Int.J.Curr.Microbiol.App.Sci (2023) *12*(03): 73-80

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 12 Number 3 (2023)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2023.1203.010

Antifungal Susceptibility Pattern of Candida Species Isolated from Sputum Samples in a Tertiary Care Hospital, Salem

R. Janani Anukraha[1] and Rajesh Sengodan[2*]

[1]Government Mohan Kumaramangalm Medical College, Salem, Tamil Nadu, India
[2]Department of Microbiology, GMKMCH, Salem, Tamil Nadu, India

**Corresponding author*

Keywords

Normal skin, oropharyngeal, mucosal membranes, respiratory specimens

Article Info

Received:
02 February 2023
Accepted:
27 February 2023
Available Online:
10 March 2023

A B S T R A C T

Candida species are by far, the most common fungal pathogens in humans. Recovery of *Candida* species from the respiratory tract usually indicates commensalism/colonisation rather than infection. However this study aims at the distribution and antifungal susceptibility pattern of different Candida species isolated form colonised and infected patients with respiratory tract infections. Methods: This prospective study was conducted at a tertiary care hospital in Salem for 6 months. A total of 200 sputum samples were examined. Species identification and antifungal susceptibility testing was performed using disc diffusion method according to CLSI guidelines. Results: Out of 200 samples, 27 samples were found to have *Candida* spp of which *Candida albicans* 11 (41%) was the most common isolate followed by *Candida tropicalis* (25.90%), *Candida parapsilosis* (18.50%), *Candida glabarata* (11.11%) and *Candida krusei* (3.7%). Candida isolates showed more susceptibility to Amphotericin B (100%) & Voriconazole (100%) followed by Itraconazole (63%) and Fluconazole (62%). They also revealed higher resistance to Ketoconazole (46%). Conclusion: In hospitalised patients, Candida infection is a significant problem and resistance to commonly used antifungal agents are increasing. So the necessity of accurate and rapid identification of Candida species and its antifungal susceptibility pattern lead to better treatment because delay can result in increased mortality and morbidity in the patients.

Introduction

Candida spp. is part of the normal skin, oropharyngeal, mucosal membranes and upper respiratory tract flora. *Candida* spp. can reach the lungs through either haematogenous dissemination or aspiration of colonised oropharyngeal or gastric contents (Murray *et al.*, 1977). The isolation of *Candida* spp. from respiratory tract secretionsis frequent in nonimmunocompromised, mechanically ventilated patients. *Candida* spp. have historically been considered commensal constituents of normal human oral microbiota, with little significance attached to their detection in respiratory specimens.

Yet in recent years, both animal experimentation and human observations have provided provocative evidence that *Candida* spp. may represent more than

an innocent bystander, both in infectious and non-infectious disease states (Kathryn *et al.*, 2017).

Candida species (spp.) are, by far, the most common fungal pathogens in humans. Of the 8%–10% of all nosocomial infections caused by fungal pathogens, 80% are attributable to *Candida* (Edwards *et al.*, 1991).

Approximately, 20 *Candida* species have been associated with causing Candidiosis in humans. The species most frequently isolated from humans and the causative agent of the majority of infections is, however, *Candida albicans* (Williams *et al.*, 2013).

Candida pneumonia is a rare lung infection with a high morbidity and mortality, commonly observed as part of a disseminated *Candida* infection and associated with predisposing clinical circumstances (i.e. long-termantibiotic use, haematologic malignancy or severe immunosuppressive states) (Silvia Terraneo and Miquel Ferrer, 2016)

Regardless of sampling site, no single test or threshold distinguishes whether the yeast is an artifact of sampling (contamination), benign and native to the patient's microbiota (commensalism), residing in a body site without causing active infection (colonization) or etiologic in an acute infection (candidiasis). Instead, the detection of *Candida* must always be interpreted within its clinical and microbiological context. (Kathryn *et al.*, 2017).

Candida albicans which is the most abundant and clinically significant representative of the *Candida* genus has a variety of microbiological traits that equip it with adaptability to colonize the mucosa alongside the bacteria and exist in a 'commensal' or mutualistic state or to become pathogenic and invasive during disease. The presence and behaviour of bacterial microbiota are believed to be key in determining *Candida*'s relative virulence.

