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Identification of *Candida* Species Isolated from Egyptians Patients with Chest Infection Using Integral System Yeast Plus

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A B S T R A C T

Pulmonary fungal infections are common potentially life-threatening conditions in immuno-compromised patients. Diagnosis represents a challenge due to the non-specific clinical manifestations. Empirical use of anti-fungal drug therapy has been a burden for years due to high cost and drug toxicity. This study was designed to determine the presence of *Candida* chest infection in immuno-compromised patients in Tanta University Hospitals. A total of 150 patients with chest infection were selected during the period from June 2015 till June 2017. Collected samples were cultured on Sabouraud's Dextrose agar media. *Candida* isolates were tested using integral system yeast plus (ISYP) for yeast typing and antifungal susceptibility. Out of 150 tested samples, *candida* was isolated from 44 samples. ISYP showed that The highest prevalence (36.4%) was for *Candida albicans*, followed by *Candida tropicalis* (25%), thirdly *Candida parapsilosis* (15.9%), then *Candida krusei* (11.4%), followed by *Candida famata*, *Candida stellatoidea* and *Candida zylanooides* with percentage as (4.5, 4.5 and 2.3%) respectively. Each candida species has different antifungal sensitivity patterns. It is concluded that *Candida albicans*, *Candida tropicalis* and *Candida parapsilosis* were the predominant *Candida* species causing chest infections in immuno-compromised patients. Further studies must be done to test the sensitivity and accuracy of ISYP.

Introduction

Fungal chest infection is a serious life-threatening infection with a 60–80% mortality rate in immuno-compromised children and adults (Hardak *et al.*, 2009).

Candida albicans is the most frequent species isolated from respiratory samples (approx. 50%) followed by *C. parapsilosis*, *C. tropicalis* and *C. glabrata*. Despite the frequent isolation of *Candida* spp. from respiratory samples, isolation in non-neutropenic patients is not considered as a diagnostic method of pneumonia regardless the species isolated (Garnacho-Montero *et al.*, 2013).

The diagnosis of fungal chest infection is challenging due to a lack of sensitive and specific clinical and radiological signs, and tissue biopsy is often precluded (and

invasive), especially among patients with thrombocytopenia (Torelli *et al.*, 2011). The traditional microbiological workup of clinical specimens is based on microscopic examination and culture on mycological media (Lass-Flörl *et al.*, 2013).

No method has proven to be sufficiently sensitive and specific to allow adequate diagnosis, and the "gold standard" consists of microscopy and culture (Pound *et al.*, 2010). Microscopic examination allows the cheap and rapid detection of fungal elements in clinical specimens. Despite this advantage of providing an early presumptive or definitive diagnosis of invasive fungal infection (IFI), fungal classification is not possible (Penack *et al.*, 2008).

In order to identify isolated yeast species, appearance and configuration of the colonies and germ tube test are used, in addition to these conventional methods, commercial kits which provided quick identification and antifungal susceptibility are used (Baran *et al.*, 2013).

The aim of the present study was to determine the presence of *Candida* chest infection in immuno-compromised patients and pneumonic patients in Tanta University Hospitals by Culture. Then we used INTEGRAL SYSTEM YEASTS Plus Test for yeast typing and antifungal susceptibility.

Materials and Methods

Study design and participants

This prospective study was carried out in the Medical Microbiology and Immunology Department, Faculty of Medicine, Tanta University during the period from June 2015 till June 2017 on 150 patients selected from Intensive Care Units (ICUs), Chest department and Oncology department of Tanta University Hospitals.

All patients were immuno-compromised (males and females) suffering from symptoms of chest infection and pneumonia. Demographic variables such as gender, age, underlying disease together with the patients' clinical data, degree of critical illness, risk factors of pneumonia, length of ICU stay, and antibiotic regimen were collected from patients' records.

Patients in this study were subjected to complete medical history with particular emphasis on the age, underlying disease, time of onset of pneumonia and through clinical examination to diagnose chest disease.

