THE BULLETIN OF THE

SRI LANKA COLLEGE OF MICROBIOLOGISTS

29th ANNUAL SCIENTIFIC SESSIONS

VIRTUAL SCIENTIFIC PROGRAMME

13 - 14 AUGUST 2020

Hosted from the
Lionel Memorial Auditorium

" Facing The Challenges of MULTIDRUG RESISTANCE "

9 PLENARY LECTURES & 4 SYMPOSIA

with online participation of world's leading experts

FREE PAPER SESSIONS

GUEST LECTURES

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The Bulletin
of the
Sri Lanka College of Microbiologists
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The Sri Lanka College of Microbiologists
Council 2019 / 2020

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The Sri Lanka College of Microbiologists

Council 2019 / 2020

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The Bulletin of the Sri Lanka College of Microbiologists is published annually with the Scientific Sessions of the College.

Address for correspondence :
Editor
Sri Lanka College of Microbiologists
No. 6, Wijerama House, Wijerama Mawatha, Colombo 7.
E-mail : slcmicrobio@gmail.com

Cover page designed by Dr. Roshan Jayasuriya

29th Annual Scientific Sessions

of the

The Sri Lanka College of Microbiologists

"Facing the Challenges of Multidrug Resistance"

Scientific Programme

13th and 14th August 2020

"Lionel Memorial Auditorium"
Sri Lanka Medical Association
Colombo 07

Concluding Ceremony

14th August 2020 at 3.15pm

"Lionel Memorial Auditorium"
Sri Lanka Medical Association
Colombo 07

MESSAGE FROM THE PRESIDENT

I am writing this message with pride because, in an unprecedented pandemic the Sri Lanka College of Microbiologists has not forgotten its main objective; to promote the advancement of Medical Microbiology, propagate information and disseminate knowledge among its members and among other groups. However, the pandemic of COVID-19 curtailed some of our planned activities such as the pre-congress and inauguration ceremony.

Considering the theme we selected for this year's Scientific Sessions "Facing the Challenges of Antimicrobial Resistance", an eminent panel of speakers from around the world and our own will be contributing to the upliftment of our knowledge. Doing this mammoth task through an online platform is by no means easy, but somehow we decided to face that challenge as well. The Bulletin of the Sri Lanka College of Microbiologists brings out the talent and research work within our membership, honing their skills and preparing them for a wider arena.

This year our editorial board, led by our hard working task master Prof. Neluka Fernado went through many hardships. During a lock down with a closed office, performing all tasks through an online platform, they did their duty which was not second to any other year.

I must mention the tireless activities of our membership in preparing lots of COVID related guidelines and engaging in many training activities, even though they are burdened with many additional activities in their work stations. I offer my humble thank you to all members, senior as well as junior, who sacrificed their time, skill and resources to the betterment of our beloved motherland through the activities of our College.

Our website is kept up to date by our able web administrator Dr. Roshan Jayasuriya and new member to the family Ms. Piume Madushanka.

In this lean year some of our sponsors did not forget the Sri Lanka College of Microbiologists. Without them we would not be able to complete the publication or conduct the sessions.

It grieves me to say that this year bestowing the fellowships to the members who served us in the past is not the glittering event we used to offer. While I offer my sincere apologies, I would like to thank my senior colleagues for their cooperation.

Special thanks also go to my two secretaries and members of the council, our two indispensable administrative secretaries Ms. Priyanga Opatha and Ms. Nilmini Weerasiri who made my life bearable in this year.

Dr. N. Shirani Chandrasiri
***President*,**
Sri Lanka College of Microbiologists

PROGRAMME AT A GLANCE

Time	13th August 2020	Time	14th August 2020
8.00 am	Registration	8.00 am	Registration
8.30 am	Free Paper Session 1	8.30 am	Symposium 3 Transplant associated parasitic diseases in Sri Lanka: Challenges in diagnosis and treatment
9.30 am	Break	9.30 am	Break
10.00 am	Symposium 1 Quality antibiotics for treatment	10.00 am	Plenary 6 Vaccines in Dengue
10.45 am	Plenary 1 TB elimination in the era of Drug Resistant TB – pipeline or pipedream	10.30 am	Plenary 7 Genes and Mycobacteria – Lessons to be learnt
11.15 am	Free Paper Session 2	11.00 am	Plenary 8 Treatment of invasive fungal infections
12.15 pm	Plenary 2 Overview of the point of need diagnostics for the SARS-CoV-2	11.30 pm	Symposium 4 Endocarditis in pacemaker
12.45 pm	Break	12.15 pm	Break
1.45 pm	Plenary 3 Diagnosis of invasive fungal infections	1.15 pm	Plenary 9 Progress towards elimination of Hepatitis C virus infection
2.15 pm	Symposium 2 Interruption of mother-to-child transmission: lessons and road map	1.45 pm	Guest lecture 2 "Changing Microbiology to support patients and to steward antibiotics"
3.15 pm	Plenary 4 Developing an antifungal stewardship programme: pharmacological options, challenges and benefits (personal experience)	2.15 pm	Photography for medical presentations
3.45 pm	Plenary 5 Facing SARS 2 with SARS 1 experience	3.15 pm	Concluding ceremony, Award and fellowship
4.15 pm	Guest lecture 1 Current and future therapies for infections due to multi-resistant Gram-negatives		

SCIENTIFIC PROGRAMME

29th Annual Scientific Session of the Sri Lanka College of Microbiologists

13th – 14th August 2020

Web Based Scientific Programme

Hosted from Lionel Memorial Auditorium, Wijerama House, Colombo 07

Day 1 – 13th August 2020

8.00 am - 8.30 am	**Registration**
8.30 am - 9.30 am	***Free Paper Session 1***
OP 1	**Development of a real-time multiplex PCR assay for the detection of *Kingella kingae* in the Children's hospital at Westmead, Sydney, NSW, Australia** *Samaranayake WAMP[1], Leung KC[1], Outhred AC[1,2,3], Kesson AM[1,2,3]* [1]Department of Infectious Diseases and Microbiology, The Children's hospital at Westmead, Sydney, Australia, [2]The Marie Bashir Institute of Infectious Diseases and Biosecurity, The University of Sydney, [3]Discipline of Child and Adolescent Health, The University of Sydney, Australia.
OP 2	**Prevalence of carbapenem resistance and molecular analysis of carbapenem resistance among clinical isolates of *Pseudomonas aeruginosa* in a tertiary care hospital** *Wijeweera KDDS[1], Piyasiri DLB[1]* Teaching Hospital Karapitiya, Galle[1]
OP 3	**Comparison of conventional culture and real time PCR based direct detection method for the identification of pneumococcal colonization** *Vidanapathirana G[1], Angulmaduwa SKL[2], Munasinghe TS[3], Ekanayake EWMA[2], Harasgama P[2], Kudagammana ST[3], Dissanayake BN[2], Liyanapathirana LVC[2]* [1]Faculty of Medicine, University of Peradeniya, [2]Department of Microbiology, Faculty of Medicine, University of Peradeniya, [3]Department of Paediatrics, Faculty of Medicine, University of Peradeniya
OP 4	**Genetic diversity among *Burkholderia pseudomallei* clinical isolates in Sri Lanka** *Muthugama TA[1,2], Jayasinghearachchi HS[2], Masakorala J[1], Silva D de[2], Corea EM[1]* [1]Faculty of Medicine, University of Colombo, [2]Faculty of Medicine, General Sir John Kotelawala Defense University
9.30 am - 10.00 am	**Break**
10.00 am - 10.45 am	**Symposium 1 – Quality antibiotics for treatment** ***Role of NMRA in providing quality antibiotics*** **Prof. Asitha de Silva** Chairman, National Medicine Regulatory Authority, Colombo ***Quality of antibiotics : How can we ensure*** **Prof. Priyadarshani Galappatthy** Professor, Head, Department of Pharmacology, Faculty of Medicine, University of Colombo

10.45 am - 11.15 am	**Plenary 1** ***TB elimination in the era of Drug Resistant TB – pipeline or pipedream*** **Dr. Chris Coulter** Microbiologist and Infectious Disease Physician, Director Queensland Mycobacterium Reference Laboratory, WHO Collaborating Centre in Tuberculosis Bacteriology, Supra National Reference Laboratory, Queensland, Australia
11.15 am - 12.15 am	**Free Paper Session – 2**
OP 5	**Development of a porcine skin model for the assessment of bacterial biofilms** *Jayasena Kaluarachchi TD[1], McBain AJ[2], Wickremasinghe R[1], Yasawardene S[3], Menike C[1], Jayathilake S[4], Ranasinghe S[1], Weerasekera MM[5]* [1]Department of Parasitology, Faculty of Medical Sciences, University of Sri Jayewardenepura, [2]School of Health Sciences, Faculty of Biology, Medicine and Health, The University of Manchester, United Kingdom, [3]Department of Anatomy, Faculty of Medical Sciences, University of Sri Jayewardenepura, [4]Department of Pathology, Faculty of Medical Sciences, University of Sri Jayewardenepura, [5]Department of Microbiology, Faculty of Medical Sciences, University of Sri Jayewardenepura
OP 6	**Preliminary study to detect mutations in UL97 gene in suspected ganciclovir resistant CMV patients' samples, presented to a diagnostic laboratory in Sri Lanka** *Thambyrajah JC[1], Jayamaha CJS[2], Ratnayake AKDVY[1], Handunetti S[1], Fernando N[1]* [1]Institute of Biochemistry, Molecular Biology and Biotechnology, University of Colombo, [2]Department of Virology, Medical Research Institute, Colombo
OP 7	**The diagnostic accuracy of a modified nested PCR-RFLP method in the diagnosis of cutaneous leishmaniasis caused by *Leishmania donovani*** *De Silva NL[1], De Silva VNH[2], Deerasinghe ATH[3], Kato H[4], Itoh M[5], Takagi H[5], Weerasooriya MV[1], YahathugodaTC[1]* [1]Department of Parasitology, Faculty of Medicine, University of Ruhuna, [2]Base Hospital, Tangalle, [3]District General Hospital Hambantota, [4]Division of Medical Zoology, Department of Infection and Immunity, Jichi Medical University, Japan, [5]Department of Microbiology and Immunology, Aichi Medial University School of Medicine, Japan.
OP 8	**Establishment of an in-house SARS CoV-2 real-time PCR and investigation of first few suspected cases of COVID-19 in Sri Lanka** *Jayamaha CJS[1], Witahnage VH[1], Jayathunga RCS[1], Ekanayake DHP[1], Wepathairage LH[1], Neelambari S[1], Chu DKW[2], Peiris M[2]* [1]National Influenza Centre, Department of Virology, Medical Research Institute, Colombo, [2]School of Public Health, LKS Faculty of Medicine, The University of Hong Kong
12.15 pm - 12.45 pm	**Plenary 2** ***Overview of the point of need diagnostics for the SARS-CoV-2*** **Dr. Ahmed Abd El Wahed** Head of the Virology Lab, Division of Microbiology and Animal Hygiene, Georg-August-University Goettingen, Germany, Guest Scientist at the German Primate Center, Visiting Professor at the University of Cairo, Egypt

12.45 pm - 1.45 pm	**Break**
1.45 pm - 2.15 pm	**Plenary 3** ***Diagnosis of invasive fungal infections*** **Prof. Cornelia Lass-Flörl,** Director, Institute of Hygiene and Medical Microbiology Director, CD-Laboratory for Invasive Fungal Infections, Medical University of Innsbruck, Austria
2.15 pm - 3.15 pm	**Symposium 2 – Interruption of Mother-to-Child transmission: lessons and road map** ***Prevention of mother-to-child transmission of HBV*** **Prof. William Irving** Professor and Honorary Consultant in Virology, University of Nottingham and Nottingham University Hospitals NHS Trust, United Kingdom ***Elimination of mother-to-child transmission of HIV and Syphilis*** **Dr. Lilani Rajapaksa** Consultant Venereologist, Deputy Director, National STD/AIDS Control Programme, Colombo ***Role of Laboratory in elimination of mother-to-child transmission of Syphilis and HIV*** **Dr. Jayanthi Elwitigala** Consultant Microbiologist, STD / AIDS Control Programme, Colombo
3.15 pm - 3.45 pm	**Plenary 4** ***Developing an antifungal stewardship programme: pharmacological options, challenges and benefits (personal experience)*** **Prof. Nelun Perera** Consultant Microbiologist, Honorary Associate Professor, Department of Infection, Immunity and Inflammation, University of Leicester, Training Programme; Director, Microbiology, Health Education, England / East Midlands, United Kingdom
3.45 pm - 4.15 pm	**Plenary 5** ***Facing SARS 2 with SARS 1 experience*** **Prof. J. S. M. Peiris** Chair Professor in Virology, The School of Public Health, University of Hong Kong
4.15 pm - 5.00 pm	**Guest lecture 1** ***Current and future therapies for infections due to multi-resistant Gram-negatives*** **Prof. David Livermore** Professor in Medical Microbiology, University of East Anglia, St Mary's Crescent, London, United Kingdom

Day 2 – 14th August 2020

8.00 am - 8.30 am	**Registration**
8.30 am - 9.30 am	**Symposium 3 –Transplant associated parasitic diseases in Sri Lanka: Challenges in diagnosis and treatment** ***Diarrhoeal pathogens: Cryptosporidium and Cystoisospora (Isospora)*** **Dr. Hasini Banneheke** Senior Lecturer, Department of Parasitology, Faculty of Medical Sciences, University of Sri Jayewardenepura ***Toxoplasmosis*** **Dr. Nilakshi Samaranayake** Senior Lecturer, Department of Parasitology, Faculty of Medicine, University of Colombo ***Strongyloidiasis*** **Prof. T. Channa Yahathugoda** Professor in Parasitology, Department of Parasitology, Faculty of Medicine, University of Ruhuna
9.30 am - 10.00 am	**Break**
10.00 am - 10.30 am	**Plenary 6** ***Vaccines in Dengue*** **Dr. LakKumar Fernando** Clinical Head, Centre for Clinical Management of Dengue & Dengue Haemorrhagic Fever, Negombo, Sri Lanka & Consultant Paediatrician, General Hospital Negombo
10.30 am - 11.00 am	**Plenary 7** ***Genes and Mycobacteria – Lessons to be learnt*** **Dr. Rajiva de Silva** Consultant Immunologist, Medical Research Institute, Colombo
11.00 am - 11.30 am	**Plenary 8** ***Treatment of Invasive fungal Infections*** **Prof. Cornelia Lass-Flörl,** Director, Institute of Hygiene and Medical Microbiology Director, CD-Laboratory for Invasive Fungal Infections, Medical University of Innsbruck, Austria
11.30 am - 12.15 pm	**Symposium 4 – Endocarditis in pace-maker** ***Pace-maker lead endocarditis – Microbiologist's perspective*** **Dr. Mahen Kothalawala** Consultant Clinical Microbiologist, National Hospital Kandy ***Challenges in treatment of pacemaker associated infective endocarditis*** ***Dr. G. I. D. Dushyanthie A. D. Athukotrala*** Consultant in Medical Microbiology, Department of Microbiology, University Hospitals of Coventry and Warwickshire NHS Trust, United Kingdom

12.15 pm - 1.15 pm	**Break**
1.15 pm - 1.45 pm	**Plenary 9** ***Progress towards elimination of hepatitis C virus infection*** **Prof. William L Irving** Professor and Honorary Consultant in Virology, University of Nottingham and Nottingham University Hospitals NHS Trust, United Kingdom
1.45 pm - 2.15 pm	**Guest lecture 2** ***Changing Microbiology to support patients and to steward antibiotics*** **Prof. David Livermore** Professor in Medical Microbiology, University of East Anglia, St Mary's Crescent, London, United Kingdom
2.15 pm - 2.45 pm	***Photography for medical presentations*** **Dr. Mangalanath Udukala** Microbiologist Kings College London, United Kingdom
3.00 pm onwards	Concluding ceremony, Awards and fellowship

CONCLUDING PROGRAMME

3.15 pm	Invitees take their seats
3.25 pm	Ceremonial Procession
3.30 pm	National Anthem
3.35 pm	Traditional lighting of the Oil Lamp
3.45 pm	Welcome Address **Dr. Jananie Kottahachchi** Hon. Joint Secretary
3.50 pm	Address by the President **Dr. N. Shirani Chandrasiri**
4.00 pm	Award of SLCM Fellowships
4.45 pm	Award of prize winners at the 29th Annual Scientific Sessions
5.00 pm	Vote of Thanks **Dr. Dushani Jyawardhana** Hon. Joint Secretary
5.05 pm	Ceremonial Procession leaves
5.10 pm	Reception

LIST OF E-POSTERS

11.08.2020

Session	Time	PP No.	Abstract
1	8.30am - 9.30am	PP 1	**Does pyrin gene influence *Helicobacter pylori* associated inflammatory changes in gastric mucosa?** *Weerasinghe GGYH[1], Gunesekara TDCP[1], Weerasekara MM[1], Jayakody S[2], Senevirathne BS[3], Weerasekara DD[4], Jayasinghe C[5], Perera MHN[5], Nisansala GGT[1], Fernando SSN[1]* [1]Department of Microbiology, Faculty of Medical Sciences, University of Sri Jayewardenepura, [2]Department of Community Medicine, Faculty of Medical Sciences, University of Sri Jayewardenepura, [3]Department of Pathology, Faculty of Medical Sciences, University of Sri Jayewardenepura, [4]Department of Surgery, Faculty of Medical Sciences, University of Sri Jayewardenepura, [5]Department of Medical Laboratory Sciences, Faculty of Medical Sciences, University of Sri Jayewardenepura
		PP 2	**Analysis of the aerobic bacterial flora in chronic wounds and the effect of locally applied acetic acid in an out-patient setting** *Galagedara WD[1], Dassanayaka KMMP[2], Wadanamby JMRW[2], Chandrasiri NS[3]* [1]Postgraduate Institute of Medicine, [2]National Institute of Infectious Diseases, [3]Colombo South Teaching Hospital
		PP 3	**Analysis of blood culture isolates of Colombo North Teaching Hospital, Ragama in 2019** *Diyalagoda DPKE[1], Senevirathne WDDN[2], Nadeeshani MV[2], Silva RKMC[2], Chanchala GHN[2], Samaranayake PMJ[2], Ranaweera DV[2], Jayasooriya JADTPS[2], Gunarathne ID[2], Namalie KD[2]* [1]Post Graduate Institute of Medicine, Colombo, [2]Colombo North Teaching Hospital, Ragama
		PP 4	**Blood stream infections of trauma patients admitted to trauma intensive care units at the National Hospital of Sri Lanka** *Diyalagoda DPKE[1], Vidanagama DS[2], Patabendige CGUA[2]* [1]Post Graduate Institute of Medicine, Colombo, [2]National Hospital of Sri Lanka, Colombo
		PP 5	**Effect of bundle care on central line associated blood stream infection at medical intensive care unit at the National Hospital of Sri Lanka** *Sugathadasa MRDN[1], Kottahachchi J[2], Patabendige CGUA[1]* [1]National Hospital of Sri Lanka, Colombo, [2]Department of Microbiology, Faculty of Medical Sciences, University of Sri Jayewardenepura

Session	Time	PP No.	Abstract
2	10.00am - 11.00am	PP 6	**The presence of inapparent dengue cases in a community and hospital** *Wing Tan[1], Jonathan Wee Kent Liew[1], Sivaneswari Selvarajoo[1], Wardha F. Refai[2], Yee Ling Lau[1], Indra Vythilingam[1]* [1]Department of Parasitology, Faculty of Medicine, University of Malaya, Malaysia, [2]Postgraduate Institute of Medicine, Colombo
		PP 7	**Sero-prevalence of antibodies to varicella zoster virus and associated factors among the undergraduates at the Allied Health Sciences Unit, University of Jaffna** *Jayamanna PCCA[1], Murugananthan K[2]* [1]Allied Health Science Unit, Faculty of Medicine, University of Jaffna, [2]Department of Microbiology, Faculty of Medicine, University of Jaffna
		PP 8	**Filarial parasites among two dog communities in selected filariasis endemic and non-endemic areas in Sri Lanka** *Rathnayake SP[1], Mallawarachchi CH[2], De Silva LAPNF[3], Gunathilaka PADHN[4], Chandrasena TGAN[4]* [1]Department of Animal Production & Health, Northern Province, [2]Medical Research Institute, Colombo, [3]Department of Community Medicine, Faculty of Medicine and Allied Sciences, University of Rajarata, [4]Department of Parasitology, Faculty of Medicine, University of Kelaniya
		PP 9	**Antimicrobial use in poultry: A field study in the Kegalle District** *Ranaweera NPNM[1], Peiris H[1], Wijayaratne WMDGB[2]* [1]Faculty of Allied Health Sciences, University of Ruhuna, [2]Faculty of Medicine, University of Ruhuna
		PP 10	**High prevalence of community-associated methicillin-resistant *Staphylococcus aureus* in patients infected with *Staphylococcus aureus* at a tertiary care center** *Kurukulasooriya MRP[1], Piyasiri B[2], WMDGB Wijayaratne[1], LG Tillekeratne[3], CK Bodinayake[1], AD de Silva[4], BP Nicholson[3], T Østbye[3], CW Woods[3], ADeS Nagahawatte[1]* [1]Faculty of Medicine, University of Ruhuna, [2]Teaching Hospital, Karapitiya, Galle, [3]Duke University, Durham, USA, [4]Faculty of Medicine, General Sir John Kotelawala Defence University

Session	Time	PP No.	Abstract
2	11.00am - 12.00 noon	PP 11	**Knowledge and attitude of food/ hand hygiene among food handlers at a University in Sri Lanka** *Senanayake NP, Malliwadu NC, Gangoda EWWMSB, Ariyaratne MN* General Sir John Kotelawala Defence University
		PP 12	**In vitro antibacterial and antibiofilm activity of *Tribulus terrestris* L. against planktonic and biofilm models of common uropathogens** *Gunasekara SP[1], Bandara DS[2], Nishadhya S[3], Aluthwaththa MG[4], Pathirana R[5], Widanagamge R[6]* [1,2,3,4]Department of Microbiology, Apeksha Hospital, Maharagama. [5,6]Department of Medical Laboratory Sciences, General Sir John Kotelawala Defence University
		PP 13	**Clinical presentation, aetiology, drug resistance and mortality of blood stream infections in elderly in a tertiary hospital, Galle, Sri Lanka** *Piyasiri DLB[1], Galhenage MN[1], Kirubaharan A[1], Nanayakkara IRS[1], Jayasekara JVG M[1], Sapukotana PM[1]* [1]Teaching Hospital Karapitiya, Galle
		PP 14	**Epidemiology of pneumococcal bacteraemia in a tertiary care center, Sri Lanka** *Piyasiri DLB, IRS Nanayakkara, Jayasekara JVGM, Galhenage MN* [1]Teaching Hospital Karapitiya, Galle
		PP 15	**Correlation of risk factors with the clinical presentation, microbiology and genetic profile of community acquired methicillin resistant *Staphylococcus aureus* in skin and soft tissue infections among patients attending a tertiary care hospital in Sri Lanka** *Liyanage N[1], Piyasiri DLB[1]* [1]Teaching Hospital Karapitiya, Galle
2	**11.08.2020** 8.30am-12.00pm	PP 16	**Ventriculoperitoneal shunt infection with *Salmonella* Enteritidis following gastroenteritis; a case report** *Ulwishewa GM[1], Piyasiri DLB[1], Jayaweera APN[1], Priyadharshana WBU[1]* [1]Teaching Hospital Karapitiya, Galle
		PP 17	**A case of invasive listeriosis following gastroenteritis in an infant** *Ulwishewa GM[1], Piyasiri DLB[1], Jayawardena P[1], Sanjeewa MAGA[1], Sapukotana PM[1]* [1] Teaching Hospital Karapitiya, Galle

Session	Time	PP No.	Abstract
		PP 18	**A case of complicated infective endocarditis following thyroidectomy: A story of treatment success** *Thushari HL[1], Wijeweera KDDS[1], Jazeel MN[1], Gunapala LHADL[1], Vidanagama DS[1], Patabendige CGUA[1]* National Hospital, Sri Lanka.
		PP 19	**A case report of infant meningitis possibly by *Salmonella* Cholerasuis** *Piyasiri DLB[1], Sajeemala WG[1], De Silva MAH[1], Dissanayake SKVP[1], Pathirage S[2]* [1]Teaching Hospital Karapitiya, Galle [2]Medical Research Institute, Colombo
		PP 20	**Viral kinetics in serial respiratory samples and clade of the virus of the first positive (SARS CoV-2) patient reported in Sri Lanka** *Jayamaha CJS[1], Narangoda E[2], Witahnage VH[1], Ekanayake DHP[1], Jayathunga RCS[1], Chu DKW[3], Peiris JSM[3]* [1]National Influenza Centre, Department of Virology, Medical Research Institute, Colombo, [2]National Institute of Infectious Diseases, Colombo, [3]School of Public Health, LKS Faculty of Medicine, The University of Hong Kong

LIST OF GUEST SPEAKERS

Prof. David Livermore

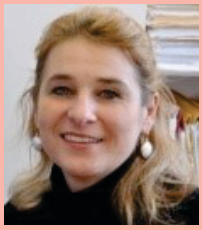
Professor in Medical Microbiology, Norwich Medical School, University of East Anglia, United Kingdom

Prof. William Irving

Professor and Honorary Consultant in Virology, University of Nottingham and Nottingham University Hospitals NHS Trust, United Kingdom

Prof. Cornelia Lass-Flörl

Director, Institute of Hygiene and Medical Microbiology
Director, CD-Laboratory for Invasive Fungal Infections,
Medical University of Innsbruck, Austria



Dr. Ahmed Abd El Wahed

Head of the Virology Lab, Division of Microbiology and Animal Hygiene, Georg-August-University Goettingen, Germany, Guest Scientist at the German Primate Center, Visiting Professor at the University of Cairo, Egypt

Prof. J. S. M. Peiris

Chair Professor in Virology, The School of Public Health, University of Hong Kong, Hong Kong

Dr. Chris Coulter

Microbiologist and infectious Disease Physician, Director Queensland Mycobacterium Reference Laboratory, WHO collaborating Centre in Tuberculosis Bacteriology, Supra National Reference Laboratory, Queensland, Australia

Prof. Nelun Perera

Consultant Microbiologist, Honorary Associate Professor
Department of Infection Immunity and Inflammation
University of Leicester, Training Programme Director
Microbiology, Health Education England / East Midlands, United Kingdom

Prof. Asitha de Silva

Chairman, National Medicine Regulatory Authority, Colombo

Prof. Priyadarshani Galappatthy

Professor, Head, Department of Pharmacology, Faculty of Medicine, University of Colombo

Dr. Rajiva de Silva

Consultant Immunologist, Medical Research Institute, Colombo

Dr. Lilani Rajapaksa

Consultant Venereologist, Deputy Director, National STD/AIDS Control Programme, Colombo

Dr. Jayanthi Elwitigala

Consultant Microbiologist, STD / AIDS Control Programme, Colombo

Dr. Hasini Banneheke

Senior Lecturer, Faculty of Medical Sciences, University of Sri Jayewardenepura

Dr. Nilakshi Samaranayake

Senior Lecturer, Department of Parasitology, Faculty of Medicine, University of Colombo

Prof. T. Channa Yahathugoda

Professor in Parasitology, Department of Parasitology, Faculty of Medicine, University of Ruhuna

Dr. LakKumar Fernando

Clinical Head, Centre for Clinical Management of Dengue & Dengue Haemorrhagic Fever, Negombo, Sri Lanka & Consultant Paediatrician, General Hospital Negombo

Dr. Mahen Kothalawala

Consultant Clinical Microbiologist, National Hospital Kandy

Dr. G. I. D. Dushyanthie A. D. Athukotrala

Consultant in Medical Microbiology,
Department of Microbiology, University Hospitals of Coventry and Warwickshire NHS Trust, United Kingdom

Dr. Mangalanath Udukala

Microbiologist, Kings College London

GUEST LECTURE 1

Current and future therapies for infections due to multi-resistant Gram-negatives

Prof. David M Livermore

It has become a truism to say that 'We have no antibiotics against problem Gram-negatives.' And, as with most truisms, it is ceasing to be true.

Two new families of β-lactamase inhibitors, diazabicyclooctanes (DBOs) and boronates have been developed, and their first combinations – ceftazidime/avibactam, meropenem/vaborbactam and imipenem/relebactam are now in clinical use. These provide answers to Enterobacterales with KPC carbapenemases and, in ceftazidime/avibactam's case, also to those with OXA-48-like enzymes; they do not, however, address Enterobacterales NDM metallo-b-lactamases (MBLs), which are more prevalent in south Asia.

In the future MBLs may be overcome too, by: (a) using a partner b-lactam that is inherently stable to MBLs together with a DBO that protects it from co-produced ESBLs, as with aztreonam-avibactam, (b) using a DBO that has direct antibacterial activity, as with cefepime-zidebactam or meropenem-nacubactam or (c) using a second-generation-boronate which, unlike vaborbactam, directly inhibits MBLs, as with cefepime/taniborbactam. One other inhibitor combination to mention, ceftolozane/tazobactam, does not address carbapenemase producers at all, but is potently active against *Pseudomonas aeruginosa* with upregulated efflux or hyperproduced chromosomal β-lactamase.

β-Lactams stable to multiple carbapenemases are reaching the clinic too, the first being cefiderocol, a catechol cephalosporin able to permeate Gram-negative bacteria efficiently by exploiting the ferric iron uptake pathway. Additionally BOS-228, a monobactam stable to all carbapenemase types, and to ESBLs, has completed Phase II development.

Among non-β-lactams, the aminoglycoside, plazomicin gave promising results in a Phase III cUTI trial and evades almost all aminoglycoside modifying-enzymes, though it does lose activity against those with ribosomal methyltransferases, which are often encoded by the same plasmids that determine NDM and (increasingly) OXA-48 carbapenemases. Lastly, the fluorocycline eravacycline is 2- to 4- fold more active than tigecycline against Enterobacteriaceae and *Acinetobacter*, including carbapenemase producers: clinical results were encouraging in intra-abdominal infection but disappointing in complicated urinary infection.

