



Co-sponsored by Kerala State Council for Science, Technology and Environment



# Proceedings of the **6 bioRADIANCE**

# The National Research Seminar in MOLECULAR BIOLOGY TECHNIQUES IN MICROBIAL RESEARCH

23<sup>rd</sup> June 2018, Senate Hall, Pushpagiri Institute of Medical Sciences & Research centre



Abstracts

# SOUVENIR



SL.No.	Table of Contents	Page
1	General Information on Bioradiance 2018	5
2	Presentation keynote speakers, panellists & Quiz master	7
3	Proceedings of Bioradiance 2018	9
4	Book of abstracts	14
5	Research facilities	41
6	The Research Advisory board	45
7	Organizing committee Bioradiance 2018	46
8	Bioradiance 2018 Sponsors	47

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# **Book of abstracts**

SL.No.	Title of Poster	Page
1	Herbal nanoparticles for mosquito control	22
2	Green synthesis of silver nanoparticles by using stem extracts of <i>Rotula</i> aquatica	23
3	Clovora – Antimicrobial herbal mouthwash for oral hygiene	24
4	Molecular characterization of <i>Culex quinquefasciatus</i> gut's microbiota to develop new strategies for the Mosquito-borne disease prevention	25
5	Detection of Cytomegaloviruses in patients with generalized chronic periodontitis and its relationship with clinical parameters.	26
6	Green synthesis of silver nanoparticles by using by using Curcuma angustfolia rhizome	27
7	Phytochemical screening and TLC profile of rhizome extracts of Curcuma angustifolia	28
8	Silver nanoparticle mediated molecular strategies for Systemic Lupus Erythematosus (SLE): clinical trials and future prospects	29
9	A review on biosurfactant production from <i>Trichoderma sp.</i> , genetics and its application	30
10	Green synthesis and characterisation of ZnO nanoparticles	31
11	Studies on the changes in the enzyme activities during seed germination in Vigna radiata	32
12	Deducing the microbial diversity of raw honey	33
13	Isolation and identification of endophytic and epiphytic bacteria from phyllosphere	34
14	Studies on the primary and secondary metabolites present in the leaf extracts of <i>Artocarpus altilis</i> and their antimicrobial activity	35
15	Hematological and clinical profile of patients with Dengue during an outbreak in Kerala 2016 at a tertiary care centre in Central Kerala	36
16	Evaluation of culture methods for isolation of <i>Mycobacterium tuberculosis</i> complex and its resistance among pulmonary tuberculosis cases in a tertiary care setting, Kerala	37
17	Isolation and molecular identification of <i>Vibrio cholerae</i> from seafood marketed in cochin area	38
18	Evaluation of anti-microbial and antioxidant effects of <i>Garcinia gummi-gutta</i> seed oil	39
19	Molecular characterisation and RAPD-PCR TYPING of <i>Aeromonas</i> hydrophila isolated from clinical samples	40



3

#### Letter from the desk of Director Academics and Research



Rev. Dr. Mathew Mazhavancheril Director Academics & Research Pushpagiri Institute of Medical Sciences and Research Centre, Thiruvalla, Kerala, 689101 Dear all,

#### Greetings from Pushpagiri Research Centre!!!

The pressing challenge faced by today's researchers is developing innovative and effective biotechnology solutions to problems encountered by us in health, environment and in agriculture. There is a need for cutting-edge research and developmental efforts in biotechnology including new strategic partnerships as this is an important interdisciplinary field. Though the main research focus is health, Pushpagiri Research Centre (PRC) has an interdisciplinary research platform recognized by DSIR with a dedicated team of scientists and supporting laboratory staff. We are encouraging and facilitating research students and researchers to take up serious studies which could benefit mankind and environment in the future.

Bioradiance is a signature annual national science seminar of PRC since 2013. This year, our target audience were bioscience postgraduates and budding researchers. Therefore the seminar was aimed to provide an in-depth knowledge in molecular biology techniques in microbial research. The seminar featured a variety of lectures from the basic to advanced techniques in a number of key sessions handled by eminent scholars of medicine. It also had panel discussion on Nipah outbreak, quiz in biotechnology, poster sessions and commercial exhibition. We are happy to have had a large participation both from biosciences as well as medical field with a total number of 228 delegates. Participants were really enthusiastic and they got a chance to interact with the stalwarts of molecular biology research both in South India as well as in Kerala state. I take this opportunity to thank all our sponsors especially the support by Kerala state council for science, technology and environment (KSCTE).

I hope the conference has sowed the seeds of inquisitiveness in our youngsters who would in-time become the future scientists of our country! I wholeheartedly pray to Lord Almighty to bless and guide them in their vocation.

> Thanking you Yours sincerely,







## General information on PRC & Bioradiance-The National Research Seminar

PRC, a unit of Pushpagiri Medical Society is a Scientific and Industrial Research Organization (SIRO) recognized by the Department of Scientific and Industrial Research (DSIR), Ministry of Science & Technology, Government of India. PRC is equipped with high-end infrastructure and skilled personnel. We have an interdisciplinary array of facilities in molecular biology, biochemistry, nanotechnology, phytoceutical sciences, microbiology, virology, cytogenetics, cancer biology, regenerative medcine & epidemiology. The centre has received funding from prestigious national agencies like DST, DBT, ICMR, KSCSTE and international agency like Bill & Melinda Gates foundation. It has been a part of many international research collaborations and with active Memorandum of Understandings with an array of institutions from government and private sectors, namely CIFT, HLL Lifecare, MG University, VIT University, SRM University and Yenapoya Universities. We have both the facilities of Institutional Review board (IRB) and the Institutional Animal Ethics **Committee** (IAEC). PRC offers PhD Programme with affiliations from Kerala University Health Sciences (KUHS), Thrissur, Kerala. We work with the vision of establishing PRC as a centre for excellence in research in our country.

'Bioradiance'is a national research conference conducted annually and is a favourite for research scholars and bio-science students to update and gain knowledge on topics of current research. Over past years Bioradiance has covered topics like Bio-informatics, recent advances in research and diagnosis of cancer, cytogenetics and its applications, demystifying statistics, frontiers in diabetic research, etc. As we all know that molecular technology has made giant leaps with varied applications in microbial research and diagnostics. It has altered the course of history in microbial isolation & identification, antimicrobial susceptibility testing and monitoring the emergence of new strains. It has greatly improved the quality of research work with direct implication in diagnosis and therapy. Keeping its increasing significance in mind, Pushpagiri Research Centre (PRC) conducted its VIth annual national conference Bioradiance-2018 with the central theme of "Molecular techniques in microbial research". Bioradiance 2018 was co-sponsored by KSCSTE.

Technology in microbial diagnostics and research has made enormous advances yet laboratories continues to rely heavily on traditional methods. For e.g. in a microbiology laboratory culture, phenotypic, and biochemical tests, to identify microorganisms present in various clinical specimens are done routinely. This is partly due to the complex and variable nature of specimens received and partly as the specimen type and test order dictate the processing and culture medium that are used for bacterial 'Bioradiance'is a national research conference conducted annually and is a favourite for research scholars and bioscience students to update and gain knowledge on topics of current research.

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and fungal culture. However much of clinical virology has shifted to tests based on molecular methods due to reduced turnaround time (TAT) and better sensitivity and specificity in comparison with viral culture. This shift has also resulted in reduced labour by eliminating time-consuming tasks, including maintenance of numerous permissive host cell lines, repeated electron microscopic examination, and chance for contamination associated with viral culture methods.

Various molecular techniques like PCR, sequencing were available for past few decades but their use in both research as well as diagnosis of viral, fungal and bacterial etiologies were largely considered as highly complicated tests by a vast majority. Thus limiting it use to molecular laboratories staffed with skilled technicians. But we hope to inspire budding research scholars and bio- science students to develop new bio-technologies and point of care tests which offer results at the earliest. With the evolution of super bugs, antimicrobial resistance is a major threat to employing conventional empirical treatment modalities. The considerable role played by molecular diagnostics in identifying them cannot be questioned. The need of the hour is to develop point of care technologies which can be of use for both diagnosis and research.

Hence we want to prompt Research scholars and bio-medical science students to keep abreast with the latest techniques and motivate them to develop point of care techniques by increasing their understanding of the most important aspects of molecular techniques thereby facilitating their opportunities as an innovator. The conference aims to highlight the research possibilities in the field of Microbiology with emphasis on using newer modalities both for research and diagnostics. It will be of great use, in particular, for postgraduate students, researchers, clinicians, academicians and Ph.D. scholars who have the interest to learn from the experts.



6



#### **Presentation keynote speakers**

#### Prof. Gopalan Sridharan, PhD, FRC Path,

Former Professor and Head, Dept. of Clinical Virology, CMC Vellore.

Prof. Sridharan is one among the pioneers in Clinical Virology and Virus Research in India. Well known for his impeccable knowledge and scientific abilities. He is a true researcher and academician who mentored several next generation scientists in the field. He has several projects funded by national aswell as international agencies.

#### Dr. Rajesh Kannangai, MD, PhD

Professor and Head, Department of Clinical Virology, CMC Vellore.

Dr. Raiesh is a post-doctoral fellow from Johns Hopkins University, USA. A well-known teacher and research guide, but a scientist to the core with special interest in HIV. He has several international/national projects to his credit and is the lead assessor of NABL.

#### Dr. Sabu Thomas, PhD

Scientist E-II, RGCB, Trivandrum.

A research scholar in Aquatic Biology after obtaining Master's in Zoology. Dr. Sabu is a lead researcher in Cholera, bacterial biofilms, probiotics etc. and a member of second Arctic Expedition.

#### Dr. Anand Ambarasu, MSc. MTech, PhD

Professor School of Biosciences & Technology, VIT, Vellore

A Microbiologist turned Bioinformatics specialist, Prof, Anand is a renowned Teacher, Scientist & Research Guide. He is one among the leading Bioinformatics scholar in the country with several funded projects and PhD students.

#### Dr. Sathish Sankar, MSc. PhD.

Associate Professor, Sri Sakthi Amma Institute of Biomedical Research, Sri Narayani Hospital & Research Centre, Vellore.

A scholar in Molecular Biology, Dr. Sathish is an expert researcher in Molecular Epidemiology, Bioinformatics, Geographical Infromation System & Vaccine Development. He has many funded projects to his credit.













#### Panellists for Nipah virus outbreak discussion

**Dr. Seema Oommen, MD, DNB,** Professor and Head of Department of Microbiology, Pushpagiri Institute of Medical Sciences and Research Centre

The moderator for the panel discussion who lead and threaded the discussion on Nipah Virus outbreak. She is a passionate and true clinical microbiologist to the core with strong background of both research and academics.

#### Prof. G Sridharan, PhD, FRC Path.

Fmr. Professor & Head, Dept. of Clinical Virology, CMC Vellore.

Chief Guest and speaker who also lend his hand for the panel discussion. He discussed in detail the laboratory diagnostic tests for detection Of Nipah Virus.

Dr. Keerthi T R, PhD, Professor & Director BioSciences

Vibrant and energetic, gave her expertise in field of Biosafety levels and processing of various agents in laboratory safely.

**Dr. A. Rajeev, MD,** Professor, Department of Community medicine, Pushpagiri Institute of Medical Sciences and Research Centre

A very dynamic and enthusiastic personality with sound knowledge and practicality. He is abreast with the latest and radiates positive energy which readily disseminates to the crowd.

