ORIGINAL ARTICLE

Characterization of Nosocomial Fungal Infection among Hepatic ICU Patients in National Liver Institute

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ABSTRACT

Background*: Fungi now represent a serious worldwide threat especially in patients with comorbidities as liver diseases with association of high mortality rates. Regular assessment of at-risk patients, diagnosis by reliable methods in addition to knowledge about antifungal susceptibility and possible sources of such infection help to contain the spread of those life threating infections among high-risk hepatic ICU patients.* ***Objective****: Determination of the incidence, morbidity and mortality of nosocomial fungal infection in hepatic ICU patients, determination of the possible risk factors and antifungal susceptibility pattern in them, tracing possible sources of infection and finally assessment available disinfectants against fungi.* ***Methodology****: The study was conducted on 150 hepatic ICU patients after 48th hours from admission and also on their related environment. Fungal cultures and Multiplex PCR were done for isolation and species identification of the isolates and antifungal susceptibility and antifungal bio typing of suspected sources of infection were performed by VITEK 2 YST–AST cards and disc diffusion method different disinfectants were tested by sampling surfaces before and after disinfection.* ***Results:*** *The incidence of NFI among hepatic ICU patients was (23.3%) with high mortality (69%). The most significant risk factors were the presence of CVC, prolonged ICU stay, COVID infection, previous fungal colonization, antibiotics and corticosteroid exposure and total parenteral nutriton. The most predominant species were C.albicans (43%) with rising incidence of Non albicans (57%).Antifungal susceptibilities were variable with increasing incidence of Azoles resistance. HCWs hands were the most common source of infection (58%). Lysoformine and Chlorine were effective against environmental fungi (100% reduction).****Conclusion:*** *NFI are obvious threat to hepatic ICU patients with high incidence and high mortality rates which is needed to be early diagnosed specifically to species level for proper antifungal targeting. Proper hand hygiene and environmental cleaning are the corner stone of prevention.*

INTRODUCTION

Nosocomial infection remains a major cause of morbidity and mortality with approximately one out of every 20 hospitalized patients developing nosocomial infection.[1]

Over the past decades, there has been a substantial increase in fungal infections worldwide. This is due to increasing in immunocompromised patients as HIV patients, organ transplantation recipients, ICUs patients and recently COVID 19 patients.[2]

Also fungal infections are recognized as an emerging problem with increased morbidity and mortality rates in patients with liver impairment diseases which are associated with a variety of host immune dysfunction.[3]

Candida spp. are the third leading cause of nosocomial bloodstream infections but rank first in mortality rates. The incidence of candidemia has increased by 50% over the past 10 years and it has been reported that mortality in ICU patients with candidemia was higher than mortality from bacteremia (42.6% VS.25.3%).[3-4]

Over the past decade there was an increasing incidence of *non-albicans Candida* (NAC) of which *C.auris is* most frightening threat, as it`s more frequent resistant to azoles ,can remain viable for long periods on environmental surfaces, can survive common cleaning detergents and processes and so colonize surfaces such as medical instruments with up to 14 days survival.[4]

The immune paralysis of hepatic patient results in an inadequate host response to potential environmental pathogens such as *Aspergillus spp*. and *Mucor spp.* which found widely in the nature, at the same time, the incidence of invasive pulmonary fungal infection has increased rapidly in recent years due to COVID-19 infection.[5]

The first step in the fight against nosocomial fungal infections (NFI) in the ICU is implementation of a reliable surveillance system to track infections and identify risk factors and possible sources of them.

Microbiology laboratories were undergoing dramatic changes in the last ten years and many techniques and specific culture media are now available .Another point of evolution is Multiplex PCR. As identification of one by one pathogen is time-consuming, one of the great advantages of the Multiplex PCR is that this technique provides a simultaneous detection of several species and mixed infections with smaller amounts of reagents and samples.[6]

Antifungal treatment is a great challenging point in hepatic ICU patients not only due to many lines affect liver function but also the rate of the resistance increased in the last few years due to misuse of both antibiotic and antifungal drugs.[7]

The aim of this work was determination of incidence, morbidity and mortality of nosocomial fungal infection in hepatic ICU patients, determination of the possible risk factors and antifungal susceptibility pattern in them, tracing possible sources of infection and finally assessment available disinfectants efficacy against fungi.

METHODOLOGY

Study design:

A descriptive study was conducted over a period of 18 months (from June 2020 to December 2021) at National Liver Institute (NLI) which was approved by the Ethical Committee of NLI,Menoufia University, Egypt (No: 00500/2020).

The study involved 150 patients (of both sex) with various hepatic diseases who were admitted to hepatology I.C.U.

