13th ANNUAL CONFERENCE AND SCIENTIFIC SESSIONS

SRI LANKAN SOCIETY FOR MICROBIOLOGY (SSM)

E-BULLETIN

7TH DECEMBER 2024

SRI LANKAN SOCIETY FOR MICROBIOLOGY

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SSM 2024

e-BULLETIN

Volume 12

7th December 2024

J Hotels, Jaffna

e-BULLETIN

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SSM 2024

Published by

Sri Lankan Society for Microbiology

Edited and designed by

Prof Vasanthi Thevanesam

Prof Veranja Liyanapathirana

Prof Faseeha Noordeen

Cover design

Prepared by Dr K Thivakar

Formatting

Prof Vasanthi Thevanesam

Prof Veranja Liyanapathirana

Prof Champa Ratnatunge

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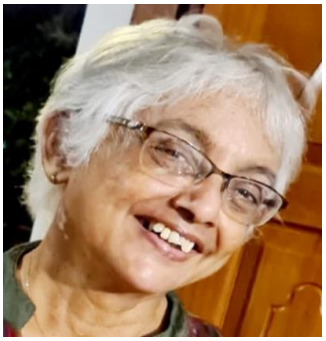

It is a great pleasure for me to welcome all of you to the 13thAnnual Conference of SSM. Taking the conference out of Kandy, its birthplace, is a 'first' and I hope it will encourage us to 'move' the conference to other venues outside Kandy in future years.

The mission of the Sri Lankan Society for Microbiology (as stated in its constitution) is to promote an integrated multidisciplinary approach towards the study and application(s) of basic and applied microbiology. It is very rewarding that even in times of hardship resulting in resource deprivation as experienced in the last few years of people, space, equipment and consumables needed for scientific endeavours, 'science' continues to progress. Thanks to the efforts of researchers – both new and those established in research, the President and Committee members of the Jaffna Chapter, and many others, we were able to organise this important calendar event for the Society this year. A heartfelt thanks to all of you.

As we look at the programme today, apart from the place change of venue, there are some changes from previous years. For the first time since the beginning of the annual SSM conference, the Jaffna Conference Committee arranged two pre-conference events – the first, is a culmination of a 4-year programme in the Jaffna peninsula of bringing awareness of use as well as misuse of antibiotics to a secondary school population. Having completed the first cycle, the Jaffna Chapter of SSM hopes to restart the second cycle to a new cohort – thereby attempting to instill an awareness of the necessity for avoiding inappropriate use of antibiotics to a younger generation. The second pre-conference event was planned to help understand the implications of the 'microbiome' – communities of microorganisms found in all environments – be it animal, human, the plant world, soil and water and any other environment we encounter. I thank the Committee and all those who worked hard to arrange these two events as well as the resource persons who have committed their time not only in preparation but also in travelling to Jaffna for these 2 events.

The presentations at the conference echo this same theme – microbes in our environment and their interactions – which are often positive and yet can also cause damage. The interaction of scientists as they study these effects and seek to modify them is of utmost importance. I hope that during today's proceedings, you will be able to meet and get to know scientists from different institutions as well as disciplines. By building an interacting scientific community, we could contribute towards fulfilling the SSM mission - to promote and practice an integrated multidisciplinary approach towards the study and application(s) of basic and applied microbiology.

Professor Vasanthi Thevanesam
President, Sri Lankan Society for Microbiology
7.12.2024

Message from Chairman, Conference Committee SSM 2024

With great pride and enthusiasm, I present to you the proceedings of the 13th annual conference and scientific sessions, held on 7th December 2024 in Jaffna for the first time, under the theme *"Microbial Marvels and Hazards: Exploring the Unseen World"*. This conference has brought together a remarkable group of scientists, researchers, and professionals to explore the dual nature of microorganisms, their extraordinary potential and their significant challenges.

From the beneficial roles of microbes in human health, agriculture, and biotechnology, to the dangers they present as pathogens and environmental hazards, our theme sought to highlight the full spectrum of microbial life. The conference sessions provided an exciting opportunity to delve into cutting-edge research on microbial ecology, antibiotic resistance, emerging infectious diseases, the human microbiome, and much more. Together, we have celebrated the marvels of microbes and confronted the complexities and hazards they present in our interconnected world.

The papers and abstracts compiled in this proceedings book are a testament to the dedication and innovation of the microbiology community. They represent the latest advances in our understanding of microbial systems and their vast, often hidden, impacts on ecosystems, human health, and global sustainability.

I would like to express my sincere appreciation to all the keynote and plenary speakers, session chairs, presenters, and participants whose contributions made this conference an enriching experience. A special thank you is also due to our organizing committee, sponsors, and volunteers, whose hard work and support ensured the success of this event.

As we continue to investigate the marvels and hazards of the microbial world, I am excited to see how the discussions and collaborations sparked during the conference will drive new research and innovations in microbiology. I hope the findings presented here will serve as a valuable resource for both current and future generations of researchers, educators, and policymakers in our shared mission to better understand and harness the power of the unseen world of microbes.

Thank you once again for your participation, and I look forward to continued collaboration and discovery in this fascinating and ever-evolving field.

Prof Gitanjali Sathiadas
Conference Chair
Faculty of Medicine/University of Jaffna

SSM Conference Organizing Committee -2024

Sri Lankan Society for Microbiology (SSM)

13th Annual Conference and Scientific Sessions

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Programme

Sri Lankan Society for Microbiology ***13th Annual conference and Scientific Sessions – 7.12.2024***		
Registration		**8.30 – 9.00 am**
Welcome – President SSM		**9.00 – 9.10 am**
Keynote Address: Prof Malik Pieris **Pandemic preparation**		**9.10 – 9.40 am**
Free paper/poster session 1: **Surveillance and diagnostics of infectious diseases in humans**		
OP1	Viral diversity and seasonal variation in acute respiratory infection in patients at Teaching Hospital, Jaffna, Sri Lanka	**9.45 – 9.52 am**
OP2	Knowledge and practices on handling of sharps among healthcare workers of two hospitals in Sri Lanka	**9.53 – 10.00 am**
OP3	Molecular detection of Rickettsia in peripheral blood of patients suspected of spotted fever rickettsiosis who tested sero-negative in indirect immunofluorescence assay	**10.01 – 10.08 am**
PP1	Preliminary study comparing smear and standard tuberculosis culture to GeneXpert MTB/Rif Ct categories in pulmonary and extrapulmonary samples in a local laboratory setting	**10.12 – 10.15 am**
PP2	Study of urine samples received for culture, and sensitivity patterns of the urinary pathogens at a tertiary care hospital in Sri Lanka.	**10.16 – 10.19 am**
PP3	Detection of carbapenemase genes in Gram negative bacterial isolates from patients with urinary tract infections	**10.20 – 10.23 am**
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PP5	Comparison of sheep blood and human blood-based media for the isolation and identification of selected pneumococcal strains	**10.27 – 10.30 am**
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TEA BREAK		**10.45 – 11.00 am**
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OP6	Nasal colonization of *Staphylococcus aureus* and *Streptococcus pneumoniae* in preschool children attending selected immunization clinics, Kandy, Sri Lanka	**11.51 – 11.58 am**
PP6	Study on antimicrobial sensitivity of staphylococci isolated from the external ear canal of cats presented to the Veterinary Teaching Hospital, University of Peradeniya	**11.59 am – 12.02 pm**
PP7	Contaminated theatre footwear: a potential source of infection for the wearer in Teaching Hospital Anuradhapura	**12.03 – 12.06 pm**
PP8	Preliminary investigation of microbial diversity and antibiotic susceptibility profiling of *Staphylococcus* spp. isolated from tick salivary glands infesting domestic animals in Kandy	**12.07 – 12.10 pm**
PP9	Bacterial diversity in water distributed under intermittent water supply	**12.11 – 12.14 pm**
PP10	Molecular detection of the spotted fever group rickettsial DNA in ticks in the Central Province of Sri Lanka	**12.15 – 12.18 pm**

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OP8	Evaluation of the antibacterial activity of *Camellia sinensis* (green tea) extract against *Streptococcus mutans*	**2.09 – 2.16 pm**
OP9	Exploring tender coconut water as a viable medium for fungal growth: A comparative study with Potato Dextrose Agar	**2.17 – 2.24 pm**
PP11	Comparison of physico-chemical properties of wine fermented with palmyrah fruit pulp and cashew apple juice	**2.25 – 2.28 pm**
PP12	Isolation and identification of potent naringinase-producing bacteria from different plant parts of *Citrus aurantium*	**2.29 – 2.32 pm**
PP13	Preliminary and potential effect of curd on management of uncomplicated urinary tract infections - A case report	**2.33 – 2.36 pm**
PP14	Alternate substrate formula for mushroom cultivation by utilizing coir waste	**2.37 – 2.40 pm**
PP15	Preliminary evaluation of in-house miniaturized biochemical test strips for identification of common enteric Gram-negative pathogens encountered in veterinary clinical practice	**2.41 – 2.44 pm**
PP16	Survival of probiotic lactic acid bacteria (LAB) in bee products incorporated bio- yoghurts	**2.45 – 2.48pm**
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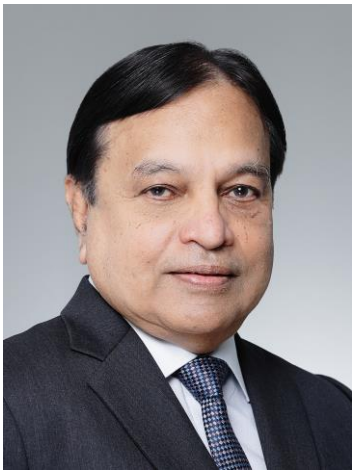
TEA BREAK		**3.00pm – 3.20pm**
Presentation of SSM scientific publication awards & best presentation awards		**3.20pm – 3.30pm**
Vote of thanks – Conference Chair		**3.30pm – 4.00 pm**
Close of Conference		

Keynote address

Pandemic preparedness



Malik Peiris,
School of Public Health, The University of Hong Kong

Pandemics emerge when animal pathogens (usually RNA viruses) spillover to humans and establish sustained transmission within human populations. Anthropogenic activities are increasing the probability of these spillovers and thus, increasing risk of pandemics. While COVID-19 had significant impacts on morbidity, mortality, social well-being and economies globally, future pandemics may well be even more severe.

Much attention is currently, rightly, being placed on capacity for rapid development of vaccines following the identification of a new pandemic pathogen. However, pandemic preparedness needs to address a range of issues, including, but not limited to, vaccines. Even if the "100-day vaccine challenge" is met, a novel vaccine whose development started after a new pandemic pathogen is detected, will not impact the first pandemic wave, even in developed countries. It will be even more delayed in reaching the developing world. Thus, pandemic preparedness should also address diagnostic capacity (including point of care diagnostics), therapeutics and epidemiological capacity to rapidly assess characteristics of a new pathogen to inform deployment (or not) of public health and social measures (to suppress transmission until vaccines become available).

Attention to measures that reduce risk of spillovers ("prevention at source") needs much more attention as well. Intensive animal husbandry practices which involve moving livestock large distances to achieve maximal production efficiency or for marketing, live game animal trade for food or as pets, live poultry markets, hunting and consumption of "wild game" provide common interfaces across which multiple pathogens have emerged to cause epidemics and pandemics. Studies at the animal-human interface carried out in a "One Health" approach is needed to identify critical intervention points where spillover risks can be generically reduced.