In short, the situation less hopeless than often portrayed, and we look set to have a life-raft of new anti-Gram-negative agents. The challenges will become 'when', 'which' and 'how' to use these, given that acquisition costs will be significant and that resistance – already seen to ceftazidime/avibactam in *Klebsiella* with mutant KPC carbapenemases and to ceftolozane/tazobactam in *P. aeruginosa* hyperproducing mutant chromosomal β-lactamases – will remain a threat.

GUEST LECTURE 2

Changing microbiology to support patients and to steward antibiotics

Prof. David M Livermore

Laboratory microbiology takes 2 days: one to grow the bacteria and a second to identify them and to determine their susceptibility. During this time the patient is treated 'empirically', carpet-bombing with antibiotics to cover all likely pathogens. This approach, necessary because the patient might have a resistant pathogen, leads to excessive treatment for the many patients whose pathogens are not particularly resistant. This, in turn, drives selection of resistance in the gut flora, and super-infection with *Clostridium difficile*.

We urgently need methods to accelerate precise diagnosis, allowing early refinement of antibiotic therapy. Three potentially useful strategies exist. Firstly, 'biomarkers' such as C-reactive protein or procalcitonin can be used to swiftly distinguish patients with bacterial versus viral infection. Secondly there is scope for molecular methods, performed directly on clinical specimens, to seek key individual resistances. This approach is already used to find MRSA and carbapenemase producers in screening swabs, informing infection control, but might be extended e.g. (i) to detect these organisms in urines clinical or respiratory specimens, guiding treatment or (ii) to identify whether *N. gonorrhoeae* (already sought by PCR in urine) is susceptible or resistant to fluoroquinolones. Last, and most tantalising, are comprehensive tests to fully profile a pathogen's resistance and susceptibilities. Several PCR-based systems are available or under development to achieve this for respiratory or urine specimens. We have shown that it is also possible, using nanopore technology, to generate comprehensive pathogen sequence data within c 4h, from infected urines, detecting all acquired resistance genes. This progress is promising but huge challenges remain, including (i) the imperfect correlation of resistance phenotypes and genotypes, and (ii) the propensity of PCR to give unwarranted significance to organisms that would be dismissed as 'mixed colonising flora' by the professional microbiologist reviewing culture plates. A final option is rapid culture, using automated microscopes to monitor early bacterial growth from specimens. This is slower than molecular methods, but will not miss novel mechanisms or ones not sought in PCR, which is limited by the number of primers that can be multiplexed.

In summary, rapid bacteriology has a great – and still largely unrealised – potential to improve antibiotic stewardship but faces many technical challenges. Overcoming these is vital if we are to advance from our present, very wasteful, use of antibiotics.

Plenary Lectures

Plenary presentation 2

Overview of the point of need diagnostics for the SARS-CoV-2

Dr. Ahmed Abd El Wahed

COVID-19 has emerged as a new viral disease, which was first reported in China in late 2019. The disease rapidly spread and developed into a global pandemic with severe health and economic impact. Approved antiviral therapeutics or vaccines are not available at the moment. Therefore, the only measures to control the pandemic are rapid detection, contact tracing, isolation and management for infected cases. Although real-time RT-PCR is the standard approach in terms of sensitivity, accuracy and safety, it however requires well-established laboratory, well trained personnel and long run time (3-5 hours). The need to develop and apply a rapid and simple diagnostic approach that maintains the performance of real-time RT-PCR and can be used in point- of-need settings is urgent. In this talk, an overview for the development of rapid molecular isothermal amplification will be presented. In addition, the advantages and disadvantages of the rapid antigen tests will be discussed.

Plenary presentation 4

Developing an antifungal stewardship programme: pharmacological options, challenges and benefits (personal experience)

Prof. Nelun Perera

Invasive fungal infection (IFI) is associated with a high mortality and morbidity amongst patients with haematological malignancies. Early diagnosis has always been a challenge to both the haematologist and the microbiologist. The mainstay of diagnosis is demonstration of typical manifestations consistent with IFI by radiology. Laboratory diagnosis by isolation of fungi in culture has a low sensitivity and can take several days. Detecting fungal biomarkers in blood offers an alternative approach to diagnosis. These tests have varying sensitivity and specificity.

Managing IFI requires a multi-disciplinary approach and requires careful consideration of local prevalence of IFI, population of patients at risk of IFI, antifungal prophylaxis and accessibility to diagnostics (i.e. radiology and mycology).

Commencing empiric antifungal drugs, when fever is not responding to broad-spectrum antibiotic treatment has a long and established history. An alternative approach is “directed therapy”. This is initiation of treatment with antifungal drugs when IFI is highly likely either due to detection of fungal biomarkers in the patient’s blood or demonstration of radiological manifestations of IFI.

The benefits of adopting a directed treatment pathway by moving away from empiric antifungal treatment, the associated antifungal cost savings (sustained year on year) in our adult haemato-oncology unit, University Hospitals of Leicester, UK will be presented.

Plenary presentation 5

Facing SARS-2 with the SARS-1 experience

Prof. Malik Peiris

The SARS outbreak in 2003 was a transformative experience. It demonstrated that an emerging infectious disease outbreak anywhere was the threat everywhere. This realization led to the implementation of the new International Health Regulations in 2005, which required every country to develop systems to detect, respond and report unusual outbreaks. However, this is not universally achieved as yet. Even more concerning was the lack of funding needed for counter-measure development, which includes antivirals and vaccines, to pathogens of significant threat. While response-systems for influenza pandemics had been enhanced, response to other emerging pathogens – corona viruses had indeed been highlighted as such – were not implemented rapidly enough. As such, when SARS-CoV-2 emerged, the world, including the developed affluent world, was woefully unprepared, in counter measures as well as with the generic public health preparedness required to deal with it. We did not learn quickly enough from SARS-1. We must make sure that we at least learn from SARS-2 for the future, because this will not be the last such emerging

infectious disease threat. Containment of SARS-1 by early patient diagnosis and isolation was possible because the virus was only transmissible 4 or 5 days after symptom onset. Furthermore, most infections were symptomatic. In contrast, it is estimated that around 40% of trans-mission of SARS-CoV-2 takes place prior to onset of symptoms, thus making testing and isolation not sufficient by themselves. Asymptomatic infections are common, especially in children and young adults. Both pre-symptomatic as well as truly asymptomatic infections are likely contributing to onward transmission. There is significant heterogeneity of transmission with around 20% of cases accounting for around 80% of new infections. These features of SARS-CoV-2 requires us to go one step further, beyond the testing and case isolation that was sufficient to contain SARS-CoV-1, to allow us to keep one step ahead of the virus transmission chain, i.e. contact tracing and quarantine, supplemented by social distancing as well as face masks/face covering and hand hygiene. Evidence of population "immunity" from sero-epidemiological studies, even in heavily affected cities, e.g. New York, show that "herd immunity" so far, remains far short of what is required for natural containment. Thus second waves are inevitable, but they do not need to be large, provided a) active surveillance is maintained at a high level to detect re-emergence early and b) public health and social interventions are reactivated promptly. Viral genetic sequencing may help understand chains of transmission and facilitate contact tracing.

Plenary presentation 6

Vaccines in dengue

Dr. LakKumar Fernando

Approximately half of the world now lives under the threat of dengue, which is responsible for approximately 390 million infections and 20,000 deaths globally each year. An effective vaccine remains the best hope against this dreaded disease and decades of research are at this task with variable hope and success. To date there is only one licensed vaccine available for use and this is approved in19 countries and Sri Lanka is not one of them. The FDA of United States who gave priority review for the same considering the need, approved it last year restricting it only for children and teens aged 9 to 16 years living in endemic areas who have had a previous, laboratory confirmed dengue infection.

There are other vaccines undergoing different phases of evaluation but are couple of years or more away from licencing.

Dengue has four serotypes and an effective vaccine should protect against all these. When one develops dengue due to one serotype there is life long immunity against that serotype and some cross protection against the other three that will not last more than about 6-18 months. Severe dengue accompanied a second (not a third or fourth) dengue infection in most patients. Antibody dependant immune enhancement is the biggest challenge against a good dengue vaccine. T-cell immunity is also crucial for effective protection against dengue.

The world badly needs a good dengue vaccine that can protect against all four serotypes that could give lasting immunity. The vaccine solution has to be equitable, affordable and ethical too.

Plenary presentation 9

Progress towards elimination of hepatitis C virus infection

Prof. William Irving

This talk will outline the WHO Global Health Sector Strategy for elimination of viral hepatitis C as a public health threat by the year 2030. Data from the UK will be used to indicate current successes and failures, and to indicate where public health efforts need to be strengthened. Issues to be discussed will include how to identify and diagnose those at most risk of infection, what regimens are most appropriate for therapy, and how drug resistance may impact on elimination targets.

Symposium 2

Prevention of mother to child transmission of HBV

Prof. William Irving

Mother to baby transmission of hepatitis B virus is one of the most important routes of spread of infection. Protocols aimed at fulfilling the WHO Global Health Sector Strategy goal of elimination of viral hepatitis B as a public health threat by the year 2030 must focus on prevention of mother-to-child transmission. Current approaches require universal antenatal screening, and appropriate institution of effective prophylaxis including antiviral therapy delivered during pregnancy, and active and passive immunisation of babies. This talk will review data on the effectiveness of those preventative measures as reported in the literature and as experienced in the United Kingdom.

Elimination of mother-to-child transmission of HIV and Syphilis

Dr. Lilani Rajapaksa

Sri Lanka is being declared as a country which has eliminated mother to child transmission of HIV and syphilis by the World Health Organization, Geneva in November 2019.

According to the reported data by the National STD AIDS Control Programme, by the end of 2018, there were 85 children who were infected with HIV due to mother to child transmission. Annually children are being newly diagnosed with congenital syphilis.

Programme for prevention of congenital syphilis commenced in 1954 and in 2002 the programme for prevention of paediatric HIV was established. In the year 2013, these two programmes were combined and further strengthened as "Elimination of mother to child transmission (EMTCT) of HIV and syphilis programme".

HIV and syphilis testing coverage among pregnant women increased to >95% by end 2017. All pregnant women with HIV or syphilis and babies exposed were managed appropriately. All pregnant women diagnosed with HIV infection and syphilis received EMTCT services and delivered uninfected children. By the end of 2018, country had achieved the set targets to be eligible to request for validation of EMTCT of HIV and syphilis status.

In July 2019, the Ministry of Health made a formal request to WHO to validate the EMTCT of HIV and syphilis status in the country. This was followed by the visit of the Regional Validation Team (RVT) of experts in September. After a thorough assessment, the Regional Validation Team presented it' s report on Sri Lanka to Global Validation Committee at WHO headquarters based in Geneva.

The WHO headquarters, based on the decision of Global Validation Committee, has formally declared that Sri Lanka has eliminated mother to child transmission of HIV and syphilis.

This programme contributed to the development of the country by assuring child health, reducing maternal illnesses and deaths and by increasing accessibility to services for women in disadvantaged situations. Further, it normalized the HIV test and promoted open discussion on HIV.

This achievement will have an inspiring impact on the resolve to end AIDS by 2025, five years ahead the global target in 2030, a commitment undertaken by the country.

Symposium 3

Diarrhoeal pathogens: *Cryptosporidium* and *Cystoisospora (Isospora)*

Dr. Hasini Banneheke

Approximately 60 people undergo an organ transplant per day in the world. Of solid organ transplantations, renal and liver transplantations are regularly performed in Sri Lanka. In 2017, 400 kidney transplantations had been performed in Sri Lanka. As of 2018, the ratio of organ transplantation per total population in Sri Lanka remains at 1: 52,477.

There is an increased risk of opportunistic infections in organ transplant (Tx) patients who are taking immunosuppressive drugs. Of those, parasitic infections are less common than viral, bacterial, or fungal infections.

Cryptosporidium and Cystoisospora belli (previously known as *Isospora belli*) are protozoan parasites causing diarrhoea in humans. Transplant recipients mostly presents with profuse diarrhoea in both infections. In Sri Lanka, both above parasitic genera are reported among animals, however cases of human infections in immunocompetent and immunocompromised persons are only described with *Cryptosporidium species*.

Both Cryptosporidium and *Cystoisospora* belong to phylum *Apicomplexa*. They infect the small intestine of humans. Both are mainly transmitted by ingesting water (or food) contaminated with mature oocysts. Parasitic oocysts are passed intermittently thus repeated samples are needed in immunocompetent persons but may not be needed in immunocompromised patients due to heavy infection. The oocysts passed out in faeces may not be identified by wet mount microscopy. Thus, suspecting the possibility of these parasites in the differential diagnosis is important to get the laboratory confirmation. Diagnosis of both parasites is primarily by microscopic identification of the oocysts in feaces stained with a modified acid-fast technique. The diagnostic challenge is to differentiate *Cryptosporidium* oocysts from yeasts that are similar in size and shape (but are not acid fast). *C. belli* is easily recognised by its characteristic shape and size. Concentration of the faecal sample (preferably using Sheather's sugar flotation technique) increases the

sensitivity. There are other techniques such as direct fluorescent antibody, enzyme immunoassays for antigens and polymerase chain reaction for the detection of *Cryptosporidium*. Even though *Cryptosporidium* is frequently detected in children and immunocompromised persons, there is no effective drug against the parasite. In AIDS patients' anti-retroviral therapy with immune restoration is associated with complete resolution of cryptosporidiosis. Kandy renal transplant unit, the biggest unit in Sri Lanka with more than 1000 post-Tx patients in follow up, treats crytposporidiosis cases by reducing immune suppression and giving probiotics. Trimethoprim-sulfamethoxazole is the drug of choice for *C. belli*. Further rehydration and repletion of electrolyte losses by either the oral or intravenous route is also important.

By looking at the literature reporting *Cryptosporidium* and *Cystoisospora* among animals and in the environment samples such as water sources in Sri Lanka, both parasites are possibly under-reported in humans. Hence it is essential to test a sample of faeces for *Cryptosporidium* and *Cystoisospora* specially in immuno-compromised persons such as post-transplant patients who develop prolonged and profuse diarrhoea.

Symposium 4

Pacemaker lead endocarditis – Microbiologist's perspective

Dr. Mahen Kothalawala

Pacemaker lead endocarditis (PME) is a potentially life threatening complication of insertion of cardiac pacemakers. Despite, the technological advancements of pacemaker design and sterile techniques, PME contributes significantly as a significant complication of pacemaker insertion.

PME carries a considerable mortality. Available data reveal an average mortality of between 0.5 to 7% in different localities. The mortality rate of 66% was reported for cases where primary pathology was not resolved appropriately.

Establishment of a firm diagnosis of PME is required for optimal management of cases. But seemingly it had become a difficult task as it may not be possible at all times. A diagnosis of pacemaker lead endocarditis should be considered in all patients presenting with fever and having a history of insertion of pacemaker.

Worldwide data shows higher Gram positive rates in PM endocarditis. Role of Gram negatives remain uncommon. Candida species and filamentous fungi too play a major role as etiological agents.

Data on PME and its etiologies are quite rare in Sri Lanka. We present data available with us at National Hospital Kandy during last five years. Out of the 224 patients with microbiologically and echocardiographically proven cases of infective endocarditis patients encountered, only 17 were confirmed as cases of PME. There was a male preponderance with 70% of the total. Average age has been around 63 years. The major pathogenic group was coagulase negative staphyloococci (53%) while, *Staphylocoocus epidermidis* (5) and *Staphylococcus intermedius* (2) and *S. heamolyticus* (1) too accounting for majority of cases. *Staphylococcus aureus* accounted for 2 cases. Candida species accounted for 2 cases with *Candida tropicalis* and *C. albicans* contributing one case each. *Filamentous Aspergillus* species accounted for one case. The patients whom where the lead was replaced early demonstrated favourable outcome.

Challenges in treatment of pacemaker associated infective endocarditis

Dr. G. I. D. Dushyanthie A. D. Athukotrala

Most of the implantable cardiac electronic devices (ICEDs) in current use have leads that connect the generator to cardiac tissue. As more devices are being implanted worldwide, though a rare complication, infections related to ICEDs including infective endocarditis (IE) are on the rise. ICED infections can be life-threatening, particularly IE, and all-cause mortality of up to 35% has been reported. ICED associated IE can be difficult to manage due to many reasons.

Due to population differences, guidelines for management of ICED associated IE in one country cannot be used as it is in other regions. Unavailability of data for ICED associated IE is a huge problem in managing patients in many parts of the world including developed countries.

Unavailability of agreed international definitions for ICED associated IE is another problem in diagnosis and management of the condition. Working parties in some countries have agreed upon definitions which will be confirmed in the future with more available evidence from patients.

Like native valve and prosthetic valve IE, diagnosis of ICED associated IE itself is difficult due to subtle signs of infection, less accurate echocardiograms and less sensitive blood cultures, making the patients at risk of

not receiving timely treatment. Unavailability of newer imaging techniques in remote facilities may play a role in missed or delayed diagnosis. Even when available, demand for cardiac imaging is such, it may not happen in a timely manner to come to an early diagnosis.

ICED associated IE is also complex to manage because there are intra-cardiac and extra-cardiac components, both of which may become infected and removal of the device can be an intricate process, with a risk of death or significant complications to patients. Some patients may decline removal of the device, leaving it for salvage of the device, which is not successful in majority of occasions. Sparsity of facilities with surgical expertise for device removal is another challenge.

Long hospital stays are inevitable due to long courses of intravenous antibiotic treatment making dependents of the patients vulnerable due to risk of losing their occupation. Out-patient Parenteral Antimicrobial Treatment (OPAT) is a measure taken in some countries to overcome this challenge. Multiple inpatient episodes are common due to persistent or recurrent infections. Biofilm formation on the device is associated with relapses. Attempts to salvage infected systems frequently result in further prolonged courses of treatment.

Peripheral venous cannulas, peripherally inserted central cannulas, mid lines and tunnelled central venous catheters are used for prolonged causes of intravenous antibiotics in managing ICED associated IE, placing the patients at risk of acquiring catheter related blood stream infections.

Management of ICED associated IE is further hampered due to increasing co-morbidities in patients such as diabetes and organ impairment especially renal impairment during the course of ICED in place. This is further complicated by use of nephrotoxic drug in the treatment of IE. Regular monitoring of renal functions and serum concentrations of antibiotics also are challenging in certain localities.

ICED associated IE has a higher mortality rate compared to generator pocket infection.

It is important to know the pathogens involved in ICED associated IE. According to 3 studies done, *S. aureus* was the most common pathogen (35%-59% of patients), followed by coagulase negative staphylococcus species (14%-32%). Gram-negative bacteria were only reported in two studies (1% and 4.5%). As around 15% of ICED associated IE patients are negative for bacterial cultures, it makes it further difficult on deciding the appropriate antibiotics in the treatment.

Furthermore, it is worth noting false positive cultures of the lead tips as a result of generator pocket infection may lead to unnecessary treatment for ICED associated IE, exposing the patient to untoward side effects of antibiotics used including *C. difficile* infection.

With availability of data and agreed definitions for ICED associated IE, the future of patients with such infections will be safe.

OP 1

Development of a real-time multiplex PCR assay for the detection of *Kingella kingae* in the Children's hospital at Westmead, Sydney, NSW, Australia

Samaranayake WAMP[1], *Leung KC*[1], *Outhred AC*[1,2,3] *Kesson AM*[1,2,3]

[1]Department of Infectious Diseases and Microbiology, The Children's Hospital, Westmead, Australia, [2]The Marie Bashir Institute of Infectious Diseases and Biosecurity, The University of Sydney, [3]Discipline of Child and Adolescent Health, The University of Sydney, Australia.

Introduction

Kingella kingae has recently been recognized as an important etiology of invasive pediatric osteoarticular infection. The prevalence of *K. kingae* infection is well defined in some setting and probably underestimated in Australia. Traditional culture methods for *K. kingae* are insensitive, and recovery from commercial pediatric blood cultures is sub-optimal.

Objectives

To develop a real-time PCR for detecting *K. kingae* in clinical specimens.

Method

We developed a multiplex real-time PCR assay using hybridisation probe techniques to simultaneously detect the *rtxA* and *cpn60* genes of *K. kingae* in nucleic acids extracted from joint fluids and tissue specimens. The assay was optimized and validated at the Molecular Pathology Laboratory at the Children's Hospital at Westmead (CHW).

Results

The PCR assay detected all nine isolates of known *K. kingae* but not other 31 bacterial strains frequently found in pediatric osteoarticular infections, showing 100% analytical specificity for this pathogen. The analytical sensitivity of the PCR was then evaluated using 10 fold serial dilution of a known isolate of *K. kingae* bacterial suspension, which was quantified by culture. It exhibited a limit of detection of one colony forming unit (cfu)/ml. As clinical joint specimens were rare, the subsequent validation studies were conducted primarily with bacterial isolates suspended in a pleural fluid specimen known to be negative of *K. kingae*. Those results compared with the standard bacterial culture on solid media. The assay showed 100% sensitivity during the laboratory validation with simulated samples. In the extended study of clinical specimens over eight months, this assay detected *K. kingae* in five joint fluids and two tissue samples that were negative for culture isolates.

Conclusion

A real-time multiplex PCR assay highly specific and sensitive for *K. kingae* was developed at CHW. This assay is fit for diagnostic purposes in detecting this microbial pathogen in patients suspected of osteoarticular infection.

OP 2

Prevalence of carbapenem resistance and molecular analysis of carbapenem resistance among clinical isolates of *Pseudomonas aeruginosa* in a tertiary care hospital

Wijeweera KDDS[1], *Piyasiri DLB*[1]

[1]Teaching Hospital Karapitiya, Galle

Introduction

Increased resistance to carbapenems among *Pseudomonas aeruginosa* has been reported worldwide and carbapenem resistant *P. aeruginosa* (CRPA) has been identified by the WHO as one of the three critical (priority1) pathogens.

Objective

To describe the antibiotic sensitivity pattern of *P. aeruginosa* in clinical isolates and to analyse the carbapenemase genes among CRPA isolates.

Method

The study was carried out from December 2018 to April 2019 at a major tertiary care hospital. Out of the clinically significant isolates suspected as *Pseudomonas*, 144 were confirmed as *P. aeruginosa* by Remel RapID™NF PLUS system. Antibiotic sensitivity test was performed by CLSI (2018) disc diffusion method.

All the carbapenem resistant isolates were further tested for metallo-β-lactamase production by imipenem/EDTA and imipenem/imipenem + EDTA tests. Molecular analysis of carbapenemases was performed by GeneXpert™Carba-R assay.

Results

Majority of the isolates were from respiratory samples (n=51, 35.4%). The highest sensitivity was shown to meropenem and imipenem (n= 119, 82.6% each) while least sensitivity was to ticarcillin-clavulanate (n=57, 39.6%). Of the sensitivity to aminoglycoside the highest sensitivity was to amikacin (n=105, 72.6%).Out of the total isolates 29.2% (n=42) were multidrug resistant. Highest cross resistance was to ciprofloxacin and the difference of resistance to ciprofloxacin between CRPA (100%) and carbapenem sensitive isolates (28.4%) was statistically significant (p<0.0001).

The prevalence of resistance to meropenem or imipenem was 16% (n=23) and considered as CRPA. Only 8 (35%) CRPA isolates yielded a positive EDTA inhibition test. A positive Gene-Xpert Carba-R assay was positive in 74% (17/23). Eleven isolates were NDM producers (64.7%), 2 were IPM producers (11.76%) and 2 were VIM producers (11.76%) while 2 were co-harbouring carbapenemases (NDM+VIM; 11.76%). There were no KPC producers. Carbapenemase producers were 100% resistant to ticarcillin clavulanate, gentamicin and ciprofloxacin.

Conclusion

While the prevalence of carbapenem resistance was 16%, the predominantly detected carbapenemase was NDM. As high levels of antibiotic resistance in *P. aeruginosa* were detected, the choice of empirical antibiotic should be carefully considered and infection control measures have to be strengthened at the concerned healthcare facility.

OP 3

Comparison of conventional culture and real time PCR based direct detection method for the identification of pneumococcal colonization

Vidanapathirana G[1], Angulmaduwa SKL[2], Munasinghe TS[3], Ekanayake EWMA[2], Harasgama P[2], Kudagammana ST[3], Dissanayake BN[2], Liyanapathirana LVC[2]

[1]Faculty of Medicine, University of Peradeniya, [2]Department of Microbiology, Faculty of Medicine, University of Peradeniya, [3]Department of Paediatrics, Faculty of Medicine, University of Peradeniya

Introduction

Nasopharyngeal colonization is a prerequisite for the initiation and transmission of pneumococcal diseases. Real time PCR (RT-PCR) is an alternative approach for the identification and quantification of pneumococci directly from samples.

Objectives

To compare pneumococcal detection rates using culture based method versus RT-PCR direct detection.

To quantify pneumococcal colonization in two study cohorts (healthy children and hospitalized children with respiratory symptoms)

Method

A total of 101 nasopharyngeal swabs (NPS) from healthy children and 183 NPSs from hospitalized children with respiratory symptoms were included in the study. All children were between 2 months to 2 years. In parallel to routine culture and identification, a RT-PCR assay targeting the *lytA* gene was done.

DNA was extracted from the NPSs using a commercial kit. A standard curve for the RT-PCR was plotted using DNA from ATCC 49619 strain of *Streptococcus pneumoniae* with a 10-fold dilution series.

Results

Considering all 284 samples tested, colonization rate by conventional culture was 41.2% (n=117) while positive colonization using RT-PCR was 43.7% (n=124). The colonization rate detected by RT-PCR in the healthy cohort was 33.7% (n=34) and it was 49.2% (n=90) in the hospitalized cohort. It was 37.6% (n=38) and 43.2% (n=79) for the two cohorts by culture. The mean Cq value for the healthy cohort is 29.61 (SD 2.85) and 28.93 (SD 3.62) for the hospitalized cohort. With the standard curve obtained from amplifying a dilution series of control DNA, the mean amount of genomic DNA copy numbers detected in children with respiratory symptoms was $\log_{10}$ 7.49 (SD 1.07) while it was $\log_{10}$ 7.30 (SD 0.23) in healthy children (p>0.05).

Conclusion

Even though the difference was not statistically significant, there was a higher detection of pneumococcal colonization using RT-PCR. This, coupled with the ability to quantify makes RT-PCR a valuable tool in detection of pneumococcal colonization.

Acknowledgements

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OP 4

Genetic diversity among *Burkholderia pseudomallei* clinical isolates in Sri Lanka

Muthugama TA[1,2], Jayasinghearachchi HS[2], Masakorala J[1], Silva D de[2], Corea EM[1]

[1]Faculty of Medicine, University of Colombo, [2]Faculty of Medicine,General Sir John Kotelawala Defense University

Introduction

Clinical presentations of melioidosis are varied and genomic differences in *Burkholderia pseudomallei* contribute to such variations. The *Yersinia*-like fimbrial (YLF) gene cluster is prevalent in Southeast Asia including Thailand (98%) but is uncommon in Australia (12%) whereas the *Burkholderia thailandensis*-like flagellum and chemotaxis gene (BTFC) is most frequently reported in Australia (88%) and is rare in South East Asia including Thailand (2%). Similarly, for the $bimA_{Bp}$ and $bimA_{Bm}$ allele variants of the actin-based motility gene *bimA*, $bimA_{Bm}$ prevalence in Thailand is 0% but it is found in 12% of strains in Australia. Prevalence of the filamentous hemagglutinin 3 (*fhaB*3) gene variant is 100% in Thailand but only 83% in Australia. The lipopolysaccharide genotype A (LPSA) is predominant worldwide.

Objective

This study describes the genetic diversity of 437 *B. pseudomallei* clinical isolates from melioidosis cases in Sri Lanka.

Method

Genomic DNA from pure cultures was extracted and BRYT green real-time PCR assays were performed to detect the following: YLF, BTFC, $bimA_{Bp}$ and $bimA_{Bm}$, LPSA and *fhaB*3 genes.

Results

The majority of isolates carried the YLF gene cluster (249/300, 83%) while BTFC was less common (51/300, 17%). While $bimA_{Bp}$ was the most prevalent *bimA* allele variant (124/146, 85%), the $bimA_{Bm}$ allele was carried in 22/146 (15%). Most of the isolates were LPSA (96/110, 87%) and *fhaB*3 (124/148, 84%) positive.

Conclusion

Our data shows that *B. pseudomallei* strains from Sri Lanka are intermediate between Thailand and Australia with an YLF rate of 83% and BTFC rate of 17%. This may be due to the original positions of the countries in Gondwanaland. Prevalence of $bimA_{Bm}$ and *fhaB*3 contrasts with data for Thailand but is similar to the rates in Australia. The variation in prevalence of these genes may contribute to variations in clinical presentation, especially the presence of neurological melioidosis which is associated with the allele variant $bimA_{Bm}$. Further studies to correlate genotypic variation with clinical presentations are planned.

OP 5

Development of a porcine skin model for the assessment of bacterial biofilms

Jayasena Kaluarachchi TD[1], McBain AJ[2], Wickremasinghe R[1], Yasawardene S[3], Menike C[1], Jayathilake S[4], Ranasinghe S[1], Weerasekera MM[5]

[1]Department of Parasitology, Faculty of Medical Sciences, University of Sri Jayewardenepura, [2]School of Health Sciences, Faculty of Biology, Medicine and Health, The University of Manchester, United Kingdom, [3]Department of Anatomy, Faculty of Medical Sciences, University of Sri Jayewardenepura, [4]Department of Pathology, Faculty of Medical Sciences, University of Sri Jayewardenepura, [5]Department of Microbiology, Faculty of Medical Sciences, University of Sri Jayewardenepura

Introduction

Biofilms contribute to delayed wound healing. Porcine skin biofilm models are ex-vivo models for biofilm investigations. As pig skin closely relates to human skin, such models could be used as an ideal platform for human cutaneous wound research. This approach has been reported in a limited number of studies and to date is not being used in Sri Lanka.

Objective

To develop an ex-vivo porcine skin biofilm model.