Any patient with symptoms and/or signs of infection on the1st 48 hours of admission (positive cultures, elevated ESR, CRP, leukocytosis and having fever) were excluded.

The clinical information of patients' medical records was reviewed by filling out a previously established epidemiological form, developed to elucidate the possible risk factors associated with NFI. All the patients included in this study were initially investigated for the presence of fungi as a cause of infection. Thus, following laboratory confirmation, all patients with NFI were treated with antifungals and clinically followed until remission of the infection and/or death.

Specimen collection:

Cultures were performed from different clinical specimens including blood (collected on BacT/ALERT® Culture Media bottles,bio Merieux, France), nasal and throat swabs, sputum, acetic fluid in case of ascites and aspiration samples for device-associated infections such as central venous catheter, endotracheal tube and indwelling catheters.

Identification of the isolates:

The collected specimens were inoculated into Sabaroud's dextrose agar (SDA) (Oxoid, England) and brilliance™ Candida chromogenic medium (Oxoid, England) which were incubated at 37 C and at 22 C for 1 week and observed daily for any growth .The growing colonies were identified by the standard microbiological features (colony morphology ,wet mount and gram staining).[8]

Identification of yeast species was done by colony color on candida chromogenic media according to manufacture color guide as shown in figure (1) and also by biochemical reactions VITEK 2C2 system yeast identification cards. Version 9.2 (bioMerieux, France).[8]

While Identification of mold species was performed by macroscopic features of the growth (front and reverse).[8]

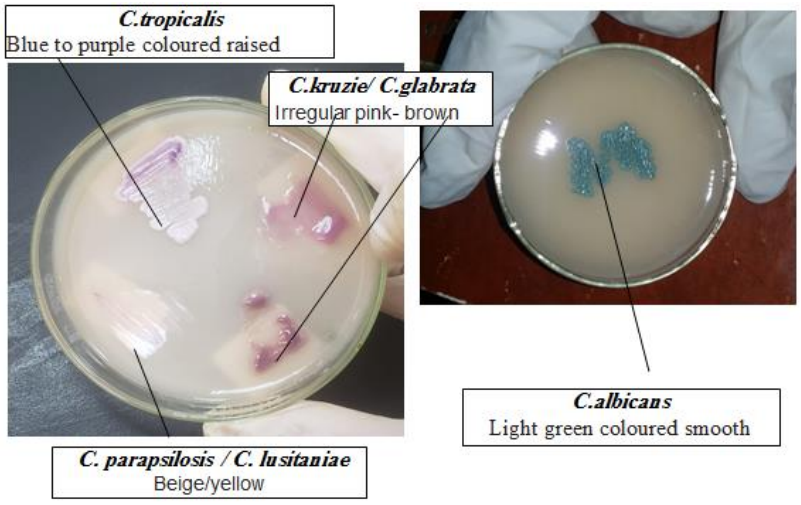

Fig. 1: A and b different *candida spp* on chromogenic media

Genotypic identification of the species causing NFI:

Multiplex PCR for different species-specific fungal primers was done to assess the agreement between different available phenotypic methods and Multiplex PCR, as genotypic method, in the diagnosis of commonest species involved in NFI**.** The target genes and their specific sequences are illustrated in table (1).

Table 1: Primers used in the study:

Fungi	Primers	Sequences	Product Size(bp)	References
Universal fungal primer	ITS1(F) ITS4 (R)	TCCGTAGGTGAACCTGCGG TCCTCCGCTTATTGATATGC	Variable according to species	**9**
Aspergillus fumigatus	AFUM1(F) AFUM2(R)	CGCCGAAGACCCCAACATGAACGC TAAAGTTGGGTGTCGGCTGGC	≈385 bp	**10**
Candida albicans	CALB1(F) CALB2(R)	TTTATCAACTTGTCACACCAG ATCCCGCCTTACCACTACCG	≈ 273bp	**10**
Candida auris	CauF (F) CauR (R)	CGCACATTGCGCCTTGGGGTA GTAGTCCTACCTGATTTGAGGCGAC	≈ 163 bp	**11**
Mucor	ZM1 (F) ZM 2 (R)	ATT ACC ATG AGC AAA TCA GA TCC GTC AAT TCC TTT AAG TTT C	≈405 bp	**12**

The PCR technique involved the following steps:

- ***DNA extraction*:**

Samples tubes were mixed thoroughly and then DNA was extracted from each sample by using DNeasy plant Mini Kit (50) (Cat.No.69104, Germany) according to the manufacture instructions.

