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ORIGINAL RESEARCH

Phenotypic characterization and detection of virulence factors of *Candida species* isolated from candidemia patients in a tertiary care hospital of North-east India

[1]Dr. Lakshyasri Baishya, [2]Dr. Nilakshi Borah, [3]Dr. Manash Jyoti Taw, [4]Dr. Ajanta Sharma

[1]Associate Professor, [4]Professor and Head, Department of Microbiology, Gauhati Medical College, Guwahati, Assam, India
[2]Associate Professor, [3]Assistant Professor, Department of Microbiology, Pragjyotishpur Medical College, Guwahati, Assam, India

Corresponding author
Dr. Nilakshi Borah
Associate Professor, Department of Microbiology, Pragjyotishpur Medical College, Guwahati, Assam, India

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ABSTRACT

Introduction: Candida species exhibits a variety of virulence factors which contribute to the pathogenesis of candidiasis.The increased incidence in Candidainfections is also associated with the emergence of drug resistant strains.Among Candida spp., expression of virulence factors may vary depending on the infecting species, geographical origin, type of infection, the site and stage of infection, and host reaction. **Aim:** To study the phenotypic characterization and to detect the virulence factors of *Candida* species isolated from blood stream infections. **Materials and methods:** Identification of the 100 consecutive *Candida*isolates from blood culture samples were done by conventional methods andVITEK 2 yeast identification system (which were not possible to identify by conventional methods). The antifungal susceptibility was tested by AST-YS08 Vitek 2 Card system.The virulence factors studied were exoenzymatic activity (coagulase, phospholipase, and proteinase), biofilm formation, and haemolysin production. **Result:** Among the 100 *Candida* spp., 34 were *C. albicans* and 66 were NAC spp.No significant differences were seen in biofilm formation capacity between *C. albicans* and NAC.Phospholipase, proteinase production and coagulase activities were predominantly observed in *C. albicans*. Most of the *C. albicans* isolates (88.23%) demonstrated potent haemolytic activity.All the enzymatic activities showed significant statistical difference between *C. albicans* and NAC species groups.Correlation was statistically significant between the biofilm formation capacity and phospholipase, proteinase, coagulase, and haemolysinproducing capacity in *Candida* spp.Antifungal drugs sensitivity are significantly associated with biofilm, coagulase, and hemolysin producing *C. albicans*and NAC spp., however, phopholipase, and proteinase producing *C. albicans*and NAC are not associated with drugs sensitivity or resistance pattern. **Conclusion:** This study highlightsthat number of hydrolytic enzyme secreting *C. albicans*is distinctly different from NAC spp. The *C. albicans* exhibits a higher prevalence of coagulase, phospholipase, proteinase, and hemolytic activity, while non-albicans*Candida* species are more prone to biofilm formation.Understanding these virulence factors is pivotal for comprehending the pathogenic nature of *Candida* species. The significance of virulence determination in rational antifungal therapy cannot be ignored and should be adopted as a routine procedure in the laboratory.

Keywords: Antifungal susceptibility tests, Biofilm, Candidemia, Coagulase, Haemolysin, Phospholipase, Proteinase

INTRODUCTION

In the recent years, Candida species has emerged as an important causative agent of blood stream infections.It is also increasingly associated with other health care associated and opportunistic infections.The increased use of intravenous catheters, total parenteral nutrition, broad spectrum antibiotics, cytotoxic chemotherapy and an increase in the population of immunocompromised patients have contributed to the increase of these infections.[1] Lately, there is an increased prevalence of infection caused by non-albicans Candida (NAC) species along with *Candida albicans.* Candida species exhibits different virulence factors like germ tube formation, biofilm formation, adhesins, phenotypic switching, thigmotropism, and production of hydrolytic enzymes which contribute to the pathogenesis of candidiasis.[2]

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In the recent times, *Candida species* have developed resistance to anti-fungal agents, in particular to the azole compounds.[3]The emergence of drug-resistant Candida species is largely attributed to the use of prolonged and inappropriate empirical therapy. Accurate species identification is important for the treatment of Candida infections, as the infections caused by NAC is also increasingly documented.[4]

Expression of virulence factors in Candida species may vary depending on the infecting species, geographical origin, type of infection, the site and stage of infection, and host reaction. Understanding these virulence factors will be an important tool to learn the pathogenesis of candidiasis and will help search new antifungal drug targets for improved therapeutic regimens. The present study was taken up with an aim to study the phenotypic characterization and to detect the virulence factors of *Candida*speciesisolated from blood stream infections.

MATERIALS &METHODS

This cross sectional study was conducted in the Department of Microbiology of a tertiary care hospital in North-East India after obtaining approval from institutional ethical committee (Registration no.MC/190/2007/pt-II/Oct-2019/21). A total of 100 consecutive isolates of *Candida* obtained from blood culture samples were included in the study. The study isolates were collected from the samples that were received routinely in the laboratory.

The blood samples showing positivity for fungal growth (yeast only) were included in the study. And those samples which showed no growth or culture positive for bacterial growth were excluded from the study.

These Candida isolates were subcultured on Sabouraud's Dextrose Agar (HiMedia Laboratories Pvt. Ltd. Mumbai, India). Identification of the isolates were done by Gram staining, germ tube test, Dalmau method, CHROM agar and Carbohydrate fermentation and assimilation tests as per the CLSI guidelines.[5]Biomerieux VITEK 2 yeast identification system was used for species which were not possible to identify by conventional methods.

The antifungal susceptibility of the isolates were tested by AST-YS08 Vitek 2 Card system (BioMerieux) as per manufacturer instructions.

The virulence factors studied were exoenzymatic activity (coagulase, phospholipase, and proteinase), biofilm formation, and haemolysin production.