Plenary address 1

A multidisciplinary approach to antimicrobial resistance: The integral role of pharmacists and healthcare teams in stewardship and surveillance

Prof. M.H.F. Sakeena B. Pharm, M. Sc, PhD
Professor in Pharmacy,
Department of Pharmacy, Faculty of Allied Health Sciences,
University of Peradeniya, Sri Lanka
Research Affiliate, Sydney Pharmacy School,
Faculty of Medicine and Health,
The University of Sydney, NSW, Australia

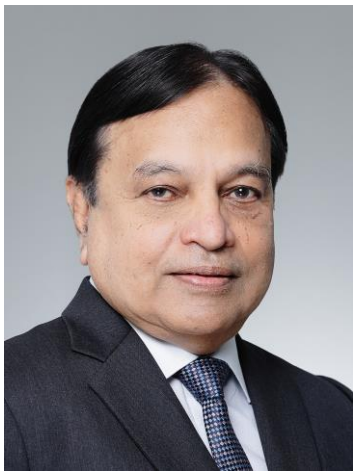
Antimicrobial resistance (AMR) is one of the most pressing challenges in modern medicine, with resistant microbial strains emerging as significant threats to global public health. These "invisible enemies" often proliferate undetected, leading to severe outbreaks. The role of pharmacists in combating this threat is crucial but often underutilized. Pharmacists, with their expertise in pharmacotherapy and patient care, are uniquely positioned to take the lead in AMR surveillance and prevention. However, the battle against AMR requires a concerted effort from a multidisciplinary team of healthcare professionals. This presentation will explore the expanding role of pharmacists in the early detection and prevention of antimicrobial resistance, focusing on their involvement in antimicrobial stewardship (AMS) and resistance monitoring initiatives. By leveraging their expertise, pharmacists contribute to the timely identification of emerging resistance patterns, ensuring that healthcare teams can respond effectively to mitigate the spread of resistant pathogens.

The discussion will highlight successful examples of pharmacist-led AMR surveillance programmes, where pharmacists collaborate closely with physicians, nurses, microbiologists, and infection control specialists. These multidisciplinary teams work together to collect, analyse, and interpret antimicrobial use and resistance data, facilitating the development of targeted interventions that optimise patient outcomes. Moreover, the presentation will emphasise the crucial role of other healthcare professionals in supporting AMS efforts. Physicians play a key role in ensuring the appropriate prescribing of antibiotics, while nurses are integral in monitoring patient adherence and educating them on proper antimicrobial use. Microbiologists contribute by providing essential data on resistance patterns and advising on appropriate antimicrobial therapies. Infection control specialists implement protocols to prevent the spread of resistant organisms within healthcare facilities.

Pharmacists also serve as vital educators, not only for healthcare teams but also for patients, ensuring that antibiotics are used appropriately and effectively. Their involvement in patient counseling, adherence monitoring, and public health campaigns is essential in reducing the misuse of antibiotics and slowing the development of resistance. The session will conclude with a discussion of the challenges faced by healthcare professionals in this evolving landscape, such as the need for additional training, institutional support, and interdisciplinary collaboration. By empowering pharmacists and integrating their efforts with those of other healthcare professionals, healthcare systems can enhance their ability to detect and respond to resistance threats, ultimately preserving the efficacy of existing antimicrobials and protecting public health.

Plenary address 11

Future of biotechnology



Malik Peiris,
School of Public Health, The University of Hong Kong

Free paper / poster (video) session I

Surveillance and diagnostics of infectious diseases in humans

9.45 am to 10.45 am

Free paper / poster (video) session II
"Microbiome" and the environment

11.35 am to 12.45 pm

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OP6 Nasal colonization of *Staphylococcus aureus* and *Streptococcus pneumoniae* in preschool children attending selected immunization clinics, Kandy, Sri Lanka HMNP Handapangoda, LPALP Ruwansiri, UPRU Dissanayake, ST Kudagammana, BN Dissanayake, LVC Liyanapathirana	13
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Free paper / poster session III
Innovative approaches
2.00pm to 3.00 pm

Free paper / poster session 1

‘Surveillance and diagnostics of infectious diseases in humans’

OP1: Viral diversity and seasonal variation in acute respiratory infection in patients at Teaching Hospital, Jaffna, Sri Lanka

JA Pradeepan*, G Selvaratnam, S Vathulan, D Pathmanathan

[1]*Department of Medicine, Faculty of Medicine, University of Jaffna, Sri Lanka*

*jebananthy@yahoo.com

Introduction and Objectives: Acute respiratory infections (ARIs) are among the most prevalent acute infections in hospitalized patients, ranging from mild, self-limiting infection such as the common cold, to severe, life-threatening lower respiratory tract infections. Common viruses associated with ARIs include influenza A and B, respiratory syncytial virus (RSV), parainfluenza, adenovirus, coronaviruses (including SARS-Co-V2), and human metapneumovirus. This study aimed to assess the viral diversity and burden of ARI in patients at Teaching Hospital, Jaffna, from June 2022 to March 2023.

Methods: A prospective longitudinal observational study was conducted among adult patients admitted with acute respiratory symptoms to the medical wards of Teaching Hospital, Jaffna. Clinical and laboratory data, including nasopharyngeal swabs for viral PCR (RespiFinder 2SMART) were collected to confirm microbiological diagnoses and guide management. Data were analysed using SPSS for Windows, version 12.0.

Results: A total of 154 patients were recruited, with 29.2% males and 70.8% females. The peak number of cases occurred in December (27.2%) and January (22%). A specific viral aetiology was identified in 47.4% of patients. Rhino/enterovirus was identified in 28.7% of cases, followed by influenza A in 17.8%, including influenza A (H1N1) in 12.1%, SARS-Co-V2 (10.8%) and parainfluenza virus (6.7%). An increase in SARS-Co-V2 cases was observed in March and April. Oseltamivir was prescribed for 35% of patients on a clinical and microbiological basis. All hospitalized patients were initially prescribed antibiotics on a clinical basis, which was later stopped based on ongoing clinical status, biochemical markers and sputum microbiology. All patients recovered and were discharged.

Conclusions: This study provides an overview of the viral diversity and clinical outcome of ARIs at Teaching hospital, Jaffna. Contrary to national trends reported in the literature, rhino/enterovirus was the predominant viral aetiology. The identification of SARS-Co-V2 highlights the continuing impact of the pandemic on respiratory health. The use of antiviral and antibiotic therapy reflects a cautious approach to clinical management. Ongoing surveillance and the implementation of tailored therapeutic strategies, including effective infection prevention measures and vaccination against influenza and COVID 19 during endemic months are essential to addressing the evolving landscape of ARIs, particularly with seasonal variations and emerging viral threats in this region.

Acknowledgement: Teaching hospital, Jaffna, ERC: S05-09-2022

Keywords: Acute respiratory infection (ARI), viral diversity, antiviral therapy

OP2: Knowledge and practices on handling of sharps among healthcare workers of two hospitals in Sri Lanka

SPGKK Jayarathna[1*], SR Liyanage[1], SPNN Senadeera[1], BMCR Wimalasiri Yapa[1], HDWS Kudagammana[2]

[1]Faculty of Health Science, The Open University of Sri Lanka, Nugegoda, Sri Lanka
[2]Faculty of Medicine, University of Peradeniya, Peradeniya, Sri Lanka

[#]kelum.gkj@gmail.com

Introduction and Objectives: This study investigates the knowledge and practices of healthcare workers (HCWs) in two hospitals in Sri Lanka concerning sharps injuries, a critical occupational health concern. The study aims to assess the level of knowledge, training, and adherence to safe practices among HCWs in relation to sharps injuries.

Methods: This cross-sectional survey was conducted at the Teaching Hospital, Peradeniya (THP) and the Base Hospital, Kamburupitiya, (BHK) involving healthcare professionals including medical officers, nursing officers, and medical laboratory technologists. Data collection utilized a structured questionnaire, developed in alignment with established guidelines and previous research. A pre-test was conducted to refine the questionnaire. Descriptive statistics were used to summarize participant characteristics, while knowledge and practice scores were computed to assess HCWs' proficiency. Group differences were analyzed using independent t-tests, and the relationship between knowledge and practice was evaluated with a paired t-test. Data were managed using Excel and analyzed with SPSS (version 21).

Results: In this study, 449 out of 481 distributed questionnaires were completed, resulting in a response rate of approximately 93.35%. The mean knowledge score for the entire population was 48.66, with THP participants scoring higher at 52.9 compared to BHK participants at 41.9, ($p < 0.05$). However, the mean practice score for the total population was 57.28, with similar scores between THP (57.2) and BHK (57.3), indicating no significant difference ($p > 0.05$). The correlation coefficient of 0.312 and a p-value of 0.00 between the mean scores of knowledge and practice in this study indicate a significant, moderate positive relationship.

Conclusions: The results highlight the need for improved training and awareness programs for HCWs, particularly in areas of infection prevention and sharps handling. Although a significant proportion of participants were knowledgeable about post exposure prophylaxis and vaccinated against Hepatitis B, there is room for enhancing knowledge levels. The study identified the importance of consistent adherence to safe practices and reveals the current state of knowledge and practices among HCWs in Sri Lanka regarding sharps injuries.

Key words: Needle prick injuries, handling of sharps, healthcare workers, knowledge and practices

OP3: Molecular detection of *Rickettsia* in peripheral blood of patients suspected of spotted fever rickettsiosis who tested sero-negative in indirect immunofluorescence assay

S Shiffana[1*], RPVJ Rajapakse[2], SAM Kularathne[2], DS Thilakarathne[2]

[1]*School of MLT (Peradeniya), Ministry of Health, Sri Lanka*
[2]*Department of Veterinary Pathobiology, Faculty of Veterinary Medicine & Animal Science, University of Peradeniya, Sri Lanka*
[2]*Department of Medicine, Faculty of Medicine, University of Peradeniya, Sri Lanka*

*****sshiffana@gmail.com

Introduction and objective: Spotted fever rickettsioses (SFR) are emerging tick-borne infections in Sri Lanka, particularly prevalent in the central hills of the country. Confirmatory diagnosis of this infection is rarely performed in referral laboratories, where imported indirect immunofluorescence antibody test (IFAT) kits are used. However, the reliability of diagnosis using imported kits is questionable, as patients with typical clinical signs often test sero-negative. Therefore, this study aimed to use molecular methods to investigate the presence of SFR-causing bacteria in peripheral blood samples from suspected patients who tested negative in IFAT.

Methods: The study encompassed peripheral blood samples submitted to the SFR diagnostic unit of the Department of Veterinary Pathobiology, Faculty of Veterinary Medicine and Animal Science, University of Peradeniya from January to December 2020 (Ethical approval No: 2019EC63). All blood samples underwent screening using Vircell® *Rickettsia conorii* immunoglobulin G (IgG) IFAT. DNA was extracted from the pelleted blood of all IFAT-negative samples using the DNeasy Blood & Tissue Kit. The extracted DNA was then subjected to nested polymerase chain reaction (PCR) targeting the 17kDa gene of the spotted fever group of rickettsia.

Results: During the specified period, a total of 144 blood samples suspected of SFR were submitted for IFAT. Of them, 104 (72.2%) tested sero-positive for rickettsia IgG, while 40 (27.8%) were identified as sero-negative. Nested PCR successfully detected rickettsial DNA in 60% (24/40) of the sero-negative samples. The percentage of rickettsial DNA positives in the IFAT-negative group was significantly higher ($p<0.001$) compared to that reported in a recent study conducted in Sri Lanka on IFAT-positive samples.

Conclusions: Although the detection of rickettsial IgG or IgM by IFAT is considered the gold standard reference, these tests have limitations, especially when using imported kits. Detection of rickettsial IgM is known to be less specific, and seroconversion to rickettsial IgG in patients can take up to two weeks. Detecting rickettsial DNA in peripheral blood of patients is less likely and expensive. However, the current study demonstrates that combining IFAT and nested PCR can efficiently confirm the diagnosis of suspected SFR patients who test negative in IFAT.

Key words: Spotted-fever, rickettsioses, sero-negative, blood, PCR

PP1: Preliminary study comparing smear and standard tuberculosis culture to GeneXpert MTB/Rif Ct categories in pulmonary and extrapulmonary samples in a local laboratory setting

K Galahitiyawa[1], S Randeni[2], S Sirimanna[3], A Kumara[1], R Karunathilake[1],T Dissanayake[1], S Jayaweera[4], K Amarasinghe[5], S Priyadarshini [5], D Madegedara[5], M Peiris[5,6], C Mendis[2] , CN Ratnatunga[1*]

[1] *Department of Microbiology, Faculty of Medicine, University of Peradeniya, Kandy, Sri Lanka*
[2] *Department of Medical Laboratory Science, Faculty of Allied Health Sciences, University of Peradeniya, Kandy, Sri Lanka*
[3] *District General Hospital, Kegalle, Sri Lanka*
[4] *Division of Bioresources, International Institute for Zoonosis Control, Graduates School of Infectious Diseases, Hokkaido University, Hokkaido, Japan*
[5] *Chest Clinic Bogambara, Kandy, Sri Lanka*
[6] *Chest Clinic Matale, Matale, Sri Lanka*

*champa.ratnatunga@med.pdn.ac.lk

Introduction and Objectives: Tuberculosis is an airborne infection caused by *Mycobacterium tuberculosis* complex (MTBC) with pulmonary and extra-pulmonary forms. The recommended Xpert MTB/Rif (Cepheid, USA) assay uses four *RpoB* and two *IS6110/IS1081* gene probes to simultaneously detect MTBC and rifampicin resistance. Results are categorised based on *RpoB* cycle threshold (Ct) values: Very Low (Ct > 28.01), Low (Ct 22.01-28), Medium (Ct 16-22), and High (Ct < 16). The relationship between smear, culture with Ct value categories in pulmonary (Pul) and extrapulmonary (EP) samples were analysed to evaluate the standard methods.