- ***PCR analysis cycle for universal primer:***

Extracted DNA was amplified using a pair of universal fungal primers ITS1and ITS4 (table 1) which used to amplify the intervening 5.8S rDNA and the adjacent ITS1 and ITS2 regions as described previously.[13]

- **Multiplex PCR program:**

After confirmation of fungal isolates by universal PCR reaction, Multiplexing reaction was performed in the form of 2 cycles**.**

The 1st cycle was performed to detect *C.albicans* and *A.fumigatus* with the reaction conditions and steps described before.[9]

The 2nd cycle was performed to detect *C.auris and Mucor* with the reaction conditions and steps described by [11-12]

- **Detection of the amplified DNA products:**

Amplicons were detected on 1.5% agarose gels by ethidium bromide staining (Sigma, USA). A DNA ladder (100-1000bp) (Fermentas, Germany) was used to estimate allele sizes in base pairs (bp) for the gel. Following electrophoresis, visualization was conducted with a UV transilluminator and the image was captured by camera.

Antifungal susceptibility of the isolates:

Antifungal susceptibility test of yeast isolates was done by VITEK2 AST-YS08 card following the manufacturer's instructions. This card tests 6 antifungal drugs: Amphotericin B, Flucytosine, Fluconazole, Voriconazole , Caspofungin and Micafungin.[13]

Mold isolates susceptibility was tested by disc diffusion method using Flucytosine 1 µg, Itraconazole 50 µg, ketoconazole 10 µg, Amphotericin B 20 µg, and Nystatin 100 U/disc and results were interpreted according to reference tables in CLSI document M60.[14]

Tracing possible hospital source of infection:

Firstly, we correlated the timing of appearance of NFI with concomitant isolation of the same organisms form patients` environment as beds,commodes,air ,A/C filter, devices as CVC and Urinary catheter and finally HCWs hands. Air samples collected by passive Sampling method described by [15]

While other samples collected by sterile saline wetted swabs and then cultured on SDA and chrome agar.[16]

Secondly we did Multiplex PCR to confirm the isolates genetically. Finally we did antifungal biogram typing with the same idea of that used in bacterial outbreak investigation.[17]

Assessment different disinfectants efficacy on environmental fungi:

We collected 2 samples (1 before and 1 after disinfection) from inanimate surfaces of as beds, commodes, walls, floors and ventilator monitors.

Samples were taken by sterile saline wetted swabs then inoculated on SDA media and chromogenic *Candida* media then incubated the plates at 37 C and at 22 C for 1 week and observed daily for any growth.[18]

Statistical methods:

Data were analyzed using SPSS software version 26. The data were described by numbers (n) and percentages. P (probability) value considered to be of statistical significance if it is less than 0.05.

RESULTS

Patient characteristics:

The age of the studied cases ranged from 23 to 81 years with mean age of 32.3±14.6 of which 93(62%) were females and 57(38%) were males.

Nosocomial fungal infection incidence, mortality and risk factors:

The total incidence rate of NFI among ICU cases was 23.3% with a mortality rate of 69% and there was a highly significant correlation between acquiring NFI in ICU cases and increasing deaths (P Value of 0.001). table(2)

Table 2: Incidence and associated mortality to nosocomial fungal infection in ICU patients:

Deaths	Group I Nosocomial fungal infection (35 cases, 23.3%)		Group II No nosocomial fungal infection (115 cases, 76.6%)		Test of sig. p-value of Deaths
	NO	percentage	NO	Percentage	X2 =10.56
Yes -	24	69%	43	37%	**P=-0.001****
NO -	11	31%	72	63%	

The clinico laboratory presentation of those NFI was variable fig (2), in which Candidemia was the highest incidence (8%) followed by oral candidiasis and catheter associated urinary tract infection (CAUTI) (5.3% for each one).In the 3rd rank came central line associated blood stream infection (CLABSI), nasopharyngeal mucormycosis and invasive pulmonary aspergillosis (IPA) which were accounted for 1.3% for each one and finally invasive fungal peritonitis (0.5%).

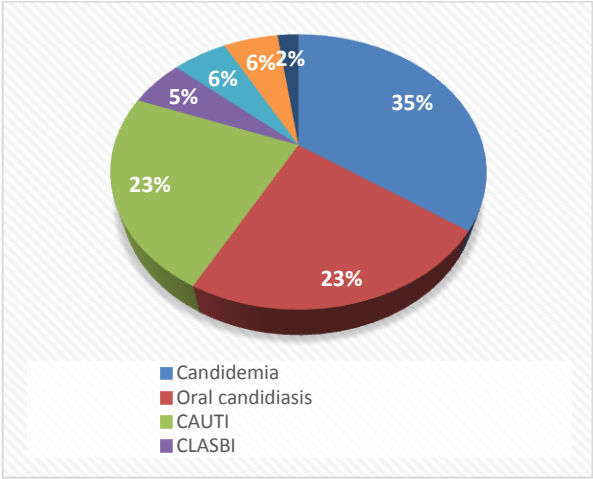

Fig. 2: Frequency of nosocomial fungal infection in hepatic ICU patients

Also the distribution of the isolates was also variable as the following: 43% *C.albicans,14% C.parapsilosis 11% C.auris, 8% C.galabrata, 6% for each of A.fumigatus , C.tropicalis* and *Mucor* and *3% for each C. guilliermondii* and *C.kruzie* fig (3)

