Bharath
INSTITUTE OF HIGHER EDUCATION AND RESEARCH
(Declared as Deemed-to-be University under section 3 of UGC Act, 1956)
(Vide Notification No. F.9-5/2000 - U.3, Ministry of Human Resource Development, Govt. of India, dated 4th July 2002)
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NATIONAL ASSESSMENT AND ACCREDITATION COUNCIL
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173, Agaram Road, Selaiyur, Tambaram,
Chennai - 600 073. Tamil Nadu.

Ref. No.SMS-2018-O-05

Date: 16.08.2018

TO

Mr. C. Naveen Kumar
Assoc. Professor/Microbiology,
BIHER.

Thro: Concern Head of the Department

Greetings!!!

We are happy to announce that the Research Advisory Committee has approved your proposal for Seed Money Scheme-2018 which was presented by you. You are requested to complete the proposal and send the progress report to the Dean Research in the prescribed time period.

Title of the Project: Virulence factors of Candida species isolated from pulmonary tuberculosis with Diabetes mellitus

Seed Money Amount: Rs.1, 00,000/- (Rupees One Lakh Only)

Approved on: 06.08.2018

Payment details:

Voucher No.50

Dated: 22.08.2018

With Regards

Dean-Research

Bharath University

SELAIYUR, CHENNAI - 600 073, TAMIL NADU, INDIA.

CASH / PAYMENT VOUCHER

Date 22/08/2018

V.No. 50

Debit _____ Amount

Rs.

PAID TO Dr. C. Naveen Kumar

RUPEES One Lakh Only

TOWARDS Seed Money Scheme - 2018

BHARATH UNIVERSITY CHENNAI 600 073. INDIA.

Authorised by **Finance Manager** **Cashier/Accountant** **Payee's Signature**

PROPOSAL SUBMISSION

1. **Details of Principal Investigator**

Name	: Dr. C. Naveen Kumar
Designation	: Associate Professor
Highest Qualifications	: Ph.D.
Department	: Microbiology
E-mail	: navin.mmb@gmail.com
Contact no	: 9047765601
Date of Joining	: 13.04.2013

2. **Details of Co-Principal Investigator**

Name	: Dr. Sandhya Rani. T
Designation	: Assistant Professor
Highest Qualifications	: M.Sc
Department	: Microbiology
E-mail	: sandhya.micro86@gmail.com
Contact No	: 8098572603
Date of Joining	: 08.04.2013

Technical details

1. Introduction:

Fungal infection caused by opportunistic pathogens have become more frequent, of these *Candida* sp. can cause a wide range of infections from mucocutaneous to systemic infections [1]. *Candida* is a major fungal pathogen of human capable of invading oral cavities and epithelial surfaces of vagina; in immunocompromised patients, it can be carried through blood to invade the internal organs [2]. Oral candidiasis occurs most frequently in immunocompromised hosts, intensive care patients and patients undergoing chemotherapy [3]. Candidiasis has also been associated with tubercle bacilli infection [4]. Tuberculosis, a major public health problem has turned to be a major threat with the worldwide epidemic of Human Immunodeficiency Virus (HIV). The prevalence of dually infected patients globally has increased greatly in the present century. It has been accounted that there are more than 14 million dually infected persons globally with India accounting for more than one million. There is substantial evidence proving that the consequences of HIV and tuberculosis co-infection are greater than the presence of either of them [5]. It is known that candidiasis is one among the symptoms of AIDS. Hydrolytic enzymes contribute to the virulence factors in *Candida albicans.* The enzymes produced are proteinase, lipase, and phospholipase; factors which are responsible for the invasiveness, and proliferation of fungi caused by the destruction of host tissues in which the organisms are provided with nutrients by the host tissues [6]. Furthermore, humans do not possess free iron and hence most pathogens obtain this indirectly from haemoglobin [7]. In HIV-positive subjects there might be selection of more virulent *C. albicans* strains and increased resistance to antifungal drugs, thus contributing to the outbreak of the disease, along with its easily relapsing nature and treatment failure [8]. There has been a rapid spread of antifungal multidrug resistance (MDR), which has become a serious public health problem in recent years [9]. There is no information about the production pattern of extracellular enzymes of *Candida* spp. isolated from HIV and TB positive patients in comparison with non-HIV and -TB patients in India. The purpose of this study was to compare the enzymatic activities, haemolysin production and antifungal sensitivity pattern of oral clinical isolates of *Candida* spp. in HIV and TB positive and non-HIV and -TB patients.

