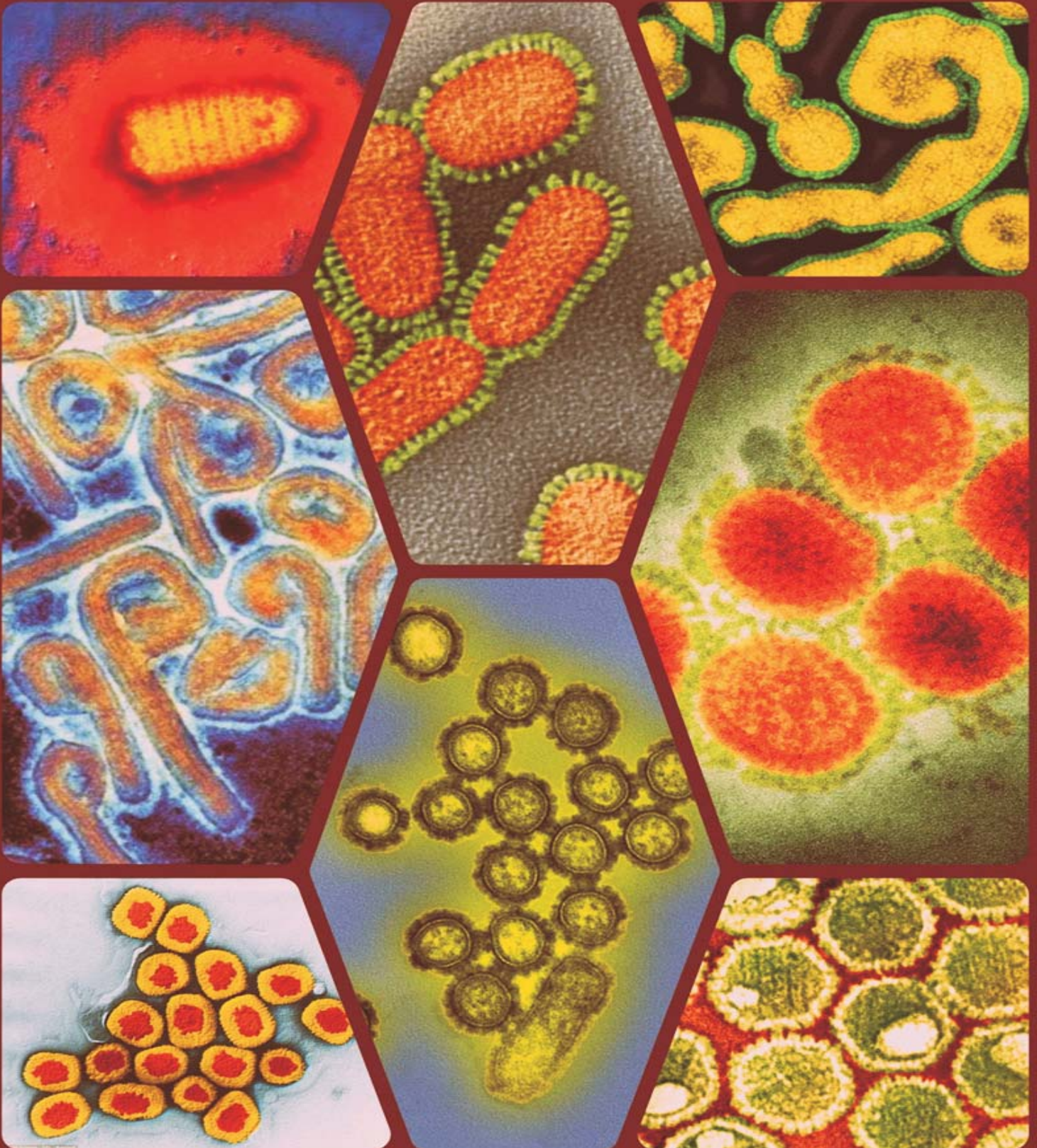
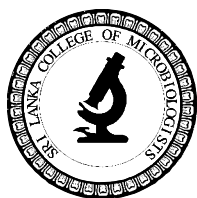


The Bulletin of the Sri Lanka College of Microbiologists

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The Sri Lanka College of Microbiologists

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Standing (L - R): Dr. Jananie Kottahachchi, Prof. Nilanthi de Silva, Dr. Kumudu Karunaratne, Dr. Ajith Nagahawatte, Dr. Surani Udugama, Dr. Dhammika Vidanagama, Dr. Samanmalee Gunasekara, Dr. Sujatha Pathirage.

The Sri Lanka College of Microbiologists

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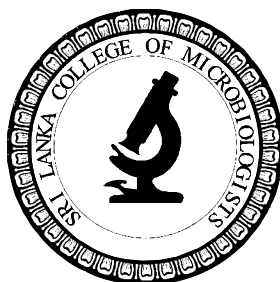
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Cover page designed by Dr. Roshan Jayasuriya

25th Annual Scientific Sessions



The Sri Lanka College of Microbiologists

Inauguration Ceremony

10th August 2016 at 6.00 pm
Sri Lanka Foundation
Colombo 7

Pre-Congress Workshop

Theme:

“Acute Encephalitis – a clinical dilemma”

10th August 2016

Scientific Programme

Theme:

“Microbial Infections: Facing Challenges and Exploring Possibilities”

11th & 12th August 2016

Sri Lanka Foundation
Colombo 7

MESSAGE FROM THE CHIEF GUEST



It gives me great pleasure in sending a congratulatory message on the occasion of the 25th Annual Scientific Sessions of the Sri Lanka College of Microbiologists.

Since its inception, the Sri Lanka College of Microbiologists has contributed immensely towards strengthening the field of microbiology in Sri Lanka. Highly dedicated efforts of the members in different specialties, have contributed immensely towards improving the microbiological services of the country by expanding its role in the diagnosis, management, prevention and control of infectious diseases, education and research.

The theme chosen this year **“Microbial Infections: Facing Challenges and Exploring Possibilities”** is very timely and appropriate in keeping with the emerging and re-emerging infectious diseases of the world and the global issue of antimicrobial resistance, an increasing problem faced by Sri Lanka too. I believe, it is very important that not only the clinicians but also the policy makers, administrators, epidemiologists and microbiologists, should work together to meet these challenges.

While congratulating the President and the Council of the College for taking timely efforts to enlighten all these stakeholders on this global health challenge, I wish the 25th Annual Scientific Sessions of the Sri Lanka College of Microbiologists every success.

Professor Mohan de Silva
Chairman
University Grants Commission

MESSAGE FROM THE PRESIDENT



Organizing the Annual Scientific Sessions and publication of the annual *Bulletin of the Sri Lanka College of Microbiologists*, is one of the most important events in the annual calendar of the college activities. The Sri Lanka College of Microbiologists celebrates an important milestone this year, where we are organizing the 25th Annual Scientific Sessions. It gives me great pleasure in writing this brief message for the bulletin.

Our College brings together several sub-specialties within the broader field of infectious diseases including medical microbiology, medical virology, immunology, medical mycology and parasitology. Considering the issue of emerging and re-emerging infectious diseases in the world and the global problem of antimicrobial resistance, the theme at this year's annual scientific sessions will be "*Microbial Infections: Facing Challenges and Exploring Possibilities*". We will also conduct a pre-congress workshop on "*Acute Encephalitis – a clinical dilemma*".

The scientific sessions will include several plenary lectures and symposia in addition to the free paper sessions and display of posters by our members. The topics for the symposia and plenary lectures were decided taking in to account, the increasing burden of antimicrobial resistance, gradually expanding population of immunocompromised patients and future plans for haemopoietic stem cell transplantation in Sri Lanka.

I take this opportunity to thank Prof Mohan de Silva, Chairman, University Grants Commission and Senior Professor and Chair of Surgery, University of Sri Jayawardenapura for accepting our invitation to be the chief guest at the inauguration ceremony. I also thank all our guest speakers, both local and foreign, for having accepted our invitations willingly to share their knowledge and expertise with us in spite of their busy schedules. I extend a special word of thanks to all the college members and the council members who have helped and contributed in numerous college activities during this year. A special mention should be made of the tireless contribution of the members who have got actively involved with the latest college activity of preparing the draft national action plan for combatting antimicrobial resistance, a multi-disciplinary activity together with the Ministry of Health and the World Health Organization.

I am indebted to the two honorary joint secretaries of the College, Dr. Primali Jayasekera and Dr. Pavithri Bandara, and Ms. Priyanga Opatha, our office secretary, who have supported me throughout the year in all our College activities.

Dr. Kanthi Nanayakkara

President

Sri Lanka College of Microbiologists

INAUGURATION PROGRAMME

6.00 pm	Invitees take their seats
6.15 pm	Arrival of the Chief Guest Introduction of Members of the Council
6.30 pm	Ceremonial Procession
6.35 pm	National Anthem
6.40 pm	Traditional lighting of the Oil Lamp
6.50 pm	Welcome Address Dr. Primali Jayasekera Hony. Joint Secretary
6.55 pm	Address by the Chief Guest Prof. Mohan de Silva Chairman, University Grants Commission
7.10 pm	Address by the President Dr. Kanthi Nanayakkara Consultant Virologist & Vaccinologist Medical Research Institute
7.40 pm	Award of SLCM Fellowships
8.20 pm	Vote of Thanks Dr. Pavithri Bandara Hony. Joint Secretary
8.25 pm	Ceremonial Procession leaves
8.30 pm	Reception



25th Annual Scientific Sessions
The Sri Lanka College of Microbiologists

Pre congress workshop

Theme:

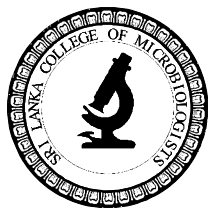
“Acute Encephalitis – a clinical dilemma”

10th August 2016

**Sri Lanka Foundation
Colombo 7**

Chairpersons – Dr. Nalini Withana and Dr. Kumudu Karunaratne

8.30 am - 8.50 am	Registration
8.50 am - 9.00 am	Welcome address Dr. Kanthi Nanayakkara <i>President SLCM</i>
9.00 am - 9.45 am	Challenges in the management of patients with acute CNS infection Dr. Udaya Ranawaka <i>Consultant Neurologist, Faculty of Medicine, University of Kelaniya, Ragama</i>
9.45 am - 10.30 am	Acute encephalitis in immune-compromised patients Dr. Nicole Gilroy <i>Infectious Disease Physician, Westmead Hospital and BMT Network, NSW, Australia</i>
10.30 am - 10.45 am	Tea
10.45 am - 11.30 am	Laboratory role in patient management Dr. Philip Rice <i>Consultant Clinical Virologist, Norfolk and Norwich, University Hospital United Kingdom</i>
11.30 am - 12.15 pm	Anaesthetist's role in managing the patient in the ICU Dr. Nilmini Wijesuriya <i>Consultant Anaesthetist, North Colombo Teaching Hospital, Ragama</i>
12.15 pm - 12.30 pm	Discussion



25th Annual Scientific Sessions
The Sri Lanka College of Microbiologists

Scientific Programme

Theme:

“Microbial Infections: Facing Challenges and Exploring Possibilities”

11th & 12th August 2016

Sri Lanka Foundation,
Colombo 7

Scientific Programme – Day 1 – Thursday 11th August 2016

8.15 am - 8.45 am **Registration**

8.45 am - 9.30 am **Free paper session I**

Chairpersons – Prof. Jennifer Perera and Dr. Madhmanee Abeywardena

OP 1 **Comparison of Community acquired and Hospital acquired methicillin resistant *Staphylococcus aureus* (MRSA) isolates in the National Hospital of Sri Lanka**

Samaranayake WAMP, Karunanayake L¹, Patabendige CGUA²

¹Medical Research Institute, Colombo, ²National Hospital of Sri Lanka, Colombo

OP 2 **Vancomycin susceptibility among *Staphylococcus aureus* isolates from a tertiary care hospital**

Shahnas MNF¹, Dissanayake BN¹, Liyanapathirana LVC¹, Ekanayake EWMA¹

¹Department of Microbiology, Faculty of Medicine, University of Peradeniya

OP 3 **MLST analysis of *Burkholderia pseudomallei* isolates from Sri Lanka reveal a large number of novel STs**

De Silva AD^{1,3}, Sathkumara HD¹, Krishnananthasivam S¹, Corea, E²

¹Genetech Research Institute, Colombo, Sri Lanka, ²Department of Microbiology, Faculty of Medicine, University of Colombo, ³Division of Vaccine Discovery, La Jolla Institute of Allergy and Immunology, California, USA

OP 4 **Prevalence of nasal colonisation with potential pathogens and associated factors in children less than 5 years**

Premaratne KKMK¹, Corea E², Karunanayake L²

¹Post Graduate Institute of Medicine, University of Colombo, ²Department of Microbiology, Faculty of Medicine, Colombo, ³Medical Research Institute, Colombo

SCIENTIFIC PROGRAMME

- 9.45 am - 10.30 am **Plenary I**
Chairperson – Prof. Vasanthi Thevanesam
Quality Assurance in Microbiology Laboratories
Dr. Jane Stockley
 Consultant Microbiologist, Worcestershire Royal Hospital, United Kingdom
- 10.30 am - 10.45 am **Tea**
- 10.45 am - 11.45 am **Free paper session – 2**
Chairpersons – Prof. N. P. Sunil-Chandra and Dr. Rajiva de Silva
- OP 5 **Serotype-Specific Detection of Dengue Viruses in a Multiplex Real-Time Reverse Transcriptase PCR Assay**
Abeynayake JI, Welmillage SU, Gunasena S, Wickramasinghe MGCN, Samaraweera B
 Department of Virology, Medical Research Institute, Colombo 08
- OP 6 **Co-infections with multiple dengue virus serotypes in patients from 3 different Provinces of Sri Lanka**
Sirisena PDNN¹, Senaratne UTN¹, Muruganathan K², Carr J³ and Noordeen F¹
¹Department of Microbiology, Faculty of Medicine, University of Peradeniya,
²Department of Pathology, Faculty of Medicine, University of Jaffna,
³Microbiology and Infectious Diseases, School of Medicine, Flinders University, Australia
- OP7 **Prevalence of subtypes of Respiratory Syncytial Virus and association of subtypes with disease severity in a selected group of children with acute lower respiratory infection**
Jayamaha CJS¹, HarshaniHBC¹, Ratnayake NR², Welmillage SU¹
¹National Influenza Centre, Department of Virology, Medical Research Institute, Colombo, ²Lady Ridgeway Hospital, Colombo
- 11.45 am - 12.45 pm **Symposium 1 – Multidrug Resistant Bacterial Infections – How to Combat?**
Chairpersons – Dr. Kushlani Jayatilleke and Dr. Pavithri Bandara
The problem from a clinician's point of view
Dr. Janake Munasinghe
 Consultant Physician, National Hospital of Sri Lanka, Colombo
Strategies to manage antimicrobial resistance – Attempting to turn the tide
Dr. Jane Stockley, Consultant Microbiologist,
 Worcestershire Royal Hospital, United Kingdom
Application of Behavioural Science in Infection Control and Antibiotic Stewardship – The Force Awakens
Dr. Rohan Chinniah
Consultant Clinical Microbiologist/Infection Control, Raja Isteri Pengiran Anak Saleha (RIPAS) Hospital, MoH, Bandar Seri Begawan, Negara Brunei Darussalam
- 12.45 pm - 1.45 pm **Lunch**

SCIENTIFIC PROGRAMME

1.45 pm - 2.30 pm

Plenary 2

Chairperson – Dr. Geethani Galagoda

The transmission, reactivation and evolution of Varicella-Zoster Virus: the solution to a 40 year-old virological mystery?

Dr. Philip Rice

Consultant Clinical Virologist, Norfolk and Norwich, University Hospital
United Kingdom

2.30 pm - 3.30 pm

Free paper session – 3

Chairpersons – Dr. Geethani Wickramasinghe and Dr. Rohitha Muthugala

OP 8

The effects of storage temperatures and evaluation of open vial policy on potency of live trivalent oral poliomyelitis vaccine (tOPV) in Sri Lanka

Senevirathne WDST, Perera KADN, Nanayakkara S, Wimalaratne OV

Department of Rabies and Vaccine QC, Medical Research Institute, Colombo 08

OP 9

An Immunogenicity Study on Intradermal Pre-exposure Rabies Vaccination in Sri Lanka

Perera KADN¹, Nanayakkara S¹, Wimalaratne OV¹, Abeynayake P², Samarakkodi PMA³, Senevirathne WD ST¹

¹Department of Rabies and Vaccine Quality Control, Medical Research Institute, Colombo 08, ²Faculty of Veterinary Medicine and Animal Science, University of Peradeniya, Peradeniya, ³Medical Center, University of Peradeniya, Peradeniya

OP 10

Comparison of BK virus viraemia and viraemia in post renal transplant patients – A single centre study

Premathilake MIP^{1,2}, Jayamaha CJS¹

¹Medical Research Institute, Colombo, ²Faculty of Medicine, University of Colombo

3.30 pm - 4.15 pm

Plenary 3

Chairperson – Dr. Omala Wimalaratne

Impact of HPV vaccination in prevention of cervical cancer

Dr. Kanishka Karunaratne

Consultant Obstetrician and Gynaecologist

4.15 pm

Tea

Day 2 – Friday 12 August 2016

8.30 am - 9.30 am

Free paper session – 4

Chairpersons – Dr. Sagarika Samarasinghe and Dr. Sujatha Pathirage

OP 11

Innate cellular immune responses in cutaneous leishmaniasis against *Leishmania donovani*

Wijesooriya MWAHD¹, Samarasinghe TN¹, Somaratne KKVN², Karunaweera ND¹

¹Department of Parasitology, Faculty of Medicine, University of Colombo,

²District General Hospital, Hambantota

SCIENTIFIC PROGRAMME

OP 12	Enterobiasis in the Western and Eastern Provinces of Sri Lanka <i>Gunawardane NK¹, Mujeeb IM², Chandrasena TGA¹, Moulana SHBMAA², Withanage S¹, Mahanama LS¹, Jayakody NK¹, de Silva NR¹</i> ¹Department of Parasitology, Faculty of Medicine, University of Kelaniya, ²Ministry of Health, Colombo
OP 13	Antifungal sensitivity profile of <i>Fusarium</i> spp. resulting keratitis - A preliminary study <i>Sigera LSM¹, Jayasekera PI¹, Malkanthi MA¹, Shabry ULF¹</i> ¹Department of Mycology, Medical Research Institute, Colombo
OP 14	Prevalence of genital chlamydia infection among attendees of the Central Sexually Transmitted Diseases Clinic, Colombo <i>De Silva RND¹, Elwitigala JP², Corea EM³</i> ¹Postgraduate Institute of Medicine, University of Colombo, ²National STD/AIDs Control Programme, ³Department of Microbiology, Faculty of Medicine, University of Colombo
9.30 am - 10.15 am	Plenary 4 Chairperson - Prof. Nilanthi de Silva The Era of "OMICS" and prospects for Malaria eradication <i>Prof. Sharmini Gunawardane</i> Professor and Consultant Parasitologist, Department of Parasitology, Faculty of Medicine, University of Colombo
10.15 am - 10.30 am	Tea
10.30 am - 11.30 am	Symposium 2 - Stem Cell Transplants - Sri Lankan perspective Chairpersons - Dr. Samanmalee Gunasekera and Dr. Janaki Abeynayake Infective complications in stem cell transplant recipients and challenges in diagnosis <i>Dr. Nicole Gilroy</i> Infectious Disease Physician, West Mead Hospital and BMT Network, NSW, Australia Overview of future stem cell transplant in Sri Lanka <i>Dr. Prasad Abeyasinghe</i> Consultant Oncologist, National Cancer Institute, Maharagama Preventing Invasive Fungal Infections in Stem Cell Transplant recipients: Writing a policy - Leicester, UK Model <i>Dr. Nelun Perera</i> Consultant Microbiologist, University Hospitals of Leicester, Hon Senior Lecturer, University of Leicester, Training Programme Director, Health Education East Midlands
11.30 am - 12.30 pm	Free paper session - 5 Chairpersons - Dr. Nadeeka Janage and Dr. Dhanushka Dassanayake
OP 15	Prevalence of subtypes of parainfluenza virus in a selected group of children with severe acute respiratory symptoms <i>Jayamaha CJS¹, Harshani HBC¹, Ratnayake NR², Karunaratne K²</i> ¹National Influenza Centre, Department of Virology, Medical Research Institute, Colombo, ²Lady Ridgeway Hospital, Colombo

SCIENTIFIC PROGRAMME

OP 16	Detection of Coronaviruses in Bat Guano Collected from the Royal Botanical Gardens, Peradeniya <i>Kudagammana HDWS¹, Noordeen F¹, Pitchai FNN, Thevanesam V¹, Daniel Chu², Peiris JSM², Eriyagama NB¹, Jayawardane PMGA</i> ¹Department of Microbiology, Faculty of Medicine Peradeniya, ²School of Public Health, University of Hong Kong, Hong Kong
OP 17	An outbreak of Influenza-A (H₁N₁) from March to August 2015 <i>Jayamaha CJS, Anuradha SL, Welmillage SU, Sanjeeva MDA, Ekanayake P, Abeywardhana MDMA, Gunathilaka GDWS</i> National Influenza Centre, Department of Virology, Medical Research Institute, Colombo
OP 18	Molecular Diagnosis of Varicella-Zoster Virus and Genotyping of Herpes Simplex Virus by a Multiplex Real-Time PCR <i>Abeynayake JL, Samaraweera B, Wickramasinghe MGCN, Hettigoda GM,</i> Department of Virology, Medical Research Institute, Colombo 08.
12.30 pm -1.30 pm	Lunch
1.30 pm - 2.30 pm	Symposium 3 - Pulmonary Aspergillosis Chairpersons – Dr. Preethi Perera and Dr. Primali Jayasekera Types of pulmonary aspergillosis and latest methods for investigation <i>Prof. Arunaloke Chakrabarti</i> Head, Center of Advance Research in Medical Mycology, WHO Collaborating Center for Reference & Research of Fungi of Medical Importance & National Culture Collection of Pathogenic Fungi, Department of Medical Microbiology, Postgraduate Institute of Medical Education & Research, Chandigarh, India Clinical scenarios and situation analysis in Sri Lanka <i>Dr. Amitha Fernando</i> Consultant Respiratory Physician, Central Chest Clinic, Colombo
2.30 pm - 3.15 pm	Plenary 5 Chairperson – Dr. Ajith Nagahawatte Interactive Case Discussion <i>Dr. Nelun Perera</i> Consultant Microbiologist, University Hospitals of Leicester Hon Senior Lecturer, University of Leicester Training Programme Director, Health Education East Midlands
3.15 pm - 3.30 pm	Award ceremony
3.30 pm	Tea

FOREIGN FACULTY



Dr. Nicole Gilroy
Infectious Disease Physician
Westmead Hospital and BMT Network,
NSW Australia.



Dr. Philip Rice
Consultant Clinical Virologist, Norfolk and Norwich, University Hospital,
United Kingdom.



Dr. Jane Stockley
Consultant Microbiologist, Worcester Royal Hospital, Worcestershire,
United Kingdom.



Prof. Arunaloake Chakrabarti
Professor and Head, Department of Medical Microbiology,
Postgraduate Institute of Medical Education and Research,
India.

LOCAL FACULTY



Dr. Udaya Ranawaka

Consultant Neurologist, Faculty of Medicine, University of Kelaniya, Ragama.



Dr. Nilmini Wijesuriya

Consultant Anaesthetist,
North Colombo Teaching Hospital, Ragama.



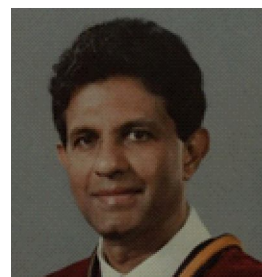
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National Hospital of Sri Lanka, Colombo 10.



Dr. Rohan Chinniah

Consultant Clinical Microbiologist / Infection Control,
Raja Isteri Pengiran Anak Saleha (RIPAS) Hospital,
MoH, Bandar Seri Begawan,
Negara Brunei Darussalam.



Dr. Kanishka Karunaratne

Consultant Obstetrician & Gynaecologist

LOCAL FACULTY



Prof. Sharmini Gunawardena

Professor and Consultant Parasitologist
Department of Parasitology, Faculty of Medicine,
University of Colombo.



Dr. Prasad Abeysinghe,

Consultant Oncologist,
National Cancer Institute,
Maharagama.



Dr. Nelun Perera

Consultant Microbiologist
University Hospitals of Leicester

Honorary Senior Lecturer
University of Leicester

Training Programme Director, Microbiology
Health Education England / East Midlands



Dr. Amitha Fernando

Consultant Respiratory Physician,
Central Chest Clinic, National Hospital of Sri Lanka,
Colombo.

Pre-Congress Workshop “Acute encephalitis – a clinical dilemma”

The challenge of treating central nervous system infections

Dr. Udaya Ranawaka

Central nervous system (CNS) infections produce large numbers of death and disability. Case fatality rates can be as high as 65-70%, and up to 50% of survivors are left with residual neurological sequelae. CNS infections are commoner and produce more deaths and disability in developing countries, but their management in these settings can be difficult due to resource constraints. Recent advances in knowledge have helped guide treatment of CNS infections, but there are many unanswered questions.

Early treatment has been shown to improve outcomes in CNS infections. Appropriate early treatment depends on rapid and accurate diagnosis, but this can be challenging. Clinical features are unreliable in arriving at a syndromic diagnosis or in detecting the underlying aetiology, and establishing the diagnosis is heavily dependent on laboratory investigations. This is especially challenging in the developing countries due to inadequate diagnostic facilities. However, isolation rates can be low even in well conducted studies in developed countries, and it is important to look beyond the traditional causative organisms.

As laboratory diagnosis is difficult, antimicrobial treatment is usually started on an empiric basis. Such empiric therapy would ideally depend on local epidemiological data, but such data are lacking from developing countries. Steroids are widely recommended to minimize the inflammation related to infection. Wider availability of diagnostic tools and newer treatment strategies would help minimise the burden of CNS infections, and there is a clear need for local epidemiological data to guide treatment in developing countries such as Sri Lanka.

Acute encephalitis in the immunocompromised

Dr. Nicole Gilroy

The clinical presentation of acute encephalitis in the immunocompromised host may be atypical, and the diagnostic clues, such as CSF pleocytosis, lacking. A consideration of the aetiology of encephalitis should take account of timing of symptom onset in relation to chemotherapy, transplantation or immunosuppression,

whether antimicrobial prophylaxis is current, exposures (zoonotic contacts, hobbies, habits), host factors such as age and comorbidities, and the intensity and duration of immunosuppression and chemotherapy. In addition, geography and the diseases endemic to places where the host, or their organ donor, may have been born, travelled, or currently reside are additional factors that should be elicited in the clinical history, and which may guide diagnosis.

Encephalitis in the immunocompromised host can follow reactivation of latent herpesviruses (eg HSV, VZV, CMV, EBV, HHV6), polyomaviruses (eg JC, BK), adenovirus, parasites (eg strongyloides) or protozoa (eg toxoplasmosis). Invasive opportunistic infections from environmentally acquired yeasts (cryptococcus), moulds (aspergillus and mucor), and atypical bacteria (nocardia, mycobacteria) are all potential differential diagnoses in the immunocompromised host presenting with symptoms of encephalitis.

The treatment and diagnostic correlates of some of the more important causes of encephalitis in the immunocompromised host will be presented.

Laboratory role in patient management

Dr. Philip Rice

The microbiology laboratory is essential to enable an accurate aetiological diagnosis of encephalitis to be made. This helps in determining the length and types of treatment, methods for the prevention of infection and allows a more specific prognosis to be made. Nevertheless, despite the advances in available laboratory tests a specific infective agent eludes diagnosis in a large proportion of cases. The history and development of laboratory diagnostic tests will be reviewed as will developments for the future. With improved diagnostics additional anti-viral drugs will follow and while some agents are on the cusp of eradication, new and emerging viruses remain a constant threat to human health as exemplified by the Zika virus outbreak.

Anaesthetist's role in managing the patient in the ICU

Dr. Nilmini Wijesuriya

Acute encephalitis is a medical emergency with 25- 50% of patients needing intensive care unit admission. The commonest reasons for ICU admission are altered level of consciousness and other associated problems.

Multidisciplinary team management is essential for a successful outcome. Early admission to preferably a neuro-intensive care unit may reduce complications associated with the course of the disease.

Reduced level of consciousness is resulted from metabolic encephalopathy, seizures, or cerebral oedema. These reasons may be accompanied by impairment of airway and respiratory drive, needing intubation and ventilation. These patients need careful fluid management to avoid or worsen cerebral oedema. Some forms of encephalitis may be associated with autonomic dysfunction necessitating haemodynamic monitoring and inotropic support.

Mass effect from rising ICP is another concern in these patients. Sedation and ventilation with measures to maintain cerebral perfusion pressure is vital. Seizures and status epilepticus are a common presentation and needs close collaboration between the neurologist and the intensivist. The goal of treatment is to control epileptic activity, but in many cases it is necessary to induce a burst-suppression pattern on EEG. The drugs used for this purpose may lead to potential side effects such as hypotension, loss of airway reflex and respiratory drive. Supportive therapy such as nutrition, prevention of aspiration pneumonia, deep vein thrombosis and bed sores as for any other intensive care unit patient is important. Early commencement of physical and cognitive rehabilitation in the ICU may help to minimize long-term sequelae.

Plenary presentations 1

Quality assurance in microbiology

Dr. Jane Stockley

Sound pathology services lie at the heart of good medical practice, and clinicians and patients alike expect the diagnostic services they use to be of the highest quality, and consistent wherever they reside or have their clinical practice. Medical microbiology is a clinical service which supports the investigation and management of patients suspected of having infection. In addition to this, microbiologists and their laboratories have wider responsibilities to public health, the monitoring of clinical disease and surveillance of antimicrobial resistance. Reliable laboratory data is of paramount importance, as is consistency of analytical process.

Quality assurance is embedded within good microbiology practice; it encompasses internal quality control, use of reliable media and reagents, sound laboratory equipment, trained scientific staff, evidence-based standard operating procedures (SOPs) and laboratory audit. External quality assurance (EQA) provides additional support, providing a benchmark and comparison with

others – and also educational material. EQA can give access to less frequently encountered clinical scenarios and isolates, and provide advice on how best to look for emerging pathogens or new resistance mechanisms. It tests the competence of individuals, but also the reliability of methods and equipment. It can provide the stimulus to update methods and acquire new equipment. Over recent years, a clinical interpretative scheme was introduced into the UK NEQAS repertoire. This gave the opportunity to evaluate ones own clinical advisory practice, and compare oneself to others. These schemes will be discussed, in terms of their benefits and limitations.

Laboratory accreditation promotes adherence to standards deemed necessary for good practice; it encompasses laboratory management systems, staffing, analytical processes and clinical practice. The use of accreditation is widespread within Europe and the United States, and although not mandatory in all countries, it has proved a useful tool for commissioners and laboratories alike to uphold standards and secure necessary resources. Clinical Pathology Accreditation (CPA) and its successor, UKAS international standard ISO 15189 will be discussed, in terms of what they hope to achieve, and implications for laboratories.

It is also important that microbiologists reach out and ensure that clinical colleagues are utilising their services optimally. New tests will be introduced, and redundant tests removed from the laboratory repertoire. The best evidence-based methods should be incorporated into patient pathways, and clinical outcomes audited. Key performance indicators need to be agreed with professional bodies, microbiologists and users of the service, so that practice continually improves – both within and outside the laboratory.

Plenary presentations 2

“The transmission, reactivation and evolution of Varicella-Zoster virus: the solution to a 40 year-old virological mystery?”

Dr. Philip Rice

It has been known for more than 40 years that chickenpox, caused by the Varicella-Zoster virus (VZV) is much more common in adults living in the tropics. Various explanations, climatic and social, have been proposed: heat, humidity, population density, competing infecting viruses etc, yet none has been subjected to a rigorous examination. There is a climatic factor which shows the largest difference between temperate and tropical zones, but which has been ignored until now. This climatic factor explains why chickenpox is more common in adults in the tropics because they do not get infected as children. This is because this climatic factor

renders chickenpox less infectious in the tropics since it inactivates the virus in skin lesions. It also explains the seasonality of chickenpox in both tropical and temperate zones. The likely implications are threefold: it explains the physical appearance of the chickenpox rash, when chickenpox actually becomes infectious and suggests how the virus might reactivate as shingles.

Plenary presentations 3

Impact of Human Papilloma virus (HPV) vaccination in prevention of cervical cancer

Dr. Kanishka Karunaratne

Cervical cancer is one of the leading causes of morbidity and mortality in women throughout the world. Persistent infection with oncogenic HPV is associated with development of cervical cancer. Infection with oncogenic HPV types is also implicated in the development of other malignancies including neoplasms of the vulva, vagina, anus, penis and oropharynx. Of the oncogenic HPV types 16 and 18 accounts for about 70% of cervical cancer. HPV is a common asymptomatic infection with an estimated 40% of sexually active women becoming infected during life.

Vaccines prevent the disease by producing high level neutralising antibodies which is several folds higher than levels produced by natural infection. The impact of the vaccination was greatest for women completely vaccinated at younger ages (9-12 years). It reduces all types of cervical abnormalities (CIN 1-3/AIS). Reduction in cervical cancer abnormalities was restricted to women who received 3 doses. It also has an impact on reducing vaginal intraepithelial neoplasia and vulval intraepithelial neoplasia.

Plenary presentations 4

Era of 'omics' and prospects for malaria eradication

Prof. Sharmini Gunawardena

Efforts to control and eliminate malaria have escalated over the past decade with increased political and financial commitment, stimulating renewed hopes for achieving global eradication of malaria. The World Health Organisation (WHO) recommends a multi-pronged strategy to control and eliminate malaria, which includes vector control interventions, preventive therapies, diagnostic testing, treatment with quality-assured

artemisinin-based combination therapies (ACT) and strong malaria surveillance. Significant progress has been achieved in malaria control over the past decade with reductions in transmission occurring in many endemic regions including Sri Lanka.

Current efforts to control and eliminate malaria relate to the combined use of antimalarial drugs, insecticide-treated bed nets (ITNs) and indoor residual spraying of insecticides (IRS), with vaccine development remaining as a longer-term goal. Mutations in the parasite population threaten to undermine these efforts, as the parasite evolves rapidly to evade host immune systems, drugs and vaccines. The success of the global research agenda toward eradication of malaria will depend on the development of new tools, including drugs, vaccines, insecticides and diagnostics. Genomic, transcriptomic, proteomic, metabolomic and glycomic projects exemplify the "omics" era, and have significantly expanded available data for biomedical research. The modern arsenal of "omics" technologies appears to offer a promising approach to engineering a long-term solution to malaria. With resistance threatening to render ineffective the mainstay of current strategies for malaria elimination, taking advantage of these technologies is vital for realising the goal of malaria elimination and eradication.

Symposium 1

Multidrug resistant bacterial infections – How to combat?

The problem from a clinician's point of view

Dr. Janake Munasinghe

The development of resistance to antimicrobials is a natural phenomenon. This is of paramount importance to us as anti-microbials are used extensively in the management of many infectious diseases. The ever increasing resistance to anti microbials will increase the burden of these diseases in multiple ways. The emergence of resistance results in a higher patient morbidity and mortality and increases the duration of hospital stay and costs of medication, thereby increasing the financial and resource burden of a free health care delivery system already stretched to its limit. The problem is further aggravated by the fact that no new antimicrobials have been discovered over past 20 or so years to replace the ones which become unusable due to the emergence of resistance.

The problem of anti-microbial resistance can be mitigated, though never eliminated, by the rational prescription of antibiotics by the *prescriber*, and the responsible use of

antibiotics by the *end user*. Adhering to guidelines and having knowledge of microbes responsible for specific diseases along with their local current sensitivity patterns will help the prescriber to select the most appropriate antibiotic. The availability of good microbiological services at all levels of health care helps to gain information on the current local sensitivity patterns and trends. When used in the outpatient setting. It is the duty of the end user to assume responsibility for correct use of the prescribed antibiotic.

Rational prescribing and responsible use should be self-initiated and motivated as much possible. However if satisfactory results are not achieved by self-restraint in prescribing and use, of antimicrobials, the National Regulatory Authority should not hesitate to intervene by implementing procedures and protocols such as formulary restriction and dual authorization in order to restrict the use of selected anti microbials.

Research into better and novel ways in the use of antibiotics with the participation of the patient, microbiologist, the clinical pharmacologist, and clinician, may help stem the alarming tide of increasing microbial resistance to antibiotics.

Strategies to manage antimicrobial resistance – attempting to turn the tide

Dr. Jane Stockley

The World Health Organisation recognises antimicrobial resistance as one of the greatest current challenges to global public health. A strong collaborative approach from countries across the world is required if we are to manage current problems caused by antimicrobial resistance, and safeguard the efficacy of these life-saving drugs for future generations. It is not only the management of infectious diseases at stake, but current practice and future advances in general medicine and surgery, including cancer treatments and organ transplantation, which are all dependent on the availability of effective antimicrobial agents.

Antimicrobial resistance is a natural biological phenomenon, but its development and spread is increased through the inappropriate use of antimicrobial agents, inadequate surveillance and infection prevention and control strategies, including immunisation and other public health programmes. Microbiology laboratory services need to be adequately supported and enhanced with high quality training for medical and scientific staff, adequate equipment and resources, educative quality assurance schemes and accreditation. Pharmaceutical companies and researchers need to be encouraged to develop novel agents; and public access to existing

antimicrobial agents needs to be controlled, with medical prescribing monitored and supported through evidence-based prescribing guidelines. The use of antimicrobial agents in animal husbandry, agriculture and veterinary practice also needs to be recognised, monitored and controlled.

National governments, healthcare policy makers, pharmaceutical companies, medical, veterinary and scientific professionals, laboratory staff, infection control practitioners, communication media and the general public all have important roles to play; and countries across the world need to have co-ordinated strategic plans in place to deal with the problem. Of course, not all countries have adequate resources or the social infrastructure to comply; many have more pressing priorities, and in many parts of the world, conflict and social upheaval compound the problem.

Microbiologists are key individuals who can bring their influence to bear at national level, educate colleagues and raise public awareness over the issues relating to antimicrobial resistance and good antibiotic stewardship.

Application of behavioural science in infection control and antibiotic stewardship

The Force Awakens

Dr. Rohan Chinniah

Antibiotic stewardship and Infection control not only need to be learned but also should be practiced diligently. Hence both these require behavioural changes among the healthcare personals for their effectiveness.

These behavioural changes should range at all organizational level including and involving all the stakeholders ranging from leadership to the end users.

