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Isolation and Identification of the Fungi that Causes Eye Infection in Wasit Province, Iraq

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ABSTRACT

Fungal ocular infection is one of the most important infections in Iraq inspite of it occcure in low incidence in compare with bacterial and viral eye infections. Two hundred(200) eye swabs samples were taken from patients suffering of eye infections. After culturing on the related selective media, results showed that 29 (15%) of the samples were positive for occurrence of microorganisms; of these, 26 (91%) were belonged to mold and 3 (9%) yeast. After the fungal isolates were identified by cultural and microscopic examinations, the following species and percentages were recorded: *Aspergillusflavus* 13 (45%), *Aspergillusterrus* 5 (17%), *Aspergillusfumigatus*4 (14%), *Aspergillus niger* 2 (7%), *Penicillium* spp. 1 (4%) and *Alternaria* spp. 1 (4%). While the yeast isolates were belonged to *Candidaguilliermondii* 1(3%), *Candidaciferrii* 1(3%) and *Cryptococcuslaurentii* 1(3%) according to their identification through the cultural, microscopic examinations, in addition to the biochemical examinations by using VITEC 2 system. Upon such findings, *Aspergillusflavus* was the predominant fungi. Regarding to the patients gender, it was found that cases of fungal eye infections in males two fold than in females. Patients group of age (50-59) years was the most infected group. Tobacco smoking and Diabetes have positive effects on infections, while there was no effects for blood pressure.

Keywords

Aspergillus flavus, Aspergillus terrus, Aspergillus fumigatus, Aspergillus niger, Penicillium spp. ,Alternaria spp., *guilliermondii*, *Candidaciferrii*,*Cryptococcuslaurentii*, VITEC 2 system.

Introduction

The eye is the most exposed part in the body to the air thatcarried dust and microorganisms such as bacteria and fungi that causing infections to it, specifically when any scratch or wound tack place in the lining tissues of the eye, as these microorganisms immidatly Settle down into the tissues that broken, resulting prominent damage to the eye(Hameed, 2020).Infections with fungi is a significant ophthalmic issue in tropical and subtropical region, although, it occasionally occur in other high-income countries with temperate climates.The most common fungal pathogens are *Aspergillus* spp. ,*Fusarium* spp. and *dematiaceous* spp.(Garg*et al*., 2016 ; Soleimani*et al*., 2020). The factors that are risk for getting fungal infections are: ocular trauma, ocular surface disease, uses of contact lens, uses of topical steroid and systemic immunosuppression (Erdem*et al*., 2017 ; Kumar*et al*., 2019). Treatment of infection has become a problematic case, particularly in sever problem, because of a few available antifungals and emerging of resistance species. On the other hand, a failure to treat can result in the vision loss (Soleimani*et al*., 2020). This study was aimed to isolate and identify the fungi that causative of eye infection in Wasit province .

Literature Review

The human eye is one of the most noticeable sensory systems.The majority of the information about the external environment gathered byHuman beingsthrough their eyes and thus depend on eyesight more than other sense, with the eye being the most sensitive organ we have (Lee ,2020).

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Eye Infection

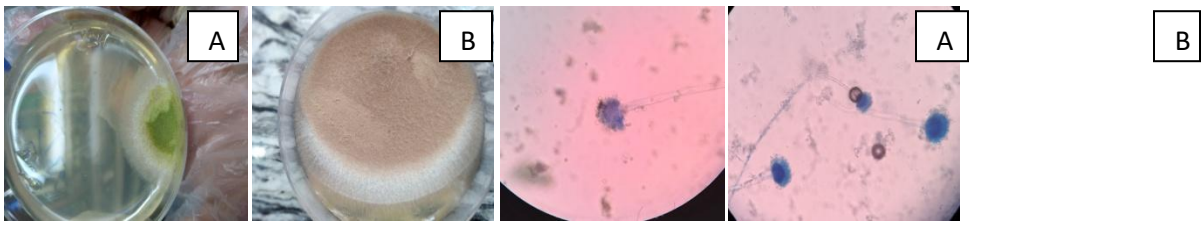
Thecommensal organisms can be seenon the external ocular surface, such as *Staphylococcus* species, *Corynebacterium* species, and *Propionibacterium* species, which are form the resident flora. Any environmental organism can be a transient flora in the eye by achance. The intraocular tissues and spaces, however, are sterile. While the conjunctiva is protected by blood supply, the cornea is a vascular; thus, the types of organisms invading these tissues may be change. The intraocular tissues are relatively immune-privileged and can be infected by any organism that manages to enter the inside of the eye(Sharma, 2012).Bacteria, fungi, parasites and viruses, can all causesocular infections and each of theses may give a sings and symptoms of disease.Furthermore, the prevalence and distribution of spasific infectious organsims related with eye infections varies widely and is dependent on variety of factors (Gautam*et al*., 2019).

