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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Ref. No.SMS-2015-O-01 Date: 17.08.2016

BHARATH INSTITUTE OF HIGHER EDUCATION & RESEARCH (Declared as Deemed to be University U/S 3 of UGC Act 1956) 17 AUG 2016 OFFICE OF THE DEAN-RESEARCH Selaiyur, Chennai-600 073. INDIA

TO

Mr. R. Srikumar
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-2015 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: Incidence Rate and Antibiotic Susceptibility Pattern of Listeria Species in High Risk Groups

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

Approved on: 10.08.2016

Payment details:

Voucher No.22 **Dated: 25.08.2016**

With Regards

Dean-Research

Bharath University

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

CASH / PAYMENT VOUCHER

Date 25/08/2016

V.No. 22

Debit _____ Amount

Rs. 1,00,000/-

PAID TO Dr. R. Srikumar

RUPEES One lakh only

TOWARDS Seed Money Scheme-2015

BHARATH UNIVERSITY CHENNAI 600 073. INDIA.

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

PROPOSAL SUBMISSION

1. **Details of Principal Investigator**

Name	: Dr. R. Sri Kumar
Designation	: Associate Professor
Highest Qualifications	: Ph.D.
Department	: Microbiology
E-mail	: rsrikumar_2003@yahoo.in
Contact no	: 9442500300
Date of Joining	: 02.01.2012

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

Name	: Dr. B. Geethavani
Designation	: Assistant Professor
Highest Qualifications	: M.Sc
Department	: Microbiology
E-mail	: gee192@gmail.com
Contact no	: 9952012724
Date of Joining	: 11.07.2013

Technical details

1. Introduction:

Listeriosis, a potentially serious invasive bacterial food borne disease caused by genus Listeria which leads to mild food poisoning in healthy individuals and severe systemic disease in certain well-defined high-risk groups.The genus Listeria includes multiple species namely L.monocytogenes, L.ivanovii, L.innocua, L.fleischmannii, L.welshmeri, L. seeligeri, L.grayi, L.marthii and L.rocourtiae [1,2]. But among different species only L.monocytogenes and L.ivanovii are pathogenic in humans [3]. Listeria monocytogenes has been found to be the causative agent in several outbreaks of food-borne Listeriosis [4]. L.ivanovii infection in humans is although rare, but there are reports on isolation of this organism from cases of AIDS and abortion [5]. Immuno-compromised individuals including transplant patients, dialysis patients, patient on immunosuppressive therapy, HIV patients [6], cancer patients [7], pregnant women [8], infants and neonates [9] are reported to be at high risk of getting Listerial infection. Listeriosis is a serious infection with high case fatality rate of about 20-30%, neonatal death rate 50% and hospitalization rate of about 91% [10]. In view of the high prevalence of Listeria monocytogenes in foods, together with the high mortality rate, this pathogen represents an important human health hazard [11]. Reports also suggest that incidence of Listeriosis has been increasing world-wide [12]. Besides, Listeriosis in India largely remains ignored. The literature reviews pertaining to the Listerial infections among immuno-compromised high risk groups in the Indian subcontinent is scarce. *L.monocytogenes* infections are usually treated with a single antimicrobial agent and combined therapies are recommended for the treatment of immuno-compromised patients [13]. Generally penicillin, ampicillin, amoxicillin, cotrimoxazole, tetracycline, chloramphenicol or aminoglycosides are recommended for the treatment of Listerial infection [14]. In 1988, the Multidrug resistant *L.monocytogenes* was first reported in France [15]. Since, then the number of drug resistant strains has been continually increasing [16]. The present study was undertaken to determine the incidence rate of Listeriosis in certain high risk groups. Furthermore, we aimed to determine the drug susceptibility pattern of *Listeria* spp. towards the common antibiotics used in the treatment of Listeriosis.

Review of status of Research and Development in the subject

Harakeh S, Saleh I, Zouhairi O, Baydoun E, Barbour E, Alwan N. Antimicrobial resistance of Listeria monocytogenes isolated from dairy-based food products. Sci Total Environ. 2009 Jun 15; 407(13):4022-7.

There has been a dearth of information on the epidemiology of listeriosis in most African countries, including India, with only few reports, when compared to Europe and USA. This is because the organism seems not to have been given attention as required. While antibiotic resistance has been reported severally in literature with clinical isolates from human beings, recent evidences however, show that antibiotic resistance traits have entered the microflora of farm animals and the food produced from them. Thus, the food microflora is not separated from its human counterpart in cases of antibiotic resistance. The occurrence of antibiotic resistance

complicates therapy and lengthens convalescence from illness. This trend has been worsened by prophylactic use of common broad spectrum antibiotics, indiscriminate usage in humans and in animal feed as growth promoters, particularly in developing nations. Despite these and the increase in the consumption of poultry products coupled with enormous untrained hands in the poultry industry in India and the associated public health implications, there is paucity of information on the prevalence and antibiotic susceptibility profiles of *L. monocytogenes* among commercial chickens as well as raw processed chicken meat; hence, this study.

