

RESEARCH ARTICLE

Co-existence of Candida Species with other Bacterial Infections in a Tertiary Care Hospital

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Article History

Received:08-10-2025

Revised:21-10-2025

Accepted:12-11-2025

Published: 01-11-2025

Abstract:

Introduction: Human body harbours numerous microbes on all the mucosal epithelial surfaces. The interactions could be direct or indirect through host response with effects on disease and host health. The microbial interaction patterns could be positive, negative or neutral.

Candida albicans, an opportunistic fungal commensal colonizes mucosal surfaces in the humans. Various bacterial species are found as commensals in the human host. These fungal and bacterial commensals co-exist in several niches of the host. Under the influence of external and internal host factors these microbial communities undergo polymicrobial interactions that result in varied host outcomes. Bacterial and fungal interactions in the human host are inevitable and its consequences are widely studied. **Objectives:** To identify the co-existence of *Candida* species with other bacterial infections, To assess the antimicrobial and antifungal resistance pattern of the identified bacterial and fungal organisms **Methods:** A descriptive study method was done. All culture samples of 1067 received in our microbiology laboratory for a period of two months was included, Microbiology department, Tertiary care hospital. The samples included sputum, urine, pus, blood cultures, body fluids and vaginal swabs. Samples were inoculated onto the following agar plates to identify the growth of pathogenic bacteria: chocolate agar, blood agar, Cystine-Lactose-Electrolyte-Deficient agar, brain heart infusion agar and MacConkey agar. The plates were incubated for 2 days at 37 °C. The colony morphology and the growth characteristics of the microorganisms detected on agar plates were observed. Gram staining and all possible basic biochemical tests were done to speciate the organism. To detect the growth of yeasts the samples were inoculated onto two selective agars to promote the growth of *Candida* spp.: Saborauds dextrose and chromogenic yeast agar (CHROMagar), which were incubated at 37 °C for 2-3 days. The growth was observed on the plates and colony morphology was noted. Gram staining was performed. If the yeast cells were seen then the identification was carried out. **Conclusion:** *Candida* organisms are observed to frequently demonstrate asymptomatic colonization. *Candida albicans* was the commonest pathogen isolated among the *Candida* species. *Acinetobacter baumannii* and *Escherichia coli* were the bacterial species that coexisted with *Candida albicans* in the two samples that showed co-infection. As the incidence of these polymicrobial infections increases, newer antifungal drugs and their pharmacodynamic properties should be explored. Long term study is recommended to identify the incidence of *Candida* and bacterial co-infections in our catchment area which might suggest the need for high index of surveillance and early recognition of these polymicrobial infections.

Keywords: *Candida albicans*, polymicrobial infections, *Candida* spp.

INTRODUCTION

Human body harbors numerous microbes on all the mucosal epithelial surfaces. The microbiota is influenced by various external and internal host factors and the cross-kingdom interaction between the microbiota are inevitable.⁽¹⁾ Although initially considered to be irrelevant, improved diagnostics and more studies have unfolded our understanding of the dynamics in the bacterial-fungal interactions (BFI). The interactions could be direct or indirect through host response with effects on disease and host health. The microbial interaction patterns could be positive, negative or neutral.⁽²⁾

One of the most often found fungi in humans, *Candida albicans*, colonizes mucosal surfaces. When the host's immune system is compromised, they can transform from commensals into opportunistic pathogens. Beneficial mutualistic connections are exemplified by

the coaggregation of *Candida albicans* and oral microorganisms. Initial trends seem to be synergistic, despite the fact that the interaction between *Staphylococcus aureus* and *Candida albicans* is still poorly understood. On the other hand, *Pseudomonas aeruginosa* and *Candida albicans* interact in a way that shows both hostile and competitive connections. Numerous studies have proposed that *Pseudomonas* sp. inhibits *Candida* sp. growth, which explains why *Candida* sp. is absent from burn wounds where *Pseudomonas* sp. is present. It's uncertain if elements like host immune status, systemic antibacterial therapy or exposure to nosocomial infections put a patient at risk of being colonized by both fungi and bacteria or not. Bacterial-fungal interactions are frequently encountered in immunocompromised individuals.⁽³⁾ Attached to natural or abiotic solid surfaces, microbial