Fungal eye infection

Fungal eye infections are considered asignificant cause of loss of vision in some regions of the world, where they are the main causes of blindness especially in Asia. Fungal etiology of ocular illness is still a diagnostic and therapeutic problem for the ophthalmologist(Behera, 2018).The most common cause of eye infections is *Candida* species, that normaly develop in immunocompromised patients that have chronic underlying systemic disease.The second most commonis *Aspergillus* species, such as *Aspergillus flavus*, *Aspergillus fumigatus*, *Aspergillus niger*, *Aspergillus terreus*, *Aspergillus glaucus*, and *Aspergillus nidulans*.There have been many reported cases that reports other emerging pathogens, such as *Fusarium* spp. ,*Penicillium* spp., *Pseudallescheria* spp., *Cryptococcus* spp., dimorphic fungi (*Histoplasma capsulatum*, *Blastomyces dermatitis*, *Sporothrix schenckii*, and *Coccidioides immitis*)(Trofa*et al*., 2008 ; Spadea, and Giannico, 2019).

Methods

Two hundred eye swabs samples were collected in Wasit province, Iraq 2020. The samples were collected from different ages and included both genders. Initial identification of each sample was performed by culture on Sabouraud dextrose agar at 30°C for at least ten days and direct microscopy using lactophenole cotton blue staining. Molds identification according to Colony morphology (color and consistency) and Microscopic characteristics (micro conidia and macro conidia, their size, arrangement and shape). Yeast identification was made by VITEK 2 system method which is a fully automated microbiology identification system.



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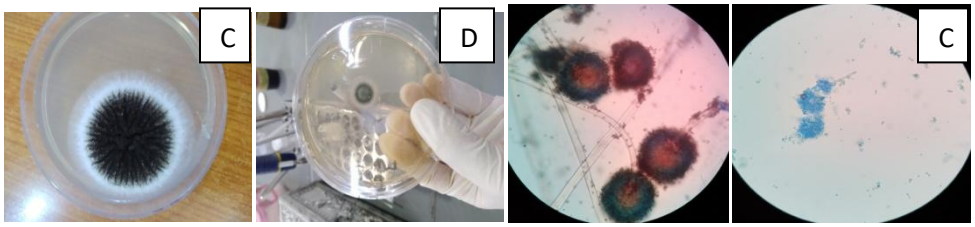

Figure 1: *Aspergillus* spp. grown on SDA

A: A.flavus, B:terrus, C: A. niger, D: A. fumigatus.

Figure 2 : Microscopic feature of *Aspergillus*

Spp. stained with Lactophenol cotton blue

A:A.flavus, B:terrus, C:A.niger, D:A. fumigatus.

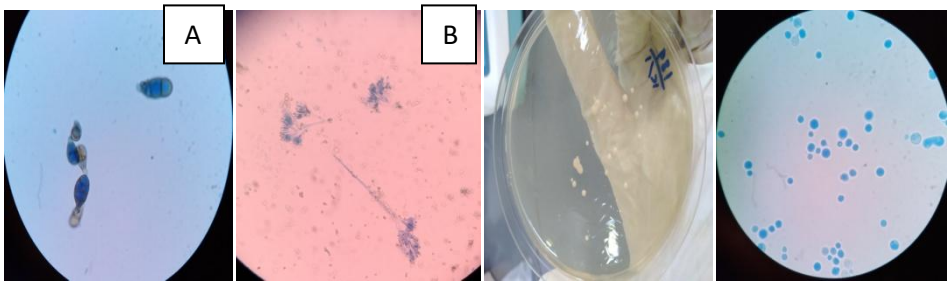

Figure 3: Microscopic feature of*Alternaria* spp.and B:*Penicillium* spp.