**Dr. Tomy Philip, MD, MRCP,** Professor, Department of General medicine, Pushpagiri Institute of Medical Sciences and Research Centre

The clinical presentations, comparisons between various viral encephalitis were discussed in detail especially highlighting their differences from Nipah viral encephalitis by this eminent physician and excellent academician.

#### **QUIZ MASTER**

**Dr. Sajan Ahmad, MD, DM,** Pushpagiri Heart Institute A cardiologist by profession, an avid reader, movie buff and quiz master with good experience in conducting various quiz in different fields of medicine

good experience in conducting various quiz in different fields of medicine. He is very enthusiastic and hosts quizzes in the most interesting manner with a skill for making even the mundane interesting.















# Proceedings of one day National Research Seminar on 'Molecular biology Techniques in Microbial Research' Bioradiance- 2018)

Pushpagiri Research Centre, a unit of Pushpagiri Medical Society supported by Kerala State Council for science, technology and environment (KSCSTE) hosted Bioradiance 2018, a one day National Research Seminar with the central theme of 'Molecular biology Techniques in microbial research' on 23rd June 2018. This national seminar of importance conducted annually since 2013 was graced by eminent scientists from South India. It was an occasion of coming together of Researchers under one roof to share knowledge, discuss important scientific advancements in research and the way forward for Research scholars, scientists and students of biosciences, medical, dental and pharmaceutical branches.

This conference commenced by invoking God's blessings and grace for endowment of true wisdom. Mr. George Varghese, General Convenor, In-charge Virology Centre, Pushpagiri Institute of Medical Sciences and Research centre welcomed all the luminaries to this one day seminar including the chief guest, dignitaries, senior faculties and of course the most vital people of the seminar, the participants. This was followed by the Presidential address by Rev. Fr. Jose Kallumalickal, CEO, Pushpagiri Group of Institutions who spoke of the importance of molecular biology in the diagnosis of various diseases especially in emerging viral infections. He mentioned about the recent Nipah outbreak and the significance of molecular techniques like PCR in its diagnosis. He proudly stated Pushpagiri Medical College as the only centre within radius of 100kms as having the unique facility for molecular viral diagnosis.

Thereafter Prof. Gopalan Sridharan delivered the inaugural address where he stressed on the commitment of faculty, staff and students towards a common goal of research and development of mankind and the significance of a 'We' culture rather than the 'I' culture. Then the conference was inaugurated by lighting of lamp symbolising the divine light by the dignitaries including Prof. Gopalan Sridharan, Rev. Fr. Jose Kallumalickal, Rev. Dr. Mathew Mazhavancheril, Dr. Vikram Gowda, Dr.George Varghese, Mr. George Varghese and Dr. George Chandy, Director, Believer Church Medical College and Hospital. Prof. Gopalan Sridharan was honoured by the CEO with a traditional Aramula kannadi. Rev. Dr. Mathew Mazhavancheril felicitated Dr Gopalan Sridharan with a ponnada signifying his immense contributions as one of the pioneers of clinical virology in the country having several publications, funded projects and also mentored several young virologists and inculcated in them a passion for research and innovations.

Rev. Dr. Mathew Mazhavancheril, Director Academics and Research spoke on why research should be undertaken and how our life is now so comfortable because of the dedication, passion and hard work of many researchers before our time. In his felicitation he also described the various facilities in PRC and urged the audience to take up serious research for the well-being of future generations. He also encouraged them to join PRC for short term training programmes as well as projects.

Next Dr. Vikram Gowda, Vice Principal Pushpagiri Institute of Medical Sciences and Dr. George Varghese, Principal of Pushpagiri Dental College felicitated the programme conveyed how honesty is essential in Research and the importance of molecular diagnosis in field of medicine especially in the current scenario with lot of emerging infections.

Finally the inaugural session came to a close by the vote of thanks delivered by Dr. Philip Mathew, Chair Publicity committee and Asst. Professor, Community Medicine who thanked all the dignitaries, speakers, panellists, sponsors and delegates. There were around 228 delegates with 19 poster presentations, 9 teams for Quiz competition, 161 pre-registered students and faculty from biosciences, dental, medical and pharmacy colleges and 34 spot registrations, apart from the judges, panellists, invited guests and speakers making the event a grand success.





The scientific sessions started with a bang by the very soft spoken yet prolific researcher with several international, national projects and also the Head of Department of Virology, CMC Vellore, Dr. Rajesh Kannangai, who spoke on 'PCR types, it's nuances and trouble shooting.' He condensed the topic in a very palatable manner for the students with various pictures showing different scenarios faced while performing PCR and discussed what can be done to prevent them. He was presented with a memento by Prof. Seema Oommen, Head of Microbiology, Pushpagiri Institute of Medical Sciences. The next invited lecture on the 'Alpha and omega of molecular typing- Sequencing' by the young researcher from Sri Sakhthi Amma Institute of Biomedical Research, Vellore, Dr. Sathish Sankar who is an expert in molecular epidemiology, bio-informatics, vaccine development and geographical information system. His talk gave young researchers an insight to the mesmerising world of sequencing and phylogenetic analysis for which a memento was presented to him by Dr. Mercy John Idikula, Professor, Department of Microbiology, Pushpagiri Institute of Sciences. The much awaited talk by the chief guest Prof. Gopalan Sridharan was on 'role of molecular testing in diagnosis and monitoring of viral infections and therapy'. He was presented with a memento by Rev. Dr. Mathew Mazhavancheril for the very

The preliminary written round for selection of teams for the Quiz competition was conducted by Dr. Cleetus Cherupanakkal under the shades of the shamiyana for selecting the best four teams out of the nine candidate teams from various bioscience colleges in Kerala. Next technical session was on 'Bio-informatics- The sixth sense in microbial research' by Dr. Anand Ambarasu from VIT, Vellore who is one of the leading bioinformatics scholars in the country with several projects and PhD students. The lucid talk covered all the basics of bioinformatics which proved to be very valuable for the budding research scholars. He was presented with a memento by Mr. George Varghese, General Convenor, In-charge Virology Centre, Pushpagiri Institute of Medical Sciences and Research centre.

The judges for the poster presentation, Prof. Mercy John Idikula, Microbiology department, Pushpagiri Institute of Medical Sciences and Prof. Anand Ambarasu, Professor, School of Biosciences & Technology, VIT evaluated the posters from students from different colleges across Kerala and interacted with them. After a sumptuous lunch the sessions were continued.

Dr. Sabu Thomas, Scientist E-II, RGCB gave an excellent lecture on 'Emerging antimicrobial resistancerole of molecular techniques in its detection.' His enthralling lecture on various microbes and their resistance genes detection in the state gave an overview of the elaborate topic in the professed time. He was presented with a memento by Prof. G Sridharan.

Next was the much awaited panel discussion on Nipah Virus outbreak. Dr. Seema Oommen, Prof. and Head of Department of Microbiology, Pushpagiri Institute of Medical Sciences was the moderator and the eminent panellists were Dr. A Rajeev, Professor, Department of Community medicine, Pushpagiri Institute of Medical Sciences, Dr. Keerthi TR, Director Biosciences, MG University, Dr. Tomy Philip, Prof and Unit Chief, Department of Medicince, Pushpagiri Institute of Medical Sciences and Dr. G Sridharan our guest of honour. An in-depth discussion on the etymology, clinical presentation, epidemiology and various diagnostic techniques of Nipah Virus ensued in the following hour. The students and faculty were entertained with various titbits of information and all panic surrounding the disease was replaced by a mature understanding of the virus and its reach. Mementoes were presented to the moderator and all the panellists by Dr. Sabu Thomas.

The final academic programme was the Quiz competition on the molecular biology techniques which was hosted by the young and dynamic cardiologist Dr. Sajan Ahmad, Pushpagiri Heart Institute. The final four teams were announced and asked to take their place on the podium and Rev. Dr. Fr. Mathew Mazhavancheril was the judge in case of any discrepancies arose. The teams were from St. Joseph's College, Irinjalakuda, KVM College of Engineering and IT, Cherthala, CMS College, Kottayam and Sree Narayana College of Technology, Vadakevila. The teams battled four rounds covering history- tribute to legends, molecular biology techniques, clinical application, & back to basics from which CMS College emerged as victors. It was a highly enjoyable event with Dr. Sajan captivating both the teams' as well as the audience with his versatility, innovative questions and gifts to the audience who answered the passed questions. Dr. Sajan was presented with a memento by Dr. Zulfikar Ahamed M, Prof. of





engaging and outstanding lecture.



Paediatric cardiology, PIMS & RC.

This was followed by the valedictory speech and announcement of the winners of Quiz and Poster competition by Dr. Sherly Antony, Organizing Secretary and Senior Resident of Microbiology, Pushpagiri Institute of Medical Sciences and Research centre. The various prizes were handed over by Dr. Tomy Philip, to the winners. The first prize was a certificate of excellance and Rs.5000 for quiz was awarded to Ms. Pooja Ghorawat and Ms. Jasrin Fathima, BSc. Biotechnology, CMS College, Kottayam, second prize of Rs.3000 was won by Mr. Anand Krishnan and Mr. Sreejith Suprasannan doing MSc. Biotechnology, Sree Nrarayana College, Vadakevila and third prize of Rs.1000 was awarded as consolation to Ms. Enrika Joy Anit and Ms. Ancy Philip from MSc. Biotechnology, KVM College, Cherthala.

Rev. Dr. Mathew Mazhavancheril handed over the first, second and third prize for posters competition with a cash prize of Rs.2000, Rs.1000 and Rs.700, awarded to Ms. Kavya Raj K (St. Josephs College, Irinjalakuda), Ms. Sreya K J (St. Josephs College, Irinjalakuda) and Ms. Athira Thomas (Mar Athanasios College for Advanced Studies, Tiruvalla). The Valedictory session concluded with a vote of thanks by Dr. Sherly Antony, Organizing secretary of this national seminar who thanked the KSCSTE for cosponsorship, the Directors of Pushpagiri Medical Society, the prominent speakers, panellists, judges, quiz master, the organizing committee members of Bioradiance 2018, faculty, students, and supporting staff for the smooth functioning of the seminar. Indeed the active participation of delegates, scientists, faculty members, students and research scholars from various Institutions were the highlight of the conference making it a successful and fruitful event. The programme ended with the national anthem and Bioradiance National Seminar was closed for this year promising to return next year with yet another important research topic.

















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## BOOK OF ABSTRACTS ABSTRACTS OF TALKS OF BIORADIANCE 2018 PCR types, its nuances and trouble shooting

Dr. Rajesh Kannangai, MD, PhD, Prof. & Head of Virology, CMC Vellore

PCR is a method to specifically amplify target sequences in a complex mixture described by Kary Mullis in 1983. The steps involved are denaturation, annealing, extension and continuation of repeated cycles. The primers determine what sequences are amplified (specificity). Contamination control is very important in laboratories performing PCR.

The different types or modification of PCR include Nested PCR, Real Time PCR – Quantitative PCR, Multiplex PCR, TAIL PCR, Hot start PCR, Asymmetric PCR, Touch down PCR, Sequence-specific PCR, Reverse-transcriptase PCR, Long-range PCR and RAPD PCR. Quantitative PCR offers the advantage of quantifying target. The limitations of PCR are contamination risk, primer complexities, primer-binding site complexities and amplifies rare species.

The approaches for standardising PCR and the various trouble shooting scenarios which may arise during standardisation is an enigma in itself. In addition to PCR, signal and probe amplification methods are available for use in the clinical laboratory. The laboratory should establish appropriate pre-examination, examination and post examination QC to generate a valid report.