Methods: A total of 39 Xpert positive [pul (n=29), EP (n=10)] samples were collected from the Chest Clinic Bogambara from January to March 2024. Decontaminated samples (Pul by modified Petroff's method and highly contaminated samples with 0.5M H2SO4) or sterile samples were directly cultured (Lowenstein Jensen) and examined weekly. Sample smears were stained with Ziehl-Neilsen (ZN) stain. MTBC colonies were confirmed using ZN stain and *Hsp65* and *GyrB* conventional PCR. Positivity of smear/ culture of each Ct category and Spearman correlation of Ct category and week of visible growth was assessed. (GraphPad Prism 10.2.3 Software).

Results: Smear and culture positivity rates were 33.33% overall, (Pul -34.48%, EP -30%). Positivity rates for Pul samples (smear and culture) were, in Ct category 'High'-n=4/7 and 3/7 ; 'Medium'-2/4 and 3/4 ; 'Low'- 3/14 and 4/14; and 'Very low'- 1/4 and 0/4 . Positivity rates for EP samples (smear and culture) were, in Ct category 'High'-n=0/1 and 1/1; 'Medium' -no samples; 'Low'-3/8 and 2/8; and 'Very low' -0/1 and 0/1, respectively. There was no significant correlation between Ct category and week of visible growth, though an increasing trend in smear/ culture positivity rate was seen.

Conclusions: Smear and culture positivity was similar in Pul and EP samples but were variable in Xpert Ct categories with poor positivity rates even in 'High' Ct samples. Improving culture sensitivity, particularly by modifying decontamination methods is required.

Keywords: *Mycobacterium tuberculosis,* molecular diagnosis, Xpert MTB/RIF Ultra, ct value, Lowenstein–Jensen medium culture

PP2: Study of urine samples received for culture, and sensitivity patterns of the urinary pathogens at a tertiary care hospital in Sri Lanka

TN Hewage[1], BWMSB Weerasooriya[1], RMKM Dayarathne[2], HDWS Kudagammana[3*]

[1]*Department of Medical Laboratory Science, Faculty of Allied Health Sciences, University of Peradeniya, Sri Lanka*

[2]*Microbiology Laboratory, Teaching Hospital Peradeniya, Sri Lanka*

[3]*Department of Microbiology, Faculty of Medicine, University of Peradeniya, Sri Lanka*

*wasanakudagammana@yahoo.co.uk.

Introduction and objective(s): Urinary tract infections (UTIs) are a significant burden to healthcare costs. Correct diagnosis of UTI is crucial to avoid irrational use of antibiotics. This study was carried out to evaluate the type and quality of urine samples received for culture and to detect the antimicrobial resistance in uropathogens at a tertiary care setting in Sri Lanka.

Methods: A descriptive cross-sectional study at Teaching Hospital Peradeniya (THP), evaluating urine samples from wards and clinics over three months.

Results: The study evaluated 1017 urine samples of which 9 were not available for evaluation. Both date and time of sample collection were mentioned in only 32 (3.17%) of the 1008 forms and 985 (97.72%) did not mention the specifications of the sample. Of the 648 containers evaluated, 12 (1.18%) were not labelled properly. Of the 250 bed-head-tickets (BHTs) evaluated 95 (39.58%) were not given antibiotics prior to collection of urine for culture. Urine for culture was taken from the catheter in 99 of the 369 patients visited (26.83%) though this was not stated in the form. A significant colony count of a single pathogen (Table 1) was found in 189 of the 1017 urine samples. Of 16 coliforms tested, 59.3% were sensitive to nitrofurantoin. Of 14 meropenem resistant isolates, serine carbapenemase was detected in 4 (28.6%), metallo-beta-lactamase in 2 (14.3%) and carbapenemase was not detected in 8 (57%) (Table 2).

Table 1: Isolated uropathogens ($\geq 10^5$/ml) ; *n=189

	Isolates	
	n	*%*
Escherichia coli	78	41.27
Klebsiella pneumoniae	40	21.16
Coliform spp.	27	14.29
Candida spp.	19	10.05
Pseudomonas spp.	7	3.7
Enterococcus spp.	5	2.65
Staphylococcus aureus	5	2.65
CoNS	5	2.65
Streptococcus spp.	4	2.12
Total	190*	

*189 = Considering two suprapubic isolates as one

Table 2: Resistance profile of isolated Gram negative bacilli to selected antibiotics

		NA			Co-AMX			NF			CXM			CTX (2nd line)**			PT (2nd line)**			MP (2nd line)**		
	Total	T*	R	%	T*	R	%	T*	R	%	T*	R	%	T**	R	%	T**	R	%	T**	R	%
E coli	78	72	51	70	75	27	36	75	10	13	45	29	64	20	17	85	20	10	50	20	5	25
K pneumoniae	40	35	19	54	37	17	46	36	20	55.5	27	18	67	13	12	92	15	8	53	15	7	47
Other Coliform spp.	27	27	22	81	27	8	30	26	5	19	22	14	64	12	9	75	13	6	46	12	2	17

T – Tested NA- Nalidixic acid; Co-AMX – Co amoxicillin/clavulanate; NF - Nitrofurantoin; CXM – Cefuroxime; CTX – Cefotaxime; PT- Piperacillin-Tazobactam; MP-meropenem *Subject to availability of antibiotic discs at the time of testing; **Subject to testing only if showed resistance to first-line options and the availability of antibiotic discs at the time of testing so resistance percentages cannot be generalized.

Conclusions: Uropathogen isolation rates were similar to local and global patterns but carbapenem resistance rate shows a rising trend. Study indicated suboptimal utilization of urine culture facilities in clinical application as interpretation of different urine samples is significantly different. This necessitates further evaluation and to improve, timing of collecting urine samples before starting antibiotics, details of labeling such as time of collection and type of samples, practices to minimize delays in collecting and transporting, and include more patient details, which will help to optimize interpretation of culture results, diagnosis of UTIs of community-acquired, hospital or catheter-related and its management.

Keywords: UTI, surveillance, uropathogens

PP3: Detection of carbapenemase genes in Gram negative bacterial isolates from patients with urinary tract infections

AWGSN Jayathilaka [1*], US Kulasekara[1], WMID Nakkawita [2], AD De Silva[2], SP Gunasekara[3], UTN Senaratne[4]

[1]*Faculty of Graduate Studies, General Sir John Kotelawala Defense University (KDU), Ratmalana, Sri Lanka.*
[2]*Department of Para Clinical Sciences, Faculty of Medicine, KDU, Ratmalana, Sri Lanka.*
[3]*National Cancer Institute, Maharagama, Sri Lanka.*
[4]*Department of Medical Laboratory Science, Faculty of Allied Health Sciences, KDU, Ratmalana, Sri Lanka.*

*shanijayathilaka2@gmail.com

Introduction and objective: Carbapenem-resistant organisms (CROs) are of critical concern, complicating the treatment of urinary tract infections (UTIs). Therefore, this study aimed to determine the presence of carbapenemase genes among different bacterial species isolated from urine.

Methods: Urinary isolates confirmed as resistant and/or intermediate to meropenem and/or imipenem were collected from University Hospital, Kotelawala Defense University and Apeksha Hospital from January to December, 2023. Ninety-seven isolates were collected and subjected to species-level identification by BD Pheonix^{TM} automated system. PCR was performed to detect carbapenemase genes using Hi-PCR® carbapenemase (Multiplex) PCR kit (Catalogue no: MBPCR132). We identified eight carbapenemase genes; *bla*$_{KPC}$, *bla*$_{NDM}$, *bla*$_{VIM}$, *bla*$_{IMP}$, *bla*$_{OXA-23}$, *bla*$_{OXA-48}$, *bla*$_{OXA-51}$, and *bla*$_{OXA-58}$. Descriptive statistics were analyzed by SPSS 25 software.

Results: Of 97 CROs, *Klebsiella pneumoniae* was the commonest species (34%). Seventy-two of 97 isolates (74.2%) were positive for at least one carbapenamase gene tested. A single gene was detected in 34 (35.05%) while, two or more genes co-occurred in 38 (39.18%) of the isolates. Overall, the highest gene occurrence was *bla*$_{OXA-51}$ (n=46; 47.4%), followed by *bla*$_{OXA-58}$ (n=40; 41.2%), *bla*$_{OXA-23}$ (n=23; 23.7%), *bla*$_{VIM}$ (n=17;17.5%), *bla*$_{OXA-48}$ (n=16;16.5%), *bla*NDM (n=9; 9.3%), *bla*$_{IMP}$ (n=3;3.1%) and *bla*$_{KPC}$ (n=1;1.0%). The highest gene diversity was observed in *K. pneumoniae*, including 11 gene combinations with a maximum of four co-existing genes (*bla*$_{NDM}$+*bla*$_{IMP}$+*bla*$_{OXA-48}$+*bla*$_{OXA-51}$, 3.0%; n=1). Table 1 shows the distribution of CRO species, and carbapenamase genes detected in the study sample.

Table 1. Distribution of CROs and carbapenemase gene detected

CRO (n=97)	%	Single genes		Gene co-occurrences	
		Gene	n (%)	Gene combination	n (%)
K pneumoniae(n=33)	34.0	*bla*$_{OXA-48}$	11 (33.3)	*bla*$_{VIM}$+ *bla*$_{OXA-48}$	3 (9.1)
P aeruginosa(n=16)	16.5	*bla*$_{VIM}$	4 (25.0)	*bla*$_{VIM}$+ *bla*$_{OXA-51}$+*bla*$_{OXA-58}$	2 (12.5)
Escherichia coli(n=15)	15.5	*bla*$_{OXA-51}$/ *bla*$_{OXA-58}$ (equal %)	8 (53.3)	*bla*$_{OXA-51}$+*bla*$_{OXA-58}$	2 (13.3)
A baumannii/ calcoaceticus complex (n=8)	8.2	*bla*$_{OXA-51}$	5;(62.5)	*bla*$_{OXA-51}$+*bla*$_{OXA-23}$	3 (37.5)
Acinetobacter baumannii(n=5)	5.2	*bla*$_{OXA-23}$/ *bla*$_{OXA-51}$ (equal %	3 (60)	*bla*$_{OXA-51}$+*bla*$_{OXA-23}$	3 (60)
Other Gram-negative bacteria (n=20)	20.6	*bla*$_{OXA-51}$	7;(35)	*bla*$_{VIM}$+ *bla*$_{OXA-51}$+*bla*$_{OXA-58}$	3(15)

Conclusions: A noteworthy diversity of carbapenemase genes were observed, with *K. pneumoniae* being the commonest species with a *high variety of resistant genes. Continuous surveillance and implementation of targeted infection control and antibiotic stewardship programs are needed to mitigate further spread of carbapenamase genes.*

Keywords: Carbapenem resistant organisms, carbapenemase genes, multiplex PCR, urinary tract infections, Sri Lanka

Acknowledgement: Financial assistance by General Sir John Kotelawala Defence University for research grant; KDU/RG/2022/FAHS/005 is acknowledged

PP4: Identifying carbapenem resistance mechanisms in blood culture isolates using mCIM and eCIM at National Hospital Kandy

KD Galgamuwa[1*], M Kothalawala[2], HDWS Kudagammana[3], LKG Nandanie[1], IP Samaraweera[1.]

