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The Bulletin of the

Sri Lanka College of Microbiologists

Scientific Programme

""Combating Sepsis "

Pre-congress
The 2nd South Asian Melioidosis Congress
"Unearthing a subterranean infection "

Plenaries

Symposia

Free Paper Sessions

Poster Presentations

Volume 15 August 2017 ISSN 1391-930X

The Bulletin
of the
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The Sri Lanka College of Microbiologists

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The Sri Lanka College of Microbiologists

Council 2016 / 2017

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Cover page designed by Dr. Roshan Jayasuriya

26th Annual Scientific Sessions

The Sri Lanka College of Microbiologists

Inauguration Ceremony

30th August 2017 at 6.15 pm

Cinnamon Lakeside Hotel

Colombo

Pre-Congress Workshop

2nd South Asian Melioidosis Congress

Theme:

"Unearthing a subterranean infection"

29th & 30th August 2017

Cinnamon Lakeside Hotel

Colombo

Scientific Programme

Theme:

"Combating sepsis"

31st August & 1st September 2017

Sri Lanka Foundation

Colombo 07

MESSAGE FROM THE CHIEF GUEST

Combatting sepsis, the theme of this year's conference, is a recognition that there is unfinished business in this fundamental aspect of infectious diseases. The advances made in diagnosis, treatment and prevention of novel and emerging infections show how far we have come in the last three decades, but we have hardly touched the impact of sepsis on the health and welfare of our communities.

It is only recently that we recognised that the burden of disease is comparable with cancer, yet the resources committed to the development of sepsis countermeasures are poor by comparison. Part of the problem is a lack of common language among the various stakeholders, compounded by differences in the definitions used. The recent consensus definition, sepsis 3, is an important step in the right direction, but its criteria have yet to gain traction with clinical laboratory medicine and public health.

One of the major drivers of the current escalation in sepsis is the emergence of multidrug resistant infections, notably the ESKAPE group. Unfortunately, the majority of accurate, laboratory based surveillance data on antimicrobial resistance has not been linked to sepsis and its clinical outcomes. Public health notification of antimicrobial resistant sepsis has yet to catch up with the widely recognised global threat.

It is therefore time to recognise that surveillance of public health challenges is now more sophisticated than the old ways of compulsory notification. Oncology has long used a registry concept to engage stakeholders in a more explicitly mutually beneficial process. We need to consider the merits of this approach to sepsis and recognise that work done here in Sri Lanka on septicaemic melioidosis provides an excellent example of what can be achieved for patients, clinical services and public health at surprisingly low cost.

There is now an urgent need to grow this capability to combat the expanding problem of AMR sepsis before we get to the last roll of the antibiotic dice. As long as the physician who has to treat a patient with sepsis is faced with a best guess antibiotic now, or a laboratory guided decision 48 hours later, they will make an educated guess. It is now up to the clinical laboratory to work out how to guide those point of care decisions, and in particular how to speed up detection and susceptibility testing of bacteria in septicaemic patients. We have some ideas how this can be done, and they will be discussed during this conference. Bringing those ideas into practice may prove to be a lengthy process, but it has never been more urgent.

Finally, I commend the organisers of this conference for their choice of topic, and thank them for inviting us all to consider what we can do to strengthen sepsis countermeasures.

Prof. Tim Inglis
Consultant Microbiologist,
School of Medicine, University of Western Australia and
Department of Microbiology, PathWest Laboratory Medicine, Nedlands, Australia.

MESSAGE FROM THE PRESIDENT

The most anticipated event in the calendar of the Sri Lanka College of Microbiologists is the "Annual Scientific Sessions". Although the beginnings of the College can be traced to 1969 (when the Ceylon Association of Microbiologists was formed) the Annual Scientific Sessions (since then called the Academic Sessions) was first held in 1991. This annual tradition has continued unbroken since then and, this year, we present the 26th Annual Scientific Sessions.