Coagulase Activity

Coagulase production was detected by the method of Yigitet al.[6] Overnight culture of *Candida* sp. was aseptically inoculated into a tube of rabbit plasma. The tubes were incubated at 35˚C and observed for clot formation after 2, 4, 6, and 24 h. The presence of a clot indicated positive coagulase test. Staphylococcus aureus ATCC 25923 and S. epidermidis ATCC 14990 were used as positive and negative controls, respectively.

Phospholipase Production

The phospholipase activity was detected by the method of Samaranayakeet al.[7]Approximately 5 μL of standard inoculum of test strain containing 10^8 Candida cells/mL was aseptically inoculated onto egg yolk agar. The plates were dried at room temperature and then incubated at 37˚C for 48 h. The plates were examined for the presence of precipitation zone around the colony. The presence of precipitation zone indicated expression of phospholipase enzyme. C. albicans ATCC 10231 was used as positive control. The phospholipase index (Pz) was defined as the ratio of the diameter of the colony to the total diameter of the colony plus the precipitation zone. A Pz value of 1 denoted no phospholipase activity; Pz< 1 indicated phospholipase production by the isolate. The lower the Pz value, the higher the phospholipase activity.[8]To minimize experimental error, the assay was conducted in duplicate on three separate occasions for each isolate.

Proteinase Activity

Proteinase activity of the isolates were screened by the method described by Staib.[9]It was measured in terms of bovine serum albumin (BSA) degradation. Approximately 10 μL of standard inoculum (10^6 Candida cells/mL) was aseptically inoculated on to 1% BSA plate. The plate was incubated for 5 days at 37˚C. C. albicans ATCC 10231 was used as positive control. After incubation, further proteinase activity was inhibited by adding 20% trichloroacetic acid and the plate was stained with 1.25% amidoblack. A zone of proteolysis surrounding the colony that could not be stained with amidoblack indicated proteinase activity. The proteinase index (Prz) was measured in terms of the ratio of the colony to the diameter of unstained zone. A Prz value of 1 indicated no proteinase activity; Prz< 1 denoted proteinase expression by the isolate. The lower the Prz value, the higher the proteinase activity.[8]To minimize experimental error, the assay was conducted in duplicate on three separate occasions for each isolate.

Haemolysin Production

Haemolytic activity was screened on sheep blood Sabouraud dextrose agar plate by the method described by Mannset al.[10] Approximately 10 μL of standard inoculum (10^8 Candida cells/mL) was aseptically inoculated onto the medium. The culture plates were incubated at 37˚C for 48 h. C. albicans ATCC 90028 was used as the control strain. The presence of a zone of haemolysis around the colony indicated haemolysin production. Haemolytic activity (Hz) was calculated in terms of the ratio of diameter of the colony to that of the translucent zone of haemolysis (in mm).

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Biofilm Formation

The ability of Candida isolates to form biofilms was assessed by two methods. [6,11,12]

Congo red agar method: Biofilm production was determined by using Brain heart infusion brothsupplemented with glucose and congo red. Plates were inoculated and incubated aerobically for 24 to 48 hrs at 37° C. Positive result was indicated by dark red colonies. Biofilm negative strains produced white or very light pink coloured colonies. The experiment was performed in triplicate and repeated three times. Candida albicans ATCC 90028 and C. parapsilosis ATCC 96142 served as controls for biofilm production.[11]

Tube method: Colonies of Candida species from SDA were inoculated in saline and incubated overnight at 37°C. 0.5 mL of this saline suspension was added into screw capped conical polystyrene tubes containing 5 mL of Sabouraud dextrose broth supplemented with glucose (8%). The tubes were incubated at 35°C for 48 h without agitation. After incubation the broth was aspirated gently using Pasteur pipette. The tubes were washed twice with distilled water and stained with 2% safranin for 10 min. The tubes were rinsed with distilled water to remove excess stain. Presence of visible adherent film on the wall and at the bottom of the tube indicated biofilm formation. Ring formation at the liquid interface was not considered as an indication of biofilm production.[6]Staphylococcus epidermidis ATCC 35984 and C. albicans ATCC 10231 were used as positive and negative controls, respectively.

Antifungal Susceptibility Testing

The antifungal susceptibility of the Candida isolates were tested by AST-YS08 Vitek 2 Card system (BioMerieux) as per manufacturer instructions.

Statistical analysis

Data were analyzed using XLSTAT, Origin 8 (Origin Lab Corporation, USA) software, and Minitab version 17.1.0 package (Minitab, LLC, USA). One-way analysis of variance (ANOVA) was used to calculate statistical differences between biofilm formation capacity of*C.albicans* and NAC spp.and other virulence features showing *C.albicans* and NAC spp., and to measure the association between virulence factors and antifungal susceptibility. The correlation between biofilm formation and other virulence features were tested using Pearson's rank correlation.Two-way ANOVA was also used to model the relationship between sensitivity and resistance patternto different antifungal agents, and virulence factors producing *C. albicans* and NAC. Level of significance was considered at *p* value less than 0.05.

RESULT

Isolation and characterization of *Candida* spp.

In the present study, a total of 100 *Candida* spp. were isolated fromCandidemia patients in a tertiary care hospital of North-east India, whichwere identified based on Gram staining, germ tube test, Dalmau method, CHROM agar and carbohydrate fermentation and assimilation tests per the CLSI guidelines, and Biomerieux VITEK 2 yeast identification system. Among the 100 *Candida* spp., 34 were *C. albicans* and 66 were NAC spp. Among the non-albicans*Candida* species (NAC), *C. tropicalis* (20/66, 30.3%) was found to be more dominant followed by *C. parapsilosis* (12/66, 18.2%), *C. auris* (12/66, 18.2%), *C. krusei* (6/66, 9.09%), *Kodameaohmeri* (6/66, 7.58%), *C. famata* (4/66, 6.06%), *C. pelliculosa* (3/66, 4.55%), *C. utilis* (2/66, 3.03%), *C. dubliniensis* (1/66, 1.52%), and *C. lipolytica* (1/66, 1.52%). All the isolated *Candida* spp. were checked for their pathogenicity which is facilitated by a number of factors, including biofilm formation, and secretion of hydrolytic enzymes (phospholipase, proteases, coagulase and haemolysin).