2.1. International Status:

Listeria species are ubiquitous in the environment and possess unique physiological characteristics that allow growth at refrigeration temperature. High-risk food items associated with listeriosis are ready-to-eat foods including refrigerated but do not undergo any substantial heat treatment before consumption. Major changes in food production, processing and distribution, increased use of refrigeration as a primary preservation method, changes in the eating habits particularly towards ready-to-eat foods, and an increase in the number of people considered to be at high risk for the disease are suggested as possible reasons for the emergence of human food-borne listeriosis.

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

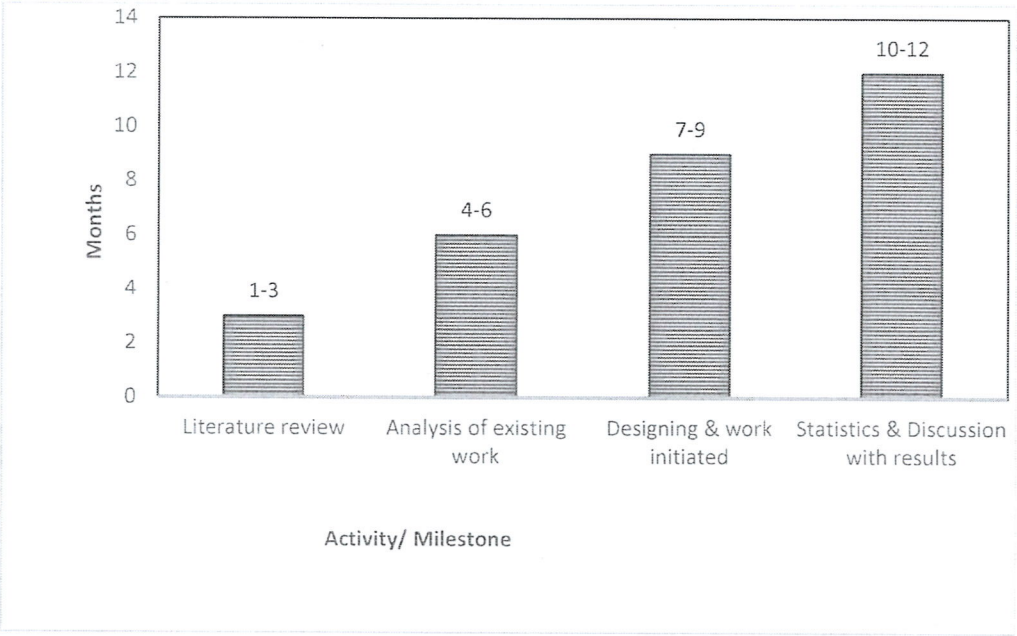
4.1 Methodology

A cross-sectional study conducted over a period of 1 year from June 2014 to May 2015 in the Department of Microbiology, Sri Lakshmi Narayana Medical College, Hospital and Government General Hospital, Puducherry, India. This study was approved by the Institutional Human Ethics Committee. Informed consent was obtained from all participants included in the study. Patient population included HIV patients, patients with malignancies, chronic liver disease, chronic renal failure, patients on long term corticosteroid therapy, infants (<12 months of age), pregnant women, women with spontaneous abortions or stillbirths and elderly patients (>65 years) presenting with either fever, flu-like illness, signs and symptoms of meningitis or diarrhea. A total of 643 clinical samples comprising 345 - blood samples, 65 - CSF, 17 - other body fluids, 49- diarrheal stool, 138 - amniotic fluid, 14 - placental bit and 15 - abortus material were aseptically collected. All the collected clinical samples were immediately transported to the laboratory and were processed for the isolation