Although great strides have been made in many areas of clinical microbiology and infectious diseases, (for example the prevention of many infectious diseases by vaccination) a number of challenges remain to be addressed. Sri Lanka is in the throes of one of the worst epidemics of dengue we have ever seen and "Dengue in Sri Lanka – Past and the Present Trends" was the topic of the Siri Wickremesinghe Oration delivered in March by Prof Faseeha Noordeen. Sepsis is one of the leading causes of death in the world and continues to pose a diagnostic and therapeutic challenge with the fourth revision of the "Surviving Sepsis Campaign: International Guidelines for Management of Sepsis and Septic Shock" being published early this year. The theme of the Annual Scientific Sessions this year is "Combating sepsis".

The busy schedule of the College continues this year with monthly CME lectures providing us with new insights and knowledge and the Task Force in Microbiology advising the Ministry of Health on policies to improve microbiology services. Work proceeds on the Infection Control Manual and other guidelines and reports commissioned by different groups. The Antimicrobial Resistance Core Group continues to serve as the unofficial secretariat of the "National Action Plan for Combating Antimicrobial Resistance" and recently launched a strategic action plan for Sri Lanka, outlining five main areas which need to be addressed if this plan is to be effective. Subcommittees of the College will soon be appointed to flesh out and implement these strategies. The two ongoing antimicrobial resistance surveillance projects of the College were combined into a single "Sri Lanka Antimicrobial Resistance Surveillance Project" that will monitor trends in antimicrobial resistance. We require and request the support and input of all our members in carrying out these projects.

I thank the Honorary Secretaries, Dr Nayomi Danthanarayana and Dr Thushari Dissanayake and our office staff, Ms. Priyanga Opatha and Ms. Imashi Abeysinghe who have worked beyond the call of duty to ensure the smooth running of all the activities of the College.

Let me welcome you all to the Annual Scientific Sessions with its smorgasbord of plenary lectures, symposia and free papers. I hope there will be something in it for every one of you. In addition, this year's pre-congress will take the form of an international conference, the "2nd South Asian Melioidosis Congress" with speakers and participants from many countries in the region as well as from Australia, Austria and the USA. We hope that this Congress will be the launching pad for many fruitful collaborations with clinicians and researchers in Sri Lanka and the region.

Dr. Enoka Corea
President,
Sri Lanka College of Microbiologists

INAUGURATION PROGRAMME

30th August 2017 at 6.15 pm

Cinnamon Lakeside Hotel
Colombo

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. Thushari Dissanayake Hony. Joint Secretary
6.55 pm	Address by the Chief Guest Prof. Tim Inglis Consultant Microbiologist, PathWest, Western Australia
7.10 pm	Address by the President Dr. Enoka Corea Senior Lecturer University of Colombo
7.40 pm	Award of SLCM Fellowships
8.20 pm	Vote of Thanks Dr. Nayomi Danthanarayana Hony. Joint Secretary
8.25 pm	Ceremonial Procession leaves
8.30 pm	Cultural Show and Reception