Biofilm formation by *Candida* spp.

Among the 100 isolates of *Candida* spp., 71% were found to be biofilm producers. On comparing the biofilm production capacity among *C. albicans* and NAC spp., biofilm production was found to occur most frequently among NAC (100% of *C. krusei*, C. *utilis*, *C. dubliniensis*; 91.6% of *C. parapsilosis*; 80% of *C. tropicalis*) in comparison to *C. albicans* (61.76%) (Table 1). No significant differences were obtained of the biofilm formation capacity between *C. albicans* and NAC ($p>0.05$).

Hydrolytic enzymes production by *Candida* spp.

We investigated the production of extracellular enzymes, such as coagulase, phospholipase, proteinase, and hemolysin, in *C.albicans*and NAC spp. We found that all these enzymatic activities were present in both *C.albicans*and NAC spp. Notably, phospholipase activity was predominantly observed in *C. albicans*, with 76.47% (26 out of 34) of the isolates exhibiting this activity, while it was absent in *Kodameaohmeri*, *C. utilis*, *C. dubliniensis*, and *C. lipolytica*(Fig.1a).

In terms of proteinase production, 82.35% (28 out of 34) of the *C. albicans* isolates displayed this activity, followed by 75% of *C. tropicalis* and 66.66% of *C. krusei*. None of the isolates of *C. famata*, *Kodameaohmeri*, *C. pelliculosa*, *C. utilis*, *C. dubliniensis*, and *C. lipolytica* showed proteinase activity(Fig.1b).

Coagulase activity was predominantly observed in *C. albicans* isolates (55.88% or 19 out of 34), with non-albicans*Candida* species like *C. tropicalis* (40%), *C. parapsilosis* (33.33%), and *C. auris* (25%) also exhibiting this activity.Most of the *C.albicans* isolates (88.23%) demonstrated potent haemolytic activity on sheep blood SDA plates, which is followed by *C. parapsilosis* (83.33%)(Fig.1c, 1d). All the enzymatic activity showed significant statistical difference between *C. albicans* and NAC species groups

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(F=44.02 at p value <0.001***).Levene's tests for comparison of variances confirmed these results.

On determining correlation between biofilm formation capacity and hydrolytic enzyme producing capacity in *Candida* spp., it was observed that, the correlation was statistically significant between the biofilm formation capacity and phospholipase, proteinase, coagulase, and haemolysinproducing capacity in *Candida* spp. (r= 0.95-0.92, p<0.05*) (Table 2).

Antifungal susceptibility of *Candida* spp.

The antifungal susceptibility patterns of the *Candida* spp. were also determined using six different antifungal drugs [Fluconazole (FLC), Voriconazole (VOR), Caspofungin (CASPO), Micafungin (MICA), Amphotericin B (AMB), Flucytosine (FLU)]. Of the *C. albicans*that exhibited biofilm formation, 100% were sensitive to CASPO, MICA, and AMB; 95% to FLU, 90% to VOR, and 76% to FLC, however, of the NAC, 100% were sensitive to CASPO, and MICA; 78% to AMB; 74% to FLU, 96% to VOR, and 63% to FLC.

The results also depicted that *C. albicans*and NAC spp.that exhibited a positive phopholipase test, 81% and 89% were sensitive to FLC, 96% and 96% to VOR, 92% and 85% to AMB, 85% and 89% to FLU, and 100% and 100% to CASPO, and MICA respectively. In regards to *C. albicans*and NAC spp. that had proteinase activity, 71% and 88% were sensitive to FLC, 93% and 96% to VOR, 75% and 92% to AMB, 79% and 92% to FLU, and both *C. albicans*and NAC spp.showing 100% sensitivity to CASPO, and MICA.

Similarly, 79% and 87% of positive coagulase activity showing *C. albicans*and NAC spp.respectively were sensitive to FLC, 95% and 100% to VOR, 84% and 93% to AMB, 89% and 80% to FLU, and both *C. albicans*and NACshowing100% sensitivity towards CASPO and MICA. Lastly, among the positive haemolysis activity showing *C. albicans*and NAC spp., majority (100%) of them were sensitive toCASPO and MICA; however, 87% and 88% to FLC, 100% and 95% to VOR, 90% and 92% to AMB, 87% and 95% to FLU (Table 3).

The significant p value of two-tailed analysis of variance (ANOVA) infers that the antifungal drugs sensitivity or resistance are significantly associated with biofilm (F=13.89, p<0.01**), coagulase (F=5, p<0.05*), and hemolysin (F=22.7, p<0.001***) producing *C. albicans*and NACspp., however, phopholipase, and proteinase producing *C. albicans*and NAC are not associated with drugs sensitivity or resistance pattern (Table S1-S5; Fig S1-S5).

Table 1: Biofilm formation capacity of *Candida* spp. isolated from Candidemia patients in a tertiary care hospital of North-east India

***Candida* spp.**	**Percentage of *Candida* spp. showing Biofilm formation**
C. albicans	61.76
C. tropicalis	80
C. parapsilosis	91.6
C. krusei	100
C. famata	75
C. auris	66.66
Kodameaohmeri	20
C. pelliculosa	66.66
C. utilis	100
C. dubliniensis	100
C. lipolytica	0

Table 2: The correlation between biofilm formation and other virulence factors tested using Pearson's rank correlation.