biofilms are organized microbial communities made up of either a single microbial species or a combination of bacterial and fungal species. Antibiotic resistance linked to biofilms is a major health concern. According to research on drug susceptibility, fungal cells have the ability to alter the activity of antifungal agents in biofilms as well as the activity of antibacterial and staphylococci agents.⁽⁴⁾ Using culture-based techniques, microbiota analysis was carried out at the molecular level. More assurance of the bacterial associates' long-term affiliation with the hosts is provided by the culture isolation of the associates from the fungal hosts. Understanding the attachment mechanism and signaling that occur during interactions between fungi and bacteria is essential for developing therapeutic techniques that would reduce the occurrence of polymicrobial infections. Awareness of these microbial interactions will help in manipulating the microbiota for disease prevention and treatment.

Polymicrobial infections and associated microbiota justifies the evolving trends of pathogen virulence and multidrug resistance patterns. Multiple bacterial associates are commonly encountered in diverse fungal isolates.⁽¹⁾ Aaron J Robinson *et al.*, showed fungi to harbor multiple bacterial associates, the diverse bacterial-fungal associations and likely complexity of the fungal bacteriome.⁽⁵⁾ Bacteria are often encountered with *Candida* species in polymicrobial biofilms *in vivo*. Shirliff *et al.*, has detailed the cross-kingdom interactions between *Candida albicans* and other bacteria.⁽³⁾ The nature of their interaction provides the basis for understanding their co-existence in the host. These polymicrobial infections had significant implications on disease management as they alter the disease course.

Numerous studies on microbial interactions and their effects have been conducted by Kruger *et al.*⁽¹⁾ These patterns of contact are influenced by the host environment and different areas of the human body. The microbiota behaves like a commensal in an environment with high microbial diversity, while the commensals turn pathogenic in an environment with limited diversity. The pathogenicity of the microbiota is changed by low diversity dysbiosis. In the human body, the results of microbial interactions differ depending on the niche. Tshikantwa and colleagues investigated the possible uses of microbial interactions.⁽²⁾ They categorized the interactions between microorganisms and explained some special interactions, such as the antifungal mechanism of 5-methyl-phenazine-1-carboxylic acid (5MPCA). They discussed elements such as pH, temperature fluctuations, oxygen concentrations, salt concentrations, and certain nutrients that have been demonstrated to affect the interactions, synthesis, structure, and composition of microorganisms.

Candida albicans enhances biofilm formation of *Pseudomonas aeruginosa* and increases macrophage response. *Candida* was identified to increase biofilm thickness through matrix-protein induction on the preformed *Pseudomonas* biofilms. Biofilm ECM

(extracellular matrix) of *Pseudomonas* with *Candida* was more prominent and it was achieved through increased alginate producing genes. ECM production of mixed organism's biofilm was inhibited by L-cysteine and that could be helpful for biofilm prevention and reduction of catheter related sepsis.⁽⁴⁾

OBJECTIVES OF THE STUDY:

1. To identify the co-existence of *Candida* species with other bacterial infections
2. To assess the antimicrobial and antifungal resistance pattern of the identified bacterial and fungal organisms.

METHODOLOGY

Type of study : Descriptive study

Study design : Observational study

Study population : All culture samples received in our microbiology laboratory were included.

Study setting : Microbiology department, Tertiary care hospital **Study period** : 2 months (August 2022 and September 2022) **Sample size** : 1067 samples.

Selection criteria:

Inclusion criteria - All culture samples received in our microbiological laboratory during the months of August and September were included in our study.