Guidelines and policies help with decision making, by providing knowledge and awareness, but they may not shift attitude or change practice. It is necessary to understand the factors that influence prescribing behaviours and decisions. Similarly understanding the factors influencing behaviours in infection control practices is paramount for their successes. Hence behaviour change or behaviour modification is a key element in optimizing antibiotic prescribing and in infection control.

We need to consider socio-cultural factors affecting behaviour in the design, implementation and reporting of any interventions for their success. Also we should use time tested behavioural modifications methods to achieve this goal.

Symposium 2

The prevention and management of Infectious complications in stem cell transplantation

Dr. Nicole Gilroy

Infectious diseases are the leading cause of preventable morbidity and mortality in stem cell transplantation. Preventing exposure to bacterial, viral, fungal and parasitic infections (primary prevention) can be achieved by appropriate donor selection, a well-designed hospital environment with filtered air, rigorous infection control practices (hand-washing) uncontaminated food and water, restricting contact with infectious cases and vaccinating patients, staff and close social contacts. When infectious exposures do occur, the emphasis shifts to preventing the development of symptomatic disease (secondary prevention) using prophylaxis or pre-emption. Prophylactic drugs (isoniazid for TB, ganciclovir for CMV, Bactrim for PCP and toxoplasmosis) and disease-specific immunoglobulins (VZIG) are examples of strategies used to neutralise infections, once they have occurred. Pre-emptive strategies rely on sensitive diagnostic methods (antigen tests, serological markers and molecular methods) to detect early, active infections and so guide the appropriate use of antimicrobial therapy to prevent the manifestations of disease. When patients develop symptoms of disease, the therapeutic aims are directed towards alleviating suffering, reducing morbidity and preventing mortality (tertiary prevention). The treatment of symptomatic disease in transplantation is complicated by high drug costs, drug toxicities, drug interactions, impaired host immunity and the frequent lack of quality evidence on which to base difficult clinical decisions.

Overview of future stem cell transplant in Sri Lanka

Dr. Prasad Abeysinghe

There are essentially two types of HSCT, based on the source of the blood stem cell – autologous or allogeneic donor transplants.

Autologous stem cell transplant is reserved for Multiple Myeloma and relapsed Lymphoma where randomized trials in both diseases, have confirmed an overall survival benefit of HSCT over standard of care. This procedure requires collection of blood stem cells via apheresis which is an intricate procedure utilising the skills of clinicians, nursing and scientific staff. The cells are subsequently cryopreserved in liquid nitrogen until required for the autologous HSCT.

Allogeneic donor transplants can be from a variety of sources such as siblings, matched unrelated donors (matched for 8/8 Human Leukocyte Antigens from worldwide donor tissue banks), haploidentical transplants (where the match is from a family member who is only half matched, HLA 4/8) or cord blood stem cell transplants. Allogeneic stem cell transplant is generally used for acute leukaemias as there is thought to be an immune attack of donor T cells on residual leukaemia remaining in the host after conditioning – the putative graft versus leukaemia (GVL) effect. This GVL effect is however offset by the toxicity of the donor's immune cells attacking the patient as well – known as graft versus host disease (GVHD). The combination of GVHD and infection confer significant risk on the recipient making allogeneic stem cell a procedure with significant potential morbidity and mortality (approximately 15-20% at 100 days post HSCT). However, allogeneic HSCT is clearly capable of curing patients with leukaemias.

A BMT unit is a long overdue facility for Sri Lanka and at present many patients needing BMT and allied services are compelled to go overseas primarily to India and Singapore incurring very high costs. National Cancer Institute combined in a bilateral partnership with the Haematology Department at St. Vincents Hospital to foster training, mentorship and professional development. Staged process over the short term with a view to long term 'twinning' between St Vincents Hospital and the National Cancer Institute (NCI) and the National Blood Transfusion Service (NBTS) in Colombo.

This program will foster leadership in haematology in SL whilst maintaining expertise and resources in Colombo. Knowledge and skills of HSCT will be exchanged between the three units creating significant local expertise in scientific, nursing and medical staff in Sri Lanka. The time frame of 1-4 months training in Australia followed by regular visits and teleconferencing with the St Vincents Hospital hosts make the objective of a stand alone HSCT unit in Colombo a reality over the next 2-5 years.

Preventing invasive fungal infections in stem cell transplant recipients: Writing a policy – Leicester, UK Model

Dr. Nelun Perera

Reducing the burden of invasive fungal infections with directed antifungal therapy in a hematopoietic stem cell transplant unit – is it cost effective; sharing the experience of Leicester, UK

Invasive fungal Infections (IFI) are associated with a high mortality and morbidity amongst patients with hematopoietic stem cell transplantation (HSCT). Diagnosing IFI early has always been a challenge to both the transplant clinician and the microbiologist. The

mainstay of diagnosis is demonstration of typical manifestations consistent with IFI by radiology. Laboratory diagnosis of IFI is sought with difficulty. Traditional culture methods have a low sensitivity and can take several days. An alternative approach is to detect fungal biomarkers in blood. These tests have varying sensitivity and specificity. Commencing empiric antifungal drugs, when fever is not responding to broad-spectrum antibiotic treatment amongst patients with HSCT, has a long and established history. The approach is known to reduce mortality due to early initiation of antifungal drugs. However, it can be associated with a high antifungal expenditure and exposure of the patients to unnecessary toxicities of the drugs. An alternative approach is "directed therapy". This is initiation of treatment with antifungal drugs when IFI is highly likely due to detection of fungal biomarkers in the patient's blood or demonstration of radiological manifestations of IFI. The approach has been shown to reduce the antifungal expenditure without compromising patient outcomes.

The challenges, benefits and the cost of introducing a robust targeted approach to managing IFI in a HSCT unit in Leicester, UK will be presented.

Symposium 3

Types of pulmonary aspergillosis and latest methods for investigation

Prof. Arunaloake Chakrabarti

With the increased prevalence and attention to pulmonary aspergillosis, the clarity on the clinical spectrum of the disease has improved. The clinical types of the disease depend on the interaction of host and the fungus. The spectrum is different in immunosuppression, structural defects and immune hyperactivity of the hosts. Acute invasive disease develops in severely immunocompromised patients. Chronic pulmonary aspergillosis affects patients with underlying lung disease like chronic obstructive pulmonary disease (COPD), sarcoidosis, prior or concurrent pulmonary tuberculosis or non-tubercular mycobacterial disease. *Aspergillus* bronchitis may develop in patients with bronchiectasis or cystic fibrosis, without any parenchymal lesion. Severe asthma with fungal sensitization (SAFS) and allergic bronchopulmonary aspergillosis (ABPA) are associated with immune hyperactivity in genetically susceptible patients. The various types of pulmonary aspergillosis may be a semi-continuous spectrum of the disease depending on immunity of the host. In recent years, the spectrum of susceptible population is changing with possible adaptation of *Aspergillus* in host and accelerated evolution of virulence in the fungus. Acute invasive disease is seen increasingly in non-neutropenic hosts like chronic liver or renal failure, critically ill patients; even in normal host after massive exposure. Therefore, a high index of

suspicion is required to diagnose the disease in non-classical patients. The disease may be silent and patients may be asymptomatic and afebrile in the early stage. The asymptomatic colonization of the respiratory tract by *Aspergillus* also requires continuous monitoring, as invasion may occur any time in ongoing immunosuppression and the disease has considerable morbidity and mortality. Early and accurate diagnosis help in timely therapy, which is crucial for survival. The present imaging and conventional diagnostic tests including microscopy, histopathology and culture face limitation due to non-specificity, difficulty in collection of samples from lung tissue, delay in turn-around time. Many transformational improvement in diagnostics are noted in last decade. The CT-pulmonary angiography has improved the specificity of diagnosis of acute invasive aspergillosis. CT guided fine needle aspiration, aggressive lung biopsy, incorporation of MALDI-TOF, extraction of DNA from tissue and sequencing have improved conventional diagnostic potentials. The biomarkers like galactomannan, beta-D glucan and improved PCR technique are now incorporated in algorithm of diagnosis of acute pulmonary aspergillosis. *Aspergillus* antibody screening helps in suspecting chronic pulmonary aspergillosis. The consensus diagnostic protocols for SAFS and ABPA have helped in better understanding of the disease. The point-of-care tests like lateral flow assay, proximity ligation assay, breadth test for metabolite signature etc. are promising and may help in early diagnosis of acute invasive disease. Despite the above improvement in diagnosis, the mortality in pulmonary aspergillosis has not come down in developing countries due to patchy accessibility of latest investigations.

Pulmonary aspergillosis clinical scenarios and situation analysis in Sri Lanka

Dr. Amitha Fernando

Aspergillus is a spore forming fungus with a worldwide distribution. Its primary route of access is by inhalation.

The spectrum of *Aspergillus* associated lung diseases comprise of three well-defined entities.

- 1) Allergic Aspergillosis
 - IgE-Mediated *Aspergillus*-sensitized asthma.
 - Allergic Broncho Pulmonary Aspergillosis (ABPA).
 - Hypersensitivity Pneumonitis
- 2) Saprophytic colonization
 - Aspergilloma
 - Chronic cavitary pulmonary aspergillosis
- 3) Invasive disease
 - Acute invasive pulmonary aspergillosis
 - Chronic necrotizing pulmonary aspergillosis

The rate of invasive and allergic *Aspergillus* infections has shown a global surge in recent decades. Those at risk include those on immune-suppressive medication post-solid organ transplant, hematopoietic stem-cell transplant and those with autoimmune diseases.

In 2010 from TB and other respiratory death rates the prevalence of post-TB chronic pulmonary aspergillosis (1,443), and all forms of chronic pulmonary aspergillosis (2,886) was estimated (1).

Based on an Acute Myeloid Leukaemia (AML) incidence of 3/100,000 and over 500 renal transplants 229 cases of invasive aspergillosis was estimated (1).

Asthma affects 2.75% of adult population, assuming ABPA prevalence of 2.5%, 10,334 cases are estimated. In those 10% with severe asthma ABPA and fungal sensitization prevalence increases to 33%, estimated cases of 13,654 patients (1).

Published data on *Aspergillus* pulmonary diseases is scarce in Sri Lanka.

Individual experiences and published case reports suggests that *Aspergillus* related pulmonary diseases are increasingly being reported and will pose serious challenges in the diagnosis and management.

It will require a concentrated effort, increase awareness, increase access to diagnostic tools, develop standardized management protocols and collect standardized data.

This will enable better patient care and health policy planners to meet present and future challenges posed by *Aspergillus* pulmonary disease.

Reference

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OP 1

Comparison of community acquired (CA) and hospital acquired (HA) methicillin resistant *Staphylococcus aureus* (MRSA) isolates in the National Hospital of Sri Lanka

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Introduction

MRSA is a bacterium that is resistant to most antibiotics. Highly virulent CA MRSA has caused infections in healthy young adults recently.

Design, setting and methods

Descriptive cross sectional study of consecutive 100 MRSA isolates from clinical and screening samples was performed to assess the demographics, antimicrobial susceptibility and PVL gene. MRSA isolates were confirmed by standard laboratory methods. CA MRSA and HA MRSA were defined according to epidemiological information. Antimicrobial susceptibility testing was performed by CLSI standards including E test for glycopeptides, MIC and double disk test for mupirocin susceptibility. The presence of PVL gene was determined by conventional PCR.

Results

Out of 100 isolates 21(21%) were CA MRSA and 79(79%) were HA MRSA isolates. No significant difference was observed among age (<45 years), gender, ethnicity (Sinhalese/non Sinhalese) between groups. Ninety two samples were clinical and eight were screening samples. Majority of samples consisted of pus. Among blood and respiratory samples only HAMRSA was found. All MRSA isolates were resistant to penicillin and all isolates were sensitive to rifampin and linezolid. Isolates were sensitive to ciprofloxacin, fusidic acid, tetracycline, cotrimoxazole and gentamicin ($p < 0.001$) among CA MRSA group. Inducible and constitutive clindamycin resistance ($p < 0.001$) and multidrug resistant phenotype ($p < 0.001$) was significant among HA MRSA group. In all isolates, vancomycin MIC₅₀, MIC₉₀ and range were 1 µg/ml, 1.5 µg/ml and 0.5 µg/ml to 2 µg/ml. Teicoplanin MIC₅₀, MIC₉₀ and range were 0.5 µg/ml, 1 µg/ml and 0.25 µg/ml to 3 µg/ml. Four percent had high level mupirocin resistance and 2% had low level resistance while 94% were sensitive to mupirocin. All mupirocin resistance isolates were among HA MRSA group ($p < 0.338$). Proportion of PVL gene among CA MRSA group was 95.23% ($p < 0.001$).

Conclusions

CA MRSA and HA MRSA strains differ according to the clinical, microbiological and genetic factors. Clinician should be aware that therapy with antibiotics and infection control practices should be based on the type of MRSA strains.

OP 2

Vancomycin susceptibility among *Staphylococcus aureus* isolates from a tertiary care hospital

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Introduction

Methicillin resistant *Staphylococcus aureus* (MRSA) is a major cause of nosocomial and community infections. Vancomycin is a commonly used therapeutic agent against MRSA infections. *S. aureus* with vancomycin MIC of ≤ 2 µg/mL is considered as susceptible, 4-8 µg/mL as intermediate and ≥ 16 µg/mL as resistant (CLSI, 2015). The effectiveness of treatment with vancomycin has been questioned as there were reports of vancomycin treatment failures.

Objectives

1. To find out the minimum inhibitory concentration (MIC) of vancomycin in clinical *S. aureus* isolates
2. To compare the vancomycin MIC values of MRSA and MSSA isolates

Design, setting and methods

A descriptive study was carried out on all laboratory isolates of *S. aureus* recovered from Teaching Hospital, Peradeniya from April to November 2015. The isolates were confirmed as *S. aureus* by using Gram stain, catalase test, slide and tube coagulase and DNase test. *S. aureus* isolates were tested for methicillin susceptibility by CLSI disc diffusion method using cefoxitin 30 µg antibiotic discs (CLSI, 2015). Vancomycin MICs were obtained by micro broth dilution method according to CLSI 2013 guideline.

Results

Total of 104 laboratory isolates of *S. aureus* including 62 MRSA and 42 MSSA were recovered during the study period. Out of that, 92(88%) were from pus specimens, 08(8%) were from blood cultures and 4(4%) from sputum samples. Vancomycin MIC values of all *S. aureus*

were range from 1 µg/mL to 2 µg/mL. 64 isolates had vancomycin MIC of 1 µg/mL and 40 isolates of 2 µg/mL. Among 62 MRSA, 37 had MIC of 1 µg/mL and 25 had 2 µg/mL. There were 27 MSSA with vancomycin MIC value of 1 µg/mL and 15 MSSA with MIC of 2 µg/mL. MIC₅₀ and MIC₉₀ of both MRSA and MSSA isolates were 1 µg/mL and 2 µg/mL respectively.

Conclusions

MIC values of ≥ 1 µg/mL were obtained for all *S. aureus* isolates irrespective of methicillin sensitivity which needs special consideration. Vancomycin resistance was not detected among our isolates. According to the MIC₅₀ and MIC₉₀ values, no significant difference was found between MIC values of MRSA and MSSA.

OP 3

MLST analysis of *Burkholderia pseudomallei* isolates from Sri Lanka reveal a large number of novel STs

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Introduction

Burkholderia pseudomallei is a Gram-negative, soil-dwelling, β -Proteobacteria bacterium with one of the most complex genomes. It is found in tropical and subtropical regions causing melioidosis, a severe disease with a broad spectrum of clinical presentations, endemic to Southeast Asia and Northern Australia. Sri Lanka is situated in the endemic belt and we started a project screening undifferentiated fever cases to identify potential melioidosis cases in Sri Lanka. This screening increased the number of culture confirmed cases dramatically and highlighted a large genetic diversity among the local strains.

Design, setting and methods

We employed multi-locus sequence typing (MLST), a molecular typing technique, to differentiate Sri Lankan isolates from each other and to others found worldwide. This data was submitted to the international *B. pseudomallei* database (<http://pubmlst.org/bpseudomallei/>).

Results

In this study a total of 76 strains from recent (years 2014-2015), all clinical isolates, were genotyped. MLST analysis revealed that they belonged to 36 different STs of which 23 STs were novel at the time of submission. 35

isolates belonged to 8 of the previously described Sri Lankan STs and, in agreement with the previous findings, ST1137 still remain the commonest ST in Sri Lanka. There were 5 shared STs (10 isolates), out of which 3 were exclusively seen in Southeast Asian region. Surprisingly, one of our isolates belonged to ST132, an exclusive Australian ST which is seen among clinical, animal and environmental isolates. A higher resolution genotyping approach (i.e., whole genome sequencing) is needed for a comprehensive comparison between these strains and to uncover as to how a dominant exclusive Australian ST is present in Sri Lanka or whether this is merely the result of homoplasy.

Conclusions

As of April, 2016, Sri Lanka has the largest representation of all the South Asian countries in the international *B. pseudomallei* database with a total of 108 isolates accounting for more than 2.4% of the entire database. The high number of STs (46) suggests that a large genetic diversity exists.

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OP 4

Prevalence of nasal colonisation with potential pathogens and associated factors in children less than 5 years

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Objectives

Peak incidence of respiratory tract infection occurs in children in the first five years of life, with the common bacterial pathogens being *Haemophilus influenzae*, *Streptococcus pneumoniae* and *Moraxella catarrhalis*. Colonisation of the respiratory tract with potential pathogens increases risk. Colonisation rates vary widely with locality. This study aimed to determine the prevalence of nasal colonisation with potential pathogens in healthy children in Biyagama and to describe factors associated with colonisation and antibiotic sensitivity patterns.

Design, setting and methods

A cross-sectional descriptive study was carried out at immunization clinics of the Biyagama MoH area between December 2013 and March 2014 among healthy children less than 5 years of age. Demographic data were collected using a pre-tested interviewer-administered questionnaire. Nasal swabs were collected, transported in Amies transport medium with charcoal and cultured on 5% sheep blood and chocolate agar. For

S.pneumoniae antibiotic sensitivity tests were performed by disk diffusion according to the Clinical and Laboratory Standards Institute guidelines. Minimum inhibitory concentrations (MIC) of penicillin for 78 of 105 pneumococcal isolates were determined using E-strips. Antibiotic sensitivity testing of *H.influenzae* and *M.catarrhalis* was done using Stoke's method and β -lactamase testing using the chromogenic cephalosporin method (nitrocefin).

Results

Of 391 children, 165 were positive for carriage of at least one potential pathogen. The prevalence of *S. pneumoniae*, *M.catarrhalis* and *H.influenzae* carriage was 26.8%, 25%, and 7.6% respectively. Only 9% of *S.pneumoniae* isolates were oxacillin sensitive but 98.7% had MICs to penicillin ≤ 2 μ g/ml. β -lactamase positive in *M.catarrhalis* and *H.influenzae* were 93.9% and 20% respectively. All isolates were sensitive to levofloxacin but sensitivity to other antibiotics varied. Nasal carriage was associated with age of child >9 months compared to 2-6 months ($p=0.000$), recent antibiotic use ($p=0.005$), having one or more sibling ($p=0.000$), and having an older sibling compared to having no or younger sibling ($p=0.003$).

Conclusions

Relatively high rates of colonisation with *S. pneumoniae* and *M.catarrhalis*, but not *H.influenzae*, were found in children less than 5 years.

Financial assistance by Medical Research Institute, Colombo is acknowledged.

OP 5

Serotype-specific detection of dengue viruses in a multiplex real-time reverse transcriptase PCR assay

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Introduction

Dengue (DENV) has re-emerged through epidemics involving all 4 serotypes. Today co-circulation results higher secondary infection rate. Since 1990s Reverse transcriptase-PCR (RT-PCR) has been used for detection; still many use original hemi-nested RT-PCR with modifications. This assay requires rounds of amplification followed by gel electrophoresis, requires one /more days, carrying risk of contamination; limits the clinical utility. The study objective is to describe a multiplex, real-time RT-PCR (rRT-PCR) that allows for detection and serotyping of DENV in a single reaction.

Design, setting and methods

This retrospective study was carried out with residual volumes of routine clinically dengue suspected samples received from September 2015 through February 2016. Considering patient data, 80 samples were (samples at day of illness 4-10) tested using below described assay. Positive samples for DENV-RNA were analysed with sample collection day and anti-DENV IgM result.

This multiplex rRT-PCR assay utilizes the Waggoner *et al* previously validated primers, probes and cycling conditions. In a single reaction (tube), all primers and probes accomplish both reverse transcription and cDNA amplification. Reaction targets, highly conserved 5' untranslated region and capsid gene, improved sensitivities. Genomic RNA from all 4 serotypes included as control strains. Viral RNA was extracted using Qlamp Viral RNA Mini kit. The assay carried out using SuperScript III Platinum One-Step qRT-PCR kit, and performed on Applied Biosystems 7500 instrument.

Results

Total 80 samples tested, 68(85%) determined the serotype, 53(66%) DENV-1, 11(14%) DENV-4, 3DENV-3 and 1DENV-2. 12(15%) did not detect /indicate the serotype. Samples were collected relatively late in the clinical course, mean day of illness was 7. All gave detectable anti-DENV IgM result with capture Dengue IgM ELISA assays.

Conclusions

Study reveals all 4 serotypes and demonstrates clinical sensitivity in patients with all 4 serotypes. Data convinced, improved sensitivities has the ability to detect DENV-RNA in samples collected relatively late in the course of illness and also with detectable anti-DENV IgM, thereby lengthening the period of time for molecular diagnosis. This sensitive single-reaction format represents a step forward in dengue diagnostics.

OP 6

Co-infections with multiple dengue virus serotypes in patients from 3 different Provinces of Sri Lanka

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Introduction

The circulation of multiple dengue viral (DENV) serotypes in a same locale has caused co-infections with mixed serotypes in individuals due to subsequent or simultaneous infections.

Objective

The objective of the present investigation was to study the clinical presentations together with reverse transcription PCR (RT-PCR) and serology of co-infections to identify pattern of disease severity in co-infected patients from 3 different Provinces of Sri Lanka.

Design, setting and methods

Clinically diagnosed dengue fever (DF)/ dengue haemorrhagic fever (DHF) patients from Teaching Hospitals, Jaffna and Kandy and General Hospitals, Gampaha and Negambo with fever days less than 5 were included in the study. Clinical and haematological data were also assessed. DENV capsid gene detection was performed followed by DENV sero-typing by a series of RT-PCR. Anti-DENV IgM/IgG detection was performed using ELISA.

Results

Of the 1249 patients, RT-PCR was positive in 329 from 2009 to 2012. Of the 329 RT-PCR positive patients, 34/329 (10.33%) had co-infections with two or more DENV serotypes. All 4 DENV serotypes were found to be co-circulating during the study period and DENV-1 was the predominant type circulated in all 3 Provinces. Highest number of co-infection (17/34) occurred with DENV-1 and DENV-2. Of the 34 co-infected patients, 24 had DF and the rest had DHF. Sixteen primary and 28 secondary infections were identified in the study cohort. Of the 16 primary infections, 12 were DF and 4 were DHF. Of the 28 secondary infections, 22 were DF and 6 were DHF. No significant difference was noted between the total white blood cell count and platelet counts in mono- and co-infections.

Conclusions

In this population DENV-1 was the dominant serotype followed by DENV-2. Presence of DENV co-infections in all 3 Provinces indicates the hyperendemicity of DENV in the country. The absence of significant association of disease severity between the mono- and co-infections points out the progression of the disease into severe forms driven by non-viral factors. The presence of DENV co-infections may lead to recombination of genetic components contributing to the emergence of new DENV strains that might be more virulent and aggressive in causing severe dengue.

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OP 7

Prevalence of subtypes of respiratory syncytial virus and association of subtypes with disease severity in a selected group of children with acute lower respiratory infection

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Introduction

Respiratory syncytial virus (RSV) causes significant morbidity and mortality among young children mainly less than 5 years of age. Molecular epidemiology is important for the ongoing vaccine development initiatives. Association between subtypes of RSV and disease severity has not been studied in Sri Lanka so far.

Objective

To detect the prevalence of RSV subtypes and association between subtypes of RSV and disease severity in a selected group of children with severe acute lower respiratory infection.

Design, setting and methods

Respiratory samples (NPS, NPA) that were positive for RSV by immunofluorescent assay and clinically suspected RSV were retrospectively analysed. Extracted RNA was subjected to real-time multiplex PCR assay to detect RSV subtypes. Demographic, clinical and disease severity data were obtained from the request forms submitted to the laboratory.

Results

Out of 101 children (mean age = 2.91 ± 2SD 6.6 years), 39 (38%) were positive for RSV A, 12 (12%) were positive for RSV B and 7 (7%) were positive for both RSV A and RSV B. Average age of RSV A, RSV B and RSV A & B positives were 35.9, 12.3 and 5.5 months respectively (range 2 months to 11 yrs.). RSV A=49%, RSV B=67%, RSV A & B = 84% positives were found in less than 1 year age group. Male: female ratio for RSV A, RSV B and RSV A & B positives was 4:3, 2:1 and 7:5 respectively (3:2 in total cohort). Complete clinical data were only available in 35 children.

RSV subtype	Total	Broncho-pneumonia	Nebu-lized	Intensive care needed	Venti-lated	Death
RSV A infection	21	13	10	6	4	0
RSV B infection	9	4	5	2	1	0
Mix (RSV A & B) infection	5	2	4	1	0	1

There was no statistical significant association between RSV subtypes and clinical severity (Chi-square chart p value > 0.5) in 35 children whose complete data were available.

Conclusions

RSV was found to be associated with severe acute respiratory infection and there was no statistical significant association of RSV subtype with clinical severity in this group of 35 children. RSV subtype A was the main subtype in this cohort, which is also the main subtype globally. Genetic sequencing is ongoing for further characterizing of genotypes.

OP 8

The effects of storage temperatures and evaluation of open vial policy on potency of live trivalent oral poliomyelitis vaccine (tOPV) in Sri Lanka

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Introduction

Live attenuated polio vaccine is the most heat sensitive of all the vaccines. The storage temperature of unopened tOPV recommended by WHO is -20°C. According to the open vial policy, multi-dose vials of opened tOPV can be used up to a maximum of 28 days stored at (5±3)°C. WHO requires thermostability of all vaccine vials at 37±1°C for 48 hours.

Objectives

1. To determine the total virus content (TVC) in unopened tOPV vials stored at different temperatures (°C); (22±1), ambient temperature (28±1), with different durations of storage (hrs.); 12, 24, 48, 72 and 7 days and at (37±1)°C after 12, 24 and 48hrs
2. To determine the TVC in opened vials of tOPV stored at (5±3)°C with different time intervals; 24hrs., 48hrs., 7, 14 and 28 days

Design, setting and methods

Two, 20 dose vials from one batch of tOPV were stored at each storage condition mentioned above. The TVC was determined with Hep-2^{Cincinnati} cell line according to the WHO recommended protocol. The TVC in CCID₅₀ per single human dose (SHD) was calculated. A working reference was used in each assay and was done in duplicate.

Results

The potency of unopened and opened tOPV vials tested at each storage condition (100%) ranged from log₁₀ 5.82 to 6.74. According to the protocol, the minimum TVC per SHD to pass the potency test of the tOPV is log₁₀ 5.8 CCID₅₀/ SHD. Therefore, the potency of all the tested vials was found to be within acceptable limits. Thermostability requirement was fulfilled after exposure to 37°C for 48hrs.

Conclusions

This study did not show a correlation between the length of time, temperature exposure and TVC within tested time and conditions. Adequate potency of vaccines tested in this study confirms the potency of tOPV even after the breakdown of cold chain within tested conditions. Opened vials maintained at (5±3)°C up to 28 days proves the safety of practicing WHO recommended open vial policy in Sri Lanka.

Financial assistance by MRI for research grant 22/2011 is acknowledged.

OP 9

An immunogenicity study on intradermal pre-exposure rabies vaccination in Sri Lanka

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Introduction

Intradermal (ID) administration of rabies pre-exposure therapy has been recommended by the World Health Organization (WHO) since 2005 and is practiced in several countries in Asia. Presently, the intramuscular rabies pre-exposure therapy is recommended for high risk groups in Sri Lanka. As more than 95% of hospitals in Sri Lanka are now practicing ID post-exposure therapy, switching over to ID pre-exposure therapy will reduce the cost and confusion created among medical officers regarding post-exposure management of these individuals following a subsequent exposure to the rabies virus.

Objective

The main objective is to assess the immunogenicity of intradermal pre-exposure rabies vaccination schedule in a Sri Lankan population before its introduction.

Design, setting and methods

This is a prospective cohort, single-center study. Ethical clearance was obtained from the Ethical Review Committee of Medical Research Institute and Faculty of Medicine, University of Peradeniya. Volunteers (70 participants) were enrolled to the study from first year veterinary students from University of Peradeniya. Single dose of 0.1mL of Purified Chick Embryo Cell Rabies vaccine was administered intradermally on Day0, Day7, Day28 (primary course of vaccination) and Day365 (1 year booster dose). Rabies virus neutralizing antibody titres (RVNAT) were assessed prior to the commencement of vaccination (Day0), 3 weeks after primary course of vaccination (Day49), before giving 1 year booster and 3 weeks after 1 year booster. Rapid Fluorescent Focus Inhibition Test was used to determine the antibody titres.

Results

None of the participants had protective RVNAT (WHO recommended protective level of RVNAT is ≥ 0.5 IU/mL) on Day0 and all of them (100%) developed adequate antibody titres (>0.5 IU/mL) on Day49. At one year, 15 participants (23%) had <0.5 IU/mL which is comparable with ID and IM studies done in other countries. All participants (100%) including ones who had titre <0.5 IU/mL developed rapid booster response with no difference, 3 weeks after the one year booster with a 25-fold rise showing an accelerated immune response.

Conclusions

This study shows, that the WHO recommended ID pre-exposure schedule of rabies vaccination is immunologically efficacious in the Sri Lankan population.

Financial assistance by Medical Research Institute for research grant 28/2012 is acknowledged.

OP 10

Comparison of BK virus viraemia and viraemia in post renal transplant patients – A single centre study

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Introduction

BK virus (BKV) nephropathy is an important cause of graft failure in post renal transplant (PRT) patients. As there is no successful treatment for established BKV nephritis, early detection and intervention is important to prevent the disease. Screening can be done with blood/urine using polymerase chain reaction (PCR). BKV had not been studied in Sri Lanka so far and the magnitude of the problem is unknown.

Objectives

1. To describe the presence of BKV in blood and urine in the sample population
2. To compare the BKV loads in urine and blood

Design, setting and methods

A hospital-based, descriptive cross-sectional study. Blood and urine samples were obtained from 95 in ward and clinic patients in a single nephrology unit at the National Hospital of Sri Lanka, within 2 years of transplantation. DNA was extracted using a commercial kit. Quantitative real-time PCR was performed using a commercial PCR assay. Statistical analysis was by SPSS.

Results

There were 54 males and 41 females, median age was 42y [Inter Quartile Range (IQR) 28-53y]. Median time duration after transplantation was 8 months (IQR 3-15). BKV was detected in urine and in blood in 58 (61%) and 10 (10.5%) patients respectively. Median viral load in urine was 2794 copies/ml (IQR 244-260,000), range 8.66- 2.7×10^9 copies/ml. Median blood viral load was 3038 copies/ml (IQR 462-9591), range: 12-61,233 copies/ml. All the patients with viraemia had urine viral load $>100,000$ copies/ml. Viral load in urine was always much higher than that in blood. The difference ranged from 2.1×10^5 to 2.6×10^9 copies/ml. There were significant positive correlations between viraemia and viruria (Pearson's correlation 0.55); and viruria and the difference between viruria and viraemia (Pearson's correlation 0.99).

Conclusions

BKV was commonly detected in urine/blood in this study sample. Viraemia was seen only at high levels of viruria. Viral load in the urine was always much higher than that in blood and the loads had a positive correlation with each other. Prospective studies are indicated to determine cut off values for the Sri Lankan population.

OP 11

Innate cellular immune responses in cutaneous leishmaniasis against *Leishmania-donovani*

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Introduction

Leishmaniasis is a neglected tropical disease caused by parasitic protozoa of the genus *Leishmania*. It has a spectrum of manifestations including cutaneous, mucosal and visceral disease. The clinical outcome of infection in humans is determined primarily by the infecting species

and the immune response by the host. Sri Lanka is endemic for localised cutaneous leishmaniasis (LCL) caused by *Leishmania donovani*; a species which usually causes visceral disease. The aim of this study was to characterize the immune response in LCL by macrophages; the cells responsible for survival as well as eventual elimination of the parasite.

Design, setting and methods

Peripheral blood mono nuclear cell (PBMC) derived macrophages from newly diagnosed LCL patients (n=8) and healthy non endemic controls (n=8) were stimulated with *L. donovani* antigen (50µg/ml) *in vitro*. The production of IL-10, TNFα and Nitric Oxide (NO) were measured by ELISA and Griess reaction at predetermined time intervals. The differences between experimental groups were analysed using the Student's t-test for parametric data and Mann-Whitney test for non-parametric data.

Results

Macrophages from patients produced more cytokines and NO at all time points. IL-10 production by patient macrophages was significantly higher (105.68 ± 26.05 vs 19.81 ± 28.24 pg/mL; $p < 0.01$) at 72 hours but did not vary markedly at 24 and 48 hours. TNFα production by patient macrophages was significantly higher at both 24 hours (23.05 ± 13.97 vs 4.01 ± 2.26 pg/mL; $p < 0.01$) and 48 hours (311.33 ± 206.29 vs 17.61 ± 21.09 pg/mL; $p < 0.01$). Levels of production of NO remained similar at 24 and 48 hours but showed increased levels by patient macrophages at 72 hours (5.40 ± 1.15 vs 2.36 ± 1.21 pg/mL; $p < 0.01$).

Conclusions

These data suggest that IL-10, TNFα and NO play a role in determining disease outcome in LCL due to *L. donovani*. In contrast to TNFα, the contribution of IL-10 and NO appear to be later in the infection. The findings should be interpreted in the context of changes in other inflammatory mediators, to better understand the underlying pathogenic mechanisms where a visceralizing *Leishmania* species is localized to the skin.

OP 12

Enterobiasis in the Western and Eastern Provinces of Sri Lanka

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Introduction

Enterobiasis caused by *E. vermicularis* is a worldwide helminth infection occurring among all socioeconomic

classes. Although Sri Lanka has achieved great strides in the control of intestinal helminthiasis, enterobiasis continues to be a problem. This is a preliminary report of an ongoing national survey.

Objectives

To determine the prevalence of enterobiasis among primary school children in the Western and Eastern Provinces of Sri Lanka.

Design, setting and methods

A cross sectional descriptive study was done among year one children in 31 selected government schools of the Western (n=18) and Eastern (n=13) Provinces from April 2013 to May 2014. Infection was diagnosed using adhesive cellophane peri-anal swabs obtained on two consecutive days.

Results

Of 722 children recruited, 423 (58.58%) and 299 (41.41%) were from Western and Eastern Provinces respectively. Of them 341 (80.6%) of Western and 285 (95.3%) of Eastern Provinces returned the swabs with an overall compliance rate of 86.7% (female: male ratio 1.03:1, mean age 6 years). The prevalence of infection by double swab examination was 27.27% and 21.05% in the Western and Eastern Provinces respectively with an overall rate of 24.44%. Single swab positivity rate was 12.78%. The prevalence according to districts was 31.17%, 26.89%, 25.52%, 23.29 and 18.71% in Kalutara, Colombo, Gampaha, Ampara and Batticaloa respectively. The rate of infection in schools varied from ≤10% (n=4) to ≥50% (n=2).

Conclusions

Enterobiasis is a common problem among primary school children in both Western and Eastern Provinces, indicating the need for more targeted control activities.

OP 13

Antifungal sensitivity profile of *Fusarium* spp. resulting keratitis – A preliminary study

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Introduction

Fungal keratitis is an important cause of visual impairment and blindness. Among the diverse fungi responsible for fungal keratitis, genus *Fusarium* is a leading cause worldwide. *Fusarium solani* is the commonest isolate and accompanied with worse outcome compared with non-solani spp. Genus *Fusarium* have been reported to exhibit higher degree of resistance to antifungal agents. Keratitis due to *Fusarium* species is a continuous therapeutic

challenge to ophthalmologists due to their therapy-refractive nature.

Objectives

1. To identify *Fusarium* isolates, causing fungal keratitis from corneal specimens received at MRI from 2013-2016, to species level.
2. To determine antifungal susceptibility pattern of *Fusarium* isolates, isolated from corneal specimens.

Design, setting and methods

All *Fusarium* isolates (51) obtained from specimens of patients with keratitis received at Department of Mycology, MRI from January 2013 to March 2016 were included in the study. Speciation of *Fusarium* isolates up to species level was done using morphological characteristics of fungi. Antifungal sensitivity testing was done according to CLSI M 51- A and epidemiological cut off values (ECV) were obtained against amphotericin B (10 µg), itraconazole (10 µg and voriconazole (1 µg). Results were analyzed using SPSS software.

Results

Fifty-one *Fusarium* isolates were sub cultured from stock cultures. Five isolates were contaminated and excluded. Majority of the isolates were *F. solani* complex (n=24) followed by *F. chlamydosporum* (n=15), *F. dimerum* (n=2), *F. nygamai* (n=1) and *F. proliferatum* (n=1). Three isolates were difficult to speciate morphologically. Forty five isolates (97.82%) were having zone diameters more than corresponding ECV for voriconazole. Forty three (93.47%) isolates had lower zone diameters compared to itraconazole ECV. For amphotericin B, 32 isolates (69.56%) had lower zone diameters compared to ECV. ECV was corresponding to MIC more than 1µg/ml for all 3 drugs. All *F. solani* complexes were resistant to itraconazole.

Conclusions

Majority of isolates belonged to *Fusarium solani* complex. Morphological identification cannot be used as the only method for speciation of *Fusarium* isolates. Antifungal sensitivity testing should be done for all *Fusarium* isolates from keratitis patients as resistance is not uncommon for commonly used antifungal agents.

OP 14

Prevalence of genital chlamydia infection among attendees of the Central Sexually Transmitted Diseases Clinic, Colombo

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Introduction

Chlamydia trachomatis (serovars D-K) is the most prevalent sexually transmitted bacterial pathogen, with the highest prevalence among youth. Many infected persons do not seek medical care as a large proportion of them remain asymptomatic in both females and males.