Figure 4: *Cryptococcus laurentii*

Figure5: *Candida*spp. grown on SDA.

A: *Candidaciferrii*, B: *Candida glumornidii*

Yeasts identified by the VITEK 2 system allows the identification of medically important yeasts and yeast-like organisms in 15 hrs due to a sensitive fluorescence-based technology and enable a result to be generated without the necessity for morphological testing(Graf*et al*., 2000). However, in this study the results included two genera Candida and Cryptococcus.

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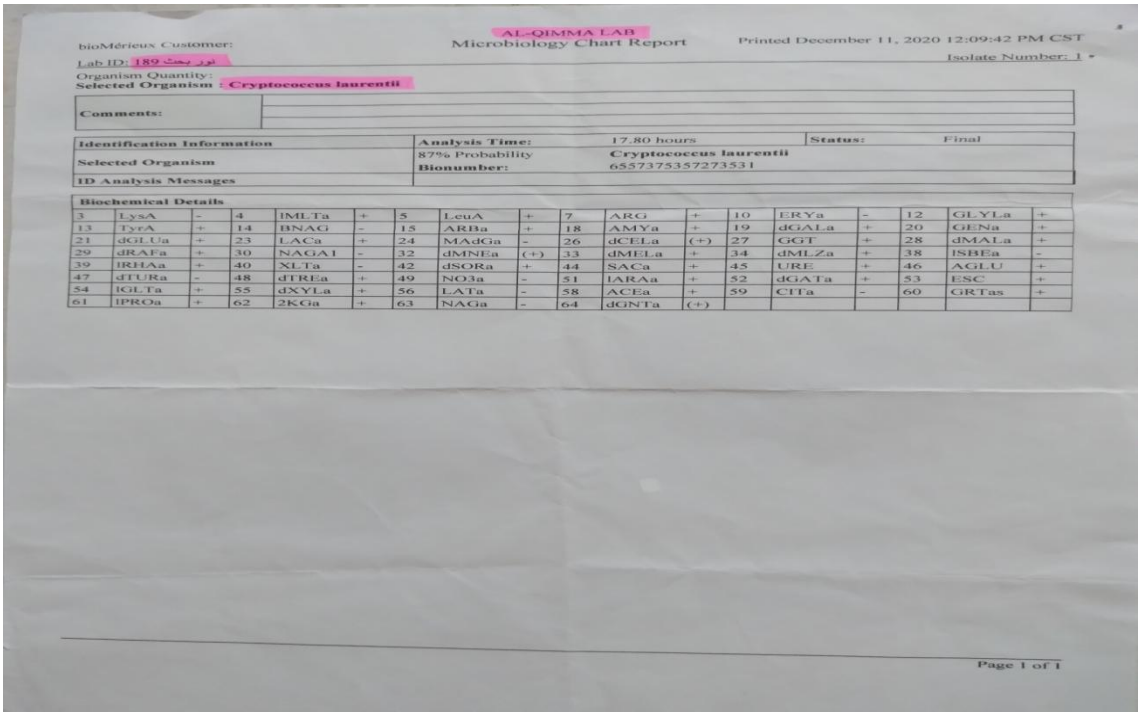


AL-QIMMA LAB
Microbiology Chart Report

bioMérieux Customer:
Printed December 11, 2020 12:09:42 PM CST
Lab ID: 189 نور بحث
Isolate Number: 1
Organism Quantity:
Selected Organism : Cryptococcus laurentii

Comments:

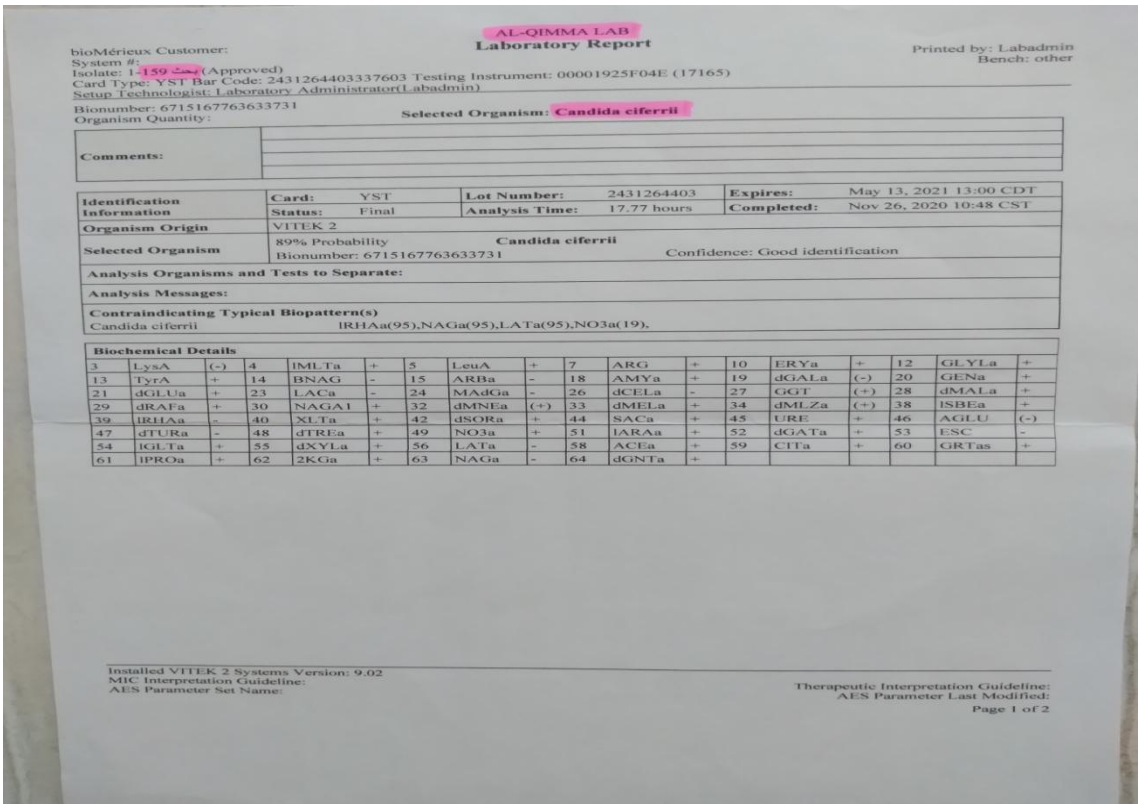
Identification Information	**Analysis Time:** 17.80 hours	**Status:** Final
Selected Organism	87% Probability **Cryptococcus laurentii**	
ID Analysis Messages	**Bionumber:** 6557375357273531	

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	(+)						

Page 1 of 1

Figure 6: Identification of *Cryptococcus laurentii* by vitek2 system



AL-QIMMA LAB
Laboratory Report

bioMérieux Customer:
Printed by: Labadmin
Bench: other
System #:
Isolate: 1-159 بحث (Approved)
Card Type: YST Bar Code: 2431264403337603 Testing Instrument: 00001925F04E (17165)
Setup Technologist: Laboratory Administrator(Labadmin)
Bionumber: 6715167763633731
Organism Quantity:
Selected Organism: Candida ciferrii

Comments:

Identification Information	**Card:** YST	**Lot Number:** 2431264403	**Expires:** May 13, 2021 13:00 CDT
	Status: Final	**Analysis Time:** 17.77 hours	**Completed:** Nov 26, 2020 10:48 CST
Organism Origin	VITEK 2		
Selected Organism	89% Probability **Candida ciferrii** Bionumber: 6715167763633731		Confidence: Good identification
Analysis Organisms and Tests to Separate:			
Analysis Messages:			
Contraindicating Typical Biopattern(s) Candida ciferrii	IRHAa(95),NAGa(95),LATa(95),NO3a(19),		

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	+						

Installed VITEK 2 Systems Version: 9.02
MIC Interpretation Guideline:
AES Parameter Set Name:
Therapeutic Interpretation Guideline:
AES Parameter Last Modified:
Page 1 of 2