	Biofilm formation	**Phospholipase**	**Proteinase**	**Haemolysin**	**Coagulase**
Biofilm formation	1				
Phospholipase	0.94*	1			
Proteinase	0.92*	0.97*	1		
Haemolysin	0.95*	0.99*	0.95*	1	
Coagulase	0.92*	0.99*	0.97*	0.98*	1

Table 3: Antifungal susceptibility pattern of the *Candida* spp. against six different antifungal drugs [Fluconazole (FLC), Voriconazole (VOR), Caspofungin (CASPO), Micafungin (MICA), Amphotericin B (AMB), Flucytosine (FLU)] were determined (S=Sensitive; R=Resistance).

Virulence factors	**Biofilm formation**		**Phospholipase production**		**Proteinase production**		**Haemolysin production**		**Coagulase production**	
Name of the organisms	***C. albicas***	**NAC**	***C. albicas***	**NAC**	***C. albicas***	**NAC**	***C. albicas***	**NAC**	***C. albicas***	**NAC**

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with the susceptibility pattern	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R
FLC	16	5	32	19	21	5	25	3	20	8	22	3	26	4	33	5	15	4	13	2
VOR	19	2	49	2	25	1	27	1	26	2	24	1	30	0	36	2	18	1	15	0
CASPO	21	0	51	0	26	0	28	0	28	0	25	0	30	0	38	0	19	0	15	0
MICA	21	0	51	0	26	0	28	0	28	0	25	0	30	0	38	0	19	0	15	0
AMB	21	0	40	11	24	2	24	4	21	7	23	2	27	3	35	3	16	3	14	1
FLU	20	1	38	13	22	4	25	3	22	6	23	2	26	4	36	2	17	2	12	3

Supplementary materials

Table S1: Two way ANOVA to compare sensitiveor resistance pattern to antifungal drugs with biofilm producing*C. Albicans* and NAC spp.

	DF	Sum of Squares	Mean Square	F Value	P Value
CAorNCA	1	1350	1350	40.08	<0.001***
SensitiveorResistance	1	4428.17	4428.17	131.46	<0.001***
CAorNCA*SensitiveorResistance	1	468.17	468.17	13.89	<0.01**
Model	3	6246.33	2082.12	61.81	<0.001***
Error	20	673.67	33.68		
Corrected Total	23	6920			

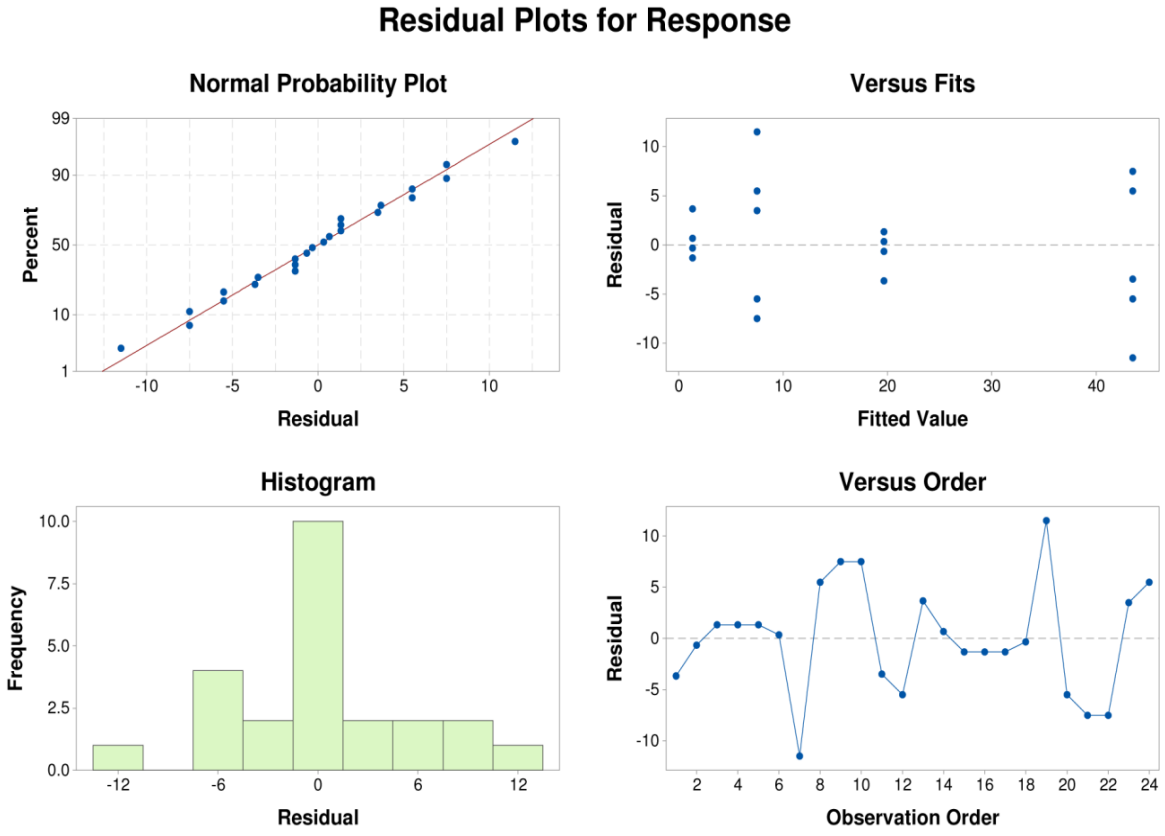

Fig S1: Residual plots of Two way ANOVA to compare sensitive or resistance pattern to antifungal drugs with biofilm producing *C. albicans*and NAC spp.

Table S2: Two way ANOVA to compare sensitiveor resistance pattern to antifungal drugs with phospholipase producing*C. Albicans* and NAC spp.