Objectives

1. To determine the prevalence of genital chlamydia infection in relation to socio-demographic characteristics, clinical presentation and sexual and STD related risk behaviour
2. To measure the significance of associated factors for genital chlamydia infection
3. To identify the most significant risk factors for genital chlamydia infection

Design, setting and methods

A cross-sectional, descriptive study was carried out on 216 female and 252 male attendees of the Central STD Clinic, Colombo. Endocervical swabs from females and urine samples from males were tested with COBAS TaqMan v2.0 Real-Time Polymerase Chain Reaction. A questionnaire was used to gather socio-demographic data, clinical features and factors associated with infection. Significance of associated factors was determined using a Chi-square test and most significant risk factors were identified by applying binary logistic model.

Results

Prevalence of genital chlamydia among female attendees was 17.1% (n=37) and among males was 5.2% (n=13). Prevalence among commercial sex workers (CSW) was 20.4% (20/98, p<0.001) and men having sex with men was 1.5% (1/65, p>0.05). Among infected females 67.6% (25/37, p=0.797) were asymptomatic with 80% (16/20, p=0.508) of infected female sex workers being asymptomatic. Among positive males, 61.5% (8/13, p=0.009) were symptomatic. Vaginal/urethral discharge was the commonest symptom (27%, 10/37) and cervical discharge (40.5%, 15/37) was the commonest clinical sign in females. Urethral discharge and dysuria were the commonest clinical presentations (both 38.5%, 5/13) in males. Female gender, age ≤ 25 years and exposure to commercial sex partners were the most significant risk factors (OR=3.942, 95% CI 1.896–8.198, OR=2.142, 95% CI 1.083–4.235 and OR=1.978, 95% CI 1.039–3.764, respectively).

Conclusions

Prevalence of genital chlamydia infection among female attendees is high and many are asymptomatic. Being a CSW and symptomatic presentation in male were significant associations. Female gender, age ≤ 25 years and exposure to commercial sex partners are significant risk factors.

National STD/AIDs Control Programme is acknowledged for financial assistance.

OP 15

Prevalence of subtypes of parainfluenza virus in a selected group of children with severe acute respiratory symptoms

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Introduction

Parainfluenza viruses (PIV) are an important aetiological agent of severe acute respiratory infection in children. Prevalence of subtypes of PIV in Sri Lanka is not known.

Objectives

1. To detect the occurrence of parainfluenza subtypes in a selected group of children admitted to Lady Ridgeway Hospital from March 2014 to January 2016 with severe acute respiratory symptoms, using rtPCR.
2. To analyze the association with clinical features, laboratory investigations, morbidity and mortality of PIV infection.

Design, setting and methods

Nasopharyngeal aspirates/swabs were obtained using an aspirator and RNA was extracted using QIAmp RNA Mini-kit. Elutes were subjected to real-time commercial PCR assay for the detection of human PIV specific RNA for the genus *Respirovirus* (PIV-1 and PIV-3) and the genus *Rubulavirus* (PIV-2 and PIV-4) and then further subtyped using an in-house multiplex PCR.

Results

Of the 75 children (mean age = $3.8 \pm 2SD 7.04$ years), twelve (16%) were positive for PIV. Subtypes prevalent were, PIV 3 = 8(66%), PIV 1 = 2(17%), PIV-2 = 1(8%), PIV1/3 = 1 (8.3%). Average age of PIV positives was 19 months (range 4m to 5 yrs), seven were less than 1 year. In the selected cohort male: female ratio was 3:2 and PIV positive male: female ratio was 7:4. Of the PIV positive cohort, haematological investigations averages were WBC = 14.2 cells/ml (range 17.6 to 387.6 cells/ml), neutrophil = 50.1% (range, 18 to 84%) lymphocyte = 44% (range, 16 to 72%), while CRP was elevated (>30) in 3 children (CRP range 13 to 384). There was no bacterial growth in sputum culture in six children while five had bacterial growth. Seven had radiological features of bronchopneumonia. Antibiotics were given to eight PIV positive children. Ten positives were nebulized while three were ventilated. One PIV-1 subtype positive child (1yr 2m) died who also had trisomy 21 anomaly.

Conclusions

Parainfluenza viruses were found to be associated with severe acute respiratory infection in 16% of children and subtype 3 was most prevalent in this cohort. Enhanced surveillance of PIV is needed to study the detailed epidemiology of PIV. To our knowledge, this is the first report of subtypes of PIV in Sri Lanka.

OP 16

Detection of coronaviruses in bat guano collected from the Royal Botanical Gardens, Peradeniya

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Introduction

Up to now four of the six known human CoVs (HCoV) have been shown to be zoonotic with three having association with bats, namely SARS-CoV, MERS-CoV and HCoV-229E. Of the 30 species of bats in Sri Lanka, only common flying fox, *Pteropodidae giganteus* forms large communal roosts on treetops. With current rates of deforestation and urbanization, resultant redistribution of bat habitats to urban and suburban areas may make bats into closer contact with livestock and humans. In that regard the significance of fruit bats in relation to the spread of CoV in Sri Lanka would be of importance.

Objective

The present study was undertaken to identify CoV from bat guano samples collected from the Royal Botanical Gardens, Peradeniya.

Design, setting and methods

A total of 50 bat guano samples were tested. About 1g of fresh bat guano was collected into viral transport medium containing screw capped tubes. Each guano sample was assumed to have originated from a different bat and collected into a separate tube. These tubes were transported in ice to the Virology Laboratory at the University of Peradeniya as quickly as possible. Viral RNA was extracted using QIAGEN RNAeasy kit (Hilden, Germany) and the cDNA was made and was subjected to a nested PCR. The assay involves the use of two sets of consensus primers that eventually resulted in the amplification of a targeted region of 440 bps common to all types of CoV. All samples that indicated a 440bp fragment were considered positive for CoV. Subsequently, the same cDNA of the CoV RNA positive samples were subjected

to sequencing at the Virology Laboratory of the University of Hong Kong.

Results

Eight out of 50 bat guano samples were positive for CoV. Sequencing of the CoV RT-PCR positive samples showed a close relationship of these bat CoV with bat CoV sequences derived from different locations in Sri Lanka and Hong Kong.

Conclusions

Sri Lankan flying foxes (*Pteropus giganteus*) from the Royal Botanical Gardens, Peradeniya appear to be infected with CoV which are phylogenitically related to bat CoV isolated from different locations from Sri Lanka and Hong Kong.

Funding: Universities of Hong Kong and Peradeniya through Grant No- RG/AF/13/38/M.

OP 17

An outbreak of Influenza-A (H₁N₁) from March to August 2015

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Objectives

To report the morbidity and mortality of an outbreak of Influenza in 2015

Design, setting and methods

Samples received at the National Influenza Centre from March to August of 2015, were processed. RNA was extracted and subjected to real-time RT-PCR (CDC protocol). The results of the PCR and demographic and clinical data were analyzed.

Results

Out of 4164 samples tested, 1156 (27.76%) were positive for influenza A/B. 1027 (88.84%) were positive for influenza A and 129 (11.16%) positive for influenza B. The affected age groups were, <1 year 75 (6.69%), 01-04 years 104 (9.28%), 05-09 years 58 (5.17%), 10-14 years 23 (2.05%), 15-49 years 643 (57.36%), 50-64 years 155 (13.83%) and >65 years, 63 (5.62%). Influenza A was further subtyped on selected samples due to limited availability of reagents and logistics. Of the influenza A subtyped, H₁N₁=202 (19.66%), H₃N₂=7 (0.68%) and untyped =30 (2.92%). From the positive females, 367/805 (45.59%) were pregnant, whom were in their 1st trimester 13%, 2nd trimester 41% and 3rd trimester 44% and post partum 2%. 199 (17.2%) positives were from ICUs and 16 of

those were positive for Influenza B, 883 positives (76.38%) were from wards. Higher positivity rate (18%-21%) was expressed when samples were obtained within 2 to 3 days of illness. There were 77 (6.66%) laboratory confirmed deaths, out of which 69 (89.61%) were influenza A, and 8 (10.39%) were influenza B. Mean, mode and range of age of deceased patients in years were 40, 42, 2 months to 90 respectively. Forty seven of the positive influenza A deaths were confirmed as influenza A/H₁N₁ and 12 were untyped. From nine maternal deaths, seven were confirmed as influenza A/H₁N₁. Strains analysis revealed that influenza A/H₁N₁ was A/California/7/2009-like and influenza/B was Yamagata lineage-B/Phuket/3073/2013-like. Co-morbidities were reported in 23 (29.8%) deaths. Most common were diabetes (8), asthma (4), heart disease (4), and chronic renal disease (2). Thirteen patients had more than one co-morbid factor.

Conclusions

Influenza A/H₁N₁ A/California/7/2009-like strain, which was the vaccine strain for the particular year, caused an outbreak with significant morbidity and mortality.

OP 18

Molecular diagnosis of varicella-zoster virus and genotyping of herpes simplex virus by a multiplex real-time PCR

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Introduction

Herpes simplex virus type 1 (HSV-1), type 2 (HSV-2) and varicella-zoster virus (VZV) infection cause mild to trivial muco-cutaneous lesions and severe encephalitis, in different population groups. Since specific antiviral drugs are available for these infections, the availability of sensitive laboratory test for the identification of these viruses are important. This study presents a comprehensive analysis of laboratory tested cases with different specimens for HSV-1, HSV-2, and VZV by a multiplex real-time PCR.

Design, setting and methods

This study included clinically suspected HSV and/or VZV samples from August 2015 to January 2016. 289 samples from both immune-competent and immune-compromised adults and children were tested. They comprised of 255(88%) CSF, 19(7%) blood, 12(4%) swabs and 3(1%) vesicular fluids. For molecular diagnosis DNA was extracted using the QIAmp Viral DNA extraction kit and the Real-Star multiplex real-time PCR kit. Included primers, probes and enzymes were used to carry out the assay. The test were performed on the Applied

Biosystems 7500 platform. The results were analysed and the samples were reviewed with sample quality, clinical profile and epidemiological data.

Results

23(8%) samples were positive, which included 17(74%) VZV, 3(13%) HSV2 and 3(13%) HSV1. 266(92%) were negative. Positives were analysed and revealed 11(48%), 5(21%), 4(17%), and 3(14%) from CSF, swabs, blood, and vesicular fluids respectively. This positive group consisted of neonates (4%), infants (14%), children 1-12 years (26%), and patients above 12 years (56%), also 19(83%) immune-competent and 4(17%) immune-compromised. Of the positives 70% obtained before starting the acyclovir or early course of treatment

(within 1-3 days) and 60% agreed with the cold chain maintenance policy.

Conclusions

The study shows that the multiplex real-time PCR approach allowed simultaneous detection and genotyping of alpha-herpes viruses in different clinical specimens such as CSF, blood, vesicular fluids and lesion-swabs, at any age in immunocompetent and immunocompromised population. Data strongly demonstrated that diagnosis using timely collected and appropriately transported samples can maximize the yield. The negative PCR results may be due to an inconsistency of sample qualities; thus one should be aware that the sample quality would grossly affect the final PCR test result interpretation.

PP 1

Neonatal bacteraemia outbreak by *Burkholderia cepacia* in a Base Hospital

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Introduction

Burkholderia cepacia is capable of surviving in nutrient-poor water. It is intrinsically resistant to most antibiotics. This is the first reported outbreak of *Burkholderia cepacia* in Sri Lanka.

Objective

Investigation of nosocomial outbreak of neonatal bacteremia in a Special Care Baby Unit (SCBU).

Design, setting and methods

This is a retrospective analysis of data obtained during the outbreak, from SCBU of a Base Hospital. Blood cultures were performed using BacT/ALERT® automated system. Environmental samples were enriched in brain heart infusion broth. All isolates were identified up to species level by API 20NE kits. Antibiotic susceptibility was carried out according to CLSI guidelines.

Results

During six days in May 2013, out of fourteen blood cultures, four grew *Burkholderia cepacia*. Four neonates had a mean birth weight of 2.26 kg and a mean gestational age of 35 weeks. Three required ventilatory support. Blood cultures became positive after a mean of 3.5 days since admission. All babies had high CRP and thrombocytopenia. All isolates showed identical antibiogram with susceptibility to meropenem, ceftazidime, ciprofloxacin and resistance to cotrimoxazole and aminoglycosides. All patients were successfully treated with intravenous meropenem.

A 10% dextrose solution prepared in the unit grew *B. cepacia*. It was revealed that the same solution was used multiple times on several babies by different nurses. Other samples that grew *B. cepacia* were normal saline bottle used for preparation of intravenous drugs, gallipot of sterile water used for suction of airways, incubator humidifier and ventilator humidifier. Antibiograms of environmental isolates were identical with patients' isolates.

It was evident during investigation that the unit was not adherent to aseptic techniques during preparation of IV fluids. Hence, unit policy was changed by preparing intravenous fluids twice a day in a clean designated place,

following aseptic methods. Use of single-use normal saline bottles, use of fresh gallipot of sterile water for airway suctioning was recommended. Humidifier cleaning technique was optimized. Outbreak was controlled with these remedial measures.

Conclusions

Discontinuation of the use of multi-dose intravenous fluid and simple hygienic measures were effective in controlling this outbreak of *B. cepacia* bacteremia.

PP 2

Epidemiology of bacterial infections of surgical sites following orthopaedic surgeries at the National Hospital of Sri Lanka

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Introduction

Orthopaedic SSI (surgical site infections) cause considerable physical limitations and reduction of health related quality of life. It makes a major impact on countries like Sri Lanka.

Local data are scarce regarding SSI following orthopaedic surgeries. Knowledge about it will lead to policies on treatment and prevention of SSI following orthopaedic surgeries.

Objectives

1. To describe the epidemiology of SSI following orthopaedic surgeries at NHSL.
2. To determine the incidence rate of SSI following orthopaedic surgeries.
3. To identify the common bacteria and their susceptibility patterns.
4. To describe the association of susceptibility pattern with antibiotic prophylaxis.
5. To identify the associated factors.

Design, setting and methods

A hospital based prospective descriptive study was conducted at NHSL for 4 months. Using CDC criteria for the diagnosis of SSI, 125 patients were studied in orthopaedic units.

Samples were taken from clinically diagnosed patients with SSI. Appropriate samples were taken (pus, tissue,

deep swabs). Gram stain of swabs was done. Swabs having pus cells more than 2 + were taken as significant. Samples collection, processing and antibiotic susceptibility tests were done according to Standard procedures.

Demographic and clinical data were collected using an internally validated data extraction sheet by re-sampling.

Results

The incidence rate of SSI is 10.4%. Majority presented with SSI are dirty wounds. 79% are following emergency surgeries. The commonest site of infection is tibia and fibular.

From 89% culture-positive infections, majority are (34%) *Pseudomonas* species. Almost all *Pseudomonas* species are sensitive to all antibiotics tested. Only 4% isolates are ESBL. MSSA are 7.5%.

93% patients are given cefuroxime as prophylaxis.

The most commonly associated factor for infection was smoking 17%. In majority of patients associated factors were not identified.

Conclusions

The incidence rate of SSI following orthopaedic surgeries at the NHSL was 10.4%.

The commonest pathogen isolated was *Pseudomonas* species which is sensitive to all antibiotics.

There is no association found in the susceptibility pattern with prophylactic antibiotics used.

The use of cefuroxime as prophylaxis is effective to reduce infections by MSSA.

PP 3

A case report of an infant presenting with bloody diarrhoea

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Introduction

Non-typhoidal salmonellae predominantly cause a self-limiting diarrhoeal illness in healthy individuals; bloodstream or focal infection is rare and mainly happens in individuals with specific risk factors. Extremes of age is a risk factor.

Salmonella Typhimurium and *Salmonella* Enteritidis account for nearly 80% of all human isolates reported globally.

Salmonella Paratyphivar java B is d – tartrate utilizing variants of *Salmonella* Paratyphi B. We report a case of *Salmonella* Paratyphi B var java bacteraemia.

Case presentation

An eight month old previously healthy girl, presented with a history of loose stools, fever of one days duration, one episode of vomiting and one episode of bloody diarrhoea. On admission, she was febrile and irritable. Hydration and perfusion were good. There was no neck stiffness.

After admission she had two episodes of frank bloody diarrhoea.

She has no contact history of diarrhoea. No history of food taken from outside. Mother usually adds scrambled eggs for her meals. There are no pets at home.

After taking blood and stool for culture, IV cefotaxime was started. In stool culture, no pathogens were isolated. Blood culture was positive for Gram negative bacilli after 4 hours of incubation. It was later identified as *Salmonella* Paratyphi B var java.

The baby was successfully treated with IV cefotaxime.

Discussion

Bloody diarrhoea is a feature of *Salmonella* Paratyphi B var java infection. Most reported cases are associated with water reptiles and are associated with outbreaks. But there is neither contact history of water reptiles nor contact history of diarrhoea in our patient. The probable portal of entry is undercooked eggs which is a common source of other non typhoidal *Salmonella*.

PP 4

Microbiological safety: How safe is milk?

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Introduction

Liquid milk for consumption is commonly either pasteurized or sterilized. Ultrahigh temperature system (UHT) is now becoming popular among the consumers worldwide.

Microorganisms can enter into milk during and/or after the milking process, the dairy environment, in transit through production facilities until consumption.

Objective

To assess the microbiological quality and safety of milk and milk products in retail shops in Colombo District.

Design, setting and methods

A total of 200 samples of liquid milk and milk products were randomly collected from retail shops in Colombo

District for 1 year period between 2012 / 2013. Samples were transported at +4°C and stored at -20°C. They were analyzed according to Sri Lanka Standard Institution methods, adopted as practicing guidelines in the Food and water laboratory, Medical Research Institute.

The aerobic plate count (APC), total coliform counts (TCC) / *Escherichia coli*, *Salmonella*, *Listeria monocytogenes* and yeasts / mold counts were done.

Results

Out of 200 samples 85.5% (n=171) was microbiologically satisfactory and 14.5% (n=29) unsatisfactory. *L. monocytogenes*, *E.coli* and *Salmonella* was not detected. Out of the liquid milk samples tested (n=67) 6 were (8.9%) pasteurized milk, 3 (4.4%) sterilized milk and 58 (86.5%) UHT treated milk. All pasteurized milk products were satisfactory. Almost a quarter (n=14, 24.1%) of UHT milk was unsatisfactory.

Furthermore, 36.8% milk added drink samples sterilized by UHT were unsatisfactory. More than a quarter (n=21, 26.5%) of UHT treated liquid milk (n=14) and milk added drink (n=7) samples was unsatisfactory.

Majority (90.19%, n=51) of yoghurt and (84.61%, n=11) cheese samples were satisfactory. All curd (n=18), butter (n=3), ice cream (n=27) and whipping cream (n=2) were microbiologically satisfactory.

Conclusions

In Colombo District, majority of liquid milk and dairy products in retail shops are satisfactory in microbiological quality and safety. The non-existence of enteric pathogens indicates no risk of fecal contamination.

Selective buying from reputed business establishments and strict monitoring by Public Health Inspectors (PHI) could have contributed to the satisfactory aspects of our results.

However, the high percentage of unsatisfactory UHT processed milk in the market is alarming and needs urgent attention.

PP 5

A case of human Brucellosis, which lead to an accidental exposure in the Microbiology Laboratory

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Introduction

Human Brucellosis is an uncommon zoonotic disease in Sri Lanka, although prevalent in neighboring countries. Brucellosis is a disease of many animal species. Four of the currently recognized species of *Brucella* can also cause human disease. Consumption of unpasteurized or raw, milk or milk products from infected animals is considered to be a main mode of transmission to humans. Direct contact with the products of infected animals and inhalation of aerosols also play a role in transmission. Brucellosis is considered to be the commonest laboratory associated bacterial infection.

Case presentation

A case of human Brucellosis was diagnosed in our laboratory when an unusual gram negative bacterium was isolated from blood culture that indicated positive in the automated blood culture machine (BacT/ALERT) after 72 hours of incubation. On sub culture small, glistening, honey droplet like colonies appeared on blood and chocolate agar after 48 hours of incubation. Scanty, powdery growth was noted on MacConkey agar after 72 hours. The isolate was oxidase and catalase positive and became urease positive within 4 hours. The patient was a 39 year old male employed in Oman and presented with a pyrexia of unknown origin. He gave a history of consumption of raw camel milk during his stay in Oman. The isolate was sent to the reference laboratory and identified as *Brucella abortus* by biochemical tests and agglutination with specific antisera. The patient's serology revealed an antibody titre of 1/1280 for *B. abortus* and 1/640 for *B. melitensis*.

Patient recovered after six weeks of doxycycline and IV gentamicin. Laboratory staff who had been exposed to the culture plates prior to identification were put on post-exposure prophylaxis with rifampicin and doxycycline for three weeks and were followed up with sequential serology.

Discussion

A high degree of suspicion in a patient with a travel history to a country with high disease prevalence and adherence to standard microbiological practices at all times in under-resourced laboratories are the key important lessons learnt. Ideally all clinical laboratories should process all blood cultures in a biosafety cabinet.

PP 6

Blood group AB is associated with dengue and dengue haemorrhagic fever in the Northern Province of Sri Lanka

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Introduction

Association between blood groups and dengue and dengue haemorrhagic fever (DF / DHF) have been described previously.

Objective

To investigate the association between blood groups with dengue fever (DF) / dengue haemorrhagic fever (DHF).

Design, setting and methods

Blood samples were collected from 465 suspected patients with DF/DHF, admitted to the medical and paediatric wards of Teaching Hospital, Jaffna from 2010 to 2012. A standard haemagglutination assay recommended by the National Blood Transfusion Services was used to group the blood samples. Dengue NS1 and anti-DENV IgM / IgG ELISA were used to confirm dengue. The distribution of blood groups ABO in Jaffna was obtained from the donors' blood group pattern from January to October 2015 and that was considered as the distribution of ABO blood groups for the population.

Results

Out of 405 patients with confirmed DF/DHF, the trend in AB blood group experiencing DF/DHF was higher than other blood groups ($\chi^2 = 39.86$, d.f. = 3, $p < 0.001$). Individuals with blood group AB appear to have 2.53 times likely to experience DHF and blood group O is less (0.62 times) affected by DHF ($\chi^2 = 12.97$, d.f. = 2, $p < 0.005$). Other blood groups with DF / DHF did not show a significant deviation from their representation in the general population (Table 1).

Table1. ABO blood groups in the general population and the chances of these blood groups experiencing DF / DHF

Blood group	Patients group (n=405)		ABO blood group among the donors (general population) (n=5348)
	% of DF (n=176)	% DHF (n=229)	
A	25.76 (59)	24.43 (43)	19.95 (1067)
B	26.6 (61)	32.95 (58)	31.11 (1664)
AB	16.59 (38)	16.47 (29)	6.51 (348)
O	31 (71)	26.13 (46)	42.43 (2269)

Conclusions

The AB blood group is largely affected by DF / DHF compared to the distribution of this group in the general population. The blood group O was less affected by DF / DHF compared to its' proportion in the general population.

PP 7

A case of melioidosis presenting as community acquired pneumonia

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Introduction

Melioidosis is known as the great mimicker as it has a variety of clinical presentations. It is caused by *Burkholderia pseudomallei*, a saprophyte in soil and water which may be endemic in Sri Lanka. Main routes of transmission are percutaneous and inhalational while aspiration and ingestion play a minor role.

Case presentation

A 64 year old male presented with fever and cough for 2 weeks. He lived in the suburbs of Colombo and went jogging frequently. He gave a history of bathing in a tank on the same day he developed fever. Clinical examination revealed right lower zone consolidation which was confirmed by chest X-ray. His white cell count was 24,000/ μ l, C-reactive protein was 96 mg/l and fasting blood sugar was 96 mg/dl. He was started on IV co-amoxycylav. As the patient remained afebrile after admission he was discharged on oral co-amoxycylav.

Blood culture yielded oxidase positive, gram negative bacilli with chalky white, β -haemolytic colonies on blood agar. On MacConkey agar non lactose-fermenting colonies were seen which later turned pink. Patient was readmitted with fever after 2 days of discharge. Isolate from blood culture was confirmed as *B. pseudomallei* by PCR and melioidosis antibody titer was found to be >10,240. He was managed with intravenous ceftazidime for 2 weeks and discharged on oral cotrimoxazole and doxycycline for 3 months. CECT abdomen was planned on follow up. His HbA1C was >9% and oral hypoglycemics were commenced.

Discussion

A high degree of suspicion of melioidosis is needed, especially in a diabetic with occupational or recreational exposure to soil or water. In this patient, the probable exposure might be inhalation with dust during jogging or aspiration. It is important to consider melioidosis in the differential diagnosis of community acquired pneumonia in endemic settings and use an antibiotic which is effective against *Burkholderia pseudomallei*.

Oxidase testing should be done even for lactose fermenters. Appropriate screening for risk factors and CT abdomen to exclude internal abscess is essential in management.

PP 8

Early and late onset neonatal sepsis at neonatal care units of a tertiary care maternity hospital in Colombo: bacteriological profile, antimicrobial susceptibility and associated factors

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Introduction

Neonatal sepsis is a major concern even with advanced neonatal care due to increasing population of very low birth weight and premature infants. Clinical presentation, risk factors and causative organisms vary with the age of onset of sepsis and the clinical setting.

Objective

To determine the occurrence, causative organisms, antimicrobial susceptibility and associated risk factors of early and late onset neonatal sepsis.

Design, setting and methods

A descriptive cross sectional study was carried out at the neonatal care units of De Zoysa Hospital for Women, Colombo, from December 2014 to March 2015. Blood cultures taken from 197 neonates who were suspected to have early or late onset sepsis was analysed in the laboratory and the relevant clinical data was collected from the clinical records.

Results

There were 94 babies who were clinically compatible with neonatal sepsis and out of which 20 were blood culture positive. The incidence of clinical cases compatible with neonatal sepsis was 36 per 1000 live births and blood culture confirmed cases were 7 per 1000 live births. The predominant organism isolated in early onset sepsis was coagulase negative *Staphylococci* (50%) followed by Group B *Streptococci* (37%) and *Staphylococcus aureus* (12.5%). In patients with late onset sepsis, coagulase negative *Staphylococci* (50%) and Gram negative bacilli (41.6%) predominated. 80% of the *Staphylococcus* spp. were cefoxitin resistant and 80% of the Gram negative bacilli were probable ESBL producers. Gentamicin resistance among *Staphylococcus* spp. was around 82% and 60% among the gram negative bacilli. Factors associated with neonatal sepsis included, prematurity, low birth weight, maternal chorioamnionitis, maternal intrapartum fever, maternal GBS colonisation, prolonged duration of labour, instrumental delivery, prolonged intubation and ventilation and indwelling vascular catheters.

Conclusions

A rise in antimicrobial resistance to empirical antimicrobial agents has been observed among the isolates causing neonatal sepsis. Therefore there is a need for continuous screening and surveillance to identify changes in causative organisms and antimicrobial resistance to ensure proper empirical therapy.

PP 9

Epidemiology of melioidosis in Sri Lanka 2006-2016

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Introduction

Melioidosis is an emerging infection in Sri Lanka. More than 100 culture positive patients have been identified in the last decade. Knowledge about the epidemiology will help to identify 'at risk' individuals for early life-saving therapy.

Objective

To describe the epidemiology of melioidosis in Sri Lanka.

Design, setting and methods

Demographic and clinical data of culture positive patients were analysed.

Results

Total of 129 cases with increasing incidence (78% after 2014) was recorded. Ninety (70%) were male. Nine were children. Age range was wide (2-92 y), reflecting ubiquity

of exposure. Commonest in middle-aged (41-60y), corresponding to frequency of soil exposure and diabetes, which are major risk factors. Majority (108/127, 85%) were rural. Highest number were from Western Province (n=36) and North Western Province (n=33). There were no cases in the hill country with a cool climate and main crop tea/rubber, not rice. Thirty six patients presented between May/July and 41 between November/January, during the monsoons (60%) and a case cluster of 10 cases was seen in Batticaloa in Nov/Dec 2015 following heavy rains. Twenty six cases were farmers and a further 38 were involved in cultivation, giving soil exposure through cultivation as 64/129 (50%). Nine patients (7%) belonged to the defence forces and 15 (12%) were drivers. While men, farmers and rural populations predominated there was representation of every group including house wives (n=24), school children (n=10), professionals (n=5), business persons (n=6), white collar workers (n=10) and blue collar workers (n=8). Diabetes was the predominant risk factor (n=86, 68.5%), 17 were alcoholics and other organ disease was seen. Three children and two adults had thalassaemia. Melioidosis was seen in healthy persons (20/129, 15.5%). Clinical presentations included community acquired sepsis and pneumonia, superficial and deep abscesses and septic arthritis. Central nervous system and genitourinary infection was reported. One had endocarditis. Mortality was 23% (30/129). However, if the 12 patients whose diagnosis was made post mortem were excluded, mortality was 15% (18/117). Eight patients relapsed.

Conclusions

Melioidosis is endemic in Sri Lanka with a wide geographic and demographic distribution. Improved diagnosis has led to reduced mortality. There is an urgent need to extend surveillance of melioidosis to under-resourced parts of the country and to populations at high risk.

PP 10

Is *Helicobacter pylori* a problem in patients with dyspeptic symptoms in Sri Lanka?

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Introduction

Prevalence of *Helicobacter pylori* (*H. pylori*) in patients presenting with dyspeptic symptoms varies between

countries. In contrast to the early studies done in Sri Lanka, recent studies show very low prevalences of *H. pylori*. Primary resistance to clarithromycin is a problem worldwide.

Objective

To determine the proportion of *H. pylori* and the proportion of resistance to clarithromycin in isolates of *H. pylori* in patients presenting with dyspeptic symptoms to a tertiary care hospital in Sri Lanka.

Design, setting and methods

A cross sectional, descriptive, prospective study was carried out in the Departments of Microbiology, Surgery and Pathology of a University and Endoscopy unit in a tertiary care hospital from March 2014 to February 2016. Ethical approval was obtained by the Ethics Committee of the University.

Hundred and thirty eight symptomatic patients who required endoscopic examination, (as decided by the surgeon) were included in the study after obtaining informed written consent. Patients less than 18 years old were excluded.

A questionnaire was filled by an investigator to gather demographic data. Biopsy specimens from all the patients were tested for the presence of *H. pylori* by rapid in-house biopsy urease test and culture. Fifty eight specimens were tested by histology.

Results

Six of the 138 biopsies were positive by in-house biopsy urease test (4.3%). Unfortunately, we were unable to isolate *H. pylori* from any of the specimens. Only 3 biopsies showed histological changes compatible with *H. pylori* infection (5.2%), of which only 1 was positive by in-house biopsy urease test.

Conclusions

Our study shows very low proportions of *H. pylori* by culture, biopsy urease test and histology, which is in line with the recent studies done in Sri Lanka and other Asian countries. Further studies are warranted to find out the aetiology in patients with dyspeptic symptoms, as unnecessary usage of antibiotics in the management of patients may increase the risk of drug resistance.

PP 11

A case of oropharyngeal histoplasmosis from Sri Lanka

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Introduction

Histoplasmosis is caused by *Histoplasma capsulatum*, a dimorphic fungus. It is endemic in North, Central, and South America and less frequently reported in Asia including Sri Lanka.

We present a case report of culture proven oral histoplasmosis.

Case presentation

A 56 year old chronic smoker having diabetes mellitus and COPD presented to the oral medicine clinic with a painful, non healing ulcer on tongue for 4 months duration. He had no history of travel abroad. An exophytic ulcer was on the left posterior tongue with indurations with no palpable regional lymph nodes or hepato-splenomegaly. Rest of the general and systemic examination was normal.

His blood counts were elevated, with normal liver function tests and renal function tests. As histopathology revealed chronic granulomatous disease, he was started with steroids which showed a little improvement.

His second biopsy revealed granulomatous inflammation with multiple granulomas and he was started with steroids again which resulted in worsening of lesions with necrosis. A third biopsy was taken due to the poor response to steroid therapy, and sent for histology and fungal studies. Histopathology revealed histoplasmosis.

Direct microscopy of the biopsy specimen was positive for yeast cells and fungal culture was positive after 14 days of incubation at 26°C for white cottony mould. Microscopy revealed hyaline, septate fungal filaments and macroconidia with tubercles. With the preliminary identification of histoplasmosis it was subcultured on blood agar, BHI agar and BHI broth at 37°C. After 7 days of incubation yeast like growth was observed and thermophilic dimorphism confirmed the identification of *Histoplasma capsulatum*.

He was started with itraconazole for 7 days and then converted to IV amphotericin B for 12 days as he was clinically deteriorating. His liver and renal functions remained normal with the treatment. His clinical condition gradually worsened with uncontrolled diabetes mellitus and high WBC count and he expired. All post-mortem samples were negative for fungal studies.

Discussion

Histoplasmosis has a wide spectrum of clinical manifestations, ranging from asymptomatic infection to severe disseminated disease. The disseminated form is common among immunocompromised individuals and could manifest as oropharyngeal or laryngeal lesions in 30-50% of patients. High index of suspicion is important for early diagnosis in immunocompetent hosts from non-endemic areas.

PP 12

A case report on an uncommon fungus in a patient with a haematological malignancy

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Introduction

Fungi which are uncommon have resulted in systemic fungal infections in patients with haematological malignancies. Opportunistic fungi such as *Geotrichum* spp. are one such example.

We present a case of systemic infection with *Geotrichum* spp. in a patient diagnosed with a haematological malignancy. Though common in the hospital environment and found in patients' endogenous gut flora due to ingestion and inhalation, pathogenic potential is yet to be assessed as few cases have been reported throughout the world so far.

Case presentation

A previously healthy eleven year old school boy presented with a 2 week history of fever, cough and neck pain to a provincial hospital. He was found to have cervical lymphadenopathy with mild hepato-splenomegaly and was pancytopenic with predominant abnormal lymphocytosis.

CT neck and chest revealed multiple cervical, axillary, mediastinal and paraaortic lymph node enlargement with right sided lower lobe consolidation and pleural effusion. Following bone marrow studies he was diagnosed to have T cell lymphoblastic leukemia and transferred to NCIM for further management.

New fever spikes occurred while being neutropenic in spite of broad spectrum antibiotic cover with ceftazidime, vancomycin and later on with meropenem and fluconazole.

Blood culture was initially negative but later became positive with fungal hyphae which grew white creamy dry colonies on SDA. Later, at the Mycology Reference Laboratory, MRI it was identified as *Geotrichum* spp. The same fungus was isolated a few days later from pleural fluid.

Patient was treated with amphotericin B after omitting fluconazole and later with voriconazole guided by literature but his condition deteriorated and he expired.

Discussion

Geotrichum spp. are filamentous ascomycetous yeasts which cause rare opportunistic invasive fungal infections with high mortality in patients with haematological malignancies. They are found worldwide in soil, water, air, sewage, plants, cereals and dairy products, and also a colonizer in the human GIT.

Identification relies on colony morphology, microscopy and biochemical reactions. Newer molecular diagnostic methods are also useful. Treatment options are limited with best available options being either amphotericin B with or without flucytosine or voriconazole.

PP 13

Disease burden and seasonality of respiratory syncytial virus in a cohort of hospitalized children with acute respiratory tract infection

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Introduction

Viral acute respiratory tract infection (ARTI) is one of the most common acute illnesses in childhood in the world including Sri Lanka. In that regard, the most commonly encountered viral aetiology of ARTI in children under 5 years are respiratory syncytial virus (RSV) based on the global literature. Although some data is available on the viral aetiology of ARTI in Sri Lanka, we do not have large studies confirming and estimating the RSV burden for a period of time with local patterns. Hence, the current study was conducted to identify the RSV burden in ARTI of children under 5 years and map the burden with local seasonality.

Design, setting and methods

Nasopharyngeal aspirates (NPA) of inward patients (1 month – 5 years) with severe acute respiratory infection (according to SARI case definition) were collected from Teaching Hospital, Gampola (THG) and Teaching Hospital, Anuradhapura (THA) from March 2013 – August 2014. NPA were initially screened using an indirect immunofluorescence assay (IFA) DAKO IMAGEN™ (UK) and then the specific viral aetiology was detected using a direct immunofluorescence assay (DFA). The RSV burden and other variables were calculated.

Results

158/443 and 165/418 children were detected to have viral ARTI from THG and THA respectively, RSV was detected in 94 children (59.96%) in THG and 85 children (51.51%) in THA. In both cohorts, RSV was detected throughout the year. In the THA, the peak RSV incidence was noted from May-July in 2013 and 2014. In the THG, two RSV peaks were observed: December 2013 to January in 2014 (major peak) and in April in 2013 and 2014 (minor peak).

The RSV incidence at THG and THA were 31.3 and 28 / 100000 person years, respectively. The RSV case fatality ratio in THG and THA were 2.1% and 2.3%, respectively.

Conclusions

Knowledge of seasonality of the occurrence of RSV in children with ARTI is important to implement early preventive measures, including RSV vaccination in childhood.

PP 14

Molecular epidemiology of dengue / dengue haemorrhagic fever in the northern part of Sri Lanka

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Introduction and objective

There was limited access to the northern part of Sri Lanka due to the war until mid 2009. This is the first study to describe the molecular epidemiology of circulating DENV serotypes and their association to DHF in the North using RT-PCR and next generation sequencing (NGS) of DENV-1.

Design, setting and methods

A total of 765 patients from the Teaching Hospital, Jaffna from 2009 to 2012 with clinically suspected DF/DHF were investigated. Identification and typing of DENV were carried out using a combination of RT-PCR and a single-tube multiplex PCR. Primers by Lanciotti et al were used to detect the C and PrM genes of the DENV and one of the DENV-1 cDNA samples was subjected to NGS. Phylogenetic trees were constructed for DENV-1 full genome using previously reported method.

Results

Of the 765 patients, 205 were positive for DENV by RT-PCR. Of these 205 patients, 64 were from 2009/2010 and the rest were from 2011/2012 outbreak. DENV-1, DENV-2, DENV-3 and DENV-4 were found in 12 (18.7%), 19 (29.6%), 25 (39%) and 1 (1.5%) patients, respectively in 2009/2010 with 7 patients having (10.9%) co-infection with DENV-2 and DENV-3. In contrast, in the 2011/2012 outbreak, DENV-1 was the dominant serotype (55.3%) and DENV-4 was not detected in any patients. In 2009/2010 outbreak, 86% of the DHF was caused by DENV-3

and DENV-2. However, in 2011/2012, more than one third of the DHF cases were by DENV-1. When one of the DENV-1 samples was subjected to NGS, high quality, consensus sequence of DENV-1 was identified and the whole genome sequence has been deposited in the NCBI Gen Bank (Acc No: KP398852). Our phylogeographic data showed that Sri Lankan DENV-1 isolate form a monophyletic group indicating a common ancestry.

Conclusions

The present study showed a shift in the transmission of DENV serotypes. Our phylogeographic analysis indicates that 2011/2012 DF/DHF outbreak in the North most likely originated from a minor variant of a DENV-1 strain that has been circulating in Sri Lanka.