Collection of samples and microbiological examination

Clinical specimens

Sputum

Fifty sputum samples were collected from Immuno-compromised patients with chest infection having expectoration, first early morning sputum samples were collected in sterile screw capped plastic container after instruction to patient to brush his teeth and rinse mouth with water, sputum should be as a result of deep cough (not saliva).

Bronchoalvolar lavage (BAL)

Fifty BAL samples were obtained from patients with chest-x-ray abnormalities complaining from unresolved pneumonia, collected aseptically in sterile screw capped plastic container, and immediately transported to the laboratory, the fluid was processed for culture and direct microscopic examination.

Endotracheal aspirate

Fifty Endotracheal aspirate samples were collected from ventilated patients suffering from ventilator associated pneumonia (VAP),

collected aseptically and immediately transported to the laboratory, the fluid was processed for culture and direct microscopic examination.

All collected samples were cultured on Sabouraud's Dextrose agar media. Identification of fungus species by Colony morphology**,** Gram stain and Germ tube test.

Integral System Yeasts Plus

Was used for yeast typing and antifungal susceptibility: which is a 24 wells system containing biochemical substrata and dried antimycotics for the identification of the most clinically important yeasts and sensitivity evaluation to antimycotics. The system is inoculated with the cell suspension and incubated at 36 ± 1°C for 48 hours.

Presumptive Identification is based on assimilation reactions of sugars; the tests for the assimilation reactions are interpreted by evaluating the color change of wells 1-GLU to 12-DUL. The combination of positive and negative reactions allows the formation of a numerical code which permits to identify the yeasts under examination, through the use of the table of codes.

The well 13-CHR contains a chromogenic substrate that permits to differentiate some yeast by evaluating the color change of the well.

Sensitivity to antimycotics, the tests are evaluated according to growth or inhibition of yeasts in media containing the antimycotic and a growth indicator in the wells 14-NY to 23-FLU.

The color change from red to orange in the wells indicates a slow growth of the yeast under examination and an intermediate sensitivity to the concentration of antimycotic in the well. The color change from red to yellow in the wells indicates a growth of the yeast under examination and resistance to the concentration of antimycotic in the well.

No color change in the well indicates no growth of the yeast under examination and sensitivity to the concentration of antimycotic in the well.

The well 24-Growth does not contain antimycotics, it contains culture medium and indicator and it works as growth control.

Statistical analysis

Data were fed to computer and analyzed using IBM SPSS software package version 20.0. (Armonk, NY: IBM Corp).Qualitative data were described using number and percent. Significance of the obtained results was judged at the 5% level.

The tests used were

Chi-square test

For categorical variables, to compare between different groups.

Fisher's Exact

Correction for chi-square when more than 20% of the cells have expected count less than 5.

Ethical approval

The study protocol was reviewed and approved by the local ethical committee of Faculty of Medicine, Tanta University, Egypt.

Results and Discussion

This work was carried out on 150 immuno-compromised patients [males (n=82) and

females (n=68)] suffering from symptoms of chest infection and pneumonia, selected from Intensive Care Units, Chest department and Oncology department (50 cases of each respectively) of Tanta University Hospitals during the period from June 2015 till June 2017.

Among 150 clinically suspected cases, *Candida* infection was detected in 44 cases (29.3%). The highest prevalence (36.4) was for *Candida albicans*, the second percentage was 25% for *Candida tropicalis*, the third one (15.9%) was for *Candida parapsilosis*, the fourth was (11.4%) for *Candida krusei*, followed by *Candida famata*, *Candida stellatoidea* and *Candida zylanooides* with percentage as (4.5, 4.5 and 2.3%) respectively (Table 1).