2. Review of status of Research and Development in the subject

Eggimann P, Garbino J, Pittet D. Epidemiology of Candida species infections in critically ill non-immunosuppressed patients. Lancet Infect Dis. 2003 Nov; 3(11):685-702.

Out of the totalll0 patients with suspected tuberculosis, 53 patients (48.1%) who were positive for acid fast bacilli by Ziehl-Neelsen stain were screened for Candida co-infection. Out of 53 Candida species tested 37(69.8%) were found to be biofilm producers. Biofilm production was most frequent among non-albicans Candida 20 (37.7%) than Candida albicans 17(32.0%). Among the non- albicans Candida species, C. parapsilosis6 (30%) was the highest biofilm.

2.1. International Status:

In the present investigation, 60 strains of Candida isolates were isolated from the oral cavity of HIV and TB patients and non-HIV and -TB patients affected with oral candidiasis. The isolates were characterised based on the colour morphology on HICHROME agar plates, staining and germ tube method. The species identified by standard methods includes C. albicans, C. glabrata, and 3 C. krusei. Enzyme analysis and antifungal sensitivity pattern were performed to conclude the virulence and resistance pattern of Candida spp. Secretion of caseinase and lipase showed maximum similarity in strains isolated from both the groups. Phospholipase activities of C. glabrata and C. krusei were similar; it was 100% in HIV and TB positive patients whereas in the non-HIV and -TB patients it was nil. It was found to be higher in C. albicans isolates. Extracellular phospholipases facilitate the ability of Candida to invade the host cells. Phospholipase and protease activity was observed in patients with invasive Candida infection

2.2. National Status:

NIL

3. Progress/ achievement so far, if any

a). Reference papers was collected.
b). Literature survey was studied.
c). Materials and methods were designed.

4. Work plan

4.1 Methodology

A total of l25 smear positive pulmonary tuberculosis patients were included in this study done during Jan- June 20l6at Sri Lakshmi Narayana Medical College Pondicherry. The study was done after obtaining informed consent from the patients and institutional ethical committee clearance. Two consecutive sputum samples were collected from each patient and subjected to gram staining and culture.

A detailed history regarding smoking, alcohol consumption, calorie intake etc.,was collected from the patients by administering a questionairre. The criteria for diagnosis of Candidiasis were based on the presence of pus cells with budding yeast cells and pseudohyphae in direct gram stain. Samples were inoculated on Sabouraud's Dextrose Agar (SDA). Colonies were identified by Phenotypic technique. Determination of Phospholipase Activity: The culture medium consisted of 1 L of SDA broth add ten microliters of previously prepared yeast suspension was inoculated onto plates, incubated at 37 °C for 5 days in aerobic condition. The presence of enzyme activity was determined by the formation of a precipitation zone around the