	DF	Sum of Squares	Mean Square	F Value	P Value
CA or NCA	1	6	6	1.63	<0.001***
Sensitive or Resistance	1	3220.16	3220.16	874.25	<0.001***
CA or NCA *Sensitive or Resistance	1	8.17	8.17	2.22	0.15
Model	3	3234.33	1078.11	292.69	<0.001***

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Error	20	73.67	3.68		
Corrected Total	23	3308			

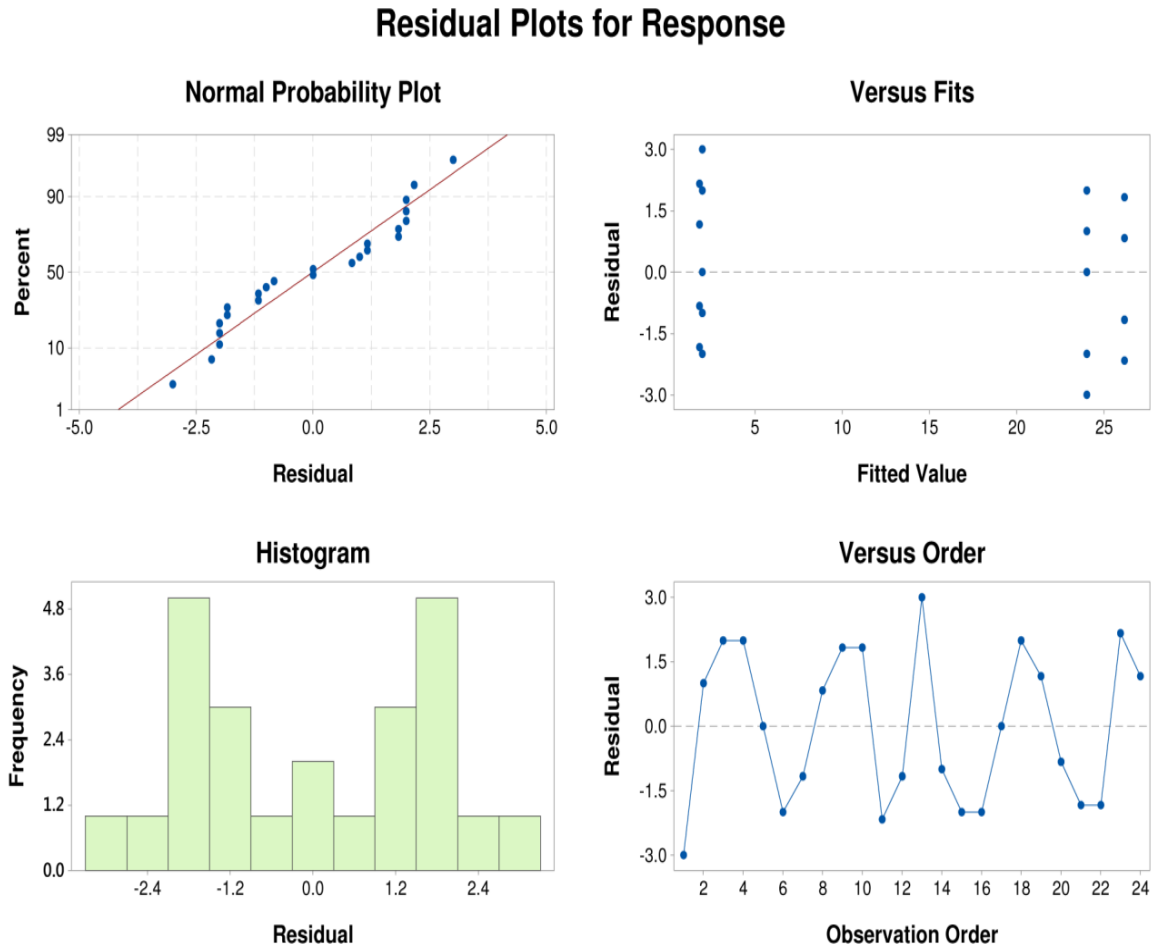

Fig S2: Residual plots of Two way ANOVA to compare sensitive or resistance pattern to antifungal drugs with phospholipase producing *C. albicans*and NAC spp.

Table S3: Two way ANOVA to compare sensitive or resistance pattern to antifungal drugs with proteinase producing*C. Albicans* and NAC spp.

	DF	Sum of Squares	Mean Square	F Value	P Value
CA or NCA	1	13.5	13.5	1.87	0.18
Sensitive or Resistance	1	2730.67	2730.67	378.38	<0.001***
CA or NCA *Sensitive or Resistance	1	6	6	0.83	0.37
Model	3	2750.17	916.72	127.03	<0.001***
Error	20	144.33	7.22		
Corrected Total	23	2894.5			