Table 2 shows Sensitivity and Accuracy for chrom method which was (59.09 for both) Antibiogram of *Candida albicans* (16 cases) reveal that the pathogens are more sensitive to Flucytosine 16 µg/mL (13cases) followed by Ketoconazole 0.5 µg/mL and Fluconazole 64 µg/mL (6 cases for each) while the highest resistance was for Clotrimazole 1 µg/mL (11 cases followed by Econazole 2 µg/mL (7 cases) (Fig. 1).

Antibiogram of *Candida tropicalis* (11 cases) reveal that the pathogens are more sensitive to Flucytosine 16 µg/mL (7 cases) while the highest resistance was for Clotrimazole µg/mL (11 cases) (Fig. 2).

Antibiogram of *Candida parapsilosis* (7 cases) reveal that the pathogens are more sensitive to Flucytosine 16 µg/mL (7 cases) while the only resistance was for Miconazole µg/mL (4 cases) (Fig. 3).

Antibiogram of *Candida krusei* (5 cases) reveal that the pathogens are only sensitive to Flucytosine 16 µg/mL, Ketoconazole 0.5 µg/mL and Voriconazole 2 µg/mL (3 cases for each) while the highest resistance was for Nystatin 1.25 µg/mL, Amphotericin 2 µg/mL, Econazole 2 µg/mL, Clotrimazole 1 µg/mL, Miconazole 2 µg/mL, Itraconazole 1 µg/mL and Fluconazole 64 µg/mL (5 cases respectively (Fig. 4).

Antibiogram shows that *Candida famata* was sensitive to Nystatin 1.25 µg/mL, Flucytosine 16 µg/mL and Ketoconazole 0.5 µg/mL (2 cases respectively) while the pathogen shows resistance to Econazole 2 µg/mL and Clotrimazole 1 µg/mL (2 cases respectively).

Antibiogram of *Candida stellatoidea* (2 cases) reveal that the pathogens are only sensitive to Flucytosine 16 µg/mL and Nystatin 1.25 µg/mL (2and1 cases respectively) while the only resistance was for Clotrimazole 1 µg/mL, (2cases).

Antibiogram of *Candida zylanooides* (1 case) reveal that the pathogen was sensitive to Flucytosine 16 µg/mL, Nystatin 1.25 µg/mL and Econazole 2 µg/mL (1 case respectively) while the pathogen shows no resistance. The present study revealed that among 150 cases: *Candida* infection was detected in 44 cases (29.3%)

In accordance with our result, a study conducted in India by Gupta *et al.,* 2016 estimated that from 210 ICU patients, 52(24.7%) were fungal pathogens, Majority of the fungal pathogens were *Candida* species 42(82.3%).

The figure was different with Chakma *et al.*, 2017 who reported that among 151 sputum samples *Candida* was isolated in 15.23% (23/151) samples. Also Yazıcıoğlu Moçin *et al.*, 2013 reported that, among the 95 case patients, the microbiology results of 63 (66.3%) revealed fungi (90.5% *Candida* species; 9.5% *Aspergillus*).

Table.1 Distribution of *Candida* species among *Candida* cases detected (n = 44)

	No.	%
Types of *Candida*		
Candida albicans	16	36.4
Candida tropicalis	11	25.0
Candida parapsilosis	7	15.9
Candida krusei	5	11.4
Candida famata	2	4.5
Candida stellatoidea	2	4.5
Candida zylanooides	1	2.3