# The Alpha and omega of molecular typing- Sequencing

Dr. Sathish Sankar, PhD, Associate Professor, Sri Sakhthi Amma Institute of Biomedical Research, Sri Narayani Hospital and Research Centre, Sripuram, Vellore.

Genomic sequencing is not a fad. It helps in classification of microbes and viruses, Identification of target genes for vaccine and therapeutic agent development, phylogeographic studies (molecular epidemiology) and pathogen discovery (eg: Hepegivirus in 2015).

The various methods of sequencing are classified under First generation sequencing and Next generation sequencing. Both Sangers method and Maxam Gilbert method comes under first generation sequencing while 454, Solexa (Illumina), Sequencing by oligonucleotide ligation and detection (SOLiD) system, Ion Torrent, Single molecule real time (SMRT) platform and Nanopore comes under next generation sequencing.

Genome sequencing can help to identify genetic patterns related to the virulence of a disease, as well as genetic factors that contribute to immunity or successful vaccine response. Genomics has the potential to improve the process of vaccine development substantially.





# Role of molecular testing in the diagnosis and monitoring of viral infections and therapy

Dr. Gopalan Sridharan, Emeritus Scientist, Sri Sakhthi Amma Institute of Biomedical Research, Sri Narayani Hospital and Research Centre, Sripuram, Vellore

Molecular diagnosis significantly improves the scope and speed of diagnosis of infections especially viral diseases. Various diagnostic tests are available for Herpes virus, arboviruses, HIV, Hepatitis viruses, CMV, etc. which are utilized for early and accurate diagnosis of several severe viral infections.

Today many commercial quantitation assays are also available to help introduce specific antiviral therapy and monitoring. Antiviral therapy monitoring is vital for appropriate treatment and infection control or elimination. The application of viromics is endless and has to be harnessed in the best possible manner to cater to our varying requirements.

## **Bio-informatics- The sixth sense in microbial research**

Dr. Anand Ambarasu, MSc. MTech, PhD, Professor School of Biosciences & Technology, VIT, Vellore

Bioinformatics deals with the methods and software tools for understanding biological data. Bioinformatics is used for *in silico* analyses of biological queries using mathematical and statistical techniques. As an interdisciplinary field of science, bioinformatics combines biology, computer science, mathematics and statistics to analyse and interpret biological data.

Gene network is the visual representation of node and edges where nodes are genes or proteins in network and edges are links or interaction in the network. The network provides a combined perspective for the collected data. It is a diagrammatic representation -concepts and relationship which reduces the complexity of the data. Network tools give the functionality for studying complex processes, analyses the network properties and identifies the key elements of sub-networks and elucidate the mechanisms of interaction.

Bio-molecular interaction data can be studied using tools belonging to Protein-protein interaction database and Gene interaction database. Then network visualization and analysis is done with functional enrichment analysis tools and network analysis tools. Thus one can do computational gene network analysis of antibiotic resistant genes in pathogenic bacteria like beta lactam resistance genes in different organisms and exploit them for new drug development.



15

# Emerging Antimicrobial Resistance-Role of molecular Techniques in its Detection

Dr. Sabu Thomas, PhD, Scientist E2, RGCB, Thiruvananthapuram.

The presentation reviewed the current problem of antibiotic resistance of bacteria, some of the practices in the food industry contributing to the evolution of resistance & overview of the mechanisms of resistance in bacteria.

The role of molecular techniques to determine the genes encoding some of the clinically relevant acquired resistance mechanisms was presented. Data on comparison of molecular determination of resistance and that of methods that directly detect the specific resistance mechanisms, in studies conducted at the institution was presented. Implications of spread of Antibiotic resistance mechanisms with example of *Vibrio cholerae* O1 strain from Haiti and India, detected by molecular methods which also enabled determination of genetic relatedness of the conjugative elements, were discussed.

# An overview of the Panel discussion and general points on Nipah Virus

#### 1. Discuss the etymology, family and lineage of the Nipah virus?

**Ans:** Nipah virus was named after the Malaysian village Kampung Sungai Nipah where it was first reported, in pig farmers in 1999. This virus belongs to the Paramyxoviridae family. It along with Hendra virus comprises a new genus designated Henipavirus in the subfamily Paramyxovirinae. One interesting factor is that both Hendra and Nipah have emerged in the last 15 years or so and both are deadly.

Two Lineages mainly identified: Malaysian and the Bangladesh strains. Both the strains have high fatality rates, between 60% and 85%. Our virus bears sequence similar to the Bangladesh strains.

#### 2. Why are Nipah and Hendra so deadly?

**Ans:** The mortality rate of humans is quite high, ranging between 50 and 100% hence making them one of the most deadly viruses known to infect humans. The important characteristics responsible for its high pathogenicity:

-Its use of highly conserved cell surface molecules (ephrin) as entry receptors.

-Its highly effective replication and fusion strategies.

-Henipavirus also encodes multiple accessory proteins which play a key role in evasion of host innate immune responses.

-Zoonotic viral disease (to which we lack antibodies).

#### 3. Though 2 strains with different lineages are known how much of homology between them?

**Ans:** Though we have two divergent NiV strains (NiV-Malaysia and NiV-Bangladesh) share 91.8% nt sequence identity.

# 4. Elaborate the major differences in outbreaks, clinical presentation and case fatality ratios between the NiVB & NiVM?

**Ans:** NiV-Malaysia emerged in 1998→Started with the outbreak of infectious respiratory and neurologic disease in commercially farmed pigs. Cases: >250 cases were reported in Malaysia and Singapore, and the case-fatality rate was 40%. No cases of human-to-human transmission were reported during the outbreak. Post which 2 cases where there was seroconversion in a healthcare worker and another case of late onset encephalitis from family member.



NiV-Bangladesh emerged in 2001 and subsequent outbreaks of disease have occurred almost annually. Cases: since 2001, >200 cases in humans have been identified in Bangladesh; the overall casefatality rate is >70%. In contrast to the rare instances of human-to-human transmission of NiV-Malaysia, human-to-human transmission of NiV-Bangladesh is a major pathway for human infection.

Clinical data from NiV outbreaks has revealed several key differences between patients infected with NiVM and NiVB.

- a. NiVB has a shorter average incubation period and a more narrow range for the incubation period than NiVM.
- b. Most cases of NiVB included respiratory symptoms while few patients infected with NiVM presented with respiratory symptoms.
- c. Few cases in the Bangladeshi and Indian NiVB outbreaks reported myoclonus, while a significant proportion of patients from the Malaysian & Philippines outbreak presented with segmental myoclonus. {Fatal cases  $\rightarrow$  presenting with an acute encephalitis syndrome.}
- d. The source of the virus in the Bangladeshi and Indian outbreaks is either unknown in some cases or has been traced to consumption of contaminated fruit or date palm sap, followed by humanto-human transmission and nosocomial spread, whereas the source of the virus in the Malaysian outbreak is known to be from pigs, which served as an amplifying host. Thus pigs were the source of infection for farm and abattoir workers, resulting in a widespread outbreak of severe febrile encephalitic disease among humans.
- e. Unlike examples found in NiVB outbreak, there were only two reported cases of potential transmission from human-to-human in the Malaysian outbreak, neither of which presented with symptoms during the outbreak, although there were some reported cases of documented human-to-human transmission in the Philippines outbreak.
- f. There is an increased rate of vomiting with NiVB infection compared with the NiVM.

#### 5. How can it be transmitted?

**Ans:** As mentioned before the transmission dynamics of NiV to humans occur via three different routes: first, pig to human, second, direct bat to human and third, human-to-human. In the Malaysian outbreak, pig to human transmission was identified as the only pathway of transmission and direct human-to-human transmission was never conclusively demonstrated to occur. Whereas in outbreaks in Bangladesh, both bat to human and human-to-human transmission occur frequently, with no intermediate host observed.

# 6. Is the natural reservoir only by bats or are other animals also vulnerable and may act as reservoir or intermediate hosts?

**Ans:** Fruit bats belonging to the genus of Pteropus are the most common natural reservoir of NiV. Other fruit bats Cynopterus, Eonycteris spp and an insectivorous bat, Scotophilus kuhlii. One interesting fact is that antibodies to Nipah-like virus have been found in sera from fruit bats collected in India, Indonesia and Timor-Leste.

Then as mentioned before that pigs which were the intermediate host in Malaysian outbreak while in the Bangladesh outbreak there were no intermediate host, it was drinking of the date palm sap which was contaminated with bat saliva and urine.

Other domesticated animal reports: horses, dogs, cats, cattle and goats.

Then last but not the least direct human-to-human transmission especially in close contacts and health care staff reported in most outbreaks so far except the Malaysian.

#### 7. How can people spread Nipah virus to each other?

**Ans:** Nipah virus is spread from person to person through contact with infectious body fluids from another person such as nasal or respiratory droplets, urine, or blood.

#### 8. Though Nipah virus was first recognised in Malaysia. Since then, there have been 12 additional



# outbreaks, all in South Asia. But before Kerala any other state in India was struck with this deadly virus? Comparatively how was the CFR there?

**Ans:** Yes, West Bengal has faced 2 such outbreaks. In Feb 2001, Siliguri, the 2nd largest city in West Bengal and also known as the gateway of North-Eastern India was reported 66 cases with 45 death, CFR of 68%.

Then borders in Apr 2007, Nadia district in West Bengal which borders Bangladesh to the east, reported 5 cases with 100% mortality. So the outbreak in Kerala is the third outbreak in India. Since May 2nd a total of 19/18 confirmed cases with 17/16 deaths i.e. CFR of 86-88.8% and last case was reported on May 17th. So despite fears of a second wave Kerala is now breathing a bit easier.

# 9. In areas of Perambra, Malapurram and in hospitals in & around Kozhikode life has come to a standstill. This intense fear surrounding Nipah especially since it has affected young adults, is it justified or are we being overly cautious?

**Ans:** Nipah is relatively a new terminology in our area with a death toll of 16 in a span of 2-4 weeks. This points to a high CFR and to be precise 86%. That is a huge number, also three more people are currently being treated for the virus at isolation wards in the Kozhikode Medical College Hospital while nine others have been kept under observation, and hence the fear is justified. But we should think of measures to prevent it.

#### 10. Is there any seasonality of NiV outbreaks in South East Asia per say? If yes, why?

**Ans:** Yes, the outbreaks of Nipah in South-East Asia are showing a strong seasonal pattern. All the outbreaks occurred during winter and spring i.e. during months of December-May. This could be associated with several factors like the breeding season of the bats, increased shedding of virus by the bats and the date palm sap harvesting season.

# 11. Is there a possibility of toddy harvesting here as a source of infection? Or is there any other theories to our outbreak?

**Ans:** It is an interesting statement but so far we have had no committed reports of what could be the source of Kerala Outbreak. A new virus suddenly lands in this part of the country away considerably from the routine epidemiological area. Many theories are being discussed:

- 1) Finding of bats in an abandoned well in Perambra near the index case home
- 2) Sabith, brother of Muhammed Salih (Index case) had worked in Malaysia→Later found that he had come back from Dubai so that theory was false.