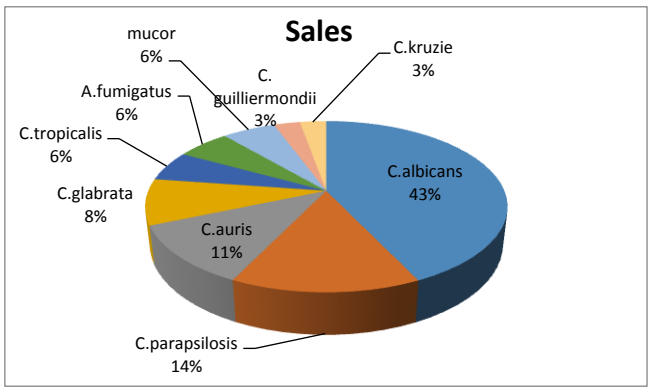

Fig. 3: Species that cause nosocomial fungal infection in hepatic ICU patients

The most common risk factor of NFI in hepatic ICU were the presence of CVC (P=0.05*), prolonged ICU stay, COVID infection, previous fungal colonization, broad spectrum antibiotic exposure, corticosteroid exposure and patient being on TPN (P= ≤0.001for each risk factor).Table (3)

Table 3: Risk factors for acquiring nosocomial fungal infection in hepatic ICU patients:

Items	Group I Nosocomial fungal infection (No=35)		Group II No nosocomial fungal infection (No=115)		Test of sig. p-value
	%	No	%	%	
Gender					
- **Male**	11	31.5%	50	43.5%	X^2 =1.02
- **Female**	24	68.5%	65	56.5%	P=0.314
- **Age** - **Mean ± SD**	42.9±12.9		34.7±21.04		X2= 0.594 P =0.79
Cause of admission					
- **DCL**	12	34%	38	33%	X^2 =1.58
- **HCC**	6	17%	23	20%	P=0.902
- **jaundice**	8	23%	31	27%	
- **GI Bleeding**	9	26%	23	20%	
Ventilation					
- **Yes**	6	17%	8	7%	X2= 1.87
- **No**	29	83%	107	93%	P=0.171
Presence of CVC					
- **Yes**	14	40%	32	28%	X2= 3.29
- **No**	21	60%	83	72%	P=0.05*
ICU stay in days					
1. **Mean ± SD**	7.0±2.9		4.1±1.6		T test=7.6
- **Min-Max**	2-13		2-8		P = ≤0.001**
COVID					
- **Yes**	28	81%	16	14%	X^2= 31.6
- **No**	7	19%	99	86%	P = ≤0.001**
Previous fungal colonization					
- **Yes**	22	62.8%	30	26%	X^2= 31.6
- **No**	13	37.2%	85	74%	P= ≤0.001**
Frequent exposure to broad spectrum antibiotic					
- **Yes**	31	88.5%	56	48.6%	X^2= 17.5
- **No**	4	11.5%	59	51.4%	P= ≤0.001**
corticosteroid exposure					
Yes	22	62.8%	20	17.4%	X^2= 27.5
NO	13	37.3%	95	82.6%	P= ≤0.001**
Patient on TPN					
-Yes	8	22%	5	4%	X^2= 11.6
NO	27	78%	110	96%	P= ≤0.001**

Phenotypic and genotypic identification of the isolates:

Universal fungal primer (1st step of Multiplex PCR reaction) was positive to 30(86%) isolates out of 35 involved in NFI while all species-specific primers (2nd step of Multiplex PCR reaction) were positive to the corresponding species. Fig (4), (5), (6)

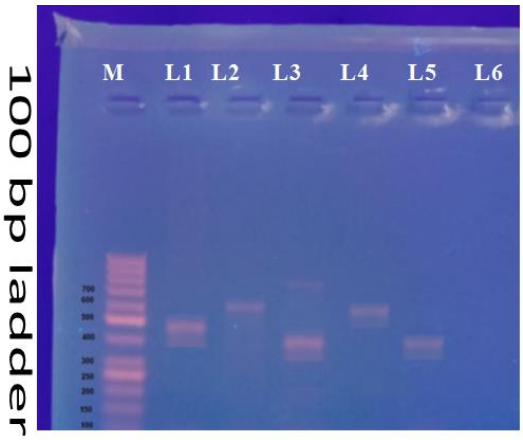

Fig. 4: Universal PCR for fungal isolates (lane 1, 2,3,4,5 are positive)