[1]National Hospital Kandy, Sri Lanka
[2]National Hospital of Sri Lanka,
[3]Department of Microbiology, Faculty of Medicine, University of Peradeniya, Sri Lanka,

*kdgalgamuwa@gmail.com

Introduction: Carbapenem resistant Enterobacterales (CRE) are a critical threat to human health. CRE identified through disc diffusion (DD) or minimum inhibitory concentration (MIC), is mediated through carbapenemase production, porin loss, efflux or hyperproduction of ESBL/Amp C coupled with porin loss. Characterization of the resistance mechanism in CREs is not routinely done in clinical microbiology laboratories. Carbapenem Inactivation Method (CIM) is simple, cost effective and highly sensitive and specific test for detecting carbapenemases.

Objectives: To describe the pattern of carbapenemase production among carbapenem-resistant Enterobacterales and *Pseudomonas spp* from blood cultures using modified CIM (mCIM) and EDTA CIM (eCIM) and to describe the associated demographic data, and bacterial species diversity.

Method: This was a laboratory based descriptive cross-sectional study conducted over four months. Thirty-four Gram negative blood culture isolates resistant to a carbapenem antibiotic by DD or MIC was speciated using VITEK 2 or Chromogenic agar and tested with the mCIM and eCIM test. Demographic and clinical data were collected from bed head tickets. Isolates confirmed as carbapenamase negative were tested with a combination disc of antibiotics (COMBI DISC D 72C) to detect ESBL, AmpC or porin loss.

Results: In this study, isolates came from patients of whom 58.4% were male (n=21) and 41.6% (n=15) were female. The median age of these patients was 58 years (5 days to 80 years). Of the 34 isolates, 24 were *Klebsiella pneumoniae* (66.6%), 6 *Escherichia coli* (16.6%), 4 *Pseudomonas aeruginosa* (11%), 1 *Stenotrophomonas spp* and 1 *Chryseobacterium spp* (both 5.5%). Of these isolates tested by the mCIM test, 6 (17.6%) were negative and 28 (82.4%) were positive for carbapenamases. Twenty-three of the 28 isolates (82%) were metallo beta lactamase (MBL) producers and 5 were serine carbapenamase producers (18%). The resistance mechanism in the 6 carbapenemase negative isolates (by mCIM test) was suspected to be ESBL/Amp C with porin loss when tested with D72C combination disc.

Conclusion: While carbapenemase production, predominantly MBL, was common, a small proportion of CRE were negative for carbapenemase production. It would be useful for laboratories to consider adding mCIM and eCIM to their testing protocols.

Keywords: CRE, carbapenem resistance, carbapenamase vs non carbapenamase, CIM test

NOTE: This was presented as a poster at PGIM Annual research Symposium 2023

PP5: Comparison of sheep blood and human blood-based media for the isolation and identification of selected pneumococcal strains

HMNP Handapangoda[1, 2], G Vidanapathirana[1], UPRU Dissanayake[2], A Ekanayake[2], LVC Liyanapathirana[2*]

[1]*Department of Medical Laboratory Sciences, Faculty of Allied Health Sciences, University of Peradeniya, Sri Lanka*

[2]*Department of Microbiology, Faculty of Medicine, University of Peradeniya, Sri Lanka*

[*]veranja.liyanapathirana@med.pdn.ac.lk

Introduction and objectives: Although the conventional culture using sheep blood supplementation is recommended for the identification of *Streptococcus pneumoniae*, developing countries use human blood as an alternative. Therefore, it is important to evaluate the impact of two different blood media on pneumococcal isolation and identification. This study aimed to compare the colony sizes, colony counts, antibiotic sensitivity (ABST) and minimum inhibitory concentration (MIC) of *S. pneumoniae* isolates on two different blood media.

Methods: Twenty strains of *S. pneumoniae* (four strains each from the five commonest serotypes found in Sri Lanka; serotypes 19F, 6B, 6A, 14 and 23F) and *S. pneumoniae* ATCC 49619 were used. Colony sizes of the isolates were measured on SBA and HBA. Proportions of colonies ≥ 1 mm, colony counts in 0.5 McFarland Standard, 10^{-1}, 10^{-2}, 10^{-3}, and 10^{-4} dilutions were compared. Disc diffusion antibiotic sensitivity testing (ABST) was performed for erythromycin, tetracycline, and levofloxacin on two types of blood-MHA. MIC for penicillin and cefotaxime were tested with micro broth dilution in two types of blood-MHB.

Results: Draughtsman appearance and alpha hemolysis were obvious on SBA. Considering all isolates together, the mean number of colonies ≥ 1 mm was 8 (±9) on HBA and 23 (±10) on SBA. In higher dilutions (10^{-3}, 10^{-4}), mean number of colonies on SBA ($1.94x10^5$, $5.36x10^4$ CFU/ml) was higher than HBA ($1.78x10^5$, $4.18x10^4$ CFU/ml). Mean ABST zone diameters of tetracycline, erythromycin, levofloxacin on human blood-MHA were 24.5, 17.5, 25.2 mm and on sheep blood-MHA were 21.2, 12.7, and 23.5 mm. MIC50 and MIC90 for penicillin were similar in both media (2 and 4 µg/ml). MIC50, MIC90 for cefotaxime in human blood-MHB was 0.5 and 2µg/ml; in sheep blood-MHB was 0.75 and 2 µg/ml.

Conclusions: Since the typical colony characteristics were not seen, there is a possibility to misidentify pneumococci on HBA. Isolation of pneumococci on HBA is less when organisms are present in lower concentrations. Larger ABST zones on human blood-MHA may alter sensitivity interpretation. Therefore, human blood cannot be recommended for the isolation and identification of *S. pneumoniae*, and alternatives need to be sought for by countries like Sri Lanka.

Keywords: *Streptococcus pneumoniae*, sheep blood agar, human blood agar, isolation and identification

Free paper / poster session II

“Microbiome” and the environment

OP4: Detection and isolation of *Salmonella* spp. in poultry meat samples at retail outlets in the Puttalam district in Sri Lanka

PRH Pathirana[1*], DPE Perera[1], S Pathirage[2], SI Fonseka[2], RMUSK Rathnayaka[1]

[1]*Department of Food Science and Technology, Faculty of Applied Sciences, Sabaragamuwa University of Sri Lanka*
[2]*Unit of Bacteriology, Medical Research Institute, Colombo 8,Sri Lanka*

*ridmipathirana123@gmail.com

Introduction and Objectives: Foodborne infections are a significant global public health concern. These are the primary causes of morbidity and mortality in humans. Among the foodborne infections, salmonellosis plays an important role worldwide. *Salmonella* spp. are often associated with food of animal origin, poultry meat, and other meat products. This study aims to determine the prevalence of *Salmonella* spp. in raw poultry meat (chicken) sold at retail outlets in MOH areas in the Puttalam district.

Methods: A pre-tested questionnaire was administered separately to wet markets, grocery shops, and supermarkets to assess the adherence of retail outlets to the Code of Hygienic Practice for the processing of poultry (SLS 516:Part 5:2017). A total of 337 raw poultry carcasses were collected representing 14.8% from supermarkets, 29.7% from grocery shops, and 55.5% from wet markets through a simple random method. Conventional culture-based methods (ISO 6579:2017 Microbiology of food and animal feeding stuff – Horizontal method for the detection of *Salmonella* spp.) were performed for detection and isolation of *Salmonella* spp.

Results: A total of 112 (33%) samples were positive for *Salmonella* spp. with a higher prevalence of 72% from the Dankotuwa MOH area. The prevalence of *Salmonella* spp. in poultry carcasses sampled from wet markets, grocery shops, and supermarkets was 71.4%, 25%, and 3.6% respectively. According to the questionnaire, 92% of wet-market retail outlets store poultry meat at ambient temperature, and none of the tested outlets monitored the storage temperature. All supermarkets and grocery shops displayed dressed meat in freezers while 79% of wet markets displayed on plastic or aluminum trays, and 21% on wooden tables used to cut the meat. None of the meat handlers in the 3 types of outlets wore gloves or protective covering in handling meat. All the supermarkets cleaned the meat contact surfaces and equipment once a week while 84% of wet markets and 27% of grocery shops cleaned less than twice a day.

Conclusion: The high prevalence of *Salmonella* spp. in poultry from wet markets underscores the urgent need for improved hygiene practices to mitigate public health risks associated with foodborne infections.

Keywords: *Salmonella* spp., raw poultry meat, retail outlets, Puttalam district, code of hygienic practice for processing of poultry

OP5: Isolation and identification of Group B Streptococcus (*Streptococcus agalactiae*) from tilapia (*Oreochromis* spp.) fish collected from selected five districts in Sri Lanka

DPE Perera[1*], PRH Pathirana[1], S Pathirage[2], RMUSK Rathnayaka[1], SI Fonseka[2]

[1] *Department of Food Science and Technology, Faculty of Applied Sciences, Sabaragamuwa University of Sri Lanka,*
[2] *Unit of Bacteriology, Medical Research Institute, Colombo 08, Sri Lanka*

*pawaniperera84@gmail.com

Introduction and Objectives: *Streptococcus agalactiae* (GBS) is an opportunistic pathogen that causes serious health complications. Recently, it was found that GBS sequence type 283 (ST 283) can act as a foodborne pathogen and cause invasive GBS ST 283 diseases in healthy adults. This phenomenon is strongly associated with consumption of raw freshwater fish. The objectives of this study were to determine the prevalence of GBS in tilapia fish for sale from selected districts (Colombo, Kurunegala, Ampara, Anuradhapura, and Rathnapura) and to assess the adherence of fish handlers to the code of hygienic practices for fresh fish (SLS 974: 1992)

Methods: Five highest freshwater fish consumption districts were selected for sampling. From each district 20 tilapia samples (n=100) were collected from 10 purchasing locations. Internal organs (eyes, brain, heart, spleen, liver, gills and guts) obtained from fish were enriched in Todd Hewitt and in BHI supplemented with gentamicin and nalidixic acid respectively as primary and secondary enrichment medias. Enriched samples were sub-cultured onto MacConkey and blood agar. Suspected colonies were identified via Gram stain, catalase, bile esculin, hippurate, CAMP test, and Lancefield's grouping test. The Phoenix (SMIC/ID-2 Pannel) automated system was used for identification and antimicrobial susceptibility testing. Adherence to hygiene practices among fish handlers was assessed through a questionnaire at sampling sites.

Results: GBS was isolated from only 2 of the 100 samples. The 2 positives were from small scale village sellers in Ampara (1/20) and Anuradhapura (1/20). All the other samples were negative for GBS. As per the survey, most of the fish handlers were not following at least the basic hygienic requirements; of 50 sampling sites, only 30% maintained the cold chain, while only 12% used sanitizers for cleaning equipment and floors. Fish are frequently placed on bare floors or wooden platforms during sales, and cutting and degutting operations are also conducted on the same surfaces.

Conclusion: There is a potential risk of transmitting invasive GBS disease through the consumption of tilapia fish available in the Sri Lankan market. The study underscores the crucial importance of enhancing fish handlers' awareness and adherence to best practices in fish handling to minimize any potential risks associated with GBS contamination.

Keywords: *Streptococcus agalactiae*, group B Streptococcus (GBS), ST283, freshwater fish, foodborne diseases

OP6: Nasal colonisation of *Staphylococcus aureus* and *Streptococcus pneumoniae* in preschool children attending selected immunization clinics, Kandy, Sri Lanka

HMNP Handapangoda[1], LPALP Ruwansiri[1], UPRU Dissanayake[1], ST Kudagammana[2], BN Dissanayake[1], LVC Liyanapathirana[1*]

[1]*Department of Microbiology, Faculty of Medicine, University of Peradeniya, Sri Lanka*
[2]*Department of Paediatrics, Faculty of Medicine, University of Peradeniya, Sri Lanka*

*veranja.liyanapathirana@med.pdn.ac.lk

Introduction and objectives: *Staphylococcus aureus* colonisation is quite prevalent among Sri Lankan children. Pneumococcal carriage is a prerequisite for pneumococcal disease. Since pre-school children are very likely to transmit pathogens to others, identification of pneumococcal and *S. aureus* colonisation rates has significant importance. The objectives of this study were to examine the prevalence, and the factors associated with nasal carriage of *S. aureus* and *S. pneumoniae* in preschool children.