3) Could the possibility of migrant labourers from WB be considered They are poorly cared for and their death may go unnoticed. (Similar to recently reported Kala-Azar from Kerala)

#### 12. What are the case definitions for Nipah?

**Ans:** Suspect Nipah Case: Person from a community affected by a Nipah virus (NiV) disease outbreak who has:

- Fever with new onset of altered mental status or seizure and/or
- Fever with headache and/or
- Fever with Cough or shortness of breath

**Probable Nipah Case:** Suspect case-patient/s who resided in the same village/ward, where suspect/ confirmed case of Nipah were living during the outbreak period and who died before complete diagnostic specimens could be collected.

Or

Suspect case-patients who came in direct contact with confirmed case-patients in a hospital setting during the outbreak period and who died before complete diagnostic specimens could be collected.





#### **Confirmed Nipah Case**

Suspected case who has laboratory confirmation of Nipah virus infection either by:

- Nipah virus RNA identified by PCR from respiratory secretions, urine, or cerebrospinal fluid.

-Isolation of Nipah virus from respiratory secretions, urine or cerebrospinal fluid.

#### 13. How do we collect the sample and which are the ideal samples to be tested?

**Ans:** During sample collection wear complete disposable Personal Protective Equipments (N 95 mask, double surgical gloves, gowns, goggles etc.). Wash hands with soap and water at least for 30 seconds and then clean hand using 1-2 ml of alcohol based hand sanitizer before and after collection of samples.

#### The appropriate samples to be collected are:

- CSF (at least 1 ml) in a sterile container
- -Throat swab to be collected in viral transport medium
- Blood in plain vial (at least 5ml)
- Urine about 10 ml in universal sterile container

Transportation and Storage of samples: Samples should be safely packed in triple container packing and should be transported under cold chain (2-8°C) to the testing laboratory with prior intimation. Before dispatching the sample, disinfect the outer surface of container using 1:100 dilution of bleach or 5% Lysol solution.

Samples should be transported at 2-8°C if they arrive at the laboratory with 48 hours; if shipping time is expected more than 48 hours, the samples should be sent using dry ice. Samples should not be held at -20°C for long periods. The sample must be stored at – 70°C if storage is required for longer periods.

#### 14. How are we able to diagnose Nipah?

**Ans:** Mainly by molecular testing for viral RNA (RT-PCR). Other tests include: Serology testing for antibodies to Nipah, Histopathology and virus isolation which is tedious and time consuming.

MRI findings: Discrete high-signal-intensity lesions, measuring 2-7 mm, disseminated throughout the brain, mainly in the subcortical and deep white matter of the cerebral hemispheres due to widespread micro-infarctions from underlying vasculitis of cerebral small vessels.

#### 15. Can serology also be used as a confirmatory test?

**Ans:** If we target a conserved antigen and also since our population is not known to harbour antibodies to this virus it may be used.

#### 16. But how can we inactivate the virus for doing serological tests?

**Ans:** Where sera are processed, should be done in a separate room from the blood separation procedure, and this room is not used for any other purpose. The only people to work in this room should be trained operators, wearing full protective gear and working in a certified biohazard cabinet. The sera are aliquoted into inactivation buffer in masterplates as outlined in the ELISA protocol supplied with the reagents. This after the heat inactivation step where sera is inactivated at 56°C for 30 minutes following a 1:5 dilution in PBS buffer containing 0.5 percent Tween20 and 0.5 percent Triton-X100, the samples are considered non-infectious. (Alternatively, irradiation is an option)

Laboratories should consider carefully what can be done safely with their facilities, and develop standard operating procedures that are written down, approved by senior management, and in which staff are regularly trained and retrained from centres like CDC.

#### 17. Can all laboratories work with this virus?

**Ans:** NiV is classified as a biosecurity level 4 agent (BSL-4) and hence tests should be carried out in special labs mainly for preventing Laboratory Acquired Infections (LAIs) which has got high potential for community spread.





#### 18. What is BSL-4? How many such facilities are there in India?

**Ans:** Biosafety Levels (BSLs) are described based on combinations of laboratory practices and techniques, safety equipment, and laboratory facilities. Each combination is specifically appropriate for the type of procedures performed and documented or suspected routes of transmission of infectious agents. BSL-1 are basic teaching laboratories, BSL-2 are diagnostic services and research laboratories, BSL-3 are high containment special diagnostic services laboratories.

While BSL-4 are maximum containment laboratories to handle dangerous pathogens i.e. Risk Group 4 (high individual and community risk) defined as a pathogen that usually causes serious human or animal disease and that can be readily transmitted from one individual to another, directly or indirectly. Effective treatment and preventive measures are not usually available. The facilities needed are As Level 3 plus airlock entry, shower exit, special waste disposal with Class III BSC, or positive pressure suits in conjunction with Class II BSCs, double ended autoclave (through the wall) and filtered air.

High Security Animal Disease Laboratory (HSADL)	Bhopal, Madhya Pradesh	1998	This facility deals with zoonotic organisms and emerging infectious disease threats.
Centre for Cellular and Molecular Biology	Hyderabad, Telangana	2009	National BSL-4 Containment Facility for Human Infectious Diseases.
Microbial Containment complex	Pune, Maharashtra	2012	National Institute of Virology

#### In India BSL-4 facilities are available at:

#### 19. How can we prevent Nipah?

**Ans:** People can protect themselves from getting Nipah virus by limiting their contact with fruit bats and sick pigs in affected areas of Southeast Asia, and by not drinking raw date palm sap/toddy. People should also avoid direct contact with body fluids from infected patients by wearing appropriate personal protective equipment such as gloves, gown, and facemask, and practicing good hand hygiene.

#### 20. Any special advisory to health care professionals?

Ans: Yes we have to be prepared for an emergency and the things we need to keep in mind:

- ↔ Wash hands thoroughly with soap and water for 20 seconds after contact with a sick patient.
- While handling Nipah cases (suspected/ confirmed), standard precautions for infection control should be practiced.
- For aerosol generating procedures, PPE such as individual gowns (impermeable), gloves, masks and goggles or face shields and shoe cover and the procedure should be performed in airborne isolation room.
- Dedicated medical equipment should be used (preferably disposable whenever possible).
- All non-dedicated, non-disposable medical equipment used for patient care should be cleaned and disinfected as per manufacturer's instructions and hospital policies.
- Safe waste disposal for potentially infected material including used PPE, linen, clothing of patient according standard biomedical waste management guidelines.
- Admit all suspected cases of Nipah to the isolation ward/ facility in the hospital. Once the case is suspected of NIPAH, attendants should not be permitted in the ward.
- Segregate all suspected cases of Nipah patients from all patients in the isolation ward/ facility.
- Avoid unnecessary contact with suspected Nipah cases or use barrier nursing. Any spillage of body fluids in the OP/Ward should be managed as per Infection control guidelines.
- Mortuary staff should wear PPE while handling corpse of Nipah. Air sealed bag should be used for transportation of the dead body.
- Use of injections and sharps should be limited.





- If the use of sharp objects cannot be avoided, ensure that the following precautions are observed:
- a. Never replace the cap on a used needle.
- b. Never direct the point of a used needle towards any part of the body.
- c. Do not remove used needles from disposable syringes by hand, and do not bend, break or otherwise manipulate used needles by hand.
- d. Dispose of syringes, needles, scalpel blades and other sharp objects in appropriate, puncture-resistant containers. Never re-use syringes or needles.
- e. Ensure that containers for sharps objects are placed as close as possible to the immediate area where the objects are being used ('point of use') to limit the distance between use and disposal, and ensure the containers remain upright at all times. Ensure that the containers are securely sealed with a lid and replaced when  $\frac{3}{4}$  full.
- f. Ensure the containers are placed in an area that is not easily accessible by visitors, particularly children (e.g. containers should not be placed on floors, or on the lower shelves of trolleys in areas where children might gain access).
- g. Closed, resistant shoes (e.g. boots) should be used by all individuals in the patient care area to avoid accidents with misplaced, contaminated sharp objects.

#### 21. When can Kerala be declared Nipah free?

**Ans:** Nipah can have a maximum incubation period (IP) of up to 21 days so only when 42 days or 2 IPs pass the last confirmed case can the state be declared NiV free.

# 22. What are emerging viral infections? For the past few years Kerala has been plagued with one or the other viral infections. Why so?

**Ans:** Emerging infections have been defined as infections that have newly appeared, that have appeared previously but are expanding in incidence and geographic range, or that threaten to increase in the near future.

Over 30 new infectious agents have been detected worldwide in the last three decades; 60 per cent of these are of zoonotic origin. Developing countries such as India suffer disproportionately from the burden of infectious diseases given the confluence of existing environmental, socio-economic, and demographic factors. In the recent past, India has seen outbreaks of eight organisms of emerging and re-emerging diseases in various parts of the country, six of these are of zoonotic origin thus showing how vulnerable India is to the threat of evolving microbes.







### **ABSTRACT 1: Herbal nanoparticles for mosquito control**



## 66

Recent advancements in nanotechnology have validated the practical applications of various phytochemical constituents including nanoparticles from medicinal plants as a potential mosquito repellent and insecticide. Kavya Raj.K, Sreedev Puthur, A.N Anoopkumar, Lakshmi Jayakrishnan, Sharrel Rebello, Embalil Mathachan Aneeshbhb

Communicable Disease Research Laboratory, Department of Zoology,

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**Introduction:** Mosquitoes are considered as the potential vectors of several severe diseases such as zika, filariasis, dengue, malaria, yellow fever, and West Nile fever. The rapid proliferation of mosquito vector population must be reduced to prevent the aforesaid drastic diseases. Recent advancements in nanotechnology have validated the practical applications of various phytochemical constituents including nanoparticles from medicinal plants as a potential mosquito repellent and insecticide. Nowadays, the developments in the synthesis of nanoparticles have been extended in mosquito vector control approaches. The present study was made to synthesize nanoparticles from medicinal plants and insecticides in future.

**Methodology:** Herbal nanoparticles of AgNPs were synthesized and systematically characterized for validating the larvicidal efficacy of nanoparticles against fourth instar larvae of Aedes aegypti as per WHO protocol. The characterization of the synthesized nanoparticles was accomplished by using the modern techniques such as UV-visible spectrophotometry, Transmission Electron Microscopy (TEM), and Scanning Electron Microscopy (SEM). Statistical analysis was done by using SPSS 24.0.0.

**Results:** Potential larvicidal efficacy of nanoparticles against *Aedes aegypti* fourth instar larvae was reported in the present study with an LC50 - 150.686 ppm with confidence limit - 97.279 - 230.101. The LC90 value of nanoparticles against fourth instar larvae of *Aedes aegypti* was 982.441 ppm with confidence limit 506.820 - 5005.002.

**Discussion:** Several medicinal plants are known to exhibit larvicidal efficacy against mosquito vector population. Ramesh Kumar et al., in 2014 reported that the synthesized nanoparticles from numerous plant extracts exhibit potential larvicidal efficacy against *Aedes aegypti* population. Hence, the larvicidal efficacy of the synthesized nanoparticles might be due to the alterations or denaturation of the phosphorous containing elements or sulfur containing proteins such as DNA. This study strongly suggests that the nanoparticles synthesized from plants might be effectively suitable to control mosquito population over the world in future.



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## ABSTRACT 2: Green synthesis of silver nanoparticles by using stem extracts of *Rotula aquatica*

#### Sreya K J, Viji M O

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**Introduction:** Kallurvanchi (*Rotula aquatica*), a member of family Boranginaceae used in traditional medicinal practices can be well exploited for various pharmacognostical studies. The roots and leaves of kallurvanchi is an important traditional medicine for kidney stone. The present study deals with the green synthesis of silver nanoparticles and pharmacognostical profile of stem extract of *Rotula aquatica*.