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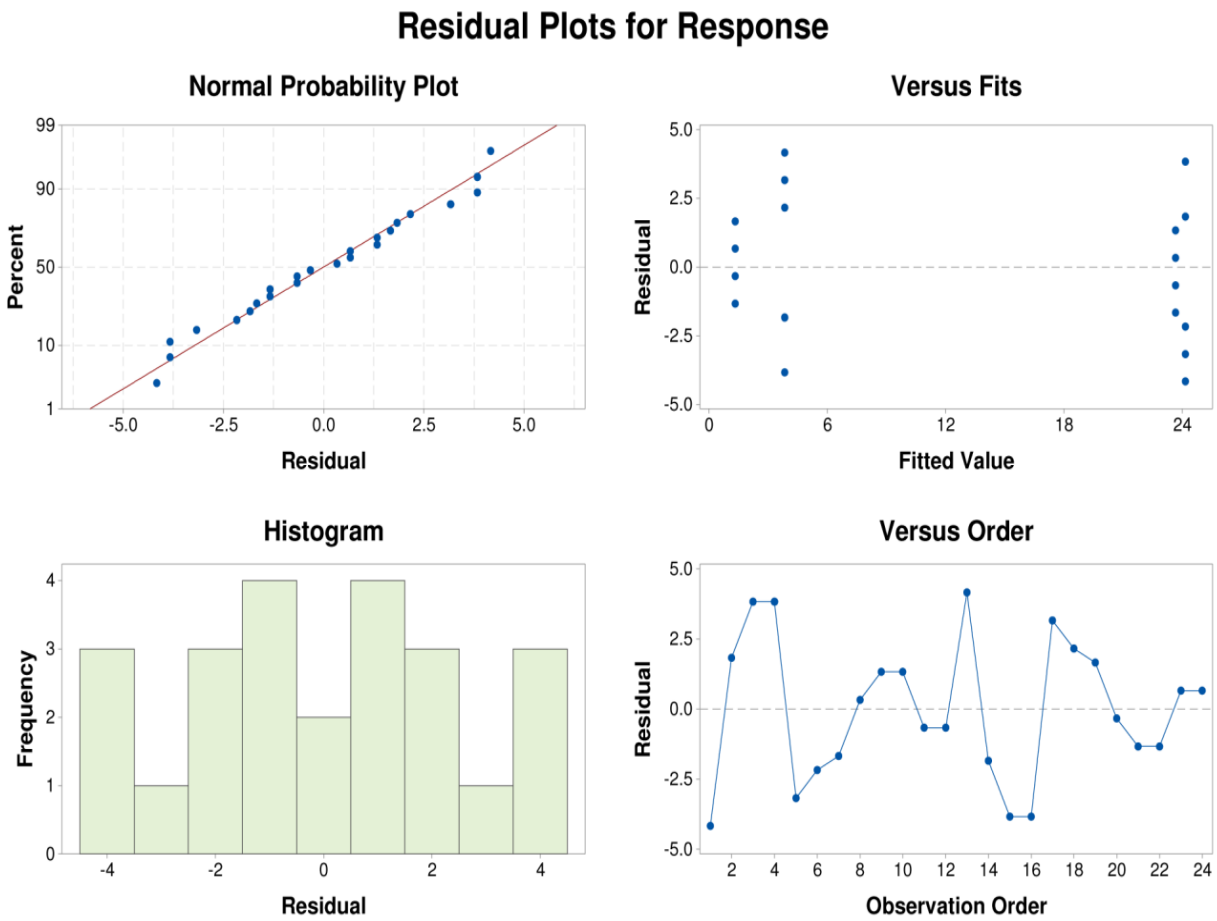

Fig S3: Residual plots of Two way ANOVA to compare sensitive or resistance pattern to antifungal drugs with proteinase producing *C. albicans*and NAC spp.

Table S4: Two way ANOVA to compare sensitiveor resistance pattern to antifungal drugs with coagulase producing*C. albicans*and NAC spp.

	DF	Sum of Squares	Mean Square	F Value	P Value
CA or NCA	1	24.00	24.00	11.25	<0.01**
Sensitive or Resistance	1	1232.67	1232.67	577.81	<0.001***
CA or NCA*Sensitive or Resistance	1	10.67	10.67	5.00	<0.05*
Model	3	1267.33	422.44	198.02	<0.001***
Error	20	42.67	2.13		
Corrected Total	23	1310.00			

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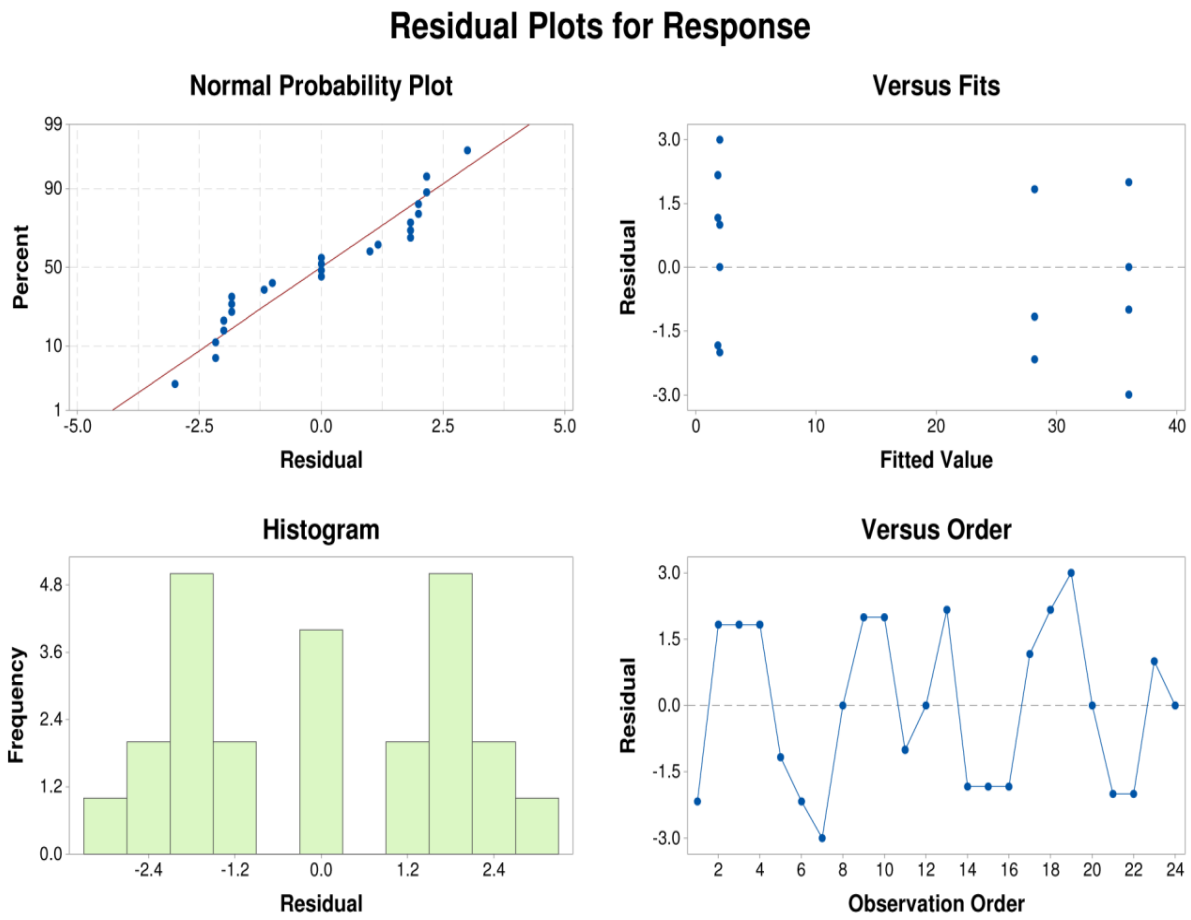

Fig S4: Residual plots of Two way ANOVA to compare sensitive or resistance pattern to antifungal drugs with coagulase producing *C. albicans*and NAC spp.