Method

Biofilm forming *Pseudomonas aeruginosa* (NTCC 10662) and *Staphylococcus aureus* (ATCC 25923) subcultures were grown in Mueller Hinton Broth (MHB) overnight. Two full thickness cubes of fresh porcine skin were washed in sterile Phosphate Buffer Saline (PBS), PBS with Tween20 (PBST), PBST with 0.6% Sodium Hypochlorite and PBST with 70% Ethanol. The epidermis of the skin cubes were removed using sterile scalpel blades. Culture growths in MHB were dripped onto one cube (positive control) and sterile MHB on to the other (negative control). Dripping was done twice daily throughout the incubation. The two cubes were incubated at 37°C for 48h in a 6-well plate humidified with PBS soaked cotton. One section of each cube was formalin fixed and paraffin embedded.

Thin sections were subjected to Gram staining and fluorescence *in situ* hybridization (FISH). To tag the bacteria in FISH, a Cyanine3 tagged Eu-bacterial probe was used and the extracellular polymeric substances (EPS) was stained with Concanavalin-A conjugated Alexa-Fluor-488. Gram staining with safranin was used to stain the EPS. Gluteraldehyde fixed sections of each skin cube were dehydrated in ethanol and subjected to scanning electron microscopy (SEM) at 5kV.

Results

Aggregations of bacteria attached to the skin tissue with EPS were observed indicating bacterial biofilm formation in the positive control. These findings were absent in the negative control. This result was confirmed by three different imaging techniques (FISH, Gram stain and SEM).

Conclusion

The experimented porcine skin biofilm model was a success and could effectively be used in broadening the avenues of ex-vivo experimenting of biofilm involvement in chronic wounds.

Acknowledgement

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OP 6

Preliminary study to detect mutations in UL97 gene in suspected ganciclovir resistant CMV patients' samples, presented to a diagnostic laboratory in Sri Lanka

Thambyrajah JC[1], Jayamaha CJS[2], Ratnayake AKDVY[1], Handunetti S[1], Fernando N[1].

[1]Institute of Biochemistry, Molecular Biology and Biotechnology, University of Colombo, [2]Department of Virology, Medical Research Institute, Colombo

Introduction

Cytomegalovirus (CMV) is one of the most common opportunistic pathogens causing infection in immunocompromised individuals. CMV resistance to ganciclovir is reported to associate with UL97 gene mutations. Seventy percent of these ganciclovir resistant CMV strains contain mutations in three specific codons (460, 594, and 595) of the UL97 gene. Ganciclovir is used to treat CMV infection and as prophylaxis in Sri Lanka. However, no studies have been carried out on mutations in antiviral genes in CMV and resistance to CMV in Sri Lanka.

Objectives

To identify the prevalence of mutations in three specific codons (460, 594 and 595) and any prevalent polymorphism of UL97 gene in suspected ganciclovir resistant CMV patients presenting to Medical Research Institute.

Method

Retrospective study was performed on suspected ganciclovir resistant patients with static or increased levels of CMV viraemia even after 2-3 weeks of ganciclovir therapy. CMV DNA was extracted and PCR was performed to amplify the target region of UL97 gene. Sequencing reactions were carried out using the BigDye® Terminator v3.1 Cycle Sequencing Kit and resolved with Applied Biosystems®3500Dx capillary sequencer. Data were analyzed using BioEdit.

Results

Out of the 340 CMV infected patients, 12 were suspected as ganciclovir resistant. No mutation was found at above specified codons of UL97 gene. Interestingly, a single nucleotide change from thymine to cytosine was detected at the 1368th nucleotide position of the UL97 gene in all 12 samples. However, this nucleotide change is not significant as both codons GAU and GAC code for the same amino acid, Aspartic acid (D466D polymorphism).

Conclusion

No mutation was found in three specific codons (460, 594, and 595) of UL97 gene in this cohort. D466D polymorphism was detected in the UL97 gene in this cohort. Studies with a larger sample size are needed to ascertain mutations in UL97 gene.

OP 7

The diagnostic accuracy of a modified nested PCR-RFLP method in the diagnosis of cutaneous leishmaniasis caused by *Leishmania donovani*

De Silva NL[1], De Silva VNH[2], Deerasinghe ATH[3], Kato H[4], Itoh M[5], Takagi H[5], Weerasooriya MV[1], YahathugodaTC[1]

[1]Department of Parasitology, Faculty of Medicine, University of Ruhuna, [2]Base Hospital, Tangalle, [3]District General Hospital Hambantota, [4]Division of Medical Zoology, Department of Infection and Immunity, Jichi Medical University, Japan, [5]Department of Microbiology and Immunology, Aichi Medial University School of Medicine, Japan.

Introduction

Southern Sri Lanka is currently experiencing a surge of cutaneous leishmaniasis. The clinical diagnosis is very challenging. Therefore, early accurate laboratory diagnosis is vital. Internal Transcribed Spacer-1 (ITS1) PCR is well established for diagnosis of *Leishmania* species.

Objectives

To assess the use of a modified nested PCR-Restriction Fragment Length Polymorphism (RFLP) method using two novel inner primers targeting ITS1 region in detecting and identifying *Leishmania* species and to use it to diagnose a subset of patients from Hambantota district.

Method

Sensitivity of the modified nested PCR was tested using serial dilutions (10^3 to 10^{-2}) of DNA extract of cultured *L. donovani* DD8 strain. ITS1 PCR was performed using outer primers-LITSR: 5'-CTGGATCATTTTCCGATG-3' and L5.8S: 5'-TGATACCACTTATCGCACTT-3' – and the product was re-amplified using – LITSR-inner:5'-CATTTTCCGATGATTACACC-3' and L5.8S-inner: 5'-TACTGCGTTCTTCAACGA-3'. HaeIII restriction digestion and RFLP analysis was performed for species identification.

A cohort of 194 patients was examined clinically by dermatologists, by smear microscopy by hospital technicians and authors and by nested PCR-RFLP. The diagnostic accuracy was measured using McNemar's Test, Kappa inter-rater agreement test, sensitivity (Sn) and specificity (Sp) and positive (PPV) and negative predictive values (NPV).

Results

The nested PCR was sensitive enough to detect 10-1 parasites compared to ITS1 PCR (10^1). Nested PCR detected significantly higher percentage (94%) of parasites in lesions whereas it was only 77.6% by microscopy ($P<0.01$). All samples (n=194) demonstrated the same RFLP pattern of *L. donovani*. Most number of negative results by both microscopy (50%) and PCR (63.6%) were observed in cases with ulcerated nodules. Negative results by microscopy and nested PCR in different clinical lesions were significantly correlated ($r^2=0.782$; $P<0.05$). Inter-rater agreements between microscopists were substantial and significant (technician vs. author[1] - Kappa = 0.720; $P<0.01$, author[1] vs. author[2] - Kappa = 0.655; $P<0.01$). A fair agreement was observed between smear positives and PCR (Kappa=0.26; $P<0.05$). Smear microscopy demonstrated Sn=80.9%, Sp=81.8% and PPV= 98.7% and NPV=20.5%.

Conclusion

Higher diagnostic accuracy of modified nested PCR allows detection of lesions with parasites in ultra-low densities.

OP 8

Establishment of an in-house SARS CoV-2 real-time PCR and investigation of first few suspected cases of COVID-19 in Sri Lanka

Jayamaha CJS[1], *Witahnage VH*[1], *Jayathunga RCS*[1], *Ekanayake DHP*[1], *Wepathairage LH*[1], *Neelambari S*[1], *Chu DKW*[2], *Peiris M*[2]

[1]National Influenza Centre, Department of Virology, Medical Research Institute, Colombo, [2]School of Public Health, LKS Faculty of Medicine, The University of Hong Kong

Introduction

An outbreak of novel coronavirus (SARS-CoV-2) which began in China in December 2019 has spread rapidly to multiple countries. At the onset of the outbreak there were many challenges due to the lack of validated assays.

Objective

To describe the establishment of an in-house real-time PCR assay for the detection of SARS-CoV-2 and to present the epidemiological analysis of the first few suspected cases of COVID-19 tested in Sri Lanka.

Method

Primers and probes of N gene (screening) and ORF1b-nsp14 gene (confirmatory) were shipped from University of Hong Kong in the freeze-dried form. Inactivated genomes of severe acute respiratory syndrome virus (SARS) were reconstituted from filter papers as positive controls (PC). A previously published PCR protocol was used. A verification assay was performed using three high PCs, three low PCs and three negative controls using a manual nucleic acid extraction method. The first 15 samples (two positives from same patient and 13 negatives) tested were sent to WHO coronavirus reference laboratory in Hong Kong, for quality assurance. Using the WHO case definition, respiratory samples collected from suspected patients were subjected to PCR. Retrospective analysis of suspected patients from 25/01/2020 to 04/03/2020 was performed using descriptive statistics.

Results

Samples sent for quality assurance gave 100% concordance results. Out of 109 samples received, 70 samples that conformed to the case definition were tested. Common presenting symptoms were fever (67%), cold (50%), cough (58.5%), sore throat (47.1%), shortness of breath (14.2%) and diarrhoea (5.7%). There were lung signs in 10%. Age range was from 4 months to 68 years, mean age 31, mode 37 years and with a male predominance (57.1%). One traveller from China became positive for SARS-CoV-2. The turn-around time for the majority of the samples was ≤36 hours.

Conclusion

Laboratory confirmation of suspected COVID-19 cases was useful in case management and in organizing public health measures.

POSTER PRESENTATIONS

PP 1

Does pyrin gene influence *Helicobacter pylori* associated inflammatory changes in gastric mucosa?

Weerasinghe GGYH[1], Gunesekara TDCP[1], Weerasekara MM[1], Jayakody S[2], Senevirathne BS[3], Weerasekara DD[4], Jayasinghe C[5], Perera MHN[5], Nisansala GGT[1], Fernando SSN[1]

[1]Department of Microbiology, Faculty of Medical Sciences, University of Sri Jayewardenepura, [2]Department of Community Medicine, Faculty of Medical Sciences, University of Sri Jayewardenepura, [3]Department of Pathology, Faculty of Medical Sciences, University of Sri Jayewardenepura, [4] Department of Surgery, Faculty of Medical Sciences, University of Sri Jayewardenepura, [5]Department of Medical Laboratory Sciences, Faculty of Medical Sciences, University of Sri Jayewardenepura

Introduction

A considerable number of dyspeptic patients are reported despite the declining proportion of *H.pylori* in Sri Lanka. Microbial, host and environmental factors play a role in disease outcome. Host protein pyrinmay modulate the inflammatory process in the gastric environment. This study investigated the role of pyrin gene polymorphism in gastric mucosal inflamation and *H. pylori* infection.

Objective

To determine the association of pyrin gene polymorphism with inflammatory changes of gastric mucosa associated with *H. pylori* infection.

Method

This comparative cross-sectional study was carried out on dyspeptic patients with any combination of four dyspeptic symptoms (postprandial fullness, early satiety, epigastric pain and epigastric burning) who presenting to the endoscopy unit, Colombo South Teaching Hospital, Kalubowila. Gastric biopsies were examined for the presence of *H.pylori* by in-house urease test or histology and to determine the severity of mucosal inflammation. EDTA anti-coagulated blood was used to test for pyrin gene polymorphism using a commercially available FMF strip assay kit (Vienna lab diagnostic, Austria). Twelve gene mutations (E148Q, P369S, F479L, M680I (G>C), M68OI (G>A), I692 del, M694V, M694I, K695R, V726A, A744S and R761H) were tested.

Results

A total of 60 specimens from thirty-five (58.3%) males and twenty-five (41.6%) females were investigated. The median age was 53 years with Inter Quartile Range 24.25. Eleven patients (18.3%) were positive for *H.pylori.* According to the modified Sydney classification system, majority of the patients [thirty-seven (61.6%)] presented with mild to moderate gastritis while fifteen (25%) patients had severe gastritis and eight (13.3%) patients had normal gastric mucosa. None of the individuals had homozygous mutations. The most common three heterozygous mutations within the population were E148Q (45%), P369S (5%), M680I (11.6%). Presence of *H.pylori* and severity of gastric inflammation were analyzed with the pyrin gene polymorphism. There was no significant association between *H.pylori* positivity, severity of gastric inflammation and presence of the gene polymorphism in this population.

Conclusion

In this population only heterozygous mutations of the pyrin gene were detected. MEFV gene mutation may not be an important determinant contributing to the severity of *H.pylori* associated gastritis.

Acknowledgement

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PP 2

Analysis of the aerobic bacterial flora in chronic wounds and the effect of locally applied acetic acid in an out-patient setting

Galagedara WD[1], Dassanayaka KMMP[2], Wadanamby JMRW[2], Chandrasiri NS[3]

[1]Post Graduate Institute of Medicine, Colombo, [2]National Institute of Infectious Diseases, Angoda, [3]Colombo South Teaching Hospital, Kalubowila

Introduction

Chronic wounds increase the burden on healthcare system and affect the quality of life. Knowledge on the microbial profile of chronic wounds is important in effective management.

Objective

To describe aerobic bacterial flora in chronic wounds and its association with selected risk factors. To determine the effect of locally applied acetic acid on chronic wounds.

Method

A descriptive, cross sectional study was carried out in three hospitals in Colombo, Sri Lanka from December 2017 to March 2018. Wound swabs were collected from 196 patients, with chronic wounds (>3 months). Patients on systemic antibiotics for seven days or antiseptic dressings for three days were excluded. Out of 196 patients, 8 were included in the acetic acid treatment component. Semiquantitave wound swab cultures were performed. Wounds in the acetic acid component were assessed with Bates Jensen Wound Assessment Tool (BWAT) and wound cultures done pre and post acetic acid dressing, two weeks apart.

Results

Majority were males (n=123, 62.8%). Mean age was 61.63 years. Mean duration of wounds was 17.55 months (95% CI). Most wounds were preceded by cellulitis (n=120, 61.2%). Number of patients with diabetes, varicose veins, eczema and chronic lymphoedema were 78 (39.7%), 52 (26.53%), 33 (16.83%), and 4 (2%) respectively.

Out of total 302 isolates, majority were *Staphylococcus aureus* (n=116, 39%). *Pseudomonas* species, coagulase negative *Staphylococcus* species, coliforms and *Streptococcus* species were 32% (n=97), 12% (n=37), 9% (n=28) and 3% (n=8) respectively. More than half of the isolates of *S. aureus* (n=69, 59%) were methicillin resistant.

S. aureus (57.7%) and *Pseudomonas* spp (53.8%) were the commonest organisms in chronic diabetic wounds. Ninety nine (50.5%) of wounds were polymicrobial. Statistically significant association observed between varicose ulcers and *S. aureus.*

In the acetic acid component, nearly 80% of wounds showed regeneration according to BWAT and nearly 70% of wounds showed reduction in microbial growth with 1% acetic acid.

Conclusion

A positive effect on wound regeneration and reduction of microbiological flora with 1% acetic acid was observed. Further in vivo studies are required to study the effect of locally applied acetic acid on wounds.

PP 3

Analysis of blood culture isolates of Colombo North Teaching Hospital, Ragama in 2019

Diyalagoda DPKE[1], Senevirathne WDDN[2], Nadeeshani MV[2], Silva RKMC[2], Chanchala GHN[2], Samaranayake PMJ[2], Ranaweera DV[2], Jayasooriya JADTPS[2], Gunarathne ID[2], Namalie KD[2]

[1]Post Graduate Institute of Medicine, Colombo, [2]Colombo North Teaching Hospital, Ragama

Introduction

Antimicrobial resistance is on the rise around the world and misuse of broad-spectrum antibiotics has extensively contributed to this. Proper collection of blood cultures is important to prevent mismanagement of patients and wastage of resources.

Objectives

To find out the rate of blood culture positivity, rate of probable contaminants and antimicrobial resistance pattern in blood cultures during the year 2019.

Method

All positive blood culture isolates were clinically evaluated and analyzed using WHONET software. Species identification was done using BD Phoenix or Remel RapID kits and antibiotic sensitivity testing was done according to laboratory standard operating procedures using CLSI 2018 standard cut offs.

Results

Blood culture positivity rate was 13.8% (1087/7876), 52.5% of that was males and 6.5% were contaminants. Significant culture positivity rate was 7.3% (577/1087). Majority of culture positive patients belongs to >61 years age group. Out of the significant isolates, 60% (346/574) were Gram negative. Gram negative isolate breakdown was coliforms (47.48%, n=274), *Acinetobacter spp* (6.5%, n=38) and *Pseudomonas* spp (32.35%, n=34). Carbapenem resistance (CR) and extended spectrum beta-lactamase (ESBL) production among coliforms were 23.72% and 22.2% respectively. Multi drug resistance among *Pseudomonas* isolates and *Acinetobacter* spp were 5.89% and 60.52% respectively.

Gram-positive isolates consisted of 18.9% (109/577) *Staphylococcus aureus* and 7.8% (n=45) *Streptococcus* spp. Methicillin resistance was 53.21% among *S. aureus*, 4.85% (n=28) were enterococci and 14.28% were

vancomycin resistant (VRE). Yeast comprised 6.58% (n=38) and majority were *Candida* spp (92.1%, 35/38) with two isolates of *Cryptococcus* spp and one *Trichosporon* spp. Majority of significant positive blood cultures were health care associated infections (71%, n=401) which included 10.34% of MRSA, 3% of enterococci and 35.8% of Gram negatives according to the clinical evaluation and sensitivity pattern. Coagulase negative staphylococci represented 59.41% (303/510) of the contaminants.

Conclusion

There were high rates of multidrug resistance among all isolates which included CR and ESBL producing *Enterobacteriaceae*, multidrug resistant *Acinetobacter* spp, *Pseudomonas* spp, MRSA and VRE. Significant percentage of isolates was yeasts. Blood culture contamination rates are unacceptably high and urgent interventions are needed to control antibiotic resistance and blood culture contamination.

PP 4

Blood stream infections of trauma patients admitted to trauma intensive care units at the National Hospital of Sri Lanka

Diyalagoda DPKE[1]*, Vidanagama DS*[2]*, Patabendige CGUA*[2]

[1] Post Graduate Institute of Medicine, Colombo, [2] National Hospital of Sri Lanka, Colombo

Introduction

Trauma patients are more prone to infections. Among those, blood stream infections are common and more harmful.

Objectives

To find the rate of bacteraemia among trauma patients, aetiology, susceptibility pattern and associated factors for culture positive blood stream infections.

Method

A descriptive cross-sectional study was carried out for four months in two accident service intensive care units (ICUs) and three neurotrauma ICUs at the National Hospital of Sri Lanka. Patients' demographic and clinical details were extracted from clinical notes, blood cultures were sent; positive blood cultures were processed according to the standard operating procedures.

Results

During the period, 251 patients admitted and blood cultures were obtained from 172 patients. Forty six blood cultures indicated positive with 7 contaminants, 16 line colonizers and 23 significant positive blood cultures, from which 18 were primary bacteraemia according to CDC criteria. Culture positive bacteraemia rate among trauma patients was (23/251, 9.2%). Majority of Gram positive isolates were enterococci (4/7) whereas *Acinetobacter calcoaceticus* (8/13) comprised majority of Gram negative organisms. All Gram positive organisms were sensitive to vancomycin, teicoplanin and linezolid whereas majority of Gram negatives were multi drug resistant organisms (MDROs) with all being sensitive to colistin.

From 12 demographic and clinical parameters and 10 laboratory parameters 6 factors found to be significantly associated with culture positive bacteraemia. Admission to an accident service ICU (POR 2.521, 95% CI 1.036-6.138), ICU admission for >5 days (POR 5.340 with 95% CI 1.881-15.164), injury severity score (ISS) >25 (POR 4.992, 95% CI-1.877-13.272), haemoglobin concentration <10 g/dl (POR 3.102 with 95% CI-1.266-7.603), albumin concentration of <30g/L (POR 6.021 with 95% CI 2.31-15.696) and AST level >3 times normal (POR 2.660 with 95% CI 1.049-6.745) were associated with bacteraemia.

Conclusion

Acinetobacter and *Enterococcus* species were common among blood culture isolates. Admitting to accident service ICU, with severe injury (ISS> 25) for more than 5 days would predict culture positivity. If blood haemoglobin is low (<10g/dL) or AST level high (>3 times normal) or a low albumin level (<30g/L) chances of culture positivity would increase remarkably.

PP 5

Effect of bundle care on central line associated blood stream infection at medical intensive care unit at the National Hospital of Sri Lanka

Sugathadasa MRDN[1]*, Kottahachchi J*[2]*, Patabendige CGUA*[1]

[1]National Hospital of Sri Lanka, Colombo, [2]Department of Microbiology, Faculty of Medical Sciences, University of Sri Jayewardenepura

Introduction

Central line associated blood stream infections (CLABSI) are a common cause of hospital acquired bacteremia.

Adherence to central line bundle care practice is associated with a significant reduction in the CLABSI rate (CLABSIR).

Objectives

To identify the CLABSIR and compliance to bundle-care (CLBC) components and the association between degree of CLBC and CLABSIR in patients admitted to medical intensive care unit (MICU).

Method

A descriptive cross sectional study was conducted at MICU for four months. A total of 69 patients with central line inserted only at MICU and kept for more than 48 hours were included. The CLBC and central line data were collected using the methodology introduced by the Institute of Healthcare Improvement 2012. The central line insertion bundle (CLIB) and central line maintenance bundle (CLMB) compliance were observed and recorded using relevant check lists prepared according to the National Health Care Safety Network (NHSN) of the Centre for Disease Control and prevention (CDC). Patient identification details were recorded to link the CLBC and CLABSI.

Two blood cultures were collected 48 hours after central line insertion and when an infection was suspected, through the central line and from a peripheral vein. When central line was removed, the tip and peripheral blood was cultured. Blood cultures were processed using BACTEC and BD Phoenix™ automated ID/AST systems.

Results

CLIB compliance rate of hand hygiene, wearing a sterile gloves and gown were 100% and least compliance (53.6%) was observed with the covering of the patient from head to toe using a sterile drape. CLMB compliance rate for all components were 100%. Overall CLABSIR at MICU was 23.66/1000 central line days.

With the improvement of CLBC from 30% to 86.6% there was reduction of the CLABSIR from 30/1000 central line days to zero/1000 central line days and had strong correlation (r = -0.953) and P value was 0.047.

Conclusion

Higher CLBC rate is associated with lower CLABSIR. The CLBC should be strengthened at other ICUs.

PP 6

The presence of inapparent dengue cases in a community and hospital

Wing Tan[1], Jonathan Wee Kent Liew[1], Sivaneswari Selvarajoo[1], Wardha F. Refai[2], Yee Ling Lau[1], Indra Vythilingam[1]

[1]Department of Parasitology, Faculty of Medicine, University of Malaya, Malaysia, [2]Postgraduate Institute of Medicine, Colombo

Introduction

Around 300 million of 390 million annual dengue infections are subclinical. Relative to symptomatic dengue, inapparent dengue appears to be more infectious to Aedes mosquitoes. Thus inapparent dengue infection plays an important role in maintaining dengue transmission and is a major health concern since the current vector control practices target areas with symptomatic cases.

Objectives

To identify inapparent dengue infection in individuals living in areas with dengue NS1 positive mosquitoes. To detect inapparent dengue infection among asymptomatic individuals living in the confirmed dengue patient's house and visitors that have stayed in the patient's house within the last 14 days.

Method

A cross-sectional study was conducted to investigate the occurrence of inapparent dengue infection amongst residents living in areas where dengue positive Aedes mosquitoes were found and among people living with dengue patients. Residents were approached within a week of detection of dengue NS1 positive mosquitoes at close proximity to dengue hot-spot apartments in Selangor, Malaysia. History and informed consent were obtained, finger-prick blood or venepunctures were performed and three blood spots were made on Whatman filter paper. Tests carried out included Dengue NS1 Ag test, real-time reverse transcription PCR, and serology (STANDARD-Q Dengue- Duo, Gyeonggi-Do). The same procedure was followed for asymptomatic individuals living in the confirmed dengue patient's house and visitors that have stayed in the patient's house within 14 days.

Results

Amongst 173 residents tested for dengue, where NS1 positive Aedes mosquitoes were found, 10/173 (5.3%) were positive for inapparent infection, RT PCR detected viremia in 10 individuals and one person with viremia was NS1 positive. From 21 household members tested for

inapparent dengue, five were positive (23.8%). Three (14.3%) were NS1 positive and two (9.5%) IgM positive, suggestive of recent infection. Four (19.0%) were IgG positive with two NS1 and one IgM positive, respectively. RT-PCR was negative in all.

Conclusion

Asymptomatic dengue infection can contribute to the burden of dengue transmission and hinder control efforts. Early, active surveillance of dengue, both in mosquitoes and humans is crucial for effective control.

PP 7

Sero-prevalence of antibodies to varicella zoster virus and associated factors among the undergraduates at the Allied Health Sciences Unit, University of Jaffna

Jayamanna PCCA[1]*, Murugananthan K*[2]

[1]Allied Health Sciences Unit, Faculty of Medicine, University of Jaffna, [2]Department of Microbiology, Faculty of Medicine, University of Jaffna

Introduction

Due to their working environment, healthcare workers and students of health professional degree programmes are at major risk for VZV infection. However, there have been very few studies conducted on the sero-prevalence of VZV and associated factors among the risk group mentioned above in Sri Lanka.

Objectives

To determine the sero-prevalence of antibodies to VZV and to assess the associated factors among the undergraduates enrolled in health professional degree programs in the Allied Health Sciences Unit of the Faculty of Medicine at University of Jaffna.

Method

This was a cross-sectional study. Three millilitres of blood and data including past history of VZV infection were collected from 182 participants including 74 males and 108 females. Serum was qualitatively analyzed using the Human IgG AccuDiag™ Varicella IgG ELISA Kit (Diagnostic Automation/Cortez Diagnostics, Inc, USA). Past history of VZV infection and risk factors were analysed using SPSS Statistics software (version 21).

Results

The sero-prevalence of antibodies to VZV in the participants was 50%. The sero-positivity of males and females were 51% and 49%, respectively. Students with a past history of chickenpox showed 9% (7/77) sero-negativity and those with no past history showed 20% (21/105) sero-positivity. Students from urban areas had a sero-prevalence rate of 56% (64/114) while those from rural areas had only 40% (27/68) which was statistically significant. Sero-prevalence rate was statistically correlated with the number of siblings. Students with no siblings, students with 1-2 siblings, and students with more than 2 siblings, showed prevalence rates of 31% (4/13), 47% (59/126) and 65% (28/43), respectively.

Conclusion

A large proportion of the study population was susceptible to VZV.

PP 8

Filarial parasites among two dog communities in selected filariasis endemic and non-endemic areas in Sri Lanka

Rathnayake SP[1]*, Mallawarachchi CH*[2]*, De Silva LAPNF*[3]*, Gunathilaka PADHN*[4]*, Chandrasena TGAN*[4]

[1]Department of Animal Production & Health, Northern Province, [2]Medical Research Institute, Colombo, [3]Department of Community Medicine, Faculty of Medicine and Allied Sciences, University of Rajarata, [4]Department of Parasitology, Faculty of Medicine, University of Kelaniya

Introduction

Zoonotic filarial infections show a rising trend in Sri Lanka with expansion to areas non-endemic for lymphatic filariasis (LF). The infections are caused by *Dirofilaria (Notchiella) repens* and *Brugia* spp. which cause human dirofilariasis and brugian filariasis respectively. Dogs are implicated as reservoirs of these parasitic zoonoses.

Objective

To study the prevalence of filarial parasites among dog communities in selected LF endemic and non-endemic areas.

Method

A veterinary clinic based descriptive study was conducted between August 2019 to February 2020 at Habaraduwa, Baddegama (Galle district) and Welioya (Mullaitivu

district). A mix of both stray and domestic canines was studied. The epizootiological data were recorded and thick blood smears (TBS) were prepared from capillary blood obtained from tip of the ear lobe. The TBS were stained with Giemsa and examined microscopically for microfilariae. Identification of microfilariae was based on morphology.

Results

Total of 295 dogs (197 and 98 from Galle and Mullaitivu, respectively) were screened. The prevalence for any filarial parasite was 60.9% (n=120) in Galle and 44.8% (n=44) in Mullaitivu. The prevalence of *D. repens* and *B. malayi* were 54.3% (n=107) and 29.9% (n=59) respectively in Galle and 43.9% (n=43) and 1.1% (n=1) respectively in Mullaithivu. The prevalence of brugian filariasis is significantly higher in Galle population ($z=6.648$, $p< .00001$) but there is no statistically significant difference in prevalence of dirofilariasis between the two populations ($z=1.689$, $p=0.0912$). Of the dogs infected in Galle, 61.9% (n=74) had mono-infections with *D. repens* while 38.3% (n=46) had mixed infections with *D. repens* and *B. malayi*. In Mullaithivu all positive canines (n=44) had mono-infections.

Conclusion

The prevalence of canine dirofilariasis was high in both Galle and Mullaitivu districts while canine brugian filariasis was significantly higher in Galle than in Mullaitivu. Raising public awareness among pet-owners and anti-filarial treatment of canines is recommended.

PP 9

Antimicrobial use in poultry: A field study in the Kegalle District

Ranaweera NPNM[1], Peiris H[1], Wijayaratne WMDGB[2]

[1]Faculty of Allied Health Sciences, University of Ruhuna, Galle, [2]Faculty of Medicine, University of Ruhuna, Galle

Introduction

Antimicrobial use and abuse in human, animal, and environmental sectors and the spread of resistant bacteria and resistance determinants within and between these sectors contribute to the increasing antimicrobial resistance across the globe.

Objectives

The aim of the research is to determine the antimicrobial activity in commercially available poultry feeds in shops and ready to serve feeds and water in selected poultry farms in the Kegalle district. Also to determine the awareness of poultry farm owners on potential effects of feeding practices in the increase of antimicrobial resistance in the community.

Method

One sample of poultry feed from four shops and 3 samples of feed and water each that were ready to serve were collected from different cages in 18 selected poultry farms. Agar well diffusion method were used to determine antibiotic effects in these samples against *Staphylococcus aureus* ATCC25923, *Escherichia coli* ATCC25922 and *Pseudomonas aeruginosa* ATCC27853. An interviewer administered questionnaire was used to collect data from farm owners.