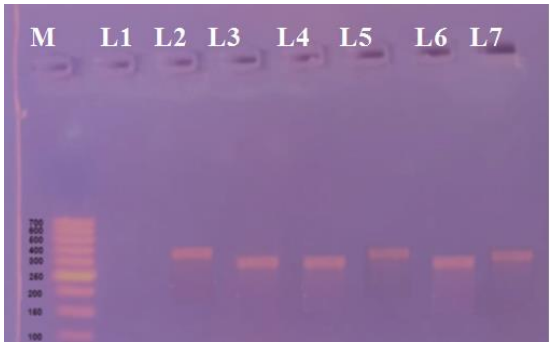

Fig. 5: Multiplex PCR for *C.albicans* (273bp) and *A.fumigatus* (385bp)
(*C.albicans* lane 3, 4,6and7 while *A.fumigatus* lane2and5)

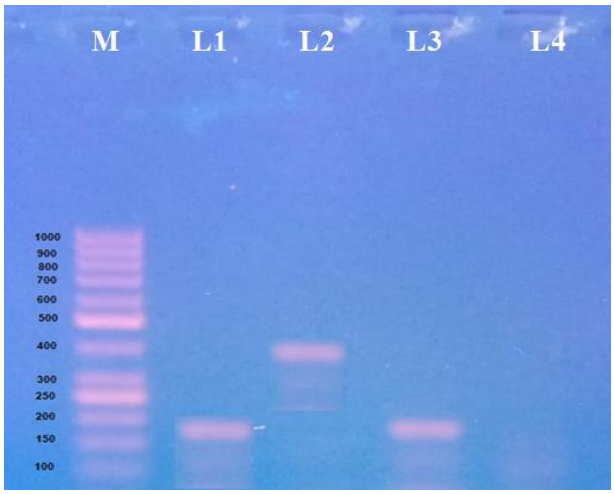

Figure (6) Multiplex PCR for *C.auris (*163 bp) **and *Mucor* (**405 bp)
(*C.auris* lane 1and3 while *Mucor* lane2)

The 5 isolates missed by PCR reaction was identified by SDA,chrome Agar and VITEK2C2 with correlation to clinical finding of having NFI.

The Agreement between phenotypic methods and Multiplex PCR in fungal isolation and species identification was variable according to the method as shown in table (4).

Table 4: Agreement between phenotypic methods and genotypic method

Phenotypic Methods		Genotypic method (PCR)		Sensitivity	Specificity	PPV	NPV	Accuracy	AUC
		+ve 30	-ve 5						
SDA	+ve	28	3	93.3%	60%	90.3%	50%	76.7%	0.667
	-ve	2	2						
CCA	+ve	26	1	85.7%	80%	96.4%	50%	82.9%	0.833
	-ve	4	4						
Vitek	+ve	26	1	85.7%	80%	96.4%	50%	82.9%	0.833
	-ve	4	4						

SDA:sabaroud dextrose agar CCA: chromogenic *Candida* agar AUC: Area under the curve

Antifungal susceptibility of the isolates:

Antifungal susceptibility was different according to the isolates as shown in table (5) and (6).

Table 5: Antifungal Susceptibility of yeast isolates causing nosocomial infections in the ICU using VITEK YST – AST card:

Antifungal drug	*C.albicans* N=15	*Cauris*[a] n=4	*C.tropicalis* N=2	*C.glabrata* N=3	*C krusei* N=1	*C.parapsilosis* n=5	*C.gluilliermondii* N=1
Amphotericin B							
Resistant	1(6.5%)	2(50%)	0	0	0	0	0
Intermediate	1(6.5%)	1(25%)	0	0	0	0	1(100%)
sensitive	13 (87%)	1(25%)	2(100%)	3 (100%)	1(100%)	5(100%)	0
Fluconazole							
Resistant	6 (40%)	3(75%)	0	1(33.3%)	1(100%)	0	NA
Intermediate	4 (26.7%)	0	0	0	0	0	
sensitive	5 (33.3%)	1(25%)	2(100%)	2(66.7%)	0	5(100%)	
Voriconazole							
Resistant	4(26.7%)	1(25%)	0	0	0	0	0
Intermediate	3 (20%)	1(25%)	0	0	0	0	0
sensitive	8(53.3%)	2(50%)	2(100%)	3(100%)	1(100%)	5(100%)	1(100%)
Micafungin							
Resistant	0	0	0	0	0	0	NA
Intermediate	1(6.5%)	0	0	0	0	0	
sensitive	14(93.5%)	4(100%)	2(100%)	3(100%)	1(100%)	5(100%)	
Caspofungin							
Resistant	3(20%)	0	0	0	0	1(20%)	1(100%)
Intermediate	3(20%)	0	0	0	0	1(20%)	0
sensitive	9 (60%)	4(100%)	2(100%)	3(100%)	1(100%)	3(60%)	0
Flucytosine							
Resistant	1(6.5%)	0	0	0	0	2(40%)	0
Intermediate	3(20%)	0	0	0	0	0	1(100%)
sensitive	11(73.5%)	4(100%)	2(100%)	3(100%)	1(100%)	3(60%)	0