Table S5: Two way ANOVA to compare sensitiveor resistance pattern to antifungal drugs withhaemolysin producing*C. albicans*and NAC spp.

	DF	Sum of Squares	Mean Square	F Value	P Value
CA or NCA	1	96.00	96.00	24.72	<0.001***
Sensitive or Resistance	1	5460.17	5460.17	1406.05	<0.001***
CA or NCA*Sensitive or Resistance	1	88.17	88.17	22.70	<0.001***
Model	3	5644.33	1881.44	484.49	<0.001***
Error	20	77.67	3.88		
Corrected Total	23	5722.00			

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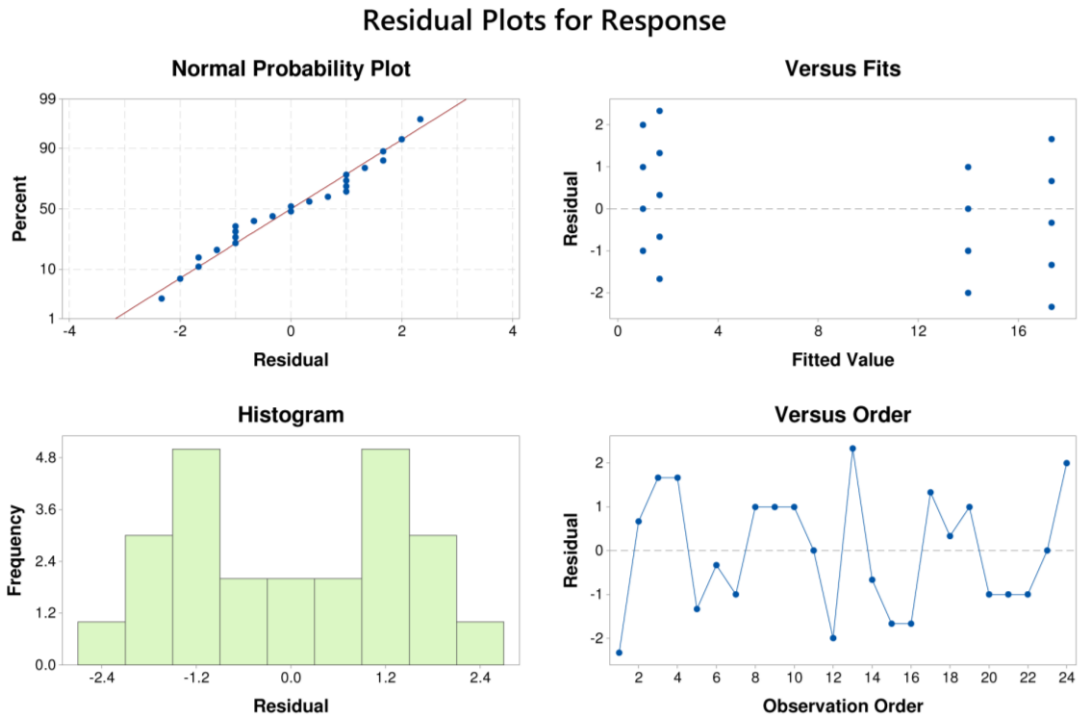

Fig S5: Residual plots of Two way ANOVA to compare sensitive or resistance pattern to antifungal drugs with haemolysin producing *C. albicans*and NAC spp.

