# RESULTS

This study was conducted at Benha University Hospitals on 45 patients with haematological malignancies who had febrile neutropenia <1000 neutophils /µl of blood with temperature of >38$^{o}$C that had failed to respond to broad spectrum antibiotics after 2 days of treatment.

They were ( 28 ) males and (17) females.Their age ranged from 15 to 74 year with mean age of 43.3±11.2 y.

***ALL THE PATIENTS WERE SUBJECTED TO THE FOLLOWING:***

1] Direct microscopic examination of urine, sputum and oropharyngeal swabs .

2 ] Fungal cultures for blood,urine, sputum and oropharyngeal swabs .

Followed by identification of Candida by :

- Subculture on Sabouraud's Dextrose Agar and colony morphology.
- Gram staining.
- Germ tube test.
- Auxacolor test .

3] Platelia$^{TM}$ Candida Ag for detection of Candida mannan antigen in serum by Enzyme Immunoassay.

4] Multiplex, Real-time PCR. for detection and identification of the common five species of Candida in blood which are (Candida albicans, Candid glabrata, Candida krusei, Candida parapsilosis and Candida tropicalis ).

**Table(1):** Results of fungal culture form different specimens

| | Positive | | Negative | | Total | |
|---|---|---|---|---|---|---|
| | No | % | No | % | No | % |
| Blood | 4 | 8.9% | 41 | 91.1% | 45 | 100% |
| Sputum | 5 | 11.1% | 40 | 88.9% | 45 | 100% |
| Urine | 5 | 11.1% | 40 | 88.9% | 45 | 100% |
| Oral swabs | 9 | 20% | 36 | 80% | 45 | 100% |

It is noted from the above table that oral swabs culture yeild the highest percentage of positivity for Candida 20% (9 cases out of 45 ) among different

specimens subjected to fungal culture followed by sputum and urine culture 11.1 % ( 5 cases out of 45 ) for each of them and lastly came the blood culture with the lowest percentage 8.9 % ( 4 cases out of 45 ) .

•In this study 23 Candida isolates were detected by culturing different specimens on Sabouraud's dextrose agar, then identification to the species level was achieved by germ tube test and Auxacolor test **(OXOID LTD., BASINGESTOKE, HAMP SHIRE, ENGLAND)**.

**Table (2):**The results of germ tube test .

| **Test** | **Positive** | | **Negative** | |
|---|---|---|---|---|
| **Germ tube test** | No | % | No | % |
| | 12 | 52.2% | 11 | 47.8% |

This table shows that 12 ( 52.2% ) out of 23 Candida isolates were Candida albicans and 11 ( 47.8% ) were Candida non-albicans species.

**Table (3):**The results of Auxacolor test at 24,48 and 72 hour.

| **Species** | No. of strains (%**)** | **No.of strains correctly Identified at :** | | | | | |
|---|---|---|---|---|---|---|---|
| | | 24 h | | 48h | | 72h | |
| | | No | % | No | % | No | % |
| **C.albicans** | 11(48%) | 11 | 100% | 11 | 100% | 11 | 100% |
| **C.tropicalis** | 5 (21.7%) | 3 | 60% | 5 | 100% | 5 | 100% |
| **C.glabrata** | 3 (13%) | 2 | 66.7 | 3 | 100% | 3 | 100% |
| **C.parapsilosis** | 2 (8.7%) | 0 | 0 | 1 | 50% | 2 | 100% |
| **C.krusei** | 1 (4.3%) | 1 | 100% | 1 | 100% | 1 | 100% |
| **Unidentified** | 1(4.3%) | 0 | 0 | 0 | 0 | 0 | 0 |

This table shows that Auxacolor test can identify 95.7% ( 22 out of 23 )of Candida isolates to the species level . with C.albicans is the commonest species identified in 11out of 23 cases ( 48% ) followed by C. tropicalis in 5 out of 22 isolates ( 21.7% ), C.glabrata in 3 cases ( 13% ), C.parapsilosis in 2 cases (8.7% ) and lastly came C.krusei in 1case out of 23 (4.3%) of isolates.

•Auxacolor test failed to identify one Candida isolates to the species level even after extension of the time of incubation to 72 hours.

•All C. albicans and C.krusei isolates can be correctly identified at 24 h of incubation and no change occurred at 48 & 72 hours.