Mucosal-associated bacteria prevent overgrowth of *Candida* via competition for epithelial cell adhesion sites, metabolic inference of hyphal transformation and induction of antimicrobial mechanisms (Huffnagle and Noverr, 2013). In the absence of indigenous bacterial microbiota, *Candida* is able to increase in number, infect and invade epithelial surface.

Therefore in this study, the distribution and antifungal susceptibility pattern of various *Candida* species isolated form colonised and infected patients with respiratory tract infections admitted in Tertiary care hospital were studied using the Clinical and Laboratory Standards Institute.

The focus of this study is to isolate *Candida* spp from sputum sample and to determine Antifungal susceptibility pattern by disc diffusion method.

Materials and Methods

Study population- Male and female patients with respiratory tract infections

Place of study – Government Mohan Kumaramangalam medical College hospital, SALEM

Period – 6 months (August 2022 to January 2023)

Study sample – Sputum sample

Inclusion Criteria

Male and female patients with above 18 years of age with respiratory tract infections.

Exclusion Criteria

Children and Patients below 18 years of age.

The present study is a prospective study carried out during 6 months period from August 2022 to January 2023 in patients with respiratory tract infection in Government Mohan Kumaramangalam medical College hospital, Salem.

Specimen collection and transport

Primary specimen Sputum

Spontaneous

Early morning specimen generated after a bout of cough. Having the patient brush his or her teeth and gargle with water immediately before obtaining the sputum specimen reduces the number of contaminating oropharyngeal bacteria. Collect specimen resulting from deep cough in a sterile screw-cap cup or other suitable sterile collection assembly of about 100 ml capacity.

To prevent contamination of the outside of the container, the patient should be instructed to press the rim of the container under the lower lip to catch the entire expectorated cough sample. Tightly screw on the cap of the container. Wipe off any spilled material on its outside with a tissue moistened with disinfectant, but take care not to let any disinfectant enter the container.

Such communication with patients can be rewarding. In addition, patients should remove dentures during the specimen collection. Early-morning sputum samples should be obtained because they contain pooled overnight secretions in which pathogenic bacteria are more likely to be concentrated.

Twenty four hour collections should be discouraged. Deliver the specimen to the laboratory as quickly as possible, preferably within 2 hours, as delicate pathogens may die out during longer delay.

Endotracheal aspirate (ETA)

Endotracheal aspiration is done with a sterile technique using a 22 inch, 12F suction catheter. The catheter is introduced through the endotracheal tube for at least 30 cm. Gentle aspiration is then performed without instilling saline solution. The first aspirate is discarded. The second aspirate is collected after tracheal instillation of 5 ml saline in a mucus collection tube. The specimens are sent to the laboratory and cultured within 1 hour of collection.

Rejection Criteria

Reject duplicate specimens received on the same day unless the initial sample was inappropriate for culture according to microscopic evaluation. Do not accept repeat cultures at intervals of less than every 48 hours. Reject the following specimens for diagnosis of lower respiratory tract disease:

24 hours sputum collection

Contaminated sputum and endotracheal specimens as per Gram stain rejection criteria

Specimens that are visually saliva only

Specimens that are visibly contaminated with toothpaste or other substances

Nasal washes or swabs of nares to diagnose sinusitis

Sputum samples are highly contaminated with normal anaerobic flora of the upper respiratory tract.

The preliminary diagnoses of specimens were performed by wet mount, Gram stain, culture on Sabouraud dextrose agar (SDA). The isolates diagnosed to be fungus other than *Candida* species were excepted from the study. For the clinical significance of *Candida* isolates from sputum, the specimens were analysed by microscopy as well for the evidence of budding yeast cell with pseudohyphae along with significant pus cells (CLSI, 2006; Yucesoy *et al.*, 2001). All samples were inoculated on Sabouraud dextrose agar (SDA) and aerobically incubated at 37 °C for 24–48 h. Any visible growth seen on SDA slope was processed for identification of the species. From an isolated colony, macroscopic examination, Gram staining, germ tube test and urea hydrolysis test was performed. The yeasty, pasty and creamy colony that showed Gram positive budding yeast cells with pseudohyphae on microscopic examination were

further processed for *Candida* speciation on CHROM agar. *Candida* species were differentiated based on type of the growth and colour of isolates on CHROM agar *Candida* (HiMedia, Mumbai, India) (Yucesoy *et al.,* 2001; Murray *et al.,* 2005). After incubation at 37 °C for 24–48 h, colour of colonies was observed on CHROM agar (*C. albicans*—green, *C. glabrata*— purple, *Candida krusei*—pink, fuzzy and C. tropicalis—blue purple, *C. parapsilosis*- mauve. Antifungal susceptibility testing was performed and interpreted for all the isolates of *Candida* using disc diffusion method as recommended by Clinical and Laboratory Standards Institute (CLSI) M44-A document guidelines (CLSI, 2006).