Results

Antibiotic activity was not detected in commercially available poultry feeds in all the shops (4/4) and ready to serve feeds in all farms (18/18). Antibiotic activity was detected in water samples collected from 33.33% (18/54) of farms. Seventy-eight percent of farm owners declared that they mix different types of additives containing antibiotics into the water served to animals. Forty-four percent of farm owners believe that the use of antibiotics in poultry is not a good practice and harmful for human health while a similar percentage claim that it is a favorable practice with economic benefits. None of the farm owners were aware that use of antibiotics in poultry can contribute to the increasing antibiotic resistance in the community.

Conclusion

Commercially available poultry feeds sold in shops in the Kegalle District did not demonstrate any antibiotic activity. Antibiotics are not added to poultry feeds given to animals. However, antibiotics containing additives are added to water served to animals in poultry farms in the Kegalle district. The awareness of farm owners on antibiotic use in poultry and its effects on the increase of antibiotic resistance in the community is unsatisfactory.

PP 10

High prevalence of community-associated methicillin-resistant *Staphylococcus aureus* in patients infected with *Staphylococcus aureus* at a tertiary care center

Kurukulasooriya MRP[1], Piyasiri B[2], Wijayaratne WMDGB[1], Tillekeratne LG[3], Bodinayake CK[1], de Silva AD[4], Nicholson BP[3], Østbye T[3], Woods CW[3], Nagahawatte ADeS[1]

[1]Faculty of Medicine, University of Ruhuna, Galle [2]Teaching Hospital, Karapitiya,Galle, [3]Duke University, USA, [4]Faculty of Medicine, General Sir John Kotelawala Defence University, Rathmalana

Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a multidrug-resistant pathogen. Based on epidemiological, genotypic, and antibiotic susceptibility patterns, MRSA can be classified as community-associated (CA) or healthcare-associated (HA). We investigated prevalence, risk factors and types associated with MRSA infection among patients admitted to Teaching Hospital Karapitiya (THK).

Method

Consecutive *S. aureus* isolates were collected from September 2019 to January 2020 from the clinical microbiology laboratory of THK, a public, tertiary care hospital with 1,500 beds. All *S. aureus* and MRSA isolates were confirmed by the laboratory using standard microbiological methods. Sociodemographic and clinical data were collected from medical records. Based on the antibiotic profile, isolates were characterized as HA-MRSA (resistance to β-lactam antibiotics together with ciprofloxacin, erythromycin and clindamycin) or CA-MRSA, according to guidelines provided by the Centers for Disease Control and Prevention, USA. Sociodemographic factors associated with MRSA versus methicillin-susceptible *S. aureus* (MSSA) infection were assessed using the Chi-square and Kruskal-Wallis tests in STATA version 13.

Results

A total of 187 *S. aureus* isolates were collected from each patient during the study period. Of all patients, 107 (57.2%) were males and 156 (83.4%) were adults (≥18 years). Isolates were obtained from blood (36, 19.3%), pus (113, 60.4%), respiratory (20, 10.7%), urine (11, 5.9%) and sterile fluid (7, 3.7%) cultures. Overall, MRSA was identified in 109 (61.6%) of *S. aureus* isolates, with most isolates cultured from pus (67, 61.5%) and blood (19, 17.4%). Out of all MRSA isolates, only 25 (23%) showed resistance to all 3 non-β-lactam antibiotics, while CA-MRSA was the leading type of MRSA infection (77%). No socio-demographic features (age, sex, sample types etc.) were significantly associated with MRSA versus MSSA infection.

Conclusion

The majority of *S. aureus* isolated from clinical cultures at THK during the study period were MRSA. CA-MRSA can be recognized as the predominant type of MRSA at THK. More robust analyses including molecular data are needed to confirm the MRSA types.

PP 11

Knowledge and attitude of food/ hand hygiene among food handlers at a University in Sri Lanka

Senanayake NP[1], Malliwadu NC[1], Gangoda EWWMSB[1], Ariyaratne MN[1]

[1]General Sir John Kotelawala Defence University, Rathmalana

Introduction

Improper practices and lack of knowledge by food handlers are contributing factors for the spread of foodborne outbreaks.

Objectives

The objective was to assess the knowledge/attitude of food/hand hygiene and observation of hand washing practices among food handlers at the General Sir John Kotelawala Defence University (KDU) and the KDU guest house, Werahara.

Method

A descriptive cross sectional study was done among 103 food handlers who are involving in food production, possessing, distribution at the General Sir John Kotelawala Defence University and the KDU guest house, Werahara. Information gathering method using a pretested validated questionnaire and direct observations of hand washing practices were used for the data collection. A relationship was tested ($P < 0.05$) between hand/food hygiene knowledge/attitude with category of service (chefs, cooks, kitchen assistants, waiters and others including food distributing staff), level of education (completed their primary education up to grade 5, completed GCE O/L and completed their GCE A/L) and duration of service (worked more than 5 years, for 1 to 5 years and for less than 1 year).

Results

Of the 103 food handlers, 86.9% had excellent knowledge on food hygiene. Category of service and level of education of food handlers were shown to be related with knowledge /attitude of food hygiene. There was no significant difference between the knowledge of food hygiene with the duration of service of the employees ($P > 0.05$). Knowledge and attitude of hand hygiene was excellent

in all the food handlers (>86%) except the knowledge for 8 steps of hand washing technique. Through direct observations, it proved that food handlers' hand washing practices significantly related with the place of work (Werahera Hotel and KDU) and category of service ($P<0.05$).

Conclusion

Most of the food handlers at the General Sir John Kotelawala Defence University have good knowledge and attitude regarding food and hand hygiene. Further educational and training programs will improve their knowledge attitude and practices of food and hand hygiene.

PP 12

In vitro antibacterial and antibiofilm activity of *Tribulus terrestris* L. against planktonic and biofilm models of common uropathogens

Gunasekara SP[1], *Bandara DS*[1], *Nishadhya S*[1], *Aluthwaththa MG*[1], *Pathirana R*[2], *Widanagamge R*[2]

[1]Department of Microbiology, Apeksha Hospital, Maharagama, [2]Department of Medical Laboratory Sciences, General Sir John Kotelawala Defence University, Rathmalana

Introduction

Bacterial biofilms play an important role in urinary tract infections. Planktonic organisms are single, floating microbial cells and a biofilm is known as a congregation of microbial cells which stick to each other and to the surfaces. Multi drug resistance of uropathogens is a matter of concern and alternatives such as local medicinal plants with antibacterial effects worth consideration.

Objective

To determine antibacterial and antibiofilm activity of aqueous and methanol extracts of *Tribulus terrestris* L. whole plant against planktonic and biofilm models of common uropathogens.

Method

Fifteen bacterial isolates from patients with urinary tract infections were selected (five each of *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Escherichia coli*).

Methanol extract and aqueous extract of the plant were screened for antibacterial activity by well diffusion method and minimum inhibitory concentration was determined using 96 well microtitre plates with alamar blue assay on planktonic cells. Minimum biofilm inhibitory concentration (MBIC) of the organisms was determined using MTT and Crystal Violet (CV) assays on 96 well microtitre plates. Absorbance was recorded at 570 nm and 590 nm for MTT and CV assays respectively using an ELISA plate reader. Triplicates were carried out to confirm the biofilm forming ability.

Results

The methanol extract showed an MIC value of 100 mg/mL against all the tested organisms except against one clinically isolated *E.coli*. Lowest MIC value of 3.125 mg/mL was observed for the standard antibiotic Amikacin against *P.aeruginosa* (ATCC 27853) and three clinically isolated *P.aeruginosa* strains.The methanol extract of the plant inhibited more than 50% of biofilms at a concentration of 1.56-6.25 mg/mL while aqueous extract showed >50% inhibition of biofilms at a concentration of 12.5 -200 mg/mL in MTT assay. Concentration of 12.5-200 mg/mL of methanol extract was required to remove >50% of bacteria from the biofilms.

Conclusion

The whole plant of *Tribulus terrestris* L. possess both antibacterial and antibiofilm activity in vitro against planktonic and biofilm causing common uropathogens.

PP 13

Clinical presentation, aetiology, drug resistance and mortality of blood stream infections in elderly in a tertiary hospital, Galle, Sri Lanka

Piyasiri DLB[1], *Galhenage MN*[1], *Kirubaharan A*[1], *Nanayakkara IRS*[1], *Jayasekara JVG M*[1], *Sapukotana PM*[1]

[1]Teaching Hospital Karapitiya, Galle

Introduction

Older adults are the largest growing sector of the population and suffer excessively from infections, representing one of the greatest challenges to the clinicians due to atypical clinical presentations, comorbidities and antibiotic resistance.

Objectives

To determine the etiology, antibiotic resistance and mortality from blood stream infections in the elderly.

Method

All the blood cultures positive for a significant bacterial pathogen from January 2015 to December 2019 of a tertiary hospital were considered and cultures from patients of age 61 years and above were analysed. Automated blood culture system was used for processing and antibiotic sensitivity was tested by CLSI method.

Results

There were 1066 positive blood cultures from elderly. Majority were males (n=606, 56.8%) and many (n=295, 27.7%) presented with sepsis of unknown origin (SUO) followed by urosepsis (n=259, 24.3%) and lower respiratory tract infections (LRTI) (n=187, 17.5%). Majority were of community origin (n=722, 68%).

Predominant pathogen isolated was *Escherichia coli* (n=527, 49%) followed by *Klebsiella* spp (n=207, 19%), *Pseudomonas* spp (n=165, 15.5%) and *Staphylococcus aureus* (n=149, 14%). Multi-drug resistance (MDR) was noted in 448 (42%) including extended spectrum beta lactamases (ESBL) producing coliforms (n=281, 26%), methicillin resistant *Staphylococcus aureus* (MRSA) (n=43, 4%), MDR *Acinetobacter* (n=40, 3.7%), carbapenem resistant enterobacteriaceae (CRE) (n=35, 3%) and other MDR organisms (n=49, 4.6%).

Comorbidities were found among 729 (68%) including diabetes (n=266, 25%), chronic kidney disease (n=157, 14.7%), and malignancies (n=134, 12.6%).

Of the total 199 (18.7%) deaths within 3 days of blood culture collection, 91 (45.7%) were due to MDR infections including CRE (42.8%), MRSA (28%), MDR *Acinetobacter* (25%), and ESBL (16.7%). A statistically significant ($p<0.001$) difference was noted between survival in non-MDR and MDR infections of nosocomial origin and between community acquired and nosocomial infections. Most deaths were due to SUO (41%) and LRTI (25%).

Conclusion

Most of the infections in this group of elderly are SUO and mainly caused by *Escherichia coli*. Community acquired infections and non-MDR nosocomial infections are associated with survival in the elderly.

PP 14

Epidemiology of pneumococcal bacteraemia in a tertiary care center, Sri Lanka

Piyasiri DLB[1], *IRS Nanayakkara*[1], *Jayasekara JVGM*[1], *Galhenage MN*[1]

[1]Teaching Hospital Karapitiya, Galle

Introduction

Invasive pneumococcal diseases cause severe morbidity and mortality especially in countries where the vaccine is not freely available. Sri Lanka is yet to introduce the pneumococcal conjugate vaccine into the national immunization programme. Positive blood culture is associated with disease severity.

Objective

To describe the epidemiology of bacteraemic invasive pneumococcal disease in the southern area of the country.

Method

All the patients with culture proven pneumococcal bacteraemia in a tertiary care center in southern province of Sri Lanka, from January 2015 to December 2019 were included. Blood cultures were processed in automated systems and subsequent tests were performed following standard operating procedures and CLSI guidelines. Duplicate isolates were excluded.

Results

There were 75 positive pneumococcal blood cultures which was 2.3% of total significant positive blood cultures during the period. The highest number was reported in 2018 (n=20, 27%) while the lowest was in 2016 (n=11, 15%). In 2018, a peak of positive cultures came in February (n=5) and the occurrence of relatively more positives in the 1st half of the year was noted which was statistically significant ($p<0.01$).

Less than 5 years age group yielded most of the positive cultures (n=32, 43%), followed by >60 years old patients (n=20, 27%). There were 3 cases of neonatal sepsis (4%). Majority were males (n=55, 73%).

Pneumonia was the underlying cause for bacteraemia in majority (n=37, 49%) and, except in 2018, it was the predominant cause in all years. In 2018, meningitis was the main focus of origin (10/20) and pneumonia contributed to 6 cases.

Eighteen patients (24%) died. Highest percentage of deaths were reported among the >60 years patients (7/18, 39%) and among males (15/18, 83%). Three children <5 years also died.

Conclusion

Increased occurrence of pneumococcal bacteraemia in the first half of the year was statistically significant but this needs to be studied further. Though the <5 year age group was mostly affected, male gender and age >60 years may be at risk of worse outcome.

PP 15

Correlation of risk factors with the clinical presentation, microbiology and genetic profile of community acquired methicillin resistant *Staphylococcus aureus* in skin and soft tissue infections among patients attending a tertiary care hospital in Sri Lanka

Liyanage N[1]*, Piyasiri DLB*[1]

[1]Teaching Hospital Karapitiya, Galle

Introduction

Community acquired methicillin-resistant *Staphylococcus aureus* (CA-MRSA) causing skin and soft tissues infections (SSTI) is a global problem, but local data are scarce.

Objective

To determine the association between risk factors and the clinical presentation and existence of PVL gene of CA-MRSA in patients with skin and soft tissue infections caused by CA-MRSA.

Method

A descriptive cross-sectional study was done in the out-patients department of a tertiary care hospital from December 2017 to March 2018. Patients with SSTI were recruited excluding possible hospital acquired infections according to CDC definition of CA-MRSA. Interviewer administered questionnaire was used to gather demographic data and risk factors. Pus samples and wound swabs were taken from 497 patients and inoculated on mannitol salt agar plates and subsequently 101 CA-MRSA were identified by CLSI method. CA-MRSA were tested for mecA, femB, and PVL genes by multiplex PCR.

Results

Out of 188 patients positive for *Staphylococcus aureus,*101 were CA-MRSA (53.7%). The commonest clinical presentation was abscess (n=86, 85.14%). Majority had infections on lower limbs (n=35, 34.66%) followed by chest (n=20, 20.8%).

CA-MRSA isolates were 100% sensitive to vancomycin, cotrimoxazole, teicoplanin, doxycycline, linezolid, and rifampicin according to the CLSI method. Resistance to erythromycin was 77.22% (n=78), while clindamycin resistance was 62.37% (n=63), which includes inducible resistance (n=53, 52.47%). All three mecA, femB and PVL genes were present in 69.30% (n=70).

Prevalence of risk factors (athletes, army personnel, team sports, hostel inmates, tattoo recipients, pets/animal contacts) were low (n=24, 23.8%).There was a statistically significant association of CA-MRSA with the presence of at least one risk factor (n=24) (p=0.0364) and age <5 years (p=0.017). No association was found between the clinical presentation and the presence of PVL gene or between the clinical presentation and the presence of risk factors (p>0.05).

Conclusion

Age <5 years and people with at least one risk factor were associated with SSTI by CA-MRSA.

PP 16

Ventriculoperitoneal shunt infection with *Salmonella* Enteritidis following gastroenteritis; a case report

Ulwishewa GM[1]*, Piyasiri DLB*[1]*, Jayaweera APN*[1]*, Priyadharshana WBU*[1]

[1]Teaching Hospital Karapitiya, Galle

Introduction

Although infection of ventriculoperitoneal (VP) shunts due to *Salmonella* spp is rare, it can occur as a result of *Salmonella* peritonitis.

Case report

A 21 month old boy with congenital hydrocephalus with bilateral VP shunts (inserted at 23 days and 6 months after birth) presented with fever for two weeks, loose stools, vomiting and excessive crying for one week.

His white cell count was 22.14x10^9/L, (neutrophils-81.4%),and platelet count of 805,000/µL with C reactive protein 59mg/L. Brain imaging was normal. The ultra sound scan of the abdomen revealed a pseudo-cyst in which the tips of the bilateral VP shunts were visible.

Shunts were externalized due to the suspicion of infection which was confirmed with cerebrospinal (CSF) full report of high protein (476mg/L), low sugar (36mg/dL), 50/mm^3 polymorphs and 120/mm^3 lymphocytes. CSF cultures were initially negative; however, became positive after 9 days for a scanty growth of non-lactose fermenting Gram negative bacillus with a reactive Kligler of *Salmonella*-pattern which was later confirmed as *Salmonella* Enteritidis by the reference laboratory with sensitivities including ceftriaxone, chloramphenicol, and meropenem with resistance to ciprofloxacin. The stool culture was negative.

The child was first treated with IV meropenem for 10 days and changed to IV ceftriaxone 100mg/kg daily after identifying the pathogen. A new VP shunt was inserted and he was discharged after 21 days of antibiotics. He remains infection-free up to date.

Discussion

The pseudo cyst formation might have been due to small bowel perforation as world literature suggests which may have occurred following gastroenteritis and caused subsequent contamination of the abdominal ends of the VP shunts leading to ventriculitis and positive CSF culture. Prompt externalization and subsequent removal of the infected shunt with targeted antibiotics for adequate duration were effective in management. To the best of our knowledge this is the first published case of VP shunt infection with a *Salmonella* species in Sri Lanka.There is a record of a similar case in a 3 month old infant from USA, in 2013.

PP 17

A case of invasive listeriosis following gastroenteritis in an infant

Ulwishewa GM[1]*, Piyasiri DLB*[1]*, Jayawardena P*[1]*, Sanjeewa MAGA*[1]*, Sapukotana PM*[1]

[1]Teaching Hospital Karapitiya, Galle

Introduction

Listeriosis is a rare and serious infection but is preventable and treatable. It is a food borne disease and manifestation ranges from gastroenteritis to meningitis/ sepsis affecting high risk groups such as pregnant, immunocompromised, elderly and infants mostly.

Case report

A 10 month old boy having low grade fever and watery diarrhoea for 2 weeks presented to a tertiary care hospital. The child was febrile without other abnormal findings. History of an episode of lactose intolerance at the age of 5 months was noted. Complementary feeding was started at the age of 6 months with dairy products, cereals and “thriposha” with apparently adequate food hygiene.

His white cell count was $15x10^9$/L, with 22.5% neutrophils and 67% lymphocytes. CRP was repeatedly normal with ESR of 24 mm/1st hr. Urine and stool cultures were negative with normal full reports. His blood culture was flagged as positive by the automated system after 25 hours incubation. Direct Gram stain revealed a Gram positive bacillus with palisades. Next day, there were beta-haemolytic, small, grey, translucent colonies on blood agar with no growth on MacConkey agar. The organism was positive for bile aesculin test and showed tumbling motility at 25°C. The isolate was sensitive to ampicillin, gentamicin and vancomycin and the identity was confirmed as *Listeria monocytogenes* at the reference laboratory. While awaiting the results from the reference laboratory, the patient was treated with IV meropenem for 7 days and discharged after recovery.

Discussion

While majority of people with *Listeria* infection clear spontaneously, it accounts for 19% of deaths due to food-borne infection. Early recognition and prompt antibiotic therapy with IV ampicillin saves lives. Though bacteraemia is reported mostly among at-risk groups, our patient did not have any risk, other than being an infant. Blood cultures often get contaminated with Gram positive bacilli like diphtheroids which are similar to *Listeria* and are disregarded. However the suggestive history and the culture characteristics led to the identification and follow-up of the patient.

PP 18

A case of complicated infective endocarditis following thyroidectomy: A story of treatment success

Thushari HL[1]*, Wijeweera KDDS*[1]*, Jazeel MN*[1]*, Gunapala LHADL*[1]*, Vidanagama DS*[1]*, Patabendige CGUA*[1]

[1]National Hospital of Sri Lanka, Colombo

Introduction

Infective endocarditis (IE) due to *Staphylococcus aureus* has an acute and rapidly progressive clinical course. Here, we report a case of complicated IE yet with a favourable outcome.

Case report

A 21-year-old female presented with low grade fever, lethargy, unsteadiness and weakness of lower limbs for three days. She had undergone a total thyroidectomy for follicular variant of papillary carcinoma 21 days before the admission. Her white blood cell count was 20,000/ mm^3 with 80% neutrophils. ESR and CRP were 55mm/ hr and 240mg/dl respectively. MRI brain revealed multiple infarctions that did not fall in to a single vascular territory. The transthoracic 2D-echocardiogram revealed a 9mm x 6mm vegetation attached to posterior mitral valve leaflet

(PMVL) while the trans-oesophageal echocardiogram revealed a 20mm x 4mm, linear, highly mobile vegetation attached to PMVL with perforation of PMVL and severe mitral regurgitation.

Her blood cultures became positive for methicillin resistant *Staphylococcus aureus* (MRSA) sensitive to vancomycin. In spite of vancomycin treatment, fever continued with a total of six MRSA positive blood cultures. The blood culture became negative with combination therapy with IV linezolid and oral rifampicin.

Following two weeks of medical management, she underwent a vegetectomy and mitral valve replacement. Both the vegetation and the pericardial fluid were sterile. Her post-operative period was complicated with hypostatic pneumonia and she was treated with IV piperacillin-tazobactam, rifampicin, linezolid and vancomycin for two, four and six weeks respectively. She was discharged with normal haematological, biochemical parameters and echocardiogram.

Discussion

Staphylococcus aureus IE in non- intravenous drug users has a mortality rate of 25% to 40%. The previous hospital stay would have been the risk factor for the infection although the entry point could not be identified, as in majority of the cases. An intensified antibiotic regimen was used here considering the possibility of further embolization. Similarly, an elective surgery was performed rather than operating in an acute condition. The timely diagnosis, early administration of appropriate antibiotics and multidisciplinary approach may have contributed to the favourable outcome of this patient.

PP 19

A case report of infant meningitis possibly by *Salmonella* Cholerasuis

Piyasiri DLB[1], *Sajeemala WG*[1], *De Silva MAH*[1], *Dissanayake SKVP*[1], *Pathirage S*[2]

[1]Teaching Hospital Karapitiya, Galle, [2]Medical Research Institute, Colombo

Introduction

Salmonella meningitis occurs more in children under 2 years than in other age groups. Disease causes up to 50% mortality especially with *Salmonella* Cholerasuis which causes systemic infection without gastroenteritis and is considered the most virulent of non-typhoidal *Salmonella*.

Case report

A 3 month old girl who presented with fever, vomiting, excessive crying for 2 days, developed convulsions and was transferred from a peripheral hospital. She was a formula-fed, adopted child.

Her white cell count was $20x10^9$/L (63.6% neutrophils) with CRP 247mg/L. Cerebrospinal fluid (CSF) full report contained 785 mg/dL protein and 140/µL polymorphs. Ultrasound scan revealed right frontal subdural empyema extending to the subarachnoid space. She had been on IV cefotaxime which was changed to IV meropenem later due to poor response, but fever continued. Both CSF and burr hole aspiration cultures were negative. After 9 hours incubation, the blood culture grew a non-lactose fermenting, oxidase negative, Gram negative bacillus with a Kligler pattern of K/A, no H_2S and gas, which was identified as *Salmonella* species by preliminary serotyping. Treatment was changed to IV ceftriaxone 100mg/kg and oral azithromycin with IV ciprofloxacin. CRP came down but fever persisted even one week after change of therapy. Ceftriaxone was replaced with IV meropenem again and other 2 drugs were continued; which resolved fever within 48 hours with remarkable clinical response. Organism was confirmed as *Salmonella* Cholerasuis (a H_2S negative variant) by serotyping at the reference laboratory with sensitivity to ceftriaxone, ciprofloxacin and meropenem. On discharge, after 28 days, empyema had resolved with normal CRP.

Discussion

Though the CSF culture was negative, clinical presentation and CSF full report were suggestive of meningitis. Antibiotics were selected on the facts such as; better meningeal penetration as with ceftriaxone and meropenem, and satisfactory intracellular concentration as with ciprofloxacin and azithromycin. Patient needs follow-up to detect any sequelae.

PP 20

Viral kinetics in serial respiratory samples and clade of the virus of the first positive (SARS CoV-2) patient reported in Sri Lanka

Jayamaha CJS[1], *E Narangoda*[2], *Witahnage VH*[1], *Ekanayake DHP*[1], *Jayathunga RCS*[1], *Chu DKW*[3] *Peiris JSM*[3]

[1]National Influenza Centre, Department of Virology, Medical Research Institute, Colombo, [2] National Institute of Infectious Diseases, Angoda, [3]School of Public Health, LKS Faculty of Medicine, The University of Hong Kong

Introduction

We report the variability of viral genes detected in respiratory samples and the genetic clade of the first reported case of SARS CoV-2 in Sri Lanka.

Case report

A 43-year-old Chinese woman was positive for COVID-19 on 26th January, 2020. She presented with a history of fever, retro-orbital pain, loss of appetite and mild sore throat of one day duration. Patient had a productive cough and lungs were clear. After day 10 of illness she became asymptomatic and recovered uneventfully. Two in-house monoplex real-time PCR targeting highly conserved regions, N and ORF1b of SARS-CoV-2 were performed for diagnosis and follow-up at the Medical Research Institute. Variability of those genes in various respiratory samples until patient was discharged is shown below (Table 1).

Table 1. Viral kinetics (as cycle threshold values) of the positive case of COVID-19

Test sample	26/01/2020		30/01/2020		02/02/2020		06/02/2020		10/02/2020		12/02/2020		17/02/2020		18/02/2020	
Date of illness	Day 3		Day 7		Day 10		Day 14		Day 18		Day 20		Day 25		Day 26	
Target genes	N	Orf 1b	N	Orf 1b	N	Orf 1b	N	Orf 1b	N	Orf 1b	N	Orf 1b	N	Orf 1b	N	Orf1b
Nasal and throat swab	-	-	ND	ND	-	-	38	ND	ND	ND	ND	ND	-	-	-	-
Nasopharyngeal aspirate	30	27	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Sputum	-	-	-	-	31	32	34	34	38	ND	36	36	ND	38	ND	ND

N - N gene, Orf1b-Orf1b gene, ND - Not detected

FELLOWSHIP OF THE SRI LANKA COLLEGE OF MICROBIOLOGISTS 2019

Fellowships of the Sri Lanka College of Microbiologists were awarded to Dr. Sagarika Samarasinghe, Dr. Preethi Perera and Dr. Omala Wimalaratne on 13th August 2019 at the Hotel Ramada Colombo.

Dr. Sagarika Samarasinghe
MBBS, Diploma in Medical Microbiology, MD Medical Microbiology

President Sir, It's my privilege to introduce Dr. Sagarika Samarasinghe; a renowned consultant in the specialty of clinical parasitology. Sagarika Samarasinghe received her primary education from Minuwangoda Central College. She won the Jathika Navodaya grade seven scholarship and later entered Visakha Vidyalaya, Colombo for her secondary education.

She obtained her MBBS in 1984 from University of Peradeniya and did her internship at National Hospital, Colombo and joined the Medical Research Institute in 1985. She obtained her Diploma in Microbiology in 1991 and MD Microbiology in 1994.

Being the only Consultant Parasitologist in the government sector of Sri Lanka she faced many challenges which sharpened her skills as a Clinical Parasitologist, a lecturer and a researcher.

In 1996, she was awarded one year training on Immuno-parasitology at Yamagata University, Japan on a JICA fellowship. Further, she received post MD training at The Institute of Tropical Medicine and Hygiene in Antwerp, Belgium. She worked on the development of resistance in malaria parasites using PCR technique and her findings were published in the *Journal of Molecular Epidemiology and Evolutionary Genetics in Infectious Diseases,* in May 2006.

Her MD research thesis was on "The prevalence of *Toxoplasma gondii* antibodies in normal pregnant mothers and mothers with a bad obstetric history" at De Soysa Maternity Hospital under the supervision of Prof. Harsha Senevirathne. The important findings of her research were published in the Asian Parasitology Journal in 2005. Further her comprehensive knowledge on cerebral toxoplasmosis has been used to treat and cure many patients presenting with cerebral tumors on the request of neurophysicians and neurosurgeons thus avoiding surgical intervention and bringing her gratification from her clinical colleagues.

The first case of *Trypanosoma evansi* in Sri Lanka was diagnosed by Dr. Samarasinghe. These organisms were cultured in mice and hamsters by her. She prescribed

the drugs to be imported and furthermore got involved in the patient management, together with the consultant physician.

Adding to the humongous work carried out by Dr. Samarasinghe; A 16 feet long tape worm segment of *Taenia saginata*; and a 11 feet long tape worm segment of *Taenia saginata*, 16 *Hymenolepis diminuta* worms, not just segments (altogether 8.32 feet long) in a child and a 30cm long *Bertiella studeri* worm were some interesting cases, where she was involved in the diagnosis, expulsion of the worms and treatment.

Identification of first two cases of *Gnathostoma spinigerum*, the first case of *Loa loa*, the first case of *Oestrus ovis* in Sri Lanka and the first case in the world literature, identification of a juvenile *Wuchereria bancrofti* worm extracted from the posterior chamber of the eye are a few among her achievements. All these were published in journals.

In 2003 a World Bank funded project was done on health status of primary school children in Sri Lanka involving all nine provinces of the country. Dr. Samarasinghe and her team conducted the survey of soil transmitted helminthes in this project.

Dr. Sagarika Samarasinghe as the President of the Sri Lanka College of Microbiologists in 2007/2008 undertook the challenge of initiating the first Antimicrobial Resistance Surveillance programme in this country with the generous sponsorship of GlaxoSmithKline. Being a Parasitologist, she accepted to go through this project among many obstacles.

Furthermore during her presidency the Health Sector Development Project of the Ministry of Health and the World Bank funded waste management project for chest and STD clinics in 5 provinces were also carried out successfully.

She is a lecturer and an examiner of the postgraduate Diploma and MD in Medical Microbiology and Medical Parasitology. She was a member of the Board of Study in Medical Microbiology. She is also a lecturer and the chief examiner in Parasitology for MLT final examinations. She is the chief examiner in Entomology as well.

