Al-Sadrya	
	Titer ($\mu.ml^{-1}$)	Inhibition Zone (mm)	Titer ($\mu.ml^{-1}$)	Inhibition Zone (mm	Titer ($\mu.ml^{-1}$)	Inhibition Zone (mm
Butter	≥256	None	≥256	None	2-22	16-128
Cream	≥256	None	≥256	None	≥256	None
Total Range	37-≥256	None-256	≥256	None	2-≥256	None-128

scale at which prominent reduction of growth is seen. Since Ezy MIC™ strip has continuous gradient, MIC values "in-between" two fold dilutions can be obtained. Always round up these values to the next two-fold dilution before categorization. For example Fluconazole showing reading of 0.75 $\mu.ml^{-1}$ should be rounded up to next concentration i.e. 1.0 µ.ml-1. If the ellipse intersects the strip in between two dilutions, read the MIC as the value, which is nearest to the intersection. When growth occurs along the entire strip, report the MIC as > the highest values on the MIC strip. When the inhibition ellipse is below the strip (does not intersect the strip), report the MIC < the lowest value on the MIC scale. Table 8 illustrate these gradient limits of susceptibility of recovered clones and Fig. 9 interpreter for that strip.

Stress adaptation cascaded by stress hardening in some forbidden clones of Polyenes-Azoles multidrug resistant *C. albicans* lineage (ARCA-PRCA) especially in acquired foreign strains entering the Iraqi ecosystem throughout food chain represent dangerous emergent hazard in man and animals. Similar cascaded researches found in other related recovered foodborne pathogens in Baghdad (Al-Shammary, 2009, 2015a, 2017 and 2019). Genetically modified or induced clones throughout conjugating plasmids bridges, dangerous integrating forbidden mycophagy (transduction prophage), transformation with forbidden residual environmental DNA or even sophisticated genes regulating proteins throughout exposure to sublethal stressors causing triggering shifting from low virulent and frequent pathogens to upgraded entities of highly virulent clones that tolerate mixed and even not exposed stress stimulus with emergent diffusion rate or logs exceeding those found normally in food. Genes sharing strategies with other microbiota cascaded by quorum sensing behavior inside enclosed biosphere of multilayered recalcitrant electromagnetic clouds of biofilm (Sordi and Muhlschlegel, 2009; Mallick and Bennett, 2013; Nogueira *et al*., 2019) create an entity of super infectious foci that affects dramatically on healthy and hygienic lifestyle as well as, prevent or reduce the efficacy of hygienic and prophylactic measurements and so on worse treatments with antibiotics. Uncontrolled importation of contaminated feeds-foods recycled contaminated Iraqi environment with foreign clones carrying strong and intelligent defense strategies called CRISPR-CAS (clustered regularly interspaced short palindromic repeats) (Rath *et al*., 2015) immune system after 2003. All these scenarios with other obscure causes leading to emergency of these resistant biofilm clones.

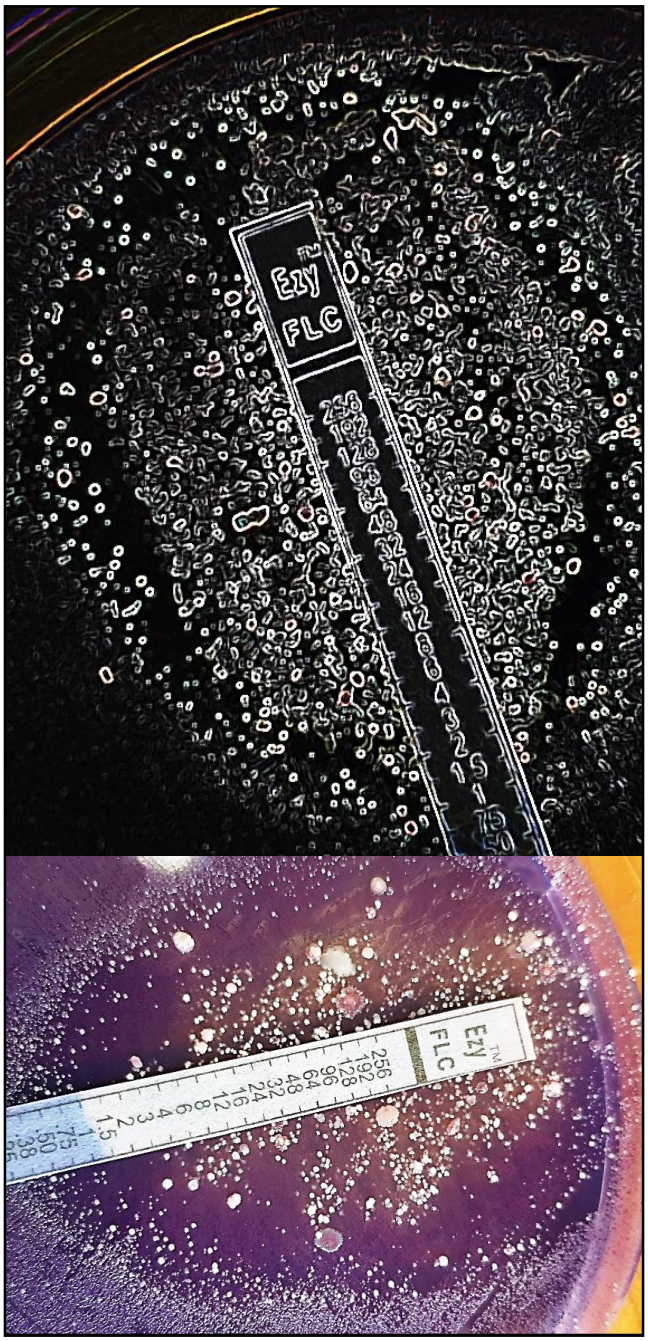

Fig. 9: Illustrate Epsilometer strip test for MIC and MFC titers of *C. albicans* clones.

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