Table 6: Antifungal Susceptibility of mold isolates causing nosocomial infections in the ICU using disc diffusion method:

	A.fumigatus N=2	*Mucor* N=2
Itraconazole		
S	2	0
I	0	1
R	0	1
Ketoconazole		
S	0	0
I	1	1
R	1	1
Flucytocine		
S	1	0
I	0	1
R	1	1
Amphotericin B		
S	2	2
I	0	0
R	0	0
Nystatine	2	
S		2
I	0	0
R	0	0

Tracing possible hospital source of infection:

The most common sources of those NFI were hands of HCWs (58%), followed by A/C filter (13%), beds, CVCs and commodes (8% for each source) and finally air (1, 5%).Fig (7)

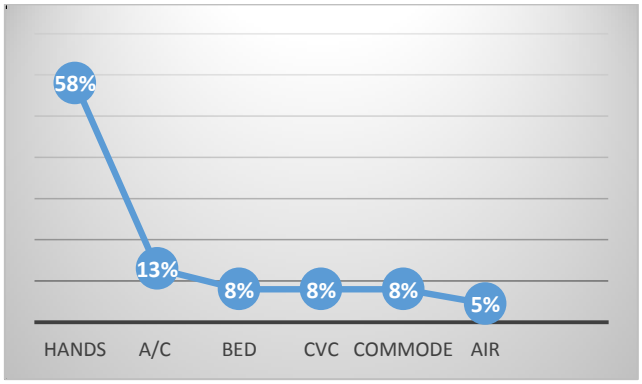

Fig. 7: Frequent sources of nosocomial fungal infection in ICU

Disinfectants efficacy on environmental fungi:

We reported that Lysoformine and Chlorine 1000ppm were more effective in killing environmental fungi than Alcohol (100% reduction vs 85.9%) with more significant p value (0.001 vs 0.01) table (7)

Table 7: Comparison the efficacy of different disinfectants on fungi isolated from ICUs` environment

Samples n=105	Alcohol	Lysoformine	Chlorine 1000 ppm
Total organisms present on surfaces before	N=51/105(48.5%)	N=40/105(38.5%)	N=38/105(36%)
Total organisms present on surfaces after	N=8/105(7.6%)	N=0	N=0
Percentage of reducation	86.9%	100%	100%
Test of significance and p value	X^2=46.4 **P=0.01***	X^2=91.3 **P=0.001****	X^2=95.5 **P=0.001****

DISCUSSION

Nosocomial infection is one of the most frequent adverse event globally. Over 4 million patients in Europe are affected every year with 7.1% prevalence rate, while in developing countries rates are higher, ranging from 5.7 – 19.1% and in critically ill patients cases reach more than 34.1%.[1-29]

Over the past decades, there has been a substantial increase in fungal infections worldwide. It is estimated that over a billion people are affected across the world resulting in approximately 11.5 million life-threatening infections and 1.5 million deaths annually.[2]

Considering the importance of nosocomial fungal infection among immunocompromised hepatic ICU patients, the present study aimed to determine the incidence, morbidity and mortality of nosocomial fungal infection in hepatic ICU patients, determine the possible risk factors and antifungal susceptibility pattern in them, tracing possible sources of infection and finally to assess available disinfectants efficacy against fungi.

In our study we reported that the incidence rate of NFI among hepatic ICU patients was (23%) (35/150 patients).

The clinico laboratory presentation of those NFI was variable in the type and incidence as shown in fig(2) in which candidemia was the commonest presentation (8%) while fungal peritonitis was the least (0.5%).

This was in agreement with *AL-Tabbakh et al.*[19] who reported that candidemia was the most common NFI in ICU with Incidence rate of 9.5%.

Our finding was somehow different from *Verma et al.*[20] who reported that the respiratory NFI was 34%, renal NFI (18%), candidemia (15%), combined renal respiratory NFI (15%), spontaneous fungal peritonitis (13%) and oropharyngeal *Candida*sis was 5%.

In the current study we have concluded that the most common source of NFI in ICU was the hands of HCWs (58%), followed by A/C (13%), beds, CVCs and commodes (8% for each source) and finally air (5%).