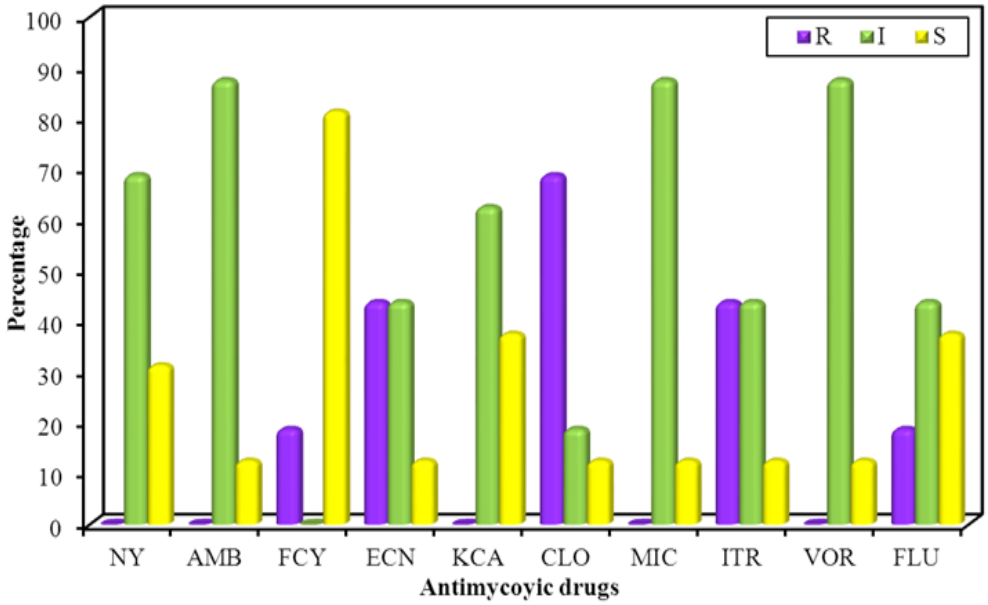
The highest prevalence (36.4) was for *Candida albicans*, the second percentage was 25% for *Candida tropicalis*, the third one (15.9%) was for *Candida parapsilosis*, the fourth was (11.4%) for *Candida krusei*, followed by *Candida famata, Candida stellatoidea and Candida zylanooides* with percentage as (4.5, 4.5 and 2.3%) respectively.

Table.2 Agreement for Chrom substrate result

		Chrom substrate standard		Sensitivity	Specificity	PPV	NPV	Accuracy
		Negative	Positive					
Chrom substrate result	Negative	0	18	59.09	-	100.0	0.0	59.09
	Positive	0	26					

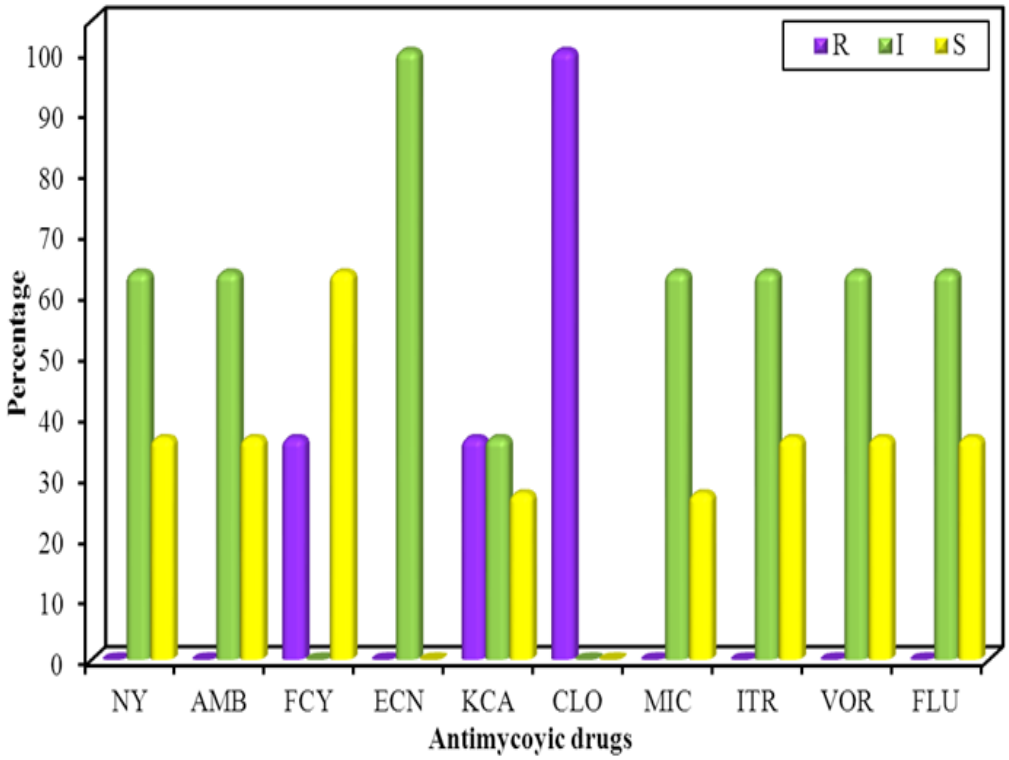
This table shows Sensitivity and Accuracy for chrom method which was (59.09 for both)