Methods: Both anterior nasal swabs and nasopharyngeal swabs (NPS) were collected from 375 children between 2 to 5 years attending immunisation clinics at Teaching Hospital Peradeniya and Yatinuwara MOH area during June 2023-January 2024. Nasal swabs were enriched in 6.5% NaCl and NPS were stored at -80 ^{o}C. *S. aureus* and *S. pneumoniae* were isolated using conventional microbiological testing. Interviewer administered questionnaires were used to collect data. Data were analyzed using SPSS version 25.0.

Results: The study group had a median age of 54 months (IQR: 36-60), with 188 (50.1%) males and 187 (49.9%) females. Among 375 participants, prevalence of *S. aureus* colonisation was 26.9% (101) and *S. pneumoniae* colonisation was 19.5% (73). Of these, 21(5.6%) were co-colonised with both *S. aureus* and *S. pneumoniae* while 52 (13.9%) carried only *S. pneumoniae* and 80 (21.3%) carried only *S. aureus*. Kindergarten attendance (OR=1.92, 95% CI=1.13-3.27), smokers at home (OR=1.85, 95% CI=1.04-3.30), having recent upper respiratory tract infections – (URTI) (OR=16.39, 95% CI=2.23-120.47), family members with URTI (OR=1.83, 95% CI=1.09-3.08) were significantly associated with pneumococcal colonisation. No significant associations were found with *S. aureus* colonisation. Age (p=0.017), weight (p=0.021), kindergarten attendance (OR=4.016, 95% CI=1.33-12.18) were significantly associated with *S. aureus* and pneumococcal co-colonisation. No significant association was found between *S. aureus* and pneumococci colonisation (p=0.694).

Conclusions: *S. aureus* and *S. pneumoniae* colonisation rates were considerably higher among children aged between 2 to 5 years in the selected population. Kindergarten attendance was a significantly associated factor for co-colonisation of *S. aureus* and *S. pneumoniae* along with age and weight.

Acknowledgement: International Society of Antimicrobial Chemotherapy (ISAC) is acknowledged for funding via a Project Grant (PI – Prof Margaret Ip).

Keywords: *S. aureus*, *S. pneumoniae*, colonisation, co-colonisation, kindergarten aged children

NOTE: Part of the work has been presented at iPURSE 2024 and KDU sessions 2024

PP6: Study on antimicrobial sensitivity of staphylococci isolated from the external ear canal of cats presented to the Veterinary Teaching Hospital, University of Peradeniya

B De Silva[2], T Manathunga[1], Y Senarath[1], K Senarathna[1], S Bandara[2], R Jinadasa[2], H Ariyarathna[1]*

[1]*Department of Veterinary Clinical Sciences, Faculty of Veterinary Medicine and Animal Science, University of Peradeniya, Sri Lanka*
[2]*Department of Veterinary Pathobiology, Faculty of Veterinary Medicine and Animal Science, University of Peradeniya, Sri Lanka*

*hsariyarathna@vet.pdn.ac.lk

Introduction and Objectives: Bacterial otitis externa (OE) is the most common ear condition in cats. However, it is less frequently reported in cats than dogs. The most common bacterial organisms in the external ear canal of cats and their sensitivity patterns are less known. This project was designed to characterize the bacterial organisms in the external ear canal and the antibiotic sensitivity of *Staphylococcus* isolates to methicillin and five other antimicrobials (co-trimoxazole, tetracycline, ciprofloxacin, chloramphenicol & gentamicin) of cats presented to the Veterinary Teaching Hospital (VTH), University of Peradeniya between May to July 2024

Methods: Both external ear canals of 120 cats were sampled. The ear swabs were cultured on blood agar aerobically and bacterial isolates were identified using routine biochemical methods. Antibiotic sensitivity was determined using disc diffusion method. Chi-square test with Marascuilo procedure for comparing multiple proportions was used for data analysis.

Results: Most of the cats were crossbred (n=108), while the rest were Persian (n=12). The age range was 2 months to 13 years. There were 64 males and 56 females. Forty-six of them were healthy, 28 had OE and 46 presented with infections/conditions other than OE. A total of 145 bacterial isolates were obtained, representing 30%, 34.5% and 35.5% from the respective groups. Most isolates (n=135, 93%) were staphylococci, representing 39, 51 and 49 isolates from the respective groups. *Staphylococcus epidermidis* predominated in all groups (53%, 74% and 68% of the *Staphylococcus* isolates respectively) followed by *S. aureus* and uncharacterized coagulase negative staphylococci. Of the 135 *Staphylococcus* isolates, 34 (25.1 %) were resistant to methicillin. Of these 34 isolates, 10 were multidrug resistant (MDR). The number of bacterial isolates and *Staphylococcus* spp. isolates obtained from cats with OE were significantly higher than healthy cats or cats with infections/conditions other than OE. No significant differences were observed regarding the methicillin resistance or MDR status between the 3 groups.

Conclusions: Staphylococci predominated the culturable aerobic bacterial flora in external ear canals of the cats tested in this study with *S. epidermidis* being the most common. Bacterial isolation rates and *Staphylococcus* spp. isolations from cats with OE were significantly higher than other two groups. Approximately 25% of staphylococci were methicillin resistant with a low incidence of MDR.

Keywords: Otitis externa, cats, *Staphylococcus*, *S. epidermidis*, methicillin resistance, MDR

PP7: Contaminated theatre footwear: a potential source of infection for the wearer in Teaching Hospital Anuradhapura

UANN Thilakarathne[1], MJH Thilakasiri[1], TMSH Thennakoon[1], TMTS Thennakoon[1], RAJS Thilakarathna[1], PKDI Vithsuka[1] , SC Illapperuma[2]*

[1]Medical undergraduate, Faculty of Medicine and Allied Sciences, Rajarata University of Sri Lanka,
[2]Senior lecturer, Department of Microbiology, Faculty of Medicine and Allied Sciences, Rajarata University of Sri Lanka.

*scillapperuma@med.rjt.ac.lk

Introduction and Objectives: Operating theatre staff must wear recommended footwear according to the jurisdiction of standard EN ISO 20347:2012 cited in the Hospital Infection Prevention and Control manual of the Sri Lanka College of Microbiologists. However, inadequate hygienic practices persist due to facility limitations and knowledge gaps among staff. Our study at Teaching Hospital Anuradhapura (THA) was conducted to determine the level of microbial contamination in theatre-footwear with potential pathogens and determine adherence to proper cleaning procedures of theatre-footwear in THA.

Methods: An interviewer-based questionnaire was conducted among 35 participating theatre staff. Swabs were taken from 35 regular theatre staff and inside of the footwear and the lower surface of the 1st and 2nd toes of the wearer were swabbed. Swabs were immersed in 10ml of sterile normal saline, vortexed and serially diluted as per in house methods. 0.1 ml of samples were spread plated in blood agar and Sabouraud dextrose agar for bacterial and fungal growth. After incubation, quantification was done using a colony-counter and identification was done using standard references. A questionnaire assessed theatre-footwear cleaning procedures of 15 cleaning staff of THA.

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Results: Of the footwear, 82.0% swabbed in the morning and evening grew bacteria, mainly *Staphylococcus spp*. and *Streptococci spp*. *Fusarium*, *Aspergillus* and *Trichophyton* were the commonest fungi isolated. Highest microbial count was observed in the evening and the number increased towards Friday. Of the theatre staff, 68.5% wore dedicated shared theatre-footwear, while 31.4% wore their own theatre-footwear and 14.3% wore slippers. Of the study population, 14.3% confirmed having previous foot infections. 86.7% of theatre cleaning staff stated “Teepol” was used to wash theatre-footwear and 46.7% stated blood-contaminated footwear is cleaned with peroxide. Several stated that insufficient time and space for drying footwear occurred due to heavy theatre list.

Conclusion: This research highlights the potential infection risks from pathogenic microbes from theatre-footwear, emphasizing the importance of proper wearing, maintenance and cleaning according to the guidelines. Malpractices can lead to infections. Theatre-footwear is a personal protective equipment for hospital staff and should be properly managed. A collective stock of dedicated theatre-footwear may reduce infection transmission risks.

Keywords: Footwear, infection, theatre

This study has been submitted to the 6th Undergraduate Research Symposium of Faculty of Medicine and Allied sciences, Rajarata University of Sri Lanka 2024

PP8: Preliminary investigation of microbial diversity and antibiotic susceptibility profiling of *Staphylococcus* spp. isolated from tick salivary glands infesting domestic animals in Kandy

KI Fazil[1,3], KSA Kottawatta[2], RPVJ Rajapakse[1], S Saheed[1], DS Thilakarathne[1*]

[1]*Department of Veterinary Pathobiology, Faculty of Veterinary Medicine & Animal Science, University of Peradeniya, Sri Lanka*
[2]*Department of Veterinary Public Health & Pharmacology Faculty of Veterinary Medicine & Animal Science, University of Peradeniya, Sri Lanka*
[3]*International College of Business and Technology, Kandy, Sri Lanka*

*dsamanthikat@gmail.com

Introduction and Objectives: Ticks pose a hazard to both humans and animals by feeding on blood and transmitting diseases, facilitated by their saliva which plays a crucial role in pathogen transmission. Heavy tick infestations in animals often lead to skin infections necessitating antimicrobial treatment. *Staphylococcus aureus* is known for causing skin and soft tissue infections. This study aimed to identify aerobic bacteria in tick saliva and assess the antimicrobial susceptibility profiles of *Staphylococcus* species.

Methods: Ten ticks (6 from dogs, 3 from cattle, and 1 from a horse) were aseptically dissected to collect salivary glands. Each salivary gland was enriched in buffered peptone water at 37 °C for 2 hours and cultured on blood agar at 37 °C for 18-24 hours under sterile conditions. Morphologically distinct bacterial colonies were subjected to biochemical tests for species identification. Gram-positive isolates were tested for catalase, slide, tube coagulase and cultured on MSA agar. Identified *Staphylococcus* spp. underwent disc diffusion assays with the following antibiotics: erythromycin (15 µg), cefoxitin (30 µg), ampicillin (10 µg), ciprofloxacin (5 µg), trimethoprim-sulfamethoxazole (25 µg), gentamicin (10 µg), and clindamycin (2 µg), following CLSI guidelines. Parallel to our experiments, ATCC *Staphylococcus aureus* 25923 was tested and zone diameters obtained were evaluated using CLSI values for QC strains, which was used as a positive control.

Results: The collected ticks represented *Boophilus* (3), *Ixodes* (2), *Rhipicephalus* (2), *Ornithodoros* (1), *Haemaphysalis* (1), and *Aponomma* (1) species. Eighteen bacterial colonies were isolated from tick saliva, consisting of 8 Gram-negative rods and 10 Gram-positive cocci. Biochemical assays identified the Gram-negative isolates as *Citrobacter species* and 5 of the Gram-positive cocci isolates as coagulase-positive *Staphylococcus (Staphylococcus aureus and Staphylococcus pseudintermedius).* All coagulase-positive *Staphylococcus* isolates were susceptible to all tested antimicrobials except clindamycin, which showed intermediate susceptibility (80%) or resistance (20%).

Conclusions: Besides medically and veterinary significant protozoans and viruses, ticks also transmit a variety of bacteria. All tested ticks carry aerobic bacteria in saliva, with *Staphylococcus* species being the most prevalent and showing susceptibility to the tested antimicrobials. A more comprehensive investigation involving a broader range of tick species from diverse hosts and geographical areas is essential for accurately identifying the risk posed to hosts.

Keywords: Ticks, saliva, bacteria, antimicrobial, susceptibility

Acknowledgment: Financial assistance given by the University of Peradeniya (URG/2022/67/V) is acknowledged.

PP9: Bacterial Diversity in Water Distributed under Intermittent Water Supply

EGW Gunawardana[1]*, BGDS Bandarawaththa[2], Yukuto Sato[3], Claudia Toma[4], CD Gamage[5], M Makehelwala[1], SK Weragoda[1], R Weerasooriya[6]

[1]*China Sri Lanka Joint Research and Demonstration Centre (JRDC) for Water Technology, Ministry of Water Supply, E.O.E Pereira Mawatha, Peradeniya, Sri Lanka*
[2]*Department of Mathematics, Faculty of Science, University of Peradeniya*
[3]*Research Laboratory Center, Faculty of Medicine, University of the Ryukyus, Okinawa, Japan*
[4]*Department of Bacteriology, Graduate School of Medicine, University of the Ryukyus, Okinawa, Japan*
[5]*Department of Microbiology, Faculty of Medicine, University of Peradeniya, Sri Lanka*
[6]*National Institute of Fundamental Studies, Kandy, Sri Lanka*

*wasanagun@gmail.com, wasanagun@jrdc.lk

Introduction and Objective(s): The fluctuation of bacteriological water quality in Intermittent Water Supply (IWS) in Drinking Water Distribution Networks (DWDNs) poses significant challenges in maintaining water safety, particularly in developing countries where IWS is common. With the prevalence of IWS in many regions of Sri Lanka, comprehensive studies are needed to address the problems associated with IWS. Consequently, the current study attempted to investigate the bacterial diversity during the supply resumption phase of IWS.