Figure 7: Identification of *Candida ciferrii* by vitek2 system

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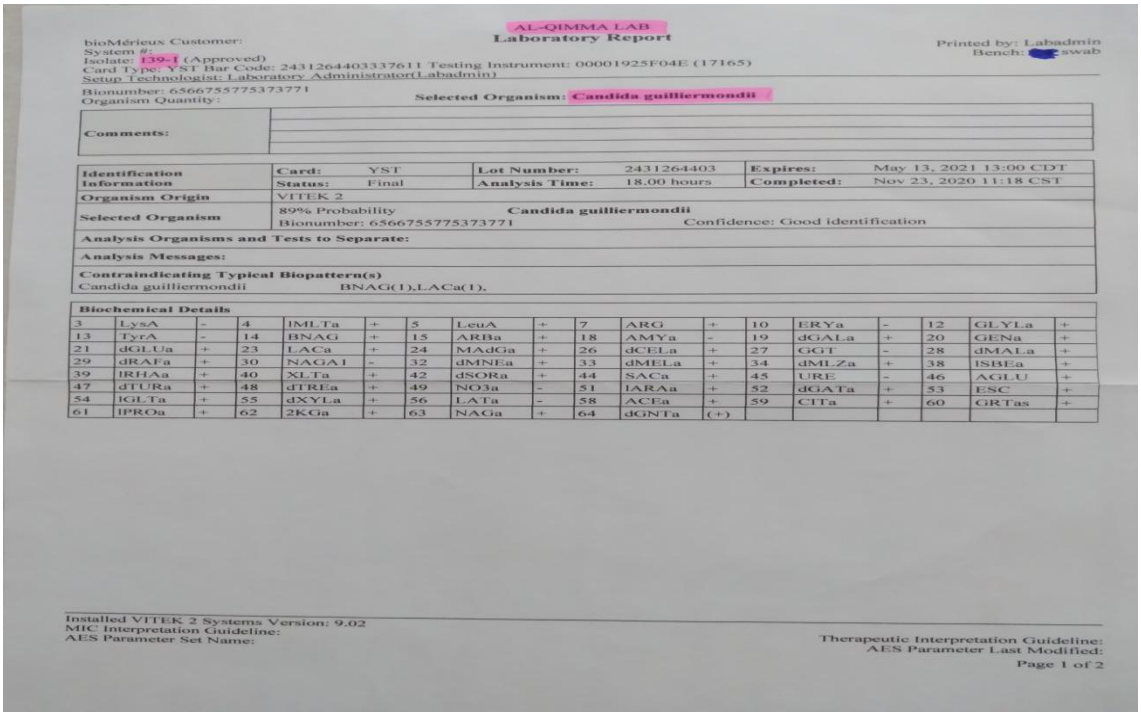

AL-QIMMA LAB
Laboratory Report

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Setup Technologist: Laboratory Administrator(Labadmin)
Bionumber: 6566755775373771
Organism Quantity:

Printed by: Labadmin
Bench: swab

Selected Organism: Candida guilliermondii

Comments:

Identification Information	Card: YST	Lot Number: 2431264403	Expires: May 13, 2021 13:00 CDT
	Status: Final	Analysis Time: 18.00 hours	Completed: Nov 23, 2020 11:18 CST
Organism Origin	VITEK 2		
Selected Organism	89% Probability	Candida guilliermondii	
	Bionumber: 6566755775373771	Confidence: Good identification	
Analysis Organisms and Tests to Separate:			
Analysis Messages:			
Contraindicating Typical Biopattern(s)	Candida guilliermondii BNAGi(1),LACa(1),		

Biochemical Details

3	LysA	-	4	lMLTa	+	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	lSBEa	+
39	lRHAa	+	40	XLTa	+	42	dSORa	+	44	SACa	+	45	URE	-	46	AGLU	+
47	dTURa	+	48	dTREa	+	49	NO3a	-	51	lARAa	+	52	dGATa	+	53	ESC	+
54	lGLTa	+	55	dXYLa	+	56	LATa	-	58	ACEa	+	59	CITa	+	60	GRTas	+
61	lPROa	+	62	2KGa	+	63	NAGa	+	64	dGNTa	(+)						

Installed VITEK 2 Systems Version: 9.02
MIC Interpretation Guideline:
AES Parameter Set Name:

Therapeutic Interpretation Guideline:
AES Parameter Last Modified:
Page 1 of 2

Figure 8: Identification of *Candida glumornidii* by vitek2 system

Data Analysis

Data entered and managed by using SPSS V 0.25 for Windows. Descriptive statistics (frequencies, mean ± standard deviation and with tables and graphs) and inferential statistics (Chi-Square Test) were used. P-value of ≤ 0.05 considered statistically significant.