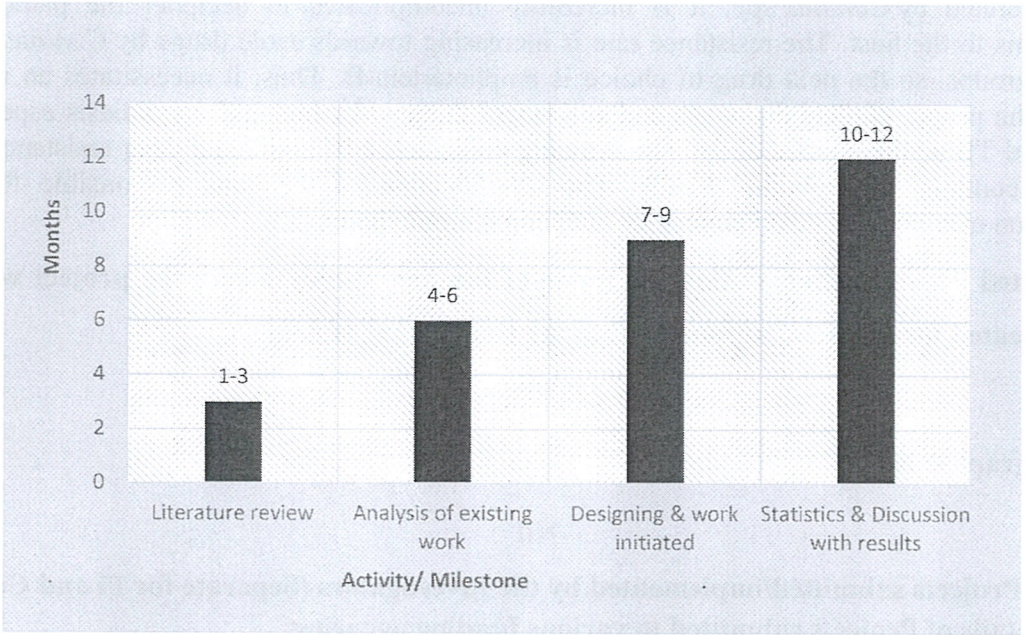
yeast colonies. Phospholipase activity (Pz) was calculated by dividing the diameter of the colony by the diameter of the colony plus precipitation zone. The Pz was scored as follows: Pz= 1, negative phospholipase activity; Pz = 0.64—0.99, positive phospholipase activity; and Pz 0.63, very strong phospholipase activity.10 Determination of Proteinase Activity: To bovine- serum albumin agar, add ten microliters of previously prepared yeast suspension and inoculated onto the plates, incubated at 37 °C for 10 days in both aerobic and anaerobic conditions. The presence of proteinase activity around the yeast colonies.11 Haemolysin Activity: Haemolytic activity was measured using blood agar plates. The Candida spp. were streaked onto SDA enriched with 5 ml of human blood at pH 5.6 + 0.2 and incubated for 5 days at 25°C. The plates were analyzed with the aid of a computerized image analysis system for all the assays and measurement of the zone of hemolytic activity.12

Esterase Activity: To determine esterase activity, Tween-80 opacity test medium was used add ten microliters of previously prepared suspension from each isolate was carefully deposited on the Tween-80 opacity test medium then incubated at 37 °C for 10 days in aerobic. Esterase activity was considered as positive in the presence of a halo pervious to light around the inoculation site.13

Biofilm Production: A loop full of organisms from the SDA inoculated into a tube containing 10ml Sabouraud's liquid medium strains supplemented with glucose. The tubes were incubated at37°C for 24 h after which the broth was aspirated out and the walls of the tubes were stained with saffranin. Biofilm production was scored as negative (0), weak positive (1+), moderate positive (2+) and strong positive (3+). Data were entered in excel worksheets and analyzed using suitable statistical methods. 14

4.2 Time Schedule of activities giving milestones through BAR diagram. (Maximum of 1/2 pages)

S. No	Activity/ mile stolen	1st Year			
1	Literature review	**1-3 month**			
2	Analysis of existing work	-	**4-6 month**		
3	Designing & work initiated	-	-	**7-9 month**	
4	Statistics & Discussion with results	-	-	-	**10-12 month**


14
12
10
8
6
4
2
0
Months
1-3
4-6
7-9
10-12
Literature review
Analysis of existing work
Designing & work initiated
Statistics & Discussion with results
Activity/ Milestone