Methods: Three water samples from the resumption phase of IWS and two samples during continuous water supply (CWS) were collected from a DWDN which experiences IWS regularly. Sterivex filters were used to send samples to the Research Laboratory, Faculty of Medicine, University of the Ryukyus, Japan, and DNA was extracted from the Sterivex filters by using the DNeasy PowerWater Sterivex Kit (Qiagen, Hilden, Germany), according to a standard protocol. The V4 region of the bacterial 16S rRNA gene was then sequenced by Illumina MiSeq sequencing.

Results: The findings revealed that most taxonomic groups were commonly present in samples collected during the CWS and supply resumption phases of IWS. However, specific taxonomic groups, such as Sphingomonadales, Actinomycetales, Enterobacterales, Burkholderiales, and Caulobacterales were prevalent in samples taken during the supply resumption phase of the IWS. The soil and environment-related bacteria groups, such as Actinomycetales and Burkholderiales were predominant in the samples taken during the supply resumption phase of IWS than in CWS which may probably be due to intrusion of outside water into the water distribution or sloughing of biofilms. In contrast, Aeromonadales, Neisseriales, Rhizobiales, and Pirellulales groups were prevalent in samples collected during the CWS phases.

Conclusions: The soil and environment-related bacteria groups, such as Actinomycetales and Burkholderiales were prevalent in water during the supply resumption phase of IWS than in continuous water supply. In contrast, Aeromonadales, Neisseriales, Rhizobiales, and Pirellulales were prevalent in CWS. Based on the results of this preliminary research, further research is recommended to elucidate whether all the IWS systems follow similar trends, the origins of the dominant bacterial groups identified during IWS, and their health-related and ecological issues.

Keywords: Intermittent Water Supply (IWS), drinking water distribution networks (DWDNs), bacterial diversity, 16S rRNA gene, health risks.

PP10: Molecular detection of the spotted fever group rickettsial DNA in ticks in the Central Province of Sri Lanka

S Ariyarathne[1,2], ME Eremeeva[3], SAM Kularatne[4], AM Nazeem[5], P Perera[1], A Dangolla[6], R Rajakaruna[1*]

[1]*Department of Zoology, University of Peradeniya, Peradeniya, Sri Lanka*
[2]*Postgraduate Institute of Science, University of Peradeniya, Peradeniya, Sri Lanka*
[3]*Department of Biostatistics, Epidemiology and Environmental Health Sciences, Georgia Southern University, USA*
[4] *Department of Medicine, Faculty of Medicine, Peradeniya*
[5]*19, Heerassagala road, Kandy*
[6]*Faculty of Veterinary Medicine & Animal Science, University of Peradeniya*

* rupika.rajakaruna@sci.pdn.ac.lk

Introduction and Objectives: Spotted fever group (SFG) rickettsioses are caused by obligate, intracellular, Gram-negative bacteria, primarily transmitted by ticks. This study aimed to identify tick species carrying SFG rickettsial DNA in the Central Province of Sri Lanka.

Method: Sampling was carried out in Kandy, Matale, and Nuwara Eliya districts (2019–2022). Ticks were manually removed from domestic animals with owner assistance, and from peri-domestic animals after sedation. They were captured using mouse cage traps. Ticks from a leopard were provided by a wildlife researcher. Tick DNA was extracted (Promega ReliaPrep^{TM}) and tested for *Rickettsia* using rOmpA gene via conventional PCR.

Results: A total of 222 ticks were collected with *Rhipicephalus haemaphysaloides* (n=75) being the most common species, followed by *Rhipicephalus sanguineus* (n=50), *Rhipicephalus microplus* (n=40), *Amblyomma integrum* (n=20) and *Dermacenter auratus* (n=9). Other species included *Hyalomma isaaci* (n=2), *Haemaphysalis bispinosa* (n=8), *Haemaphysalis turturis* (n=2) and *Haemaphysalis intermedia* (n=3), with 13 *Haemaphysalis* ticks identified only to genus level. *R. haemaphysaloides* was collected from dogs (n=35), cattle (n=22), pigs (n=3), mongoose (n=2), and leopard (n=1). Most *R. sanguineus* and all *D. auratus* were from dogs, while *Hy. isaaci* was collected from buffalo. No ticks were found on rats (n=9), bandicoots (n=5), or mice (n=14). Overall, 78.8% of the ticks tested positive for *Rickettsia* DNA, with a frequency of 90.5% in Matale, 77.6% in Kandy and 63.2% in Nuwara Eliya. *Rickettsia* DNA was detected in 8 out of 9 tick species, including 92.4% of *R. haemaphysaloides*, 88.9% of *D. auratus*, 84.6% of *R. sanguineus*, 65.0% of *A. integrum* and 57.5% of *R. microplus*. All *Hy. isaaci*, *H. turturis* and *H. intermedia* tested positive, while all *H. bispinosa* were negative. Among the domestic animals, 83.5% of ticks from dogs and 66.2% from cattle were positive. Among peridomestic animals (n=37), ticks were found only on mongooses (n=7) and squirrels (n=2), all of which were positive for *Rickettsia* DNA.

Conclusion: *Rickettsia* DNA was detected in ticks feeding on various mammals. Further research is needed to confirm if the *Rickettsia* are pathogenic, if they are present in questing ticks, and whether the detection reflects pathogens from the ticks' blood meals or independent infection.

Keywords: spotted fever, rickettsial DNA, *Rhipicephalus*, *Dermacentor*, *Hyalomma*

Acknowledgment: National Science Foundation, Sri Lanka (RG/2019/BT/01)

Free paper / poster session III

Innovative approaches

OP7: Investigation of anti-inflammatory effect of *Cinnamomum verum* and *Piper nigrum* extracts against *Escherichia coli* induced inflammation in zebrafish model

DMCB Dissanayake[1], IMN Molagoda[2], S Hettiarachi[1]*

[1]*Department of Biological Sciences, Faculty of Applied Sciences, Rajarata University of Sri Lanka, Mihintale, Sri Lanka*
[2]*Department of Bioprocess Technology, Faculty of Technology, Rajarata University of Sri Lanka, Mihintale, Sri Lanka*

*sanath@as.rjt.ac.lk

Introduction and Objectives: Inflammation is a defense mechanism initiated by the immune system in response to various adverse stimuli including infections and cellular damage. It protects the host by recognizing and responding to intrinsic and extrinsic factors by activating sophisticated, finely tuned mechanisms. Anti-inflammatory drugs are widely used to reduce inflammation despite their many harmful side effects. This study evaluated the anti-inflammatory potential of cinnamon (*Cinnamomum verum*) (Sri Gamunu variety) and black pepper (*Piper nigrum*) (Panniyur 1 variety) extracts against *Escherichia coli*-induced inflammation in zebrafish larvae.

Methods: Cold maceration with ethanol followed by concentration and freeze-drying were used to prepare the extracts. Third day post-fertilization (3dpf) zebrafish larvae (n=10) were exposed to different concentrations of herbal extracts and determined three nontoxic concentrations to the zebrafish model as cinnamon 20, 10, 5 µg/mL, and pepper 1000, 100, 10 pg/mL. The 3dpf zebrafish larvae were exposed to 10^8 CFU/mL *E. coli* as the inflammatory agent. The post-treatment infected 4dpf larvae were treated with selected concentrations of plant extracts as test drug material. Biomarker analysis included malformations, heartbeat, and macrophage migration assessment. The neutral red (NR) staining experiment relative densities were quantified by Image J Software. The data were analyzed using one-way ANOVA with Bonferroni correction.

Results: *E. coli* infection significantly triggered inflammatory responses in zebrafish larvae, leading to inflammation-induced mortality. Malformations observed were necrotic yolk, bent spine, and swollen pericardial sac. The following table shows the data on heart rate and NR accumulation and compares the effect of the post-treatment with the control.

Parameter	Control	Treatment		
		E. coli alone	Black pepper	Cinnamon
Heart rate (beats/ min.)	161.80 ± 2.95[a]	263.00 ± 16.21[b]	165.50 ± 2.41[a]	162.20 ± 5.26[a]
NR Accumulation	100 ± 4.14%[a]	111.43 ± 1.66%[b]	97.70 ± 0.87%[a]	107.87 ± 3.61%[a]

The differences of values denoted by different letters in a row are statistically significant ($p = < 0.001$). The plant extracts reduced both parameters to a level not significantly different from the control.

Conclusions: These findings reveal the anti-inflammatory potential of cinnamon and black pepper extracts. This research opens promising avenues for developing effective, natural plant-based anti-inflammatory treatments. The importance of zebrafish model in evaluating plant-based therapies is also emphasized.

Keywords: Anti-inflammatory drugs, animal model, drug screening model, *Escherichia coli*-induced inflammation, macrophage migration .

OP8: Evaluation of the antibacterial activity of *Camellia sinensis* (green tea) extract against *Streptococcus mutans*.

MMF Mufeena[1], AMA Wathooth[1], MRF Ashra[1], TJ Gnanakarunyan[1*], FSS Mafras[1], JAMS Jayatilake[2], R Ramachandran[3], T Thayalini[4]

[1] *Department of Medical Laboratory Sciences, Faculty of Allied Health Sciences, University of Jaffna, Sri Lanka,*
[2] *Department of Oral Medicine and Periodontology, Faculty of Dental Sciences, University of Peradeniya, Sri Lanka*
[3]*Teaching Hospital Jaffna, Sri Lanka*
[4]*Unit of Siddha Medicine, University of Jaffna, Sri Lanka*.

*thevakijg@univ.jfn.ac.lk

Introduction and Objectives: *Streptococcus mutans* is a common oral commensal responsible for dental caries and systemic infections in compromised patients. Green tea (*Camellia sinensis*) is a popular beverage which contains catechins with antibacterial properties. The aim of this study was to evaluate the antibacterial activity of green tea extract against *S. mutans* isolated from dental caries.

Methods: *Camellia sinensis* powder (gun powder 1) was obtained from a tea factory in the Central Province, Sri Lanka. Ethanolic extract of *C. sinensis* was prepared and used to screen the antibacterial activity. A total of 85 isolates of *S. mutans* were used in the study. This included 84 isolates collected from caries lesions of patients attending the Dental Clinic, Teaching Hospital Jaffna between September to October 2023 and the standard strain of *S. mutans* (ATCC 700610). There were 18 erythromycin resistant and 14 clindamycin resistant *S. mutans* isolates within the 84 clinical isolates. Erythromycin (15 µg) and distilled water were used as positive and negative controls. Well diffusion method was used to check the antibacterial activity. Accordingly, after inoculation of *S. mutans* onto Mitis salivarious agar medium, 9cm wells were cut, loaded with tea extract (2.5 mg/mL) and controls before incubating at 37 ^{o}C at 5-10% CO2 incubator. Diameter of the zone of inhibition (ZOI) was measured after 24 hours of incubation.

Results: Ethanolic green tea extract demonstrated a 13.08% yield and inhibited all 84 clinical isolates with the diameter of ZOI ranging from 19±3.2 mm to 32.67±3.2 mm, demonstrating an anti-bacterial potential. Mean ZOI against standard *S. mutans* strain (ATCC 700610) was 29±0.3 mm. Erythromycin and clindamycin resistant isolates were also inhibited by green tea extract with mean diameters of ZOI 21.92±3.27 mm and 22.12±3.61 mm respectively.

Conclusions: Sri Lankan green tea showed anti-bacterial activity against *S. mutans* including the isolates that are resistant to erythromycin and clindamycin in-vitro. Hence, the green tea extract could serve as a potential ingredient in the formulation of oral hygiene products after some subsequent investigations.