We supposed that the high risk of fungal contamination of A/C is due to A/C filters is missed in most times of terminal cleaning and high fungal loads built up by the time.

Our results was in accordance to *da Silva et al* [21] who reported that 52.83% HCWs were involved in cases of nosocomial fungal infection in the ICU.

Similar to our results, *Pilmis et al* [22] *reported* two cases of pulmonary aspergillosis after a routine air filter of A/C change had occurred in the same ICU.

In the current study we reported that Species that caused nosocomial fungal infection in ICU patients were 43% *C.albicans*,14% *C.parapsilosis*,11% *C.auris*,8% *C.galabrata*,6% for each of A.*fumigatus* , *C.tropicalisand Mucor* and finally 3% for each *C. guilliermondii* and *C.kruzi*.

these results was in accordance *to Poissy et al.*[23] who reported that infections with *Candida* spp. are the most common NFI in the ICU accounting for 19% of all nosocomial infections.They also showed that the most common species was C.*albicans* (45 %), although the incidence of non-*albicans Candida* (NAC) has risen dramatically as the following *C.glabrata* (41.9%), *Ctropicalis* (8.6%), *C.parapsilosis* (0.9%), *C krusei*(0.9%). They reported also that *A.fumigatus* prevelance was (1.9%) and *Mucor* was (0.8%).

In the current study the associated mortality rate due to NFI was 69% with a highly significant P Value of 0.001**.**

This finding was similar to *Suleyman and Alangaden*[2] who reported that mortality rate in the ICU due to nosocomial fungal infections was 65%.

Regarding the risk factors associated with NFI ,we reported that there was significant association between development of NFI in the ICU patients with presence of CVC (P=0.05*) ,prolonged ICU stay, COVID infection, previous fungal colonization, broad spectrum antibiotic exposure, corticosteroid exposure and patient being on TPN (P= ≤0.001for each risk factor).

This was in accordance to *Bartoletti et al.*[3] who reported that there was no significance association between nosocomial fungal infections and specific age, gender, GI bleeding, liver failure, HCC and diabetes

Our finding also was in accordance to *Avkan et al* [24] who showed that all patients with nosocomial fungal infections were had CVC insertion (p value 0.0001), 93 % of them were previously colonization and also on broad spectrum antibiotics during their hospitalization (p value 0.005), 82% of them were on steroids (p value 0.035) and they had long ICU stay with mean days 17.4+_ 14.0 (p value 0.0001).

Also *Cighir et al* [6] reported that in the case of SARS-CoV-2 positive patients, associated fungal infections were more common than bacterial infections

(28.57% vs. 14.29%) with associated mortality rate of 71.43% (p value of <0.0001).

The accurate diagnosis of different fungal species remains a challenge. For this purpose, we compared the rate of detection of NFI cases by different available phenotypic methods (SDA, Chrome agarandVITEK 2c2 system) and multiplex PCR as a genotypic method.

We founded that universal fungal primer (1st step of multiplex PCR reaction) was positive to 30 isolates out of 35 involved in NFI while all species-specific primers (2nd step of multiplex PCR reaction) were positive to the corresponding species. The 5 isolates (1 *mucor*,1 *C.albicans* ,2.*C.parapsilosis*and*1C.kruzie*) missed by PCR reaction were identified by SDA,chrome Agar and VITEK2C2 with correlation to clinical finding of having NFI which was diagnosed by physician and improvement of cases after initiating antifungal therapy.

We supposed that those negative PCR results may be due to *Mucor* hyphae were much less amenable to direct amplification, because of more intractable cell walls &abundant endogenous nucleases.

Also Candida *spp.* DNA may be were obtained from older cultures which decrease the chance of amplicon gain.

Our result was in accordance to *Luo et al* [10] who tested 242 fungal isolates using multiplex PCR and reported that only 53.2% of the molds were positive to the universal primer but those entire positive isolates were all positive to species-specific primers. However their results in yeast isolates were little different to ours as all yeast isolates in their study were positive to universal fungal primer.

In our study we reported that *C.albicans* showed high resistance to fluconazole (40 %) , while the highest sensitivity was to micafungin (93.5%) followed by amphotericin B(87%), flucytocine (73.5%) ,caspofungin (60%) and finally voriconazole (53.3%).

Zheng et al [4] reported that *C.albicans* strains showed the lowest susceptibility rates for miconazole, ketoconazole, and fluconazole; 52.95%, 85.3%, and 91.60%, respectively with a sensitivity to polyenes (98.4%) and echinocandins (100%).

In the present work *C.auris* showed high resistance to fluconazole (75%) and amphotericin B (50%) but it showed highest sensitivity to micafungin (100%), caspofungin (100%), flucytocine (100%) and 50% were voriconazole sensitive.