**Materials and Methods:** Extraction procedure was soxhlet based extraction with different solvents namely chloroform, ethyl acetate and methanol. Microbial pathogens used in the determination of antibacterial activities of different plant extracts were *Staphylococcus aureus, Escherichia coli, Klebsiella pneumonia, Bacillus subtilis,* and *Proteus mirabilis* collected from MTCC. Microbial pathogen used in the determination of antifungal activities of different plant extracts were *Mucur indicus, Rhizopus stolonifer* and *Aspergillus niger*. Phytochemical constituents were analysed qualitatively using standard testing procedures. The green synthesis of silver nanoparticles using stem extract of *R. aquatica* and silver nitrate solution was accomplished by microwave assisted approach. UV- visible spectroscopy analysis, FTIR analysis and SEM analysis were performed. Brime shrimp lethality test (BSLT) was used to predict the cytotoxic activity in the plant extracts.

**Results and Discussion:** Qualitative analysis reflects the presence of Alkaloids, Flavanoids, Tannins, Phenolics, Glycosides, Carbohydrates and Anthraquinones. The nanoparticles were characterized by UV-visible spectrophotometer, FTIR and SEM. In UV spectrum analysis maximum absorption occur at 420nm. The absorption bands corresponding to O-H stretches, C=C stretches, C-N stretches and C-H bending were observed. Other minor bands were also observed. SEM analysis revealed the size of silver nanoparticles to be ranging from 200-250nm. In antibacterial and antifungal screening silver nanoparticle extract of stem showed significantly higher activity compared to other extracts of plant. LC50 values ranged from  $0.61-10.33\mu g/ml$ , silver nanoparticles showed high cytotoxic effect when compared to other extracts. The present study offers a new platform for green nanotechnology with a cost effective, eco-friendly and sustainable approach.



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Qualitative analysis reflects the presence of Alkaloids, Flavanoids, Tannins, Phenolics, Glycosides, Carbohydrates and Anthraquinones.





## ABSTRACT 3: CLOVORA – antimicrobial herbal mouthwash for oral hygiene



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The agar well diffusion method and disk diffusion method were used to study the antibacterial activity of mouth wash samples towards Escherichia coli, Streptococcus mutans, Staphylococcus aureus and Candida albicans. Athira Thomas, Amal PK, Anu anna Joy, Jessiya Jacob

Department of Phytomedical Science and Technology, MACFAST Thiruvalla

**Introduction:** Natural Mouthwash means the refined treatment for our mouth. The natural mouthwash is prepared using the natural ingredients. Using the natural mouthwash in conjunction with the brushing and flossing is a great way to reduce oral bacteria and even maintain the optimal oral health and hygiene. Here we taken some of the natural ingredients and formulated a mouth wash which have antimicrobial effects. It also has polyphenolic contents and pH level up to. And these all properties can improve our oral hygiene

**Materials and methods:** The extraction was done with both aqueous ethanol and water. 20g Clove, 20g Cinnamom and 5g Cardamom are weighed separately. After that it is crushed and divided into two part. The first part is mixed with 1litre water and second part is mixed with 500ml water + 500ml Ethanol. The extraction was filtered in a Buchner funnel under vacuum separately. The separated two samples were put into the rota vapour (60oc) for concentration. The concentrated samples are collected in a beaker and 1g Sodium bi-carbonate was added to each samples and marked separately.10ml of each samples given to Microbiology lab for further evaluation. Second extraction was done with 500ml water in first sample and 250ml ethanol and 250ml water for another sample. Both samples were filtered separately and concentrated for crystallization. The crystals were powdered and tested for the presence of polyphenols and the pH value.

**Oral anti-bacterial study:** The agar well diffusion method and disk diffusion method were used to study the antibacterial activity of mouth wash samples towards *Escherichia coli, Streptococcus mutans, Staphylococcus aureus* and *Candida albicans*. Cultured bacteria were mixed with agar media (150 ml/750µl).these were poured into sterilized petri plates. After solidification of agar medium, wells were made using well borer.100µl from each samples were added into the wells in different petri plates using micropipettes. And in the disc diffusion method discs were added instead of well. These petri plates were incubated at 37oc.

**Result:** From the above observation it is clear that sample 1 which is prepared in water solvent is better than sample 2 in aqueous ethanol extract. And also its pH is more appropriate to the oral pH. Then we formulated a mouthwash with sample 1.



### ABSTRACT 4: Molecular characterization of *Culex quinquefasciatus* gut's microbiota to develop new strategies for the Mosquito-borne disease prevention.

**Elsa Devassy,** Lekshmi Jayakrishnan, Sreedev Puthur, A.N Anoopkumar, Sharrel Rebello, Embalil Mathachan Aneesh

Communicable Disease Research Laboratory, Department of Zoology, St. Joseph's College Irinjalakuda, 680121

**Introduction:** The microbial fauna inhabiting the gut of mosquito vectors has play vital impact in the host-parasite interaction and upsurge the vectorial capacity of mosquito-borne diseases such as Japanese encephalitis, West Nile fever, and Lymphatic filariasis. Hence, mosquito gut is renowned as an important site of host-pathogen interaction and the survival of the pathogen is believed to be an outcome of the aforesaid interaction. Recent research reveals that the diverse bacterial fauna in the gut of mosquito vectors might expressively affect the development, metabolic activities together with the immunity of their hosts. The characterization and isolation of *Culex quinquefasciatus* guts microbiota will definitely contribute towards good perception of mosquito biology including mosquito-pathogen interaction to develop an effective mosquito vector control strategy in future.

**Methodology:** The dissections of the gut from field collected Culex quinquefasciatus larvae were performed using Labomed stereomicroscope with sterilized apparatus as per prescribed protocols. Larval gut dissections were carried out in sterilized and hygienic environment and dissected sections were homogenized separately. The isolated homogenates were cultured by pour-plated on nutrient agar media. The molecular identification and characterization of the isolated bacterial population from the gut of field-collected *Culex quinquefasciatus* mosquito vectors were accomplished by using 16S rRNA gene sequences. The prominent identification of isolated bacterial population in genus level was done using gram staining techniques and species level was based on Gen-Bank sequence comparison and the phylogenetic tree was constructed by using the MEGA7 software.

**Results:** The characterization and identification of gut bacterial fauna in *Culex quinquefasciatus* collected from 15 locations led to the identification of 2 bacterial genera including two species. Based on the Gram staining technique a total of two genera such as Bacillus (Gram positive), and pseudomonas (Gram negative) were identified. We constructed a phylogenetic tree to determine the relationship between isolated bacterial sequence and known sequence downloaded from The molecular identification and characterization of the isolated bacterial population from the gut of field-collected *Culex quinquefasciatus* mosquito vectors were accomplished by using 16S rRNA gene sequences.

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NCBI, Gen Bank. 16S rRNA gene sequence analysis confirmed the presence of two bacterial species such as *Bacillus thuringiensis* (Firmicutes) and *Pseudomonas aeruginosa* (Proteobacteria) in the Culex quinquefasciatus larval gut.

**Discussion:** Apte-Deshpande et al., in 2014 reported that bacterial fauna in the larval gut of mosquitoes might play significant impact over the host and parasite interaction. The present study reported the presence of two bacterial species within the gut of *Culex quinquefasciatus* larvae. Hence, the present study will help to understand the gut microbiota of mosquitoes and could be employed for the development of novel strategies to reduce the mosquito-borne disease transmission in future.

# ABSTRACT 5: Detection of Cytomegaloviruses in patients with generalized chronic periodontitis and its relationship with clinical parameters.

#### Annu Elizabeth Joseph1, Heera Pillai2, Thomas George V1

# 66

As bacterial etiology could not support and explain various aspects of periodontal disease, herpes virus is now proposed to be one of the causative factors responsible for the periodontal destruction. initia Enzabeth Josephi, neera Pinaiz, mornas George Vi

Pushpagiri Dental College, Thiruvalla 1

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**Introduction:** As bacterial etiology could not support and explain various aspects of periodontal disease, herpes virus is now proposed to be one of the causative factors responsible for the periodontal destruction. The purpose of present study is to detect the presence of Cytomegalovirus (CMV) in patients with chronic generalized periodontitis.

**Material and Methods:** A total of 5 patients were consecutively selected for the study, out of which 3 were diagnosed with moderate to severe chronic generalized periodontitis. Sub gingival material was taken from the deepest pocket of the dentition from every study subject, before the commencement of any procedure and polymerase chain reaction (PCR) assay were done to detect the presence of CMV.

Results: CMV DNA was detected qualitatively in 3 of the samples.

**Conclusion:** This study is in support to the previous studies and further emphasis on the proposed pathogenic role and clinical relevance of viruses in periodontitis.







# ABSTRACT 6: Green synthesis of silver nanoparticles by using by using *Curcuma angustfolia* rhizome

#### Reshna K R, Viji M O

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**Introduction:** *Curcuma angustifolia* is a rhizomatous herb which also known as white turmeric, narrow arrowroot, East Indian arrowroot, Bombay arrowroot etc. The genus Curcuma, a member of the *Zingiberaceae* family. It been used in traditional systems of medicine for long time. The present study deals with the green synthesis of silver nanoparticles and pharmacognostical profile of rhizome extract of *Curcuma angustfolia* 

#### Materials and methods:

• Sample preparation

Extraction procedure was soxhlet based extraction with different solvents namely chloroform, ethyl acetate and methanol.

• Synthesis of silver nanoparticles

The green synthesis of silver nanoparticles using stem extract of *Curcuma angustfolia* and silver nitrate solution was accomplished by microwave assisted approach.

- 1. UV-Visible spectroscopy analysis
- 2. SEM analysis
- 3. FTIR analysis
- Determination of antimicrobial activity

Microbial pathogens used in the determination of antibacterial activities of different plant extracts were *Staphylococcus aureus, Escherichia coli, Klebsiella pneumonia, Bacillus subtilis,* and *Proteus mirabilis* collected from MTCC. Microbial pathogen used in the determination of antifungal activities of different plant extracts were *Mucur indicus, Rhizopus stolonifer and Aspergillus niger*.

• Cytotoxic effect of the plant: Brime shrimp lethality assay

**Result and discussion:** The green synthesis of silver nanoparticles using rhizome extract of Curcuma angustifolia and silver nitrate solution gives synthesis of silver nanoparticles. The nanoparticles were characterized by UV-vis Spectrophotometer and SEM. In UV spectrum analysis maximum absorption occur at 420 nm. From SEM analysis it is found that the size of silver nanoparticles is ranging from 200-250 nm.

Screening test of rhizome extracts of *Curcuma angustifoli*a under study shows antibacterial activity against *Staphylococcus aureus, Escherichia coli, Klebsiella pneumonia, Bacillus subtilis* and *Proteus mirabilis* and

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The green synthesis of silver nanoparticles using rhizome extract of *Curcuma angustifolia* and silver nitrate solution gives synthesis of silver nanoparticles. The nanoparticles were characterized by UV-vis Spectrophotometer and SEM.





compared it with Tetracycline. In antibacterial screening of, silver nanoparticle extract of rhizome showed significantly higher activity compared to other extracts of plant.

In the brine shrimp lethality test, the silver nanoparticle extract of *Curcuma angustifolia* under study exhibited maximum inhibition. The minimum concentration at which the rhizome extract was found to exhibit cytotoxic potential was confirmed to be silver nanoparticle extract. The study provides evidence that the plants can be used in medicinal preparations to treat diseases and disorders.