PP 15

Are 'band-forms' useful in supporting a clinical diagnosis of infection in elderly patients?

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Introduction

Laboratory investigations may not always be helpful in supporting diagnosis of sepsis in elderly patients. For example white blood cell count (WBC) may not be raised and c reactive protein (CRP) is only moderately elevated. It is conceivable that detection of immature WBC or 'band-forms' in peripheral blood film may aid in the diagnosis of sepsis even when WBC is normal or CRP is inconclusive.

Objective

To study the correlation between band-forms, WBC, CRP, microbiology and radiological investigations in clinically septic elderly patients.

Design, setting and methods

Study design: Prospective cross-sectional study.

Subjects: Patients aged ≥ 70 years with clinical diagnosis of sepsis were admitted to Northwick Park Hospital, London in the period October 2015-December 2015.

Blood specimens sent for WBC were examined for band-forms. Band-forms were expressed as a percentage of WBC.

Data analysis: Correlation between percentage of band-forms, WBC, CRP, blood and urine cultures and radiology results using Pearson correlation coefficient.

Results

Forty patients were reviewed during the study period. All, except two patients had $>10\%$ band-forms in the blood film.

The average WBC was $10.9 \times 10^9/L \pm 6.4$ SD (range: 2.4-36.6); CRP was 132.37 ± 86.42 SD (range: 16-337); Band-forms $34.8\% \pm 18.6$ SD (range: 8-79). In six patients (15%), both CRP and WBC were normal but blood film showed $>10\%$ band-forms. In these patients there was clinical evidence of pneumonia or UTI, supported by new radiological changes on CXR or significant urine or positive blood cultures. The correlation coefficients between band-forms and CRP, band-forms and WBC and WBC and CRP were 0.82, 0.46 and 0.74 respectively.

Conclusions

Presence of $>10\%$ band-forms in the blood film is a reliable indicator of sepsis even in patients with normal WBC and CRP.

PP 16

The aerobic bacteriological profile and antibiograms of deep-seated collections of pus at the National Hospital of Sri Lanka

Gunasekera GCS, Patabendige CGUA

National Hospital of Sri Lanka, Colombo 10

Introduction

Deep-seated collections of pus occur in areas beneath skin which are usually not visible with features of inflammation. Most are caused by infections with aerobic or anaerobic bacteria, fungi or parasites, former being the majority. Mixed bacterial infections also play a role. The causative organisms vary and may reflect the body site. The infective aetiologies are hardly looked into as a whole and local data are limited.

Objectives

1. To study the aerobic bacteriological profile of deep-seated collections of pus and the antibiotic susceptibility patterns at the National Hospital of Sri Lanka.
2. To identify the causative aerobic bacterial agents, to describe their antibiotic susceptibility patterns, to identify the associated factors and to determine the outcome of patients.

Design, setting and methods

This descriptive cross-sectional study conducted at NHSL from 01.12.2014 to 31.03.2015 had all patients with deep-seated collections of pus undergoing drainage/aspiration, accounting to 185 samples. Following routine microbiological processing, further identification of isolates was done with RapID manual identification systems. Antibiotic susceptibilities were determined by CLSI disc diffusion method and MIC where applicable. A data extraction sheet was used to assess the associated factors and outcome.

Results

Aerobic bacterial growth was seen in 54.59% of samples. This resulted in 125 isolates. Poly-microbial growth was seen in 18.8% of the positives. The most frequent organism was *Escherichia coli* (14.4%). Most samples were cerebral. Most yield was from gastro-intestinal and intra-peritoneal samples (95.65%) followed by renal and peri-nephric collections (83.33%). 50% of *Staphylococcus aureus* were MRSA and 35.14% of relevant enterobacteriaceae were probable ESBL producers; acquisition was equally from community and hospital. Among all Gram negatives 6.9% and 2.3% were carbapenem resistant and multidrug-resistant respectively; all were hospital-acquired. Diabetes mellitus and alcoholism were significantly associated with positive growth. One patient succumbed to the pathogen.

Conclusions

In a 54.59% of aerobic bacterial growth were enterobacteriaceae, Highest yield was from gastro-intestinal and peri-nephric collections. MRSA, probable ESBL producing enterobacteriaceae and carbapenem and multidrug resistant Gram negatives were detected with notable hospital acquisition.

PP 17

Clinical audit on external ventricular drain management at the National Hospital of Sri Lanka

Samaranayake WAMP¹, Patabendige CGUA¹

National Hospital of Sri Lanka, Colombo

Introduction

Cerebrospinal fluid diversion through an external ventricular drain (EVD) is an important procedure for patients with elevated intracranial pressure.

Objective

To establish demography, microbiology, current practices, outcomes and infection rates of EVD at the National Hospital of Sri Lanka (NHSL).

Design, setting and methods

A prospective clinical audit in neurotrauma units at NHSL was conducted for a period of 6 weeks. Patients of all ages who had undergone EVD during that period were enrolled using theater lists. Data was collected with data extraction form. They were followed up for 90 days from insertion, to assess the need of VP shunt. EVD related infection rate was calculated as primary outcome. GCS at day 30, mortality, need for permanent CSF diversion were taken as secondary outcomes. Qualitative and quantitative data analysis was done with SPSS system.

Results

Seventy one patients underwent EVD insertions. Majority of them (53) presented following trauma and forty seven of them were male. Fifty five insertions were done as emergency procedure. Twenty five patients developed EVD related infections (25/71, 35.21%). Coagulase negative *Staphylococci* and *Acinetobacter* spp. were the most common organisms isolated from CSF and the rest were coliforms, *Enterococcus* spp and *Pseudomonas* spp and most showed multidrug resistance phenotype.

There was no significant difference among gender, ethnicity, presentation (trauma/non trauma) and age between EVD infected and non-infected groups. The guidelines for prophylaxis and treatment of EVD related infections were followed. Low GCS level (<8) on day 30 ($p<0.01$) and mortality ($p<0.001$) were significant among those who developed EVD infections. Multiple EVD insertion ($p<0.001$) and the need for permanent VP shunt ($p<0.005$) were prominent among them. Prolonged duration of EVD ($p<0.001$), peri operative CSF sampling ($p<0.005$) and CSF leak ($p<0.04$), concurrent other body site infections ($p<0.001$) and prior CNS infection ($p<0.001$) were the other factors that increased the risk of EVD related infections.

Conclusions

EV related infection rates are high at NHSL. This study highlights the need for restriction of frequent manipulation of EVD, proper treatment, early removal and good infection control practices.

PP 18

Microbiological quality in ground water of the Kelani river basin, Sri Lanka

Mahagamage MGYL¹, Pathirage MVSC², Pathmalal M Manage¹

¹Department of Zoology, University of Sri Jayewardenepura, Gangodawila, Nugegoda, ²Food and Water Laboratory, Medical Research Institute

Introduction

Global estimates suggest that nearly 1.5 billion people lack safe drinking water and that at least 5 million deaths

per year can be attributed to waterborne diseases. Bacterial diseases are frequently linked to consumption of drinking water contaminated with *Shigella* spp., *Salmonella* spp., and *Campylobacter* spp. Kelani river basin is the third largest watershed and fourth longest river of the country and provides 80% drinking water for greater Colombo area. Majority of the people who live within the river basin, use ground water for drinking and other daily needs.

Objective

The present study was carried out to assess the microbiological quality including presence of *Salmonella* spp., *Shigella* spp. and *Campylobacter* spp. of ground water from Kelani river basin.

Design, setting and methods

Water from seventy two ground water sampling locations in the river basin was tested for total coliform, fecal coliform, *Salmonella*, *Shigella*, and *Campylobacter*.

Samples were collected and transported according to the PHI manual, Ministry of Health, Sri Lanka (2010). Samples were analyzed following Health Protection Agency, National Standard Method for detection of *Salmonella* spp., *Shigella* spp. and *Campylobacter* spp. Sri Lanka Standard Institute test methods were used for the detection of fecal coliform and total coliform in groundwater.

Results

Ninety six percent of (70) ground water samples in the Kelani river basin were contaminated with total coliform and 17% (12) of samples were contaminated with *Salmonella* spp. Three percent of samples were contaminated with *Campylobacter* spp.

Conclusions

The results showed that ground water of the river basin is not suitable for direct consumption as drinking water and therefore it is important to educate the public of the need to use boiled cooled water for drinking.

PP 19

Retrospective analysis of laboratory data of foodborne outbreaks

Pathirage MVSC¹, Jayalal TBA², Wikramasinghe TW¹, Ganegoda GSS¹, de Silva TBYA¹

¹Medical Research Institute, Sri Lanka, ²Ministry of Health, Sri Lanka

Introduction

Outbreaks due to consumption of contaminated food can adversely affect the health of the population in a country.

This study was carried out to analyze data on food borne outbreaks, reported to Medical Research Institute to understand the present situation of food borne outbreaks in Sri Lanka based on the laboratory evidence.

Objectives

1. To analyze the laboratory data related to food borne outbreaks.
2. To identify the food borne bacterial pathogens from the samples sent from the food borne outbreaks.
3. To identify the gaps related to the food samples sent from food borne outbreaks.
4. To determine the hygienic quality of the food.

Design, setting and methods

Retrospective analysis was carried out by collecting data from request forms and laboratory work sheets for the samples received from food borne outbreaks during 2012-2015. Per each outbreak a data extraction sheet was completed to record necessary data. Summary information was recorded and analyzed using Microsoft Excel 2013.

Gaps were identified in the process of submitting samples e.g. lack of implicated food samples, samples not submitted within the time and etc. when analysing the collected data. Using laboratory result sheets microbiology quality of food were analysed comparing to the standard levels.

Results

Total 46 outbreaks were reported during April 2012 – December 2015. 14 outbreaks were reported in 2012, 12 in 2013, 11 in 2014 and 9 in 2015. Highest number of outbreaks have been reported from Colombo district (12), followed by Gampaha (8) and Batticaloa (5) respectively. Out of 46 outbreaks nearly one third were reported from schools. There were 6 outbreaks associated with industrial zones and 4 in military establishments.

According to the data out of 46 outbreaks, 61 samples from 146 food samples received were unsatisfactory including 13 having potentially hazardous pathogens. *Salmonella* was isolated from 3 outbreaks, unsatisfactory levels of *Bacillus cereus* were isolated from 5 outbreaks and *Staphylococcus aureus* was isolated from 2 outbreaks.

Conclusions

Highest number of outbreaks were reported from schools. Bacterial pathogens identified during this period from implicated food were *Salmonella* sp. *Staphylococcus aureus* and *Bacillus cereus*.

FELLOWSHIPS OF THE SRI LANKA COLLEGE OF MICROBIOLOGISTS

Fellowships of the Sri Lanka College of Microbiologists were awarded to Dr. Maya Atapattu, Prof. Lalitha Mendis, Dr. Nalini Withana, Prof. Manel Wijesundara and Prof. Vasanthi Thevanesam on 12th August 2015 at the Sri Lanka Foundation, Colombo 7.



Dr. Maya Chandrani Attapattu

MBBS (Ceylon), D. Bact. (Manchester), Ph.D. (London), MD (Colombo)

Maya Chandrani Attapattu obtained her MBBS from University of Ceylon in 1961. She joined the Medical Research Institute after a short stint as an anesthetist and remained there until her retirement from government service in the year 2000.

Her career in Microbiology started in 1967. While at Medical Research Institute she proceeded to Manchester University for her Diploma in Microbiology in 1971 and later obtained the Ph.D in Medical Mycology from the University of London in 1975. In 1982 she obtained the MD in Microbiology from the Post Graduate Institute of Medicine, University of Colombo.

She served the MRI for 35 long years. During this period her contribution to the field of microbiology was immense. Her dedicated service was of lasting benefit to MRI and to the country.

One of the greatest gifts from her to the country was in 1994, when she was able to stop the issue of the rabies vaccine prepared in the MRI from the brain tissue of goats. This resulted in preventing the hardships the patient had to undergo as several painful injections and the possibility of neurological complications of the brain tissue vaccine including paralysis.

The other was a very special achievement which she holds very close to her heart even now and that is the immense task of building the division of Mycology single handedly. It involved building the image of mycology from a mere skin disease to the detection of deep infections. In this

process she introduced many sophisticated techniques, and as a result she diagnosed and published many papers on new systemic mycotic diseases.

She pioneered research into diseases caused by fungi. She has won awards from the National Science Council of Sri Lanka for her research work on pulmonary mycotic disease in Sri Lanka, from Sri Lanka Medical Association for research on pulmonary aspergillosis and she was awarded the Sir Marcus Fernando Oration of Sri Lanka Medical Association for her paper on "New Frontiers In fungal infections". She has also been invited as guest speaker to many national and international conferences.

Her teaching and training experiences are vast. She was an examiner in microbiology, chairperson of the Board of Study in Microbiology of the PGIM and she was in charge of the School of Medical Laboratory Technology in the Medical Research Institute.

She was the acting director of the MRI, the deputy director and finally its director.

Outside the MRI she served as the Consultant Bacteriologist to the Respiratory Diseases Control Programme from 1984 – 2000. During this time she high-lighted the prevalence of drug resistant tuberculosis. For ten years from 1985 she served as consultant to the Sri Lanka Standards Institute on microbiological standards. She was a member of the Food Advisory Committee of the Ministry of Health, Chairperson of the Steering Committee of

Medical and Veterinary Sciences of the National Science Council of Sri Lanka, President of the College of Microbiologists for two terms and the President of the College of Medical Administrators.

The College of Medical Administrators awarded her a fellowship in 2002 for her contributions as editor, council member and President.

After retirement from public service, she joined the WHO as the National Consultant for Laboratory Services which took her to cleared and uncleared areas of the North and East of the Country. Where she was involved in improving the overall diagnostic services in these areas. Later after

the tsunami, in her capacity as the National Consultant for Laboratory Services, WHO, she also helped to rejuvenate the laboratory services in the tsunami affected areas in the north and the south of Sri Lanka.

She served as a WHO International Consultant for Assessment of Laboratories in Myanmar and the Maldives. Madam President, I present Dr. Maya Chandrani Attapattu for the Honorary Fellowship of the Sri Lanka College of Microbiologists.

Citation read by Dr. Preethi Perera
Consultant Mycologist



Vidyajyothi Professor Lalitha Mendis

MBBS (Cey), MD (Col), PhD (Lond), Dip.Bact (Manch),
Fellow of the National Academy of Science of Sri Lanka
Emeritus Professor of the University of Colombo

It is my great honor and pleasure to introduce Professor Lalitha Neelangani Mendis, an outstanding administrator, scientist and, above all, teacher and mentor extraordinaire.

Having had her primary education at Girls High School, Kandy, she entered the Colombo Medical Faculty in 1961. She joined the staff of the same faculty in 1973. Her desire to embark on a career in Virology led her to the prestigious Medical School at the University of Manchester where she obtained the Diploma in Bacteriology and St Thomas' Hospital, London where she obtained a PhD in Virology from the University of London. She later obtained the MD in Microbiology from the Postgraduate Institute of Medicine, University of Colombo.

Her postgraduate assignments included training in world renowned institutions such as Johns Hopkins and Emory universities in the United States and also provided her with the opportunity of associating with many pioneering scientists, such as Professor Nahmias, Professor JE Banatvala and Professor Ivan Roitt.

After returning from postgraduate training Prof. Lalitha Mendis's prowess as a teacher and a researcher became evident. Her research included the first ever Sri Lankan studies on rubella, human papilloma virus and MRSA and extensive studies on rotavirus infections in Sri Lanka.

With over 140 publications and presentations to her credit and a dozen students obtaining PhD's, MPhil's and MD's under her supervision her research abilities are masterful.

Her teaching skills, which some of us here were fortunate enough to experience were simply unparalleled. When it came to her lectures, the introduction, the body of it, the summing up and the overall delivery were all seemingly slow and flowing yet full of effortlessly graspable facts. Her ease of imparting practical skills at lab sessions created an atmosphere of ease and enthusiasm among the students.

Having served the Department of Microbiology as the Professor and Head, Professor Lalitha Mendis was appointed to the post of Dean of the Faculty of Medicine, Colombo in September of 1996 where she continued to serve for a period of 2 terms. The most remarkable achievement during her tenure as the Dean was the implementation and stabilization of the student centered, system-based curriculum; a bold, groundbreaking deviation from a century old traditional curriculum which stimulated many other Faculties of Medicine in this country to re-evaluate and make changes in their curricula. She was responsible for setting up and staffing the Medical Education and Research Centre at the Faculty of Medicine, Colombo as well as other units to stabilize the curriculum.

She was next appointed the Director of the Postgraduate Institute of Medicine. Her achievements at the PGIM are many, for example setting up of a medical educational research center, computer assisted laboratory for IT studies, MD in Transfusion Medicine, and Distance Learning Programs. Her efforts at providing a lasting

solution towards the financial crisis prevailing at the time at the PGIM paved the way for a much needed upgrading of the infrastructure at the PGIM.

The first ever Student Scientific Sessions at both the Faculty of Medicine, Colombo and PGIM were organized by her. Also the first ever International Conference on Postgraduate Education in South Asia.

Having served as the President of the Sri Lanka Medical Association in 2008, Prof. Lalitha Mendis was appointed as the President of the Sri Lanka Medical Council in 2009, which certainly projected her into the limelight. It was a time of serious debate on establishment of a private medical schools in the country. Suffice it to say she was a fine skipper to steer that institution through troubled waters. In the midst of all this stress she was able to publish a book on Guidelines and Specifications for Accreditation of Medical Schools in Sri Lanka. As you would comprehend this was certainly no easy task given the wide consultation compiling a book of this nature

requires. It was the first time that the concept was introduced of Accreditation of Local Medical Schools.

I feel that Prof. Lalitha Mendis has developed a wonderful skill of bringing calm to chaos. Her demeanor certainly is of that nature. The latest job of work she was called upon to do as the Competent Authority of the University of Colombo called for these particular skills and the UGC and the whole University of Colombo are extremely appreciative of the calm she brought to the University, as its Competent Authority. I cannot guess what's next in line for her.

Madam President it is my great pleasure and privilege to present an outstanding academic and a great and much loved lady – Emeritus Professor Lalitha Mendis for the award of the “Honorary Fellowship of the Sri Lanka College of Microbiologists”.

Citation read by Dr. Channa Senanayake
*Consultant Virologist, Faculty of Medicine,
University of Colombo*



Dr. Nalini Withana

MBBS, Diploma in Bacteriology - with Distinction (Manchester), M Sc (Liverpool) and MD

Dr. Nalini Withana graduated MBBS from the University of Peradeniya in 1968. She joined the Medical Research Institute in 1975 and her career as a virologist commenced in 1982 at the Colombo South National Polio and Influenza virus laboratory. In 1990 this laboratory shifted to the new laboratory complex at the MRI and she was given the added responsibility of managing all units of the Department of Virology.

Dr. Withana's hard work made Sri Lanka proud when in 1992, the World Health Organization designated the National Polio laboratory MRI, as a Regional Reference Laboratory in the Global Polio Laboratory Network.

Dr. Withana was a founder member of the Sri Lanka College of Microbiologists. She was a joint secretary in 1990 when the College organized its first academic sessions. She was the President of the College in 1998/99, and delivered the second Siri Wickremesinghe memorial oration in 2005. She was the President of SLAAS Section A in 1997. She also received a Presidential award for research in 2002 and was a recipient of the Outstanding Health Professional award from the Sri Lanka Medical Association, in 2012.

After serving as Head of the Department of Virology for 5 years, Dr. Withana took up a position in WHO SEARO as the Regional Polio Laboratory Network Coordinator to manage 17 polio laboratories. Polio Lab Network

Quarterly Update in September 2005 had this to say (I quote) "Dr. Withana retired last month as the SEA Regional Laboratory Coordinator. The Region and the Global Laboratory Network lost a tireless worker and an enthusiastic booster with uncompromising goals, unflinching principles, and unfailing ability to meet the challenges no matter the obstacles. During her tenure the SEAR Network emerged and remains on a global scene as a model in test proficiency, specimen volume, laboratory efficiency and reporting timeliness, despite logarithmic increase in demand. In tribute to her modesty and true mentoring skills, she always gave credit for successes to the laboratories, never to herself. She leaves behind a remarkable legacy in polio eradication and a role model for all of us" (unquote).

In 2008 the WHO appointed Dr. Withana as a Member of the SEAR Certification Commission for Polio Eradication and her signature is on the polio free certificate dated 27 March 2014 issued to the WHO and the SEAR Member countries declaring that SEAR is free of Polio.

Madam President, it is my privilege to present Dr. Nalini Withana for award of the Honorary Fellowship of the Sri Lanka College of Microbiologists.

Citation read by Dr. Omala Wimalaratne
Consultant Vaccinologist and Virologist



Professor Manel Kalyani de Silva Wijesundera

MBBS (Ceylon), PhD (Peradeniya), MD (Colombo)

Manel Kalyani de Silva Wijesundera nee Ratnavibhushana is the Emeritus Professor of Parasitology of the University of Peradeniya. She graduated MBBS from the University of Ceylon in 1967, followed by a Masters in Medical Parasitology from the London School of Hygiene and Tropical Medicine in 1975, and a PhD from the University of Peradeniya in 1985. In 1988, she was awarded MD and Board Certification in Microbiology, specializing in Parasitology by the Postgraduate Institute of Medicine of the University of Colombo.

Prof. Wijesundera joined the Department of Parasitology in the University of Peradeniya in 1976. She was appointed to the Chair in 1990, and became Senior Professor of Parasitology in 1996; which post she held until her retirement in 2008. During her long career in the University, she has been awarded many WHO and Commonwealth Fellowships, and she has engaged in research relating to a wide variety of different parasites of medical importance. She has received several prizes and awards for research presentations at the sessions of the Kandy Society of Medicine, the Annual Research Sessions of the University of Peradeniya, and the sessions of our own College.

She has contributed towards national development in many different ways, including serving as the President

of the Sri Lanka College of Microbiologists in 1999/2000. As I see it, however, Prof. Wijesundera's signal contribution was through the Postgraduate Institute of Medicine. She was largely instrumental in initiating and running the MD Parasitology training programme during the 1990s, and served as Chairman of the PGIM's Board of Study in Microbiology from 1998 to 2006, guiding all of us in the development of systematically organized training programmes and rigorous assessments. It was a great privilege for me to have been one of her first MD trainees, and to have served as Secretary to the BoS in Microbiology under her chairmanship. Her warm, generous, personality which combined an unwavering commitment to whatever task she undertook, with a strong sense of integrity, was an example to us all.

Members of the Council, it is my honour and privilege to present to you, Prof. Manel Wijesundera for award of the honorary fellowship of the Sri Lanka College of Microbiologists.

Citation read by Prof. Nilanthi de Silva
*Senior Professor of Parasitology and Dean,
Faculty of Medicine,
University of Kelaniya*



Professor Vasanthi Thevanesam

MBBS (Ceylon), DM (Peradeniya), MRCP (UK), FRC Path (UK)

Vasanthi Thevanesam acquired her MBBS degree in 1972. Subsequently she travelled to the United Kingdom where she obtained her MRCP followed by the MRCPATH, a unique combination of qualifications that laid the foundation for her abiding interest in Clinical Microbiology and Infectious Diseases. She went on to hone her research skills by doing a DM degree at the University of Peradeniya.

Since then she has enhanced and developed her skills as a teacher, academic, clinician and researcher and generously shared her expertise in these areas with her colleagues, peers, students and patients. Along the way she has held positions of responsibility in many institutions and organizations, each of which she has touched and changed irrevocably due to her farsighted and visionary leadership.

She has been attached to the Department of Microbiology, Faculty of Medicine, University of Peradeniya since 1989, becoming the cadre Chair in 2001 and Senior Professor in 2008. As many present tonight would testify, she is an enthusiastic and committed teacher and examiner of undergraduates, postgraduates and para clinical staff. At Peradeniya she has established a model Department with democratic values, ethics and justice for all. She has mentored many staff members who remain immensely grateful and fiercely loyal to her. In addition her trainees have gone on to contribute to higher education by taking up teaching positions at the underserved Faculties of Medicine in Jaffna, Rajarata and at the Eastern University.

As a member and Secretary of the Board of Study in Microbiology she spearheaded the fundamental change in the emphasis in training from laboratory based to clinical microbiology. As part of her vision for higher education she designed and launched an MSc in Medical Microbiology that filled a long standing void in microbiology training for science graduates and university academics.

In spite of her busy academic career she has never lost her clinical touch and has been the Honorary Consultant Microbiologist at the Teaching Hospitals in Peradeniya and Kandy and offsite consultant to numerous Base and General Hospitals spanning the length and breadth of Sri Lanka. Her close links to the Ministry of Health enabled her to establish the Task Force in Microbiology that still remains the key forum providing technical advice for development of microbiology services throughout the island.

As a researcher she has published widely, received many Presidential awards and has supervised a large number of MPhil and PhD students, even obtaining a SUSRED award from the NSF. As a recognition of her contribution to the advancement of science in Sri Lanka she was inducted as a Fellow of the Sri Lankan Academy of Sciences in 2008.

She was the President of this College in 2000/1. As part of her vision for a greater microbiology community in Sri Lanka that is inclusive of other disciplines such as plant, soil, environmental, industrial microbiology etc she pioneered the setting up of the Sri Lankan Society for Microbiology which inter alia publishes the Sri Lankan Journal of Infectious Diseases of which she is the Chief Editor.

Her dedication, integrity, courage, perseverance, positive attitude and generosity are worthy of emulation. Her legacy to the field of Microbiology is unparalleled.

Madam President, I present Professor Vasanthi Thevanesam to receive a Honorary Fellowship of the Sri Lanka College of Microbiologists.

Citation read by Dr. Enoka Corea

*Senior Lecturer, Department of Microbiology,
Faculty of Medicine, Colombo.*

PRIZE WINNERS AT THE 24TH ANNUAL SCIENTIFIC SESSIONS 2015

Following presentations were awarded first, second and third places at the 24th Annual Scientific Sessions of the Sri Lanka College of Microbiologists held on 13th & 14th August 2015.

Oral presentations

1st prize

- OP 5 - **Human metapneumovirus (hMPV) infection in a selected group of children with severe acute respiratory symptoms**

Noordeen F¹, Pitchai FNN¹, Jayawardana PMGA¹, Kudagammana ST², Abeykoon AMSB¹

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

2nd prize

- OP 1 - **Patterns and predictive factors of long-lasting impregnated bed net usage in a previously high malaria endemic area in Sri Lanka: a cross-sectional survey**

Fernando SD¹, Whidden CE², Jayanetti SR³, Senanayake MDNC⁴, Epasinghe GP⁴, Premaratne Rishintha G⁵

¹Department of Parasitology, Faculty of Medicine, University of Colombo, Sri Lanka, ²Lincoln College, University of Oxford, Turl St, Oxford, United Kingdom, ³Office of the Regional Director of Health Services, Anuradhapura, Sri Lanka, ⁴Faculty of Medicine, Colombo, Sri Lanka, ⁵Anti-Malaria Campaign, 555/5 Elvitigala Mawatha, Colombo 5, Sri Lanka.

3rd prize

- OP 12 - **HIV INNO-LIA HIV I/II assay: modified minimum criteria for HIV-1 diagnosis**

Janage SN, Sudhanva M, Zuckerman M

South London Specialist Virology Centre, Kings College Hospital NHS, Foundation Trust, Denmark Hill, London, SE5 9RS, United Kingdom

Poster presentations

1st prize

- PP 2 - **Efficacy, safety and cost-effectiveness of thermotherapy, a novel mode of treatment for *Leishmania donovani*-induced cutaneous leishmaniasis: A randomized controlled clinical trial**

Refai FW¹, Madarasingha NP², Weerasingha S³, Senarath U⁴, De Silva A⁵, Fernandopulle R⁶, Satoskar A⁷, Karunaweera ND³

¹Postgraduate Institute of Medicine, Colombo, ²Teaching Hospital, Anuradhapura, ³Department of Parasitology, Faculty of Medicine, Colombo, ⁴Department of Community Medicine, Faculty of Medicine, Colombo ⁵Department of Economics, University of Colombo, ⁶Department of Pharmacology, Kotelawala Defence University, ⁷Department of Pathology, The State University of Ohio

2nd prize

- PP 9 - **Seroprevalence of measles, mumps and rubella antibodies in infants in Colombo district**

Nadhikala M¹, Pathirana PPSL¹, Peiris S², Handunnetti SM³, Galagoda GCS⁴

¹Department of Parasitology, Faculty of Medicine, University of Colombo, ²World Health Organisation, ³Institute of Biochemistry, Molecular Biology and Biotechnology, University of Colombo, ⁴Department of Virology, Medical Research Institute

3rd prize

- PP 10 - **Microbiological diagnosis of a rare surgical case**

Asanthi MAI, Patabendige CGUA

National Hospital of Sri Lanka, Colombo 10

Presidential address delivered at the inauguration of the 24th Annual Scientific Sessions of the Sri Lanka College of Microbiologists on 12th of August 2015



Prof. Nilanthi de Silva

Senior Professor of Parasitology and Dean, Faculty of Medicine, University of Kelaniya

Good evening, ladies and gentlemen. The Chief Guest, Dr Palitha Mahipala, Director General of Health Services, Past Presidents of the College, members of the College Council, our invited speakers from overseas and from Sri Lanka, College members, distinguished invitees, ladies and gentlemen, thank you for being here with us this evening. Your presence is a great source of strength to us, and I truly appreciate it.

My Presidential Address this evening is in two parts. In the first, I will talk about our College, particularly our activities over the period since the present Council and I assumed office at the beginning of November 2014. In the second half, I will introduce the theme for this year's sessions, and present to you a few highlights of my own work on one group of the Neglected Tropical Diseases.

The Sri Lanka College of Microbiologists has evolved over a period of nearly 50 years. The parent organization was called the Ceylon Association of Microbiologists and it was established in 1969 with 16 founder members, two of whom are still with us. In 1974, in accordance with the country's change of name, the Ceylon Association of Microbiologists became the Sri Lanka Association of Microbiologists. Soon after this, it was decided that the Association should become a fully fledged College, and so the Sri Lanka College of Microbiologists was born in 1979, with a new Constitution. The College did not, however, begin to have Annual Scientific Sessions until 1991, when the College membership was a little wider

and stronger. The Siri Wickremesinghe Memorial Oration, which is the other major fixture on our College calendar, was instituted in 2004.

We have grown and expanded over the years, and now we have about 180 members. They include medical, dental and veterinary professionals, with a common interest in medical microbiology and related disciplines. Thus we have Bacteriologists and Clinical Microbiologists, as well as medical mycologists, virologists, parasitologists and immunologists amongst our members. The large majority work for the Ministry of Health, but we also have a significant number of university academics, as well as members working in the private health sector in Sri Lanka, and overseas.

I would now like to spend a few minutes elaborating on our College activities over the past 9 months.

Since the present Council took office at the Annual General Meeting held on 31 October 2014, we have had two Ordinary General Meetings in January and May 2015, and we have had regular monthly Council meetings on the 3rd Friday of every month.

At the last AGM, the membership resolved that the College should introduce a formal, ceremonial induction of its President, and so that took place for the first time this year, on the 13th of March at the Aldo Castellani Auditorium of the Medical Research Institute. The Siri Wickremesinghe Memorial Oration for 2015 also took

place on the same day, when Dr Harsha Perera spoke on Influenza Surveillance in Sri Lanka. The award of the Oration was made after an open call for submission of manuscripts from our College membership, unlike in previous years, when the Oration was delivered on invitation.

At the last AGM, our members also resolved to begin another tradition from this year onwards: the award of Honorary Fellowships in recognition of outstanding service to the College and the profession. Accordingly, four of our senior-most Past Presidents were awarded Fellowships in March: Prof Ivy de Fonseka, Prof Mahroof Ismail, Dr Tissa Vitarana and Prof Emil Wijewantha. Today, it will be our pleasure and honour to award another five Fellowships to the next senior-most Past Presidents.

Members of the College have continued to work hard on publications that support and strengthen our Sri Lankan health care system. The Biosafety Manual, which was first published by the MRI, was revised and updated by College members and the 2nd edition was launched at the last AGM in 2014. The Ministry of Health, which supported the College in publication of this Manual, has now distributed it to all state hospitals with large diagnostic laboratories. The College has additional copies available for any other institution or person who may find it useful. At the OGM in January 2015, we agreed on the need to revise the Hospital Infection Control Manual which was first published by the College about 10 years ago. The Editorial Board and College members have been very active since then, and many of the chapters have been re-written, and new chapters added. We hope that it will be possible to publish the 2nd edition within the next few months, because Infection Control is indeed a major problem in all our hospitals.

Another activity that has demanded a great deal of effort and commitment from our College membership is the drafting of National Antibiotic Guidelines for Empirical Treatment of Infections, in consultation with all other relevant Colleges and Associations. This activity was begun by the last Council, under the able leadership of our then President, Dr Philomena Chandrasiri. The task has taken somewhat longer than anticipated, mostly because of the need to ensure that the other Colleges were fully engaged and in agreement with the final guidelines. However, all 19 guidelines have now been finalized. Most of them have been issued as circulars by the Ministry of Health. We hope to compile all of them together into a single handy booklet that can be referred to easily by practicing clinicians. The WHO office in Colombo has generously supported us in this activity, and we hope that they will continue to support the College in the next biennium too, in disseminating these guidelines and raising awareness around the country.

The College also strives to provide regular opportunities for the continuing professional development of its

members. The monthly CME lectures and case presentations which are organized by our Joint Secretaries is one such activity. The Editor of our College publications, Dr Dhammika Vidanagama has continued to produce regular newsletters for our College members: the first issue for this year was circulated in February, and the 2nd issue in June. The 3rd issue will be circulated in late October, just before the next AGM. And of course, this year too, the annual Bulletin of the Sri Lanka College of Microbiologists has been published in time for the Sessions. The Bulletin serves as a record of the proceedings of the annual scientific sessions as well as other significant events and scientific writing by College members. I have to make special mention of the tremendous effort made by the Editor, the Editorial Board and all contributors, in producing the Bulletin, as well as extend a special word of thanks to Dr Roshan Jayasuriya who has outdone himself this year in producing this beautiful graphic for the cover page of this year's Bulletin, using images that people sometimes find quite revolting! College members have also continued with 2 projects that monitor antimicrobial resistance: the Antibiotic Resistance Surveillance Project that was started in 2009, to collate data on antibiotic sensitivity patterns in organisms isolated from blood cultures, and the National Laboratory Based Surveillance of Antimicrobial Resistance project, which was started a couple of years later, to collate data on significant urinary culture isolates. Several consultant microbiologists in hospitals around the country have participated in the two projects and a summary report was submitted to the DGHS earlier on this year. Results indicate that antimicrobial resistance is indeed a major problem, and urgent action is needed to deal with this issue.

As in the past, the College has continued to support the Ministry of Health in a variety of different ways. College members meet regularly with the DGHS at the monthly Microbiology Task Force meeting, and a range of different aspects pertinent to strengthening of the Microbiology services in the Health Ministry are discussed at this forum. College members continue to provide off-site consultancy services to those hospitals that do not have on-site consultants – this year, nearly 40 hospitals are being served in this manner. College members have also assisted the Ministry in developing indicators of the quality of healthcare in state hospitals, through measures of healthcare associated infections, and adoption of hand hygiene. We hope that these will be implemented and made effective in the near future in all state hospitals.

I would like to mention one other Health Ministry project in which College members have sought active engagement – there is an on-going World Bank funded project to develop a Hospital Information Management System, and we would very much like to see the development of a Microbiology Laboratory Information Management module within this system, to enable digital record keeping of samples received for microbiological

diagnosis and report generation, as well as to manage stores and inventory, and even facilitate surveillance of antimicrobial resistance.

You might have noticed that almost all of the activities that I have mentioned up to now have been related to Clinical Microbiology. That is a simple reflection of the specialization of the majority of our members. However, we do have other specialists as well, including Parasitologists like me, and this year we organized an activity that was much appreciated by all those who teach Parasitology and Entomology in our medical schools.

In the last 2-3 decades, Sri Lanka has progressed from low income to low-middle income country status, and that has been accompanied by drastic changes in the epidemiology of many parasitic and vector-borne infections. In the same period, many medical schools have changed their curricula, and these two factors together have resulted in a situation where the Parasitology and Entomology curricula in our medical schools are quite different from each other. As a national body, the College was able to organize a very successful workshop in May, which brought together teachers of Parasitology and Entomology from all medical schools, to identify a core curriculum. The resulting consensus statement has now been sent out to all Deans of the 9 state-run medical faculties. The College plans to do the same for Bacteriology, Virology and Mycology as well, over the coming year. I now move on to the 2nd half of my Presidential Address. The theme we have selected for this year's sessions is '*Neglected Tropical Diseases in Sri Lanka – towards elimination*'.

'Neglected Tropical Diseases' is a term that was coined about 10 years ago by the WHO to refer to a group of very diverse infectious diseases, all bound by the commonality that they were largely neglected in public health interventions funded by global organizations (that is, compared with diseases like malaria, TB and HIV). They are also strongly associated with poverty and social exclusion. I am not going to say much more about them this evening, because I am sure that Dr Dirk Engels, Director of the Dept for Control of Neglected Tropical Diseases at WHO Headquarters in Geneva will tell us much more about them tomorrow in his plenary lecture. For now, I will just say that our country is fortunate in that we have to contend with only 6 of them, and during the course of the next 2 days, we will have speakers talking to us about the state of control of each of them. So for the next 15 minutes or so, I will confine myself to talking about my own favourite parasites, the soil-transmitted helminths, and work that we have done on these infections in Sri Lanka.

Let me first introduce the beasts – and I will ask for a little patience from those who remember this stuff from their medical school days. The term 'soil-transmitted helminths' encompasses 5 species of worms that

parasitize the human gut, but in Sri Lanka, only 3 of them pose major public health problems: the common roundworm, the whipworm and one of the hookworm species. Roundworms are large – growing up to about 30 cm in length – and are often passed out by infected children. So when people talk about children and worms, this is the one they often refer to. However, hookworm, which is much smaller, sucks blood from the intestinal wall, and causes anaemia in infected persons, and so is a lot more problematic. This is a scanning electron micrograph of the mouthparts of the hookworm, but these two other pictures are ordinary photographs.

I will touch on a few basic salient points in the biology of the soil-transmitted helminths before I move on to talk about our research. The adult worms of all five species live in our gut. The female worms produce microscopic eggs that are passed out in faeces, and require a period of development in the soil in order to develop to the next stage – hence the term soil-transmitted helminths. People acquire new infections by either ingesting infective eggs with food or water or soil, or in the case of hookworms, when the larval stages in the soil come into contact with bare skin. Each egg that is swallowed or each larva that enters the body, will grow into an adult worm, which lives out its given life span – about a year in the case of roundworms, and 3 – 5 years in the other two species.