She is a member of the Research and Ethics Committees of the Medical Research Institute while being an external examiner for Medical Parasitology examinations at Kotelawela Defence University. She is a life member of the Indian Academy of Tropical Parasitology and Asia Pacific Bio safety Association as well.

She is the head of both Parasitology and Entomology departments of the MRI. She is a member of the Pesticide Subcommittee in the Ministry of Health. In addition to conducting surveillance activities, she attends to epidemic situations due to insects and mosquitoes.

She is in a constant struggle to prevent registering low quality insect repellants, mosquito repellants and larval control products which are locally manufactured as well as imported from foreign countries as it is her responsibility to issue bio efficacy reports for these products.

Her determination and the courage made it possible for her to face the intimidating challenges which came through her way. An exemplary leader to both Parasitology and Entomology departments at MRI, an inspiring teacher to her students, a beloved wife, a loving mother to her two daughters and as a dedicated doctor, she has made her mark in this world.

President Sir, I present Dr. Sagarika Samarasinghe to receive an honorary fellowship of the Sri Lanka College of Microbiologists.

Citation read by **Dr. Jananie Kottahachchi**
Senior Lecturer, Faculty of Medical Sciences, University of Sri Jayawardenepura

FELLOWSHIP OF THE SRI LANKA COLLEGE OF MICROBIOLOGISTS 2019

Dr. Preethi Perera

MBBS (Cey), Diploma in Medical Microbiology, MD Medical Microbiology

Preethi Devika Perera had her primary education at Visakha Vidyalaya Colombo from nursery to advanced level. She excelled both in studies and extra-curricular activities. She represented the school, as a member of its athletic team in both the junior and senior school and also in school English dramas. She was the House vice-captain and a school prefect in both the junior and senior school. She carried away class prizes and subject prizes. She was the Honorary Doctor to the hostel for many years. She was the first Visakhian to have 4 generations along with her 2 daughters.

She graduated from the Faculty of Medicine University of Colombo. After her internship in Colombo she joined the Blood Transfusion Service at the Central Blood Bank and was the medical officer in charge of the blood bank at General Hospital Kurunegala for 2 years. Thereafter she worked at Base Hospital Nawalapitiya for 6 years.

After her outstation stint she joined the Medical Research Institute in 1991 as Medical officer at the Department of Mycology. She obtained the Diploma in Microbiology in 1993 and MD Microbiology in 1998 from the Postgraduate Institute of Medicine, University of Colombo.

While at Medical Research Institute she proceeded to Japan in 1991 where she had a six months training in quality control of culture media and reagents at the Kumamoto Public Health Laboratory.

In 1994 she had special training in mycology for one year at Department of Microbiology and Immunology, Teikyo University Medical School, Tokyo, under a very eminent Mycologist Prof Hideyo Yamaguchi.

Her Post MD training in Mycology from 1999-2000 was at St John's Institute of Dermatology, St Thomas's Hospital, London under Prof Rodrick Hay, an authority on Mycology and at Mycology Reference Laboratories in Bristol and Leeds, United Kingdom.

Taking over the Department of Mycology, Medical Research Institute as Consultant in the year 2000, she established the antifungal sensitivity testing method and PCR for identification of candida species directly from blood samples. An exotic fungus *Saksenea vasiformis* was identified in a patient for the 1st time in Sri Lanka. She collaborated research with Department of Chemistry, University of Colombo and other researchers into the antifungal properties of Sri Lankan flora where some of our flora was found to possess very promising antifungal properties. She has publications in local and international journals. She received the presidential award in 2007.

As a postgraduate trainer from 2000-2013 she taught mycology to all the postgraduate trainees in the Diploma and MD Microbiology courses of the Post Graduate Institute of Medicine and has lectured and supervised the mycology training of all the medical laboratory technologists during this period and was an examiner as well. Postgraduate trainees in Dermatology have also undergone mycology training under her.

She was a supervisor of research projects for MD Microbiology and Dermatology of the Postgraduate Institute of Medicine.

She has been a member of the Board of Study in Microbiology from 2004 to 2014 during which time she was the Diploma and MD Microbiology course coordinator from 2003-2005 and 2006-2013 respectively. She has been an examiner for the Diploma and MD Microbiology from 2003-2014 and 2005-2015 respectively and the chief examiner for MD Microbiology in 2013.

From 2013 todate she has been the lecturer for the fungal module in the MSc course at the Institute of Biochemistry and Biotechnology, University of Colombo and developed the Mycology course module for medical laboratory sciences degree program 2018/19 at the Open University, Sri Lanka.

She has participated at international mycology conferences in Japan, India, Paris and Rome.

She delivered presentations at the invitation of the College of Pathologists, Association of Otorhinolaryngologists, Ceylon College of Physicians, Sri Lanka Society of Critical Care and Emergency Medicine, Sri Lanka Medical Association and the 4th SAARC ENT Congress.

Joining the Sri Lanka College of Microbiologists in 1994 she held the posts of council member from 2005-2010, Secretary 2005/06 and its President for the year 2008/09. The Health Sector Development Project of the Ministry of Health funded by the World Bank was successfully completed and the final report handed over to the Ministry of Health during her Presidency. At the 2013 Annual Scientific Sessions of the College she delivered a Plenary lecture on “Invasive fungal infections – when and how to treat”.

She contributed to the Mycology component of the Laboratory Manual compiled by the College and distributed through the Ministry to state sector laboratories. She also contributed to the Infection Control Manual which was also compiled by the College.

After retirement she was attached to the Postgraduate Institute of Medicine, Colombo as a senior lecturer from 2015-2018.

President Sir, it is my pleasure and privilege to present Dr. Preethi Devika Perera for the award of Honorary Fellowship of the Sri Lanka College of Microbiologists.

Citation read by **Dr. Primali Jayasekera**
Consultant Mycologist, Medical Research Institute, Colombo.

FELLOWSHIP OF THE SRI LANKA COLLEGE OF MICROBIOLOGISTS 2019

Dr. Omala Vajreshwari Wimalaratne
MBBS, Diploma in Medical Microbiology, MD Medical Microbiology

Dr. Omala Wimalaratne was educated at Visakha Vidyalaya, Colombo where she excelled in her studies. She graduated from the Faculty of Medicine, University of Colombo in 1978 and completed her internship at the Castle Street Hospital for Women and Lady Ridgeway hospital. She obtained her post graduate qualifications, the Diploma in Medical Microbiology in 1990 and MD in 1994 from the Post Graduate Institute of Medicine, University of Colombo. She is a senior consultant Vaccinologist and Virologist who has more than 25 years' experience in her speciality. She was the first Vaccinologist in Sri Lanka and was the head of the Department of Vaccines and Rabies, Medical Research Institute from 1995-2014. She was responsible for the establishment of the rabies specialist advice clinic at the MRI in 1995 and a 24 hour hot line which is accessible to all medical officers in the island on management of rabies post exposure therapy.

Dr. Wimalaratne was the head of the National Control Laboratory (NCL) for vaccines from 1997-2014 and was responsible for technical evaluation of all human vaccines brought to Sri Lanka before registration. As head of the NCL, in 1997 on WHO recommendation, she introduced the lot release system for all vaccines procured by the MOH to ensure their quality and safety.

She has had training in virology and vaccinology at WHO approved centres in Japan, UK, France, Switzerland, Thailand, South Korea and India. On numerous occasions, she has been an invited guest speaker at international conferences and has represented Sri Lanka at WHO meetings. Since 2000 to date, she has been a WHO advisor and was a member of the WHO pre-qualification board for Influenza vaccine in 2012. From 2003 - 2006 She was a member of the WHO Global Advisory Committee on Vaccine Safety, a member of the Rabies Expert Committee from 2004 - 2014 and South East Asian Rabies Forum from 2012 - 2014.

Dr. Wimalaratne is a senior member of several important committees in the Ministry of Health where policy decisions are made. Some of them are Advisory Committee on Communicable Diseases, Adverse Events following Immunization Monitoring Committee and National Rabies Expert Committee.

In 2002, Dr. Wimalaratne was invited to deliver the prestigious 12th Susan George Pulimood memorial oration which is awarded to a distinguished old girl of Visakha Vidyalaya for bringing credit to the school during her career in her chosen speciality.

Dr. Wimalaratne took a lead role in convincing the policy makers for the stoppage of nerve tissue rabies vaccine in 1995 and to switch over to the safe and effective cell culture rabies vaccine. Her greatest contribution in her chosen field is the introduction of intradermal administration of rabies cell culture vaccine for rabies post exposure treatment in 1997. As a result of this the Ministry of Health was able to save a large sum of foreign exchange for the country. Currently this technique is practiced in all government hospitals for the management of animal bite victims seeking post exposure treatment. She was responsible in training a large number of medical officers, nurses and paramedical staff all over the country in rabies and vaccinology and has established anti rabies treatment units in major hospitals for correct management of animal bite victims. This has enabled to minimize wastage of anti-rabies serum and vaccine.

She was a trainer and an examiner for both undergraduate and postgraduate medical personnel and was a member of the Board of Study in Microbiology for several years. She was the President of the College of Microbiologists in 2009/2010 and was the President of the Vaccine Forum of Sri Lanka 2016/2017.

She authored several chapters and was a co-editor in the book "SLMA guidelines and information on vaccines" published in 2008, 2011, 2014 and 2017.

She authored the chapter on "Rabies Post-Exposure Management" in the Immunization Hand Book published by the, Ministry of Health in 2012 and was also the author of the chapter "Medical Research Institute" in the book on "History of Medicine in Sri Lanka 1988-2018" published by the SLMA in 2018.

She has been the recipient of the Presidential Award for research on seven occasions and has more than 40 research papers published in local and foreign peer reviewed journals.

She has received several awards both nationally and internationally – some of these are

- "Citation of Excellence" in the Journal of Travel Medicine for the best brief communication 1997-1999
- Hector C. Perera award in 1999 and 2001 at the Annual Scientific Sessions of the Sri Lanka Veterinary Association
- Co-author for the best scientific paper at the Annual Scientific Sessions of the Sri Lanka College of Microbiologists in 2001 and 2011
- National award for Science and Technology (NASTA award) 2014

Since her retirement from government service in 2014, she conducts animal bite clinics and immunization in the private sector and is an advisor to the MOH on rabies and vaccinology.

President Sir, I have the privilege and honour to present to you Dr. Omala Vajreshwari Wimalaratne to confer her with the highest honour of the Sri Lanka College of Microbiologists and admit her as a honorary fellow.

Citation read by **Dr. Kanthi Nanayakkara**
Consultant Virologist and Vaccinologist, Head / Department of Rabies and Vaccine QC, National Control Laboratory, Medical Research Institute, Colombo.

FELLOWSHIPS OF THE SRI LANKA COLLEGE OF MICROBIOLOGISTS 2020

Dr. Pranitha Somaratne

Dr. Philomena Chandrasiri

Dr. Geethani Wickramasinghe

2ND SOUTH ASIAN MELIOIDOSIS CONGRESS 2017

First Prize

P02 - Platelets aid in host defense during melioidosis

E Birnie[1], TAM Claushuis[1], GCKW Koh[2], LEH van der Donk[1], AE Grootemaat[3], DI Picavet[3], NN van der Wel[3], J Ware[4], B Hou[5], AF de Vos[1], T van der Poll[1,6], C van't Veer[1], WJ Wiersinga[1,6]

Second Prize

P03 - Prevalence of environmental *Burkholderia pseudomallei* in Sri Lanka

K Assig[1], E Corea[2], B Folli[1], M Abeykoon[3], N Mubarak[4], T Kumanan[5], T Senanayake[6], B Piyasiri[7], I Steinmetz[1]

Third Prize

P09 - Melioidosis in northern Sri Lanka: filling the gap in the map

FN Mubarak[1], S Thavapalan[1], EM Corea[2], AD De Silva[3], BJR Cooray[4], S Ghetheeswaran[5], K Indranath[4], T Kumanan[6], T Peranantharajah[5], JA Pradeepan[6], GJ Pratheepan[7], G Selvaratnam[6], S Sivansuthan[5], N Suganthan[6], S Uthayakumaran[5]

PRIZE WINNERS AT THE 28TH ANNUAL SCIENTIFIC SESSIONS OF THE SRI LANKA COLLEGE OF MICROBIOLOGISTS AND 50TH ANNIVERSARY CONFERENCE ON INFECTIOUS DISEASES AND INFECTION PREVENTION AND CONTROL

Following oral presentations were awarded first, second and third places at the 28th Annual Scientific Sessions of the Sri Lanka College of Microbiologists and 50th Anniversary Conference on Infectious Diseases and Infection Prevention and Control held on 14th and 15th August 2019

Oral presentations

1st prize

OP 2

Laboratory diagnostic methods comparison, antibiotic susceptibility pattern and serotyping of invasive and colonizing group B Streptococcus isolates in a selected group of hospitals in Sri Lanka

Chinthamani PR[1], Chandrasiri NS[2], Pathirage S[3]

[1]Postgraduate Institute of Medicine, University of Colombo, [2]Colombo South Teaching Hospital, Kalubowila, [3]Medical Research Institute, Colombo

2nd prize

OP 16

Sero-prevalence and genotype distribution of Hepatitis C infection, among patients with Haemophilia A and B, in four selected tertiary care hospitals

Fernando MAY, Abeynayake JI

Department of Virology, Medical Research Institute, Colombo

3rd prize

OP 12

Pneumococcal colonization in two groups of Sri Lankan children between 2 months to 2 years

Vidanapathirana G[1], Angulmaduwa ALSK[2], Munasinghe TS[3], Ekanayake EWMA[2], Harasgama P[2], Kudagammana ST[3], Dissanayake BN[2], Liyanapathirana LVC[2]

[1]Faculty of Allied, Health Sciences, University of Peradeniya, [2]Department of Microbiology, Faculty of Medicine, University of Peradeniya, [3]Department of Paediatrics, Faculty of Medicine, University of Peradeniya

Following poster presentations were awarded first, second and third places at the 28th Annual Scientific Sessions of the Sri Lanka College of Microbiologists and 50th Anniversary Conference on Infectious Diseases and Infection Prevention and Control held on 14th and 15th August 2019.

Poster presentations

1st prize

PP 9

Evaluation of rapid immunochromatographic assays for the detection of selected respiratory viruses during an outbreak

Danthanarayana NS[1], Madusha SAE[1], Lakmali JPR[1], Jayamaha J[2], Udara GKJN[1], Sooriyaarachchi PGPR[1], Kumara MKR[1]

[1]Teaching Hospital Karapitiya, Galle, [2]National Influenza Centre, Department of Virology, Medical Research Institute

2nd prize

PP 5

Molecular epidemiology of antimicrobial resistance of *Salmonella enterica* isolated from different parts of Sri Lanka

Pathirage S[1], Tay MYF[2,3] Chandrasekaran L[2,3], Fonseka S[1], Sadeepanie N[1], Waidyarathna KDK[3], Liyanage LRDC[1], Seow KLG[2,3], Hendriksen RS[4], Takeuchi MT[5], Schlundt J[2,3]

[1]Medical Research Institute (MRI), Sri Lanka , [2]Nanyang Technological University Food Technology Centre (NAFTEC), [3]Nanyang Technological University (NTU), Singapore, [4]National Food Institute, Technical University of Denmark, WHO Collaborating Center for Antimicrobial Resistance in Food borne Pathogens and European Union Reference Laboratory for Antimicrobial Resistance, Kongens Lyngby, Denmark, [5]Food and Agriculture Organization Regional Office for Asia and the Pacific, Thailand

3rd prize

PP 1

Epidemiology and clinical presentation of culture positive melioidosis in the Southern Province of Sri Lanka

Piyasiri DLB[1], Corea EM[2], Ulwishewa GM[1], Sapukotana PM[1], Wijeweera KDDS[1], Gamage TSH[1], Jayasundera MCT[1], Priyarangani P[1], Priyadharshana U[1], Jayasekara JVGM[1], Nanayakkara IRS[1]

[1]Teaching Hospital Karapitiya, Galle, [2]Faculty of Medicine, Colombo

Dr. C. Palasuntheram Prize

OP 4

Molecular characterization and laboratory detection of carbapenemase producing Enterobacteriaceae

Kumudunie WGM[1], Wijayasinghe YS[1], Wijesooriya WRPLI[1], Namalie KD[2], Sunil-Chandra NP[1]

[1]Faculty of Medicine, University of Kelaniya, [2]Colombo North Teaching Hospital, Ragama

Presidential address delivered at the Induction of President and Dr. Siri Wickremesinghe Memorial Oration 2020 of the Sri Lanka College of Microbiologists on 29th February 2020

Protecting the most vulnerable: keeping the lid down

Dr. N. Shirani Chandrasiri
Consultant Clinical Microbiologist,
Colombo South Teaching Hospital,Kalubowila

Past presidents, Mrs Rangani Wickremasinghe and family members, distinguish invitees, friends and colleagues, thank you very much for gracing this occasion.

Let me introduce you to the Sri Lanka Colleague of Microbiologists, SLCM in short. The parent organization of the Sri Lanka College of Microbiologists was started in 1969 with 16 founding members as the Ceylon Association of Microbiologists which was subsequently changed to The Ceylon Association of Microbiologists. This was changed to the Sri Lanka Association of Microbiologists in 1974 and in 1979 this association was renamed as the Sri Lanka College of Microbiologists with a new constitution. Last year we celebrated 50 years since the inception, with a strength of 267 life members.

These are the objectives our founding members focused on:

- Promote the study of microbiology.
- Disseminate knowledge in microbiology among the members and other healthcare personnel and the public.
- Highlight the importance of microbiology in relation to the development and requirements of the country.
- Advice and initiate action on issues related to microbiology which may arise in the country.
- Discuss matters of scientific and professional interest pertaining to microbiology.

- Promote research and actively assist in such work and publish original work in microbiology.

In keeping with these objectives our members;

- Work closely with the Ministry of Health, Sri Lanka.
- Conducts CMEs, workshops and annual academic sessions.
- Represent boards of study in Microbiology and are dedicated teachers.
- Publish the Annual Bulletin of SLCM.
- Take part in teaching, examinations and work-shops for medical laboratory technologists for the development of the discipline.
- Update infection control activities with infection control nursing officers and other disciplines.
- Carry out research activities.
- Upgrade microbiology knowledge in the general public.
- Act as technical experts for the Sri Lanka Accreditation Board.
- Partnered with Country Grant – Fleming fund to improve hospital microbiology services.

Our membership include microbiologists, virologists, mycologists, immunologists and parasitologists.

Our proud contribution to upliftment of Microbiology is our publications

Books published and guidelines developed by Sri Lanka College of Microbiologists

Year	Book / Guideline
2002	Laboratory Manual in Microbiology – 1st Edition
2002	Collection and transport of clinical specimens
2004	Bio safety Manual – 1st Edition
2005	Hospital Infection Control Manual
2005/2006	Handbook on waste management of chest and STD clinics
2005/2006	Code of practices on waste management of chest and STD clinics
2006/2008	National guidelines on 10 topics in Microbiology as 3 books, under Health Sector Development Project
2011	Laboratory Manual in Microbiology – 2nd Edition
2014	Bio safety Manual – 2nd Edition
2016	Empirical and prophylactic use of antimicrobials – National guidelines 2016. In collaboration with other Professional Colleges in healthcare and the Ministry of Health
2017	Guidelines on microbiological investigations, antimicrobials and infection preventive and control practices in stem cell transplantation
2017	National strategic plan for combating antimicrobial resistance in Sri Lanka 2017 – 2022, in collaboration with the Ministry of Health and WHO

We have our sessions planned under the theme of "Facing the challenges of multi drug resistance" from 11-13 August this year.

Today I would like to talk about protecting the most vulnerable: keeping the lid down.

Ladies and gentlemen I am the microbiologist who has served the longest in the same station in a government hospital which is Colombo South Teaching Hospital (CSTH). With this post I inherited a Pandora box called the special care baby unit.

To understand my presentation you will have to remember that a premature baby is a baby born before 37 weeks and any term or preterm baby is vulnerable to infection due to many reasons. Premature babies need special care more than mature babies and babies in special care usually have more than one problem.

Hospitals in the Colombo district have the highest number of live births followed by Kurunegala and Kandy districts. Data from CSTH show that in the last four years births are gradually decreasing but the number of premature births are increasing.

An article by Professor Harendra de Silva says NICUs developed in mid 1980s: I think one of the two from Colombo must be our SCBU although we don't call it a NICU. In an audit by Yatawatta et al., published in 2011, the number of NICUs are mentioned as three [1,2].

Colombo South Teaching Hospital was opened as a Base hospital in 1960 by Mrs Srimavo Bandaranayake, the first female prime minister in the world. With the growth of the hospital into Colombo South Teaching Hospital in 1995, our SCBU may have started. I became acting microbiologist at CSTH in February 2004. To my eyes, just returning from overseas, was whether any planning was undertaken before constructing the SCBU. The problems encountered there included.

- The isolation room opening in to the general SCBU
- No space for more than 2 septic babies in the isolation area
- Babies coming for observation being put with other babies
- Minimum staff facilities

Over time I started to see our strengths:

- The SCBU nurses and doctors were dedicated.
- We got our first neonatologist in March 2008, Dr Kumudu Weerasekera followed by Dr Rajeeva Sabanantharaja in October 2012.
- The first permanent consultant microbiologist, Dr Philomina Chandrasiri was appointed in October 2004.
- The infection control nursing officer allocated to SCBU was the senior most infection control nurse in the unit.
- The hospital administration was always supportive.

Time and experience showed me more pitfalls: sometimes pushing an incubator and putting in another baby because the number inside was not defined and minimal isolation facilities leading to external transfers not being isolated. These babies were the focus of infections. Some of these problems we could have addressed with proper planning.

Today I am not telling you about our many close shaves but actual closures of SCBU in the past.

However, closing this SCBU was always a problem. This was our only facility and some hospitals in the peripheries also depend on our SCBU. We have to inform the Ministry of Health and these hospitals about any closures. Sometimes the paediatrician calls me and says this baby is deteriorating and needs special care whilst I hear the obstetrician reasoning out why he did the LSCS without in-utero transfer. I can understand their problems: I am part of the team but have to take the decision although unpleasant.

Our first closure was in 2011 and was caused by an ESBL forming coliform, an organism which could destroy most antibiotics: for these pathogens meropenem is the only treatment. I was unable to find records about the number of affected babies but there was a document where the correct dose of meropenem was stressed along with other things such as on admission blood cultures. This early document was not an internal circular but issued by the microbiologist.

In 2012 we had another coliform outbreak due to *Klebsiella pneumoniae*; here again meropenem was the saviour since it was sensitive. Although we do not have affected numbers, we have gone to town with the introduction of an antibiotic policy and an admission policy for the SCBU. However, the number of cots was not restricted in this policy.

These outbreaks were traced to transfers but our isolation facilities were not improved.

In 2012, we decided to analyze the antibiotic usage and identified the microbiologist and chief pharmacist as

responsible. I thought we had closed the lid properly. In retrospect, I was reminded of this saying by emperor Napoleon Boneparte; treaties are observed as long as they are in harmony with interests.

In late November of 2013 we started getting meropenem resistant isolates that we identified as *Acinetobacter* spp. which were only sensitive to co-trimaxazole. On top of that there was an ESBL producing coliform outbreak.

We again closed the unit, cleaned it and opened it after assessing air quality using settle plates. We had several recommendations; an admission policy was reissued with antibiotic policy, some structural modifications came in to place, we used to clean the SCBU with alcohol because of the concern about neonates but the meropenem resistance alarmed me to such an extent that we started the final clean with TCL. We always did settle plates before opening without cutoffs but clean room cutoffs were adopted to interpret the results. From the Ministry of Health, there was a one member investigation – He could not understand the difficulties we had without automated blood cultures, therefore this issue was not included in the report.

Two pathogens affecting one after the other was missed because only a microbiologist could interpret the laboratory data. I was at fault for not following the full outbreak protocol in this outbreak. The meropenem resistance in SCBU frighten me and we never found much in our previous outbreak investigations. Another internal circular was issued.

I was determined to analyze the antibiotic consumption because high consumption is one factor for antibiotic resistance. Analysis of antibiotic consumption was started from 2015. In the latter part of 2017, an automated blood culture machine arrived to CSTH, just in time for another outbreak.

In October 2017 one of the babies blood culture grew an unusual looking pathogen. It was sensitive to meropenem and ceferperazone-sulbactam. In September 2016 the Medical Research Institute (MRI) had gotten a state of the art automated identification and sensitivity system called BD Phoenix™. We sent them the isolate which was identified as *Rhizobium radiobacter*. Even I hadn't heard the name before.

Between 3rd and 30th October, seven premature babies were affected; Four died in spite of the isolate being meropenem sensitive and the original brand of meropenem being used for treatment. Seventeen cultures were positive from these 7 babies.

I wanted to find the source since it was unlikely to be from gut flora; possible sources were listed and tested for bacterial contamination at our laboratory as well as in the reference laboratory at MRI, which included medical devices, parenteral preparations and environmental samples. Unfortunately, we failed to identify the source. I discussed this outbreak with our external examiner of the MD 2017 and he told me that a certain amount of luck is needed in outbreak investigations. However the IV cannulae were found to be contaminated with *Pseudomonas aeruginosa*. It was noted that cannulae and infusion sets used during the period of outbreak, had been purchased from a local manufacturer and the particular brand had not been used in the hospital before. The cannula and infusion sets were replaced with previous known products. The SCBU was thoroughly cleaned and reopened for admission after 30 days.

In 2018 we faced an outbreak due to a meropenem resistant organism for the second time. This time it was *E. cloacae*. The isolate was susceptible only to levofloxacin, amikacin and colistin and identification was done using BD Phoenix™ system from MRI. Because of our past experience we investigated everything possible to cause an outbreak with the help of the MRI. An opened 3% saline solution yielded an isolate similar to the blood culture isolates in appearance and sensitivity. It was found that a 500ml opened saline container was kept for 24 hours. When unopened 3% saline solutions were tested, they were sterile. Our antibiotics and other solutions were not kept after opening but the saline issue had somehow occurred.

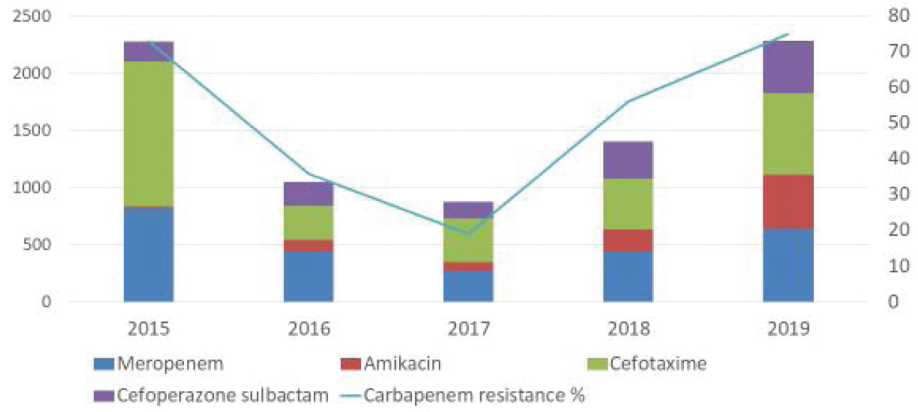
A Sri Lankan microbiologist who was a friend of mine, helped me to analyze these isolates in Australia. MALDITOF was used for the first time to identify an outbreak causing pathogen. Our isolates had many resistant genes [3]. The Sydney team was tackling a *Serracia marcescens* outbreak in their NICU with deaths but those isolates did not have this many resistant genes. *E. clocae* contamination is not that uncommon in neonatal units as shown by this outbreak report from Turkey [4].

Our admission and antibiotic policy was reviewed. We introduced on admission deep ear swabs with blood cultures. Due to limited space, it was decided that < 28 weekers would be cared for separately. We initiated regular hand hygiene audits in the SCBU every 3 months and instead of 500 ml 3% NaCl solutions 10 ml solutions were ordered. Until such time it was decided that an open NaCl solution would be kept for only 6 hours. However one day I overheard a physician telling about the price difference of 500ml 3% NaCL and small volume containers. In good faith he had not approved it. For 3

months the SCBU was closed for construction, an extension was undertaken and our isolation facility was further improved.

We analyzed our antibiotic use in the SCBU for a five year duration. Antibiotic consumption was assessed using the number of vials since it was done at the end of the year and was a variable that was easy to collect. You can see how our consumption went down and gradually coming back to initial high levels; So did the resistance in our isolates. We discussed the reasons in our perinatal mortality morbidity meeting. Though our antibiotic consumption is like this, our antifungal stewardship is a success story.