# ABSTRACT 7: Phytochemical screening and TLC profile of rhizome extracts of Curcuma angustifolia

#### Sruthy Dinesh V, Viji M O

#### Dept. of Biotechnology, St Joseph's college Irinjalakuda

Introduction: Plants are a rich source of many natural products in India.

# 66

The qualitative analysis of phytochemicals present in the rhizome extract of Curcuma angustifolia under study showed the presence of alkaloids, tannins, flavanoids, polysterols, phenols, saponins, coumarins, resins, sterol, carbohydrate and polysaccharides.

Most of which have been used for traditional human health care. Kattu koova (Curcuma angustifolia) is a rhizomatous herb and it is a member of family zingiberaceae used in traditional medicinal practices can be well exploited for various pharmacognostical studies. It is an excellent diet in the form of conjee in case of Dysentery, Dysuria, and Gonorrhoea etc. It is essential oil used as antifungal medications and antibacterial. The present study deals with the phytochemical screening and TLC profile of rhizome extract of Curcuma angustifolia.

#### Materials and methods:

- Plant extract preparation: Extraction procedure was soxhlet based extraction with different solvents namely chloroform, ethyl acetate and methanol.
- Qualitative analysis of phytochemical: Phytochemical constituents were analysed qualitatively using standard testing procedures. Qualitative analysis reflects the presence of Alkaloids, Flavanoids, Tannins, Phenolics, Glycosides, Carbohydrates and Anthraquinones.
- Thin layer chromatography: The extracted samples were analysed by TLC using silicagel plate
- Determination of antifungal activity: Microbial pathogens used in the determination of antifungal activities of different plant extracts were Mucor indicus, Rhizopus stolonifer and Aspergillus niger.

**Result and discussion:** Curcuma angustifolia rhizome was chosen for present study. It was analysed with various aspects such as phytochemical screening and TLC profile. The qualitative analysis of phytochemicals present in the rhizome extract of Curcuma angustifolia



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under study showed the presence of alkaloids, tannins, flavanoids, polysterols, phenols, saponins, coumarins, resins, sterol, carbohydrate and polysaccharides. Thin Layer Chromatography analysis of rhizome extract in methanol, ethyl acetate, and chloroform extracts gave different Rf values shows the presence of various compounds. The intensity of the bands in the ethyl acetate rhizome extract of Curcuma angustifolia was more compared to the other extracts of plant.

Curcuma angustifolia under study shows antifungal activities against Mucor indicus, Aspergillus niger, Rhizopus stolonifer. The study confirmed the presence of phytochemical screening and isolation of active constituents in from the plant Curcuma angustifolia.

## ABSTRACT 8: Silver nanoparticle mediated molecular strategies for SYSTEMIC LUPUS ERYTHEMATOSUS (SLE): clinical trials and future prospects

Nidhilangelo M M, Sajeshkumar N. K

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**Introduction:** The prognosis of patients with Systemic Lupus Erythematosus (SLE) has greatly improved since treatment regimens combining corticosteroids and immunosuppressive medications as therapeutic strategies. Immune suppression is efficient but leads to higher susceptibility to other infectious and malignant diseases especially cancer and fungal diseases. Toxic effects and unexpected dramatic complications of current therapies have been progressively reported.

**Aim and objectives:** Identifying novel molecular strategies therefore remains an important issue in the treatment of SLE. The aim of this research work is to highlight the emerging pharmacological options and new therapeutic avenues for SLE with a particular focus on non-antibody molecular strategies.

**Materials and methods:** The green synthesized nanoparticle using egg fruit seed (*Pouteria campechiana*) fused with silver nanoparticle could be conjugated with protein in immunity like antibodies. This novel approach could reduce the symptoms and eventually cure SLE. Stock solution was prepared by dissolving 1mM sliver nitrate (AgNO3; Merck, Mumbai, India) and volume made up to 250 ml with distilled water. 10 ml of Egg fruit (*Pouteria campechiana*) seed extract was added to 90 ml of 1mM AgNO3 solution and allowed to react at room temperature. SEM-EDX Analysis was carried out in instrument JSM 6390 with acceleration voltage 20 kV.

**Results and Discussion:** A colour change was observed from colourless to dark brown. This occurred due to the reduction of silver ions present

A colour change was observed from colourless to dark brown. This occurred due to the reduction of silver ions present in the solution. Synthesized silver nanoparticles were characterized by UV-VIS

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Spectrophotometry.

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in the solution. Synthesized silver nanoparticles were characterized by UV-VIS Spectrophotometry. The maximum peak was found to be 435 nm (A max) for *Pouteria campechiana*.

**Conclusions:** The results showed that seed extracts of *Pouteria campechiana* could be effectively used for the green synthesis of silver nanoparticles. The nanoparticle antibody conjugate could effectively be used in the treatment of SLE and has promising leads in various therapeutic strategies.

## ABSTARCT 9: A review on bio surfactant production from Trichoderma sp., genetics and its application

#### Allie N U, Midhusha Johny

Department of Biotechnology, St. Joseph's college, Irinjalakuda, Thrissur

**Introduction:** Bio surfactants are the surface-active biomolecules produced by microorganisms with wide-range of applications. They were used in several industries including organic chemicals, petroleum, petrochemicals, mining, metallurgy (mainly bioleaching) etc. The field of production of bio surfactants by bacterial species is well explored, but relatively rare species of fungi are known to produce bio surfactants. Sophorolipids is the major type of bio surfactants produced by fungal species. *Trichoderma species* are cosmopolitan fungi. Their dominance in soil may be attributed to their diverse metabolic capability and aggressive competitive nature. The most frequently isolated species is *T. harzianum*, identified as an effective agent for solid waste bioconversion using spore suspension.

**Materials and Methods:** Sabouraud Dextrose broth can be used for the maximum production of bio surfactant. The bioprocess conditions for the maximum production can be optimized. Genotypic characterization of Trichoderma on the basis of macroscopic and microscopic morphology and 18S rDNA gene sequence homology. The bio surfactant-producing capability of the fungal isolates can be screened using oil displacement activity, emulsification index assay. The bio surfactant produced may be sophorolipids or glycolipoprotein based on the estimation of macromolecules and TLC analysis. The partially purified bio surfactant can also showed antimicrobial activity.

**Conclusion:** *Trichoderma sp.* can be analysed for bio surfactant production as it is widely spread in all types of soil. It also play important role in environmental biotechnology as a bioremediation agent. Sophorolipids are mainly the bio surfactant produced by fungi cells which is coded by sophorolipid gene cluster that involves five genes.

Sophorolipids are mainly the bio surfactant produced by fungi cells which is coded by sophorolipid gene cluster that involves five genes.

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# ABSTRACT 10: Green synthesis and characterisation of ZnO nanoparticles

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The rapid biological synthesis of zinc oxide nanoparticles is a simple and efficient route for synthesis of nanoparticles. The structure, morphology and optical studies of prepared ZnO NPs were examined by FT-IR, XRD and Photoluminescence. Athira P Anil, Biju Dharmapalan

Department of Bioinformatics, School of Bioscience, MACFAST, Thiruvalla

**Introduction:** Among all the inorganic semiconducting nanoparticles, zinc oxide nanoparticles have attracted increasing attention because ZnO is a green material that is biocompatible, biodegradable, and nontoxic for medical applications and environmental science. The Green synthesis of nanoparticles mainly concerns the elimination of hazardous wastes and the utilization of sustainable processes, implementation of environmental friendly chemicals, solvents and renewable materials. Biosynthesis of nanoparticles is a kind of bottom-up approach where the main reaction is occurring is reduction/oxidation. The microbial enzymes or the plant phytochemicals with antioxidant or reducing properties are usually responsible for reduction of metal compounds into their respective nanoparticles. These methods have slower kinetics and they offer better manipulation and control over crystal growth and their stabilization.

**Materials & methods:** The raw materials used are zinc nitrate, Capsicum frutescences leaves, distilled water, filter paper. Zinc nitrate is an inorganic chemical compound with the formula Zn (NO3)2. It is typically encountered as a hexahydrate Zn (NO3)2.6H2O, is used as substrate and the extract of Capsicum frutescences leaves used as reducing agent. The structural characterization of obtained product was done using XRD and FTIR analysis. The optical characterization was carried out by using photoluminescence studies

**Results & discussion:** The rapid biological synthesis of zinc oxide nanoparticles is a simple and efficient route for synthesis of nanoparticles. The structure, morphology and optical studies of prepared ZnO NPs were examined by FT-IR, XRD and Photoluminescence. The average grain size lies between 20–30 nm were found from XRD study as well as FT-IR spectra revealed the functional groups of stretching bands for ZnO NPs were found around 600–400 cm-1. This method of nanoparticle synthesis is inexpensive, stable and nontoxic, safe and eco-friendly without side effects of human beings.



# ABSTRACT 11: Studies on the changes in the enzyme activities during seed germination in *Vigna radiata*

#### Melna Mary.C.J, Dr. Kavitha.O

Dept. of Biotechnology, St.Joseph's College, Irinjalakuda

**Introduction:** Seed germination can be defined as the active growth of embryo within a seed, that result in rupture of seed coat and emergence of the radical and plumule constituting a young plant. Here we using *Vigna radiata* seeds study the changes in the enzyme activity and analyse the protein, carbohydrate and amino acid content of the germinating seeds of *Vigna radiata* at different stages of germination.

#### Materials and methods:

- Sample preparation: Extraction procedure was soxhelt based extraction with different buffer with different PH. (phosphate buffer 6.7, 7.5 &acetate buffer4.6).
- Estimation of carbohydrate by anthrone method, Estimation of protein by using Folin's Lowry's method, Estimation of amino acid using Ninhydrin and Assay of amylase activity, Assay of protease activity, Assay of invertase activity are used.

**Result and discussion:** During germination of seeds, a massive breakdown of the reserve substance being with the help of amylotic, proteolytic and lipolytic enzymes and the products are transported to the growing seedling for their development. The enzyme activity was found to be highest in the control. The activity was continuously decreasing during germination. Also protein, carbohydrate, amino acids content was decreasing during germination. During germination catabolism of lipids and carbohydrates produces energy for the germinating seeds and also contribute to the loss of the dry weight.

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During germination of seeds, a massive breakdown of the reserve substance being with the help of amylotic, proteolytic and lipolytic enzymes and the products are transported to the growing seedling for their development.

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## **ABSTRACT 12: Deducing the microbial diversity of raw honey**



Two bacterial colonies appeared after 24 hours of incubation. Good intact DNA was obtained from the DNA isolation and visualized under UV. 100ng of DNA was used for amplification and the products were visualized under UV.

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Sweety James <sup>1</sup>, Dr.Beena P.S<sup>2</sup>

Department of Biotechnology, St.Joseph's College Irinjalakuda, Thrissur<sup>1</sup>

Omics Gen LifeSciences, BIONEST, KINFRA HITECH PARK, Kalamassery, Kochi<sup>2</sup>

Introduction: Microorganisms in honey can influence quality or safety of honey. The microbes of concern in honey are primarily yeasts and spore-forming bacteria. Standard industry practices control yeast growth. Bacterial spores, particularly those in the Bacillus genus, are regularly found in honey. The spores of *Clostridium botulinum* are found in a fraction of the honey samples at low levels. No vegetative forms of disease-causing bacterial species have been found in honey. Bacteria do not replicate in honey and as such high numbers of vegetative bacteria could indicate recent contamination from a secondary source. Honey can be expected to contain low numbers and a limited variety of microbes. The use of honey in products that receive no or limited heat treatment may require quality tests .Here the study is a curiosity to find the bacterial population present in honey collected from premise of Hi-tech Kinfra Park, squeezed out of honey comb by apiarist. The present study is carried out to evaluate bacterial population in natural honey even after collection of one month and identify those observed bacteria.