•3 out of 5 isolates of C.tropicalis ( 60% ) can be correctly identified at 24 hour & the other two isolates need extension of incubation time to 48 hour.

•Also2 out of 3 C.glabrata isolates ( 66.7% )can be identified correctly at the end of the first 24 hour and the third one was detected at the end of 48 hour.

•Non of the two C.parapsilosis isolates could be identified at 24 hour & only one of them can be detected at 48 h and the other one was identified after extension of incubation time to 72 hours.

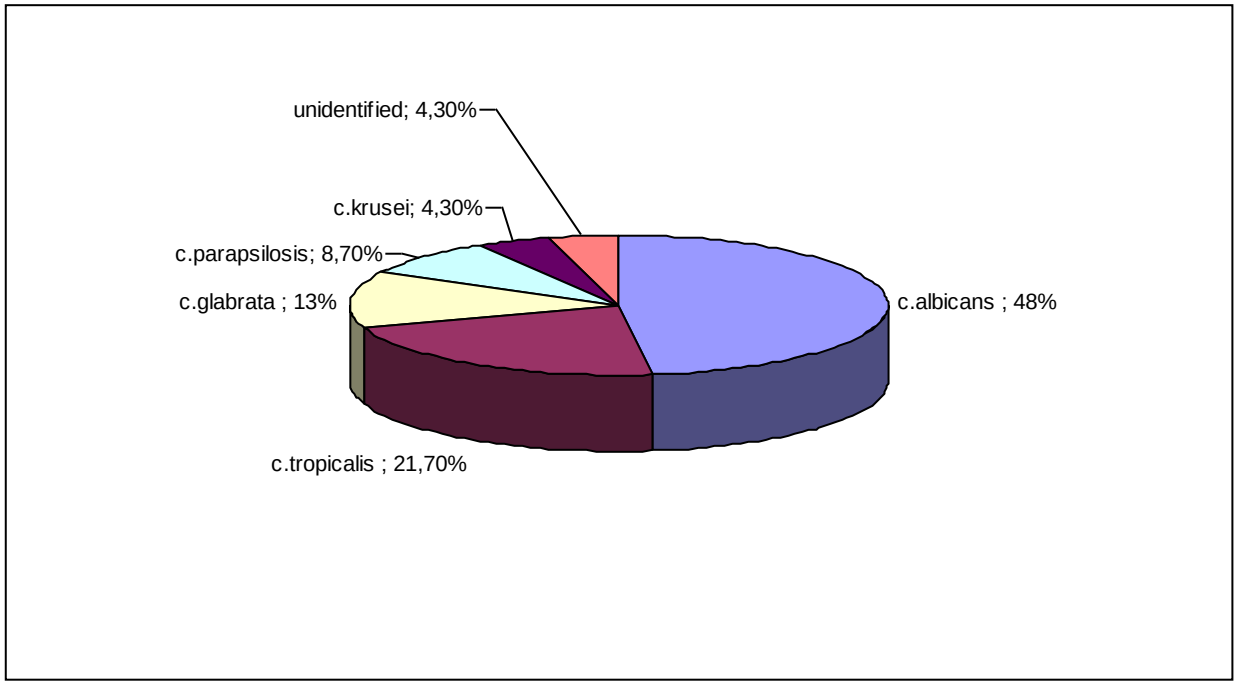


***Fig (1)*** : Pie chart: Percentage of different Candida Species identified by Auxacolor ™test

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**Table(4):**Performance of germ tube test in comparison with Auxacolor test for diagnosis of Candida albicans.

| | | Germ tube test | | |
|---|---|---|---|---|
| | | positive | negative | Total |
| **Auxacolor test** | positive | 11 | 0 | 11 |
| | negative | 1 | 11 | 12 |
| | total | 12 | 11 | 23 |
| **Chi-Square($x^2$)** | 19.32 | | | |
| **Significance(p)** | 0.00< 0.01 | | | |

P > 0.05 ( non significant difference )
P < 0.05 (significant difference )
P < 0.01( highly significant )

It is noted from the above table that there is high significant statistical difference in the performance of germ tube test in comparison with Auxacolor test for diagnosis of Candida albicans. And the agreement between the two methods was 91.7%.