2nd South Asian Melioidosis Congress 2017

29th & 30th August 2017

Cinnamon Lakeside Hotel, Colombo

Day 1 – 29th August 2017

8.00 am - 9.00 am	Registration and Inauguration
	History
9.00 am - 9.30 am	**Historical background of melioidosis** Prof. David Dance University of Oxford, UK
	Epidemiology
9.30 am - 10.00 am	**Global epidemiology of melioidosis** Dr. Direk Limmathurotsakul Mahidol University, Bangkok, Thailand
10.00 am - 10.30 am	**Epidemiology of melioidosis in South Asia** Prof. Chiranjay Mukhopadhyay Kasturba Medical College, Manipal University, India
10.30 am - 11.00 am	**Tea Break**
11.00 am - 11.30 am	**Nationwide epidemiology of melioidosis in Sri Lanka** Dr. Enoka Corea University of Colombo, Sri Lanka
11.30 am - 12.00 am	**Epidemiology of melioidosis in Bangladesh** Prof. Md Shariful Alam Jilani Ibrahim Medical College, Dhaka, Bangladesh
12.00 noon - 12.30 pm	**One health initiative to uncover epidemiology of melioidosis in southern Thailand** Dr. Apichai Tuanyok University of Florida, USA
	Immune response
12.30 pm - 1.00 pm	**Host and pathogen specific biomarkers for melioidosis disease management** Dr. Mohan Natesan Division of Molecular and Translational Sciences, USAMRIID
1.00 pm - 2.00 pm	**Lunch**
2.00 pm - 2.30 pm	**Immune responses in *B. pseudomallei* infection** Dr. Dharshan de Silva Kothalawala Defence University, Sri Lanka

Pathogenesis

2.30 pm - 3.00 pm **Fatal attraction: host-pathogen interactions in *B. pseudomallei* infection**
Prof. Natkunam Ketheesan
Australian Institute of Tropical Health and Medicine

3.00 pm - 3.30 pm **Insights into the pathogenesis of *B. pseudomallei***
Prof. Joost Wiersinga
University of Amsterdam, Netherlands

3.30 pm - 4.00 pm **Mechanisms of human susceptibility to *B. pseudomallei* infection**
Dr. Ganjana Lertmemongkolchai
Khon Kaen University, Thailand

4.00 pm - 4.30 pm **Tea Break**

4.30 pm - 5.30 pm **Case Presentations**

Day 2 - 30th August 2017

8.00 am - 9.00 am **Meet the Experts**

Clinical Features

9.00 am - 9.30 am **The clinical correlates of barefoot bacteraemia**
Prof. Tim Inglis
PathWest Laboratory, Australia

9.30 am - 10.00 am **Septicaemic melioidosis: The best approach to diagnose**
Dr. K. E. Vandana
Kasturba Medical College, Manipal University, India

Diagnostics

10.00 am - 10.30 am **Laboratory diagnosis of melioidosis**
Dr. Narisara Chantratita
Mahidol University, Bangkok, Thailand

10.30 am - 11.00 am **Tea Break**

Molecular epidemiology

11.00 am - 11.30 am **Molecular epidemiology of *B. pseudomallei* in South Asia**
Dr. T. A. K. Chaitanya
Kasturba Medical College, Manipal University, India

11.30 am - 12.00 noon **Molecular epidemiology of *B. pseudomallei* in Sri Lanka**
Mr. Adam Merrit
PathWest Laboratory, Australia

PRE-CONGRESS WORKSHOP PROGRAMME

	Antibiotic Resistance
12.00 noon - 12.30 pm	**Antibiotic resistance mechanisms in *B. pseudomallei***
	Dr. Herbert P. Schweizer
	University of Florida, Emerging Pathogens Institute, USA
	Management
12.30 pm - 1.00 pm	**Update on treatment of melioidosis**
	Dr. Wirongrong Chierakul
	Mahidol University, Bangkok, Thailand
1.00 pm - 2.00 pm	**Lunch**
	Soil Surveillance
2.00 pm - 2.30 pm	**Update on soil surveillance**
	Dr. Ivo Steinmetz
	Medizinische Universität Graz Österreich
2.30 pm - 3.00 pm	**Soil epidemiology in SW India**
	Mr. Tushar Shaw
	Kasturba Medical College India, Manipal, India
	Future areas for study
3.00 pm - 3.30 pm	**'Omics' of *B. pseudomallei***
	Dr. Tim Inglis
	PathWest Laboratory, Australia
3.30 pm - 4.00 pm	**Tea Break**
4.00 pm - 4.30 pm	**Valedictory**

26th Annual Scientific Sessions

The Sri Lanka College of Microbiologists

Scientific Programme

Theme:

"Combating sepsis"