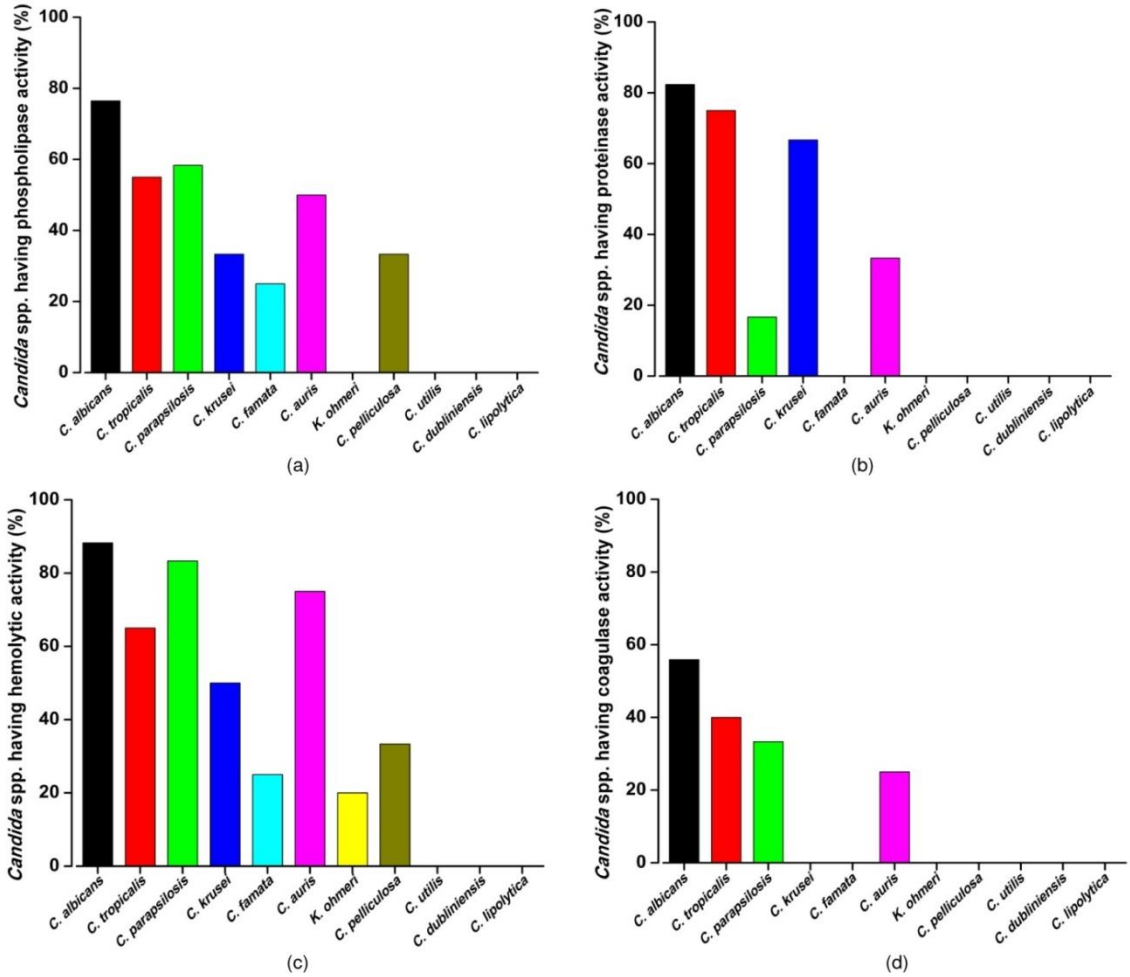

Fig 1: Percentage of *Candida* spp. having (a) phospholipase activity, (b) proteinase activity (c) coagulase activity, and (d) haemolytic activity

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DISCUSSION

In the present study, NAC (66%) outnumbered the *Candida albicans* (34%) which co-relates with many of the previous studies.[13,14]

Biofilm production was found to occur most frequently among NAC in comparison to *C. albicans*which correlates with the findings of some other studies[14-17] but it is in contrast to findings of Deorukhkar*et. al* and Tellapragada*et. al* where higher biofilm production was seen in *C. albicans*.[18,19]Biofilm formation in *Candida*speciesis associated with increased risk of resistance to antifungal agentswhich may be due to several mechanisms like less penetration of drug through biofilm matrix, decrease in the rate of *C. albicans* need for nutrition and increase rate of expression of resistance genes.[20] This factor also helps to evade host immune responseleading to treatment difficulty requiringdifferent lines of treatment. [21,22]

Extracellular hydrolytic enzymes play a significant role in the pathogenesis of candidiasis.[23]These enzymes helpsthe Candida to adapt to different types of infection and enhance the pathogen survival.[24]

Phospholipases helps in host cell penetration by hydrolyzing ester linkages andlyses host cells facilitating adhesion and penetrationcontributing to microbial virulence.[22,25-29]In this study, Phospholipase activity was predominantly observed in *C. albicans* than with NAC. Phospholipase production in NAC is a rareentity as reported in few other studies.[30-33]

Aspartic proteinases damages the surface proteins like albumin and keratin [34,35]which helps in tissue invasion and resistance to the antimicrobial attack by the host.[26]Proteinases also helps *Candida* to resist cellular and humoral immunity by degrading antibodies, complement, and cytokines.[34]In our study, highest proteinase expression was seen in C. albicans followed by the NAC species as has been reported in previous studies.[36-38]

Coagulasecauses clotting of plasma by binding to plasma fibrinogen and activating a cascade of reactions.[39]In this study, coagulase activity was predominantly observed in *C. albicans* isolates than with NAC which correlates with findings of other studies.[40]

Haemolysin secretion helps the deeper tissue invasion by *Candida*.[41]In our study, most of the *C. albicans* isolates (88.23%) demonstrated potent haemolytic activity on sheep blood SDA plates, which is followed by *C. parapsilosis* (83.33%).Fewother studies also showed similar findings.[40,42]

All the enzymatic activity showed significant statistical difference between *C. albicans* and NAC species groups.

Antifungal susceptibility testing is still less developed and utilized than antibacterial testing. Antifungal drugs sensitivity or resistance are significantly associated with biofilm, coagulase and hemolysin producing *C. albicans*and NAC spp., however, phopholipase, and proteinase producing *C. albicans*and NAC are not associated with drugs sensitivity or resistance pattern

CONCLUSION

This study imparts essential insights into the pathogenicity of *Candida* species responsible for Candidemia in North-east India. The research highlights that number of hydrolytic enzyme secreting *C. albicans*is distinctly differ from NAC spp. The *C. albicans* exhibits a higher prevalence of coagulase, phospholipase, proteinase, and hemolytic activity, while non-albicans*Candida* species are more prone to biofilm formation. Notably, the study reveals a correlation between biofilm production and enzyme secretion. Understanding these virulence factors is pivotal for comprehending the pathogenic nature of *Candida* species. In light of the mounting evidence supporting the necessity for improved *Candida* species management due to increasing antifungal resistance, the study also detects sensitivity or resistance to various antifungal agents. Importantly, it's reassuring that biofilm, coagulase, and hemolysin producing*C. albicans* and NAC spp. are significantly associated with the sensitivity or resistance to antifungal drugs. This finding offers solace to clinicians and microbiologists, eliminating concerns about the pathogen's virulence impacting drug responses.In recent years, a gradual increase in the fungal diseases and the widespread use of empirical antifungals caused the emergence of resistant strains of fungi. Thereby, the need for in vitro antifungal susceptibility testing are increasing nowadays to select appropriate and effective antifungal therapy. Hence, the significance of virulence determination in rational antifungal therapy cannot be ignored and should be adopted as a routine procedure in the laboratory.

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