The inoculum was prepared by suspending five colonies of growth in 5 ml of sterile saline and compared the turbidity to 0.5 McFarland Standard. A cotton swab was dipped into the inoculum suspension and evenly streaked onto Mueller–Hinton agar supplemented with 2% glucose and 5 µg/ml methylene blue (Ishan Pandita and Wyawahare; Kaur *et al.,* 2016). *C. albicans* ATCC 90028, *C. tropicalis* ATCC 750 were used as controls. Antifungal discs containing fluconazole (10 µg), ketoconazole (10 µg), Amphotericin B(20µg), Voriconazole(1µg), Itraconazole (10µg) were placed on the inoculated media. Zone of inhibition around the disc was measured after incubating the media at 37 °C for 24 h (Lee *et al.,* 2001; Pfaller *et al.,* 2004).

Results and Discussion

A total of 200 sputum samples were obtained from various wards and were examined of which 27 samples were found to be positive for yeast cells with pseudohyphae on doing gram stained smears and on wet mount preparation.

Age-wise distribution

11 cases were of age group more than 60years, 10 were of age group 40-60 years and 6 cases were of age group 18-40 years.

Sex wise distribution

15 cases were male and 12 cases were female patients.

Germ tube production

11 isolates out of 27 isolates shows formation of germ tube on germ tube test.

That is isolation rate of Non-*Candida* albicans was higher as compared to *Candida albicans*. Then all the *Candida* isolates were inoculated onto CHROM agar and growth and colour change were observed.

Candida species are emerging as a potentially pathogenic fungus in patients with broncho-pulmonary diseases. *Candida* infections are on rise worldwide due to various factors like immunosuppression, uncontrolled antibiotic usage, increase in transplant etc (Ishan Pandita and Wyawahare *et al.,*). Due to this emergence of disease, it is important to isolate and speciate the *Candida* species from sputum samples and to identify their antifungal susceptibility pattern. In our study, among the 27 *Candida* spp isolated from the sputum, 11 isolates produced germ tube indicating *Candida albicans* and 16 isolates did notproduced germ tube indicating non *Candida albicans. Candida albicans* (40.74%) was the most common isolate followed by *Candida tropicalis* (25.90%), *Candida parapsilosis* (18.50%), *Candida glabarata* (11.11%) and *Candida krusei* (3.7%) that is consistent with study of Kaur *et al.,* (2016).

Among the non *Candida albicans, Candida tropicalis* was the most common isolated species that is consistent with study of Kaur *et al.,* (2016). On demographic distribution, about 41% of *Candida* were isolated form >60 years of age and 55% of isolates were from male patients. Our study showed *Candida* species were found to be more susceptible to Amphotericin B (100%) and Voriconazole (100%) followed by Itraconazole (63%), Fluconazole (62%), 46% of isolates were resistant to Ketoconazole(46%). In this present study 29% of

total isolates were found to be resistant to Fluconazole by disc diffusion method. Highest rate of resistance was found in *C.glabarata* (33%) followed by *C.tropicalis* (29%) and *C.parapsilosis* (20%) which is consistent with Khadka *et al.*, (2017) study.

Table.1

***Candida* isolates**	**No. of isolates**	**Percentage**
***Candida* albicans**	11	41.00%
Non *Candida* albicans	16	59.00%
Total	27	100%

Table.2

Colony appearance	**Species**	**Number of isolates**	**Percentage**
Green	Candida albicans	11	40.74%
Blue purple	Candida tropicalis	7	25.90%
Mauve	Candida parapsilosis	5	18.50%
Purple	Candida glabarata	3	11.11%
Pink	Candida krusei	1	3.7%