Sample processing:

All the culture samples sent to our department were included in the study. The samples included sputum, urine, pus, blood cultures, body fluids and vaginal swabs. Samples were inoculated onto the following agar plates to identify the growth of pathogenic bacteria: chocolate agar, blood agar, Cystine-Lactose-Electrolyte-Deficient agar, brain heart infusion agar and MacConkey agar. The plates were incubated for 2 days at 37 °C. The agar plates mentioned above are standard medias used for the detection of pathogenic bacteria in our laboratory. The colony morphology and the growth characteristics of the microorganisms detected on agar plates were observed. The colonies from the blood agar plates were assessed by Gram staining. Gram negative organisms were further analyzed by biochemical tests like oxidase, catalase, indole, methyl red, Voges-Proskauer, citrate, urease, triple sugar iron, nitrate reduction, sugar fermentation and oxidative fermentative test. All possible basic biochemical tests were done to speciate the organism. If it was a gram positive organism, we did catalase, slide and tube coagulase, Hugh and Leifson oxidative fermentative test, urease, and mannitol salt agar for *Staphylococcus* growth. For *Streptococcus pneumoniae*, catalase, optochin test, Gram staining and bile solubility was performed. Antibiotic sensitivity testing was done by disc diffusion method as per CLSI guidelines M100 2022 edition.

To detect the growth of yeasts the samples were inoculated onto two selective agars to promote the growth of *Candida* spp.: Sabouraud's dextrose and chromogenic yeast agar (CHROMagar), which were

incubated at 37 °C for 2-3 days. The growth was observed on the plates and colony morphology was noted. Gram staining was performed. If the yeast cells were seen then the identification was carried out. In addition, all the colonies that are grown on Sabouraud's dextrose and CHROMagar were analyzed further by lactophenol cotton blue, KOH wet mount, Calcoflour white staining, germ tube test, chlamyospore production in corn meal agar, sugar assimilation, sugar fermentation and other tests for the identification of *Candida* spp. Germ tube test was performed in normal human serum. Yeast colonies were inoculated in 0.5 ml of pooled human serum and incubated at 37°C for 2-4 hrs. The suspension was observed under microscope for the presence of germ tubes. Positive control (*Candida albicans*) and negative control (*Candida tropicalis*) was used. Urease test was done. Corn meal agar (Dalmau culture plate technique) was incubated for 2-3 days at 25° C to demonstrate chlamyospores. In chrome agar we identified yeast species in 24 hrs. For sugar fermentation tests for yeast, glucose, maltose, sucrose and lactose was used. The carbohydrate broth with Durham's tube was inoculated with 0.1 ml of yeast inoculum and incubated at 25°C for a week. The tubes were examined every 48-72 hrs interval for production of acid and gas. Yeast nitrogen base was used for sugar assimilation and all the basic and special sugars discs commercially purchased in our lab were used. Antifungal susceptibility testing (AFST) was performed by disc diffusion as per CLSI guidelines M44/A2 and ICMR SOP 2nd edition 2019.^(6,7) We inoculated 4-5 yeast colonies in a test tube with 5 ml of normal saline. The inoculum is compared with 0.5 McFarland turbidity standard and a lawn culture with sterile cotton swab on AFST media (Mueller Hinton+ GMB agar) was done. The procedure was repeated twice by rotating the plate 60° each time and finally the rim was swabbed. Antifungal disk was placed and the plates were incubated at 37°C for 24 hrs. The plates were observed for zone of inhibition and sensitivity pattern to antifungals. The recommended quality control strains used were *Candida albicans* ATCC 90028. *Candida albicans* was tested for fluconazole, voriconazole, and capsosfungin.

Data collection procedures & instruments used: Data was collected appropriately and entered in excel format and analysed.

Confidentiality: Patient's confidentiality was maintained throughout the study period.

Informed consent: Not applicable.

Statistical methods: Data was entered in Microsoft Excel software in codes & analysis was done with SPSS-23 Computer package under statistician guidance.