31st August & 1st September 2017

Sri Lanka Foundation
Colombo 7

Scientific Programme – Day 1 – Thursday 31st August 2017

8.15 am - 8.45 am ***Registration***

8.45 am - 9.45 am **Free paper session 1**

Chairpersons – Dr. Malka Dassanayake and Dr. Nadisha Badanasinghe

OP 1 **An audit on antimicrobial consumption in the special care baby unit (SCBU) at Colombo South Teaching Hospital (CSTH) during 2015 and 2016**

Wickramasuriya UAGH, Chandrasiri NS, Premaratne KKMK, Sathanandaraja R, Thabrew H, Waniganayake YC, Sutharson A, Wijethunga GSB, Amarasinghe SD

Colombo South Teaching Hospital

OP 2 **The microbiology of bacterial peritonitis due to appendicitis**

Fernando R, Silva KRS, Nathaniel DA, Silva W, Amarasinghe R

Microbiology Department and Surgical Department, District General Hospital, Negombo, Sri Lanka

OP 3 **Typing of Gram negative bacteria obtained from respiratory specimens in an Intensive Care Unit**

Tissera K[1], Tennakoon M[1], Adasooriya D[1], Pinto V[2], Dissanayake N[1], Ekanayake A[1], Nanayakkara D[1], Liyanapathirana V[1]

[1]Department of Microbiology, Faculty of Medicine, University of Peradeniya, [2]Department of Anaesthesiology and Critical Care, Faculty of Medicine, University of Peradeniya

OP 4 **Use of antibiotics for acute respiratory tract infections in children: practice, irrational use and contribution to the emergence of antimicrobial resistance**

Jayaweera JAAS[1,2], Noordeen F[2], Joseph A[1], Croos S[1], Rayes MLM[3]

[1]Department of Microbiology, Faculty of Medicine and Allied Sciences, Rajarata University of Sri Lanka, [2]Department of Microbiology, Faculty of Medicine, University of Peradeniya, [3]Department of Paediatrics, Faculty of Medicine and Allied Sciences, Rajarata, University of Sri Lanka and Teaching Hospital, Anuradhapura

9.45 am - 10.30 am

Plenary 1

Chairperson – Prof. Vasanthi Thevanesam

Sepsis: Panacea or Pandora's box?
Dr. Prabath Nanayakkara
Head, Section Acute Medicine, Department of Internal Medicine
VU University Medical Centre, Amsterdam

10.30 am - 10.45 am Tea

10.45 am - 11.45 am

Free paper session 2

Chairpersons – Dr. Rohini Wadanamby and Dr. Jananie Kottahachchi

OP 5 **Vancomycin resistant enterococcal colonization in Intensive Care Unit of a tertiary care hospital**

Dassanayake DMGA[1], *Dissanayake BN*[2], *Pinto V*[3], *Liyanapathirana LVC*[2], *Shahnas MNF*[4], *Ekanayake EWMA*[2]
[1]Post Graduate Institute of Science, University of Peradeniya, [2]Department of Microbiology, Faculty of Medicine, University of Peradeniya, [3]Department of Anaesthesiology and Critical Care, Faculty of Medicine, University of Peradeniya, [4]Teaching Hospital, Peradeniya

OP 6 **Risk factors and outcome of infections caused by *Acinetobacter* spp. among critically ill patients in a tertiary care hospital in Sri Lanka**

Priyaranganie WKAP[1], *Piyasiri DLB*[1], *Nagahawatta A*[2], *Vidanagama DS*[3], *Perera B*[4]
[1]Teaching Hospital Karapitiya, Galle, [2]Department of Microbiology, Faculty of Medicine, University of Ruhuna, [3]The National Tuberculosis Reference Laboratory, Welisara, [4]Department of Community Medicine, Faculty of Medicine, University of Ruhuna

OP 7 **A multicenter study to determine the prevalence and the associated factors of New Delhi Metallo-β lactamase- 1 (NDM-1) strains among Gram Negative Bacilli in clinical isolates**