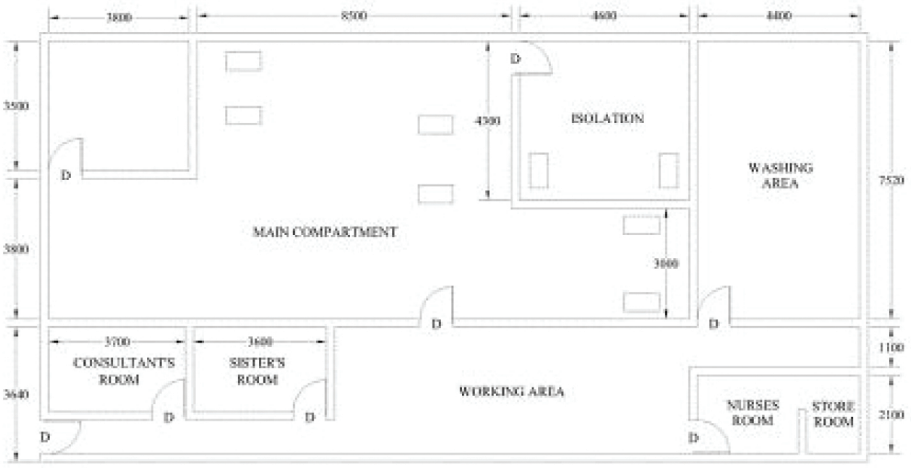
Correlation between antibiotic consumption and meropenem resistance



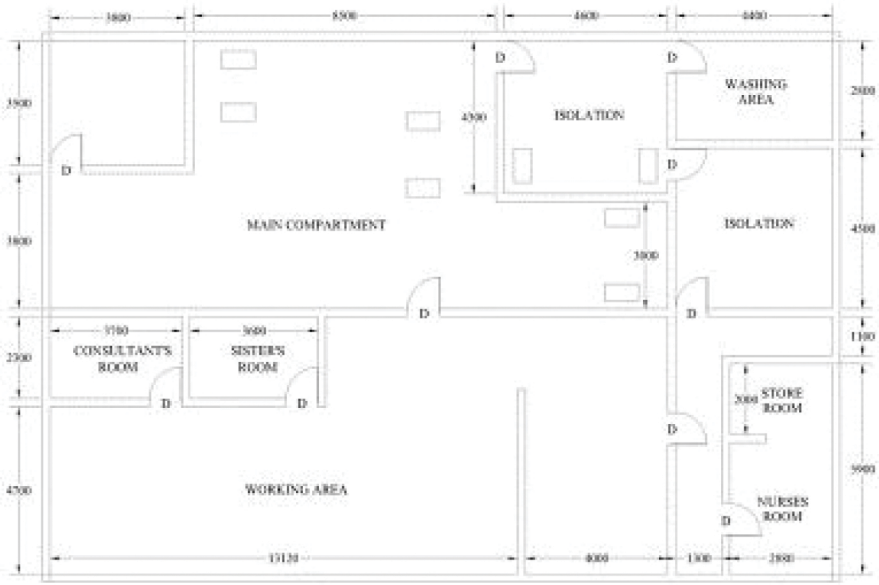
These are some Sri Lankan outbreaks published in the literature. This is just the tip of the iceberg.[5,6,7]

Ladies and gentlemen, as you can see in next few slides, we have done our best for these vulnerable babies.

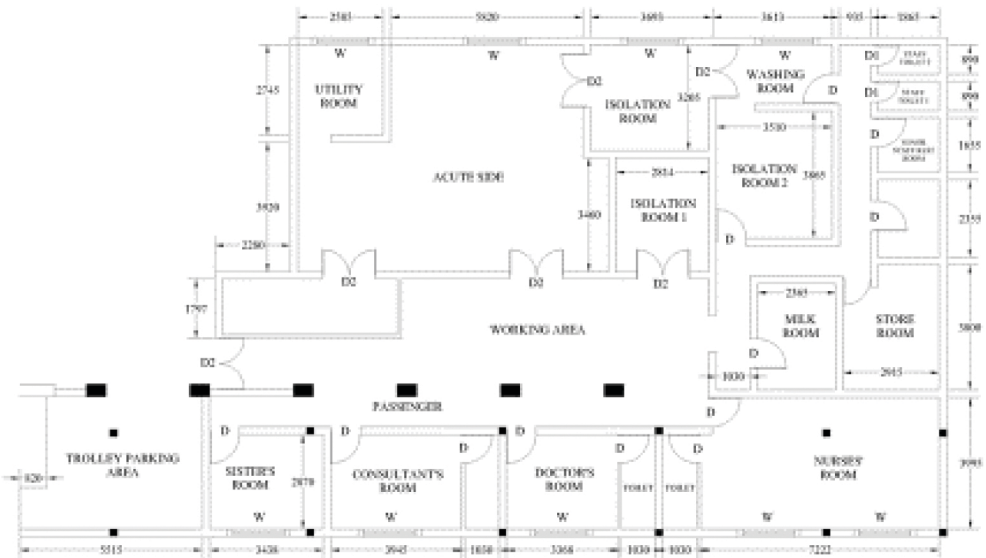
Original floor plan of the Special Care Baby Unit – CSTH



Floor plan following renovation in 2014
Special Care Baby Unit – CSTH



Present floor plan of the Special Care Baby Unit – CSTH



This is a multifactorial problem and we all have a role to play. Planning of SCBU or NICU is very important and proper infrastructure facilities are needed. Eg: need to isolate transfers till screening is complete, premature babies (< 28 weeks) should be separated from others, expected long stays like congenital problems should ideally stay in isolation, separate designated space for sterile/ special procedures, staff facilities and feeding/ gavage feed collection area for mothers.

The staff of the unit have an important role. Eg: attention to hand hygiene, follow aseptic procedures, adherence to unit protocols, good antibiotic stewardship and audits.

All SCBUs should be supported by automated blood culture and ID systems with an onsite microbiologist. We need introduction of CPE selective agar for screening and early detection of colonized babies. We can all play a role in carrying out surveillance, audits including hand hygiene/ antibiotic consumption, antibiotic MDTs and antibiotic stewardship.

The obstetricians too can help by identifying at risk mothers and giving special care, aiming for maximum possible maturity, in utero transfer whenever special care is needed, minimum vaginal examination during labour and avoiding antibiotics as much as possible. Choosing single embryo transfer as appropriate when undergoing in vitro fertilization is also important because being pregnant with multiples (twins, triplets, or more) has a higher risk of preterm birth.

Regulatory bodies have a role in providing quality antibiotics (even meropenem and vancomycin was withdrawn due to quality failure in 2019) and high quality devices for all invasive procedures, preventing shortages so that poor quality products are not able to enter the system, providing us adequate staff: patient ratios, investigating outbreaks with a panel consisting of epidemiologist, neonatologist and microbiologist.

As for the society, a pregnant mother needs all the help she can get during pregnancy. If a mother's work is physically strenuous preterm labour is more likely. Women with a history of spontaneous preterm delivery are 1.5 to two times more likely to have a subsequent preterm delivery, therefore we need to identify and follow-up these mothers in centers with adequate facilities. Ensuring women have access to health care before and between pregnancies will reduce preterm labour and identifying women at risk for preterm delivery and offering effective treatments to prevent preterm birth is also important. We need to prevent unintended pregnancies and teen pregnancies and ensure that expectant mothers wait at least 18 months between pregnancies. We need health education in these aspects.

My sincere gratitude to you all for sharing this morning with me and for enabling me to share my experiences on the microbiology and infection control aspects of managing this very vulnerable patient group, the neonates.

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Towards polio free world – contribution from Sri Lanka

Dr. Sunethra Gunasena
Senior Lecturer and Consultant Virologist
Department of Microbiology, Faculty of Medicine, University of Ruhuna, Karapitiya, Galle

Madam President, Past Presidents, Members of the Council, Members of the College, Mrs. Ranganie Wickremesinghe, Members of the Family of late Dr. Siri Wickremesinghe, distinguished invitees, Ladies and Gentlemen.

I thank the President and the Council for inviting me to deliver the Siri Wickremesinghe Oration – 2020. It is a privilege to stand before you to deliver the oration to honor the memory of late Dr. Rakkitha Sirimal Bandara Wickremesinghe, a Medical Microbiologist, a Scientist, a Teacher, and a Gentleman.

I am not sure when I first met Dr. Siri Wickremesinghe. It is most likely at the interview held in mid-1989, to select the candidates for the Diploma in Medical Microbiology Program. Of the many memories I have of Dr. Wickremesinghe, most vivid one is the way he used to tell stories when the members went out to lunch after the College AGM. He used to tell many stories; some may be true, but others were too good to be true.

Dr. Wickremesinghe served as the Consultant Medical Microbiologist for many years at the Medical Research Institute. He was the Director during the period 1996 to 1998. He was the first Secretary to the Board of Study in Microbiology of the Postgraduate Institute of Medicine of the University of Colombo, a much-respected teacher and mentor of many postgraduate trainees in Microbiology. He was my teacher when I started the training the way back in 1990. Dr. Wickremesinghe was one of the earliest members of the Sri Lanka College of Microbiologists and became the President in 1993/1994.Other than Medical Microbiology, Dr. Wickremesinghe had many interests: History, Geography, Literature, Cricket and Wildlife and particularly for the turtle conservation project at Kosgoda. Ladies and Gentlemen, to honor the memory of late Dr. Siri Wickremesinghe I like to present on “Towards polio free world – contribution from Sri Lanka”.

My presentation will be in four parts. First, I will briefly discuss the events that led to the launching of the Global Polio Eradication Initiative (GPEI) and the polio in Sri

Lanka together with the control activities. This will be followed by the key milestones to-date achieved by the GPEI. Third part will be the center of my presentation. I will discuss some of the challenges facing the GPEI and how Sri Lanka has contributed and managed these challenges. Presentation will be supported by the information, data and findings of studies originated in Sri Lanka. Lastly, I will very briefly touch on future interests of GPEI which are directly related to the challenges I have presented.

1. Introduction

1.1 History of polio

Paralytic poliomyelitis commonly known as "Polio" has been the scourge in the early 20th century. The causative agent, poliovirus was first identified by Karl Landsteiner and Erwin Popper in 1908 [1]. Breakthrough came in 1948, when John Enders and his team were successful in cultivating the poliovirus in the laboratory. This development greatly facilitated the vaccine research against polio [2]. The first effective polio vaccine was developed in 1955 by Jonas Salk. The Salk vaccine, or inactivated polio vaccine also referred as IPV was licensed for use in 1957. Oral polio vaccine, better known as OPV containing attenuated strains of the virus was developed by Albert Sabin in 1961 and was licensed for use in 1962 [3].

1.2 Global Polio Eradication Initiative

With the introduction of vaccine, industrialized countries were able to control the disease, but it remained a major public health problem in developing countries. In 1988, World Health Organization (WHO) estimated 350,000 polio cases occurring in more than 125 endemic countries. As a result, at the 41st World Health Assembly in 1988, member states adopted the resolution for global eradication of poliomyelitis by the year 2000. Following the resolution, Global Polio Eradication Initiative (GPEI) was launched [3].

GPEI was led by the National governments, in partnership with the World Health Organization, Rotary International, Center for Disease Control, United States of America (US CDC), United Nations International Children's Emergency Fund (UNICEF), Bill & Melinda Gate Foundation and GAVI the vaccine alliance. GPEI adopted four strategies namely: High coverage of routine immunization, supplementary immunization, lab-based AFP surveillance and the outbreak response to achieve this target [3].

1.3 Polio in Sri Lanka

Paralytic poliomyelitis (Polio) was made a notifiable disease in Sri Lanka in 1944. The first major epidemic of polio occurred in 1962 with 1810 cases and 180 deaths. Since then the disease appeared as epidemics every six years (1968, 1974 and 1980). Though a epidemic was expected in 1986, only 9 cases were reported. However, unexpected epidemic of polio reported during the latter half of 1987 from the Jaffna Health Division without any cases from rest of the island. Prevailing unsettled conditions, disruption of the immunization activities in this area, frequent movement of adult and children to and back from India and the outbreak of polio in South India all contributed to this epidemic [4].

Sri Lanka conducted a pilot project to immunize children using trivalent OPV (tOPV) in 1961, followed by the introduction of the vaccine in Colombo and its suburbs in 1963. Trivalent OPV was included in the routine immunization of infants in 1964 [4].

The Polio Eradication Program of Sri Lanka was initiated in 1988. The Epidemiology Unit functions as the national focal point to coordinate the activities under the strategies adopted by the program. Polio Regional Reference Laboratory (Polio RRL), Medical Research Institute (MRI) provides the laboratory support for the surveillance activities. With the commitment to eradicate polio in Sri Lanka, acute flaccid paralysis (AFP) as a suspect case of polio, was made a notifiable disease in 1990. As the next step, the case-based, lab-based AFP surveillance was initiated in 1991. On the same year. 5th dose of OPV prior to school entry was introduced to the Immunization Program [4]. With these strategies Sri Lanka was able to eliminate polio. The last case of polio which was caused by wild poliovirus (WPV) type 1 was reported in November 1993.

Sri Lanka introduced changes to the polio immunization in concurrence with strategies adopted by the GPEI. In readiness for the planned withdrawal of Sabin virus type 2 from OPV, Sri Lanka introduced single intramuscular (IM) dose of IPV to the immunization program starting from July 2015. With the globally coordinated vaccine switch from tOPV to bivalent OPV (bOPV) in April 2016, Sabin virus type 2 was withdrawn from OPV. Immunity to type 2 was conferred by the dose of IPV which contains inactivated poliovirus type 1, 2 and 3. With the introduction of IPV to the immunization schedules in OPV using countries, increased the demand that in turn led to the global shortage of IPV. After considering the recommendations of the WHO Strategic Advisory Group of Experts (SAGE) on Immunization, Sri Lanka introduced fractional dose IPV to the immunization schedule in July 2016 [5, 6].

Current polio immunization schedule includes 5 doses of bOPV at completion of 2, 4, 6, 18 months and at school entry together with two doses of fIPV at completion of 2 and 4 months [5].

2. Milestones to-date achieved by the GPEI

With the implementation of eradication strategies, case load of polio has reduced from estimated 350,000 in more than 125 countries in 1988, to 173 cases from two countries Pakistan (144) and Afghanistan (29) in 2019. Though Nigeria is still considered polio endemic country it has not reported cases during 2017 to 2019. Four of six WHO regions namely American region (1994), Western Pacific region (2000), European region (2002) and South East region (2014) were certified free of polio. This makes 80% of world population living in polio free regions [7]. The Global Commission for the Certification of Poliomyelitis Eradication declared the global eradication of WPV type 2 on 20th September 2015 [8]. Global eradication of WPV type 3 was declared on 24th October 2019, World Polio Day [9].

3. Meeting challenges

Even though there was a rapid reduction of 99.9% of the polio case load from 1988, the goal of polio eradication was not achieved by year 2000 not even by 2019. During next 10 minutes or so I like to identify some of the factors that challenge achieving the final goal of polio eradication. I will discuss how Sri Lanka is trying to meet these challenges with the supportive information, data and study findings.

Maintaining high immunization coverage and continued AFP surveillance which is most important for the continued control of the polio situation has become a major challenge for the country programs. Risk of importation of WPV from endemic country to a polio free country, outbreaks due to circulating vaccine derived poliovirus (cVDPV) and potential poliovirus spread from long term excreters are also identified as some of the challenges to achieving the goal of polio eradication [10]. Lastly, I take up the IPV and its effect on gut immunity in OPV primed children.

3.1 Maintaining high immunization coverage

Annual birth cohort of Sri Lanka in 2018 was about 320,000 surviving infants. In 2018, the Immunization Program has used nearly 1.6 million doses of OPV and 600,000 doses of fIPV, while achieving 95% to 96% immunization coverage across 5 doses of OPV and 2 doses of fIPV (Personal communication). Sri Lanka maintained high immunization coverage over the years through the National Immunization Program [11].

Studies have shown that high level of population immunity was also maintained across the country. A cross sectional, community based sero survey was conducted in 2014 in 3 districts (Colombo, Badulla, Killinochchi) in covering four age groups namely 9-11 months, 3-4 years, 7-9 years and 15 years.

Study findings showed seropositive rate for poliovirus type 1 and type 2 were more than 95% in all age groups. Seropositive rate for PV type 3 varied between 95% in the 9 to 11 months age group to 75% in 15 years age group. Study finding showed that high level of immunity persists even 10 years after the last dose of OPV [12].

Findings of the study which was conducted after withdrawal of Sabin virus type 2 from OPV much more important in current context. A sero survey was conducted during the period December 2017 to March 2018 to compare the prevalence of polio antibodies specifically antibodies to poliovirus type 2, following the change from bOPV with full dose of IPV to bOPV with two fractional doses of IPV schedule [13].

The study findings showed that antibody prevalence to PV type 1 and type 3 was 100% in all 3 districts. Antibody prevalence to PV type 2 following IPV full dose ranges between 90-93% while the same following fIPV ranges from 78-100% (p=0.22). This study confirmed that Sri Lanka is maintaining high seroprevalence rate for all poliovirus types. Further it also showed that implementation of fIPV to the immunization schedule has not significantly decreased the antibody prevalence to PV type 2 [13].

3.2 Continued AFP surveillance

With increasing technological support for the clinical diagnosis of AFP cases, maintaining the interest in the AFP surveillance has become a challenge. During the period 2015 to 2019, 75-87% of AFP cases has been investigated with adequate stool samples in Sri Lanka. During this period, wild poliovirus was not isolated [14].

3.3 Risk of importation of WPV

With increasing global travel and the largely sub clinical nature of PV infection favour the transmission of the WPV from an endemic country to a polio free country by an individual with suboptimal intestinal mucosal immunity. WPV type 1 was detected in environmental samples from Israel in 2013 and later, on the same year in environmental samples from West bank and Gaza strip. Though the virus detected from environmental samples for several months, fortunately there were no polio cases reported [3].

More recently in May 2019 Iran reported detection of WPV type 1 also in environmental samples from a province that borders both Pakistan and Afghanistan. Genetic sequencing studies have shown that this virus was originally from Sindh province in Pakistan. These importations to countries that have been free of polio for years for example Iran since 2001, is of great concern and shows that the risk of importation would remain as long as there is circulation in endemic countries [3].

3.4 Outbreaks caused by circulating VDPV

Vaccine poliovirus circulate longer in community with poor immunization coverage and low immunity level acquiring mutations to become Circulating VDPV (cVDPV) which behave as wild poliovirus causing outbreaks of polio (10). During 2019, 3 countries experienced circulating VDPV type 1 outbreaks. 15 countries experienced circulating VDPV type 2 outbreaks. During this period outbreaks due to circulating VDPV type 3 was not reported [3]. DR Congo was experiencing circulating VDPV outbreaks since 2017 and several other countries in the African region have also reported multiple outbreaks. This showed that unless it was promptly interrupted, circulation of the virus could lead to continuation of the outbreak with the possibility for spread to other countries across the borders [3, 15]. Therefore, it is of great importance to maintain high level of immunization coverage, high level of population immunity and maintain high quality surveillance to prevent the development of circulating VDPV and to protect country from consequences of an importation of WPV [3].

3.5 Long term poliovirus excreters

Persons with primary immune deficiency disorders (PIDD) especially those with B cell defects tend to excrete polioviruses including the vaccine polioviruses from OPV longer. During the prolonged multiplication in the gut vaccine viruses undergo mutation to develop in to immune deficient VDPV (iVDPV). They are not related to any reported outbreaks but can become important source of poliovirus following OPV withdrawal and in the post eradication era [10, 16].

Seven country study series on PV excretion among patients with PIDD was conducted involving low- income and middle-income countries. Study in Sri Lanka was conducted during the period 2009 to September 2011. Of the 942 patients investigated by the Department of Immunology, MRI, 51 patients were detected with PIDD and were included in the study. Poliovirus excretion was studied at the Department of Virology, MRI [17].

Five patients excreted PV including 2 patients who excreted iVDPV [17, 18]. Three patients with severe combined immune deficiency (SCID) including the one who excreted the iVDPV expired, before taking follow up samples. One patient with X linked agammaglobulinemia (XLA) cleared the virus within few months as revealed by the follow up negative stool at 3 months and at 11 months. Other child excreted the iVDPV for 7 months [18].

I will talk about the two children who excreted iVDPV in little more details. Immune deficient VDPV type 2 was detected from an 8-month-old child with SCID. Two isolates from this child had 8 and 12 nucleotide changes in the VP1 region from the parent Sabin virus thus fitting to the definition of iVDPV type 2 (more than 6 nucleotide changes for type 2). This child has received OPV at the scheduled age of 2, 4, and at 6 months [17, 19]. Development of a VDPV required the circulation of the Sabin poliovirus strain for a minimum period of 12 months [16]. Detection of iVDPV with 8 and 12 nucleotide changes during maximum period of 6 months in this child raises the possibility of faster rate of mutation with increase rate of replication of the PV in PIDD patients or whether he has acquired the virus long before the age of 2 months when he received first dose of OPV.

Immune deficient VDPV type 3 with 12 nucleotide changes was isolated from a child with common variable immune deficiency (CVID). Follow up samples collected at 2 months, 4 months, and 7 months, yielded iVDPV with 18, 19 and 23 nucleotide changes. It was interesting to note how the virus changed acquiring more mutations during the period of 7 months. This child was considered a “Prolonged Excreter of iVDPV” according to WHO definition as she continued excretion for more than 6 months. Fortunately, the sample collected 9 months later did not yield an isolate [17] showing that she had stopped the excretion. She remained “Negative” up to 2018 in her follow up samples (Personal communication). Summary of the seven-country study series showed that of the 562 persons with PIDD, 17 persons including 5 from Sri Lanka excreted the poliovirus. However, two iVDPV detected in this study series came from Sri Lanka [18]. None of the persons in this series had paralysis including the ones who excreted PV and VDPV and thus not captured by the AFP surveillance. Persons with PIDD who excrete for longer period, has the potential to introduce the virus during polio free situations. Recognizing the importance of this risk, WHO recommended to develop national VDPV surveillance as supplementary to well established AFP surveillance [17, 18, 20]. Sri Lanka initiated the VDPV surveillance as a supplementary surveillance to AFP surveillance in 2012. Patients with PIDD are regularly monitored for PV excretion through this surveillance (Personal communication).

3.6 IPV on gut mucosal immunity

Studies have shown the boosting of humoral immune response following IPV administration. However, there was limited data available on the effect of IPV on mucosal immunity in children immunized with OPV. This study was conducted to find the effect of IPV administration on children who has previously received multiple doses of OPV. Group 1 was given single full dose of IPV. Group 2 was given single fractional dose of IPV. Group 3 was the control which did not receive IPV. Challenge dose of OPV given to all groups one month later [2]). Blood sample was taken before giving the IPV and before giving the challenge dose of OPV to detect the antibody prevalence. Stools samples were collected to detect the excretion of PV as an indicator of mucosal immunity.

Study findings showed that both full dose IPV and fractional dose IPV increased antibody prevalence to almost 100% for all 3 serotypes. Poliovirus excretion following the challenge dose of OPV was 16% in Group 1 who received full dose of IPV. 9% in Group 2 who received fractional dose of IPV, 76% in Group who did not receive IPV. When PV excretion in IPV group and fIPV group was compared, there was no statistically significant difference *(P=0.1)*. However, when the PV excretion of IPV or fIPV group was compared with "No IPV" group, there was statistically significant difference *(P=0.001)*. Relative decrease in virus shedding was 80% for IPV group and 88% for fIPV group when compared to "No IPV" group [21]. This study confirmed the findings that single full dose of IPV or single fractional dose of IPV was equally effective in boosting the serum immunity in OPV primed children. More importantly the finding showed that single full dose of IPV or single fractional dose of IPV was equally effective in boosting gut mucosal immunity as shown by the decrease in virus excretion following OPV challenge dose [21].

Currently, IPV is restricted to use in routine immunization because of the global shortage. Findings of our study that fractional dose of IPV is equally effective boosting the gut mucosal immunity in previously OPV immunized population provide the support for use of fractional dose in outbreak situations in current context of IPV shortage.

4. Planning forward

The Polio Endgame Strategy 2019-2023 of the GPEI has identified key research areas of interest [22]. I will briefly discuss two areas that are relevant to my presentation. Development of a safer OPV that can be used in the supplementary immunization activities is one area of interest. Novel live attenuated OPV type 2 (nOPV2) which is more stable than the current vaccine thus reducing the risk of development of VDPV has undergone a preclinical evaluation [23].

Use of antivirals are anticipated in 3 instances namely for patients with PIDD who are excreting the PV, for management following accidental exposure and to use with IPV during CVDPV outbreaks in post eradication era. Multiple candidates have been screened without a promising one [23].

Ladies and Gentlemen, I like to conclude my presentation with the goal of the Global Polio Eradication Initiative: To complete the eradication and containment of all wild, vaccine-related and Sabin polioviruses, such that no child ever again suffers paralytic poliomyelitis.

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CHEMOPROPHYLAXIS FOR MALARIA: A SRI LANKAN PERSPECTIVE

Weerasekera CJ [1] , Fernando SD[2]

[1]Department of Parasitology, Faculty of Medical Sciences, University of Sri Jayewardenepura,
[2]Department of Parasitology, Faculty of Medicine, University of Colombo

Background

Malaria, continues to ravage the world, causing disease in 228 million people in 2018, and resulting in more than 400,000 deaths [1]. Ten Sub-Saharan nations and India are identified as greatly burdened by the disease by the World Health Organization (WHO) [1]. Sri Lanka eliminated the disease in 2016. Yet, with 378 imported malaria cases reported between 2013-2019 and the presence of the primary vector *Anopheles culicifacies,* the country faces a threat of reintroduction of the disease.

Unlike in the era prior to elimination when indigenous transmission was ongoing, today, both Sri Lankan travelers and foreigners form a major source of imported malaria. Migrant workers originating from malaria endemic Asian countries and working on development projects throughout the country, form a major reservoir of infection. An Indian national, the probable index case in the first introduced case of malaria reported from Sri Lanka after elimination, was a migrant worker in the Moneragala district [2]. Asylum seekers [3,4], Sri Lankan pilgrims returning from travel to India and other Asian nations [3], fishermen [4], gem miners [5] and military personnel travelling to the African continent [6] are also major sources of imported malaria. The threat of malaria resurgence increased after *Anopheles stephensi* was reported from the Northern Province [7]. Unlike *A.culicifacies*, a rural vector, *A.stephensi* is an urban vector currently causing disease transmission in South India [8]. A container breeder similar to the *Aedes* mosquito, it has now been reported from Kalmunai in the Eastern Province [7]. With an influx of daily flights from Chennai to Palali International Airport, the threat of reintroduction of malaria in to Sri Lanka remains high.

Malaria prevention in travelers is dependent on increased awareness, personal protection against mosquito bites and chemoprophylaxis [9]. Chemoprophylaxis is recommended by WHO for those travelling to the endemic nations with indigenous transmission. The Anti Malaria Campaign (AMC) of Sri Lanka recommends chemoprophylaxis to Sri Lankan travelers as per WHO guidelines and issues drugs free of charge. This review aims to highlight the importance of chemoprophylaxis in the Sri Lankan context, AMC-recommendations of chemoprophylactic drugs for Sri Lankan travelers, factors affecting adherence to chemoprophylaxis and the role of mefloquine in long-term chemoprophylaxis.

Recommendations of the Anti Malaria Campaign

The AMC of Sri Lanka currently recommends mefloquine and doxycycline for travelers to countries with risk of *Plasmodium falciparum* malaria or chloroquine-resistant *P.vivax* malaria. List of such countries includes all malarious countries except, India, Nepal and Haiti with *P.vivax* malaria transmission [9]; chloroquine is prescribed for chemoprophylaxis for travelers to these named countries. Chemoprophylaxis should commence one week prior to departure and continue for up to four weeks upon return.

Chloroquine

Chloroquine, prophylaxis is administered as a weekly dose [9], and can be safely given to pregnant and lactating women as well as to children for short term use. It is well absorbed by mouth and has an enormous volume of distribution [10]. Although generally well tolerated, it may induce gastrointestinal disturbances and pruritus affecting the palms, soles and scalp. An increased risk of retinopathy is associated with long-term usage [10].

Mefloquine

Mefloquine, recommended as a weekly dose is safe in pregnancy and lactation, but it is not recommended for children under 3 months of age or those with body weight less than 5 kg [9]. It is moderately well absorbed, extensively distributed and slowly eliminated from the liver. Gastrointestinal, sleep disturbances and severe neuropsychiatric reactions are the main adverse effects when given for prophylaxis [10]. Several contraindications

include active psychiatric illness, epilepsy, and history of cerebral malaria [10].

Doxycycline

Doxycyline is given as a daily dose [9]. It is contraindicated in liver dysfunction, pregnancy, lactation and in children under 8 years [9]. It should be taken with plenty of water, well before going to sleep to avoid gastric or oesophageal irritation [9].

Chemoprophylaxis to India

The percentage of travelers to India and other Asian countries who obtain chemoprophylaxis from the AMC prior to departure is reported to be extremely low [11]. Even though more than 200,000 Sri Lankans travel to India each year [6], and approximately 2/5 of imported malaria cases between 2013 to 2017 have originated from India [6], the risk of a Sri Lankan acquiring malaria while there is low [11]. If chloroquine is issued for all travellers to India (cost per tablet 2.00 LKR), it would consume 0.65% of the annual budget of the AMC. The alternative treatment mefloquine will cost 250 LKR per tablet, which is a greater expense to the AMC. Although chloroquine resistant *Plasmodium falciparum* is found in India, the risk of infection is low [9]. Thereby it has been suggested that while chemoprophylaxis is important, it should not be considered as a major strategy to prevent reintroduction of the disease through travellers to India [11].

Chemoprophylaxis to Africa

Sri Lanka Tri-forces and Police Department are continuously sending troops on United Nations Peace Keeping missions to African destinations. Supplying sufficient anti-malarial medicines for these groups (approximately 200-400 individuals each contingent) has become a challenge to the AMC. Mefloquine is issued to security forces personnel (other than pilots of the Sri Lanka Air Force who are issued doxycyline) deployed in African nations. Medicines are issued for 6 months by the AMC with instructions to purchase the balance required for the duration of stay which is usually one year. However, in some instances, troops spend over one year in the African nations.

There is currently a dilemma about the duration for which mefloquine can be taken safely without significant adverse effects. A Cochrane review revealed that the relative risk of insomnia, depressed mood, anxiety and abnormal thoughts and perceptions were higher among recipients of long term prophylaxis (6 months or longer) compared to short term [12]. It also revealed that participants who took mefloquine were more likely to discontinue chemoprophylaxis compared to those who obtained atovaquone-proguanil (AP).

A study targeting a group of Sri Lankan security forces personnel who returned to Sri Lanka in 2017 following a period of deployment in South Sudan for 12 months revealed that the compliance for mefloquine was 100% [6]. Further, a majority (121/144, 84%) reported no adverse effects. Of the adverse effects reported, two people complained of neuropsychiatric symptoms (and thus the chemoprophylactic drug was replaced with doxycycline), while the others mainly complained of abnormal sweating and gastrointestinal disturbances; none of these were serious enough to discontinue mefloquine. In contrast, only 3/5 of a group of Air Force personnel, returning to Sri Lanka in late 2015, after 14 months of deployment in the Central African Republic, had adhered to chemoprophylaxis [13]. The better adherence to chemoprophylaxis in the recent past may be attributed to awareness programmes conducted by the AMC staff, better educational levels of the travellers and institutional supervision using a directly observed treatment strategy.