Statistical variables:

Qualitative – frequency; Quantitative – mean (SD)

Gender

Male: 796 (75%)

Female: 271 (25%)

Mean age = 55.3 ± 45.5

Positive growth = 219

Negative growth = 848

Ethical consideration: Study was conducted only after getting prior consent from Scientific Research Committee and Institutional Ethics Committee of the college (Ref.no: 01/SVMCH/IEC-Cert/June22, dated 19/07/2022). This study was performed in microbiological laboratory and did not include human participants. The clinical samples underwent standard microbiological analysis. No additional samples were collected for this study.

OBSERVATION AND RESULTS

Of the 1067 samples collected in our laboratory, organisms were grown in 219 samples. The samples ranged from urine, pus, blood, sputum, body fluids to vaginal swabs. Bacteria alone was grown in 217 samples. Bacteria and *Candida albicans* were isolated in 2 samples. *Acinetobacter baumannii* (0.4%) and *Escherichia coli* (0.4%) were the bacterial species grown in this co- infection. *Klebsiella pneumoniae* (15.9%), *Acinetobacter baumannii* (8.6%), *Escherichia coli* (17.8%), *Pseudomonas aeruginosa* (16.8%), *Enterococcus faecalis* (6.4%), *Citrobacter freundii* (6.8%), *Proteus mirabilis* (9.1%), *Staphylococcus aureus* (13.6%) and *Streptococcus pneumoniae* (3.6%) were grown as pure bacterial cultures. *Staphylococcus* showed catalase and coagulase positive reaction. In mannitol salt agar it appeared as yellow colonies due to mannitol fermentation. Phosphatase test was positive and urea was hydrolysed. Antibiotic sensitivity testing was done as per CLSI guidelines M100 2022 edition.⁽⁸⁾

Gram staining and lactophenol cotton blue mount showed yeast cells. The yeast cells appeared as bluish white in colour under fluorescent microscope following Calcofluor white staining. The germ tubes are seen as long tube like projections extending from the yeast cells without any constriction as seen in pseudohyphae. Germ tube test was positive. On cornmeal agar, large thick walled chlamyospores were demonstrated. CHROMagar showed bluish green colonies which confirmed growth of *Candida albicans*. Urea was hydrolysed. *Candida albicans* fermented glucose and maltose with gas production. In *Candida albicans*, sugar assimilation was observed in glucose, maltose, sucrose, lactose, galactose, trehalose and in xylose. Presence of growth around the sugar discs was considered as positive. Sugar assimilation was negative in raffinose. Antifungal susceptibility testing (AFST) was performed by disc diffusion as per CLSI guidelines M44/ A2.⁽⁶⁾ *Candida albicans* showed sensitivity pattern to fluconazole, voriconazole and capsosfungin.

In the samples that had coexisting fungal and bacterial microbiota, *Acinetobacter baumannii* was isolated in sputum from a patient with respiratory tract infection. It appeared as gram negative coccobacilli in gram staining, and was catalase positive, oxidase negative, non-motile, indole negative, methyl red positive, voges proskauer negative and citrate positive. It showed no hemolysis on blood agar, no H₂S on triple sugar iron agar, nitrate negative and non-fermenter. It showed high sensitivity to cefoperazone, imipenem and gentamicin,

and intermediate sensitivity to Amoxicillin/Clavulanic acid. There was no resistance pattern observed. *Escherichia coli* isolated from pus in a diabetic foot patient. It appeared as gram negative bacilli in gram staining. It demonstrated motility and was oxidase negative, catalase positive, indole and methyl red positive and voges proskauer and citrate negative. It produced hemolysis on blood agar and lactose fermenting colonies on MacConkey agar. Urea was not hydrolysed, and triple sugar iron showed A/A with acid and gas production, no H₂S and all sugars were fermented, except sucrose.