Sajeevan TR[1], *Karunanayake L*[1], *Patabendige CGUA*[2], *Mubarak FN*[3], *Ravikumar R*[4]
[1]Medical Research Institute, Colombo 8, [2]National Hospital of Sri Lanka, Colombo, [3]Teaching Hospital, Jaffna, [4]Department of Neuromicrobiology, National Institute of Mental Health and Neuro Sciences (NIMHANS), Hosur Road, Bangalore, India

OP 8 **Methicillin-resistant *Staphylococcus aureus*: high prevalence of suspected nosocomial colonization in the surgical, medical and orthopaedic wards of Teaching Hospital Karapitiya**

Kurukulasooriya R[1], *Wijayaratne WMDGB*[1], *Tillekeratne LG*[2], *Bodinayake CK*[1], *Rajapakshe MD*[1], *Umesha BGJ*[1], *Abewickrama TDR*[1], *Anuradha KVT*[1], *de Silva AD*[3], *Nicholson BP*[2], *Østbye T*[2], *Woods CW*[2], *Nagahawatte A*[1]
[1]Faculty of Medicine, University of Ruhuna, Galle, Sri Lanka, [2]Duke University, Durham, North Carolina, USA, [3]Genetech Research Institute, Colombo, Sri Lanka

11.45 am - 12.45 pm

Symposium 1 – Combating Antimicrobial Resistance

Chairpersons – Dr. Jayanthi Elvitigala and Dr. Shirani Chandrasiri

National Strategic Plan for Combating AMR
Dr. Kumudu Karunaratne
Consultant Microbiologist, Lady Ridgeway Hospital for Children, Colombo

Behavioural Aspects of Antimicrobial Stewardship
Dr. Carolyn Tarrant
Associate Professor, Sapphire, Department of Health Sciences, College of Medicine, Biological Sciences and Psychology, University of Leicester

Detection of Carbapenemases in the Clinical Microbiology Laboratory
Prof. Balaji Veeraraghavan
Professor and Head, Department of Clinical Microbiology, Christian Medical College and Hospital, Vellore, India

12.45 pm - 1.45 pm Lunch

1.45 pm - 2.30 pm

Plenary 2

Chairperson – Prof. Sharmini Gunawardena

Toxoplasmosis
Prof. Sarman Singh
Professor and Head, Division of Clinical Microbiology and Molecular Medicine, Department of Laboratory Medicine, All India Institute of Medical Sciences, New Delhi

2.30 pm - 3.30 pm

Free paper session – 3

Chairpersons – Dr. Ajith Nagahawatte and Dr. Hasini Banneheke

OP 9 **Prominent T helper (Th)-1 response to *Leishmania donovani*-induced cutaneous leishmaniasis in Sri Lanka**
Manamperi NH[1], Oghumu S[2], Pathirana N[3], de Silva MVC[4], Abeyewickreme W[1], Satoskar AR[5], Karunaweera ND[6]
[1]Department of Parasitology, Faculty of Medicine, University of Kelaniya, Ragama, Sri Lanka, [2]Department of Environmental Health Sciences, College of Public Health, Ohio State University, Columbus, OH, USA, [3]Department of Dermatology, Army Hospital, Colombo, Sri Lanka, [4]Department of Pathology, Faculty of Medicine, University of Colombo, Sri Lanka, [5]Department of Pathology, Wexner Medical Center, Ohio State University, Columbus, OH, USA, [6]Department of Parasitology, Faculty of Medicine, University of Colombo, Sri Lanka

OP 10 **Genomic analysis of Sri Lankan cutaneous *Leishmania donovani* isolates from poor responders to Sodium stibogluconate**
Samarasinghe SR[1], Samaranayake TN[1], Karunaweera ND[1]
[1]Department of Parasitology, Faculty of Medicine, University of Colombo