Fig.1

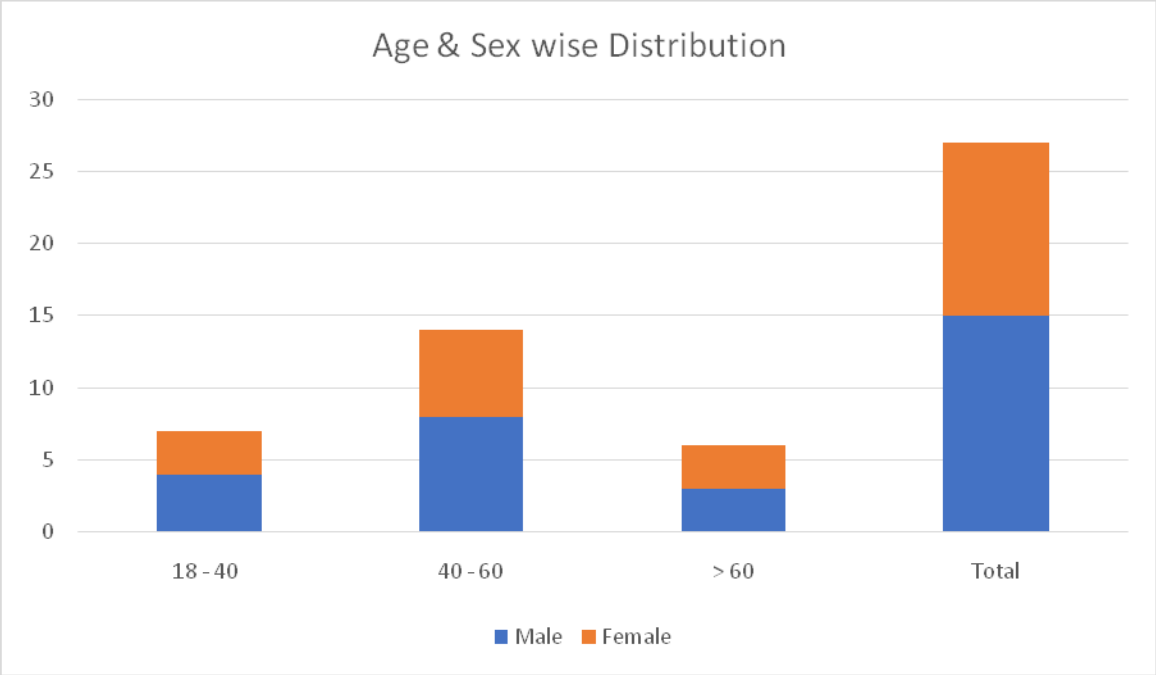


Fig.2

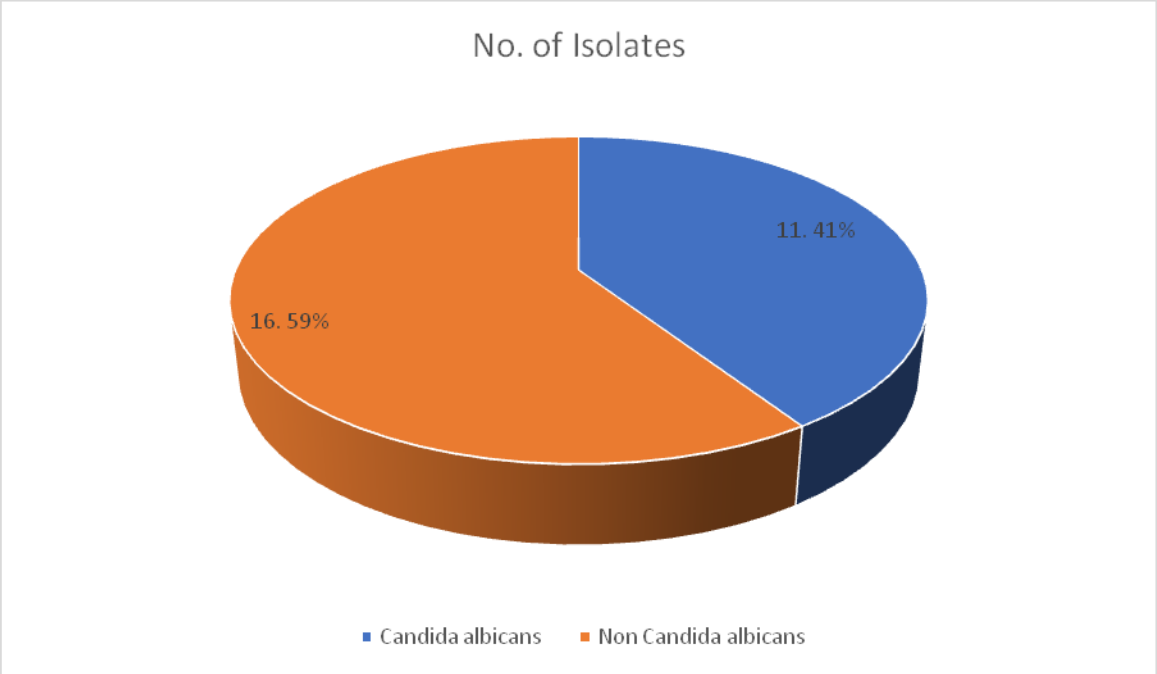

No. of Isolates
11. 41%
16. 59%