Nitrate was reduced and phenyl alanine deaminase was negative. It showed sensitivity to cefipime, imipenem and amikacin, and intermediate sensitivity to gentamicin. It was resistant to cefotaxime. *Candida albicans* grown from this co- infection samples showed sensitivity to fluconazole, voriconazole and capsosungin.

Graph-1: Total number of samples received for bacterial culture in August and September 2022

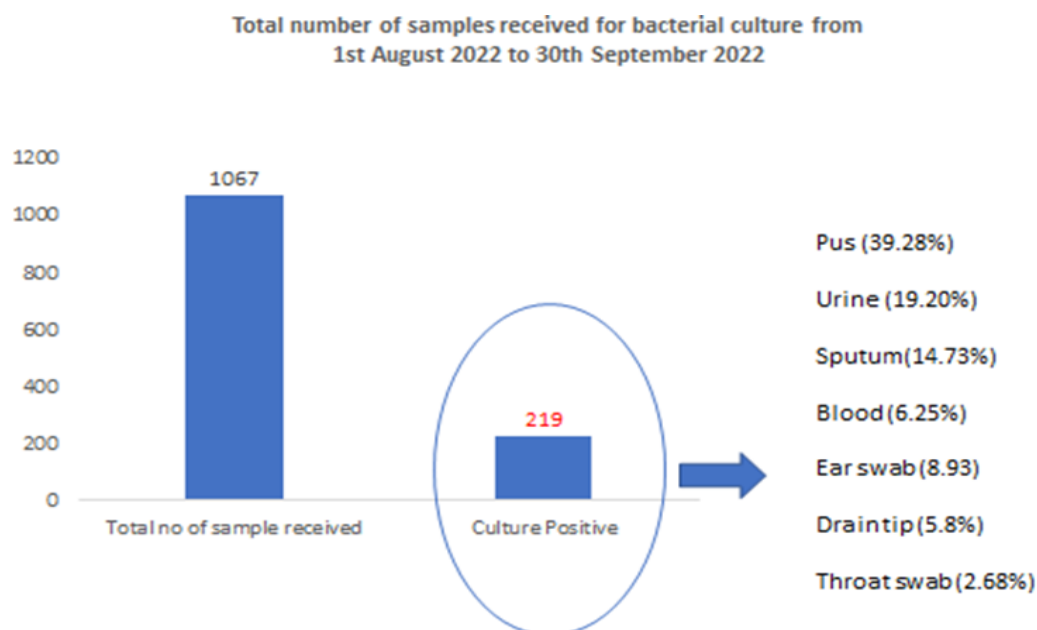


Table-1: Number of isolates identified from August 2022 to September 2022

Organism	Number of isolates	Percentage (%)
<i>Acinetobacter baumannii</i>	19	8.6
<i>Citrobacter freundii</i>	15	6.8
<i>Enterococcus faecalis</i>	14	6.4
<i>Escherichia coli</i>	39	17.8
<i>Klebsiella pneumoniae</i> ss. <i>pneumoniae</i>	35	15.9
<i>Proteus mirabilis</i>	20	9.1
<i>Pseudomonas aeruginosa</i>	37	16.8
<i>Staphylococcus aureus</i> ss. <i>aureus</i>	30	13.6
<i>Streptococcus pneumoniae</i>	8	3.6
<i>Acinetobacter baumannii</i> + <i>Candida albicans</i>	1	0.4
<i>Escherichia coli</i> + <i>Candida albicans</i>	1	0.4
Total	219	—