OP 11 **Brugian filariasis in Sri Lanka: a preliminary report on survey in Gampaha District**
Mallawarachchi CH[1], Chandrasena TGAN[2], Samarasekara SD[3], Mallawarachchi SMNSM[1], de Silva NR[2]
[1]Post Graduate Institute of Medicine, University of Colombo, Sri Lanka, [2]Department of Parasitology, Faculty of Medicine, University of Kelaniya, Sri Lanka, [3]Quarantine Unit, Ministry of Health, Sri Lanka

OP 12 **Do we have antifungal resistance in invasive candida isolates? A study by the National Mycology Reference Laboratory, Sri Lanka**
Jayawardena MN, Sigera LSM, Jayasekera PI
Department of Mycology, Medical Research Institute

3.30 pm - 4.15 pm **Plenary 3**

Chairperson – Dr. Primali Jayasekera

Microbiome with a Focus on the Mycobiome
Dr. Nelun Perera
Consultant Microbiologist, University Hospitals of Leicester, Honorary Associate Professor, Department of Infection, Immunity and Inflammation, University of Leicester, Training Programme Director, Microbiology, Health Education England, East Midlands

4.15 pm Tea

Scientific Programme – Day 2 – Friday 1st September 2017

8.30 am - 9.30 am **Free paper session – 4**

Chairpersons – Dr. Omala Wimalaratne and Dr. Rohitha Muthugala

OP 13 **Epstein-Barr virus (EBV) infection and associated factors for Post-Transplant Lymphoproliferative Disorder (PTLD) in a group of renal transplant patients**
Mahanama AIK, Abeynayake JI
Department of Virology, Medical Research Institute, Colombo 08, Sri Lanka

OP 14 **Prospective study to ascertain the relationship between rabies infection and anti-rabies immunization status of dogs and cats in Sri Lanka**
Kumarasinghe KADM, Nanayakkara S, Balasubramaniam R, Jayasinghe AU, Udara GKJN, Perera KADN
Department of Rabies and Vaccine QC, Medical Research Institute, Colombo 8

OP 15 **An outbreak of concurrent infections with dengue virus serotypes in Kinniya, Trincomalee, Sri Lanka**
Abeynayake JI[1], Fernando LK[2], Gunasena S[1], Hettigoda GM[1], Fernando MAY[1], Welmillage SU[1], Wickramasinghe MGCN[1]
[1]Department of Virology, Medical Research Institute, Colombo 08, [2]Centre for Clinical Management of Dengue and Dengue Haemorrhagic Fever, District General Hospital, Negombo

OP 16 **Human bocavirus infection in a selected group of children with severe acute respiratory symptoms: Preliminary survey**
Jayamaha CJS, Sanjeewa MDA, Ekanayake P
National Influenza Centre, Department of Virology, Medical Research Institute, Colombo

9.30 am - 10.15 am **Plenary 4**

Chairperson – Dr. Nadeeka Janage

Viral Infections in the Immunocompromised
Dr. Mark Atkins
Consultant Virologist (Specialty Lead), Ashford and St. Peter's Hospitals, Surrey, United Kingdom

10.15 am - 10.30 am Tea

10.30 am - 11.30 am **Symposium 2 – Advances in Combating Sepsis**

Chairpersons – Dr. Kushlani Jayatilleke and Dr. Geethika Patabendige

PHANTASi Trial: (Pre)Hospital Management of Sepsis: Opportunities and Challenges
Dr. Prabath Nanayakkara
Head, Section Acute Medicine, Department of Internal Medicine
VU University Medical Centre, Amsterdam

Diagnostic Stewardship
Prof. Balaji Veeraraghavan
Professor and Head, Department of Clinical Microbiology, Christian Medical College and Hospital, Vellore, India

Antimicrobial Susceptibility Profiling; Slow or Fast?
Prof. Tim Inglis
Consultant Microbiologist, School of Medicine, University of Western Australia and Department of Microbiology, PathWest Laboratory Medicine, Nedlands, Australia.