Candida albicans
Non Candida albicans

Fig.3

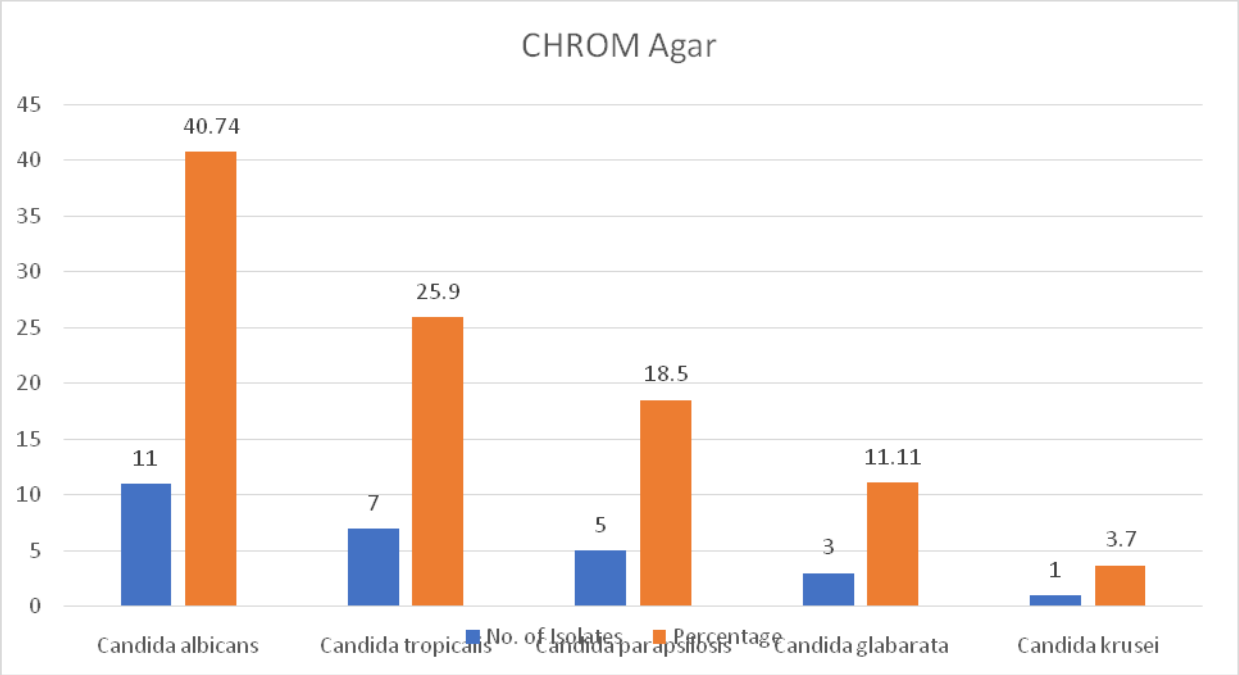
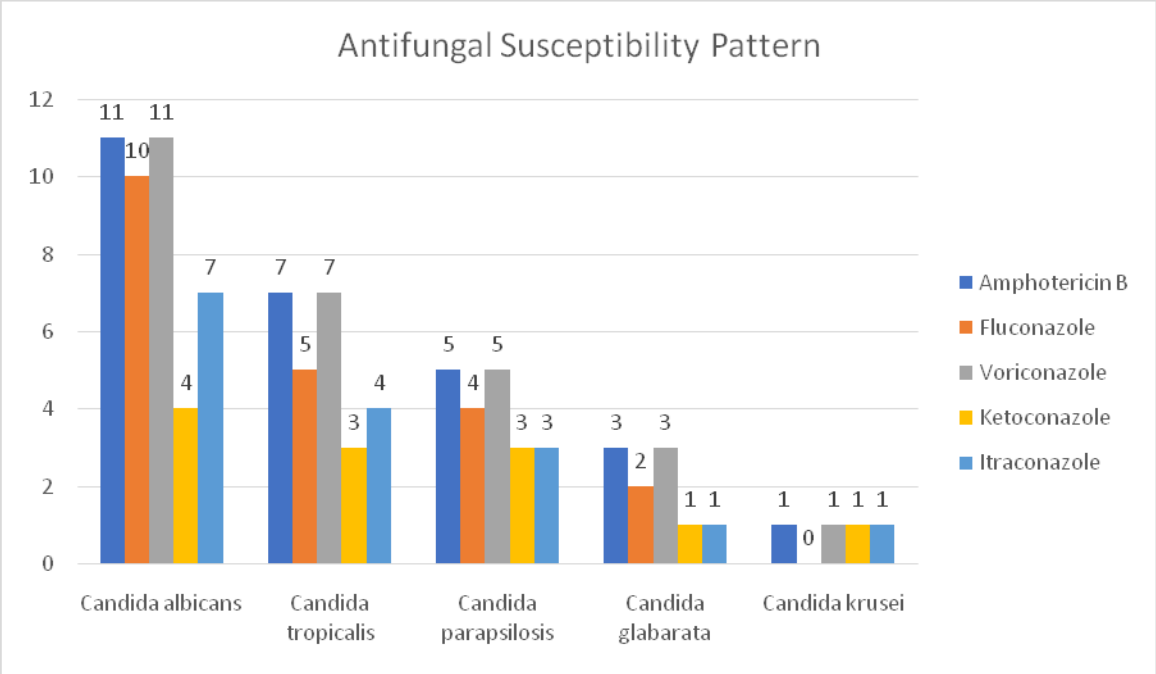