**Table(5):** Specificity, Sensitivity, PPV, NPV, False+ve and False-ve results of Auxacolor test considering Germ tube test as a reference method.

| Method | Specificity | Sensitivity | PPV | NPV | False +ve | False - ve |
|---|---|---|---|---|---|---|
| Auxacolor test | 100% | 91.7% | 100% | 91.7% | 0 | 1 |

It is noted from the above table that the diagnostic validity test done for Auxacolor considering Germ tube test as a reference method shown specificity of 100% while the sensitivity was 91.7% as it failed in identification of one of Germ tube positive isolate.

**Table (6 ):** Frequency of Candida species isolated from different specimens.

| Candida species | Number | % | Type of sample | | | |
|---|---|---|---|---|---|---|
| | | | Blood | Sputum | Urine | Oral swabs |
| C.albicans | 12* | 52.2% | 2 | 2 | 2 | 6* |
| C.tropicalis | 5 | 21.7 % | 1 | 1 | 1 | 2 |
| C.glabrata | 3 | 13% | | 1 | 1 | 1 |
| C.parapsilosis | 2 | 8.7 % | | 1 | 1 | |
| C.krusei | 1 | 4.4% | 1 | | | |
| Total | 23 | 100% | 4 | 5 | 5 | 9 |

This table shows that out of 180 clinical specimens equally collected from (blood , urine, sputum and oral swabs ) 23Candida species were isolated with percentage of 12.8% .

The most common Candida species isolated from these clinical specimens was C. albicans with percentage of 52.2 % ( 12 out of 23 ) with 6 strains equally isolated from blood , sputum and urine ( 2 for each ) and 6 strains from oral swabs . Followed by C. tropicalis with percentage of 21.7 % ( 5 out of 23) . with 3 strains equally isolated from blood , sputum and urine ( one for each ) and 2 strains from oral swabs .

Also followed by C. glabrata with percentage of 13% ( 3 out of 23 ) and C.parapsilosis with percentage of 8.7% ( 2 out of 23 ) both of them were equally isolated from sputum and urine ( two for each ) . And the remaining one of C.glabrata was isolated from oral swabs.

And lastly comes C.krusei with percentage of 4.4% ( 1 out of 23 ) isolated from blood. .

*Out of Those twelve C.albicans species one of them failed to be identified by Auxacolor test as it was previously detected positive by germ tube test.

**Table (7):** Data of patients with positive blood culture .

| Patient (no) | Number of cases | Sex | Disease | Results obtained with fungal blood culture |
|---|---|---|---|---|
| 4 | 1 | M | AML | C. albicans 50% |
| | 1 | F | | |
| | 0 | M | ALL | C. tropicalis 25 % |
| | 1 | F | | |
| | 0 | M | Lymphoma | C. Krusei 25 % |
| | 1 | F | | |
| 41 | 14 | M | AML | - |
| | 20 | F | | |
| | 2 | M | ALL | |
| | 0 | F | | |
| | 4 | M | Lymphoma | |
| | 1 | F | | |

It is noted from the above table that only 4 patients out of the 45 enrolled were positive for Candida by blood culture with percentage of 8.9%. 2 of these 4 Candida isolates were Candida albicans, both were isolated from patients with AML, one was Candida tropicalis and was isolated from patients with ALL and the last one was Candida krusei and it was isolated from patient with Lymphoma.

**Table (8):** Results of Platelia™ Candida Ag For Detection of Candida Mannan Antigen in Serum By Enzyme Immunoassay.

| Test | No of examined cases | | | | | |
|---|---|---|---|---|---|---|
| | Positive | | Negative | | Total | |
| **Candida Ag detection** | No | % | No | % | No | % |
| | 13 | 28.9% | 32 | 71.1% | 45 | 100% |

The above table shows that 13 patients ( 28.9%) out of the 45 enrolled were positive by Platelia™ Candida Ag detection test. Ag detection test can detect all the blood culture positive cases and 9 of the blood culture negative cases (21.9%).

**Table ( 9):** Results of Multiplex, Real-time PCR for detection of Candida in blood.

| Test | Total cases examined | | | | | |
|---|---|---|---|---|---|---|
| | Positive | | Negative | | Total | |
| **Multiplex,Real time PCR** | No | % | No | % | No | % |
| | 14 | 31.1% | 31 | 68.9% | 45 | 100% |

It is noted from the table that Multiplex, Real time PCR was successful in detecting Candida in all positive blood culture samples and in detecting 10 positive cases out of 41 negative blood culture patients.