11.30 am - 12.30 pm **Free paper session – 5**

Chairpersons – Dr. Geethani Galagoda and Dr. Pavithri Bandara

OP 17 **Validation of multiplex PCR in the detection of pathogen causing acute bacterial meningitis**
Devakanthan B[1], *Dissanayake N*[1,2], *Liyanapathirana V*[2], *Harasgama HDRPD*[2], *Punchihewa PHJP*[1]
[1]Department of Microbiology, Teaching Hospital, Peradeniya, [2]Department of Microbiology, Faculty of Medicine, University of Peradeniya

OP 18 **Comparison of real time PCR with the culture method for detecting *Salmonella* spp in raw chicken**
Wickramasuriya UAGH, Pathirage MVSC, Jayamaha CJS
Medical Research Institute Colombo

OP 19 **Comparison of Direct Fluorescent Test with Real-time PCR for Diagnosis of Respiratory Virus Infections in Children**
Jayamaha CJS[1], *Harshani HBC*[1], *Ratnayake NR*[2]
[1]National Influenza Centre, Department of Virology, Medical Research Institute, Colombo, [2]Lady Ridgeway Hospital for Children, Colombo

OP 20 **Detection and genotyping of Human Papillomavirus in patients with oral and oropharyngeal carcinomas**
Samaraweera B, Abeynayake JI
Department of Virology, Medical Research Institute, Colombo 08, Sri Lanka

12.30 pm - 1.30 pm Lunch

1.30 pm - 2.30 pm

Symposium 3 – Blood Borne Viruses

Chairpersons – Dr. Jude Jayamaha and Dr. Janaki Abeynayake

Dilemmas in the Diagnosis of Blood Borne Viral Infections
Dr. Mark Atkins
Consultant Virologist (Specialty Lead), Ashford and St. Peter's Hospitals, Surrey, United Kingdom

Clinical Dilemmas in Managing HIV Patients
Dr. Ananda Wijewickrema
Consultant Physician, National Institute of Infectious Diseases, Angoda

2.30 pm - 3.15 pm

Plenary 5

Chairperson – Dr. Dhammika Vidanagama

Interactive Case Discussion
Dr. Nelun Perera
Consultant Microbiologist, University Hospitals of Leicester, Honorary Associate Professor, Department of Infection, Immunity and Inflammation, University of Leicester, Training Programme Director, Microbiology, Health Education England, East Midlands

3.15 pm - 3.30 pm Award ceremony

3.30 pm Tea

LIST OF GUEST SPEAKERS

Dr. Prabath Nanayakkara
Head, Section Acute Medicine,
Department of Internal Medicine,
VU University Medical Centre,
Amsterdam

Dr. Kumudu Karunaratne
Consultant Microbiologist
Department of Microbiology,
Lady Ridgeway Hospital for Children,
Colombo 8

Dr. Carolyn Tarrant
Associate Professor,
Sapphire, Department of Health Sciences,
College of Medicine,
Biological Sciences and Psychology,
University of Leicester

Prof. Balaji Veeraraghavan
Professor and Head,
Department of Clinical Microbiology,
Christian Medical College and Hospital,
Vellore, India

Prof. Sarman Singh
Professor and Head,
Division of Clinical Microbiology and Molecular Medicine,
Department of Laboratory Medicine,
All India Institute of Medical Sciences,
New Delhi

LIST OF GUEST SPEAKERS

Dr. Nelun Perera
Consultant Microbiologist
University Hospitals of Leicester

Honorary Associate Professor,
Department of Infection, Immunity and Inflammation
University of Leicester

Training Programme Director, Microbiology
Health Education England /East Midlands

Dr. Mark Atkins
Consultant Virologist (Specialty Lead),
Ashford and St. Peter's Hospitals,
Surrey, United Kingdom

Prof. Tim Inglis
Consultant Microbiologist,
School of Medicine, University of Western Australia and
Department of Microbiology,
PathWest Laboratory Medicine,
Nedlands, Australia.