**Materials and methods:** In this study the curiosity was to find the microbial diversity of raw honey collected from the premises of KINFRA HITECH PARK, Kochi. The honey sample was diluted in 1:15 ratio with distilled water and spread plated on to nutrient agar and incubated for 24 hours in an incubator at 37°C.Two colonies of bacteria was observed. The DNA isolation followed by the amplification of V3-V4 regions of 16S rRNA of the observed bacteria was carried out. The PCR products were sequenced using Sanger's Dideoxy method in a 3500 ABI machine (KRIBS-BIONEST, KINFRA HITECH PARK, Kalamassery). The sequences were analysed online using Basic Local Alignment Search Tool (BLAST) software.

**Result and conclusion:** Two bacterial colonies appeared after 24 hours of incubation. Good intact DNA was obtained from the DNA isolation and visualized under UV. 100ng of DNA was used for amplification and the products were visualized under UV. After sequencing the sequences were obtained in ABI format and FASTA format and utilized for analysis. It is confirmed that the organism OG\_03\_H1 observed is Bacillus cereus with 99% identity with'0' E values and 100% query coverage according to nucleotide homology and also confirmed that the organism OG\_04\_H2 observed is Bacillus toyonensis with 99% identity with '0' E values and 100% query coverage according to nucleotide homology and also confirmed that the organism of uncleotide homology. This study confirmed the presence of bacteria capable of survival in honey which justifies previous reports on organisms in honey. This result will be an insight to the microbes that can withstand high sugar content and survive for long.





# ABSTRACT 13: Isolation and identification of endophytic and epiphytic bacteria from phyllosphere

#### Silpa Mohandas, Dafini Mendez

Dept. of Biotechnology, St Joseph's college Irinjalakuda

**Introduction:** Main focus of the present study deals with the isolation identification and pattern of diversity of endophytic and epiphytic bacteria from phyllosphere of Arutha (*Ruta graveolens*), Nilamparanda (*Desmodium triflorum*), Marigold (*Tagetes erecta*), and Nettle (*Urtica parviflora*). Multitrophic interaction in phyllosphere will be the basic step to develop techniques for plant protection in future and understanding roles and importance of indigenous bacteria, the better is to predict and protect plant against pathogen infection.

**Materials and methods:** For the isolation of endophytic and epiphytic bacteria, healthy leaves were collected from Irinjalakuda and Wayanad. Then sterilized and extracts were made and used for preparing standard plate count method. The heterotrophic bacterial strains were isolated and identified by using macroscopic, microscopic features like Gram staining ,Spore staining and biochemical tests like catalase test, oxidase test, DNase test , Triple sugar iron test , Antibiotic sensitivity test (Kirkby - Bauer method) upto generic level.

**Result and discussion:** The present study reveals the bacterial diversity in the phyllospheric region of four different medicinal plants. Total heterotrophic cultivable bacteria showed a progressive increase in endophytic region than in epiphytic region. 12 different bacterial genus were identified, of these gram positive bacteria were dominant group found in the phyllospheric region of the plants. Precisely gram positive cocci-23%, gram positive rod-40%, gram negative rod-37%. The study reveals that Bacillus were found to be the dominant genus and Chromobacter, Aeromonas, Staphylococcus, Sarcina, forms the least occurred genus. In endophytic bacterial distribution pattern Micrococcus was found to be the dominant group and in epiphytic bacterial distribution pattern Klebsiella was the most occurring one. Phyllospheric bacteria also have potential for application in industrial biotechnology because of their ability to produce enzymes of industrial importance.

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The present study reveals the bacterial diversity in the phyllospheric region of four different medicinal plants. Total heterotrophic cultivable bacteria showed a progressive increase in endophytic region than in epiphytic region.

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# ABSTRACT 14: Studies on the primary and secondary metabolites present in the leaf extracts of *Artocarpus altilis* and their antimicrobial activity

Sreelakshmi T B, Kavitha O

Department of Biotechnology, St. Joseph's college, Irinjalakuda, Thrissur

**Introduction:** Artocarpus altilis (bread fruit) is tropical plant belongs to the family *Moraceae*. The extracts and metabolites from leaves, stem, fruit and bark contain numerous beneficial biologically active compounds which can be used for antimicrobial, antifungal, tyrosinase inhibitory and cytotoxicity activities. The present study is based on the properties of bread fruit (*Artocarpus altilis*) plant leaf.

#### Materials and methods:

- Plant extract preparation: Extraction using methanol and hot water. The water extract was used for quantitative estimation of primary metabolites while methanol extract was used for qualitative analysis of secondary metabolites.
- Quantitative estimation of primary metabolites: The primary metabolites like carbohydrates, proteins and free amino acids were estimated quantitatively by standard procedures such as Anthrone method, Folin-Lowry method and Ninhydrin test respectively.
- Qualitative analysis of secondary metabolites: The preliminary phytochemical analysis was carried out using methanolic extract by standard procedures like Salkowshi's test, Liebermann Burchard test, lead acetate test, ferric chloride test, foam test, etc.
- Antimicrobial activity: Antimicrobial activity of the extract was analyzed by Kirby Bauer (agar disc diffusion method) using various test organisms like Escherichia coli, Klebsiella pneumoniae, Bacillus subtilis and Staphylococcus aureus.

**Result and discussion:** The estimation of the primary and secondary metabolites showed that the extract contain considerable amount of carbohydrates (5.6 mg/g), protein(4.4mg/g), free amino acids (4.2mg/g), secondary metabolites like phenol, flavonoids, steroids, saponin and tannin. The methanolic extract given positive results for antimicrobial activity against the test organisms used with the following zone diameters: *Staphylococcus aureus* (10mm), *Escherichia coli* (4.5mm), *Klebsiella pneumonia* (16mm) and *Bacillus subtilis* (11mm). The results were very much encouraging but scientific validation is necessary before being put into practice.

The estimation of the primary and secondary metabolites showed that the extract contain considerable amount of carbohydrates (5.6 mg/g),protein(4.4mg/g), free amino acids (4.2mg/g), secondary metabolites like phenol, flavonoids, steroids, saponin and tannin.

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## ABSTRACT 15: Hematological and clinical profile of patients with Dengue during an outbreak in Kerala 2016 at a tertiary care centre in Central Kerala

#### Kavitha R Nair1, Dr. Seema Oommen1, Dr. Vidya Pai2

Department of Microbiology, Pushpagiri Medical College1, Department of Microbiology, Yenepoya Medical College2

**Introduction:** Dengue fever has become a major national health problem. The spectrum of dengue is wide ranging from asymptomatic to symptomatic disease which further manifests as classical dengue fever, dengue hemorrhagic fever and shock syndrome. Transmission is more intense during rainy and post monsoon periods often leading to outbreaks. During each outbreak demographic and clinical profile of patients varies. This is mostly dependent on the circulating serotypes. This study was aimed to study the hematological profile and clinical manifestations of dengue during an outbreak in Kerala, 2016.

**Materials and methods:** A prospective study was conducted in Pushpagiri Medical College, Tiruvalla, Kerala from May 2016 to August 2016. A total of 236 adult patients who were NS1 positive were further analyzed for their biochemical, hematological and clinical profiles.

**Result:** Out of 236, 183(77.5%) were diagnosed as primary dengue and 53 (22.5%) as secondary dengue infection. Common clinical symptoms were fever (100%), generalized body ache (53%), headache (42%), vomiting (22%), and abdominal pain (10%). Thrombocytopenia, leucopoenia and elevated liver enzymes were observed. All patients improved clinically with improvement of biochemical and hematological parameters. Case fatality amongst these patients were nil.

**Conclusion:** Most common form of clinical presentation was primary dengue. Presence of thrombocytopenia and elevated liver enzymes are more indicative in secondary infection.

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Out of 236, 183(77.5%) were diagnosed as primary dengue and 53 (22.5%) as secondary dengue infection. Common clinical symptoms were fever (100%), generalized body ache (53%), headache (42%), vomiting (22%), and abdominal pain (10%). Thrombocytopenia, leucopoenia and elevated liver enzymes were observed.

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# ABSTRACT 16: Evaluation of culture methods for isolation of *Mycobacterium tuberculosis* complex and its resistance among pulmonary tuberculosis cases in a tertiary care setting, Kerala



Culturing of the organism in addition to smear, increases the possibility of microbiological diagnosis and offers a possibility for drug susceptibility testing. Sreeja Nair1, Dr. Seema Oommen1, Dr. Vidya Pai2

Department of Microbiology, Pushpagiri Medical College 1, Department of Microbiology, Yenepoya Medical College 2

**Introduction:** Tuberculosis is an important public health problem in India and globally. Prompt detection and susceptibility testing of *Mycobacterium tuberculosis* from clinical specimens is essential for appropriate management of patients with tuberculosis. With this in background, this study aimed at the comparison of isolation of *Mycobacterium tuberculosis* complex from clinical specimens of patients suspected of pulmonary tuberculosis using BACTEC Micro MGIT and Lowenstein Jensen (LJ) media with detection of resistance.

**Materials and Methods:** A prospective study was carried out for one year from November 2016 to October 2017 in the Department of Microbiology, Pushpagiri Medical College, Tiruvalla, Kerala. A total of 81 samples were processed for Ziehl-Neelsen (Z-N) staining and was then cultured using liquid Mycobacterium growth indicator tube (BDTM BACTECTM Micro MGIT) and solid (Lowenstein Jensen's) media. Isolates confirmed as Mycobacterium tuberculosis complex were subjected to drug susceptibility testing (DST) using modified proportion method by MGIT for 1st line drugs i.e. streptomycin ( $1.0\mu g/ml$ ), isoniazid ( $0.1\mu g/ml$ ), rifampicin ( $1\mu g/ml$ ), ethambutol ( $5\mu g/ml$ ) and pyrazinamide ( $100\mu g/ml$ ).

**Results:** Of the total 81 samples that were cultured, 7(8.6%), were positive by both MGIT and Lowenstein Jensen's. Three (3.7%) were positive by MGIT only and not by LJ. Only 4/10 (4.9%) culture positive samples were positive by direct smear examination. Turnaround times for culture positivity by MGIT vs LJ was an average of 15.1 days vs 23.8 days. Sensitivity to all drugs was observed in 4/10 (40%) isolates while, monoresistance was documented to pyrazinamide and streptomycin in 4/10 (40%) and 1/10 (10%) respectively.

**Conclusion:** Culturing of the organism in addition to smear, increases the possibility of microbiological diagnosis and offers a possibility for drug susceptibility testing. This would result in better administration of appropriate antimycobacterial therapy thereby decreasing morbidity and mortality as well as preventing the spread of infection in the community.