Fig.1 Antibiogram of *Candida albicans* Cases (n = 16)



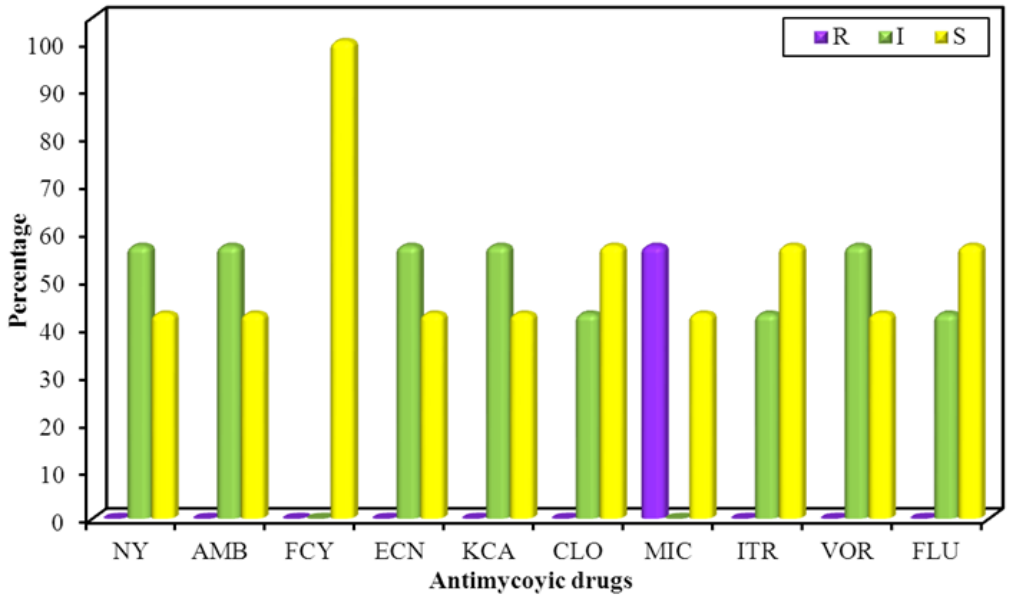
Antibiogram of *Candida albicans* (16 cases) reveal that the pathogens are more sensitive to Flucytosine 16 µg/mL (13cases) followed by Ketoconazole 0.5 µg/mL and Fluconazole 64 µg/mL (6 cases for each) while the highest resistance was for Clotrimazole 1 µg/mL (11 cases followed by Econazole 2 µg/mL (7 cases).