The adult worms cannot multiply and increase their numbers inside the human host, unlike in other infections where viruses or bacteria multiply inside us. The question of how many worms someone has at a given point in time is important, because that is generally what determines if the person will suffer ill effects from the worm infection or not. Another important point to note is that roundworm and whipworm eggs can remain viable in the soil for many years, if the environmental conditions are suitable (like in most of our country), and re-infection is very common among those whose behavior puts them at risk of infection.

To set our work in its proper context, let me also mention a few salient facts about these infections in Sri Lanka. STH eggs are relatively large, and easily identified through microscopic examination of a simple saline smear of a faecal sample. So the first surveys in Sri Lanka were carried out nearly a hundred years ago, soon after the parasites were described by scientists in Europe. Those surveys showed that virtually everyone who was examined had worms (1). Heavy infections and complications such as severe hookworm anaemia and intestinal obstruction due to roundworms, were also very common. Mass deworming was introduced in the 1930s and although the drugs which were used then were not very effective, the general population accepted that regular deworming is desirable. Deworming also became part of the annual school medical inspections several decades ago as did routine antenatal deworming with mebendazole, to combat maternal anaemia.

So over the course of the last century, there was a slow decline in prevalence of STH infections, until about the 1980s, and then there was a much sharper drop. There are several factors that probably contributed to the accelerated decline. Improved living conditions with better sanitation and hygiene definitely has been one factor - the infections cannot be transmitted from one person to another if water-sealed latrines are used for disposal of faeces. The discovery of mebendazole and albendazole was another, because they are both very effective in getting rid of STH infections; and once the patent on the branded variety expired, our local State Pharmaceuticals Manufacturing Corporation started producing a good quality, low cost, generic preparation of mebendazole. Somewhere along the way, the belief that good parents regularly deworm their children became part of our national psyche.

I will now spend a few minutes talking about some of the research that my co-workers and I have done on STH infections in Sri Lanka.

I stumbled into working on these infections when I was young probationary lecturer in the University of Peradeniya. For my MD research project, I chose to look at intestinal parasitic infections among pre-school children in the Mahaiyyawa slums in Kandy, under the able guidance of Prof Manel Wijesundera. I thought we would find a lot of amoebiasis and giardiasis, but we didn't. Instead, we found that about one-fourth of the children had worm infections. And even within that underprivileged community where most people were very poor, and not very educated, we found that the children of mothers who had a little bit more education, were much less likely to have worm infections (2).

After passing my MD exam in 1994, I went to talk to Prof Mahroof Ismail, who had just returned from a WHO meeting on hookworm infection and anaemia in girls and women. He suggested that a study to establish the safety of mebendazole use for deworming in pregnancy would be very timely, because Sri Lanka seemed to be the only country that was doing routine antenatal deworming at that time. I had moved from Peradeniya to Kelaniya University by then, and the medical faculty at Ragama was in its infancy. A small team of us, which included a physician, an obstetrician, and a paediatrician, got together and designed a study to compare birth outcomes in women who had received routine mebendazole while pregnant, with those who had not received it. We examined the babies of about 7000 mothers who delivered in Ragama and Peradeniya hospitals over a 6-month period. We were able to demonstrate that the incidence of major birth defects was not significantly different in the two groups and so we concluded that mebendazole use in pregnancy was safe (3).

The paper that described this study was published in the *Lancet* in 1999, and it was one of the pieces of evidence

that led to the WHO recommendation that all women in hookworm endemic regions should be offered routine antenatal anthelmintic treatment after completion of the 1st trimester of pregnancy.

A few years later, my colleague Pathmes and I had the privilege of being part of a large, intersectoral team that conducted a national study of the health status of primary school children. The survey team, which was supervised by Dr Sagarika Samarasinghe from the MRI, examined about 2500 nine-ten year old children in 144 schools located in all 9 provinces of Sri Lanka. The survey methods included examination of a stool sample for soil-transmitted helminth infections. We found that only about 7% of children harboured one or more species of worms. The prevalence was highest (i.e. between 10 and 15%) in the conflict-affected Eastern and Northern Provinces, as well as in the Western Province, because of the urban slum areas (4).

A few years later, another team of us from Ragama carried out a survey that looked more closely at the situation in the plantation sector, because although we found that at a national level, the prevalence of STH infections was low, we know that infection is closely associated with poverty and lack of sanitation. We examined nearly 2000 children from 114 estate sector schools in those districts that had lots of tea or rubber plantations. There we found that the overall combined prevalence was nearly 30%. The commonest infection was roundworm. Interestingly, we found no hookworm infections in Nuwara Eliya and Badulla districts, probably because the high altitudes make it too cold for the hookworm larvae to survive (5). This map, which was drawn by my colleague Kithsiri Gunawardena, shows the predicted prevalence of STH infection in the 5 districts that we studied. This area in red, in Kegalle district, had a predicted prevalence of over 50%, whereas this area in blue, which covers most of Kandy and Badulla districts, predicts prevalence of less than 20%. This photograph down here shows part of the reason why STH infections are still common in the estate sector – even if people have latrines, they do not understand the value of using them for disposal of faeces. They are perceived to be of greater value for storing firewood, or even tethering livestock.

This study in the estate sector combined assessment of prevalence rates with a cluster randomized study of the impact of deworming and iron supplements on the cognitive abilities of school children. We randomized schools to treatment or control: treatment consisted of a single dose of mebendazole and weekly iron supplements for 24 weeks, while the control group received placebos. Children were assessed for STH infection, Hb levels and in tests of concentration ability, at baseline and follow up after 6 months (6).

At the 6-month follow up we found that there was a significant reduction in the prevalence and intensity of

roundworm and whipworm infections in the treated group, but we could not demonstrate any impact on Hb levels or cognitive abilities.

Spurred on by the recommendations that have been issued by the WHO's Dept for Control of Neglected Tropical Diseases, and based on data from several of the studies that I just described, the Family Health Bureau of the Ministry of Health has stepped up efforts to control STH infections. However, the WHO recommendations and the Health Ministry circular both have the goal of merely controlling the transmission of STH infections, i.e. bringing the levels down to a certain specified prevalence.

I feel we should be bolder, and work towards a goal of eliminating STH infections from Sri Lanka, even in the high risk populations in the estate sector and the urban slums. It will require a much more focused effort than at present but I am certain that a strong triple-pronged effort of regular deworming, improved sanitation and hygiene, and good health education, can bring about a Sri Lanka which is largely free of STH infections in the near future. I hope the DGHS who has so graciously accepted our invitation to be the Chief Guest tonight will give serious consideration to my suggestion.

There are many, many people that I must thank tonight, but I will not take up your time to identify everyone by name. However, I am compelled to tell you how much our Joint Secretaries and my Council have supported me in the last few months; and our Administrative Assistant Ms Priyanga Opatha is truly worth her weight in gold!

I have to also mention how much Prof Kamini Mendis, Prof Mahroof Ismail and Prof Manel Wijesundera, who I consider my gurus, have inspired me, and if I have achieved anything at all in Parasitology in this country, it is because I have stood on the shoulders of giants.

Over the years I have had the privilege and pleasure of working on a large number of research projects, with many different people from many different institutions: the University of Peradeniya, where I began, and in Kelaniya for the last 22 years. The team at WHO Headquarters, which is now the NTD Dept in its present reincarnation, has been a tremendous source of support, as has been Don Bundy at the World Bank, and Lesley Drake and the Partnership for Child Development at Imperial College London. Our research projects have been

funded by many different agencies, and I am grateful to all of them. But none of it would have been possible if not for the willing cooperation and enthusiastic participation of the communities that we worked in: the children, their families and their teachers.

Above all, I have to thank my family because their support is the rock on which I have built my life: my parents, who brought me up to believe that I could achieve whatever I wanted, if only I put my mind to it, and worked hard; my husband Janaka who has been a co-researcher in some of this work, but a partner in much more than that, for nearly 30 years now; Janaka's mother and father, and his brother Asita; and last, but not least, my daughter Tiloka and my son Manodha, who have had to learn to live with all my eccentricities!

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Primary immune deficiency in Sri Lanka – The Long March

Madam President, Past Presidents, Members of the Council, Members of the College, Mrs. Ranganie Wickremesinghe and members of the Family of the late Dr. Siri Wickremesinghe, friends, Ladies and Gentleman.

It is a great honour to stand before you to deliver the Siri Wickremesinghe oration – 2016, to commemorate a Medical Microbiologist, a great scientist, and, what is most important, a gentleman. I first met Dr. Siri Wickremesinghe in 1979 when my parents and I went to the centenary Battle of the Blues with him, my father being a colleague of his at MRI. Subsequently, I met him as a Visiting Lecturer in Microbiology at the Faculty of Medicine, Colombo, when I was a student. What struck me at that time was his silvery grey hair and his ability to crack jokes without moving a single facial muscle. Later, while visiting the MRI as a House Officer, I passed a black haired gentleman who looked vaguely familiar. It was Dr. Wickremesinghe in another incarnation!

All of us know him as an eminent Microbiologist with facts on microbiology, natural history, history, turtles, cricket etc, in other words an encyclopedia, within one frame. I once mentioned to him that I had seen a golden oriole (kaha kurulla to most people). He asked me whether it was the Black-hooded Oriole, Black-naped Oriole or... I was lost. However, it was not only his skills or his ability that made me respect him. In a world where many values have been lost, where financial gain is the epitome of success, he stood for other, more decent values. We miss him for these qualities, as well as for his knowledge.

To strike a personal note, he was my teacher, and he also introduced me to the management of Durand's Hospital, when he was the Resident Pathologist/

Laboratory Manager. I am merely repaying a great debt by delivering this oration.

"From moments after birth, we constantly breathe, eat, and come into physical contact with millions of microorganisms until we are consumed by them at death." (Jones and Falco, 1996) [1]. The immune system is geared to protect the host from pathogenic microbes. Cells, tissues and chemicals of the immune system act in concert to tackle the microorganisms besieging the body. Traditionally, the immune system is divided into the innate immune system and the specific immune system. The innate system comprises cells, such as the monocyte and its derivative the macrophage, the neutrophil and the dendritic cell, and chemicals such as complement. When a microbe breaches the external barriers including the skin and mucous membranes, it is immediately confronted by the macrophage. The macrophage ingests or phagocytose the microbe, resulting in damage or death to it. In addition, the macrophage produces a host of chemicals, called cytokines, which results in inflammation. Inflammation leads to cells such as neutrophils and anti microbial chemicals such as complement reaching the site of infection from the blood stream. This happens within hours and helps keep the infection in check. These immune mechanisms are part of the innate system and are non specific, in other words does not depend on a particular micro organism. A more focused or targeted response is necessary, geared against the specific microbe, to eliminate the pathogen. This is mediated by the specific immune system, specifically the lymphocytes. There are 2 main lymphocytes, the B lymphocyte and the T lymphocyte. B lymphocytes produce immuno-

globulins or antibody, IgM, G, A and E. These attack microbes living outside cells. There are microbes who live inside cells. Some microbes such as viruses live in the cytoplasm, while others live in membrane bound vesicles, an example being *M tuberculosis*, the cause of TB. The second kind of lymphocyte, the T lymphocyte is important in attacking these intracellular microbes. As intracellular microbes live in different niches there are different types of T lymphocytes, identified by certain markers found on the cell surface. The 2 main ones are the CD 4 T cell the helper T cell, and the cytotoxic CD 8 T cell. The CD 4 T cell is further divided into other types, but suffice it to say at this juncture, the CD 4 cell acts as a conductor of an orchestra. As the term “helper” implies, the CD4 T cell helps B cells to produce antibodies which deal with extracellular pathogens. It also helps CD 8 T cells kill microbes that live in the cytoplasm, such as virus infected cells. This occurs by destroying the cell that harbours the microbe. It also helps macrophages to kill microbes that live inside vesicles, such as the TB bacillus. In this case, the entire cell is not killed, rather, the bacteria is killed while it is inside the cell.

Antibodies kill microbes with the help of other chemicals. For example, extracellular bacteria are coated by antibody and complement, making the microbe “more tasty” – ie it opsonizes the microbe so that neutrophils find it palatable. Therefore, extracellular microbes, such as *S pneumoniae* and *H influenzae* are eliminated by antibody and complement opsonizing the organism for subsequent ingestion and killing by neutrophils. Virus

infected cells are killed by NK cells, a third kind of lymphocyte, and cytotoxic T cells (CD 8+), whereas intravesicular pathogens such as *M tuberculosis* are dealt with by CD4+ T cells (TH1 cells) activating macrophages. Thus, defects involving antibodies, complement or neutrophils lead to infections with extracellular pathogens, while infections with intracellular organisms are observed in cell mediated immune deficiency or defects of the type 1 cytokine pathway (which will be described later). Primary immune deficiency diseases (PIDD) are due to genetic defects in the immune system, in contrast to the more common secondary immune deficiencies such as HIV/AIDS. Patients with PIDD have recurrent infections or infections with unusual microbes. Some patients, paradoxically have an immune system that attacks its own tissue, a process described as autoimmunity. In addition, some patients develop various malignancies.

Around 200 such PIDD have been described to date. The International Union of Immunological Societies (IUIS) has classified these into 9 groups (Table 1) [2]. Contrary to popular belief, PIDD are not rare, ranging from often asymptomatic IgA deficiency (1:500) to the very rare (1:500,000). The prevalence in the US may be as much as 1:2000 [3]. While most people think that PIDD occurs only in childhood, they may present in adult life as well. The European Immune Deficiency Registry (ESID) which collates PIDD in European countries has published the following pie chart on its web site (Figure 1). The corresponding data from 73 patients diagnosed with PIDD

Table 1 [2]

Category	Examples
Combined immunodeficiencies	SCID, CD 40 L deficiency
Combined immunodeficiencies with associated or syndromic features	AT, WAS, HIGE syndrome
Predominantly antibody deficiency	XLA, AR agammaglobulinaemia, CVID
Diseases of immune dysregulation	HLH, X linked lymphoproliferative syndrome, Gricelli syndrome
Congenital defects of phagocyte number and/or function	Severe congenital neutropenia, CGD
Defects of innate immunity	ED with ID, IRAK 4 deficiency, chronic mucocutaneous candidiasis
Autoinflammatory syndromes	FMF, TRAPS
Defects of Complement	
Phenocopies of PID	ALPS

in a 4 year period in Sri Lanka at the MRI is given in comparison [4]. In both registries, antibody deficiencies are the most common, along with combined immune deficiency, phagocytic defects and other well defined PID. Immune dysregulation is relatively more common in Sri Lanka, whereas complement and innate defects, and auto-inflammatory disorders were not seen (Table 2), due to lack of diagnostic facilities.

The aim of this talk is to give a snap shot of the immune deficiencies prevalent in Sri Lanka, and the measures taken to diagnose and treat the affected patients.

Figure 1. PID in Europe and Sri Lanka

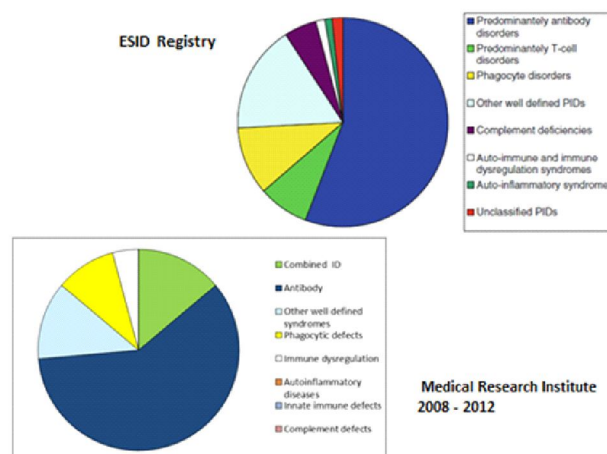


Figure 1a. ESID registry and MRI data on PID [4]

Table 2 [4]. Spectrum of primary immune deficiency

Disease Combined	Number % 10 (13.6)	Sex		Age					
		M	F						
				< 1 Years	1- < 5 Years	5 < 12 Years	12 < 18 Years	18 - <30 Years	≥ 30 Years
Severe combined immuno deficiency (including x linked = 02 Omenn syndrome=01)	10 (13.6)	04	06	10	-	-	-	-	-
Well defined syndrome	10 (13.6)								
Ataxia telangiectasia	06 (8.2)	02	04	-	03	03	-	-	-
Di George syndrome	02 (2.7)	01	01	02	-	-	-	-	-
Hyper IgE syndrome (autosomal dominant)	02 (2.7)	01	01	-	-	01	01	-	-
Antibody deficient	44 (60.27)								
X Linked agammaglobulinemia	15 (20.54)	15	0	01	07	05	02	-	-
Autosomal recessive agammaglobulinemia	02 (2.7)	0	02	-	01	01	-	-	-
Common variable immune deficiency	21 (28.76)	10	11	-	01	04	04	04	08
Partial Ig A deficiency	01 (1.36)	0	01	-	-	-	01	-	-
Hyper IgM syndrome (including CD 40 deficiency = 01)	05 (6.8)	03	02	-	02	03	-	-	-
Immune dysregulation	03 (4.1)								
Criscelli syndrome	03 (4.1)	02	01	03					
Phagocytic defects	06 (8.2)								
Chronic granulomatours disease	04 (5.4)	04	0	01	02	01	-	-	-
Leucocyte adhesion deficiency type 1	02 (2.7)	0	02	02	-	-	-	-	-
Autoinflammatory	0	-	-	-	-	-	-	-	-
Innate immune defects	0	-	-	-	-	-	-	-	-
Complement defects	0	-	-	-	-	-	-	-	-
Total	73	42	31	19	16	18	08	04	08

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1. Combined immune deficiency (T and B cell defects)

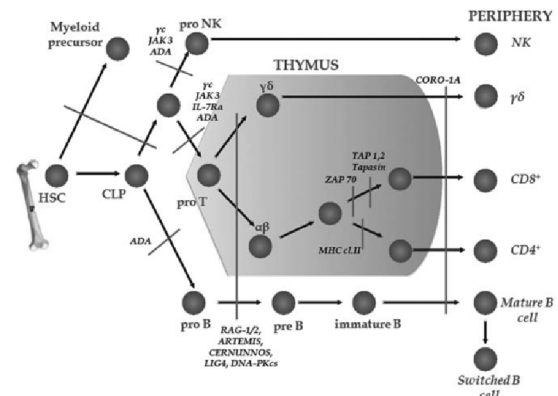
(a) Severe combined immune deficiency

A 4 month old boy presented with an unexplained family history of infant deaths in 2005. There was a history of 15 deaths in 3 generations in his family, all infants, including his elder sibling. All had died following infections. Could they be having an immune deficiency which was X linked?. This was possible, as all 15 children were males. If the deaths were due to immune deficiency, severe combined immune deficiency, which kills in infancy, was on the top of the differential diagnosis. We looked at his immunoglobulin levels, and they were normal, which did not confirm a SCID. However, flowcytometry revealed an absence of T lymphocytes, including both CD 4 and 8 cells, and also NK cells. Absent T lymphocytes and NK cells, but normal numbers of B lymphocytes indicated a T- B + SCID. As all affected children were male, I diagnosed an X-linked SCID due to a deficiency of the common γ chain. Unfortunately, it was too late to intervene to save this baby.

5 years later the parents presented at 20 weeks of pregnancy with a male foetus, diagnosed by ultra sound scanning. Foetal blood samples revealed a T-B+NK- SCID, and genetic diagnosis at the Genetics Unit/University of Colombo and Asiri Hospital, Colombo revealed a substitution in exon 5. The mutation was modeled in the UK which revealed a unique receptor. The baby underwent stem cell transplantation (SCT) in India before 3 months of age and is now almost 6 years old, off all medications. Combined immune deficiencies comprise disorders with impairment or dysfunction of T cells and low T cell numbers. Some patients have normal numbers of B lymphocytes, as the genetic defect is confined to the T lymphocyte. However, as mentioned earlier, B lymphocytes need T lymphocyte help for efficient functioning, and in spite of a normal B cell count, the antibody responses are not adequate. In other instances, both T and B lymphocytes are affected, when the defect affects both cell types (Figure 2) [5]. If the condition is severe, it is called severe combined immune deficiency (SCID). Patients with SCID develop infections within the first few months of life, due to viral, bacterial and fungal pathogens. Diagnosis of SCID is a medical emergency, as early stem cell transplantation is curative, whereas those not offered this intervention succumb to infections by the first year of life. It is our hope that patients with severe combined immune deficiency will be offered this therapeutic option in the near future.

A total of 10 patients have been diagnosed with SCID by our group. Two patients (20%) have been identified as having X linked SCID, by genetic testing. In the West, 50% of all SCID are due to X linked mutations in the γ chain, which cause a T-B+ phenotype [6]. In our population it is less common [4].

Figure 2. Severe combined immune deficiency



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(b) Omenn syndrome (OS)

A two month old baby boy, the only child of consanguineous parents, presented with an erythematous papular rash involving the whole body progressing to a seborrhoeic type which was treated as seborrhoeic dermatitis, though the response was poor. He had severe hair loss, along with sparse eyebrows. At the age of 45 days he developed bronchopneumonia, blood culture growing *Pseudomonas*, which was sensitive to the given antibiotics. On day 5 of illness there was no clinical improvement and he developed a greenish mucoid diarrhea with fever, vomiting and abdominal distension. He had hepatosplenomegaly but no lymphadenopathy. The skin rash progressed to a diffuse erythematous scaly ichthyotic one making the baby oedematous.

He was not febrile, though the inflammatory markers remained persistently above range (CRP = 356 – 282 mg/L). White cell counts were initially very high ($40 \times 10^9 / \text{mm}^3$) with eosinophilia ($4 \times 10^9 / \text{mm}^3$), high ALT and AST levels (100-305 / 195-122 IU respectively), low serum proteins and normal renal function.

Erythroderma, hepatosplenomegaly, oedema and eosinophilia made me suspect Omenn's syndrome (OS). His serum immunoglobulins were done, and found to be low. Flowcytometry was then performed, which showed absent B cells, a low CD 8 count, but increased CD 4 counts. The CD 4 cells were activated however, with increase DR positivity, indicating that the patient was indeed having Omenn syndrome, a type of leaky SCID. The same genes responsible for SCID are involved, but the phenotype, ie the clinical picture is different to that of SCID. He passed away following a protracted course, in spite of third line antibiotics and supportive care at the age of 62 days.

OS is a disease where oligoclonal T cells (ie T cell with receptors with a limited V β repertoire) are present unlike in classical SCID, where there is a T cell lymphopenia. These patients have erythroderma, alopecia, hepato

splenomegaly, lymphadenopathy with eosinophilia and increased IgE levels. The pneumonitis and enteropathy they manifest are due to inflammatory rather than infective causes. The patient presented was diagnosed with OS, but could not be saved. Another sibling was born with OS to these parents, who in turn died before the age of 1 year. Two more children with OS, from different families, have been diagnosed by us. These children were B-, most probably due to a Rag1/2 mutation, which is the commonest cause of OS [5]. SCT is necessary for survival.

(c). Hyper IgM syndromes

A 1 year old son of consanguineous parents, a Sri Lankan born US citizen, presented with persistent skin infections, *Pneumocystis jirovecii* pneumonia, scabies and abnormal gait. He had neutropenia, and a negative HIV test, IgG and IgA was absent, IgM normal. T and B cell counts were normal. T cell proliferation to mitogen was normal. The presence of *Pneumocystis*, a fungus that lives within cells, indicated a cell mediated immune defect. It is commonly seen in HIV/AIDS. However, the T cell count, and function was normal. IgG and IgA were also absent, indicating a combined immune deficiency (CID), where both cell mediated and humoral immune defects were present. A CID with normal T and B cell counts indicated hyper IgM syndrome as the most likely cause. I suspected X-linked HIM and he was started on IVIG and cotrimoxazole prophylaxis at 12 months and he had no more infections. He was investigated further in the US as he is a citizen of that country, where he was found to have a normal CD40 ligand expression but absent switched memory B cells. Importantly, his CD40 expression was absent confirming the diagnosis of AR-HIGM, rather than Xlinked HIGM. Neurological evaluation showed microcephaly, a retrocerebellar cyst but no evidence for ataxia and his frequent falls resolved with tailor-made shoes. A role for CD40 in neurological development (microcephaly) has not been established but impaired neurological development was reported in another patient. He underwent SCT in the US [7].

A similar patient, who developed bronchopneumonia at 5 months of age, whose clinical history was suggestive of hyper IgM syndrome was reported previously by us [8]. Subsequently a total of 9 patients were diagnosed with HIGM by our group. The clinical characteristics are presented in the slide.

The HIGM syndromes comprise a class of PIDD with low or absent IgG, A and E, with normal or increased IgM [9]. B lymphocytes need the help of CD 4+ T cells, for certain functions. IgM antibody with a poor fit or low affinity, may be produced without T cell help. However, T cell help is needed to produce antibodies with a better fit (ie higher affinity) for which a process called somatic hypermutation and affinity maturation needs to take place. In addition, different isotypes of antibody, such as IgG, A and E need

to be produced, to serve different functions (this is called class switch). For this purpose, T - B cell cooperation is necessary, partly supplied by CD40 - CD40 L interactions. The same CD 40 - CD 40 L interaction between T cells and macrophages leads to intravesicular pathogens, such as *Pneumocystis jirovecii* being dealt with efficiently by the macrophage. Depending on the defect, patients would have a pure B cell defect, or have combined immune deficiency, similar to our 2 patients. The majority of HIGM (70%) have a CD 40 L defect and have X linked HIGM, in boys, and <1% have a CD 40 defect and AR recessive HIGM, similar to our patient who underwent a SCT. These patients have infections with capsule bacteria, similar to patients with pure B cell defects, as well as with intracellular microbes such as *Pneumocystis* and *Cryptococcus*. The rest have a pure B cell defect.

2. Combined immunodeficiencies with associated or syndromic features

(a). AD hyper IgE syndrome

A 9 year old boy of non consanguineous parents presented with fever, cough and haemoptysis of 32 days duration. He gave a history of recurrent skin abscesses and lymphadenopathy from the 3rd day of life, with surgical drainage instituted 37 times, with the isolation of *S aureus* on a few occasions. He developed a pruritic dermatitis from day 10, and recurrent bronchopneumonia from the age of 5 months. He had developed lobar pneumonia with encysted pleural effusion at 3 years, and paronychia and oral thrush from infancy. He had abnormal (dysmorphic) facies and a double row of teeth due to absent shedding of primary dentition. Chest xray revealed a pneumatocele.

His immunological markers, including immunoglobulin levels, lymphocyte subsets and T cell function were normal. Sero conversion following immunization with the typhoid Vi vaccine was absent, and his IgE level was > 2000 IU/mL (normal < 100).

The presence of a neonatal skin rash, recurrent skin abscesses, dysmorphic facies, absent shedding of his primary dentition, candida infection and pulmonary features, suggested a diagnosis of autosomal dominant hyper IgE syndrome (HIGE) [10].

At that point of time, the genetic cause for the disease was unknown. However, today, most patients with AD HIGE have a mutation in Stat 3. To understand the pathogenesis, an outline of the immune defenses dealing with *Candida* infections need to be appreciated (Figure 3). "So went Satan forth from the presence of the LORD, and smote Job with sore boils from the sole of his foot unto his crown." (King James Bible). Initially termed Job's syndrome, like the character in the bible who suffered severe boils on the skin, it is a multi system disorder with dermatitis, *Candida* infections and connective

Subsequent to the first case from Sri Lanka, we described 5 more patients with HIGE, and confirmed the diagnosis by identifying mutations in the Stat 3 gene, carried out at Royal Free Hospital, UK by Dr. Suranjith Seneviratne and his team. The mutations were all known. HIGE is rare, and only a few patients have been described with the mutation in Asia. The features in Asians, compared to Europeans is noted in a paper by us (in preparation).

(b). Chromosome 22q11.2 deletion syndrome

Thymic aplasia, hypoparathyroidism, cardiac defects and facial dysmorphisms are cardinal features of DiGeorge syndrome (DGS), due mainly to deletion of chromosome 22q11.2 [12]. Ninety percent have the deletion, involving many genes, including the gene TBX 1, which codes for a transcription factor. Rarely, deletions in chromosome 10 may also result in the same phenotype. Most patients have a normal or mildly compromised immune system.

A 9 year old girl was referred with a history of unsteady gait from the age of 2 years. She also had recurrent lower respiratory infections, including one episode of pneumonia at the age of 5 years. She had dilated blood vessels in her eyes (telangiectasia of bulbar conjunctiva). On examination she had abnormal muscle tone (dystonia) and movement (dyskinesia), as well as jerky movements (ataxia). A diagnosis of ataxia telangiectasia was made. Investigations confirmed the diagnosis. It included elevated α fetoproteins (AFP). CT brain showed isolated cerebellar atrophy, consonant with the diagnosis. However, her IgA level was normal.

(d). Wiskott Aldrich syndrome

3. Predominantly antibody deficiency

5 year old boy presented with recurrent infections from the age of 5 months. These included pneumonia and seborrhoeic dermatitis at 5 months, 4 episodes of lower respiratory infection, one episode of bronchopneumonia and 2 episodes of meningitis. His IgG and IgA levels were low, with absent isohemagglutinin levels and a reduced B lymphocyte count. A diagnosis of agammaglobulinemia was made. 85% of patients with agammaglobulinemia have a mutation in the Bruton's tyrosine kinase (Btk) gene. Single sequence conformational polymorphism (SSCP) analysis of m RNA of his Btk gene showed a mutation.

A diagnosis of X linked agammaglobulinemia was made, and he was commenced on IVIG.

Bruton's agammaglobulinaemia is one of the first immune deficiencies identified. Colonel Ogden Bruton was a physician in the US Army. He found that an 8 year old boy with recurrent pneumonia due to *S pneumoniae* had an absent gamma globulin fraction on serum electrophoresis. He coined the term agammaglobulinaemia for this condition and connected this with the clinical condition. He started the patient on subcutaneous injections of gammaglobulin, with good results. This was in 1952. It was subsequently discovered that the patient did not have B cells, and a linked mutation in the Bruton's tyrosine kinase was responsible. 85% of patients with agammaglobulinemia and absence of B cells have this disease. Of the remainder, 1/3 have genetic defects in 6 genes, and have AR agammaglobulinemia. These mutations lead to a block in B cell development. Patients with agammaglobulinemia develop infections in the first year of life, after waning of maternal antibody, with respiratory and gastrointestinal infections predominating [14].

We did SSCP analysis of 10 patients with agammaglobulinaemia. In this test, mRNA is extracted and the copy DNA (cDNA) that is produced is amplified by polymerase chain reaction (PCR), which is then digested by restriction enzymes. These digests are then run on a gel and conformational changes that occur due to mutations are identified by the electrophoretic pattern. All 10 patients tested had a mutation of the Bruton's tyrosine kinase, leading to a diagnosis of XLA. A total of 19 patients with agammaglobulinemia have been diagnosed, 10 with XLA, 4 with AR agammaglobulinemia and 5 boys with an uncertain genetic diagnosis.

Early detection and treatment would enable these children to lead a normal life, with survival compared to normal children.

(b). Common variable immune deficiency disorders

A 30 year man presented with a chronic cough of over 1 year duration. He was perfectly well till the age of 26 years of age when he developed chronic diarrhoea, and he had an episode of pneumonia at 28 years of age. He was HIV negative. His IgG and IgA levels were low, while his IgM levels were normal. He did not have isohemagglutinins, and did not seroconvert following immunization with the typhoid Vi vaccine. His CD 4 count was low, while his CD 8 and B cell numbers were normal.

This patient was over 2 years of age, had frequent infections, with low IgG and IgA levels. His functional antibodies were low. No other cause could be identified to explain his symptoms and thus, diagnosis of common variable immune deficiency disorder (CVID) was made.

CVID is the commonest clinically manifesting PID [15], in the West as well as in Sri Lanka [4]. In our study,

28.76% of PID were due to CVID, while 20% were due to X linked agammaglobulinemia [4]. CVID is a diagnosis of exclusion [15, 16, 17]. Patients with CVID have recurrent infections, mainly respiratory, but also gastro intestinal. They are also prone to autoimmune disease, such as thrombocytopenia and neutropenia [18]. One of our patients presented with neutropenia. We have a total of over 30 patients with CVID, on IVIG therapy. In addition, patients may develop lymphoproliferative disease and malignancy [15].

The cause of CVID is uncertain in the vast majority. Antibody production is always reduced in CVID, due to B cell dysfunction, but may result from lack of T cell help for antibody production [16]. A number of genes have been identified as being responsible in about 5% of CVID [16, 19]. These include the TACI [19], BAFF R, ICOS, CD 19, CD 20, CD 81 and LRBP. Our group, in collaboration with the Institute of Biochemistry, Molecular Biology and Biotechnology (IBMBB) University of Colombo, has sequenced the TACI, ICOS and Baff R genes in our patients with CVID. A novel homozygous mutation has been identified in the TACI gene in one patient, which is however, clinically silent [20]. Another missense mutation, seen in 4/19 patients, may be clinically significant. None of the controls had the mutation. Three patients with a similar homozygous mutation in the 3' UTR region of the ICOS gene, have also been identified in this cohort, but not in other cohorts of patients with CVID. While we are yet uncertain as to its clinical significance, it is note worthy that this same mutation has been seen in a significant proportion of Chinese patients with macular degeneration. Analysis of Baff R sequencing is on going.

A 52-year-old man presented with severe local destruction of his upper and lower lip and total destruction of the anterior nasal septum and was diagnosed with mucosal leishmaniasis. The causative organism was confirmed to be *Leishmania donovani*. In addition he had tuberculous lymphadenitis. This patient was diagnosed with CVID. He was successfully treated with intramuscular sodium stibogluconate. Even though leishmania and tuberculous co-infection has been reported in association with HIV, co-infection had not been reported previously in inherent immune deficiency [21].

(c). Antibody deficiency and polio

A 10 year old girl, the only child of first cousins, had been having recurrent infections from the 2nd year of life. Subsequently, she developed quadriparesis ie all 4 limbs became paralysed, due to an enteroviral infection attacking the nervous system. Subsequently, she was diagnosed as having CVID and was started on intravenous immune globulin (IVIG). She was enrolled in a multi country (10 countries) study sponsored by WHO. A mutated polio virus, derived from the oral polio vaccine virus was found in her stools, and was continuously

excreted for 8 months. This virus, an immune deficiency associated polio virus (iVDPV) had a total of 23 nucleotide changes in its VP 1 region [22, 23].

The Global Polio Eradication Initiative, established in 1988, has made substantial progress toward achieving polio eradication, with only 3 countries never having eliminated wild poliovirus. This has been attributed to the oral polio vaccine (OPV). However, persons with primary immune deficiency disorders exposed to OPV are at increased risk of vaccine-associated paralytic poliomyelitis (VAPP) and of prolonged excretion of Sabin polioviruses. However, the risk for prolonged excretion is not known. Therefore, we studied the prevalence of PIDD with long-term poliovirus excretion in Sri Lanka, a middle income country currently using OPV.

We stimulated the referral of patients under the age of 35 years, with clinical features suggestive of immune deficiency [22, 23]. Stool samples from patients with PIDD were cultured for the presence of poliovirus (PV). Poliovirus isolates were tested for intratypic differentiation (ITD). The VP1 region of all poliovirus isolates was sequenced in India. Of 942 patients investigated, 51 (5.4%) were diagnosed with PIDD. Five (10.2%) patients excreted poliovirus. Three patients excreted vaccine derived polio virus. However, more significantly, 2 patients excreted viruses that have substantially changed, or mutated, from the parent OPV strain. One patient with SCID excreted a P2 vaccine-derived poliovirus (VDPV 2) [24], and another with common variable immune deficiency (CVID) excreted a VDPV 3. The CVID patient with VDPV 3 excreted for 7 months, and has developed a 23 nucleotide divergence in VP1 (~ 900 nucleotides) from the parental Sabin virus. These 2 patients excreted viruses which had mutated sufficiently to be identified as iVDPV. These vaccine derived polio viruses are potentially virulent, and may pose a threat after wild polio virus infection is eradicated. With improving health care quality patients with CVID and XLA may survive longer especially with provision of intravenous immune globulin. Regular screening of patients with PIDD for excretion of poliovirus is necessary to identify chronic excretors and make available specific therapies.

Subsequent next generation sequencing of the entire genome of the virus with 23 nt variations has been performed in the Polio Reference Centre in India (manuscript in preparation).

Moving away from clinical cases, I will present a few research projects related to immune deficiency.

(d). Respiratory infections in antibody deficiency

The respiratory tract is the commonest site of infection in antibody deficiency. Recurrent pneumonia may lead to bronchiectasis, and chronic colonization of the lung with various bacterial pathogens. In cystic fibrosis (CF), there is an ordered progression of colonization, initially intermittent and then persistent, with *S pneumoniae* and

H influenzae, followed by *S aureus* and finally, in those with severe lung damage, *P aeruginosa* [25]. There is little data on PIDD, and generally, immunologists extrapolate data derived from CF patients. A study, aimed to identify the bacterial respiratory colonizers of patients with PAD and whether these colonizers have an orderly pattern of progression similar to patients with CF. While we have not yet evaluated HRCT scores with the progression of pathogens, a tentative pattern of persistent *S. pneumoniae* increasing with the duration of symptoms and *H. influenzae* colonization changing from intermittent to persistent colonization may be hypothesized. It is possible that initial *H. influenzae* colonization is intermittent and that the organism subsequently becomes persistently present in the respiratory tract [26]. Optimal treatment is problematic in patients with pseudomonal colonization, with even tobramycin nebulization having variable outcomes.

(e). The use of typhoid Vi vaccine to detect Specific Antibody Deficiency Syndrome (SPAD)

Individuals who present with recurrent infections, particularly of the airways and air passages, may have an underlying immunodeficiency. Defective production of specific antibodies in response to polysaccharide antigens is a major cause of infection in immunodeficient patients. Currently the response to Pneumovax® is assessed in individuals presenting with recurrent respiratory tract infection (RRTI) to identify a possible deficiency in the production of antibodies raised in response to a polysaccharide antigen. This is an essential investigation that has to be done to evaluate the immune system. Treatment may depend on it. In addition, one of the commonest immune deficiencies, specific antibody deficiency, where the immunoglobulin levels are normal, but defective antibody responses to polysaccharides are seen, is diagnosed by assessing the response to Pneumovax®. The standard assessment involves the measurement of the fold increase in concentration pre and post vaccination (FI) and achievement of a particular post vaccination concentration. **However, there are major problems with this assessment, including interpretation as well as the use of the conjugate pneumococcal vaccine which will interfere with the results.** One other problem is that there are only in house assays available for this test.