of *Listeria* spp. following the US Department of Agriculture (USDA) method [17]. The samples were enriched by two step enrichment procedure by inoculating in University of Vermont medium (UVM)-1 and incubated at 30°C for 24 hours followed by (UVM)-2 and incubation at 30°C for 24 hours. A loopful of inoculum from enriched (UVM)-2 was cultured on to selective medium PALCAM agar and then incubated at 37°C for 24 hours. Grayish glistening colonies surrounded with a diffuse black zone were identified as *Listeria* colonies [Table/Fig-1] [17, 18]. The identified *Listeria* colonies were examined morphologically for Gram positive coccobacilli [Table/Fig-2] and its characteristic tumbling motility at 20-25°C, which was then confirmed for genus *Listeria* on the basis of Latex agglutination test using LK07- Hi *Listeria* Latex Test Kit {Hi-Media, India}. The identified *Listeria* isolates were further subjected to biochemical characterization using KB012A-Hi *Listeria* identification kit which includes catalase test, nitrate reduction test, esculin hydrolysis, Voges-Proskauer test, methyl red test and sugar fermentation tests. The kit contained sugars like xylose, lactose, glucose, alpha-methyl-D mannoside, rhamnose, sucrose and mannitol to differentiate various species of *Listeria*. Isolates exhibiting catalase, methyl red and Voges Proskauer test positive and nitrate negative reactions were considered as "presumptive" *Listeria* isolates. These "presumptive" *Listeria* isolates were further differentiated up to the species level into *L. monocytogenes* and other *Listeria* species based on sugar fermentation pattern. Isolates which showed glucose, α-methyl - D mannoside, rhamnose, lactose and sucrose positive, xylose and mannitol negative were considered as *L.monocytogenes*. The recovered isolates were subjected to antimicrobial susceptibility testing by Kirby Bauer disc diffusion assay, as per CLSI guidelines 2012, with the following antibiotics generally recommended for the treatment of the *Listeria* infection such as ampicillin (10 mcg), penicillin G (10 units), tetracycline (30 mcg), chloramphenicol (30 mcg), trimethoprim/sulfamethoxazole (co-trimoxazole) (25 mcg), gentamicin (10 mcg), amikacin (30 mcg), erythromycin (15 mcg) and clindamycin (2 mcg).

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**



4.3 Expected outcome within the time period of See Money Scheme

Out of 643 samples tested for Listeriosis, 32 samples showed the presence of Listeria spp. giving an overall incidence rate of 4.98%. Invasive Listeriosis had an incidence rate of 4.2% compared to Non-Invasive Listeriosis which had an incidence rate of 0.8%. In view of the increased incidence of Listeriosis, this disease should be considered an important differential diagnosis in clinical practice especially in high risk individuals. Maternal Listeriosis should be considered in all stages of pregnancy and due diligence must be followed in laboratory diagnosis. Being a food borne pathogen, strong efforts have to be made to ensure food safety. The increased drug resistance in this genus to the commonly prescribed antibiotics against Listeriosis is an area of concern and judicious use of antibiotics is to be encouraged to prevent further increase in resistance.

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.	Naveen Kumar C1, Ponniah M2, **Srikumar R3***, Vijayakumar R4, Chidambaram R4, Jayalakshmi G1, Prabhakar Reddy E5, Manoharan A6,	Incidence of Dengue Fever in Febrile Patients and Co-Infection with Typhoid Fever in South India	Annals of Medical and Health Sciences Research	7:	111-113	2017

	Sai Ravi Kiran B5					
2.	S Latha, R Venkataramanan, **R Srikumar**, RV Kumar	Effect of Triphala on noise stress induced alteration in glucocorticoid and carbohydrate metabolism.	International Journal of Pharma and Bio Sciences	6(2)	1-15	2015
3.	Manikandan Sundaramahalingam, **Srikumar Ramasundaram**, Sheela Devi Rathinasamy, Ruvanthika Pulipakkam Natarajan, Thangam Somasundaram	Role of Acorus calamus and alpha-asarone on hippocampal dependent memory in noise stress exposed rats.	Pakistan journal of biological sciences: PJBS	16(16)	770-778	2013

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	Others 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. Dr. G. Muthu** Research Scientist, ICMR, Thirunelveli **Mobile No**: 98843 04202 **E-mail id:** gopalmuthukrishnan@gmail.com	2. **Dr. Manikandan** Associate Professor in Physiology Tagore Medical College and Hospital, Chennai **Mobile No**: 9444434725 **E-mail id:**manikandanphysio@gmail.com

CERTIFICATE FROM THE INVESTIGATOR

Project Title: Incidence Rate and Antibiotic Susceptibility Pattern of *Listeria* Species in High Risk Groups

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-2015.
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-2015, BIHER, and Chennai.

Name and signature of Principal Investigator

Name and signature of Co-Principal Investigator

Date: 04.07.2016
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. R. Srikumar
Name of the Co-Principal Investigator	Dr. B. Geethavani
Name of the Department	Microbiology
Title of project	Incidence Rate and Antibiotic Susceptibility Pattern of *Listeria* Species in High Risk Groups
Recommendation of the evaluation committee (Recommended/Revision/Not Recommended)	Recommended
Financial allocation recommended	Rs. 1,00,000/-

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.

(Dr. P. Jayakumar)