Keywords: Dental caries, *Streptococcus mutans*, green tea

OP9: Exploring tender coconut water as a viable medium for fungal growth: A comparative study with Potato Dextrose Agar

S Shayanthavi[1*], R Kapilan[2], I Wickramasinghe[1]

[1]*Department of Food Science and Technology, Faculty of Applied Sciences, University of Sri Jayewardenepura, Nugegoda, Sri Lanka.*
[2]*Department of Botany, Faculty of Science, University of Jaffna, Jaffna, Sri Lanka*

*shayanthavi@sci.sjp.ac.lk

Introduction and Objectives: Tender coconut water (TCW) is a nutrient-rich and readily available resource in tropical regions, offering an alternative to traditional fungal growth media such as Potato Dextrose Agar (PDA). The current study explores the viability of TCW as a fungal growth medium by comparing the growth of various fungi in TCW of different concentrations and PDA.

Methods: Tender coconut water aged 5-7 months was collected from local coconut farms in Jaffna. The TCW was filtered through Whatman filter paper and TCW-agar media were prepared by incorporating 1.5% agar into the fresh TCW. The media were then autoclaved for sterilization. The fungi evaluated in this study included one species each of *Fusarium, Penicillium, Aspergillus*, and *Mucor*. These fungi isolates were obtained from the Department of Botany, University of Jaffna. Fungi were incubated at room temperature for 6 days and the fungal growth was assessed through colony diameter and fungal dry weight measurements.

Results: The results indicated that 100% TCW concentration yielded significantly higher colony diameters for *Fusarium* (7.48 cm), *Mucor* (8.45 cm), *Aspergillus* (8.17 cm), and *Penicillium* (3.58 cm) compared to PDA, which showed colony diameters of 6.15 cm, 5.05 cm, 7.25 cm, and 2.65 cm, respectively. In terms of dry weight, 100% TCW concentrations resulted in 80.90 mg for *Fusarium*, 162.93 mg for *Mucor*, 143.10 mg for *Aspergillus*, and 72.90 mg for *Penicillium*. Comparatively, PDA yielded higher dry weights of 201.50 mg, 346.90 mg, 460.73 mg, and 97.90 mg for *Fusarium, Mucor, Aspergillus, Penicillium*, respectively. These findings demonstrate that full-strength TCW supports greater fungal colony diameters than PDA, suggesting superior surface growth. However, PDA outperformed TCW in terms of fungal biomass production, as indicated by higher dry weight measurements.

Conclusions: The study finds that TCW-agar media promote significant fungal colony growth, while PDA is better for biomass production. However, limitations include the absence of standard fungal strains, pathogenic fungi like *Candida* spp. and dermatophytes, and a lack of colony morphology and microscopic comparisons essential for fungal identification. Further optimization and validation are required before it can be considered a full alternative to PDA.

Keywords: Colony diameter, dry weight, fungal growth media, tender coconut water

PP11: Comparison of physico-chemical properties of wine fermented with palmyrah fruit pulp and cashew apple juice

R Tenuja[1], N Sobini[2]*, RS Dassanayake[1], A Kirushanthi[2]

[1] *Department of Biosystems Technology, University of Sri Jayewardenepura, Sri Lanka*
[2] *Palmyrah Research Institute, Kandy Road, Kaithady, Jaffna, Sri Lanka*

* sobinithi30@gmail.com

Introduction and Objectives: Palmyrah fruit and cashew apple are underutilized seasonal fruits in Sri Lanka, reported to possess important nutritional and medicinal properties. This study was conducted to develop wine from three different formulations of palmyrah fruit pulp (PFP) and cashew apple juice (CAJ) with an aim of giving a better commercial value for the above fruits.

Methods: Initially, tannin in cashew apple was removed using 0.77% of gelatin. PFP was extracted manually (50ml water per seed). Different ratios of CAJ and PFP (100:0, 80:20, 50:50 %) were mixed and fermented by Baker's yeast (*Saccharomyces cerevisiae*). The optimum fermentation time was determined for formulated wine based on the production of ethanol which was monitored by gas chromatography. The changes in ethanol content, total soluble solids (TSS) and pH were analyzed for 21 days. Physicochemical, and sensory tests were done in order to compare the variations. Sensory test was done using 9-points hedonic scaling using twenty trained panelists and data was analyzed using friedman test.

Results: Ethanol content resulted using CAJ and PFP ratios of 100:0, 80:20, 50:50 % were 9.47 ± 0.02, 7.72 ± 0.06 and 7.30 ± 0.03 % respectively, obtained at the optimum fermentation time of 18^{th}, 15^{th} and 11^{th} day respectively. Wine with CAJ and PFP (50:50) was selected as the best formulation based on the sensory evaluation. Methanol was absent in all three types of wine. TSS, titratable acidity, pH, total ash, fructose and glucose content of wine with CA juice and PFP (50:50) were 6.24 ± 0.060^{o}, 2.180 ± 0.016 g/L, 3.47 ± 0.01, 0.29 ± 0.01%, 2.70 ± 0.00 g/L, 0.86 ± 0.00 g/L respectively. It was observed that titratable acidity of wine with CAJ and PFP (50:50) is lower than others and it has a higher level of glucose and fructose contents than others.

Conclusion: The wine fermented with 50:50 CAJ: PFP had better sensory and physico-chemical properties compared to the other two formulations of wines processed in this study and, it has a potential to further develop in to a commercially viable product.

Key words: Baker's yeast, ethanol content, titratable acidity, total soluble solids

Acknowledgement: Palmyrah Research Institute

PP12: Isolation and identification of potent naringinase-producing bacteria from different plant parts of *Citrus aurantium.*

P Kavashana[1]*, R Kapilan[2], PN Yapa[1], AN Dunuweera[3]

[1] *Department of Biological Sciences, Faculty of Applied Sciences, Rajarata University of Sri Lanka*
[2] *Department of Botany, Faculty of Science, University of Jaffna, Sri Lanka*
[3] *Department of Basic sciences, Faculty of Allied Health Science, University of Peradeniya, Sri Lanka*

*kavashana@gmail.com

Introduction and Objectives: Naringinase is a versatile and fascinating study subject since it is produced by various sources in nature, resulting in variance in reaction specificity. It has numerous important applications in the food and pharmaceutical industries. This study aimed to isolate, characterize, and identify potent naringinase-producing bacteria from various parts of the *Citrus aurantium* plant.

Methods: Forty-five bacterial strains were isolated from *Citrus aurantium* fruit, leave, stem and root by spread plate method and qualitatively assessed for naringinase activity using 1% $FeCl_3$. Among these isolates, nine strains (BC1-BC9) showed naringinase activity indicated by a distinct reddish-brown colour change with $FeCl_3$. These nine strains were then selected for a quantitative test using both submerged (SMF) and solid-state (SSF) fermentation methods. The SMF medium contained naringin (2.0g/L), glucose (2.0g/L), peptone (7.0g/L), $MgSO_4.7H_2O$ (0.1g/L), KH_2PO_4 (0.5g/L), $ZnSO_4.7H_2O$ (0.07%), $CuSO_4.7H_2O$ (0.07%), $FeSO_4.7H_2O$ (0.07%) and for the SSF, **rice bran (20%)** was added to the existing SMF medium, making the mixture a solid medium rather than a liquid one. The measurement of naringinase enzyme activity relied on monitoring changes in absorbance. Best naringinase producing isolate (BC2) was identified based on the morphological,biochemical screening and molecular assay using 16S rRNA sequence analysis.

Results: All nine isolates produced significantly higher naringinase enzyme on SSF media than the SMF media. Bacterial isolate BC2 exhibited significantly higher (p value<0.05) naringinase activity (497.890U/g±1.107) of the dry weight of the substrate than the other isolates under SSF condition. The yield of the enzyme was calculated by determining the amount of reducing sugars released during the enzymatic reaction. The absorbance changes were monitored at 550 nm wavelengths using a spectrophotometer. The morphological, biochemical screening and mmolecular study using 16S rRNA sequence analysis revealed that isolate BC2 was *Bacillus megaterium* CP026736.

Conclusions: This study identified *Bacillus megaterium* CP026736, isolated from *Citrus aurantium* fruit, as a potent naringinase-producing bacterium. Comparative assessments showed that SSF resulted in significantly higher naringinase activity than SMF across nine strains. Among them, strain BC2 demonstrated the highest enzyme activity under SSF conditions, indicating its industrial potential. Molecular assays confirmed BC2 as *Bacillus megaterium*, providing valuable insights for future applications in the food and pharmaceutical industries.

Keywords: *Bacillus megaterium*, *Citrus aurantium*, naringinase activity, solid-state fermentation.

PP13: Preliminary and potential effect of curd on management of uncomplicated urinary tract infections - A case report

GCS Gunasekera*

Department of Medical Microbiology and Immunology, Faculty of Medicine, University of Colombo, Sri Lanka

*chathurigcs@yahoo.com

Introduction: Recurrent urinary tract infections (UTIs) are culture proven UTIs that have occurred at least twice within 6, or thrice within 12 months. Uncomplicated UTI is common in women. Several studies done on the effect of vaginally administered probiotics and yoghurt in the prevention of UTIs have been found to be promising as a supplementary method of treatment. This case report is of a girl who had recurrent uncomplicated UTIs who tried local application of curd as a treatment method.

Case report: An 18-year-old single girl presented with mild dysuria for 2 days. She had 4 episodes of UTI in the 2 years prior to this presentation with urine cultures growing $>10^5$ CFU/mm^3 coliforms each time. She denied any sexual contact. Physical examination and Xray KUB and USS abdomen were normal.

After obtaining urine for culture, she was advised on non-medical methods (increased hydration, double micturition and not holding up urgency to urinate, wiping/cleaning perineum from front to back). Antibiotics were not given pending culture results which grew a coliform $>10^5$ CFU/mm^3 sensitive to all tested antibiotics. Although her symptoms had markedly reduced within 48 hours, she was very concerned about recurrence. Having done a literature search on application of curd over the genitourinary area she was willing to try this in preference to antibiotic prophylaxis

Having discussed her concerns on applying curd (presence/absence of pathogens in curd, frequency, area and duration of application), a reputed local brand of curd with expiry date of 24 hours from production was chosen. About 3 full tablespoons of the curd were applied daily before bedtime for 7 days over the genitourinary area using her fingers from front to back following hand washing and washing of the area with soap and water. She was advised to maintain a diary and seek medical advice in the event of a recurrence of symptoms. She was reviewed monthly for 6 months with no recurrence. Two urine cultures 3- and 6-months following curd application were negative.

Discussion/ Conclusions: The source of urinary pathogens in females is the perineal microbial flora (the vaginal microbiome). The effect of curd on the vaginal microbiome is not well studied. Follow-up of this patient over a longer period and setting up a well-designed study to determine the protective value of this intervention may be useful in management of this common condition in women.

Keywords: Recurrent UTIs, vaginal microbiome, curd

PP14: Alternate substrate formula for mushroom cultivation by utilizing coir waste

R Rafaya[1], N Jeyagowri[1*]

[1]*Department of Bio science, Faculty of Applied Science, University of Vavuniya*

*kjgowri@vau.ac.lk

Introduction and Objectives: Mushroom cultivation traditionally relies on sawdust as a primary substrate due to its high carbon content. However, the scarcity of sawdust in Sri Lanka imposes a search for alternative materials. Abundant availability of coir, a by-product of coconut processing, has potential substrate for mushroom cultivation with sufficient mineral and cellulose contents, and restored water holding capacity. This study explores the potential substrate formula using coir waste as an alternative substrate to sawdust.

Methods: Cultivation of oyster mushroom (*Pleurotus ostreatus*) was conducted in a mushroom hut under 20-30 ^{o}C temperature and 80-90% humidity. The experiment was carried out in a complete randomized design (CRD) with four treatments, and each treatment had five replicates. The substrate was prepared by including coir dust, sawdust, and rice bran in various proportions on the dry weight basis, while constant amounts of $CaCO_3$ (2%) and $MgSO_4$ (0.2%) were maintained for all treatments. The treatments were sawdust and rice bran (10:1) (T1-standard), coir dust and rice bran (10:1) (T2), sawdust, coir dust, and rice bran (5:5:1) (T3), sawdust, coir dust, and rice bran (6:3:2) (T4). The growth parameters were observed daily. The means value for mycelium invasion, pinhead formation, fruiting body formation, stipe length, pileus diameter, fresh weight, and biological efficiency were compared for the first harvest using Duncan's Multiple Range Test (DMRT) using SPSS 26.