In countries with endemic typhoid fever, Typhi Vi vaccine is administered to at risk populations and so there is the potential to use the response to the Vi polysaccharide to assess the production of polysaccharide antibodies in individuals with RRTI.

We assessed the measurement of Typhi Vi antibodies for the identification of individuals with defective polysaccharide antibody production in a cohort of patients presenting with RRTI. 66 controls and 74 patients with recurrent respiratory infections were given the typhoid Vi vaccine and the response measured. In patients with

RRTI, the 5th percentile identified 7 patients with the defective ability to produce Typhi Vi antibodies and the 10th percentile, 13 patients including two with Specific Antibody Deficiency. Seven further patients were identified that may warrant monitoring.

(a). Chronic granulomatous disease

A 27 day old boy was referred with a history of a scrotal abscess. He presented again at 4 months of age with severe pneumonia, and ultra sound scan of the liver indicated multiple abscesses. The presence of infective abscesses in the internal organs, particularly the liver, necessitates exclusion of chronic granulomatous disease. The nitroblue tetrazolium assay (NBT) was carried out on the patient, and was positive. A diagnosis of chronic granulomatous disease was made. As the mother was found to have 2 populations of neutrophils, ie, indicating that she was a carrier, a diagnosis of x linked CGD was given. He was started on co trimoxazole and itraconazole prophylaxis. However, he continued to develop infections, including pneumonia and skin abscesses. He has also been diagnosed with mycobacterial disease.

NADPH oxidase, an enzyme that catalyses the transfer of a free electron cytoplasmic NADPH to molecular oxygen, comprises a number of proteins, mutations in 5 leading to CGD [27]. Following the formation of superoxide, further steps lead to the production of bleach and other chemicals. These are necessary for the killing of microorganisms, and also for activating other antimicrobial peptides.

The organisms implicated in the US are *S aureus*, *Burkholderia cepacia*, *Serratia*, *Nocardia*, and *Aspergillus fumigatus*. Mycobacterial infections are very rare in patients with CGD in the US. However, it is an important pathogen in Asia.

The first patient with CGD was reported by us in 2000 [28]. Subsequently, a total of 10 patients, 8 males and 2 females have been diagnosed with this condition.

(b). Leukocyte adhesion deficiency 1

A ten day old baby girl diagnosed with severe umbilical sepsis with septicaemia. The umbilicus stump was resected surgically at the local hospital on day eight of life. She was born at term to a healthy Sri-Lankan couple with second degree consanguinity with a birth weight of 2.9kg. Their four-generation pedigree did not reveal inherited disorders including primary immunodeficiency syndromes. History did not favor an acquired immunodeficiency. There were no miscarriages, stillbirths, neonatal or infant deaths in the family.

The child was febrile, lethargic with skin mottling on admission, had a circumumbilical erythema and a

significant ulcer (2 x 3 x 0.5 cm, with a leathery base) at the nape of the neck with some local cellulitis. Her peripheries were warm, she had a hepatomegaly of 4-5 cm below the right costal margin with no splenomegaly. Rest of the clinical examination was normal.

The complete blood count revealed, leukocytes 95,000/mm³ with 83% (accounting for a 78850/mm³) absolute neutrophil count. The values were confirmed by a peripheral blood film. The C reactive protein was >72mg/l, the CSF was normal. Blood cultures were sterile while the wound swab from the ulcer isolated a *Pseudomonas* species.

The absence of pus and a very high neutrophil count, specially in a neonate raised the possibility of leukocyte adhesion deficiency (LAD) type 1. Type 2, with neurological features figures only in isolated case reports. In LAD, another characteristic feature is the delay in separation of the umbilical cord, but in this instance, it had been resected.

Prof. Chandy, a Consultant Haematologist from CMC, Vellore, sent a monoclonal antibody against CD 18, enough for 2 tests, the control and the patient. The flow cytometry was performed, the CD18 level of the patient was <1% while the control levels were – 98%. Leucocyte adhesion deficiency type 1 was the diagnosis.

One other suggestive feature was a biopsy report from the active margin of the ulcer. It revealed numerous dermal blood vessels with entrapped neutrophils within the lumen; tissue neutrophils and plasma cells were inconspicuous. We tried to arrange a stem cell transplantation, but unfortunately, the patient died before it could be arranged. Even more tragically, the parents gave birth to another child. We used a surrogate marker, CD 11c, which is also not expressed in LAD 1. This baby too, had the severe variant of LAD 1.

Leukocytes need to enter tissues from the blood stream to combat microorganisms. This entails adhesion to the endothelium, and occurs in a well regulated manner. One such group of adhesion molecules associated with leukocyte trafficking are the integrins. In LAD 1, a very rare PID, with only about 200 cases described world wide, the β integrin (CD 18) is mutated, leading to non expression or expression of an abnormal protein on leukocytes which are unable to exit the blood stream, and accumulate in numbers in the blood, but absent at sites of infection [29]. Patients with LAD 1 have recurrent, life-threatening bacterial and fungal infections of the skin and mucous membranes. A characteristic feature is the absence of pus, as seen in our patient. In patients with <1% expression, ie severe phenotype, stem cell transplantation is necessary to save life. Our department have diagnosed 3 patients with this condition, who, unfortunately could not undergo SCT.

(c). Mendelian susceptibility to mycobacterial disease (MSMD)

A 5 month old boy presented with left sided axillary adenitis, ie inflammation of his lymph nodes (from 2nd month) and fever (from 3rd month). He had failed to gain weight and hepato-splenomegaly. The chest x ray revealed hilar lymphadenopathy, a lymph node biopsy revealed necrotizing lesions without granulomata. PCR for TB from the biopsy was negative.

A history of failure to thrive, prolonged fever, and enlargement of lymph nodes, both in his axilla and thorax, indicates a possibility of infection due to *M bovis*, the attenuated bacillus used as the BCG vaccine to protect against severe TB. Unfortunately, in patients with immune deficiency, this attenuated organism may cause problems. The left axilla is important, as infection by the BCG bacillus causes problems in the draining lymph nodes, which is in the left axilla. While secondary immune deficiencies were excluded, including HIV/AIDS and lymphoma, the possibility a PIDD remained. These were, in order of likelihood, SCID, CGD and what was at that time named type 1 cytokine deficiency. His lymphocyte subsets evaluated by flowcytometry were normal, but T cell function, using the mitogen concanavalin A was reduced. However, there was partial function, so SCID was probably excluded. CGD can cause localized mycobacterial disease; the NBT test was normal, and CGD was excluded. The type 1 cytokine system had to be assessed which was done at the University of Cambridge, and an IFN γ deficiency, probably AR and complete, was diagnosed. This patient was subsequently lost for follow up. He would have needed a SCT to survive. Today, this condition is labeled Mendelian susceptibility to Mycobacteria disease (MSMD), meaning genetic diseases, 9 of them at present, which specifically predispose to infections with mycobacteria.

MSMD is due to defects of the type 1 cytokine pathway [30]. Intra vesicular pathogens, such as the TB bacillus, invade the vesicles of macrophages, which in turn present mycobacterial peptides to CD 4+ T cells. They also secrete IL 12, which acts via the IL 12 R on the T cell. This results in activation of the lymphocyte and production of IFN γ , which then activates the macrophage via the IFN γ R. Activation leads to killing of the invading pathogen. Nine genes responsible for MSMD have been identified, leading to infections with non tuberculous mycobacteria and Salmonella. Some mutations are complete, giving rise to severe illness, while others are partial, with milder symptoms. The patient described had a severe illness.

05. Disorders of Complement

(a). Mannan binding lectin deficiency

Sepsis remains a major cause of morbidity and mortality among neonates, especially very low birth weight infants.

Physiological immaturity of the specific immune system may be a major risk factor for neonatal infections. The innate system may play a relatively more important role in this age category. However, genetic variations in the innate immune system may play a role in modulating severe infections.

The complement system includes a series of proteins involved in inflammation, anti microbial defense and disposal of immune complexes. The complement system is activated by 3 mechanisms, including the mannanose binding lectin pathway (MBL). Mannose binding lectin, a member of the collectin family, is an acute phase protein produced by the liver. It acts as a pattern recognition receptor, binding to mannanose and N acetyl glucose amine, present in a wide range of micro organisms. MBL is an opsonin, as well as an activator of complement. A few studies have been carried in developed countries, on the association of low MBL levels with sepsis in neonates, who have an immature immune system [31]. Our group, evaluated whether low MBL plasma levels at birth was associated with early onset sepsis (EOS) during the first 72 hours after birth, and with culture proven sepsis and pneumonia during the first month of life, in 78 neonates admitted to a NICU at birth. To our knowledge, this is the first such study in the setting of a developing country. The results are presented in the table 4 [32] (AISSL biennial sessions 2012).

Low plasma MBL levels has a significant association with severe sepsis in first month of life but not with EOS. We concluded that MBL measurements may be used to identify infection prone neonates.

Conclusion

Antibody deficiency is the commonest PIDD in this country, and CVID is the commonest clinically manifesting disease. This is similar to the situation in the West. However, clinically significant IgA deficiency, and specific antibody deficiency is uncommon in Sri Lanka. γ chain deficiency as a cause of SCID is relatively uncommon, compared to the situation in the West; the AR varieties predominate, because of more intermarriage in Sri Lanka compared to the West. We have seen very rare defects, ie the hyper IgE syndromes, the LAD 1, the CD 40 deficiencies. These diseases are rare in the West. Unfortunately, comparison with neighbouring countries such as India is not satisfactory as data from that country is scarce. For example, details of only 4 HIGE patients with Stat 3 mutations have been published in India. Only Iran, Japan and China, of the Asian countries publishes on PIDD with some regularity.

In the 80s and 90's, few immune deficiencies were diagnosed, and that too of diseases which could be identified by phenotype. Many patients died undiagnosed. In the past 1½ decades, in the new millennium, our knowledge has expanded, and with it the diagnosis and

Table 4. MBL levels and sepsis

Variable	Sepsis				Possible infection			
	n	OR	95% CI	Pvalue	n	OR	95% CI	Pvalue
MBL plasma level								
Low ($\leq 0.7\mu\text{g/ml}$)	12	3.4	1.1-10.7	0.3	11	12.4	0.8-7.3	ns
Normal ($>0.7\mu\text{g/ml}$)	7	Ref			9	Ref		
MBL level ($\leq 0.2\mu\text{g/ml}$)	10	4.3	1.3-14.1	0.01	7	2	0.6-6.9	ns
($>0.2\mu\text{g/ml}$)	9	Ref			13	Ref		

the management of patients have improved in Sri Lanka. Today, patients with antibody deficiency and certain other PIDD are diagnosed early, treated with costly but life saving drugs and are leading normal lives. Many of their elder siblings/relations are dead. Hopefully, their life expectancies would be no different from the “normal” population.

I have taken you through a brief survey of the state of PIDD in Sri Lanka, and it has been a long march. We have travelled through deserts and mountains, hills and valleys, tasting success and also failure. Some patients have died but many have survived. And it is not over. Much needs to be done. But we will get there.

The most urgent need today is to offer some hope to all patients with PIDD. To provide timely diagnosis, prompt treatment and support. It is a matter of regret that patients with SCID, and a few other PIDD do not survive, because we cannot offer them stem cell transplantation. This may be a reality in the near future. Diagnostic aids have improved tremendously in the past few years. Sanger sequencing, where individual genes are sequenced has been a technique with a long history. As I mentioned earlier, these techniques have been used in some of our patients. However, this technique is cumbersome, expensive and not practical to do in one unit. In the UK, with multiple Immunology Departments, sequencing individual genes have been farmed out to different units. However, there is a new kid on the block – next generation sequencing (NGS), where the entire genome, the exome (coding regions) or defined genes can be sequenced without trouble, at a cheaper cost. At present, the DNA of 19 of our patients are being subjected to next gen sequencing in the UK, at the Royal Free Hospital, at no cost to us.

It is my hope, that we will be able to offer our patients with PIDD, the same services that patients in the affluent West receive.

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CARBAPENEMASES IN *ENTEROBACTERIACAE*: A REVIEW

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Introduction

Carbapenems have a broad spectrum of activity, ranging from Gram positive cocci to Gram negative bacilli such as *Pseudomonas* and *Acinetobacter*. They are usually reserved for nosocomial infections caused by antibiotic resistant Gram negative bacteria. They have excellent pharmacokinetic and pharmacodynamic properties, showing good tissue penetration, high stability to enzymes, high binding to penicillin binding proteins (PBP) and reduced susceptibility to efflux. Unfortunately, resistance to carbapenems has emerged, resulting in multi-drug resistant (MDR) pathogens. The spread of these MDR pathogens is now a major worldwide public health threat.

Although a few bacteria, such as *Stenotrophomonas maltophilia*, are intrinsically resistant to carbapenems, most other forms of resistance are acquired. The mechanism of resistance may be limited entry of the drug (e.g. loss of OprD in *Pseudomonas aeruginosa*, loss of outer membrane proteins OmpK 35/36 combined with AmpC β -lactamases in *Klebsiella*), increased efflux, alteration of PBPs or production of carbapenemases (1).

While chromosomally-coded carbapenemases have been described in isolates of *Serratia marcesans* (SME) and *Enterobacter cloacae* (IMI/NMC), integrin-coded carbapenemases in isolates of *Pseudomonas* and *Acinetobacter* (IMP,VIM) and plasmid-coded carbapenemases in *Acinetobacter* (OXA), these enzymes are species specific, and strains remained confined to restricted geographic locations resulting in only local clonal spread (1). The escape of these genes onto plasmids and transposons of *Enterobacteriaceae*, conferring transferable resistance, is a more alarming development. Since these plasmids disperse freely, they can be acquired by commensal intestinal flora of animals and healthy people and spread between humans or enter the food chain. *Enterobacteriaceae* are among the most common causes of community and hospital-acquired infections so the potential impact on public health is much greater than when such resistance was restricted to *Pseudomonas* and *Acinetobacter*. In the past two decades there has been a significant worldwide increase in reports of carbapenem-resistant *Enterobacteriaceae* (CRE) causing difficult-to-treat infections with a high

mortality rate. This is due to the emergence and horizontal spread of both plasmids coding for carbapenemases and successful epidemic clones of CRE.

This article summarises the common groups of plasmid-mediated carbapenemases described in *Enterobacteriaceae*, their geographical distribution and the current status of laboratory detection. It will not deal with screening for carriers of CRE using selective agar media such as Chromagar.

Classification and epidemiology of carbapenemases

Carbapenemases are a heterogenous mixture of β -lactamases belonging to different Ambler classes (2). Classes A, C, and D are serine enzymes and Class B are metallo- β -lactamases (MBL). Classes A, B and D contain carbapenemases (Table 1).

Table 1. Ambler classes of β -lactamases
(carbapenemases are in **bold italics**)

A	B	C	D
Serine penicillinases	Metallo- β -lactamases	Cephalosporinases	Oxacillinases
TEM-1, TEM-2, SHV-2, ROB			OXA-1
TEM-3-26, CTX-M		AmpC	OXA-163
SME, NMC/IMI, GES, KPC	IMP, VIM, GIM, SPM, NDM		OXA-23, OXA-48, OXA-181

The first reports of *Klebsiella pneumoniae* carbapenemases (KPC) were in the USA (3). KPCs are Class A enzymes and have a broad-spectrum of activity, hydrolysing penicillins, cephalosporins, carbapenems and aztreonam (1). A single, highly transmissible *K. pneumoniae* clone, ST258, emerged and spread to Europe, China and Israel causing nosocomial outbreaks (3).

Sporadic infections and hospital outbreaks due to *Enterobacteriaceae* expressing plasmid-coded VIM and IMP have been reported worldwide, especially in southern Europe and Asia (3). A novel MBL, dubbed New Delhi metallo- β -lactamase-1 (NDM-1), was detected in 2008.

Unlike KPC, where global spread was due mainly to a single clone, the NDM gene proved to be highly mobile, transferring to many unrelated species and disseminating to many countries (3,4). NDM-producers are the most important type of CRE in South Asia, including Sri Lanka. Unlike KPC producers, which almost exclusively cause nosocomial infections, NDM-producing *Enterobacteriaceae* are found as colonizers in healthy people and are widespread in the environment and in the food chain.

Class D carbapenemases or oxacillinases were originally found only in *Acinetobacter* sp. (1). Since they were relatively weak carbapenemases they did not cause much alarm. A more disturbing development was the emergence of plasmid-coded oxacillinases in strains of *Enterobacteriaceae*, resulting in another group of CRE, carrying the OXA group of carbapenemases. While OXA-48 is found in North Africa, Middle East and Turkey, OXA-181 originated and is widespread in Asia (3,4). Since these strains remain susceptible to cephalosporins and aztreonam, and have only low level resistance to carbapenems with minimum inhibitory concentrations (MIC) in the intermediate or even sensitive range, they are difficult to detect and their true prevalence is probably underestimated (5). As with NDM, OXA-181 is found in multiple, clonally unrelated isolates (5).

In general, KPCs are found mainly in the USA, VIM in Greece, OXA-48 in Middle East, North Africa and Turkey and NDM and OXA-181 in Asia, including South Asia (3).

The first report of a MBL-producer in Sri Lanka was in 2010, where the enzyme was detected using EDTA as an inhibitor in a combined disc test (6). However, at that time, no facilities were available to identify the enzyme further. A further paper, published in 2014, described 22 distinct strains of carbapenemase-producing *K. pneumoniae* producing OXA-181 and/or NDM-1 enzymes. The authors emphasized the surprising diversity of bacterial genotypes and β -lactamases encountered in these strains (7). No KPCs were found in this study, compatible to other studies in South Asia (8).

Detection of carbapenemases

Rapid and accurate detection of CRE is essential for effective antibiotic treatment and infection control to limit spread. However, detection of such isolates is challenging due to the multiple types of carbapenemases and strains with relatively low MICs. Carbapenem resistance can also be caused by overexpression of extended spectrum (ESBL) or AmpC β -lactamases in combination with porin loss. Therefore a series of screening and confirmatory, phenotypic and genotypic methods have to be used in parallel.

Screening for carbapenem resistance

The first step in the detection of CRE is to screen all isolates for resistance to carbapenems by disc susceptibility testing or a MIC method. Sensitivity can be improved by using both meropenem and ertapenem

(9,10). Strains showing resistance or reduced susceptibility (MICs higher or zones of inhibition below a screening breakpoint) should be subject to further testing (3,9). However, producers of OXA-type carbapenemases often have quite low MICs and may be missed when these breakpoints are followed (5).

The modified Hodge test (MHT) is useful to screen for KPC and OXA producers (1,3,5,11) but is unreliable to detect NDM producers (false negatives) and can be falsely positive in strains with high level AmpC/CTX-M production combined with porin loss (3,4,9,12). Therefore, it is not suitable as a screening test in Sri Lanka where KPCs are not seen and NDM is common.

Use of the antibiotic sensitivity test pattern (antibiogram)

The antibiogram of the strain can be used to identify which carbapenemase is responsible for resistance. Strains producing KPCs show resistance to carbapenems, cephalosporins and aztreonam. MBLs are unable to hydrolyse aztreonam, so strains are resistant to carbapenems and cephalosporins but sensitive to aztreonam. However, concomitant ESBL production can often result in aztreonam resistance. Strains with OXA carbapenemases may appear sensitive to carbapenems (due to low MICs) and are sensitive to cephalosporins and aztreonam but resistant to betalactam/beta-lactamase combinations and have high MICs to temocillin (3,5). It has been suggested that temocillin and piperacillin-tazobactam sensitivity can be used to rule out the presence of OXA enzymes (12).

Inhibitor patterns

Combined disc tests (CDT) or double-disc tests (DDT) using enzyme inhibitors specific to each type of carbapenemase have been developed. For KPC, phenylboronic acid (PBA) or aminoPBA (APBA) is used. Since strains producing AmpC may also be positive by this test, the absence of inhibition by cloxacillin should be shown simultaneously (3,9). For MBLs the inhibitor incorporated into these tests is EDTA or dipicolinic acid (DPA). An Etest MBL strip, based on inhibition by EDTA, is available (1,9,10). Although both compounds are equally sensitive, DPA may be more specific than EDTA, which may give false positives (12). One study recommended the use of five discs on a single agar plate, meropenem, meropenem+DPA, meropenem+EDTA, meropenem+APBA and meropenem+cloxacillin to distinguish between KPC, MBL and AmpC/ESBL hyperproducers with porin loss (12).

OXA carbapenemases are not inhibited by any of these compounds and cannot be detected using these methods (3). They can be suspected when an isolate is carbapenem non-susceptible, MHT positive, negative in the inhibitor tests and has a high level of resistance to temocillin. A new phenotypic OXA-48 disc test, using two imipenem discs loaded with the test strain and impregnated with EDTA and EDTA+PBA respectively placed on either side of a plain imipenem disc on a lawn

of susceptible *E. coli*, has been proposed to distinguish between all three types (KPC, MBL and OXA) (11).

A limitation of all phenotypic assays is their inability to detect co-production of multiple carbapenemases.

Molecular methods

Using the polymerase chain reaction (PCR) to detect carbapenemases requires the use of a panel of primers to detect NDM-1, VIM, IMP, KPC, OXA-48/181, GES etc. (1,3,4,7,9). Several single and multiplex PCR methods have been described, both in the conventional and RT-PCR format (9). PCR is very rapid, sensitive and specific and can reduce reporting time considerably. It can also detect OXA-type enzymes that may be missed by phenotypic testing and can identify the presence of multiple carbapenemase genes. However, PCR is costly and requires expertise. Also it is unable to detect novel carbapenemases.

Advances in the phenotypic detection of carbapenemases in the clinical laboratory

Because of the risk of non-detection of some CRE using current breakpoints and the difficulties and delays in performing and interpreting multiple phenotypic tests in the clinical laboratory, newer methods for direct detection of all types of carbapenemases have been developed.

The Carba NP test is based on the hydrolysis of imipenem by a lysis extract of the test strain resulting in a drop in pH detected by an indicator (13). While this is a very rapid (<2h), bench-top test, the preparation of a fresh pH adjusted solution containing imipenem, ZnSO₄ and phenol red is required and the bacterial suspension has to be incubated in a lysis buffer and centrifuged to obtain the supernatant containing the enzyme.

The carbapenem inactivation method (CIM) is a very low cost, low-tech method, where a loopful of isolate is suspended in water in an eppendorf tube, a meropenem disc (20µg) is added and incubated at 35°C for 2 hours. Subsequently the disc is placed on a Mueller Hinton agar plate inoculated with *E. coli* ATCC25922 and the plate is incubated overnight. If the isolate produces carbapenemase the disc will be inactivated and no zone of inhibition will be seen (14). The CIM has proven to be a very good phenotypic method able to detect almost all groups of carbapenemases (KPC, NDM, OXA-48, VIM, IMP) except the rare GES type. It is an easy and rapid tool to identify CRE in clinical laboratories in resource poor settings.

Advanced and reference laboratory methods

The matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) method for speciation of bacteria has also been used to detect carbapenemases (9). Carbapenemases undetectable by routine testing may be detected by reference laboratory methods such as spectrophotometry, isoelectric focusing and immunochromatography (1,3,9).

Difficulties in detecting carbapenemases using current techniques

The breakpoints of carbapenems for *Enterobacteriaceae* were reduced considerably by CLSI and EUCAST to improve the detection of CRE. According to these guidelines, treatment decisions can be made on MIC/zone sizes alone, without special testing. Special testing is recommended only for epidemiological and infection control purposes. However, it is likely that some carbapenemase-producers are missed because their MICs are within the sensitive range. While any type of carbapenemase-producer may be misclassified as susceptible, the OXA group are the most difficult to identify as they only hydrolyze carbapenems weakly and are susceptible to cephalosporins and aztreonam (3). Since data on the effectiveness of carbapenems in treatment of infections due to carbapenemase-producers within the susceptible range is scarce, it is probably more prudent to detect and report such isolates to the clinician to ensure careful monitoring of the patient and stringent infection control (15).

Conclusion

While not more virulent, CPE give rise to infections that are difficult to treat. It is important to identify these strains quickly to institute appropriate therapy and infection control. However, this is complicated by the absence of standardized sensitive and specific tests. There is an urgent need for a cheap, rapid, sensitive and specific test to detect carbapenemases in the clinical laboratory and a screening medium capable of detecting all types of carbapenemases, including the OXA group that are susceptible to the cephalosporins used in most commercial screening agars (5).

Resistance genes are found naturally in environmental bacteria and in a small number of strains of pathogens. However, the use (and abuse) of antibiotics 'selects' for such resistant genes (and strains) as they confer a survival advantage. Some of these genes become mobilized on plasmids and transfer rapidly within and across species. In environments where large amounts of antibiotics are used in human, veterinary and agricultural practice these resistant strains expand. Antibiotic stewardship strategies aim to reduce the selection pressure exerted by antibiotics. The other mode of spread of resistance is through the emergence of resistant clones with enhanced fitness that effectively spread locally and globally. Such spread can only be interrupted by rigorous infection control measures, including early identification of carriers.

While these strategies may be useful to reduce nosocomial infections caused by *Enterobacteriaceae* (especially *K. pneumoniae*) producing KPC, NDM, OXA or IMP/VIM, they have limited effectiveness in a setting where *E. coli* with NDM and OXA-48 or-181 spread rapidly in the community giving rise to community acquired infections, reminiscent of the spread of ESBLs previously (3).

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Review article:

ZIKA VIRUS: EMERGING THREAT AND GLOBAL TOUR

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Introduction

Zika virus (ZIKV), causative agent of Zika fever is an *Aedes* mosquito-borne flavivirus that emerged in Brazil in 2015 and then rapidly spread throughout the tropical and subtropical Americas. This review details ZIKV, in particular discovery and emergence, transmission, clinical presentation and potential human risk, diagnosis and prevention.

Discovery and emergence of Zika virus

ZIKV was first isolated in 1947 by Dick, Kitchen, and Haddow from a serum of a febrile rhesus monkey that

had been caged on a forest as part of a sylvatic yellow fever study in the Zika Forest in Uganda [1]. The first human isolate of ZIKV was obtained a decade later around 1960 from the serum of a 10-year-old Nigerian girl who presented on day 5 of illness with fever and headache during a suspected outbreak of yellow fever [2].

In 1966, the first case was confirmed in South East Asia, and in the late 1970s it was documented in Pakistan, India, Malaysia and Indonesia. Following its discovery in 1947, ZIKV remained an obscure, confined to areas of Africa and Asia. The first official epidemic out of Africa and Asia, almost after 3 decades, was on the isolated

island of Yap, Micronesia in 2007. It hit French Polynesia in 2013, with a huge outbreak. In 2014, ZIKV arrived in northern Brazil and spread slowly throughout Brazil for around a year, before the WHO reported the first outbreak outside the country, (Colombia) on October 2015. In early December 2015, the disease spread to Mexico for the first time, raising concerns in the US. By the start of January 2016, cases were found from Caribbean countries. A few imported cases have been reported locally in the US, the UK, Denmark, Germany and Australia [3].

ZIKV is an enveloped, positive-sense, single-stranded RNA virus that is a constituent of the *Flaviviridae* family, *Flavivirus* genus, and is one of two members of the clade containing Spondweni virus [4].

Zika virus transmission

The primary mode of ZIKV transmission to humans is via the bite of *Aedes* species mosquitos, including *Ae. aegypti* and *Ae. albopictus*, the most important vectors globally for the transmission of DENV. In early mosquito studies in Uganda, including the manuscript describing its discovery, ZIKV was isolated from *Ae. africanus* mosquitos [1]. Importantly, these vectors are endemic throughout the tropics, subtropics, and many parts of the globe including southeastern United States [3].

Potential non-vector-borne modes of ZIKV transmission include sexual [5], transfusion-associated [6], and perinatal [7] transmission. Most notably, the ZIKV pandemic in the Americas corresponded with a dramatic upsurge in the number of reported cases of fetal and pediatric microcephaly throughout Brazil [8], suggesting intrauterine transmission. This hypothesis was supported by the work of Oliveira Melo et al., who described two cases of fetal microcephaly in which ZIKV RNA was detected in amniotic fluid, from two women who reported symptoms consistent with Zika fever during their pregnancy [8].

Clinical presentation of zika infection

Zika fever, an undifferentiated systemic febrile illness may also go undetected or be confused with other causes of febrile illness like dengue or chikungunya. The clinical presentation of patients with acute ZIKV infection typically includes a combination of fever, headache, retro-orbital pain, conjunctivitis, maculopapular rash, myalgia, and/or arthralgia [9]. Fever is often low grade (~38°C), though cases with fever of up to 40°C has been reported [9]. Symptom persists generally for 2 to 7 days, but the rash and arthralgia may last 2 weeks or longer [9]. Notably, it is estimated that 80% of patients with a ZIKV infection remain asymptomatic or develop only mild clinical manifestations [9]. However, there is no evidence that pregnant woman are more susceptible to or experience more severe disease during pregnancy.

Beyond dengue and chikungunya, the differential diagnosis for patients with ZIKV is broad and includes infections with herpes simplex-virus, cytomegalovirus,

Epstein-Barr virus, human herpesvirus-6 and other arboviral infections such as West Nile virus and yellow fever virus infections [10].

Clinical concern and potential complications of ZIKV infection

Though ZIKV was initially thought to cause only a mild febrile illness, with limited morbidity and without mortality, reports from Brazil indicate that infection during pregnancy may be associated with severe birth defects, most notably fetal microcephaly [9]. The clinical concern was raised due to the unusual number of births of infants with microcephaly, coinciding with the spread of ZIKV across Brazil [9]. The preliminary findings suggest that exposure to ZIKV at any stage of pregnancy is at risk for the developing fetus as it can cause microcephaly or intracranial calcification in the fetus [11]. During the French Polynesia outbreak 2013, a number of cases of fetal microcephaly were also noted and are being further investigated to establish linkage with ZIKV infection [8]. However, new studies strengthen the connection between Zika and an array of birth defects.

The increased incidence of Guillain-Barre syndrome (GBS) and other severe neurologic complications during the French Polynesia outbreak 2013, suggested the association of ZIKV infection. During 2015/2016 outbreaks in South and Central Americas, also reported higher number of GBS cases leading considerable link to neurological disorders [13].

Zika virus diagnostics

There is relatively limited number of diagnostic assays for ZIKV identification. However, laboratory diagnosis of acute ZIKV infection currently relies upon the detection of ZIKV RNA in patient specimens since serological assays have strong cross-reactivity, especially in patients with prior flavivirus infection or immunization history. Further testing required providing higher specificity, using neutralization assays [1].

ZIKV has been detected in whole blood (also serum and plasma), urine, cerebrospinal fluid, amniotic fluid, semen and saliva. There is accumulating evidence that ZIKV is present in urine and semen for longer periods than in whole blood or saliva [13].

For ZIKV RNA testing specimens need to be collected in a dry tube from patients presenting with the onset of symptoms ≤ 7 days, whereas for serology, IgM detection sample need to be collected in a dry tube from patients presenting with onset of symptoms ≥ 7 days. Wherever possible, paired serum specimens should be collected at least 2-3 weeks apart [13].

During 2013–2014 French Polynesia outbreaks ZIKV RNA was more frequently detected in saliva than in serum when rRT-PCR assay was used to test paired specimens. The rRT-PCR was also used to detect ZIKV RNA in the amniotic fluid from the two cases of fetal microcephaly [8].

Viral culture-based methods for ZIKV detection are used in public health and research laboratories but are not generally available for clinical purposes. The reference method for the isolation of ZIKV is intra-cerebral mouse inoculation [1]. ZIKV is also culturable in several cell lines including African green monkey (Vero) and rhesus monkey kidney (LLC-MK2), as well as *Aedes pseudo-scutellaris* (MOS61 or AP- 61) and *Aedes albopictus* (C6/36) [1].

Prevention

Prevention is of utmost important since there is no vaccine or antiviral to prevent or treat ZIKV disease. Very simply prevention from mosquito bites and reducing the mosquito density is very important to prevent ZIKV infection.

The current guidelines by the U.S. Centers for Disease Control and Prevention (CDC) suggest that pregnant women should avoid travel to areas of active ZIKV infection. For those individuals who must travel to such areas, available safe insect repellants can be used during pregnancy. Women who return from areas of active ZIKV infection should undergo fetal ultrasonography to detect cranial abnormalities, but only symptomatic women and women with abnormal ultrasound findings should undergo specific testing for ZIKV infection [14].

To minimize ZIKV contamination, CDC guidelines recommends to defer blood products donating for at least 4 weeks if they visit to active ZIKV areas and/or confirmed with ZIKV infection, as well as if had sex with a man with known ZIKV infection [15].

Perspectives

The spread of ZIKV to the Americas and its association with a marked increase in the incidence of fetal neurologic abnormalities and other neurological disorder, Guillain-Barre syndrome have led to unprecedented interest in this once esoteric pathogen. Large epidemiologic studies and intensification of basic and translational research will result in increased understanding of ZIKV pathogenesis and immunology as well as in important breakthrough in sorely needed ZIKV therapeutics and vaccines. The sensitive and specific diagnosis of patients with Zika fever is critical to this work, to ongoing epidemiologic surveillance, and to the care of patients with an undifferentiated systemic febrile illness. However, as a way forward, further work will be required to clearly detail test performance characteristics in these patient groups and to determine whether these recommendations meet the needs of a pregnant women and their neonates in countries effected by ZIKV.

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INFECTIONS IN HAEMATOPOIETIC STEM CELL TRANSPLANT (HSCT) RECIPIENTS

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Haematopoietic Stem Cell Transplant is a medical procedure used to treat diseases once thought incurable. It is a potentially curative procedure for malignant and non-malignant diseases of the bone marrow, immune deficiency and metabolic disorders. HSCT has been developed over past 50-60 years since the first human experimental transplants were performed in the 1950s. One of the earliest curative allogeneic bone marrow HSCT was performed in a young child with immune deficiency syndrome in 1968. Currently over 50,000 procedures are performed worldwide each year as estimated by the Centre for International Blood and Marrow Transplant Research (CIBMTR).

Indications for transplant

Malignancies

Leukaemia, neuroblastoma, lymphoma, Ewing's sarcoma, brain tumours, Wilms' tumour, rhabdo-myosarcoma.

Non-malignant diseases of the marrow

Severe aplastic anaemia, Fanconi anaemia, thalassaemia and other haemoglobinopathies, Wiskott -Aldrich syndrome, haemophagocytic syndrome.

Immune deficiency

Severe combined immunodeficiency syndrome (SCIDS), chronic granulomatous disease, severe autoimmune diseases.

Metabolic diseases

Adrenoleukodystrophy, Hurler's syndrome – HSCT involves the intravenous administration of haematopoietic cells to a recipient whose haematopoietic and immune systems have been ablated by a cytotoxic preparative regimen (conditioning regimen) alone or in combination with radiation, given over the 4-10 days before transplantation. Haematopoietic stem cells are obtained from bone marrow, peripheral blood or umbilical cord.

Types of transplant

Autologous transplant

In this type, stem cells are collected from the patient at a specific time in the treatment of their disease and cryopreserved for future use. Following high dose chemotherapy and/ or radiation the cells are re-infused to the patient.

Allogeneic transplant

In this type, stem cells come from a donor – someone other than the patient. A donor may be a sibling or other family member, or someone who is unrelated.

The most common cause of death after stem cell transplantation is disease relapse, which accounts 73% of deaths after autologous HSCT and 33%-42% of deaths after allogeneic HSCT. Other causes of morbidity among HSCT recipients are infection, graft versus host disease (GVHD), regimen related toxicities and graft failure. Despite recent advances, infection still accounts for 16-19% of deaths after allogeneic transplant and 6% of deaths after autologous transplants

Graft – Versus Host Disease (GVHD)

GVHD is a major life threatening complication of allogeneic transplantation, developing in 40% to 80% of patients. The risk is higher in older patients and with partially matched and unrelated donors. Donor T lymphocytes mount an immune attack against recipient's tissues.

Clinical manifestations of acute GVHD include rash, cholestatic hepatitis, nausea, vomiting and diarrhea. GVHD itself and the immunosuppressive drugs (tacrolimus, cyclosporine, methotrexate and corticosteroid) used in the treatment can cause prolonged immunosuppression after HSCT. Patients with acute and chronic GVHD have splenic dysfunction and they are at risk of developing infections caused by encapsulated bacteria such as *Streptococcus pneumoniae*, *Haemophilus influenzae* and *Neisseria meningitidis*.

Patterns of immunosuppression at different times after HSCT

Three risk periods of immunodeficiency have been identified in recipients of HSCT (fig. 1).

Pre-engraftment period (2-6 weeks)

Early post-engraftment period (until day 100)

Late post-engraftment period (after day 100)

Pre-engraftment risk period

This period begins with the onset of conditioning therapy and continues until approximately 20-42 days after transplantation. Bacterial infections are common during this time of profound neutropenia and lymphopenia, necessitating promptly administered empirical systemic

antibiotics. Prophylactic systemic antibiotics (often a fluoroquinolone such as levofloxacin) can be administered when the neutrophil count drops to less than 500 cell/mm³ and continued until the neutrophil count recovers to prevent bacterial infection. However prophylactic antibiotic use has shifted the spectrum of the aetiological agents of bacteremia to more gram positive organisms; in particular, coagulase negative staphylococci and viridans group streptococci.

Mechanical barrier defects caused by mucositis and central catheters predispose patients to bloodstream infections by allowing access for skin colonising organisms and gastrointestinal flora to sterile body sites. During this period, HSV is predictably reactivated in 80% of patients who are HSV seropositive. Prophylactic acyclovir 400 mg twice daily (5mg/kg twice daily for children) has minimized this clinical infection.

Candidemia and early onset aspergillosis occur in fewer than 5% of patients with neutropenia. The risk is greater in patients with slow engraftment or extended neutropenia before transplantation. With fluconazole (200 to 400mg/day) prophylaxis this has been mostly eliminated. However *Candida krusei* and *Candida glabrata* have emerged as fluconazole resistant pathogens.

If transplant recipient is at higher risk for mold infection, they should be given mold prophylaxis.

These patients include those with acute myelogenous leukaemia, who have undergone serial chemotherapy before transplantation, those with myelodysplastic syndrome those with aplastic or Fanconi anaemia or if a patient have a history of aspergillosis within 4 months of transplantation

Use of haematopoietic growth factors has reduced the incidence of bacteremia by shortening the duration of neutropenia. Adjunctive therapy with granulocyte transfusion has been used for treatment of serious infection that develops in patients with neutropenia.