Results

A sample of 200 participants involved in this study to isolate of fungal pathogens from patients with eye infections. The sample mean ±SD of age was 45.2±20.8 years (ranged 1-88 yrs.), 63.5% of them aged ≥40 years old. Female to male ratio was 1.1:1 ; as outlined in the table (1).

Table (1): Demographic description of study sample (N= 200).

Variables		Frequency	%
Gender	males	95	47
	females	105	53
Age (years)	1-9	10	5
	10-19	13	6.5
	20-29	29	14.5
	30-39	21	10.5
	40-49	33	16.5
	50-59	37	18.5
	60-69	27	13.5
	70-79	20	10
	80-89	10	5

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Tobacco smoking prevalent in 32 person (16%) of sample, figure (9). Nearly one-half of sample (104) present with Blood pressure, and 68 (34%) of them present with diabetes mellitus, figure (10).

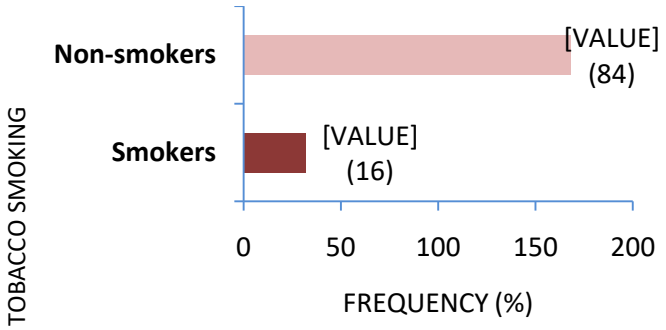

Figure9: Distribution of tobacco smoking.

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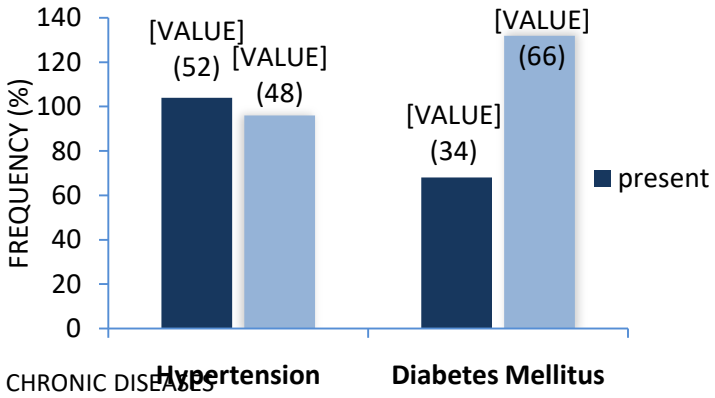

Figure10: Distribution according topresent/ absent of chronicdiseases

During the current study period, laboratory investigations indicated that 29 (15%) of the total samples diagnosed with a fungus species, 13 (45%) of them present with *Aspergillusflavus* followed by five cases (5) 17% with *Aspergillusterrus* , four cases (4) 14% with *Aspergillusfumigatus*, two cases (2) 7% with *Aspergillus niger* and one cases (1) 4% of each *Penicillium* spp. and *Alternaria* spp. , (1) 3% for each *Candidaguilliermondii* , *Candidaciferrii* and *Cryptococcuslaurentii* as outlined in the figure (11).