Fig.2 Antibiogram of *Candida tropicalis* cases (n = 11)



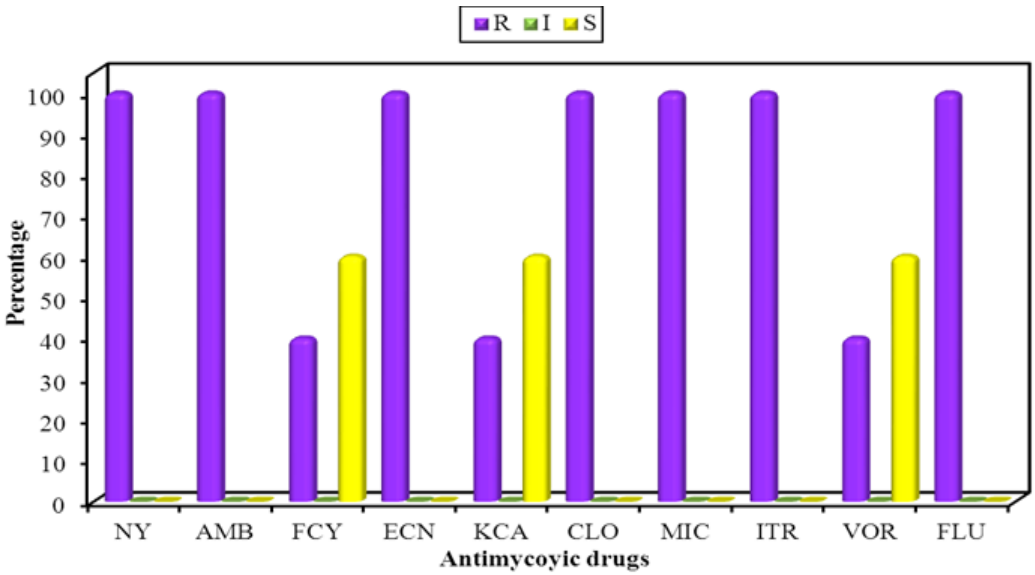
Antibiogram of *Candida tropicalis* (11 cases) reveal that the pathogens are more sensitive to Flucytosine 16 µg/mL (7 cases) while the highest resistance was for Clotrimazole µg/mL (11 cases).

Fig.3 Antibiogram of *Candida parapsilosis* cases (n= 7)



Antibiogram of *Candida parapsilosis* (7 cases) reveal that the pathogens are more sensitive to Flucytosine 16 µg/mL (7 cases) while the only resistance was for Miconazole µg/mL (4 cases).

Fig.4 Antibiogram of *Candida krusei* cases (n= 5)



Antibiogram of *Candida krusei* (5 cases) reveal that the pathogens are only sensitive to Flucytosine 16 µg/mL, Ketoconazole 0.5 µg/mL and Voriconazole 2 µg/mL (3 cases for each) while the highest resistance was for Nystatin 1.25 µg/mL, Amphotericin 2 µg/mL, Econazole 2 µg/mL, Clotrimazole 1 µg/mL, Miconazole 2 µg/mL, Itraconazole 1 µg/mL and Fluconazole 64 µg/mL (5 cases respectively).

Our study use CHROM substrate *Candida* as a method of typing of *Candida* which Sensitivity was (59.09) and positive predictive value (PPV) was 100%.

Against our result, Ozcan *et al.*, (2010) who reported that Sensitivity of chrom agar *Candida* was 87.5 and positive predictive value (PPV) was 100%.

Also Peng *et al.*, 2007 reported that the sensitivity and specificity for CHROM substrate *Candida* in C. *albicans* identification were 100% and 94.6% respectively.

Regarding antibiogram of *Candida albicans* (16 cases) reveal that pathogens are more sensitive to Flucytosine 16 µg/mL (13cases) followed by Ketoconazole 0.5 µg/mL and Fluconazole 64 µg/mL (6 cases for each) while the highest resistance was for Clotrimazole 1 µg/mL (11 cases followed by Econazole 2 µg/mL (7 cases). According to the study of Njunda *et al.*, (2013), C. *albicans* isolated reported high susceptibility to ketoconazole (80.2%) which is in agreement with our study while they stated that Nystatin was the least susceptible antifungal drug to *Candida albicans* which is against our study which shows lowest sensitivity to Econazole.