Graph-2: Percentage of isolated *Candida* species along with bacteria

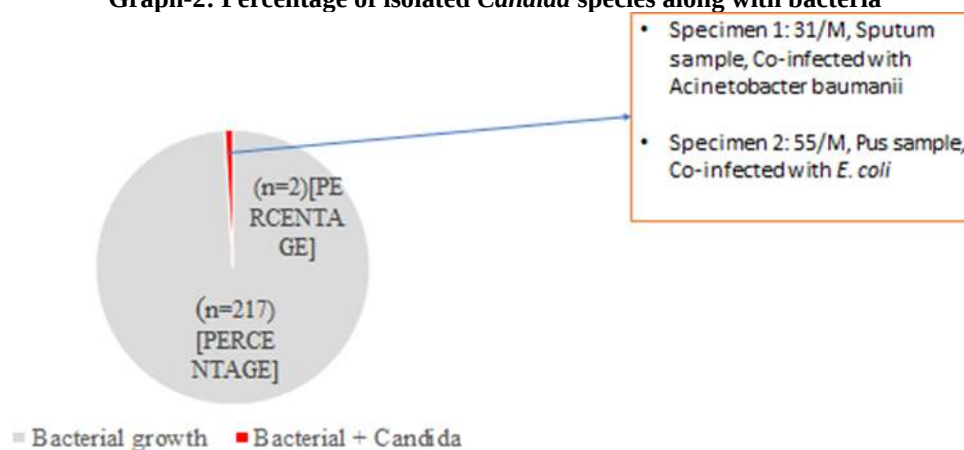


Table-2: Sensitivity, Intermediate and Resistant pattern of *Acinetobacter baumannii* associated with *Candida albicans*

Antibiotic name	Antibiotic class	Antibiotic subclass	%R	%I	%S
Cefoperazone	Cephems	Cephalosporin III	0.0	0.0	100.0
Imipenem	Penems	Carbapenems	0.0	0.0	100.0
Gentamicin	Aminoglycosides	—	0.0	0.0	100.0
Amoxicillin/Clavulanic acid	Beta-lactam + Inhibitors	—	0.0	100	0.0

Table-3: Sensitivity and Resistant pattern of *Escherichia coli* associated with *Candida albicans*

Antibiotic name	Antibiotic class	Antibiotic subclass	%R	%I	%S
Imipenem	Penems	Carbapenems	0.0	0.0	100.0
Amikacin	Aminoglycosides	—	0.0	0.0	100.0
Cefotaxime	Cephems	Cephalosporin III	100.0	0.0	0.0
Amoxicillin/Cla	Beta-lactam +	—	100.0	0.0	0.0
vulanic acid	Inhibitors				

Table-4: Sensitivity pattern of *Candida albicans* coinfectd with *Acinetobacter baumannii*

Antifungal tested against <i>Candida albicans</i>	Zone of diameter	Interpretation
Caspofungin	20	Sensitive
Fluconazole	24	Sensitive
Voriconazole	23	Sensitive

Table-5: Sensitivity pattern of *Candida albicans* coinfectd with *Escherichia coli*

Antifungal tested against <i>Candida albicans</i>	Zone of diameter	Interpretation
Caspofungin	22	Sensitive
Fluconazole	21	Sensitive
Voriconazole	23	Sensitive



Figure-1: CHROMagar showing bluish green colonies of *Candida albicans*

DISCUSSION

Human mucosal surfaces are colonized by the opportunistic fungus commensal *Candida albicans*. The human host harbors a variety of commensal bacterial species. These commensals of bacteria and fungi coexist in a number of host environments. These microbial communities experience polymicrobial interactions under the impact of internal and external host stimuli, leading to a variety of host outcomes. Interactions between bacteria and fungi in the human host are unavoidable, and their effects are extensively researched. These microbial relationships may be antagonistic (predation, parasitism, amensalism, competition), beneficial (mutualism, synergism, commensalism), or nonexistent. Numerous diseases brought on by polymicrobial infections are the result of these interactions. There are ramifications for elements such as oxygen concentrations, nutrient availability, adhesion sites (interaction with the immune system), and biofilm formation in the host environment implications on these interactions. Further these factors vary in different niches of the human body.^(1,2) Fungal-bacterial interactions occur through multiple mechanisms in host. Synergistic interactions potentiate pathogenesis and antagonistic interactions restrict microbial virulence. We studied the co-existence of *Candida* species with other bacterial infections in a total of about 1067 clinical samples received in our laboratory. There are about 200 species in the genus *Candida* and *Candida albicans* accounts for about 75% of all candida infections.