# 38

# ABSTRACT 17: Isolation and molecular identification of *Vibrio cholerae* from seafood marketed in Cochin area

Larlyn Katharpi, **Preenanka. R,** Anu Ruby Benny, Rinsila Kareem K A, Safeena M P

Kerala University of Fisheries and Ocean Studies (KUFOS), Panangad, Ernakulam

**Introduction:** Choleragenic *V. cholerae* is an important public health concern in developing countries like India because of its ability to produce cholera toxin (CT). *V. cholerae* is autochthonous to aquatic environment and it is well recognized that seafood is frequently contaminated with this pathogen and thus it plays a major role in the disease transmission. Hence this study aimed to investigate the occurrence of *V. cholerae* in seafood marketed in Cochin area by PCR.

**Methodology:** The seafood samples were collected from markets in Cochin and V. cholerae was isolated by following USFDA-BAM method. After extracting the DNA by CTAB method, it was subjected to PCR for differentiating the strains into toxigenic and non-toxigenic. Molecular differentiation of the strain was done by targeting the ctxA gene for toxigenic strains and ompU gene for non-toxigenic strains using predesigned primers.

**Results and discussion:** Out of 20 samples of fish collected, 8 (40%) samples showed typical colonies of *V. cholerae* on TCBS agar whereas out of 13 samples of shrimps and 10 samples of clams collected, 10 (77%) and 8 (80%) samples showed presumptive *V. cholerae* colony on TCBS agar respectively. The isolates were biochemically confirmed by IMViC tests, Oxidase test, Catalase test and TSI test. Among the 22 enrichment lysates, none of them showed positive bands for ctxA gene (564 bp), whereas two isolates were shown positive bands for ompU gene (896 bp). It has been suggested that ompU gene is involved in conferring bile resistance, which may be required for survival in the human gastrointestinal tract. In earlier studies of ompU with *V. cholerae* O1 strains, reported that ompU was detected in most of the environmental non-O1/non-O139 strains of *V. cholerae*.

**Conclusion:** *Vibrio cholerae* isolated from seafoods marketed in Cochin was confirmed by biochemical analysis. Molecular identification of ompU gene in 2 strains among 22 isolates revealed the presence of non-toxigenic strains in seafood.

Vibrio cholerae isolated from seafoods marketed in Cochin was confirmed by biochemical analysis. Molecular identification of ompU gene in 2 strains among 22 isolates revealed the presence of non-toxigenic strains in seafood.

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# ABSTRACT 18: Evaluation of anti-microbial and antioxidant effects of *Garcinia gummi-gutta* seed oil

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G. gummi-gutta seed oil significantly inhibited the growth of pathogens such as Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus, Salmonella typhi and Bacillus subtilis.

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#### Shiney George, Krishnakumar N M

Department of Biotechnology, Presentation College of Applied Sciences, Puthenvelikkara

**Introduction:** *Garcinia gummi-gutta* (L.) N. Robson is commonly known as 'Malabar tamarind' or 'Kodampuli' (Family: *Clusiaceae*) is a multipurpose tree naturally found in the evergreen and semi-ever green wild forests of Western Ghats, and also grown in the home gardens of Kerala. The fruit rind is extensively used in culinary preparations as flavoring agent and it is a rich source of anti-obesity compound, hydroxy citric acid.

**Materials and Methods:** *G. gummi-gutta* seeds were collected, washed thoroughly, sun-dried, finely powdered and heated with water to extract the oil. The anti-microbial and antioxidant effects of the seed oil were evaluated by standard methods.

Results and Discussion: G. gummi-gutta seed oil significantly inhibited the growth of pathogens such as Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus, Salmonella typhi and Bacillus subtilis. The maximum percentage of inhibition of microbial growth (94.19%) was observed in S. aureus by the seed oil (100 µL) treatment. The seed oil exhibited a dose dependent inhibitory effect on the growth of microorganisms selected for the study. G. gummi-gutta seed oil (100 µL) showed maximum growth inhibition percentage of 27.40% and 36.36% against fungi Aspergillus niger and Candida albicans respectively, compared to the control. The seed oil also exhibited significant free radical scavenging effect in vitro. The GC-MS analysis of the seed oil revealed the presence of fatty acids such as 9-octadecenoic acid, stearic acid and palmitic acid. Therefore, it could be used as a renewable natural resource for the development of natural anti-microbial for the treatment of different bacterial and fungal diseases. The phytochemical compounds present in the seed oil are responsible for its anti-microbial and antioxidant activities and detailed study is needed to isolate the active compounds responsible for the anti-microbial and antioxidant effects and also to elucidate the exact mechanism of action.



# ABSTRACT 19: Molecular characterisation and RAPD-PCR typing of *Aeromonas hydrophila* isolated from clinical samples

#### Midhusha Johny1, and R Subashkumar2

1. Department of Biotechnology, St. Joseph's College, Irinjalakuda, Thrissur.

2. Department of Biotechnology, Sri Ramakrishna College of Arts & Science, Coimbatore.

**Introduction:** Aeromonas hydrophila is a Gram negative, facultative anaerobic, rod shaped bacteria found in different environments. It is associated with intestinal and extra intestinal infections in human. Multifactorial virulence genes plays important role in Aeromonas pathogenesis. It is also considered as the etiological agent of wide spectrum of diseases in human and animals. RAPD-PCR is a molecular approach used for typing of clinical isolates of *A. hydrophila* from various disease outbreaks. Nowadays, *A. hydrophila* poses variety of challenges to public health and scientific community.

**Materials & Methods:** In the present study, total of 42 different clinical samples were collected from Thrissur, Kerala. We aimed to identify and characterize *A. hydrophila* isolates using phenotypic method .16s r RNA PCR used in this study for genotypic characterization of *A. hydrophila* strains using specific primer. All the positive strains were subjected to RAPD-PCR for determination of genetic relationship among Aeromonas isolates.

**Result & Discussion:** Increasing prevalence of *A. hydrophila* were observed from different clinical samples. An intact band positioned at 1050 bp in gel electrophoresis were showed the 16 s rRNA-PCR amplification of *A. hydrophila* isolates. All the positive isolates produced bands with different molecular weights in RAPD-PCR. So, the study point out that RAPD-PCR act as a powerful molecular marker in molecular diagnostics.

Increasing prevalence of A. hydrophila were observed from different clinical samples. An intact band positioned at 1050 bp in gel electrophoresis were showed the 16 s rRNA-PCR amplification of A. hydrophila isolates.

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# Phytoceuticals and Molecular Biology Research Lab Scientist: Dr. Yogesh Dalvi, PhD, yogesh\_dalvi@pushpagiri.in

The lab is involved in the collection of Medicinal Mushrooms and its bioactivity. These mushrooms ore collected from different parts of Western Ghats of India including Maharashtra, Karnataka and Kerala states. The identification is carried out using micro/macro morphology and molecular identification through a range of DNA conserved regions followed by evolutionary-relationship using Phylogenetic study.

The extracts are made using various polar/non-polar solvents and each extract is characterized by HPLC, HPTLC, GC-MS and LC-MS. These extract are further explored for their various activities like anti-inflammatory, anti-hyperglycaemic, anti-angiogenic, anti tumurogenic and wound healing by biochemical, cellular and molecular analysis using in vitro and in vivo models.

# Pushpagiri Centre for Virology Scientist: Mr. George Varghese, MSc Medical Microbiology (CMC Vellore), surugv@pushpagiri.in

Involved in providing diagnostic services and cutting-edge research on viruses and viral diseases in humans. Aims at maintaining surveillance and developing affordable technologies for diagnosis of viral diseases. Pushpagiri centre for virology has been groomed into a premier centre for viral diagnosis and research in Central Travancore. It is equipped with state of the art facilities. The mission of the centre is to initiate diagnostic services and research on viruses and viral diseases of humans.

**Diagnostic Viral Serology Facilities:** The laboratory is handling various types of specimens, primarily from Pushpagiri Medical College Hospital. Serological diagnosis of HIV, Hepatitis viruses, including various HBV markers, Dengue Fever, Japanese Encephalitis, Measles, Rubella, Herpes Simplex Viruses, Cytomegalovirus etc. Apart from ELISA's, the volume of work is managed by fully automated systems like Vidas Biomerieux using fluorescence method, ELFA. The lab is giving valuable services during seasonal outbreaks of Influenza, Dengue etc.

**Diagnostic Molecular Virology Facilities:** The hall mark of Pushpagiri Centre for Virology is the diagnostic molecular virology. The lab is equipped with Conventional and real time PCR facilities. Standardized PCR's available for several viral disease. Qualitative PCR has been The mission of the centre is to initiate diagnostic services and research on viruses and viral diseases of humans.

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standardized for Hepatitis B, Hepatitis C, Herpes Simplex Virus 1&2, Japanese Encephalitis, Dengue, Respiratory Syncitial Virus etc. Real time PCR is used for quantification of Hepatitis B & C and Enterovirus diagnosis. Few bacterial diseases of importance like Leptospirosis, Tuberculosis are also been done on real time PCR mode. The quality of the lab is evaluated with internal quality controls and external quality controls (EQAS) regularly. Though in its infancy, we do have a good virus cell culture laboratory too.

Research Facilities: Research at virology has been aimed at elucidating the epidemiology in the local community, initiating and maintaining surveillance, studies in the genetic variations of viruses and developing affordable technologies for the provision of diagnostics for diseases. It also offers consultancy services for the evaluation of commercial kits. The centre also provides training programmes in PCR and RT-PCR.

# Regenerative Medicine and Tissue Engineering Lab Scientist: Dr. Nebu George Thomas, MDS, PhD, nebugeorgethomas@pushpagiri.in

Involved in the development of scaffolds, using principles of engineering and life-sciences, which may help regenerate tissue lost due to various diseases or injuries. The goal of the lab is to develop scaffolds that have the ability to induce cellular response and stimulate regenerative cells. Focus on bone and skin regeneration in general, and periodontal tissue in particular. Also involved in studying interaction of various biomaterials with tissue structures in the body.

# Pushpagiri Clinical Epidemiology Unit Scientist: Dr. Philip Mathew, MD, pushpagiriceu@pushpagiri.in

Involved in providing statistical support to research projects, apart from services like proposal writing and study designs. The unit is affiliated to Indian Clinical Epidemiology Network and conducts various accredited short term training programmes in Biostatistics and Epidemiology.

# Metabolic Disorders and biochemistry Research Lab Scientist: Dr Kannan Vaidyanathan, MD, drkannan@pushpagiri.in

Involved in research and diagnosis of metabolic disorders, and characterizing molecular aspects of human genetic disorders including cancer. The lab also offers diagnostic services in inborn errors of metabolism and genetic disorders, apart from training programmes in techniques like HPLC.



43

# Microbial Technology Research & Infectious disease laboratory Scientist: Dr. Sherly Antony, MD, drsherly@pushpagiri.in

Involved in the testing of antimicrobials from natural products as well as synthetic materials that can lead to drug innovations and remediation strategies. The emergence of antimicrobial resistance is a global health problem. Hence solutions encouraging and facilitating the evolution of new antimicrobials are the necessity of the hour. Also new emerging & re-emerging infectious diseases are scrutinised with in depth analysis of the agents of infectivity, their molecular characterisation and co-relation to severity of disease manifestations. Short term training programmes in basic clinical microbiology are offered.

## Animal House Experiment Facility: Dr. Santosh Pillai, MD, drsantosh74@pushpagiri.in

Animal house in Pushpagiri Institute of Medical Sciences and Research Centre was established in the year 2008. It is registered with Ministry of Environment and Forests (Animal Welfare Division), Govt. of Indio. The Institutional Animal Ethics Committee (IAEC) was established in the Institute, in accordance with the standards established by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). The IAEC scrutinizes each project to ensure that it.









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