4.3 Expected outcome within the time period of See Money Scheme

Our findings indicate that *Candida* spp. isolated from HIV and TB (immunocompromised) patients has significantly higher production of phospholipase, caseinase and lipase, than non-HIV and -TB (Immunocompetent) patients. The production of these proteolytic enzymes is responsible for the proliferation and demolition of the host tissues. Alpha haemolytic activity was observable in all the isolates. A statistically significant difference in its production between the groups was also observable. The production of haemolysin in both the groups may be responsible for virulence to the host tissue. If we could find out the nature of the haemolytic factors secreted by *Candida* spp. it is incredibly uncomplicated to decipher the pathogenic mechanisms to the host. The resistance rate is increasing towards azole drugs by *C. albicans* of both the groups, so the next drug of choice is amphotericin-B. Thus, it necessitates an urgent need for the proper clinical diagnosis and antifungal therapy for mucosal candidiasis especially in HIV and TB positive individuals. The growing prominence of antifungal drug resistance will probably continue through the decade. The identification of the gene responsible for the colonization may provide novel targets for antifungal prophylaxis.

5. Suggested Plan of action stating the name of funding agency where the project will be communicated for financial support within the time period of project.

Nil

6. Bibliography: Nil

Nil

7. List of Projects submitted/implemented by the Investigators (Separate for Pi and Co-PI)

7.1 Details of Projects submitted to various funding agencies:

S.No	Title	Cost in Lakhs	Month of Submission	Role as PI/Co-PI	Agency	Status
1	NA	NA	NA	NA	NA	NA

7.2 Details of Projects under implementation

Sl. No.	Title	Cost in lakhs	Duration	Role as PI/ Co-PI	Agency
1	NA NA	NA	NA	NA	NA

7.3 Details of Projects completed during the last 5 years

Sl. No.	Title	Cost in lakhs	Duration	Role as PI/ Co-PI	Agency
1	NA	NA NA	NA	NA	NA

8. List of publications published by the Investigators, if any:

a) Principal Investigator

S. No	Author names	Title of paper	Name of Journal	Vol (Issue)	Page No.	Year
1.	**Naveenkumar C1,** Swathi S2, Srikumar R3, Sairavikiran B4, Prabhakarreddy E5	Comparative Study on Normal Symptomatic Medication with Andrographis Paniculata (Nila-Vembu) Extract on Platelets Count in Thrombocytopenia Patient affected from Dengue Fever	Indian Journal of Public Health Research & Development	9(8)	16-20	2018
2.	**Naveen kumar C1,** Srikumar R2, Swathi S3, Chidambaram R4, Muthukrishnan G5, E Prabhakar Reddy6	Phytochemical Analysis and Antifungal Activity of Ganoderma lucidum	Indian Journal of Public Health Research & Development	9(120	130-135	2018
3.	**Naveen Kumar C,** Sri Kumar R, Swathi R, Prabhakar Reddy E, Chidambaram R.	Role of Ganoderma lucidum against trizole drugs resistant Aspergillus species	International Journal of Research Pharmaceutical Sciences	9(4)	1189-1195	2018
4.	S Ayyappan, Sachu Philip, N Bharathy, V Ramesh, **C Naveen Kumar,** S Swathi, A Arun Kumar	Antioxidant status in neonatal jaundice before and after phototherapy	Journal of pharmacy & Bioallied sciences	7(1)	S16-19	2015
5.	KP Shiva Govindan, Saleem Basha, V Ramesh, **C Naveen Kumar,** S Swathi	A comparative study on serum lipoprotein (a) and lipid profile between rheumatoid arthritis patients and normal subjects	Journal of Pharmacy & Bioallied Sciences	7(1)	S22-25	2015
6.	Jayaranjani.K Jayarani.K, Sandhyarani.T, **Naveen Kumar.C,** Swathi.S	Detection of MBL Producing Pseudomonas aeruginosa in Tertiary Care Hospital, Pondicherry	International Journal of Recent Scientific Research	5(8)	1460-1463	2014