Although, Francuzik *et al.*, (2015) reported that C. *albicans* strains were the most resistant against antimycotics. On the other hand Gupta *et al.*, (2016) found that 11(34.3%) and 7(21.8%) of C. *albicans* were resistant to fluconazole and nystatin respectively.

Regarding antibiogram of *Candida tropicalis* (11 cases) which reveal that the pathogens are more sensitive to Flucytosine 16 µg/mL (7 cases) while the highest resistance was for Clotrimazole µg/mL (11 cases) but it is nearly comparable to that reported by Njunda *et al.*, (2013) who stated that *Candida* tropical is recorded high susceptibility to ketoconazole (94.7%) followed by voriconazole (78.9%) and miconazole (73.7%). Nystatin was least resistant. Antibiogram of *Candida parapsilosis* (7 cases) reveal that the pathogens are more sensitive to Flucytosine 16 µg/mL (7 cases) while the only resistance was for Miconazole µg/mL (4 cases). These results differed from

that of Njunda *et al.,* 2013 who reported that *Candida parapsilosis* recorded 100% susceptibility to all 10 antifungal agents

Regarding antibiogram of *Candida krusei* (5 cases) reveal that the pathogens are only sensitive to Flucytosine 16 µg/mL, Ketoconazole 0.5 µg/mL and Voriconazole 2 µg/mL (3 cases for each) while the highest resistance was for Nystatin 1.25 µg/mL, Amphotericin 2 µg/mL, Econazole 2 µg/mL, Clotrimazole 1 µg/mL, Miconazole 2 µg/mL, Itraconazole 1 µg/mL and Fluconazole 64 µg/mL (5 cases respectively).

This was in agreement with Pfaller *et al.,* 2008 who found that C. *krusei* exhibited decreased susceptibility to voriconazole (82.9% S, 7.8% R) also Francuzik *et al.,* (2015) found that all isolated C. *krusei* is resistant to Fluconazole.

On the other hand Njunda *et al.,* (2013) reported that *Candida krusei* which were isolated exclusively from the oral cavity recorded 100% susceptibility to all 10 antifungal agents.

Antibiogram shows that *Candida famata* was sensitive to Nystatin 1.25 µg/mL, Flucytosine 16 µg/mL and Ketoconazole 0.5 µg/mL (2 cases respectively) while the pathogen shows resistance to Econazole 2 µg/mL and Clotrimazole 1 µg/mL (2 cases respectively). Controversially, this result disagreed with Njunda *et al.,* (2013) who found that the four *Candida famata* isolates exhibited 100% susceptibility to ketoconazole, amphotericin B and fluconazole. Resistance was recorded with nystatin (50%) and flucytosine (25%).

Antibiogram of *Candida zylanooides* (1 case) reveal that the pathogen was sensitive to Flucytosine 16 µg/mL, Nystatin 1.25 µg/mL and Econazole 2 µg/mL (1 case respectively) while the pathogen shows no resistance. This is in agreement with Njunda *et al.,* 2013 who found that *Candida* zeylanoides, which were isolated exclusively from the oral cavity, recorded 100% susceptibility to all 10 antifungal agents

Comparison between C. *albicans* and *Candida* non *albicans* to different antifungal agents showed that resistance to clotrimoxazole was significantly detected in *Candida* non *albicans* isolates compared to C. *albicans* isolates (Francuzik *et al.,* 2015).

In conclusion, Studies that determine the incidence of fungal infections in all departments of our hospital should repeatedly be performed in order to stand the magnitude of the problem.

Also Fungal cultures and antifungal susceptibility testing for all suspected cases of fungal chest infection or pneumonia should be performed to confirm the etiology and administer the proper treatment to reduce the morbidity and mortality rate.

Establishment of antifungals treatment protocols to avoid overuse and misuse of antifungals drugs to prevent the emergence of resistant fungal strains especially in hospital environment.

Further studies must be done to prove importance of Integral System Yeasts Plus for yeast typing and antifungal susceptibility and also to test the sensitivity and accuracy of it

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