Post-engraftment risk period

This period begins with neutrophil recovery and continues until day 100, when early B and T-lymphocyte functional recovery may be initially apparent. Reconstituted T lymphocytes have abnormal function for approximately 18 months. Also T lymphocyte reconstitution may be blunted by effects of GVHD or CMV and their treatment (corticosteroids, cyclosporine, anti T lymphocyte therapy and ganciclovir). In patients with GVHD develop disruption of the gastrointestinal mucosa which can cause transmural entry of pathogens and can lead to bacteraemia and fungaemia.

Late onset aspergillosis may also occur during this period especially those with GVHD, those receiving high dose corticosteroids. Careful surveillance is required for high risk patients.

Prophylaxis for *Pneumocystis jirovecii* with trimethoprim – sulfamethoxazole, atovaquone or aerosolised pentamidine is required for first 6-12 months after transplant or longer if chronic GVHD is continuing.

Reactivation of CMV occurs in 20-60% of patients who are CMV seropositive. Surveillance for CMV reactivation needs to be done by using either pp65 antigenaemia assay or PCR testing for serum DNA. Ganciclovir therapy initiated preemptively if there is an indication for reactivation.

Late risk period

This period begins at approximately day 100 and ends when the patient regains the normal immunity, 18-36 months after HSCT. In general patients regain their normal immunity one year after HSCT. However who take immunosuppressive treatment for longer period and recipient with chronic GVHD this period persists as long as the therapy is required. VZV reactivation, infections with encapsulated bacteria, and invasive aspergillosis and other invasive mold infection may develop in this period. CMV disease may develop; therefore CMV surveillance must be continued in seropositive patients with chronic GVHD. Sinusitis, bronchitis, pneumonia and otitis media caused by respiratory viruses are common in the late risk period.

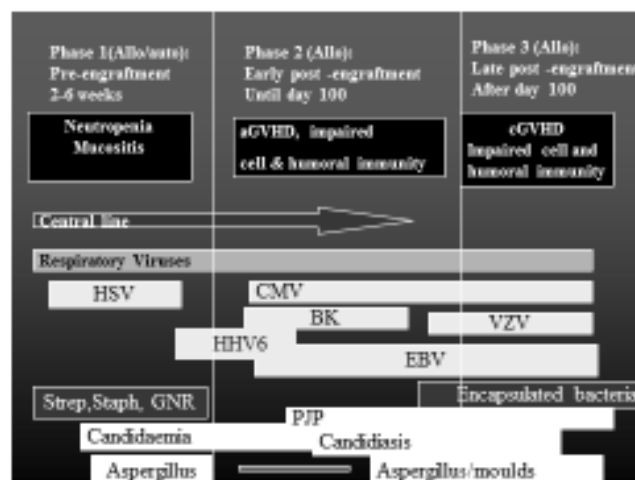


Figure 1. Phases of predictable opportunistic infections among patients undergoing HSCT.

HSV, herpes simplex virus; CMV, cytomegalovirus; BK, BK virus; VZV, varicella-zoster virus; HHV6, human herpes virus 6; EBV, Epstein-Barr virus; PJP, *Pneumocystis jirovecii* pneumonia.

Advances in HSCT have given hope to those who suffer from once uniformly fatal diseases. Infectious complications have been associated with significant morbidity, mortality and increases cost of care after the procedure. Specific measure to prevent infection has been described for the pre transplant as well as the different

phase of post-transplant period. These include patient care evaluations, vaccines and prophylaxis and pre-emptive treatment for many bacterial, fungal and viral infections.

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Review article:

DIAGNOSIS AND MANAGEMENT OF PROSTHETIC JOINT INFECTIONS

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Summary

The prosthetic joint infections (PJI) have to be diagnosed (or excluded) before surgery, which enables starting targeted antimicrobial treatment preoperatively, deciding on duration of antimicrobial therapy and allows planning of the most appropriate surgical management.

Introduction

Joint replacement is a life-enhancing procedure for millions of people worldwide. The most challenging complication of this procedure is the PJI. *S. aureus* and coagulase-negative staphylococci contribute to 50% to 60% of PJI, while streptococci and enterococci together account for 10% of cases (1) and aerobic Gram-negative bacilli are involved in 10% of cases (1).

Diagnosis

Clinical and laboratory studies

The presence of a sinus tract that communicates with the prosthesis is a definitive evidence of PJI (2). Blood leukocyte count and differential are not sufficiently

discriminative to predict the presence or absence of infection. C-reactive protein (CRP) repetitive measurements are more informative than a single value in the postoperative period while a low CRP may have a role in helping to rule out infection. CRP of less than 13.5 mg/L had a negative predictive value of 88.5% in the diagnosis of late prosthetic knee infection (3). A CRP above this had a positive predictive value of only 59.2% (3). A test for sedimentation rate (ESR) or CRP should be performed in all patients with a suspected PJI when the diagnosis is not clinically evident. The combination of an abnormal ESR and CRP seems to provide the best combination of sensitivity and specificity (2). The role of procalcitonin in patients with prosthetic joint infection has not yet been defined.

Microbiological, histological and imaging studies

Preoperative diagnosis

In PJI where the patient is medically stable, withholding antimicrobial therapy for at least 2 weeks prior to collection of synovial fluid for culture increases the likelihood of recovering an organism (2).

Culture of a superficial wound or sinus tract often represents microbial colonisation from the surrounding skin and can therefore be misleading but only isolation of *S. aureus* from sinus tracts is predictive of the causative pathogen (4). A diagnostic arthrocentesis should be performed in all patients with acute PJI unless the diagnosis is evident clinically and surgery is planned and antimicrobials can be safely withheld prior to surgery. Arthrocentesis is also advised in patients with a chronic painful prosthesis in whom there is an unexplained elevated ESR or CRP level or in whom there is a clinical suspicion of PJI. It may not be necessary if in this situation surgery is planned and the result is not expected to alter management. Synovial fluid analysis should include a total cell count and differential leukocyte count, as well as culture for aerobic and anaerobic organisms. Culture of aspirated synovial fluid detects the infecting microorganism in 45-100% and may be further improved by inoculation into a paediatric blood culture bottle (5). Synovial fluid leukocyte count and differential represents a simple, rapid and accurate test for differentiating PJI from aseptic failure. A synovial fluid leukocyte count of $>1700/\text{mm}^3$ and differential of $>65\%$ neutrophils had a sensitivity for diagnosing prosthetic joint infection of 94% and 97%, and specificity of 88% and 98%, respectively (6). Blood cultures for aerobic and anaerobic organisms should be obtained if fever is present, there is an acute onset of symptoms, or if the patient has a concomitant infection that would make the presence of a bloodstream infection more likely.

Plain radiography of the affected joint is unhelpful in early infections but may show bone loss and evidence of loosening around an implant in chronic infections which are not specific to infection. Bone scans, leukocyte scans, magnetic resonance imaging (MRI), computed tomography (CT), and positron emission tomography (PET) scans should not be routinely used to diagnose PJI. Ultrasonography may detect fluid effusions around the prosthesis and can be used to guide joint aspiration and drainage procedures. CT is more sensitive in the imaging of joint space and may assist in guiding joint aspiration and selecting the surgical approach. MRI displays greater resolution for soft tissue abnormalities and greater anatomical detail.

Intraoperative diagnosis

When possible, any antimicrobial therapy should be discontinued at least 2 weeks prior tissue sampling for culture and perioperative prophylaxis at revision surgery should not be started until after tissue specimens have been collected for culture.

Intraoperative histopathological examination of periprosthetic tissue samples is a highly reliable diagnostic test (2). The presence of acute inflammation as seen on histopathologic examination of periprosthetic tissue at the time of surgical debridement or prosthesis removal is highly suggestive evidence of PJI (2). The presence of purulence without another known aetiology

surrounding the prosthesis is definitive evidence of PJI. Periprosthetic tissue cultures provide the most accurate specimens for detecting the infecting microorganism (7). It is recommended that five or six periprosthetic tissue specimens should be taken because of its low level of sensitivity (7) and the isolation of an indistinguishable microorganism from three or more independent specimens was highly predictive of infection (sensitivity, 65%; specificity, 99.6%;), while Gram staining was less useful (sensitivity, 12%; specificity, 98%) (7). Explanted prosthesis itself also can be submitted for aerobic and anaerobic culture following sonication. Sonication fluid cultures represent a cheap and easy diagnostic modality demonstrating increased sensitivity but it lacks specificity due to contamination (10). The combination of preoperative aspiration and intraoperative cultures that yield the same organism (indistinguishable based on common laboratory tests including genus and species identification or common antibiogram) may also be considered definitive evidence of PJI (2).

Growth of a virulent microorganism (eg, *S. aureus*) in a single specimen of a tissue biopsy or synovial fluid may also represent PJI (2). One of multiple tissue cultures or a single aspiration culture that yields an organism that is a common contaminant (eg, coagulase-negative staphylococci, *Propionibacterium acnes*) should not necessarily be considered evidence of definite PJI and should be evaluated in the context of other available evidence (2).

Antibiotic and surgical management

The empirical antimicrobial therapy should be guided by local organism susceptibilities and must be active against staphylococci including MRSA and wide range Gram-negative organisms (for example, a glycopeptide and a carbapenem). It should be modified according to culture results and sensitivities. The use of local antibiotics in addition to the administration of systemic antibiotic agents is a debatable issue.

Debridement antibiotics and implant retention (DAIR)

The reported success rates of DAIR are around 60%-80% (8). This is more successful when a patient presents with a well-fixed implant, no sinus tract, within 30 days of implantation and less than 3 weeks of onset of symptoms (9). Whether debridement should be an open surgical procedure or may be performed as effectively arthroscopically is a matter of some debate. More recent data suggest higher rates of failure when arthroscopic washout is used compared with open debridement, particularly in those cases where *S. aureus* is isolated (9). According to some studies cases with sinuses have achieved higher success rates by adequate debridement and soft tissue reconstruction using muscle flaps. Those with rheumatoid disease, undergoing debridement of a previously revised joint or infected with *S. aureus* specially MRSA may do less well (9).

For Staphylococcal infections, 2 to 6 weeks of a pathogen-specific intravenous antimicrobial therapy in combination with rifampin 300-450 mg orally twice daily followed by rifampin plus a companion oral drug (ciprofloxacin, doxycycline, cotrimoxazole, linezolid and daptomicin) for a total of 6 months for a knee infection and 3 months for a total hip, shoulder and ankle PJI (2). Experimental data support the use of regimens based on rifampicin, as this is an agent with excellent oral bioavailability that achieves high concentrations in biofilms. While on rifampicin, the patient should be warned about orange red urine/ tears/ contact lenses, weekly liver function tests should be done and carefully watch for drug interactions. For other organisms, 4 to 6 weeks of pathogen-specific intravenous or highly bioavailable oral antimicrobial therapy is recommended (2) but some units extend the oral antibiotics for 6 months in cases of comorbidities, complex rerevision and short life expectancy. Many units use up to 2 years of oral antibiotics and in selected high-risk patients there may be individualized decisions to go longer, or even indefinitely.

For Gram negative infections, intravenous agent for 4-6 weeks (for example ceftriaxone, ertapenem or meropenem) according to identification and susceptibility and, where indicated, should continue with an oral agent. The combination of ceftazidime and ciprofloxacin has been successful in the treatment of *Pseudomonas aeruginosa* infections (10).

One-stage exchange

This may be considered in patients with a hip PJI who have a good soft tissue envelope or bone stock, identified causative organisms preoperatively and susceptible to highly bioavailable oral agents. Also appropriate for those too frail to withstand two procedures and the demanding rehabilitation that follows a long period of relative immobility. This involves explantation of all total knee components, thorough debridement, copious irrigation and reimplantation of new appropriate total knee components with antibiotic impregnated cement. For Staphylococcal infections 2 to 6 weeks of pathogen-specific intravenous antimicrobial therapy in combination with rifampin and for other organisms to 4-6 weeks of pathogen-specific intravenous or highly bioavailable oral antimicrobial therapy is recommended (2).

Two-stage exchange

This is suitable for patient with poor soft tissue support, difficult to treat micro-organisms, prior failed two-stage exchange, delayed reimplantation technically feasible and when anticipating a good functional outcome. Two-stage revisions are the most widely favoured and successful (75%-85%) (8). However, they are demanding for health-care facilities and the patient in terms of the repeated procedures and the limited mobility between stages. The first stage involves removal of all total knee components and cement, thorough debridement and irrigation followed by implantation of an antibiotic cement depot/spacer in the joint. Antibiotics in common usage for this purpose

are gentamycin, vancomycin, tobramycin and cefuroxime. The antibiotic cement depot releases antibiotics locally at high concentrations helping to eradicate the infection. In patients with extensive infection, antibiotic impregnated beads may be used as well. In some cases a second or even further debridement may be required to achieve adequate surgical clearance. Specific antibiotic therapy is then given for up to 6 weeks, usually intravenously but orally if an suitable agent with good oral bioavailability is available (11). An antibiotic-free period of around 2 weeks may be considered following completion of therapy prior to the re-implantation second stage to allow microbiological sampling at operation. Empirical antibiotics are given peri-operatively and stopped if cultures are negative. If there are significant microbiology cultures at re-implantation it may be reasonable to give oral antibiotics of suitable bioavailability for 3-6 months postoperatively (11).

Permanent resection arthroplasty

This is considered, when there is massive bone or soft tissue loss, highly resistant microorganisms, unacceptable medical or surgical risks from another reconstructive attempt or patient preference. Four to six weeks of pathogen-specific intravenous or highly bioavailable oral antimicrobial therapy is recommended (2).

Amputation

This is considered when there is necrotizing fasciitis, severe bone loss, inability or failure to provide soft tissue coverage, prior failed attempts at resection arthroplasty or arthrodesis to control infection, no medical therapy available, functional benefit expected from amputation over resection arthroplasty or arthrodesis. Pathogen-specific antimicrobial therapy should be given until 24-48 hours after amputation and 4 to 6 weeks of pathogen-specific intravenous or highly bioavailable oral antimicrobial therapy is recommended if, despite surgery, there is residual infected bone and soft tissue (2).

Arthrodesis is indicated as salvage after failed treatments. Intramedullary nailing and external fixators are used in order to fuse the joint.

Antibiotic suppression alone is considered only under special circumstances because the prognosis for infection eradication is poor with only low success rate. It may be considered if the implant is stable, the microorganism has low virulence and is susceptible to oral antibiotics (12).

Prevention

The incidence of PJI has been reduced by improvements in ultraclean air, preoperative preparation, surgical technique, theatre design, prophylactic antibiotics, hand hygiene and wound care (13). MRSA screening and decolonization is now mandatory for all elective ortho-

paediatric admissions in the UK (14). There has been controversy over the role of dental prophylaxis for patients with joint replacements (15).

Discussion

The pathogenesis which is associated with biofilm formation allows difficulty in diagnosis and eradication of PJI. Accurate timely diagnosis and appropriate antimicrobial and/or surgical management are crucial. The mitigation of intraoperative and haematogenous infections is the basis for prevention of PJI.

Conclusion

A combination of clinical, laboratory, histopathology, microbiology and imaging studies are usually required for diagnosis of PJI. Debridement antibiotics and implant retention (DAIR), one stage, two stage, resection arthroplasty and amputation are the popular surgical options. Targeted long term antimicrobial therapy is crucial for successful outcome. Key preventive measures have to be considered in prevention of PJI.

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CONTROVERSIES IN THE SCIENCES

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Introduction

Two areas constitute our intellectual enterprise, the Humanities and the Sciences. Science, in its pristine sense, is an objective inquiry meant to reveal the truth in natural phenomena. "The idea that the same experiments always get the same results, no matter who performs them, is one of the cornerstones of science's claim to objective truth" (1). Yet, as these examples in the sciences show, science, in its practice and recording, is not the Holy Grail that naïve scientists believe it to be.

Spontaneous generation of life

There was the classical debate on "Spontaneous origin" in microbiology, in which Louis Pasteur, and André Gratia in France featured prominently, having established the axiom – *Omne vivum ex vivo*, 'life from life'. This problem, especially the role of J. Tyndall in Britain, was discussed by Friday (2). The misinterpretation in the 'spontaneous generation theory' occurred before the modern era of microbiology.

Where do viruses come from?

Nobel Laureate Peter Medawar (1980) wrote: "Astronomer Fred Hoyle and mathematician Chandra Wickremasinghe's theory was that viruses come to earth from outer space. One of the authors of *Diseases from Space* is a famous astronomer, the other an applied mathematician. Neither, then, is a biologist, although they wrote together an earlier book, *Lifecloud*, claiming that life arrived on earth from outer space.

Epidemiological considerations lead the authors to question whether the common cold and influenza are indeed infectious in character – that is to say, they question the widely prevalent notion – that the causative agents may be propagated from one person to another. The authors believe that person to person transmission would be too slow to account for the sometimes pandemic character and speed of onset of so many outbreaks of influenza and the common cold. These phenomena are much more easily explained, they believe, by a fall-out of virus from the atmosphere. The authors do not give adequate attention to the various factors that effect the transmission from person to person of disease-causing agents. One such factor is the varying degrees of pre-existing acquired immunity, and a second is the existence of inborn differences in susceptibility to bacterial and viral diseases. Arguments that do not give these factors adequate weight are sure to be misleading". (3)

Vaccination against small-pox

Who invented it? Was it the Chinese who, many centuries ago, used snuff made from small-pox crusts? Was it Edward Jenner or Benjamin Jesty, in England? The most common public, and medical view is that it was Edward Jenner. Wikipedia states: "The first successful vaccine to be developed was introduced by Edward Jenner in 1798". However the Dorset Page states the first recorded smallpox vaccination was by Benjamin Jesty, an English farmer: "With the point of a stocking needle, he scratched his wife's arm just below the elbow and inserted the pus" from a cow pox lesion. "The first authenticated vaccination had taken place. He then repeated the process on his two sons". (4)

The cause of AIDS

AIDS is widely prevalent in South Africa; an important politician there disputed the role of the HIV virus as the cause of AIDS. There surely would have followed serious blocks to medical measures for the identification and treatment of the disease in South Africa, if this politician's view prevailed.

Who discovered the AIDS virus?

The TIME100 list of the most important people of the 20th century, published on 31 December 1999, said: "On a spring day in 1984, Dr Robert Gallo stood before a press conference at the National Cancer Institute and announced that he had discovered the virus that causes AIDS. What he neglected to mention was that Dr Luc Montagnier of the Pasteur Institute in Paris had also identified what turned out to be the same virus. The two institutes had previously shared samples; they agreed to publish together and even made a joint announcement. But when the press got wind of the news, the NCI felt compelled to proceed without the French" (5).

Antibiosis and penicillin

Friday stated: "There are many examples of unrecognized precursors of great discoveries"(2). Sidebottom stated: "Many myths surround the origin of penicillin....the most important and difficult of these, particularly it is what most school children are taught, is that Alexander Fleming not only 'discovered' penicillin (which he did essentially by accident in 1928) but he gave the antibiotic ready to treat the grateful, waiting world" (6). Preceding workers on antibiosis and penicillin included Roberts in England in 1874, Tyndall and Huxley in England in 1875,

Caminhoa in Brazil in 1877, Tiberio in Italy in 1895, Duchesne in France in 1897, Gratia in France in 1924, and Twilight in Costa Rica in 1927 (from Arseculeratne & Arseculeratne 2016, A re-appraisal of the conventional history of antibiotics and penicillin (unpublished).

Waller commented on the four major claims in the Fleming-penicillin story; these include "...Second, that this was the first time the antibacterial properties of the mould *Penicillium* had been noticed. Third, that Fleming was quick to grasp the therapeutic importance in curing infectious disease. Finally, that he worked hard to realize the potential of penicillin as a mass-produced wonder drug" (7).

Tyndall in his 1876 paper on the "Optical Department of the Atmosphere" described the antibiotic action of *Penicillium glaucum* on bacteria, and Tyndall's observations are the earliest noted by Florey et al (8)." Joaquim Monteiro Caminhoa, a Professor of Botany and Zoology of the Faculty of Medicine of the Federal University of Rio de Janeiro in Brazil, also recognized the antibacterial activity of *Penicillium* and other fungi in 1877."... "In 1895, Vincenzo Tiberio, University of Naples, published research on a mould (*Penicillium*) in a water well that had antibacterial action" [Wikipedia – Penicillin].

O'Connor noted of Ernest Duchesne (1874-1912), a physician in France, "While Fleming generally receives credit for discovering penicillin, in fact technically, Fleming rediscovered the substance. In 1886, the French medical student Ernest Duchesne originally discovered the antibiotic properties of *Penicillium*, but failed to report a connection between the fungus and a substance that had antibacterial properties, and *Penicillium* was forgotten in the scientific community until Fleming's rediscovery" (9).

The problem of priority again enters the penicillin story: "A Costa Rican, Clodomiro Picardo Twilight working at the Pasteur Institute in Paris was the first (sic) "to record the action of the fungal genus *Penicillium* sp. on the growth of bacteria and the work was published in 1927".

"In a communication to the Belgian Society of Microbiology, Gratia and Dath reported their success in isolating from a completely clarified culture another mould, a variety of *Penicillium glaucum*, which completely dissolved *B. anthracis*" (10).

Controversies also exist on the first use of penicillin as a therapeutic agent. On February 12, 1941, a moribund "43 year old policeman, Albert Alexander, became the first person to be treated with penicillin"; he had a septic wound which led to generalized sepsis with an eye that had to be removed. Charles Fletcher who administered the penicillin recalled "Four days later there was a striking improvement"; but with exhaustion of the stock of penicillin, treatment stopped and he died a month later (11).

"What is almost uniformly unappreciated, however, is the fact that penicillin was first used clinically in 1930 by

Cecil George Paine (a pathologist) and Albert Boswell Nutt (an ophthalmologist) at the Sheffield Royal Infirmary in four cases of neonatal conjunctivitis, two infections were due to gonococci and two to staphylococci..." (12).

"On March 14, 1942, John Bumstead and Hess became the first doctors in the world to successfully treat a patient (Anne Miller) with penicillin"; Grossman (2008) gave the date as 12 March 1942, when at New Haven Hospital at Yale, John Bumstead gave penicillin to a patient moribund with beta-haemolytic streptococcal sepsis. Grossman wrote: "Dr Wilder Tileston, a senior consultant, looking at the temperature chart, muttered to those of us close enough to hear 'black magic'" (13).

Rhinosporidiosis

The next three examples relate to rhinosporidiosis on which this author has researched.

This author submitted a paper titled "The effects of biocides on the endospores of *R. seeberi*", to a leading Western microbiological journal. Its referee criticized it because the author had not tested an antibiotic (ciprofloxacin). The work dealt with biocides while the referee's ciprofloxacin was an antibiotic and not a biocide. The paper was published elsewhere.

An Asian author identified the causative pathogen of this disease as a mycelial fungus. It is not; it is in fact a non-mycelial Mesomycetozoon. This author's initial sample was from a nose, that was inevitably, heavily contaminated with other micro-organisms including mycelial fungi, hence the confusion.

Another Asian author regarded the spherical bodies, the germinal units, commonly seen in rhinosporidial tissue as lysosomes filled with starch from excessive eating of tapioca. This author boldly titled the paper 'a study the resolves all controversies'. This author used sophisticated instruments and techniques, e.g. confocal microscopy, organic chemistry. It is perhaps an instance of *idola machinorum*, a variant of *idola quantitatis* (the fondness for quantification) of Nobel Laureate Peter Medawar, in addition to illogical interpretation, as exemplified by David Bostock's comment on faulty logic, $A = B$, $B = C$, therefore $A = C$, the syllogism of Aristotle, viz., *Rhinosporidium* has round bodies, lysosomes are round bodies, therefore *Rhinosporidium*'s round bodies are a lysosomes. This same author next concluded that *R. seeberi* is rather *Microcystis aeruginosa*, a common ground water bacterium. The natural habitat of *R. seeberi* is also ground water but is totally different, taxonomically and biologically.

Misapplication of scientific terminology

Other examples of misinterpretations in the sciences are derived from misapplications of scientific terminology such as pathogenesis/pathogenicity, and infectious/infective. Pathogenicity refers to the capability of a micro-organism to establish disease while pathogenesis refers

to the pathological and immunological mechanisms that result in a disease. Pathogenetically, a disease can be caused solely by exotoxins e.g. botulism, while typhoid is essentially an infective disease i.e. caused by the presence of the pathogen without exotoxins in the infected tissues; the endotoxins in the microbial body could, however, elicit pathological host reactions. The word 'infectious' refers to a disease that can be transmitted from patient to person. Two journals (J. Infectious Diseases [USA], Sri Lanka J. Infectious Diseases) that publishes papers on microbial causes of disease, use the word 'infectious' in an improper sense since the diseases included are infective but not infectious diseases. 'Infective' diseases can however also be infectious, e.g. tuberculosis.

Causes of controversies

Some causes of these controversies can be identified
Immaturity of science at the time. 1 – Spontaneous generation of life.

'Foraging in a foreign field'. 2 – The Hoyle – Wickremasinghe theory; an astronomer and a mathematician commenting on a topic in biology.

'One-upmanship' and individual prejudice. 4 – AIDS, the Microcystis and starch theories in rhinosporidiosis arose as a result of illogical inferences and conclusions while ignorance of scientific literature is exemplified in the history of antibiosis and penicillin.

Improper or questionable terminology led to the lysosome-starch theory in rhinosporidiosis, and the other examples quoted above. The starch-lysosome theory in rhinosporidiosis is also an example of failure of the synthesis of theory and methodology.

Peter B. Medawar used the term *idola quantitatis* (idolatry of quantification) to describe the fondness for sophisticated use of figures to impress readers. The authors invent a new variant *idola machinorum* to describe cases such as cited above, in which the author used sophisticated technology, ('confocal' microscopy) to reach

questionable conclusions. In these examples of misinterpretations on Rhinosporidium, Nobel Laureate Peter Medawar's comments are relevant: "In the ordinary course of events scientists very often guess wrong, take a wrong view, or devise hypotheses that later turn out to be untenable".

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THE STORY OF THE MICROBIOLOGISTS' ARMAMENTARIUM WITH AN UPDATE ON NEWCOMERS

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This article takes you through a brief overview of the pre-antibiotic era, antibiotic era and post antibiotic era. Process of antibiotic development and new antibiotics that are recently approved by the US Food and Drug Administration (US FDA) will also be outlined.

Pre antibiotic era

During the nineteenth century pneumonia, tuberculosis, diarrhoea and diphtheria were considered the main causes of death in children and adults. *Streptococcus pyogenes* caused half of all postpartum sepsis and deaths.

Staphylococcus aureus was fatal in eighty percent of infected wounds and bacterial pneumonia was a famous killer (1).

Antibiotic era

Father of chemotherapy, Paul Erlich and his concept "Magic Bullet" was that the chemicals selectively target only disease causing microbes but not the host cells. In 1906 he discovered compound 606 and in 1909 found that it could cure syphilis infected rabbits. In 1910, it was publicly released as salvarsan (2). In 1928 Alexander Fleming (1881-1955) found that fungus *Penicillium* on a culture plate had an antibacterial action and his findings were presented in 1929 and penicillin as a possible antibiotic. Howard Florey (1898-1968) and Ernest Chain (1906-1979) resumed the study of penicillin, analyzed the biochemistry and delivered the penicillin to humanity in 1940s (2). According to Mark S Butler *et al*, "over the next 40 years, now seen as the "golden era" of antibiotic research, the majority of antibiotic drug classes in use today were discovered. From 1970 to 1999, most newly approved antibiotics were based on these known scaffolds" (3).

Antibiotic resistant era

In the 1940s some experts including Alexander Fleming had seen the red light of resistance. Alexander Fleming stated "The greatest possibility of evil in self-medication

is the use of too small doses so that instead of clearing up infection the microbes are educated to resist penicillin and a host of penicillin fast organism is bred out which can be passed to other individuals and from them to others until they reach someone who gets a septicemia or pneumonia which penicillin cannot save" (1). By the 1950s when antibiotics were still new there was ample evidence of the emergence of resistance but till 1960s, resistant bacterial strains seemed to matter very little.

An assessment made in 1999 by Australia's top experts announced that "Resistance has emerged for all known antibiotics in use (Figure 1: Antibiotic time line till 2010 with resistance) and antibiotic resistant genes have entered the bacterial populations in the domains where antibiotics are used regularly (for example in hospitals, farms and aqua culture ponds)" (4).

Post antibiotic era

Rapidity of bacteria to develop resistance to antibiotics and in contrast the slowness of new drug development led some experts to warn of a 'Post antibiotic era'. Better infection control practices in hospitals and more rational prescribing may help to preserve the effectiveness of the currently available antibiotics (5).

The rate of clinical drug development is at a considerably low success rate. For instance, only one in five drug

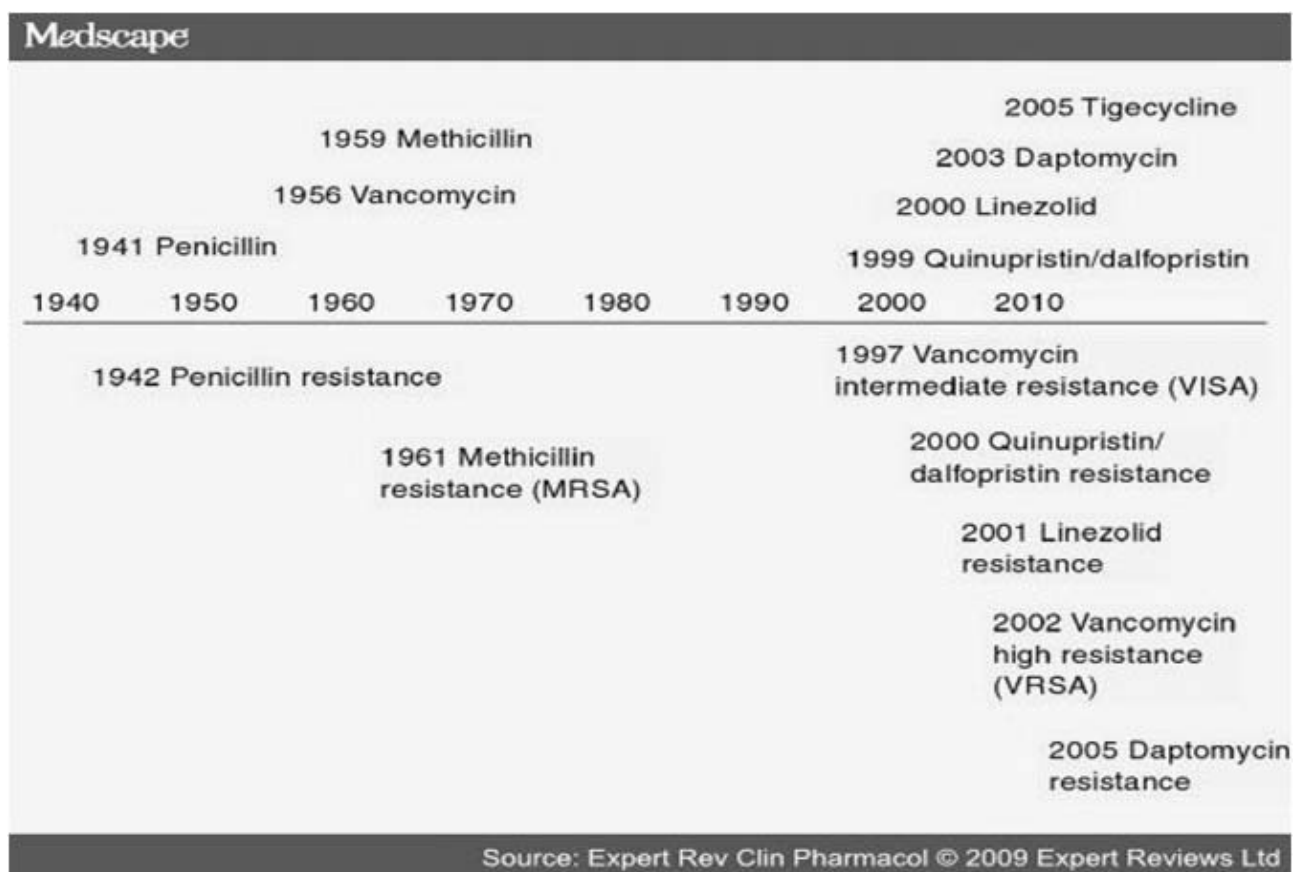


Figure 1. Antibiotic timeline till 2010 with resistance.

developers that enter human testing (phase 1 clinical trials) is accepted for use in patients (3).

Drug development and approval process

After a new drug compound is developed an approval of the Investigational New Drug Application (IND) by an agency such as US FDA, European Medicines Agency (EMA), Japanese Pharmaceuticals and Medical Devices Agency (PMDA) or equivalent agency is an essential prerequisite to proceed to the next step, clinical trials. In general, this approval for an antibiotic is granted for the following indications; *C.difficile* infections / associated diarrhoea, complicated skin and skin structure infections (cSSSI); Community/Hospital acquired pneumonia (CAP/HAP); Urinary tract infections (UTIs); complicated intra-abdominal infections (cIAI) and tuberculosis. Once phase III trials are completed successfully a New Drug Application (NDA/FDA and PMDA) or Marketing Authorization Application (MAA/EMA) must be submitted to get approval to sell the drug (3).

Current status in the field

A program supported by the Infectious Disease Society of America (IDSA) named “the ‘10 × 20’ initiative in 2010 planned to develop 10 new systemic antibacterial drugs within 2020, targeting mainly the so called ESKAPE pathogens. (*E.faecium*, *S.aureus*, *K.pneumoniae*, *A.baumannii*, *P.aeruginosa* and *Enterobacter* spp.). By December 2015 there were 39 new antibiotics with probable activity against serious bacterial infections which were in the clinical development for the US market (6, 7).

An account on newcomers

Antibiotics which came to the market from year 2000 onwards are, linezolid (2000), telithromycin (2001), doripenem, tigecycline (2005), telavancin (2009), the first lipoglycopeptide, ceftaroline (2010), fidaxomicin (2011), bedaquiline (2012), tedizolid, dalbavancin (2014), ceftolozane/tazobactam and ceftazidime /avibactam (2015). Antibiotics that are in phase III trials by December 2015 are delafloxacin, eravacycline, plazomicin, solithromycin, surotomycin, omadacycline, lefamulin, imipenem/cilastatin+relebactam, lclaprim, cadazolid, taksta and carbavance (3,7,8).

Telithromycin: Approved to use for mild to moderate CAP. The first ketolide antibiotic and it is a semisynthetic erythromycin derivative. Tablet form is available and fairly rapidly absorbed and diffused into most tissues and phagocytes. It is active against macrolide resistant pneumococcus.

Doripenem: Approved for use in pyelonephritis, complicated UTI and cIAI. It has been noted to increase mortality in people who have ventilator-associated bacterial pneumonia (VAP), hence no longer recommended for VAP. The fourth carbapenem and has similar properties to meropenem. It is more active against *Pseudomonas aeruginosa* than other carbapenems.

Doripenem is highly active against methicillin-susceptible *S.aureus* (MSSA), but not effective against methicillin-resistant *S.aureus* (MRSA), *E.faecium* and vancomycin resistant enterococci (VRE). The action against *E.faecalis* is inferior to that of imipenem, but it has excellent activity against pneumococci and other streptococci.

Fidaxomicin: Approved for *C.difficile* associated diarrhoea. It belongs to a family of actinomycete-derived macrolactone and impedes the de novo initiation of RNA synthesis. It has shown higher global cure rate than vancomycin and a lower recurrence rate with selective eradication of pathogenic *C. difficile*.

Ceftaroline: Approved for CAP and cSSSI. A 5th generation cephalosporin and acts against Gram-positive bacteria, MRSA and Gram-negative bacteria. It does not have activity against *Pseudomonas* spp. *Acinetobacter* spp. or Gram negative anaerobes.

Bedaquiline: Approved for use only in cases of multi-drug-resistant and extensively drug resistant tuberculosis (TB). It interferes with the bacterial energy metabolism by specifically inhibiting the mycobacterial ATP synthase. It is the first new medicine for TB after more than forty years. The drug also has a black-box warning for death and arrhythmias, as it may induce long QT syndrome.

Telavancin: Approved for cSSSIs. In HAP it should be used only when alternative treatments are unsuitable or unavailable. It is a lipoglycopeptide and semi-synthetic derivative of vancomycin. It is administered once-daily. Telavancin has an excellent activity against MRSA, coagulase negative staphylococci (CoNS) with reduced susceptibility to glycopeptides, and both vancomycin-susceptible and resistant enterococci. It is not affected by pulmonary surfactant. Telavancin has a “substantially higher risk for death” for patients with kidney problems or diabetes compared to vancomycin.

Tedizolid: Approved for cSSSIs. It is a second-generation oxazolidinone, bacteriostatic and active against MRSA, MSSA, Streptococci and *E. faecalis* and is more potent against staphylococci and enterococci compared to linezolid. Oral and intravenous preparations are available.

Dalbavancin: Approved for: cSSSIs. It is a novel second-generation lipoglycopeptide that act upon MSSA, MRSA, and *S. pyogenes*. It is given as intravenous once-weekly antibiotic. Dalbavancin also shows in vitro activity against vancomycin susceptible *E. faecium* and *E. faecalis* and may exhibit activity against enterococci expressing VanB or VanC phenotype of resistance. Serious adverse reactions included hematologic disorders, hepatotoxicity, *C. difficile* colitis, bronchospasm, infusion-related reactions including Red Man Syndrome.

Ceftolozane/tazobactam:

Approved for: cUTI, cIAI, acute pyelonephritis and HAP/VAP. A 5th generation cephalosporin and act against multi-drug resistant *Pseudomonas aeruginosa* and coliforms but somewhat poor activity is observed for *Klebsiella* and *Enterobacter* species with extended spectrum beta lactamase expression.

Ceftazidime+Avibactam: Approved for: cUTI, cIAI, acute pyelonephritis and HAP/VAP. Avibactam is a novel non- β -lactam β -lactamase inhibitor that inhibits KPCs, AmpC, and some Class D beta lactamases, but not metallo beta lactamases and NDM-1. In cIAI, it is used in combination with metronidazole. The activity against the *P. aeruginosa* is variable, due to the potential presence of other resistance mechanisms in addition to beta lactamase production. Regarding *Haemophilus*, *Moraxella*, *Neisseria* and *Acinetobacter baumannii*, it offers little or no advantage over ceftazidime monotherapy, due to the widespread expression of resistance mechanisms other than beta-lactamase production.

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WHAT IS THE POTENTIAL RISK OF ACQUIRING THE FIFTH MALARIA SPECIES, *PLASMODIUM KNOWLESI* IN SRI LANKA?