Long term chemoprophylaxis prescribed differs from country to country. The French military uses doxycycline as first-line chemoprophylaxis for both short and long term deployments since 2002 [14]. The USA followed suit in 2011 by recommending atovaquone-proguanil (AP) and the US drug regulatory authorities issued a 'boxed' warning that mefloquine should be discontinued if psychiatric and neurological symptoms occur during prophylactic use [15]. In 2016, the UK parliament instructed its Ministry of Defence to prescribe mefloquine only as the last resort [16]. Mefloquine is known to be associated with an increased risk of mental disorders, suicide and violence towards others including homicide [15]. Taking the above into consideration, the AMC is also in the process of recommending AP for long term prophylaxis in military personnel.

Factors affecting adherence to chemoprophylaxis

Older travellers, travellers to African destinations or individuals travelling with an organized tour by a reputed organization are more likely to adhere to chemoprophylaxis than young travellers, business travellers, backpackers and those travelling to India [17]. As education, pre-travel advice and increased awareness have shown to increase adherence to prophylaxis [17], AMC staff at its Headquarters and Regional Malaria Officers are attempting to understand the level of the travellers' knowledge of malaria and tailor the advice based on the endemicity of the destination.

Currently, the AMC works in collaboration with other departments such as the Ports Health Authority and the military to promote chemoprophylaxis [6,18]. It is compulsory for those travelling to Africa to take the Yellow Fever vaccine from the Ports Health Authority or Medical Research Institute. From here, individuals are directed to the AMC to obtain prophylaxis for malaria. Provision of printed material and visual display of messages on malaria prophylaxis at the Bandaranaike International Airport (BIA) are other measures implemented to minimise the risk of reintroduction of malaria to the country by international travel. Further, an office of the AMC is in-situ at the BIA so as to enable travellers to obtain chemo-prophylaxis free of charge [18].

Consequences of non-adherence: case histories

Malaria has been reported amongst individuals who have returned to Sri Lanka with a history of non-adherence to the recommended chemoprophylaxis regime. In 2016, an army officer returning from military training in Malaysia, presented with fever for 10 days and was treated as viral flu, prior to malaria was suspected. He had not taken chemoprophylaxis and was subsequently diagnosed with *P.knowlesi*, a zoonotic infection, prevalent in South East Asia [18]. It has the ability to cause severe malaria in humans, and is difficult to distinguish morphologically from *P.malariae* [18]. Fortunately, the natural macaque hosts *Macaca fascicularis* and *Macaca nemestrina* are not found in Sri Lanka, thus preventing the onward transmission and resultant high morbidity and mortality.

A Sri Lankan gem miner, a frequent visitor to Madagascar since 2005, initially obtained chemoprophylaxis, but subsequently defaulted [5]. He has had several episodes of malaria, thus in 2018, he self referred himself to AMC four days after return to Sri Lanka and was tested negative. Eleven days later, he was diagnosed in a private sector laboratory, when he requested for screening upon developing fever. He was treated for severe *P.vivax* malaria from which he recovered completely.

In 2015, a Police Officer, upon return from India, developed fever. He was diagnosed with vivax malaria [19]. However, molecular testing gave a diagnosis of *P.ovale* infection. A travel history to Liberia prior to India was elicited; the officer had been on chemoprophylaxis (with mefloquine) in Liberia, but not in India. Since Liberia is endemic for *P.ovale* in contrast to India and since mefloquine prophylaxis cannot prevent relapses due to *P.ovale*, it was concluded that this patient got infected in Liberia.

In 2015, an Air Force personnel succumbed to severe malaria whilst on a Peacekeeping mission in the Central African Republic [13]. He had not adhered to chemoprophylaxis. Among the group of 120, 37 individuals reported 44 episodes of malaria; all of them admitted poor adherence to prophylaxis, the main reason being forgetfulness during long-term field deployment. The relationship between non-adherence and acquisition of malaria was statistically significant [13].

Current gaps and way forward

A formal referral mechanism does not exist for those travelling to India and other malarious nations in Asia to obtain chemoprophylaxis from the AMC. A study conducted at the AMC revealed that out of the civilians who obtained chemoprophylaxis, only 5% to Asian destinations [6]. After its elimination, malaria has now become a forgotten disease [11,18]. Increasing awareness among primary level health-care providers and first contact medical officers in both the public and private sectors regarding chemoprophylaxis will ensure referrals prior to departure to endemic countries.

Sri Lanka, which nearly eliminated malaria in the 1960s, faced a resurgence and it took nearly half a century to attain the elimination status [20]. The cost for prevention of reintroduction is around 0.6 USD per citizen per annum, 12 million USD per year. Should malaria resurface, it is estimated that over 169 million USD over five years would be needed for treatment and control [20]. Thus, it is a responsibility of all citizens of this country to maintain the status of malaria elimination in Sri Lanka.

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THE NATIONAL EXTERNAL QUALITY ASSESSMENT SCHEME IN CLINICAL MICROBIOLOGY, MEDICAL RESEARCH INSTITUTE: REVIEW OF THE NATIONAL PROGRAMME IN SRI LANKA

Kulatunga KAKC[1], Karunanayake L[1], Rangama BNLD[1], Rajapakshe RRN[1], de Silva PGSM [2]

[1]Department of Bacteriology, Medical Research Institute, Colombo, [2]Medical Research Institute, Colombo

Abstract

Background: National EQA programmes are organized to improve the performance of testing sites to ensure benefits to patient care. In Sri Lanka at present, the Department of Bacteriology at the Medical Research Institute conducts the National EQA Scheme (NEQAS) in clinical microbiology. Our aim is to review the existing national programme in clinical microbiology to support improvements in the microbiology services in Sri Lanka.

Material and methods: Data was obtained from the NEQAS laboratory network between 2001-2018. Two performance indicators were analysed: Bacterial culture identification and antimicrobial susceptibility testing (AST).

Results: A majority of NEQAS participating microbiology laboratories belonged to public sector hospitals. Active participation among enrolled laboratories has improved since 2011 and was at its highest (90%) in 2018. Correct identification of bacterial cultures and AST have significantly improved over the years. A remarkable improvement in the category 'Very Good' AST results was seen in public sector microbiology laboratories in 2018 compared to 2014-2017 (p=0.001).

Conclusion: The reorganizing of the NEQAS programme in clinical microbiology has made a significant positive impact on microbiology services in Sri Lanka. EQA package delivery to hospitals and initiation of a web-based EQA program should be implemented, to further improve the national programme.

Key words: EQA, Sri Lanka, clinical microbiology, Quality Assurance, national EQA

Background

External Quality Assessment (EQA) is a systematic assessment conducted by an external organization, where proficiency test items are sent to participating laboratories and their responses are evaluated. It is a tool that measures the testing process (the analytical phase), including the quality of results, generated by the laboratory [1,2]. Participation in an EQA programme provides evidence of laboratory competency for users, accrediting bodies and regulatory bodies, improves the quality of the service and facilitates the comparability of results among different laboratories [3].

The Medical Research Institute (MRI) is the main reference laboratory in the country and is under the purview of the Ministry of Health (MOH), Sri Lanka. The Quality Control Laboratory in the Department of Bacteriology is the sole organizer of the National External Quality Assessment Scheme (NEQAS) for clinical microbiology in Sri Lanka. This programme was initiated in 1997 by two eminent bacteriologists of the MRI, late Dr. R.S.B. Wickremesinghe and Dr. Maya Atapattu, with voluntary participation of 14 public sector microbiology laboratories. Between its inception and today, the programme has continued uninterrupted for 23 years.

Briefly, NEQAS surveys are conducted quarterly. Laboratory-prepared, pre-tested lyophilized cultures and /or smears for Gram stain are sent as identical specimens to all participants with printed instructions for the survey and a results sheet. It is expected that proficiency testing (PT) specimens are processed by the same method used for the processing of routine clinical specimens received in their laboratories. Thereafter, the completed results sheet is sent to the organizer laboratory before the given deadline. Individual laboratory data are kept confidential and only known by the participating laboratory and the NEQAS organizer [4].

The performance indicators tested are the identification of bacterial cultures, their antimicrobial susceptibility and interpretation of the Gram stain. The EQA organizer evaluates the results and assigns a score which can be viewed by the relevant laboratory. Specific feedback is also provided which can only be viewed between the EQA organizer and the relevant laboratory. At the end of each survey a preliminary report with the proficiency test results of the samples is shared with participating laboratories.

The annual evaluation report is sent to participating laboratories which includes the mean score in each survey of participating laboratory, the mean annual score of all participants, their annual rank in previous and current years, to enable comparison of results.

The objectives of the present study are to review the NEQAS programme in clinical microbiology and evaluate the performance of participating laboratories in Sri Lanka in order to improve its use in the future.

Material and method

Data were obtained from the NEQAS laboratory network for the period between 2001-2018. Two performance indicators were analysed: culture identification and antimicrobial susceptibility testing.

The definitions used in this study are:

1. Correct identification: When bacterial cultures are correctly identified with 50% or more score per survey by the laboratory, it is labelled as 'correctly identified'. Cultures not identified or partially identified with a score of < 50% per survey were not taken for the analysis.

2. Antimicrobial Susceptibility Test (AST) results: Only the correct results were taken for the analysis. The results were calculated by 3 categories: percentage of correct results more than 90% (Very Good), between 50-90% (Good) and less than 50% (Poor).

Statistical analyses were performed using MSEXCEL software version 2016 and IBM® SPSS® software version 20. Independent samples t-test was used for the analysis and the significance was set at $p<0.05$.

Data for correct bacterial identification and AST was obtained by calculating the mean per survey and dividing it by the number of active participations. Percentage of annual active participation was calculated as a mean from number participated in each survey divided by the total number of enrolled laboratories.

Ethics approval for the study was obtained from the Ethics Review Committee of Medical Research Institute.

Results

We extracted data from 5400 reports received from participating laboratories in the past 18 years (2001-2018). Majority (71%) of microbiology laboratories participating in the NEQAS are from the MOH. In addition, clinical microbiology laboratories in the private sector and the university microbiology laboratories are enrolled in the National EQA programme.

1. Active participation in the National EQA program by microbiology laboratories

We analysed the active participation of the microbiology laboratories in NEQAS surveys.

The bar chart illustrates the annual percentage of laboratories actively participating in NEQAS surveys from 2001-2018.

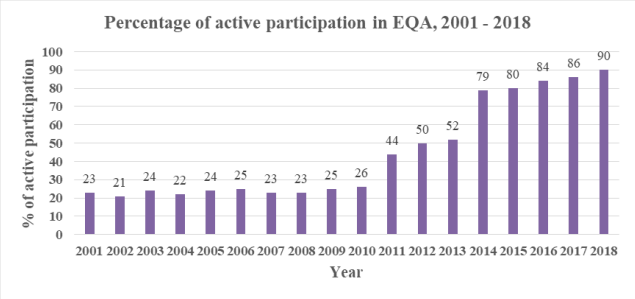

Figure 1. Percentage of annual active participation by laboratories between 2001 to 2018.

Participation from 2001-2010 has been remarkably low (mean 23.6%). A steady increase is seen from 2011 with a sharp rise in 2014. Significant improvement in active participation is seen over the years ($p=0.000$).

2. Identification of bacterial cultures by participating microbiology laboratories

This bar chart describes the percentage of correct bacterial identification results obtained by participating laboratories annually.

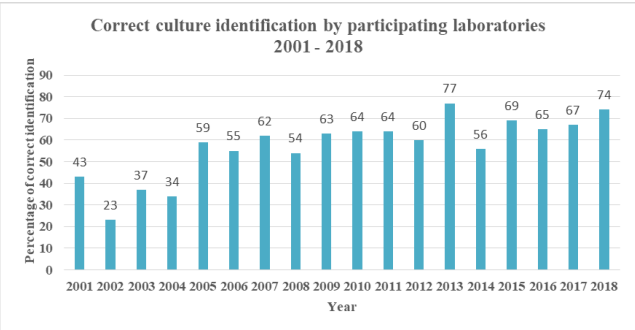

Figure 2. Correct identification of bacterial cultures by participating laboratories.

The bacterial identification results have improved since 2005 and stabilized over 60% with slight fluctuations. After 2009, reporting of correct identification results have significantly ($p=0.009$) improved (mean 65%) compared to 2001- 2009 (mean 45%).

3. Antimicrobial susceptibility testing by participating laboratories

The stacked bar chart compares three different categories of correct AST results by year. Each segment from top to bottom represents correct AST results 'over 90%', between '50-90%' and 'less than 50%' for the participating laboratories.

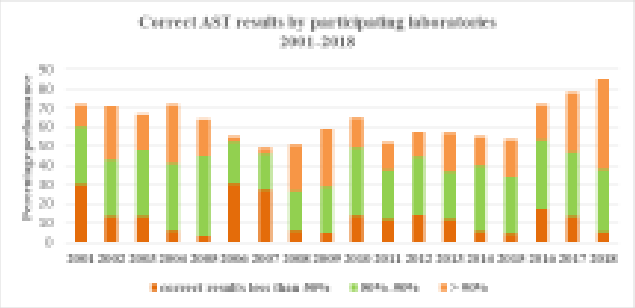

Figure 3. Performance of antimicrobial susceptibility test by participating laboratories.

There is a significant reduction in correct AST results '<50%' after 2009 (p=0.002) and significant differences are not seen for other 2 categories: 50-90% (p=0.085), >90% (p=0.854). In 2018, 'Very Good result >90%' increased significantly (p<0.05).

4. Performance of public sector microbiology laboratories under MOH

4.1 Performance of public sector microbiology laboratories under MoH on 'Correct Identification and AST Results' for 5-year period (2014-2018).

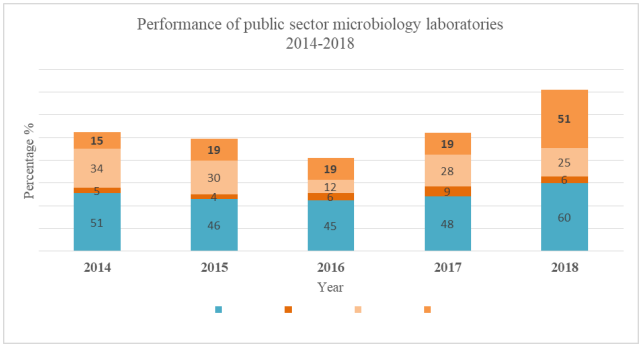

Figure 4. Performance of public sector microbiology laboratories on correct identification and AST results: <50%, 50-90% and >90% between 2014-2018.

The stacked bar chart above shows the performance of the public sector microbiology laboratories with correct culture identification and AST results for 5 years (2014-2018).

Segments from bottom to top represent percentages for correct culture identification and AST result categorized as <50% (Poor), 50-90% (Good) and >90% (Very Good).

A significant improvement was seen in 2018, with correct identification of bacterial cultures (p=0.024) and correct AST >90% (p=0.001) compared to other years.

4.2 The table below shows NEQAS participation by different levels of public sector microbiology laboratories, provision of in-hospital and off-site microbiology services and distribution of microbiologists in 2018.

Table I. Microbiology laboratories in the Ministry of Health participating in NEQAS in 2018

Category	Number of hospitals	Number of microbiology labs *n*	Off-site microbiology services *n*	EQA Participation *n*	Microbiologist present *n*
BH-A[1]	27	12 (44.4%)	14 (51.8%)	08 (66.6%)	01
DGH[2]	19	16 (84.2%)	03 (15.7%)	16 (100%)	15
PGH[3]	03	03 (100%)	00 (0.0%)	03 (100%)	03
TH*[4]	20	19 (95%)	01 (95%)	19 (100%)	14
Institutions[5]	03	03	00	03 (100%)	04
Total	73	52	23	49 (94.2%)	37

Source: Medical Research Institute and Ministry of Health, Sri Lanka

*Dental Hospital Peradeniya not included. [1]Base hospital -A, [2]District General Hospital, [3]Provincial General Hospital, [4]Teaching Hospitals, [5]public institutions with clinical microbiology services

In BH-A, 12 had in-hospital microbiology laboratories and only eight (66.6%) laboratories participated in NEQAS. All THs, PGHs and DGHs with in-hospital laboratories and institutions with microbiology services were enrolled in NEQAS programme. NEQAS participation by public sector hospitals in MOH was 94.2%.

Discussion

Microbiology laboratory testing and services have an important role in patient care and outcomes. Evaluation of the clinical impact of laboratory errors is not always straightforward. However, a majority of laboratory errors is preventable and amenable to laboratory-based interventions such as increased education or close supervision [5].

Yuan et al. (2005) showed that in microbiology laboratories, most errors occur in the analytical phase in contrast to other specialties such as molecular genetics and chemistry. This reflects the uniqueness of work in the microbiology laboratory, with its heavier reliance on skilled manual work and subjective interpretation [5].

Ninety four percent of the microbiology laboratories under the MoH participates in NEQAS (Table 1). Participating in EQA provides confidence that the service meets user expectations and denotes a system for continuous improvement, reliability and efficiency of the services in the laboratory. Furthermore, it helps administrators and regulatory bodies gain insight into the performance of the laboratories across the country.

We retrospectively analysed NEQAS data to review the national programme and the performance of participating laboratories between 2001-2018.

In 2012, the National EQA programme was reorganized. All participating laboratories were categorized according to the availability of resources in hospitals using General Circular 1-13 / 2005 issued by the MoH. THs, PGHs, public sector institutions, universities and private sector hospitals were included in category 1 and BHs and DGHs in category 2. A new marking scheme was developed and negative points were given for key determinants in the reliability of microbiology reports such as major errors in AST results and incorrect identification of the organism. In addition, points were added for on-time submission and completeness of tables.

Following a stationary phase for over a decade, active participation in NEQAS by the clinical microbiology laboratories significantly improved since 2011 with a steep rise in 2014 (Figure 1). Enrolment in the Northern and Eastern Province hospitals after the end of Sri Lanka's civil war in 2009, and the introduction of the year-end participant certificate in 2014 may also have contributed to this improvement.

In 2013, a 5-year programme was introduced for the three National EQA programs at MRI: bacteriology, hematology and biochemistry, through the 2nd Health Sector Development Project, MOH. The organizer laboratory was strengthened by provision of essential equipment and Certified Reference Material (CRM) for quality control of AST and media. To ensure homogeneity and stability of PT material, lyophilization was introduced in 2014. The laboratory staff underwent short-term fellowships and local training on proficiency testing based on ISO/IEC 17043: 2010 conducted by the Sri Lanka Accreditation Board.

Laboratory visits were arranged for the participating laboratories to identify on-site deficiencies, especially in internal quality control measures, and annual workshops were organized to improve their bench skills and promote new techniques to detect antimicrobial resistance. A 'Handbook on National External Quality Assessment Scheme in Bacteriology' was published with the support of World Health Organization, Sri Lanka. It described the objectives of the organizer laboratory and future changes to the program.

Overall, a significant improvement was shown by the participating laboratories in the correct identification of bacterial cultures with slight fluctuations over the years (Figure 2). In contrast, a steady improvement in AST results was shown by the laboratories after 2016 (Figure 3). Introduction of automated and rapid identification and susceptibility systems, availability of good quality antibiotic disks and media may also have contributed to the immense improvement in microbiology services. Additionally, communication through NEQAS laboratory network was enhanced by feedback comments and distribution of handouts as an educational stimulus.

Facilitation of information exchange is a vital part in a National EQA program [2]. One such event was when we identified a batch of quality-failed optochin disks while evaluating responses from participating laboratories. A majority had wrongly identified pneumococcus as viridans streptococci in one survey. The information was immediately shared with the laboratory network and relevant officials for corrective action.

Moreover, in the public sector laboratories, the significant improvement shown for 'Very Good' AST category (51%,

p=0.005) in 2018 is a result of many factors including the increase in the number of microbiologists in public sector laboratories, availability of automated identification and susceptibility systems and the reactivation of the Antimicrobial Resistance (AMR) surveillance program which made EQA participation compulsory for those laboratories identified as AMR surveillance sites (Figure 4, Table 1).

Conclusion

The reorganizing of the NEQAS programme in clinical microbiology has made a significant positive impact on microbiology services in Sri Lanka. Further improvement in identification of bacterial isolates and refining of antimicrobial susceptibility testing is warranted. EQA package delivery to hospitals and initiation of a web-based EQA program should be implemented, to further improve the national EQA programme.

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AN APPROACH TO THE MANAGEMENT OF BACTERIAL ENDOPHTHALMITIS

Ranasinghe RATK[1], Vidanagama DS[2]

[1]Department of Microbiology, National Eye Hospital,

[2]Department of Microbiology, National Hospital of Sri Lanka

Endophthalmitis is an inflammation of the eye involving the vitreous with or without aqueous humour. It is usually caused by infection due to bacterial or fungal pathogens and can develop very rapidly or develop slowly and persist for long periods. Prompt attention of an ophthalmologist is essential to make an appropriate diagnosis and initiate treatment to save the vision.

Bacterial endophthalmitis

Most cases of endophthalmitis have bacterial aetiology which present acutely as a medical emergency. Delayed or insufficient treatment may cause irreversible damage to the vision. The infection is termed endogenous when pathogens are introduced via the blood stream [1].

Exogenous infections are the commonest and occur as a complication of cataract and other ophthalmic surgery, intravitreal injection, penetrating ocular trauma or as an extension of corneal infection [2,3].

Acute post-cataract endophthalmitis complicates cataract surgery in about 0.1%-0.2% of cases [1,4]. Patient usually presents one week post operatively with decreased vision, red eye and eye pain. Examination reveals slightly swollen eyelids, conjunctival injection and a hypopyon (Figure 1). The microflora on patient's own eyelid or conjunctiva that contaminates the aqueous at the time of surgery is usually responsible. Predominant pathogens are coagulase negative staphylococci which account for 70% of culture-positive cases [1,2].

Chronic post-cataract endophthalmitis, a persistent low-grade inflammation in the anterior chamber, is usually caused by *Propionibacterium acnes*. A few cases have been caused by coagulase-negative staphylococci and diphtheroids [1,4].

Up to 95% of the post-injection endophthalmitis is caused by Gram positive cocci, mainly coagulase-negative staphylococci causing around 60% of cases and viridians streptococci causing around 25% [2,4].

Bleb-related endophthalmitis is related to a surgically created scleral defect, covered only with conjunctiva. When the eye becomes colonized by virulent bacteria acute endophthalmitis may occur at any time. *Streptococcus pneumoniae* causes 50% of cases. *Haemophilus influenzae*, *Moraxella catarrhalis*, *S. aureus* and coagulase-negative staphylococci are among the other pathogens [1,4] (Figure 2).

Post-traumatic endophthalmitis occurs in 3% to 10% of patients who sustain penetrating trauma to the eye especially by metal objects, retained intraocular foreign bodies and delay in primary repair. Main pathogens are *Bacillus* species and coagulase-negative staphylococci [1,4,5].

Endogenous endophthalmitis is the result of seeding of the organism into the eye from an infection elsewhere. It has the risk of bilateral infection in 15-25% of cases. Fungal pathogens are responsible for half of the endogenous cases. Approximately 32-37% of all endogenous endophthalmitis cases are caused by Gram negative organisms and they typically have poor visual outcomes because they are difficult to treat [2,5,6].

Diagnosis

The diagnosis of endophthalmitis is made based on clinical features and risk factors followed by attempts to confirm the pathogen with cultures of the vitreous or the aqueous. Systemic symptoms are not prominent except in endogenous endophthalmitis, when there is another focus of infection with haematogenous spread or panophthalmitis, in which infection has spread from the globe to the orbit. Panophthalmitis is caused by virulent pathogens and characterized by marked local signs and symptoms suggesting orbital cellulitis. Blood cultures may become positive only in these two occasions [1,2]. Only 40% to 50% of Gram stains of vitreous samples from bacterial endophthalmitis cases are positive for organisms. In an inflamed eye, melanin, released from the iris or retinal pigment epithelium, creates pigmented granules in the Gram stains of aqueous or vitreous samples. They appear football shaped or spherical and may be mistaken for Gram positive cocci. Pigment granules are highly refractile and appear copper coloured with focus modulation, whereas bacteria do not [2,4,7].

Molecular diagnostics (multiplex polymerase chain reaction) on intraocular fluids has the advantage of rapid identification and high sensitivity in culture-negative cases. Currently molecular detection tests for ocular samples are available mainly in research laboratories [2,4].

Antibiotic management

In any type of endophthalmitis, bacteria are introduced into the intraocular environment which is an 'immune-privileged site'. It is devoid of vascularization and inflammatory mediators and cells that are essential to fight infections. With the absent or delayed initial immune response, there will be an optimal growth medium for organisms that reach the area. The function and integrity of ocular tissues can be damaged within the first 24 hours, due to the multiplication of organisms and release of toxins and enzymes. It could happen rapidly even before proper diagnosis is made or antimicrobials are prescribed [5,6,8]. In the process of infection, during the early exponential growth of bacteria, the efficacy of antimicrobial action is greater, in contrast to the late phase with stationary growth and high microbial density. Therefore it is essential to select the correct antibiotic considering the pathogen and

Figure 1. Acute-onset post-operative endophthalmitis (sutured corneal wound and hypopyon) From Ref 11.

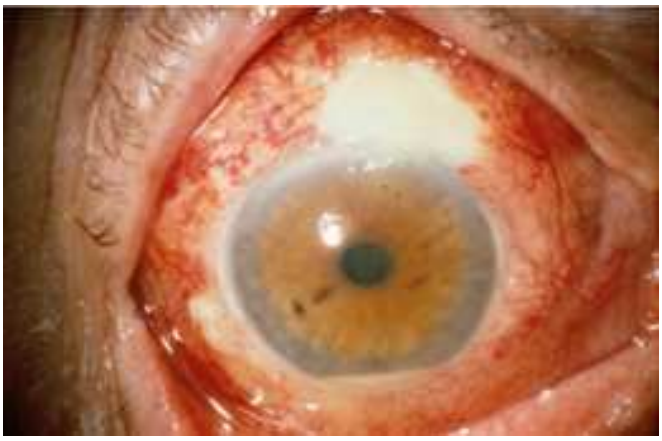

Figure 2. Bleb-related endophthalmitis (purulent filtering bleb and hypopyon) From Ref 11.

probable antimicrobial sensitivity pattern and to make the effective antibiotic concentration available at the bio-phase [5,6].

Since most cases of exogenous endophthalmitis are directly caused by the habitual flora that normally exists in the conjunctiva, eyelid, or eyelashes, the organisms have low levels of antibiotic resistance. With the irrational use of antibiotics there is a trend of normal flora of healthy people becoming resistant to antibiotics [6].

Approximately 20% of *S. aureus* and more than 50% of coagulase-negative staphylococci could be resistant to beta-lactams in some European countries, especially in the hospital environment. Resistance to glycopeptides (vancomycin and teicoplanin), linezolid, daptomycin and tigecycline exists at low levels among staphylococcus species. The resistance rate of *S. pneumoniae* for penicillin and 3rd generation cephalosporins is growing. More worrying is the resistance pattern of enterobacteria such as *E. coli* or *Klebsiella* spp, showing high level of resistance to cephalosporins and quinolones. Even though *B. cereus* shows resistance to beta-lactams, most strains are sensitive to carbapenems, vancomycin and clindamycin [6].

The small diffusion surface and poor vascularization of the eye globe results in a very limited access of antibiotics, especially antibiotics with a high protein binding rate or low liposolubility. As in the blood-brain barrier, ocular inflammation increases the drug permeability across the blood-retinal barrier [6,9,10].

Antibiotics are eliminated from the vitreous humour by two main mechanisms.

I. Some antibiotics are eliminated by passive diffusion across the vitreous to the anterior chamber: Anterior route

II. Some antibiotics are eliminated by active transport, via capillaries and the retinal pigment epithelium: Posterior route: [6,7]

In an inflamed eye anterior route (passive transport) is facilitated while posterior route (active transport) is blocked. Vancomycin, aminoglycosides, macrolides and rifampicin are usually removed through the anterior route. Beta-lactams, clindamycin and fluoroquinolones are removed through the posterior route. Therefore pharma-cokinetic parameters are altered in the presence of inflammation and the half-life elimination of a drug may not be a critical point [6,7].

There is only infrequent data on the intravitreal concen-trations of systemic antibiotics. When targeted therapy is intended, antibiotics like vancomycin, cefazolin, ceftriaxone, ceftazidime, imipenem, daptomycin and trimethoprim/sulfamethoxazole display good activity. Rifampicin may be considered for combination therapy in complicated staphylococcal infections. There is some evidence supporting the use of systemic moxifioxacin for most infections caused by both Gram positive and negative organisms due to its excellent intraocular penetration and broad coverage but literature mentions the need for more convincing data [10].

Systemic antibiotics alone are not effective in treating bacterial endophthalmitis. Intravitreal injection ensures direct access to the intraocular space, by-passing the blood-retinal barrier and generating a high ocular concentration of drugs for an extended duration. For effective treatment of an infection by resistant organisms this becomes a critical factor [1,2,9].

For endogenous endophthalmitis treatment includes systemic therapy for the underlying infection and intravitreal antibiotics.

The most important component of treatment is direct injection of antibiotics into the vitreous by the ophthal-mologist. The recommended empirical intravitreal antibiotics are vancomycin, plus either ceftazidime or amikacin. Ceftazidime is preferred over amikacin as there are reports of macular infarction following injected aminoglycosides [1,3,5].

Sri Lankan recommendation

Sri Lankan national guidelines recommend intra-vitreal vancomycin and ceftazidime or amikacin as the primary therapy for acute bacterial endophthalmitis. For penetrating ocular trauma, ciprofloxacin or intravenous ceftriaxone is recommended. This is the routine practice while oral moxifloxacin is used when the clinical response is poor.

Surgical management

Vitrectomy is often used to eradicate dead bacteria, damaged tissue, the inflammatory exudate and other toxic substances from the interior of the eye. In severe cases of endophthalmitis clearing of the posterior segment aids faster recovery of vision [1,5].