In general, *Candida albicans* is regarded as commensal and functions as an opportunistic pathogen in response to modifications in the host defensive mechanism. *Candida* commonly colonizes the vagina, rectum, and oral cavity. A total of 219 isolates (n=1067) were cultivated from the total samples. The several locations from which the samples were taken are displayed in Graph 1. Microbiota was frequently seen in the pus sample. The different organisms that were cultivated and their percentage growth are listed in Table 1. *Streptococcus pneumoniae* was the least common (3.6%) while *E. coli* was the most common (17.8%). There was both pure and mixed growth (Graph 2). Two samples—one from the pus of a patient with diabetic foot ulcers and the other from the sputum of a patient with pneumonia—showed bacterial and fungal interactions. *C. albicans* forms mixed species communities with bacteria with its spectrum of adhesion capabilities. Coaggregation and coadhesion reactions of microorganisms help in the formation, stabilization, and maintenance of these complex communities. Adhesion processes is the key event in the initiation of the polymicrobial biofilms.

Table-2 and Table-4 shows the anti-bacterial and anti-fungal susceptibility patterns respectively that observed in *Candida albicans* and *Acinetobacter baumannii* coinfection isolated from sputum. Physical interaction in *Candida albicans* and *Acinetobacter baumannii*

coinfections appear to have antagonistic relationship in the host environment. It is known to induce fungal apoptosis and thereby limit microbial virulence. This patient responded well with cefaperazone, imipenem, gentamicin and fluconazole regimen. Table-3 and Table-5 shows the anti-bacterial and anti-fungal susceptibility patterns observed in *Candida albicans* and *Escherichia coli* coinfection isolates from the pus sample. *Candida albicans* and *Escherichia coli* chemical interaction and metabolic byproducts release has antagonistic relationship by inhibiting fungal biofilm formation and by killing *Candida albicans*. This patient was treated with piperacillin-tazobactam, meropenem and fluconazole. Hospital stay for these two patients was relatively prolonged for about 3 weeks. Among all *Candida* isolates, *C. albicans* was found to be a predominant organism to cause candidiasis followed by *C. glabrata*. The only isolate in the co-infections in our investigation was *C. albicans*. The *Candida albicans* colonies on CHROMagar are displayed in Figure 1. In immunocompromised patients, fungal-bacterial co-infections prolong healing and increase costs. *Candida*-bacteria interactions can be altered by extrinsic factors such as pH and nutrient availability. The results of polymicrobial biofilm infections, drug tolerance, and virulence are all impacted by these interactions.⁽⁹⁾ Understanding these intricate colonization processes of bacteria and *Candida* will aid in the creation of new procedures to prevent sticky responses and regulate the growth of biofilms. Effective clinical and financial management of polymicrobial illnesses will be facilitated by greater knowledge and a predictable strategy to early detection.

CONCLUSION

Candida organisms are observed to frequently demonstrate asymptomatic colonization. 40% of the healthy adults carry *Candida albicans* in their oral cavities and 20 to 25% of healthy women carry *Candida albicans* in the vagina. *Candida* spp. generally considered as colonizers and regarded as clinically irrelevant. As pathogens they were underdiagnosed. Increased awareness and research led to the reporting of fungi and bacteria co-infections. Fungal- bacterial interactions and their impact on the humans were studied. As the incidence of these polymicrobial infections increases, newer antifungal drugs and their pharmacodynamic properties should be explored. Studying the incidence and outcomes of fungal-bacterial co- infections over long term period, will throw light on the frequently encountered co-infections, their antibiotic and antifungal susceptibility patterns and the clinical course following early intervention.

Funding: This project is funded by ICMR-STC (Ref. No.:2022-10902)

Acknowledgement: The authors are grateful for the contribution of Department of Microbiology, Research Cell and Statistician.

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