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Abstract

Plasmodium knowlesi is the fifth species causing malaria in humans. Macaque is the natural host of the parasite. Infection is transmitted from monkey to humans by the bite of mosquitoes of *Anopheles leucosphyrus* group. Symptoms are similar to other malarial infections and also cause fatalities. *P.knowlesi* is frequently misdiagnosed as *Plasmodium malariae* during microscopy and molecular biological tests are useful in establishing the diagnosis. The vector and the reservoir hosts are present in Sri Lanka but fortunately neither category nor human is infected with *P.knowlesi*. Potential risk of introduction of *P.knowlesi* malaria to Sri Lanka is low.

Introduction

Prevalence of many parasitological diseases is low at present in Sri Lanka for variety of reasons such as upgrading in quality of life, improvement in health related knowledge, better health system and effective control

programmes etc. However certain parasitic infections emerge or re-emerge from time to time. Considering the facts such as global warming, change of lifestyle, urban development, deforestation and increase of immune suppressed individuals, certain parasites have tendency to thrive.

Autochthonous cases of malaria in Sri Lanka have been successfully brought to zero since October 2012 and thus Sri Lanka is currently in the elimination and the prevention of re-introduction phase (1). Meanwhile efforts are also being taken to detect and treat imported cases of malaria. Certification of malaria elimination by World Health Organization (WHO) pertains only to four species of human malaria (2). Experience of other countries such as Brunei and Singapore indicates that elimination of human malaria and prevention of re-introductions not necessarily keeps the 'knowlesi malaria' away. Singapore received the malaria certification in November 1982 (3). Later, after two decades of stillness, Singapore started

reporting both autochthonous and imported cases of *P.knowlesi* since 2007. Brunei which was declared as 'malaria-free' by WHO in 1987 reported the first case of *P.knowlesi* malaria in 2013. Thus one needs to be vigilant about knowlesi malaria while on guard against human Plasmodium species. Is Sri Lanka at risk of acquiring the fifth malaria species, *P.knowlesi*? Do we have the reservoir hosts and vectors of *P.knowlesi* in Sri Lanka?

A recent case history of *Plasmodium knowlesi* quoted from ProMed (4).

"Two cases of *P.knowlesi* malaria that occurred after a camping trip involving 24 teenagers and 3 adults in Temburong National Park, Brunei. The trip occurred between 2nd and 9th November 2015 and the onset of illness was on 20th November 2015 for both individuals. Diagnosis of *P.knowlesi* was made by PCR speciation. None of the participants of the trip received malaria prophylaxis."

What is *Plasmodium knowlesi*?

P.knowlesi is considered the fifth *Plasmodium* species causing malaria in humans (5). The natural host of the parasite is the long tailed and pig-tailed Macaque (*Macaca fascicularis* and *Macaca nemestrina* respectively) monkeys. Infection is transmitted from monkey to humans through forest dwelling mosquitoes of *Anopheles leucosphyrus* (6) group. Several vector species (*A. balabacensis*, *A. latens*, *A. cracens*, *A.dirus* etc) have been implicated as having the ability to transmit *P.knowlesi* to humans (6). Biological characteristics such as peak biting time, host preference, survivorship and sporozoite rate etc differ widely in vectors implicated in transmission (7). Human to human transmission of *P.knowlesi* has still not been demonstrated but suspected in a region of Vietnam (6).

In the early days, *P.knowlesi* was misdiagnosed as *Plasmodium malariae* by microscopy until molecular biological tests established the correct diagnosis (8-9). Morphology of early trophozoites of *P.knowlesi* resembles that of *Plasmodium falciparum* while rest of the stages look alike *P.malariae* causing this confusion (6). Careful examination may detect the minor differences of certain stages such as having 16 merozoites in *P.knowlesi* compared to 6-12 in *P.malariae*. However such differences are easily overlooked in a busy diagnostic laboratory and hard to detect with limited knowledge and experience in malaria diagnosis. As a result WHO has recommended to report *P.malariae* cases as *P.malariae* /*P.knowlesi* (10).

P.knowlesi has been considered for malariotherapy to treat neurosyphilis patients (6) as the researchers thought it only causes a mild infection. Later they found that it can cause a spectrum of clinical manifestations from mild infection to fatal disease. Overall case fatality rate of *P.knowlesi* malaria is 3.08 deaths/1000 cases in Malaysia (compared to 4.83 and 0.87 deaths per 1000 cases for

P.falciparum and *P.vivax* respectively). All fatalities have been in over 15 years age category (11). Most cases are mild to moderate not requiring any treatment (12).

P.knowlesi parasite has a short (24 hours) erythrocytic phase compared to *P.malariae* which has a 3 day cycle. Thus parasitaemia increases rapidly causing a fast tract malaria infection by *P.knowlesi* in humans. Initial symptoms are similar to other malarial infections with fever, chills, rigors, headache, myalgia, arthralgia, malaise, poor appetite etc. Cough, abdominal pain and diarrhoea are the other manifestations. Clinical examination may find hepatomegaly and splenomegaly. Severe cases have had feature of respiratory distress, hypotension and jaundice. However cerebral malaria like syndrome has not been reported among knowlesi malaria patients (6). Thrombocytopenia has been the most commonly noted laboratory finding and this may complicate the decision making of the attending physician due to existence of dengue in these *P.knowlesi* endemic areas. Severe anaemia is not seen as in *P.falciparum*. Yet liver and renal functions can get affected. Since *P.knowlesi* is difficult to distinguish from *P. falciparum* and *P. malariae* by microscopy molecular biological techniques are essential to confirm the diagnosis. Treatment of *P.knowlesi* differ from country to country and Malaysia uses chloroquine or ACT (artemether lumifantrine combination) for treatment (13).

Which countries are burdened with *P.knowlesi* malaria?

P.knowlesi was first detected in macaque brought to India from Singapore in 1931. First case of *P.knowlesi* in humans was reported in Malaysia in 1965. Knowlesi malaria is prevalent in several countries in the Asian region namely Malaysia, Thailand, The Philippines, Myanmar, Singapore, Vietnam, Indonesia, Brunei and Cambodia (6). Since the report of first case of *P.knowlesi* from Malaysia in 1965, it accounts for the highest reported (38% of all) among all cases of malaria (13).

What is the potential risk of acquiring *Plasmodium knowlesi* in Sri Lanka?

There are over 20 species of *Plasmodium* infecting monkeys (14). Out of them certain species have the ability to cause human infections (12). Thirteen species of *Plasmodium* infecting non-human primates are present in Southeast Asia (7). Of those some species (*P. fragile*, *P.simiovale*, *P. cynomolgi* and *P. inui*) are present in Sri Lanka (7). However to date *P.knowlesi* has not been detected in macaques, mosquitoes or humans in Sri Lanka.

The countries reporting human knowlesi malaria cases have natural distribution of long tailed and pig-tailed macaques, infected macaques with the *P.knowlesi* parasite and natural distribution of mosquitoes of the *Anopheles leucosphyrus* group (6). Luckily even though present, long tailed and pig-tailed macaques in Sri Lanka are not known to be infected with *P.knowlesi* (6).

Transmission of vector borne diseases is dependent on the bionomics and the distribution of the vectors (6). Sri Lanka has natural distribution of mosquitoes of the *Anopheles leucosphyrus* group (*A. mirans*) (6),(15). The other important aspect of transmission of vector borne diseases is contact of vector (mosquitoes in this case) and humans. Overlapping of animal and human habitats caused by invasion of forest areas by humans for agricultural purposes, development activities, jungle trekking for leisure, wild game can increase the risk of acquiring *Plasmodia* of simian origin (5,14). Farmers, log cutters, wild game hunters, travellers to jungles are at risk (6). In Malaysia 78% of patients are males indicating the link to exposure (16). Duration, frequency, timing of occupation exposure is not known (16). Changes in land usage practices have been highlighted as key drivers to acquire *P. knowlesi* (16). Overlapping of animal and human habitats and encroachment into forest is not uncommon in many areas in Sri Lanka.

Frequent travelling occurs between Sri Lanka and *P. knowlesi* endemic countries. Naturally occurring knowlesi malaria cases have been reported in travellers from New Zealand, USA, Finland, Sweden, Spain, France, Germany, Australia and Netherland (17). Suspicion and consideration of malaria left alone knowlesi malaria, in a patient even with suggestive features in the differential diagnosis by the Sri Lankan physicians is low. Travel history may be missed out during history taking. Even when it is suspected and a sample is sent to the diagnostic laboratory, the limited experience in malaria blood smear examination of the microscopists and technicians in Sri Lanka may prevent arriving at the correct diagnosis. Even in areas where malaria is common in rest of the world, misdiagnosis is common for all malaria species when compared with molecular techniques (9). Molecular biology facilities are not readily available for most parasitic diseases currently existing in Sri Lanka.

Plasmodium malariae is the species which share similar morphologies to *P. knowlesi*. Since 1969 *Plasmodium malariae* cases has not been present (18) in Sri Lanka except one for two cases; one sporadic case in 1984 and another imported case of mixed infection with *P. falciparum* in 2008. The second case of *Plasmodium malariae* had been confirmed using Polymerase Chain Reaction (PCR) (19).

A series of maps have been developed by combining data on the occurrence of *P. knowlesi* parasite in humans, known and presumed macaques hosts and vector species and human malaria cases in the region. It illustrates the potential geographical range of the *P. knowlesi* parasite pertaining to each country. As for Sri Lanka, it shows that western and Sabaragamuwa provinces have the higher risk compared to rest of the country (20). This risk is due to the presence of macaque host and mosquito vectors. However these potential risks for those two provinces are at a point which is below the level of weak evidence range.

Conclusion

Frequent travelling occurs between Sri Lanka and countries endemic for *P. knowlesi*. These countries have natural distribution of long tailed and pig-tailed macaques, infected macaques with the *P. knowlesi* parasite and natural distribution of mosquitoes of the *Anopheles leucosphyrus* group (6). Fortunately except for having *Anopheles leucosphyrus* group (6) mosquitoes Sri Lanka do not have any of the other favourable factors similar to South East Asian countries burden with knowlesi malaria. Meanwhile Sri Lanka has been placed below the level of weak evidence range for potential geographical range of *P. knowlesi* parasite. Nevertheless we need to be watchful about knowlesi malaria not only because *P. knowlesi* is able to cause severe and fatal malaria in humans but due to the fact that Sri Lanka do not need any further vector borne diseases than what we currently struggle to control.

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MELIOIDOSIS: WHAT MORE SURPRISES AWAIT US? A VARIETY OF CLINICAL PRESENTATIONS

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Introduction

Melioidosis is a multi-spectrum disease in which almost any organ or system in the body can be infected by the causative organism *Burkholderia pseudomallei*. It is endemic in Malaysia, Thailand, Singapore and Australia and although several cases have been reported in Sri Lanka it is still probably under-reported [1]. Common presentations in melioidosis include septicaemia, pneumonia, lung and other deep seated abscesses and cutaneous lesions like abscesses and cellulitis. During 2014 and 2015, several patients with varying presentations of melioidosis were successfully managed at Teaching Hospital, Karapitiya and the mistakes, difficulties and challenges in the diagnosis and management of those patients are described here.

Case 1

A 73 year old male paddy and chena cultivator from Wanduramba, Galle, presented in September 2015 with cough and shortness of breath for 4 days and loss of appetite and loss of weight for 3 weeks. Being a diagnosed patient with chronic obstructive pulmonary disease (COPD), this was treated as an infective exacerbation. His blood culture, taken on admission, was reported as positive for *Pseudomonas* spp and he was managed with intravenous (IV) ceftazidime and IV ciprofloxacin according to the antibiotic sensitivity pattern (ABST).

Due to a poor response to the antibiotics and in the light of a pan systolic murmur detected on examination, 2D echocardiogram was performed which revealed multiple aortic vegetations (1x1 mm) and the same antibiotics were continued for 4 weeks, as for endocarditis.

When the clinicians sought the microbiologist's opinion regarding oral substitutes for the intravenous antibiotics prior to discharge, it was decided to re-visit the initial blood culture with fresh subcultures and the isolate was presumptively identified as *B.pseudomallei* on colony appearance and ABST. Identification was later confirmed by PCR for the LpxO gene. Patient's antibodies to *B.pseudomallei* were only marginally positive at a titre of 40. Several transthoracic echocardiograms were done to

confirm the diagnosis as endocarditis in melioidosis had not been reported in Sri Lanka before.

The patient was treated as having endocarditis due to *B. pseudomallei* with IV ceftazidime 1g 8 hourly for 6 weeks and oral cotrimoxazole 960mg 12 hourly for 20 weeks with weekly reviews which included C reactive protein (CRP), full blood count and 2D echocardiograms.

Case 2

A 33 year old primigravida from Ahangama who had undergone elective caesarian section 6 days previously for severe oligohydramnios, presented with shortness of breath on 24th January 2016. She was treated with enoxaparin for presumed pulmonary embolism in the light of a positive D dimer test. Fever was present since admission with a CRP >96 mg/l, white cell count (WBC) of 11400/μL and low platelet count (74000/μL). She did not have any co-morbidity.

She developed pulmonary haemorrhages 3 days after admission (possibly enoxaparin induced) and had to be intubated in the intensive care unit for respiratory failure. Her blood culture, taken on admission, became positive for an extended spectrum beta lactamase (ESBL) producing *Escherichia coli* which was sensitive to meropenem, imipenem and amikacin. Even with broad spectrum coverage with antibiotics such as IV meropenem 1g 8 hourly (later IV imipenem 500mg 6 hourly), IV amikacin (for *E.coli* in blood culture), cotrimoxazole 1920 mg 12 hourly (for a pure growth of *Stenotrophomonas maltophilia* in the culture of bronchial wash), IV teicoplanin and IV fluconazole (for high candida colonization index), fever did not respond.

Her CT abdomen indicated pyelonephritis with early renal abscess in the right kidney and CT brain was reported as acute demyelinating encephalomyelitis (ADEM). Meropenem was re-started and the dose was increased to 2g 8 hourly to give maximum cover, including penetration of the central nervous system, and an antibody test for *B.pseudomallei* was requested.

Fever started to subside gradually with high dose meropenem and the melioidosis antibody test became

positive with a titre of 2560. Oral high dose cotrimoxazole was continued. In the light of severe sepsis she was given granulocyte colony stimulating factor (G-CSF) for 3 days. Patient gradually recovered over the next few weeks with IV high dose meropenem for 3 weeks and oral cotrimoxazole for 12 weeks. Her melioidosis antibody titer came down to 640 in 12 weeks. The baby's antibody test was negative.

Case 3

A 46 year old housewife from a chena cultivator family from Boossa presented in December 2014 with a thigh abscess for one week with recent anorexia and weight loss. She had been on oral prednisolone for systemic lupus erythematosus (SLE) for the last 10 years and on warfarin for deep vein thrombosis. She had sustained bilateral femur fracture following an accident and had become wheelchair bound 8 months prior to this episode. The patient was febrile (101°F) and had a WBC of 19000/ μ L with 90% neutrophils, CRP of 97mg/l (which later went up to 375mg/l) and an ESR of 133 mm/hr. Ultrasound scan revealed a 10 x 4 cm abscess in the right vastus lateralis muscle. The abscess was drained and pus culture was reported as coliform spp while a positive blood culture was reported as *Pseudomonas* spp.

The patient did not respond to standard treatment with cloxacillin and started to develop more abscesses on the right forearm and a left sided knee joint septic arthritis. All pus, joint fluid and repeat blood cultures yielded the same organism with a similar ABST which was identified as *B.pseudomallei*. Patient was started on IV ceftazidime 2g 6 hourly with oral cotrimoxazole 1920 mg 12 hourly and responded very well.

Case 4

A 44 year old male, from Wakwella, Galle, presented in December 2015 with evening pyrexia for 2 weeks and recent loss of appetite and loss of weight. He was a known diabetic for 3 years but had defaulted treatment. He had been working as an attendant in the soil laboratory of the Engineering Faculty for the past few years and was an ex-alcoholic and an ex-smoker.

After admission, he was diagnosed to have a right liver abscess of 4x4cm and was found to have a high CRP (202mg/l), WBC 14280/ μ L and ESR 74mm/hr. He did not respond to empirical treatment with IV ceftriaxone and IV metronidazole and was started on IV ceftazidime 2g 8 hourly on microbiologist opinion. The abscess was drained and the pus culture isolate identified as *B.pseudomallei*. The patient started to respond once the dose of IV ceftazidime was increased to 2g 6 hourly and oral cotrimoxazole 1920mg 12 hourly added. His melioidosis antibody test was positive with a titre >10240.

Discussion

This case series illustrates the variety of clinical presentations of melioidosis, including abscesses in

many different organs, septic arthritis, septicaemia and endocarditis.

The difficulties in clinical diagnosis of melioidosis are exemplified by the delayed diagnosis seen in all 4 patients due to clinical unfamiliarity and failure to include it in the differential diagnosis of acute fevers in Sri Lanka. The presence of underlying risk conditions (COPD, SLE on prednisolone therapy and diabetes) and exposure to soil (farming, chena cultivation, working in a soil laboratory) were clues that may have been elicited by careful history taking. However, as seen in these patients, the exposure may be many months or years previously as *B. pseudomallei* may remain latent in the body for long periods of time [2].

Further delay was caused by late laboratory identification of clinical isolates. Correctly identifying *B. pseudomallei* in culture from patients' specimens is a challenge encountered frequently by microbiology staff. In addition to the atypical colonial and microscopic morphology that may sometimes be seen on primary isolation, lack of experience with the organism, non-availability of reagents and absence of reliable commercial identification kits are factors that contribute to misinterpretation of the culture isolate as *Pseudomonas* spp or coliform spp, as happened in our cases. It is interesting to note that in case 3, identical isolates from different sites were misidentified differently. However, vigilance, knowledge, a high index of suspicion and persistence helped us to correct the initial mistakes and to diagnose melioidosis correctly.

The colony morphology of *B.pseudomallei* is variable, ranging from smooth to dry, haemolytic to non-haemolytic and lactose fermenting to non-fermenting up to the 'typical' wrinkled colonies with an earthy smell that appear only after a few days of incubation. Gram stain may show a 'safety pin' appearance, but this may be obvious only after 48-72 hours of incubation. The Kligler pattern is non-reactive and it is oxidase positive, although this reaction may be weak in some isolates. The most typical feature is the characteristic ABST pattern, with sensitivity to coamoxiclav and resistance to gentamicin and colistin. It usually shows a sensitive zone around a cotrimoxazole disc but absence of the zone does not exclude the identity. Confirmation is done by PCR testing for specific genes of the isolate. A positive antibody test is usually used to determine exposure to the organism, rather than acute infection [2] but in culture negative cases it may be helpful in the diagnosis, as in the post-partum patient.

Empiric therapy of acute community acquired infections according to standard guidelines is not effective in melioidosis, as seen in all the patients in our case series. Antibiotic treatment of melioidosis should be based on published guidelines and depends on the site and severity of the infection. The mainstay of treatment consists of acute phase antibiotics such as intravenous ceftazidime, imipenem or meropenem for 2-6 weeks. Often, maximum

doses of the antibiotics are necessary, as in Cases 2, 3 and 4. Further, in the septic arthritis and liver abscess cases, high dose cotrimoxazole was required, in addition, for a good response. When long term treatment courses are mandatory, as in these cases, the continuous availability of antibiotics is a concern in our setting. Also the patient's willingness to tolerate high dose antibiotics for a long duration can affect optimum treatment, as in the case of the patient with endocarditis as the patient refused to have IV antibiotics and insisted on discharge. Other therapeutic agents, such as G-CSF, can be tried in severe sepsis but the response may vary [3].

Upon completion of the acute phase, treatment with oral cotrimoxazole is given for 12 weeks or more in the eradication phase [3]. The MERTH trial done in Thailand [4] compared combined therapy with cotrimoxazole and doxycycline with cotrimoxazole therapy alone and proved that the latter is equally effective provided the correct dose, according to body weight, is given. We used this regime in our patients with good results to date.

In conclusion, when diagnosing this multi-spectrum disease which can mimic any other suppurative condition or any kind of chronic infection, such as tuberculosis [5], clinicians should have a very high degree of suspicion and the microbiology team should have the necessary training and adequate facilities to correctly identify the

culture isolate. Counseling the patients regarding the nature and the management of the disease will improve their compliance to treatment. Early diagnosis and optimum antibiotic therapy will improve the survival rate.

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Case Report:

MISINTERPRETATION OF A SEROLOGICAL TEST LEADING TO MISUSE OF ANTIBIOTICS

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Introduction

SAT (Widal test) is a serological test, done in patients suspected of having typhoid fever. Though it is used as a tool to diagnose enteric fever in several developing countries with limited facilities, it has several significant limitations and shortcomings which were clearly mentioned in the literature. This is an account of a woman who had been repeatedly treated for typhoid fever based on the positive SAT result. She had a history of typhoid vaccination in the past. It is an eye opener for the clinicians from this part of the world as there were several incidences with misinterpretation of SAT, reported in the past.

Case history

A 31 year old lady presented to the Department of Microbiology of Sri Jayewardenepura General Hospital

with the history of repeatedly positive SAT results for 11 years with frequent episodes of treatment for typhoid and paratyphoid fever.

She received typhoid vaccination in 2004. Then, she developed fever after 2 weeks of vaccination and SAT was done to investigate the cause. It became positive and she was treated for typhoid fever with antibiotics.

Again in 2006 and in 2007, her SAT test results were found to be positive and she was treated with IV antibiotics for typhoid. Then in 2014 and in 2015 also, she had the same experience.

Lastly in October 2015, she was tested positive for SAT, though she did not have any symptoms. As it seemed to

be a never ending story, eventually she consulted the Consultant Microbiologist of Sri Jayewardenepura General Hospital regarding her illness and for further management.

Discussion

Widal test (SAT)

It is an agglutination reaction which demonstrates the presence of lipopolysaccharide (LPS) somatic (O) and protein flagellar (H) antibodies to *S. enterica* ser. Typhi in the serum of a patient using suspensions of O and H antigens.

It is used extensively in the sero-diagnosis of typhoid fever and it remains the only practical test available in most developing countries, though it has several limitations.

Limitations

- The cut-off for a positive Widal, chosen in a particular community depends on the background level of typhoid fever (i.e., the prior exposure) and the level of typhoid vaccination. Both may vary with time.
- It may lack sensitivity and specificity particularly in a community with endemic typhoid fever. It can be falsely positive in patients with previous vaccination or infection [2].
- The test has significant cross-reactivity with other infectious agents which give false-positive results [3].
- A single test in an endemic area is of no value.
- It should not be used as a screening test for asymptomatic individuals.
- A negative test does not rule out typhoid fever in patients with signs and symptoms of the disease. But it has good negative predictive value.
- It should not be used as a basis for deciding duration of treatment.
- It has low positive predictive value [1].
- Numerous studies indicate that sensitivity, specificity, and predictive values of the test vary dramatically among laboratories.

As discussed above, the patient under discussion might have positive antibody titres due to one of the many reasons given above. None of the episodes were confirmed by a positive culture or a rising titre of antibodies. The infections were probably of viral aetiology and none of them were episodes of typhoid fever.

Conclusion

Misdiagnosis based only on Widal test resulted in several episodes of over-treatment cases and might also perpetuate the perception that typhoid is common. It can also lead to unnecessary use of antibiotics and promote development of drug resistance. Highly fatal febrile illnesses such as malaria, endocarditis and urinary tract infection may be missed leading to increased mortality. Therefore introduction of newer rapid methods with higher sensitivity and specificity for early diagnosis of typhoid fever remains critical.

Both laboratory technicians and clinicians should understand the limitations of the Widal test interpretation. Blood culture before initiating antimicrobial therapy remains the diagnostic method of choice.

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Case Report:

A NEW BORN WITH RECURRENT SEPSIS

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Introduction

Chronic granulomatous disease (CGD) is a rare primary immune deficiency disease in which neutrophils fail to produce the respiratory burst due to lack of components of NADPH oxidase which catalyse oxygen to oxygen radicals. Patients are susceptible to life-long infections from bacteria and fungi which can be life threatening.

Case report

A case report of a baby boy is presented here, who was born after one healthy girl who is 8 years old now, and a baby boy who died of Gram negative sepsis 11 days of life and a third pregnancy which ended in a miscarriage at 20 weeks. Parents are apparently healthy and deny consanguinity.

He was born normal following an uncomplicated pregnancy. BCG was given. Baby was clinically well at birth except for poor weight gain and had no dysmorphisms. All systems were normal on examination. Baby developed fever on day 6 which settled without complications only to recur at day 14 and had white cell count (WBC) of 27×10^9 /L, C reactive protein (CRP) of 188mg/l with positive urine full report. Blood and urine cultures isolated coliforms sensitive only to meropenem. Ultra Sound Scan (USS) of abdomen was suggestive of left sided pyonephrosis. After successful treatment with meropenem for 14 days he developed cellulitis of right elbow which was resolved with flucloxacillin.

On investigation retroviral studies were negative, USS of thymus gland, absolute lymphocyte count and immunoglobulin levels were normal. Flow cytometry revealed a low B cell count (9%). He was discharged pending further investigations. On clinic visit, there was a positive urine culture with coliforms, with late onset fever, high CRP and high WBC. Repeat blood culture on day 10 isolated multi-resistant *Burkholderia cepacia*. Later he developed cervical lymphadenopathy suggestive of mycobacterial infection on biopsy and was started on anti-tuberculous treatment. Culture of lymph node and bone marrow and quantiferon test were negative for *M.tuberculosis*. Later blood culture isolated *Candida non-albicans* which was treated with intravenous (IV) amphotericin B after failure with (IV) fluconazole for 7 days.

Genetic studies for SCID were negative and flow cyto-

metry of CD 11c showed 96% expression on gated cells excluding leucocyte adhesion deficiency.

Nitrobluetetrazolium test (NBT) gave 0% result on three occasions to arrive at a diagnosis of CGD. He is now awaiting bone marrow replacement therapy while on chemoprophylaxis with cotrimoxazole. At 5 months of age he developed pus discharge at the BCG scar and moderate hepatosplenomegaly with abdominal mesenteric adenitis.

Discussion

CGD has an overall prevalence of 1 in 250,000 with a high morbidity which has improved in the recent years due to advancement of facilities (1,2). These patients have a normal immune response to viral infections.

It is inherited as X linked recessive disease in a majority and 90% due to a mutation in the gp91phox gene, but also inherited as an autosomal recessive disorder causing milder disease (1,2).

Patients usually present before 2 years of life but can present at adult age. These patients develop Severe, Persistent infections due to Unusual organisms and Recurrent infections commonly denoted by SPUR. Infections are mostly of epithelial surfaces in contact with the environment. Commonest infections in CGD patients are pneumonia, adenitis, liver abscess, and osteomyelitis. Pneumonia is caused by *Aspergillus* spp in 41%, followed by *Staphylococcus aureus* (12%), *Burkholderia cepacia* (8%), *Nocardia* spp (8%)(3).

Commonest organisms causing infection in general in order of frequency are *Staphylococcus* spp., *Aspergillus* spp., *Serratia* spp., *Nocardia* spp., *Burkholderia* spp., *Klebsiella* spp., and *Candida* spp. Increasing frequency of *Burkholderia cepacia* and *Nocardia* and decreasing frequency of *Salmonella* infections have been noted recently. *Aspergillus fumigatus* and *A. nidulans* are the commonest fungi causing infections. Infections which suggest a diagnosis of CGD are liver abscess due to *Staphylococcus aureus* and *Serratia* and osteomyelitis due to *Serratia*. Unusual organisms like *Chromobacterium violaceum*, *Mycobacterium* spp., and *Legionella* spp warrant an evaluation if encountered (3).

CGD patients have a higher risk for inflammation due to lack of NADPH, a critical factor for down regulation of inflammation. Differentiation of inflammatory complications is important with normal CRP as they require steroid treatment (1,2).

Diagnosis is by NBT which demonstrate the oxidative burst of the neutrophils by stimulation of neutrophils with phorbolmyristate acetate and incubating with nitrobluetetrazolium dye where the normal phagocytes reduce the dye to blue colour. Flow cytometry can diagnose by specific fluorescence probes which increase fluorescence upon oxidation. Genetic testing is required to identify mutations.

Management is by minimizing infection with antibiotics and antifungal prophylaxis and treatment, immunization and surgical prophylaxis (4). Definitive treatment is with interferon gamma, bone marrow transplant or gene therapy (5).

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Case Report:

NEONATAL BACTERAEMIA – FROM COMMON TO UNCOMMON

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Introduction

Neonatal sepsis is an infection with high mortality and morbidity. Accurate data regarding incidence is not available in Sri Lanka. Due to limited availability of culture facilities aetiological agents are not identified in some instances. Group B Streptococci and *Escherichia coli* are the common pathogens followed by coliforms but rarely other organisms can cause neonatal sepsis.

Case 1

A 4 day old baby was admitted with fever, poor sucking and jaundice. Born by normal vaginal delivery, his birth weight was 2.9kg. Mother's recovery was uneventful in this first confinement. She was treated for a urinary tract infection at 32 weeks of gestation without culture evidence. She did not have any other risk factor.

Baby was started on benzyl penicillin and gentamicin after collecting specimens for microbiological investigations and underwent exchange transfusion at special care baby unit. Two blood cultures became positive for Group B *Streptococcus* which was sensitive to penicillin.

Discussion

Group B *Streptococcus* (GBS) is a leading infection causing neonatal morbidity and mortality. Maternal carriage is a major risk factor. Early-onset GBS infection occur within first 7 days. It's primarily manifested as non-focal sepsis, pneumonia and less often as meningitis (1). In a 2 year retrospective study in a single centre 48% of the babies developed neurological sequelae and mortality was 4.7% (2).

Two strategies for identifying women at increased risk for an affected baby are universal antenatal screening and risk-based approach (3).

The risk factors for Group B Streptococcal sepsis in neonates are; a previous GBS-infected baby, GBS-bacteriuria during current pregnancy, preterm labour and imminent birth, intra-partum fever $\geq 38^{\circ}\text{C}$, membrane rupture ≥ 18 hours and GBS carriage. Intra-partum antibiotic prophylaxis prevents vertical transmission. Benzyl penicillin is the drug of choice. Ampicillin, clindamycin and vancomycin are alternatives (2). Considering the mortality and morbidity associated with this disease, an approach of risk based prophylaxis will

help in lowering of the incidence. A trivalent vaccine against GBS is in the pipeline (1).

Case 2

A mother who had normal vaginal delivery and uneventful recovery 11 days ago, got admitted to a post-natal ward with per-vaginal discharge and fever. She was septic, with neutrophilic leucocytosis, raised platelet counts and C reactive protein levels. No retained products were seen ultrasonically and mother was started on IV cefotaxime but without prior blood cultures. Baby developed fever after admission. He had intra uterine growth retardation which was detected antenatally and his birth weight was 1.75kg. His blood culture became positive for group A *Streptococcus* (GAS). Meningitis was excluded. Baby was started on benzyl penicillin and gentamicin. Then cefotaxime was added. Since there were no cultures done on the mother it is difficult to establish the maternal infection as GAS.

Discussion

When a mother develops peri-partum invasive GAS infection, the infant needs a full diagnostic evaluation. If positive, a minimum of 10 days of IV benzyl penicillin should be given. Gentamicin may be added until cultures

become sterile and clinically well (4). Infection control is essential in the management. Ideally, patients should be isolated in a single room until 24 hours on appropriate antimicrobials (5).

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‘The Unlucky Ones’; severe manifestations of common viral infections

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The nature as well as the severity of the host response vary widely in viral infections even with the same virus. Immunosuppression is one most important determinant for the difference in the clinical presentation.

Enteroviral infections are common and self limiting specially during childhood, but the case history of this 12 year old boy was contrary to the usual presentation. He developed posterior reversible encephalopathy syndrome following enteroviral encephalitis leaving him with long term motor and hearing defects.

Chickenpox presenting with complications without a vesicular rash is uncommon. A 35 year old immunocompetent patient initially presenting with severe chickenpox pneumonitis without the rash highlights the importance of having a high degree of suspicion if the patient has an exposure history.

Invasive adenovirus infections can be severe with suppressed immunity. Fatal hyper acute liver failure due to adenovirus hepatitis in a 40 year old bone marrow transplant patient demonstrates that early diagnosis and prompt treatment is critical when managing severe immune-suppressed patients.

Although uncommon, multi-drug resistant Cytomegalovirus can emerge with long term use of antivirals in

an immunosuppressed individual. A 41 year old immunocompromised patient developed resistance to ganciclovir, foscarnet and cidofovir while on treatment and died due to complicated CMV colitis.

Complicated infections due to common viral pathogens are not uncommon and early diagnosis and prompt treatment is life saving. Strengthening diagnostic laboratory services and looking in to the availability of alternative treatment options, specially with regard to the growing immunosuppressed population in the country is urgently needed.

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Professor Mahroof Ismail

On 13 June 2016, Sri Lanka lost another doyen in the field of Medical Parasitology. Prof Ismail was one of the earliest members of the Sri Lanka College of Microbiologists, serving as its 11th President, in 1987. In 2015, when the College decided to honour those who have served the College and our profession by awarding honorary Fellowships, the Council was unanimous in deciding that Prof Ismail should be among the first recipients of a Fellowship.

Mohamed Mahroof Ismail obtained his MBBS from the University of Ceylon and his PhD in Medical Parasitology from McGill University, Canada. He also spent a post-doctoral year at the London School of Hygiene and Tropical Medicine, in the UK. After his return to Sri Lanka, he worked at the MRI for several years and became its Director in 1983. In the same year, he joined the Faculty of Medicine, University of Colombo as the Professor of Parasitology and later served as Dean of the Faculty from 1994 to 1996. Throughout his working life, Prof Ismail engaged in research, mostly on lymphatic filariasis and soil-transmitted helminths. His work was of such quality that it had significant impact on national as well as international health policies in relation to control of both groups of infections. The most significant body of work that he and his collaborators produced was to demonstrate for the first time that albendazole combined with diethylcarbamazine citrate or ivermectin has a pronounced and sustained effect of reducing microfilaraemia for over two years. This combination is currently being successfully used by the WHO and the Ministries of Health in 83 endemic countries as part of the global strategy to eliminate filariasis.

Prof Ismail also held many eminent posts, serving as a member of the University Grants Commission, as the Chairman of the Board of Management of the Post-Graduate Institute of Medicine; and as external examiner in Parasitology of the University of Malaya as well as the National University of Malaysia. He served the WHO at its Headquarters in Geneva, and in the South East Asian Regional Office in New Delhi in many different capacities: as Chairman of the WHO Expert Committee on Soil-Transmitted Helminthiasis; as a member of the WHO Expert Committee on Lymphatic Filariasis and the WHO Technical Advisory Group for Lymphatic Filariasis; as a WHO Consultant to Egypt and Bangladesh to revise their



National Filariasis Control Programmes; and Chairman of the South East Asian Programme Review Group for the elimination of lymphatic filariasis from 2002 until 2006.

I am just one of many who owe an immense debt of gratitude to Prof Ismail. I learnt much of my parasitology from him, as a medical student, as a postgraduate student, and even after that. He was one of the examiners at my MD examination in 1994. I still recall very clearly, the occasion when I went to thank him after passing the exam. I was a young probationary lecturer back then, who had just started working at Ragama, in a medical faculty that was virtually in its infancy. My husband Janaka and I met Prof Ismail in the Dean's Office in the Colombo Medical Faculty. We talked of this and that, and then I asked him if he had any suggestions for research. He immediately shared with us an idea that had occurred to him while attending a WHO meeting a few weeks previously. He said that this study could only be done in Sri Lanka because of the confluence of circumstances at that moment in time, but that it had the potential to transform international policy with regard to deworming programmes. Together with other colleagues from Ragama, we turned this idea into a study that was eventually published in one of the foremost medical journals. This little episode is only one example of the unassuming generosity and supportiveness that Prof Ismail extended to all who came into contact with him. He probably never thought twice about what he did, but for me, it was a landmark in my academic career.

Over the decades since then, we came into contact at regular intervals, especially at Parasitology oral examinations for medical students. Those times when I was his co-examiner were days that I really enjoyed, because Prof Ismail somehow turned them into learning experiences for both students and me, and his unfailing sense of humour lessened the tedium of coping with medical students who seemed to view Parasitology oral exams as an instrument of torture.

As he gradually withdrew from the professional arena, Janaka and I tried to stay in touch by visiting him at home. He was an unfailingly courteous and considerate host, and we learnt that he was an excellent cook, who also enjoyed good food. We also saw a marriage that seemed to have been made in heaven. Prof Ismail and

his wife Jezima, an equally eminent figure in her own right, in the field of education, lived their life together, not only in bringing up a family, but in many other ways that sought to support the underprivileged and disadvantaged.

I have had the privilege of following in Prof Ismail's footsteps, in that my research has been largely in the same fields. At international meetings, mention of my Sri Lankan nationality often results in enquiry after Prof Ismail. World-renowned experts speak of him with much respect and affection. One of them characterized him as a 'gentleman and a scholar' – a phrase which struck me as a particularly appropriate description of Prof Ismail. May he rest in peace!

By Prof. Nilanthi de Silva

The Bulletin of the Sri Lanka College of Microbiologists

The Bulletin of the Sri Lanka College of Microbiologists is the annual publication of the Sri Lanka College of Microbiologists issued along with the Annual Scientific Sessions of the College. The Bulletin includes the summaries of the speeches/lectures/symposia and abstracts of oral/ poster presentations to be made during the Annual Scientific Sessions in addition to review/ research articles and case reports relevant to microbiology and infectious diseases sent by the membership. The aims of the bulletin are to encourage the membership to conduct and publish good quality research to support and improve the practice of microbiology in Sri Lanka and to share experiences to enrich and upgrade the professional standards.

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.

TYPES OF CONTRIBUTIONS

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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.

Articles

These articles should be limited to 1500 words and 12 references. Journal will give priority to articles dealing with topics of interest and importance in microbiology and infectious diseases in Sri Lanka.

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These should not exceed 750 words and 5 references and should be structured as Introduction, Case report and Discussion. Abstract is not required. Editorial board will be paying attention to the significance of the case report to the practice of microbiology in Sri Lanka.

Abstracts of presentations to be made at Annual Scientific Sessions

These should be limited to 250 words. May be accompanied by no more than five references or suggested further reading.

Photo quiz

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

Please see separate guidelines issued with the notice calling for abstracts.

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Previous publication of some content of a paper does not necessarily mean that the paper will not be considered for publication in the *Bulletin*, but the Editorial Board should be made aware of this in the cover letter that accompanies the manuscript.

Authors should include all those who have contributed to the work described, including supervisors and if applicable, those interpreting and analyzing data used in the study to be presented. Only persons who contributed to the intellectual content of the paper should be listed as authors. Authors should meet all of the following criteria, and be able to take public responsibility for the content of the paper:

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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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