Conclusion

In spite of advances over the past 100 years, endoph-thalmitis remains an important sight threatening condition. Timely diagnosis, appropriate use of anti-microbial agents

for treatment and surgical intervention may optimize visual outcomes [3,12]. Intravitreal antibiotics are more effective than systemic antibiotics unless there is a distant focus.

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WHOOPING COUGH IN CHILDREN

Karunanayake L[1], Gunawardena KDN[1], Mannapperuma B[2], Somaratne D[1], Jayasinghe A[2]

[1]*Department of Bacteriology, Medical Research Institute, Colombo,* [2]*Colombo North Teaching Hospital, Ragama*

Introduction

Whooping cough is caused by *Bordetella pertussis* which infects only humans. It is a highly communicable bacterial acute respiratory disease transmitted person-to-person by airborne droplets. Whooping cough-like disease can be caused by *B. parapertussis* and *B. holmesii* and other agents such as adenoviruses, respiratory syncytial viruses, *Mycoplasma pneumoniae*, human parainfluenza and influenza viruses [1]. The infection has a worldwide distribution and remains an important cause of infant morbidity and mortality in developing countries [1].

Pertussis is under-diagnosed due to limited laboratory facilities and lack of awareness of disease existence. It is a notifiable disease in Sri Lanka and epidemiological surveillance is carried out only by clinical suspicion [2]. In recent years, <50 clinically-suspected whooping cough cases have been reported [2]. Until recently, laboratory-confirmed cases have not been reported in Sri Lanka due to limited laboratory facilities.

The incubation period of whooping cough ranges from 7-10 days. In non-immune patients, there are 3 clinical phases each lasting 1-3 weeks [3]. Following the incubation period, the catarrhal phase begins, with non-specific symptoms such as rhinorrhea, nasal congestion and sneezing with no or low-grade fever. At this stage, high index of suspicion is required as the clinical features mimic those in other viral infections [3]. This is followed by the specific paroxysmal phase and is characterized by coughing spasms, inspiratory whoop and vomiting. Finally, in the convalescent phase the frequency of cough decreases slowly [3]. Typically, neonates and nonimmune infants may present with nonspecific cough and apnoea as the only symptoms. Delay in clinical recognition is more likely to result in complications [3].

We describe two infants who presented to a tertiary care hospital with 'whooping cough-like' illness.

Case 1

Seven week old male infant presented with nasal congestion, milk regurgitation, paroxysmal cough and cold for 4 days duration. The mother and the siblings had similar symptoms since previous week. On examination, the child was afebrile with an unremarkable physical examination.

Case 2

Four month old male infant presented with an acute episode of paroxysms of cough, breathing difficulty and excessive crying for 1 day. He has had persistent cough and cold for 2 weeks. He received the 2nd dose of pentavalent vaccination 2 days prior. He was afebrile with an unremarkable physical examination.

In both cases, the full blood counts showed peripheral leukocytosis with high absolute lymphocyte counts. Nasopharyngeal swab and throat swab respectively were positive for *B. Pertussis* and negative for *B.parapertussis* DNA by real time polymerase chain reaction (rtPCR). Both responded to erythromycin and the follow-up was uneventful. Close contacts were given prophylaxis / treatment.

Discussion

Pertussis is more dangerous in infants with high morbidity and mortality [4]. Timely and accurate diagnosis can distinguish between other etiological agents and early antibiotic therapy mitigates symptoms which may help prevent transmission. Resurgence of pertussis observed in the 1990's highlighted the importance of standardized, sensitive and specific laboratory diagnosis [5]. Laboratory confirmation is encouraged to monitor disease burden and the impact of immunization in whooping cough [2].

For many decades, the Medical Research Institute (MRI) used the Bordet-Gengou medium with cephalexin as the standard medium for the isolation of *Bordetella* spp with

no positive growth. Thus, in 2018, multiplex rtPCR for *Bordetella* spp. was established at the Microbiology Reference Laboratory, MRI, Sri Lanka.

Between February 2018 and February 2019, we received 33 specimens from inward patients suspected of whooping cough. *B. pertussis* DNA was detected in 15 (45.45%) samples but were negative for *B. parapertussis.* Majority of the infected patients were males (11/15, 73.3%) with a median age of 8 weeks (range 4-60 weeks). Eleven were from hospitals in the Western Province and one each from the Central, Northern, North-Western and Southern Provinces.

Critical steps towards ensuring high sensitivity include accurate specimen collection and rapid specimen transport within 24 hours [5]. Low sensitivity (45%) of PCR in our patients were mainly due to inappropriate sampling and unfavorable transport conditions samples taken from posterior nasopharynx are optimal for culture and molecular testing [5]. Swabs with thin, flexible shafts that can reach the posterior nasopharyngeal area should be used. For best results from culture Dacron or nylon swabs are recommended as cotton swabs can be toxic to the organism thus affecting its viability. Calcium alginate swabs are appropriate only for culture and should not be used for molecular testing because it inhibits PCRs [5]. For PCR, samples should be sent dry (without transport media) and in a cool environment.

Culture is the 'gold standard' but has a low sensitivity. Ideally, specimens should be sent in transport media such as the Regan-Lowe transport medium or the Stainer Scholte broth in room temperature [5]. Culture sensitivity is high in early disease in the catarrhal phase, in infants and unvaccinated children. Culture may be less useful for older and vaccinated children, adolescents and adults [5].

Molecular testing for *Bordetella* has become an established method for the detection and identification of the organism. Development of multiplex rtPCR has largely replaced conventional PCR due to its high sensitivity, quicker results and speciation [5]. Unlike culture, it does not require the presence of viable organisms in the specimen. DNA can be detected even 14 days after the onset of paroxysmal cough, which is 1-2 weeks longer than a positive culture result. Since PCR has good sensitivity, it can be used at any time regardless of the stage of the disease [5].

Despite vaccination pertussis continues to be a problem in the developing and developed world. Adults represent a significant source of infection for infants and transmission has been reported from mothers and adolescents [1,3]. Secondary attack rate of 80-100% among unimmunized household contacts and 20% of well-immunized contacts has been reported [1]. Immunity after disease or vaccination does not confer long lasting protection [2,3]. Hence, adult vaccination for whooping cough has a place in the prevention and control of pertussis in high risk population.

Sigera et al (2016) showed low level of seropositivity among an immunized study population (age 4-24 years) in Sri Lanka [6]. It is known that the immunity is short lived with whole cell vaccine and it wanes off in 10-12 years [1]. In a case-control study, the unadjusted estimated vaccine efficacy for 1-2 whole cell vaccine doses was 83.6% and with 4 doses the efficacy rose to 97.7% [7].

Several developed countries have commenced vaccination of adolescents and pregnant mothers to reduce infant morbidity and mortality due to pertussis [1,4]. Pneumonia Etiology Research for Child Health (PERCH) study data in developing countries has shown the importance of neonatal immunity through 2nd trimester vaccination of mothers to prevent disease of unimmunized infants [4].

In conclusion, pertussis is present as a childhood disease in many provinces in Sri Lanka. Awareness of the disease in clinical practice, early recognition and appropriate treatment is important to reduce childhood morbidity and mortality. A large-scale study is warranted to understand the burden of the disease and to evaluate vaccination strategies.

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CORYNEBACTERIUM DIPHTHERIAE ENDOCARDITIS

Galagedara WD[1], Dassanayaka KMMP[1], Karunanayake L[2], Yapa DWK[1], Perera S[1], Jayasundara MCT[1]

[1]*Lady Ridgeway Hospital for Children, Colombo,* [2]*Medical Research Institute, Colombo*

Introduction

Corynebacterium spp are frequently encountered organisms in microbiology laboratories and are considered as skin contaminants when isolated from clinical specimens including blood cultures. Although infective endocarditis due to *Corynebacterium diphtheriae* has been reported in patients with structural cardiac anomalies, it is uncommon in the structurally and functionally normal heart [1].

Case history

A 7 year old previously well child presented to Lady Ridgeway Hospital with a history of prolonged fever. There were two admissions to the local hospital during the course of fever. During the first admission, systemic examination was normal. The full blood count revealed neutrophil predominance with low platelets. C reactive protein level was high with a value of 205 mg/dl. A diagnosis of dengue fever was made as dengue IgM was positive. Child was treated with intravenous cefotaxime for four days and discharged as CRP reduced to 14.5 mg/dl. Fever recurred and the child got re-admitted to the local hospital, where it was treated as secondary sepsis following dengue fever. Blood culture yielded diphtheroid, which was reported as a probable skin contaminant. The child was treated with intravenous cefotaxime for 4 days and discharged with oral cefixime.

As fever recurred, the child was admitted to Lady Ridgeway Hospital for further investigations where he was started on intravenous cefotaxime after collecting a blood culture. The patient's echocardiogram revealed multiple vegetations on the tricuspid and mitral valves including a large vegetation on the septal leaflet of the tricuspid valve (10mm × 2.5 mm). The patient was started on intravenous ceftriaxone and gentamicin after obtaining three blood cultures as suggested by the Microbiology team. On the next day, the first blood culture sent before starting intravenous cefotaxime signaled positive and Gram stain revealed Gram positive bacilli. The other three blood cultures also signaled positive subsequently. All four blood cultures grew the same organism, which was identified as *Corynebacterium* species. The isolate was sent to the reference laboratory at Medical Research Institute where it was identified as *Corynebaterium diphtheriae* by the BD Phoenix system. It was susceptible to vancomycin and showed intermediate susceptibility to ceftriaxone. As the fever was continuing while on ceftriaxone and gentamicin, vancomycin was added and gentamicin was omitted. Fever settled and child clinically improved after adding vancomycin. On day 25 of antibiotics, vancomycin had to be withheld due to development of a generalized urticarial rash. Ceftriaxone was continued. Echocardiogram was performed by day 28 of ceftriaxone and it revealed no vegetations on the anterior leaflet of tricuspid valve and the size of the vegetations on the septal leaflet of tricuspid valve and mitral valve had reduced. The child was treated with ceftriaxone for 6 weeks. The echocardiogram performed after completion of intravenous ceftriaxone for six weeks revealed a structurally and functionally normal heart.

Discussion

Infective endocarditis caused by *C. diptheriae* in patients with a structurally and functionally normal heart is uncommon [1]. Underlying cardiac anomalies and presence of prosthetic heart valves are recognized risk factors. None of these risk factors were identified in this patient.

Majority of invasive diseases are caused by non-toxigenic *C. diphtheriae* which has been associated with an aggressive course with high morbidity and mortality. Endocarditis caused by *C. diphtheriae* results in valvular destruction and dysfunction with heart failure and frequent embolic complications [2]. This patient was successfully managed with the medical treatment alone under close supervision without any of the above complications. The toxigenecity study in our isolate was not performed due to unavailability of resources.

The patient's immunizations were up to date. Toxin induced classic diphtheria is rare in countries like Sri Lanka with high vaccine coverage. There are concerns over an increase in invasive disease caused by non-toxigenic strains of *C. diptheriae* in populations with high vaccine coverage [3].

C. diphtheriae endocarditis has been successfully treated with beta-lactam antibiotics alone or in combination with an aminoglycoside. There are reports of a few cases treated with vancomycin alone or in combination with an aminoglycoside [1]. This patient was treated with ceftriaxone and vancomycin as fever did not respond to ceftriaxone and gentamicin.

The diphtheroid isolated from initial blood culture at the local hospital was considered a skin contaminant. This is an important lesson for the microbiology laboratories and highlights the necessity of correlating culture results with the clinical information even if the isolate is considered as a contaminant [4]. A diagnosis of dengue fever was made as the dengue IgM was positive at the first admission. This could have been a concurrent dengue fever or a false positive result. The delay in diagnosis of infective endocarditis in the patient could have been prevented with appropriate correlation of clinical and laboratory data.

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COMPLICATED MRSA BACTERAEMIA FOLLOWING DENGUE FEVER

Chinthamani PR[1], Shanthakumara WDDI[1], Thushari HL[1], Perera BNGK[1], Kumara JALU[1], Vidanagama DS[1], Patabendige CGUA[1]

[1]Department of Microbiology, National Hospital of Sri Lanka

Introduction

Dengue is among the most significant viral diseases in the world for which Sri Lanka is hyper-endemic. Generally dengue patients experience uneventful recovery and minority complicates with haemorrhage and shock. Disseminated MRSA infections including endocarditis [1] have been described in dengue patients in Sri Lanka.

Both dengue and infections due to MRSA can be fatal on their own. We describe three patients who were treated at National Hospital for metastatic MRSA infections following recent dengue management at three other hospitals.

Case 1

A 32-year old male who was recently treated for dengue, presented with fever, acute onset left-sided hemiparesis and reduced conscious level. Inflammatory markers indicated severe bacterial infection and blood culture was

positive for MRSA. Treatment started with IV vancomycin. He was diagnosed to have aortic valve endocarditis with persistent MRSA bacteraemia. Imaging revealed multiple cerebral emboli and large splenic abscess. Intravenous linezolid and oral rifampicin were started due to poor response. Patient developed rhabdomyolysis and linezolid was discontinued after 7 days. Patient responded clinically and biochemical markers were improved. Valve replacement surgery was postponed until the patient was stable for a major surgery. Fifteen days later he developed multi-drug resistant *Klebsiella pneumoniae* bacteraemia and IV colistin was started. Patient gradually deteriorated and IV fluconazole was started due to candidaemia. Despite maximum therapeutic supports he expired on day 24 of hospital admission.

Case 2

A 30-year old male healthcare worker admitted with a cannula site infection following discharge from a local hospital after managing dengue fever. Despite IV flucloxacillin fever persisted and trans-oesophageal echocardiogram (TOE) revealed a mitral valve vegetation measuring 24x10mm. Vancomycin was added and valve replacement surgery was performed immediately. Both the blood culture and resected valve yielded MRSA. Linezolid and rifampicin were added due to poor response. On post-operative day 14 patient developed pneumonia with pleural effusion and respiratory specimen grew ESBL coliform. Massive pericardial effusion was noted on CT chest. IV meropenem and colistin nebulization was started. Repeated TOE revealed 4x4 mm vegetation in the prosthetic valve. Repeat blood culture was positive for *Pseudomonas* species and started IV imipenem. He developed massive haemoptysis with high INR and succumbed to the illness despite six weeks of aggressive therapy.

Case 3

A 28-year old mother of three, transferred from a private hospital with high fever, abdominal pain and dyspnoea. She gave a recent history of dengue fever and thrombo-phlebitis due to cannula site infection. Two blood cultures were positive for MRSA. Chest X-ray revealed bilateral pleural effusions.TOE had no evidence of vegetations but suggestive of pericardial effusion with 2 cm thick exudate causing congestive cardiac failure. Fever recurred with rising WBC and CRP while on vancomycin. Linezolid IV was started after detection of multiple cystic lesions in the spleen by CECT chest and abdomen. Pericardiectomy was done and pleural and pericardial aspirations were sterile. Later she developed pneumonia which was treated with IV imipenem. Treatment was completed as possible endocarditis according to modified Duke criteria. Since there were no residual lesions in the spleen patient was discharged after completing eight weeks of antibiotic therapy.

Discussion

Dengue management requires frequent venepunctures and vascular access. Therefore, cannula site infection is a common finding in post dengue period. Concurrent neutropaenia makes patients more vulnerable to infections. Poor hand hygiene and skin antisepsis, inadequate training and lack of awareness in healthcare workers and deficiencies in resources are contributory factors. Because of the fact that 2% of population carries MRSA in skin [2] and 10% of the staff were at risk of transmitting infection [3], the potential source of MRSA in these three cases could be the hands of healthcare workers or the patient's skin flora. MRSA bacteraemia is a common complication of long term IV cannulae [4]. These bacteria can lodge in the damaged endocardium and cause endocarditis and its complications.

Endocarditis necessitates prolonged therapy with longer hospital stay eventually leading to more hospital acquired infections (HAI). Even though the patients recover from the primary MRSA infection they acquire other HAIs due to multi-drug resistant organisms. Two patients died despite timely broad-spectrum antibiotics. It highlights the importance of HAIs including MRSA bacteraemia in post dengue infection period. Infrastructure development and increasing awareness among health care workers in peripheral hospitals regarding prevention of HAIs and implementing outpatient parenteral antibiotic therapy should be considered in conditions which need prolonged antibiotic therapy.

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Clinical significance of bacteriuria by *Staphylococcus aureus*

Piyasiri DLB[1], Priyadharshana WBU[1]

[1]*Teaching Hospital Karapitiya, Galle*

Staphylococcus aureus (SA) is a relatively uncommon cause of urinary tract infection (UTI) in the general population without a history of catheterization or risk factors like obstruction, calculi, malignancy or chronic carrier status [1]. Patients with SA bacteriuria can often be asymptomatic and the positive culture may be ignored due to lack of awareness. However, SA bacteriuria can be secondary to concomitant bacteraemia or ongoing invasive infection; and in such cases, treatment for the bacteriuria alone will mask the underlying infection and might lead to more severe sepsis.

We summarized two cases presented with SA bacteriuria and how their underlying pathology was identified.

A 62 years old diabetic male presented with severe back ache for 2 weeks without fever or urinary symptoms. Apart from hospitalization for dengue fever 3 weeks before (without subsequent cannula site infection or catheter associated UTI) there was no history of renal calculi or trauma. Examination findings were unremarkable.

His white cell count (WCC) was $24x10^9$/L with 88% neutrophils and thrombocytosis ($422x10^9$/L), and had CRP 231mg/L and ESR 105mm/1st hr. Ultra sound scan (USS) was suggestive of bilateral acute pyelonephritis and patient had been started on ciprofloxacin empirically after taking urine for culture which grew *Staphylococcus aureus* (>10^5 CFU/mL), sensitive to cloxacillin, clindamycin and cotrimoxazole.

Patient developed severe abdominal pain and difficulty in walking with mild paresthesia after 4 days of admission. Blood culture yielded the same SA with same sensitivity. Contrast CT revealed destructive lesions involving T11 and T12 vertebrae and intervertebral disc with bilateral paraspinal and epidural masses. Tuberculosis and malignancy were excluded and the 2D echocardiogram was normal.

He was started on IV flucloxacillin 2g 6 hourly and after 3 weeks WCC reduced to $12x10^9$/L with platelets $292x10^9$/L and CRP became 44mg/L with ESR 40mm. However, patient died of secondary nosocomial sepsis due to *Klebsiella pneumoniae.*

Next case was a 59 years old diabetic male with chronic renal disease who presented with recurrent UTI and had several positive urine cultures with methicillin resistant SA (MRSA) for the last 1 year and each time received symptomatic treatment with oral nitrofurantoin or cotrimoxazole. This time he presented with fever for 1 week, loss of weight and loss of appetite for several months and a small abscess on left foot.

Both blood and urine cultures grew MRSA with sensitivity to cotrimoxazole, linezolid and vancomycin and the patient was started on IV vancomycin adjusted dose and oral cotrimoxazole. USS showed multiple renal abscesses. 2D echocardiogram was negative. Aspirated pus from both foot and renal abscesses grew similar MRSA. After 3 weeks his WCC reduced from $29.5x10^9$/L to $12x10^9$/L while CRP reduced from 132mg/L to 7mg/L with radiological clearance of the abscess.

Although urinary SA may be the source of staphylococcal bacteremia, the proportion of patients with chronic SA bacteriuria who subsequently become bacteremic is unknown [2]. The mechanism of staphylococcal bacteriuria during SA bacteremia is not clearly defined; however, studies suggest that microscopic cortical abscesses are common in patients with staphylococcal bacteremia. These lesions usually heal spontaneously and only rarely progress to macroscopic abscess, but micro-abscesses may have caused bacteriuria [3].

Chihara et al [4] described SA bacteriuria as a prognosticator for outcome of SA bacteremia showing that patients with SA bacteremia and a SA positive urine

culture had significantly higher mortality than did bacteremic patients with a negative urine culture. This was true even after adjustment for multiple covariates including catheterization, lower UTI symptoms, recent urological surgeries, line infection or phlebitis or presence of comorbidities. We found a case which may suggest the same.

A 43 years old diabetic female with end-stage-renal-disease undergoing regular dialysis, admitted with accidental displacement of the neck line and subsequent site infection. Her WCC was $29x10^9$/L with 93% neutrophils and CRP was 211mg/L. Two blood cultures and the wound swab culture grew MRSA with similar sensitivity. She was started on adjusted dose of IV vancomycin.

Urine culture was collected as a part of septic screening which became positive for similar MRSA. She developed endophthalmitis and subsequently shortness of breath after 4 days and died on the 9th day despite the treatment. Considering the facts, it can be concluded that SA bacteriuria might be due to underlying spondylitis, spinal or renal abscess, or even endocarditis as suggested by Lafon et al [1] or could be a prognostic factor [4]. It warrants at least the basic investigations like WCC, CRP, ESR and further investigations including blood cultures, USS, 2D echo, with CT depending upon the results of basic investigations.

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CHALLENGES IN BONE AND JOINT INFECTIONS IN CHILDREN

Ranasinghe RATK[1], Perera BNGK[1], Welagedara PGRIS[1], Weerakoon DN[1], Pathirage S[2], Chandrasiri NS[1]

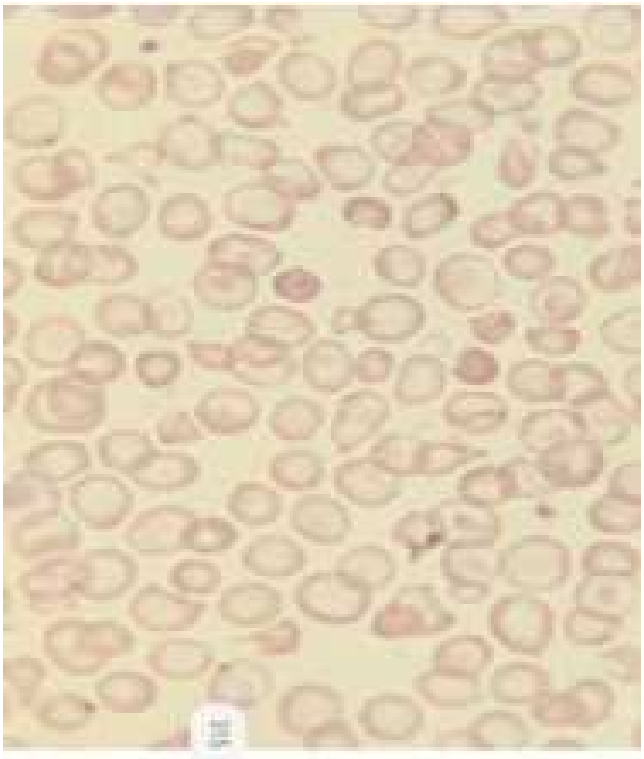
[1]*Department of Microbiology, Colombo South Teaching Hospital,*
[2]*Department of Food and Water, Medical Research Institute*

Introduction

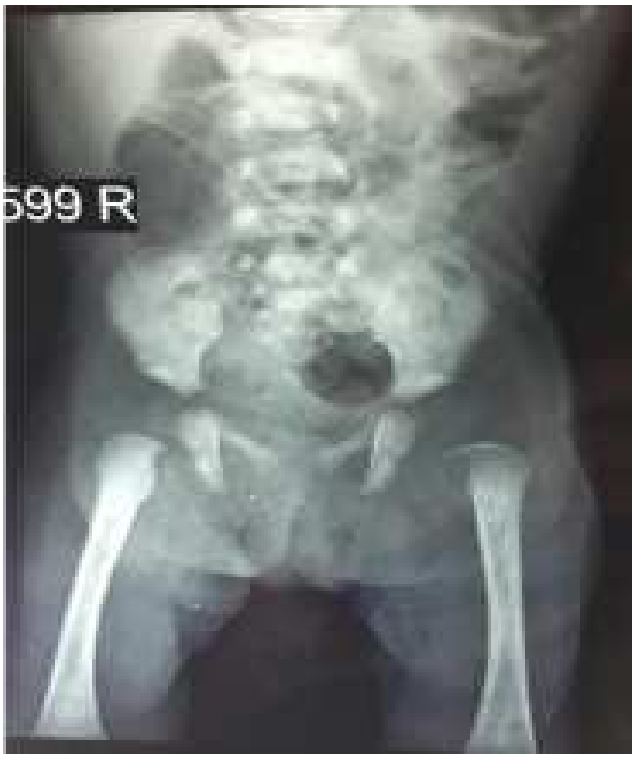
Paediatric osteoarticular infections consist of a spectrum of disorders such as osteomyelitis, septic arthritis, spondylodiscitis or combinations of them. They endure to bring management difficulties for the clinicians despite advances in investigations and interventions.

Case History 1

A thirteen-month-old previously well boy presented with high fever for three days with insignificant trauma following falling from the stroller. On the day six he developed pain and restricted movements in his left shoulder. The aspirated pus sample grew *Salmonella Typhimurium* sensitive to ampicillin, cefotaxime, ceftriaxone and trimethoprim-sulfamethoxazole. His blood picture showed mild anaemia with red cell changes suggestive of Thalassemia carrier state. He was started on intravenous cefuroxime, given for 9 days and following the availability of the culture result intravenous meropenem was given for 28 days. Left shoulder joint arthrotomy revealed joint cavity filled with pus that was drained and washed. The child responded and therapy was converted to oral trimethoprim-sulfamethoxazole and oral ciprofloxacin till the completion of six weeks.



Case No 1. Blood picture showing mild anaemia + red cell changes suggestive of Thalassemia carrier state.



Case No 2. X-ray of bilateral hip joints showing left hip joint changes.

Case history 2

A twenty-eight-day old baby girl, born by an elective caesarian section presented with restricted movement of the left lower limb. At the age of day 13, the baby was hospitalized for three days due to poor weight gain and was managed as breast feeding failure. Sepsis was excluded and was managed with intravenous fluid via a cannula in the left leg. On the day of discharge from the hospital she developed fever and left lower limb swelling, starting from the cannula site. Gradually the left lower limb movements got restricted and the examination revealed swelling of the left hip. The ultra-sound scan of the left hip showed developmental dysplasia of the hip with dislocation and evidence of infection. Hip arthrotomy was performed twice. At the second arthrotomy superficial abscess was drained. Hip joint aspirate and superficial abscess aspirate both grew ESBL producing *Klebsiella pneumoniae*, intermediately sensitive to meropenem and sensitive to trimethoprim-sulfamethoxazole. The baby was treated with intravenous meropenem for 6 weeks and converted to oral trimethoprim-sulfamethoxazole.

Discussion

The source of the osteoarticular infection may be haematogenous in origin, extension of a contiguous focus of infection or secondary to direct inoculation from trauma and surgery. The commonest age for bone infection is less than three years and septic arthritis predominates [1].

The commonest pathogen is *Staphylococcus aureus*. Group B Streptococcus and *Escherichia coli* are important pathogens in newborns [2].

Bone and joint infections commonly occur in primarily healthy children who do not have predisposed conditions but the following associations are described [2].

- Upper respiratory infection – *Kingella kingae*
- Wounds, erosions, varicella infection – Group A *Streptococcus*
- Sickle cell disease – *Salmonella* spp, *S. aureus*
- Prosthetic joints: Coagulase negative *Staphylococcus*
- Immunodeficiency – (ex: Chronic granulomatous disease) – *Serratia*, *Aspergillus*
- Penetrating wounds – anaerobes and *Pseudomonas* [2]

Due to the evolving immune response of infants and neonates signs and symptoms of the infection may be minimal. Often they present with recent minor injury and prodromal symptoms [1].

The therapy is guided by inflammatory markers, microbiology specimens and imaging modalities. Infants and neonates rarely produce a leukocytosis as the white blood cell count response is age related. The highest sensitivity is reported (98%) when both the ESR and CRP are raised [1].

Blood, tissue and fluid sampling are sent for cultures. Specimens should also be sent for histopathology as childhood malignancies can present similarly. Polymerase chain reaction and other molecular diagnostics recently have significantly increased positive results [3].

Up to 90% of patients with an early osteomyelitis can be cured with conservative treatment of antibiotics. Surgery should be reserved for cases, not responding to medical treatment. In septic arthritis, prompt clearance of the inflammatory products from the joint space is required other than the antibiotics [1,3].

Total duration of antibiotic therapy should be an average of 2-3 weeks for septic arthritis and 3-4 weeks for osteomyelitis. Longer therapy around 4-6 weeks is required in infections by resistant or unusual pathogens like MRSA and Salmonella, significant bone destruction or complications such as abscesses, involvement of pelvis or spinal column, for immunocompromised children and in neonates and very young infants (< 3 months) [2,3].

There is evidence on association between Thalassemia and Non-Typhoidal Salmonella joint infection [4]. Poor infection control practices have resulted in serious peripheral catheter-related bloodstream infections and significant morbidity, prolonged hospital stay and increased healthcare system costs [5].

Challenges we faced during management of these cases were evaluation of risk factors, continuation of the long term antibiotic treatment, and prevention of complications. Early diagnosis with prompt treatment is essential to reduce the distressing sequelae such as septicaemia, growth arrest, discrepancy in limb length and chronic infection.

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All manuscripts will be subjected to review before acceptance and will be accepted with the understanding that the work is not being submitted simultaneously to another journal and has not been already published / accepted for publication elsewhere.

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Editorial board selects one or more from the articles submitted as review articles. This should contain less than 2000 words and address a microbiologically significant topic of current interest. This article should be supported by no more than 20 key references.

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These should be in the format of introduction/background including the purpose of the study, materials and methods, results, discussion and conclusions. Each manuscript must have a structured abstract of 200 words giving the background, materials and methods, results and conclusions. The text should be limited to less than 2000 words and 15 references. Discussion should be clear and limited to matters arising directly from the results.

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This should be accompanied by a clear photograph and text. Limit your references to three for the answer. (Those submitted without references may be accepted if editors decide as suitable for publication).

Abstracts of research presentations (oral / poster) at Annual Scientific Sessions

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1. Dellit TH, Owens RC, McGowan JE et al. Infectious Diseases Society of America and the Society for Healthcare Epidemiology of America guidelines for developing an institutional program to enhance antimicrobial stewardship. *Clinical Infectious Diseases* 2007; **44**: 159-77.

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