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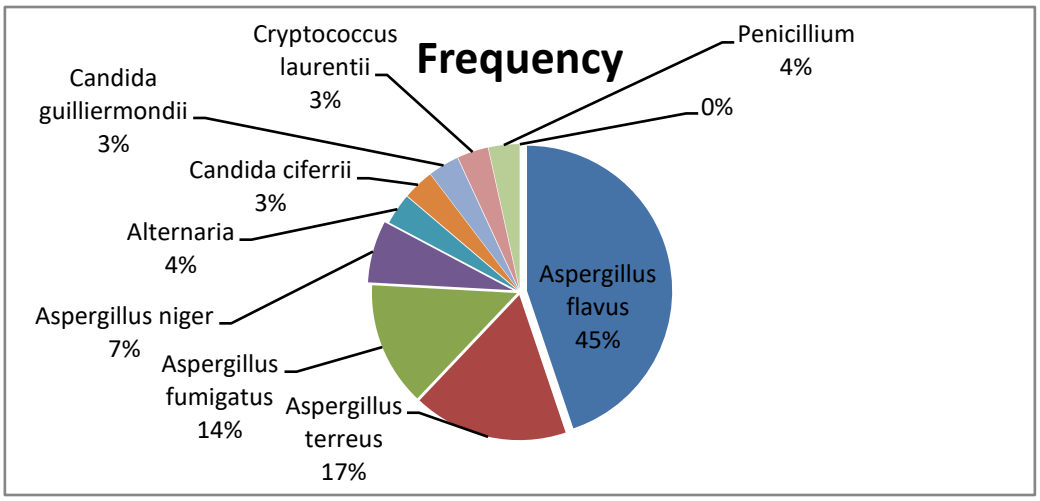

Figure (11): Distribution of fungus species.

Table (2): Relation of culture result with demographic characteristics, tobacco smoking and chronic diseases.

Factors		Culture result		Statistic	
		Positive (n=29)	Negative (n=171)	Chi-Square Test	Sig.
Gender	males (n=95)	20 (21%)	75 (79%)	6.267	0.012
	females (n=105)	9 (9%)	96 (91%)		
Age groups	<40 yrs. (n=73)	9 (12%)	64 (88%)	0.437	0.509
	≥40 yrs. (n=127)	20 (16%)	107 (84%)		
Tobacco smoking	smoker (n=32)	10 (31%)	22 (69%)	8.621	0.006*
	non-smoker (n=168)	19 (11%)	149 (89%)		
Type 2 Diabetes mellitus	present (n=68)	18 (26%)	50 (74%)	11.909	0.001
	absent (n=132)	11 (8%)	121 (92%)		
Blood pressure	present (n=104)	12 (12%)	92 (88%)	1.533	0.216
	absent (n=96)	17 (18%)	79 (82%)		

Discussions

According to the results there was statistically significant association between gender and the culture results of fungus (P 0.012), the fungus spp. detected in males two fold than in females this result was agreed with Thomas, (2003). The effects of smoking on the eye and ophthalmic diseases was first reported in the late 1970s. Current smokers may develop the disease about 10 years earlier than nonsmokers Zisimopoulos *et al*., (2021).the relation of healthy risky behavior with fungus culture data analysis indicated that there was a significant association between tobacco smoking and detection of fungus (P 0.006); fungus spp. identified in smoker patients three fold than in non-smokers this was agreed with Nita and Grzybowski,(2017),which found that exposure to tobacco smoke suppresses the activation of innate immune responses to

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infection. Diabetes increases the risk of infection and damages multiple organs, which may affect the ability of protection against variety of pathogens. Poor glycemic control and chronic diabetes mellitus cause several complications like micro- and macrovascular complications, diabetic foot ulcers, eye infection, nephritis, and nerve infections, which are responsible for high morbidity and mortality Pearce *et al*., (2019).In addition, the results in the table (2) show that infection with fungus species associated with present of type 2 diabetes mellitus in which patients with type 2 diabetes mellitus have fungus infections three times more than in patients without diabetes (P 0.001) this result agree with Saud *et al*., (2020), who found that hyperglycemic state causes immune dysfunction which leads to local and systemic infection due to overgrowth of microflora and causes an opportunistic infection. However, there was no statistically significant association between the culture results of fungus and Blood pressure (P 0.216).

Conclusion

According to the results of the current study, we conclude that : *Aspergillus* spp. was reported as a high incidence in patients with eye infections, the relation between the fungal infections by any species was increased with diabetic patients, smoking has an effect with fungal eye infection and the blood pressure has no effect on fungal eye infection.

Limitations and Future Studies

Fungal eye infection should be put in mind by clinician inspite of its occurrence in low incidence because of their complications such as loss of vision if not successfully treated, studying of the predisposing factors those affecting the incidence of fungal eye infection and finally studying fungal eye infections on other people groups such as (pregnancy, AIDS, thalassemic patients,burns patients, dialysis patients and patients with hematological malignancies).

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