**Table (10) :** Different Candida species identified by Multiplex , Real time PCR .

| | **No. of Candida species identified by Real-time PCR** | |
|---|---|---|
| **Organisms** | NO | % |
| **C.albicans** | 7 | 50% |
| **C.tropicalis** | 2 | 14.3% |
| **C.glabrata** | 2 | 14.3% |
| **C.parapsilosis** | 2 | 14.3% |
| **C.krusei** | 1 | 7.1% |

It is noted from the table that C.albicans was the most commonly isolated Candida species 50% ( 7out of 14 ) followed by C.tropicalis, C.glabrata, C.parapsilosis with equal percentages of 14.3% ( 2 of 14 ) for each one of them and lastly came C.krusei with the lowest percentage 7.1% ( 1 out of 14).

**Table (11)**: Results of different methods used for diagnosis of Candidemia.

| **No of examined cases** | **Methods for diagnosis of Candidemia** | | | | | |
|---|---|---|---|---|---|---|
| **45 ( 100% )** | Blood culture | | Ag detection | | Multiplex,Real-ime PCR | |
| | No | % | No | % | No | % |
| | 4 | 8.9% | 13 | 28.9% | 14 | 31.1% |

The present study shows that only 4 cases ( 8.9% ) were diagnosed as Candidemia by the use of blood culture. This number increases to 13 ( 28.9% )

by the use of Ag detection test and further increase to 14 cases out of the examined 45 patients was by Multiplex, Real-time PCR.

**Table (12) :** Agreement between blood culture, Ag detection and multiplex real-time PCR tests used for diagnosis of Candidemia.

| Test | Agreement % | Kappa | P value |
|---|---|---|---|
| Blood culture versus Ag detection test. | 30.8% | 0.38 | 0.00< 0.01 |
| Blood culture versus multiplex, real-time PCR. | 28.6% | 0.35 | 0.00< 0.01 |
| Ag detection versus multiplex, real-time PCR | 92.9% | 0.94 | 0.00< 0.01 |

P > 0.05 ( non significant difference )
P < 0.05 (significant difference )
P > 0.01( highly significant )

| value of K | Strength of agreement |
|---|---|
| 0.2 | poor |
| o.2-0.4 | fair |
| 0.41-0.6 | moderate |
| 0.61-0.8 | good |
| 0.81-1.00 | very good |

This table shows that in the diagnosis of patients with Candidemia the agreement between the results of blood culture and Ag Detection Test of Candida mannan antigen in serum by enzyme immunoassay was 30.8% (kappa= 0.38 ), the agreement between the results of blood culture and multiplex, real-time PCR was 28.6% (kappa= 0.35 ) and the agreement between the results of Ag detection test of Candida mannan antigen in serum by enzyme immunoassay and multiplex, real-time PCR was 92.9% ( kappa= 0.94).

So it is concluded that in the diagnosis of Candidemia there was high statistical difference between blood culture and both of Ag detection test and multiplex, real-time PCR and there was high statistical difference between Ag detection test and multiplex, real-time PCR.

**Table (13):** Specificity, Sensitivity, PPV, NPV, False+ve and False-ve results of Ag detection test and blood culture test methods for detection of Candida in blood taking Multiplex,real-time PCR as gold standard.

| Method | Specificity | Sensitivity | PPV | NPV | False+ ve | False - ve |
|---|---|---|---|---|---|---|
| **Ag detection** | 100% | 92.9% | 100% | 96.9% | 0 | 1 |
| **Blood culture** | 100% | 28.6% | 100% | 75.6% | 0 | 10 |

The above table shows the diagnostic validity test done for Ag detection test and blood culture method considering Multiplex,real-time PCR as gold standard method. As shown in the table the specificity of blood culture method and Ag detection test was 100%, while the sensitivity of blood culture method was low as it failed in identification of 10 of positive cases detected by Multiplex, real-time PCR.

**Table (14):** Time taken by each method to detect Candidemia.

| | Method | | |
|---|---|---|---|
| **Time taken by each method** | Blood culture | Ag detection | Multiplex,Real-time PCR |
| | 3-5 days up to 2 wks. | 3-4 hour | Less than 60 minutes after extraction procedures. |

It is noted from the above table that blood culture takes the most time to detect Candidemia,while Multiplex,real-time PCR takes the least time after extraction Procedures. .