7.	Janani.S Sandhyarani T, Jayarani.K, Sai Ravikiran B, **Naveenkumar.C**	Microbiological Profile and Spectrum of Drug Susceptability In Asymptomatic Bacteriuria Among Antenatal Women	Universal Research Journal Of Medical Sciences	1(1)	13-16	2014

9. Budget

SI. No	Head	Amount (Rs.)
1	BP Apparatus, Stethoscopes, Body weight weighing machine, SPSS version 16 Chicago, IL, USA, ECG machine	45000
2	Consumables (gels bottles, cotton, sprit, testing charges, tools, etc.)	10000
3	Travel support for the purpose of research work.	10000
4	Contingency	25000
5	Other's consumables	10000
	Total	**1,00,000**

*In case of any joint proposal for purchasing a same equipment, each of the associated PLs is also required to give separate budget (without any clubbing) to avoid any ambiguity, if all the associated projects are not awarded by committee.

10. Name of at least two subject experts from the Institute and one from the outside Institute with their contact details:

1. **Dr. Florida,** Research Scientist, Sree Balaji Medical College and Hospital, Chennai **Mobile No**: 9940027169 **E-mail id:** biozonediagnostic@gmail.com	2. **Dr. Suba,** Professor in Microbiology, Rela Transplantation Institute, Chennai **Mobile No**: 9962526457 **E-mail id:** subamicro@gmail.com

CERTIFICATE FROM THE INVESTIGATOR

Project Title: Virulence factors of Candida species isolated from pulmonary tuberculosis with Diabetes mellitus

It is certified that

1. I do hereby agree to submit a complete proposal for financial support to the external funding agency within the time period of SMS-2018.
2. I undertake that spare time on equipment procured in the project will be made available to other users.
3. I agree to submit a certificate from Institutional Biosafety Committee, if the project involves the utilization of genetically engineered organisms. I also declare that while conducting experiments, the Biosafety Guidelines of Department of Biotechnology, Department of Health Research, GOI would be followed in to.
4. I agree to submit ethical clearance certificate from the concerned ethical committee, if the project involved field trails/experiments/exchange of specimens, human & animal materials etc.
5. I agree to abide by the terms and conditions of SMS-2018, BIHER, and Chennai.

Name and signature of
Principal Investigator

Name and signature of
Co-Principal Investigator

Date: 30.07.2018
Place: Pondicherry

Forwarded by Head of the Department

Signature of the Head

DEAN
SRI LAKSHMI NARAYANA INSTITUTE OF MEDICAL SCIENCES
OSUDU, AGARAM VILLAGE,
KOODAPAKKAM POST,
PUDUCHERRY - 605 502

PROJECT EVALUATION FORMAT

Recommendation sheet

Name of the Principal Investigator	DR. C. Naveen Kumar
Name of the Co-Principal Investigator	Dr. Sandhya Rani. T
Name of the Department	Microbiology
Title of project	Virulence factors of Candida species isolated from pulmonary tuberculosis with Diabetes mellitus
Recommendation of the evaluation committee (Recommended/Revision/Not Recommended)	Recommended
Financial allocation recommended	Rs - 100,000/-

SI. No.	Head	Amount
1	BP Apparatus, Stethoscopes, Body weight weighing machine, SPSS version 16 Chicago, IL, USA, ECG machine	45000
2	Consumables- Gel bottles, cotton, sprit, testing charges, tools, etc.	10000
3	Travel support for the purpose of research work.	10000
4	Contingency	25000
5	Others consumables	10000
	Total	**1,00,000**

Name and Signature of the Research Advisory Committee members with date.

Internal Quality Assurance Cell * SLIMS * IQAC Pondicherry-07

(Dr. G. Jayalakshmi)