CHROM Agar
45
40
35
30
25
20
15
10
5
0
40.74
25.9
18.5
11.11
11
7
5
3
1
3.7
No. of Isolates
Percentage
Candida albicans
Candida tropicalis
Candida parapsilosis
Candida glabarata
Candida krusei

Table.3 Antifungal Susceptibility Pattern

Isolates	Amphotericin B	Fluconazole	Voriconazole	Ketoconazole	Itraconazole
Candida albicans	11(100%)	10(91%)	11(100%)	4(36.3%)	7(63.6%)
Candida tropicalis	7(100%)	5(71.4%)	7(100%)	3(42.8%)	4(57.1%)
Candida parapsilosis	5(100%)	4(80%)	5(100%)	3(60%)	3(60%)
Candida glabarata	3(100%)	2(66.6%)	3(100%)	1(33.3%)	1(33.3%)
Candida krusei	1(100%)	0	1(100%)	1(100%)	1(100%)

Fig.4



This study also reveals high rate of resistant to Ketoconazole (46%) with highest rate in *C.glabarat* (66%) and *C.albicans* (64%) followed by *C.tropicalis* (58%), *C.parapsilosis* (40%). This may be due to overuse of antifungal agents or due to empirical therapy (Khadka, 2017). The above findings suggest that there is the rapid increase in resistant for ketoconazole among *Candida* species and need of speciation and antifungal susceptibility before starting antifungal treatment.

In hospitalised patients, *Candida* infection is a significant problem worldwide. Resistance to commonly used antifungal agents are increasing among *Candida* species. These problems contribute to the necessity of accurate and rapid identification of *Candida* species better treatment because delay can result in increased mortality and morbidity in the patients. Based on the results of our study, routiene species identification of *Candida* isolates and detection of resistant strains by antifungal susceptibility test is essential. Also to monitor the changes and antifungal susceptibility, there should be continuous surveillance.

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How to cite this article:

Janani Anukraha, R. and Rajesh Sengodan. 2023. Antifungal Susceptibility Pattern of *Candida* Species Isolated from Sputum Samples in A Tertiary Care Hospital, Salem. *Int.J.Curr.Microbiol.App.Sci.* 12(03): 73-80. **doi:** https://doi.org/10.20546/ijcmas.2023.1203.010