C. auris strains from around the world exhibit a clade-specific resistance to fluconazole but varying susceptibility to other triazoles, amphotericin B, and echinocandins *Du et al* [25]

El-Kholy et al.[26] published the 1st academic report about *C.auris* in Egypt and they reported that the isolate showed high resistance to fluconazole and amphotericin B, but it was susceptible to echinocandins.

In our study all *C.tropicalis* isolates were sensitive to all antifungal drugs in VITEK 2 AST card while *C.glabrata* showed highest sensitivity (100%) to amphotericin B, voriconazole, micafungin, caspofungin and Flucytocine with 33.3% were resistant to fluconazole**.**

In the study done in Egypt by *El-Kholy et al* [27] (36.62%) and (7.04%) of *C.tropicalis* were resistant to fluconazole and voriconazole, respectively but no resistance to echinocandins, amphotericin B, nor 5-flucytosine was detected.

Different Arab countries reported different results regarding azole susceptibility. *Eddouzi et al* [28] in Tunisia reported a low incidence of azole resistance while *Ibrahim et al* [29] in a Saudi Arabia reported a high rate of fluconazole and voriconazole resistance (62.5% and 25%, respectively).

In the United States, *C.tropicalis* isolates were >97% susceptible to fluconazole while in Latin America, Asia -Pacific regions, nearly 90% of *Candida* isolates were azole susceptible *Arastehfar et al* [30]

In our work , we reported that *C krusei* was sensitive to all antifungal drugs in VITEK 2 AST card except fluconazole (intrinsic resistant) and *C.parapsilosis* showed highest sensitivity (100%) to amphotericin B, fluconazole, voriconazole and micafungin with some resistance to flucytocine (40%) and caspofungin (20%).

Kajihara et al [31] reported that *C krusei* had high resistance (40%) to amphotricin B and high sensitivity (100%) to caspofungin and micafungin.

Also *Mohamed et al* [32] stated that C. *parapsilosis* showed high susceptibility to the three azoles i.e. voriconazole, itraconazole and fluconazole. While, *Mahdavi et al* [33] showed that all *C.parapsilosis* isolates (100%) were susceptible to flucytocine.

We also observed antifungal susceptibility pattern to mold isolates by disc diffusion method and reported that all *A.fumigatus* isolates were highly sensitive to Itraconazole, Amphotericin B and Nystatine .Ketoconazole sensitivity was variable as 50% of those isolates showed intermediate sensitivity while the remaining 50% were resistant.Flucytocine sensitivity was also variable between the isolates as 50% of them were highly sensitive while the remaining 50% were resistant.

Yang et al [34] in their study showed that all *A.fumigatus* isolates were sensitive to Amphotericin B and caspofungin while Voriconazole exhibited more efficacy than Itraconazole and Posaconazole.

In *Mucor* isolates, we reported that all were highly sensitive to,Amphotericin B and Nystatine but 50% of the isolates were intermediate sensitive to Flucytocine ,Itraconazole, and Ketoconazole while the remaining 50% were resistant.

Wagner et al.[35] eported that *Mucor* sensitivity to Amphotericin B and Terbinafine was 100% with

variable sensitivity to Isavuconazole, Itraconazole, and Posaconazole.

In the current study we investigated different disinfectants efficacy against fungi and reported that Lysoformine and Chlorine 1000ppm were more effective in killing environmental fungi than Alcohol (100% reduction vs. 85.9%) with more significant p value (0.001 vs. 0.01).

Our results was in accordance to *Gunar et al* [36] who showed that alcohol was not effective in killing all fungal species on contrast ,they reported that sodium hypochloride was most effective within a maximum of 3 min contact time.

CONCLUSION

Our study highlights the high incidence and mortality rates among hepatic ICU patients associated with nosocomial fungal infection.

In this setting, the choice of species specific therapy is paramount, and risk factors for nosocomial fungal infection should be taken into consideration to optimize patients'management.

Colonization by fungi is an important cause of NFI in hepatic ICU patients and it depends on long ICU stay, broad spectrum antibiotics and previous steroid exposure.

Also the presence of devices (as central venous catheters and urinary catheters) and total parenteral nutrition increase patient risk to NFI.

Recommendation:

Fungal outbreaks have been difficult to control, due to its faulty detection by routine diagnostics, rapid transmission, and their ability to survive in adverse conditions and resistance to removal by environmental disinfection procedures.

Timely diagnosis by rapid and reliable identification methods help to prevent the spread of NFI.

Information on species distribution and their sources could help to develop guidelines for preventive strategies. Furthermore, molecular biology techniques can help in the identification of resistant fungal species as *C.auris* which is defined by CDC as an emerging fungus that presents a serious global health threat.

No conflict interest

There is no conflict of interest

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