Results: The time required for growth phase, growth parameters, and yield parameters varied with the treatments (p value<0.05) as shown in Table 1.

Table1: Growth phase, growth parameters and yield parameters of oyster mushroom

Parameters	T1	T2	T3	T4
Mycelium invasion(days)	18.80±0.44^{a}	20.80±1.78^{b}	18.40±0.54^{a}	**18.40±0.54^{a}**
Pinhead formation(days)	46.20±2.28^{b}	51.20±1.30^{b}	43.00±1.22^{a}	**41.20±1.64^{a}**
Fruiting body formation (days)	49.60±2.30^{b}	53.20±1.30^{c}	45.20±1.30^{a}	**43.20±1.64^{a}**
Stipe length(mm)	35.40±1.67^{b}	33.80±1.78^{b}	36.20±1.30^{ab}	**38.40±1.51^{a}**
Pileus diameter(mm)	60.20±5.93^{b}	59.20±2.58^{b}	63.40±4.66^{ab}	**66.80±3.27^{a}**
Fresh weight (Kg)	31.77±6.35^{b}	28.40±3.11^{b}	30.54±9.16^{b}	**42.93±7.21^{a}**
Biological efficiency (%)	5.61	5.01	5.39	**7.58**
Cost (Rs)	38.00	23.00	30.50	**36**
Benefits (Rs)	1.71	12.50	7.67	**17.66**

Note: Each value represents mean ± SD of sample (n=5), values with the same superscript letter along the column are not significantly different (positive results order: a>b>ab>c). T1:sawdust + rice brane, T2:coir dust + rice brane, T3: sawdust + coir dust + rice brane, T4: sawdust + coir

Conclusion: As per the study, all studied growth and yield parameters were better in treatment T4 compared to other treatments and standards. Further, coir dust can be mixed with sawdust instead of standard traditional farming practices adding sawdust as the primary carbon source. The formula 6:3:2 of sawdust, coir dust, and rice brane is a potential alternative to reduce dependence on sawdust and utilize coir dust as a better resource.

Keywords: Alternate substrate, coir dust, mushroom production, sawdust, substrate combination.

PP15: Preliminary evaluation of in-house miniaturized biochemical test strips for identification of common enteric Gram-negative pathogens encountered in veterinary clinical practice

RAKA De Alwis[1], HRN Jinadasa[2*], RMUSK Rathnayaka[1]

[1]*Department of Food Science and Technology, Faculty of Applied Science, Sabaragamuwa University of Sri Lanka*

[2]*Department of Veterinary Pathobiology, Faculty of Veterinary Medicine and Animal Science, University of Peradeniya, Sri Lanka*

*rnjinadasa@vet.pdn.ac.lk

Introduction and Objectives: Microorganisms are routinely identified using biochemical tests. Consequently, miniaturization of biochemical reaction makes the identification more economical. In this study, citrate, urease and triple sugar ion (TSI) tests were miniaturized for the identification of selected bacterial isolates encountered in veterinary clinical practice .

Methods: Citrate and urease tests were conducted using 4 mL (Standard), 0.40 mL and 0.20 mL (strips). For TSI, 5 mL (Standard), 0.50 mL and 0.30 mL (strips) volumes were tested. Media from the same batch were used in all experiments. Bacterial isolates were selected to test both positive and negative phenotypic observations. *Klebsiella spp.* was selected to observe citrate and urease test positive colour changes and TSI positive gas formation. *E. coli* was selected to observe citrate, and urease test negative colour changes and TSI positive gas formation. *Salmonella spp.* was selected to observe TSI positive colour changes and H_2S formation. The same bacterial suspension was used to inoculate each set of standard and miniaturized test preparations. All experiments were incubated aerobically at 37 °C. All tests were conducted in three replicates. Observations taken by five independent observers were analyzed with one way ANOVA. Results were also compared using an artificial intelligence aided color-coding system.

Results: For all three organisms, citrate and urease tests produced expected colour changes in all miniaturized volumes without any significant difference compared to standards. For the TSI test, the gas production was not accurately observable for *E. coli* and *Klebsiella spp.* beyond 0.50 mL, though the colour changes in the media were not significantly different compared to standards. H_2S gas production was observable, but the colour changes were significantly different in the TSI test for *Salmonella spp.* beyond 0.50 mL miniaturization.

Conclusion: These preliminary findings show that both citrate and urease tests, the miniaturizing was successful up to 0.20 mL with a 95% cost reduction for identification of the *E. coli*, *Salmonella spp.*, and *Klebsiella spp*. However, the TSI test was successfully miniaturized only up to 0.50 mL with 90% cost reduction for these three isolates. Most importantly, results in 0.20 mL test strips were obtained within 6 hours. These findings need to be evaluated among a wider group of known isolates as well as in clinical isolates.

Keywords: Miniaturizing, urease, citrate, triple sugar ion, food pathogens

PP16: Survival of probiotic lactic acid bacteria (LAB) in bee products incorporated bio-yoghurts

E Pavithira[1], N Jeyagowri[1*]
[1]*Department of Bio science, Faculty of Applied science, University of Vavuniya, Sri Lanka*

[*] kjgowri@vau.ac.lk

Introduction and Objectives: Bee products incorporated bio-yoghurts possess functional properties due to the presence of probiotics, prebiotics and bioactive compounds. Therapeutic minimum of 6-7 log CFU/ g of viable probiotic bacteria should be sustained in the food product for effective health benefits. Inclusion of bee products such as honey, pollen and royal jelly to the dairy products potentially exerts distinct positive effects on the activity of key lactic acid bacteria (LAB). Therefore, the current study was focused to evaluate the survival of probiotic LAB species in the bee product incorporated bio -yoghurt to confirm the therapeutic minimum during storage

Methods: The research was designed to incorporate three bee variants as treatments (0.6% royal jelly 0.6% royal jelly (T1), 5% bee honey (T2), and 0.5% bee pollen (T3) and control (C)). Dairy yoghurts were prepared following standard procedures. Probiotic starter culture (2% ABT-5) was added after cooling the yoghurt mixture at 45 ^{o}C and incubated at 42 °C for 4-5 hours and refrigerated. Samples were drawn from each treatment and the control with replicates and diluted up to eight levels, inoculated on MRS media and incubated at 37 °C for 72 hours under anaerobic conditions. The colony forming units of LAB were determined using the standard plate count technique on MRS agar and the pH was measured at one-week interval for 28 days. Bacterial colonies were confirmed as LAB by conducting Grams staining, spore staining, and catalase tests.

Results: Bio-yoghurt enriched with bee honey exhibited significantly ($P< 0.05$) higher number of survival (log10 values 9.25 to 6.58) compared to control yoghurt which showed the lowest survival, (log10 values 8.14 to 5.56) from first to 28 days of storage period. It was observed that there was a significant ($p<0.05$) decrease in LAB counts during storage in all treatments, possibly linked to the formation of lactic acid and decline in pH during this period.

Conclusion: The improved bacterial survival in bio-yoghurts highlights the potential prebiotic benefits of bee products which maintained the survival of probiotic LAB conceivably within the therapeutic minimum range throughout the storage period.

Keywords: Yoghurt, bee products, lactic acid bacteria, survival

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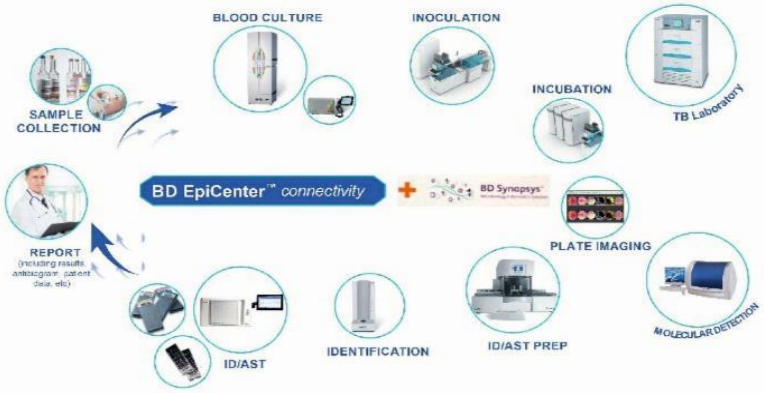

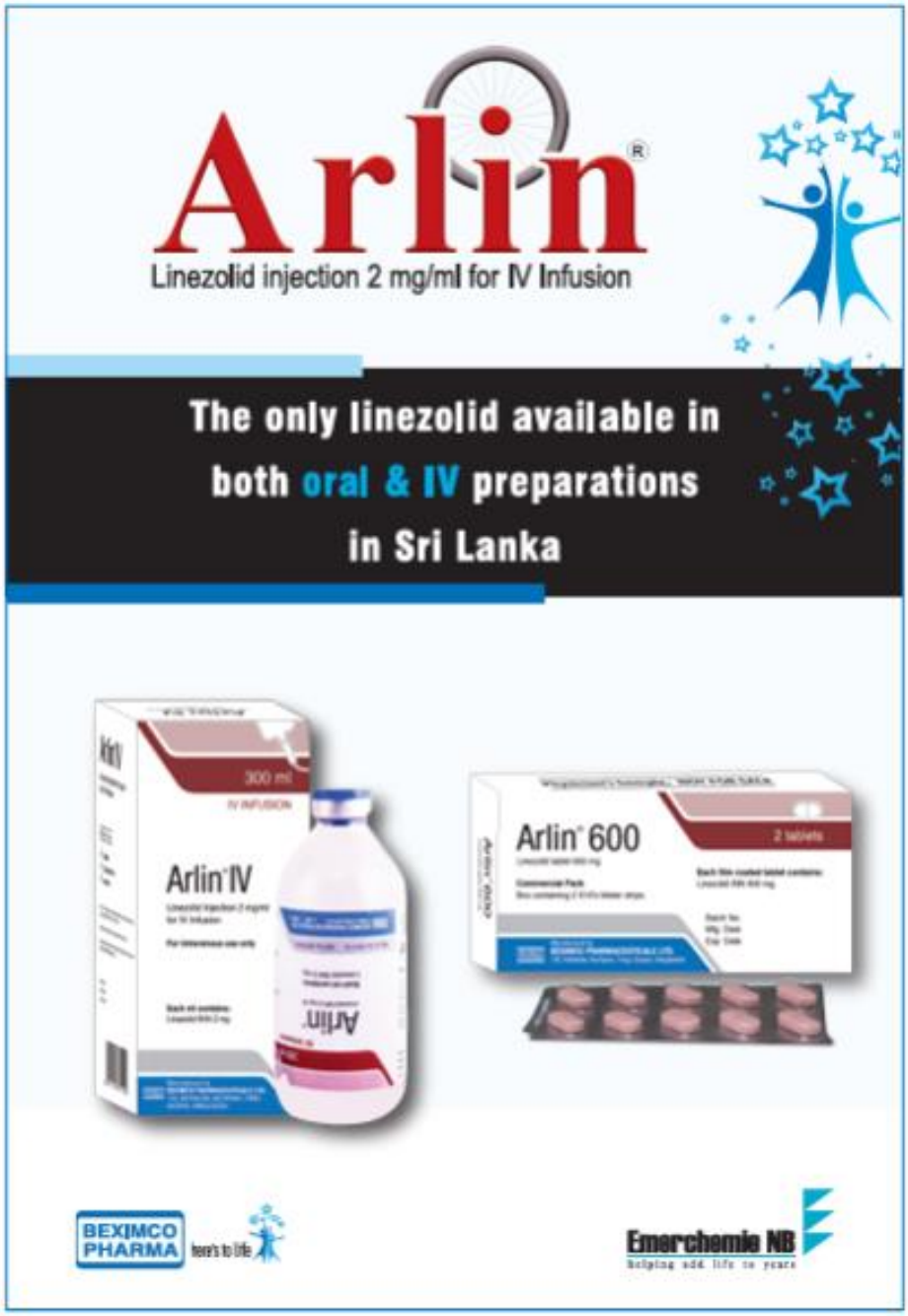
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