Dr. Ananda Wijewickrema
Consultant Physician
National Institute of Infectious Diseases,
Angoda

ABSTRACTS OF THE PLENARY LECTURES AND SYMPOSIA

Plenary Lectures

Plenary presentation 1

Sepsis: Panacea or Pandora's box?

Dr. Prabath Nanayakkara

Sepsis is a common, life threatening illness affecting millions of people globally, causing more deaths than AIDS, breast cancer and prostate cancer put together. Sepsis is a complex syndrome rather than a disease with a golden diagnostic standard. It is difficult to identify in early stages, where treatment is still possible, while in later stages it may be easier to recognize but more difficult to treat. Hence the major variations in the reported incidence and mortality rates. Reported incidence estimates are highly variable ranging from 50 to 100 cases per 100,000 individuals, while the real incidence is probably much higher, partly due to under-reporting and the absence of incidence rates from countries where good hospital and intensive care is scarce. The case fatality rates depend on setting, severity of disease, patient-specific characteristics and the definition that is being used. For young patients without co-morbidity, reported mortality is less than 5% and for intensive care patients with severe sepsis or septic shock mortality ranges from 25% to 45%. In spite of the advances in the facilitatory care especially in the critical care setting, early recognition and treatment still remains the cornerstone of therapy.

Sepsis was defined as a proven or suspected infection associated with a generalized inflammatory response, the "systemic inflammatory response syndrome" (SIRS). However, In early 2016, the new Sepsis-3 Taskforce, consisting of members of the European Society of Intensive Care Medicine and the SCCM, proposed the new definition for sepsis. The new definition resulted from a better understanding of the pathophysiology of sepsis and the need to adapt the definition here. In addition, there always had been criticism on the specificity of the SIRS criteria. However, the Sepsis-3 criteria has never been prospectively validated in the emergency department setting, where majority of the sepsis patients are treated, which is a major limitation.

Plenary presentation 2

Toxoplasmosis in Immunocompromised Hosts

Prof. Sarman Singh

Toxoplasmosis is caused by a coccidian protozoa T*oxoplasma gondii*. Even though this parasite can infect any metazoan, the infection has major consequences in humans and farm animals. If acquired during the early part of gestation, the sequelae of the infection in the foetus or later in the infected child can be devastating. It can infect both immunocompetent as well as immunocompromised human hosts. It is reported that that systemic infection with *Toxoplasma gondii* triggers not only a transient increase in activated CD4+cells but also parasite-induced destruction of the thymic epithelium and overall architecture of the lymphoid organ. Suggesting that *T. gondii* infection itself can lead to an immunocompromised state. However, in already immunocompromised patients, the clinical presentations can be atypical, more severe and even fatal. Most common risk group is HIV/AIDS patients followed by solid organ and haemopoietic stem cell transplant recipients, patients with autoimmune diseases and cancer patients. Very high fatality is reported in liver and cardiac transplant recipients. The post transplant mortality increases among the *T. gondii* seropositive recipients suggesting that in most cases re-activation of the dormant cysts takes place during the post-transplant immune suppression. Encephalitis, chorioretinitis, pneumonia and disseminated toxoplasmosis are common manifestations in these patients. Unfortunately very limited data is available from Asia or Africa. In severely immunocompromised patients, diagnosis is difficult, mainly because in majority of cases the disease is not suspected till a very fulminant form. Serology in this group of patients, especially if relying on IgM is not very rewarding. Pre- and post- transplant or IgG seroconversion can be important diagnostic markers but most sensitive and specific diagnostic method is PCR and sequencing. Treatment is more aggressive than given in immune competent patients.

Plenary presentation 3

Microbiome with a Focus on the Mycobiome

Dr. Nelun Perera

Introduction of Next Generation Sequencing technologies have led to new insights into our understanding of microbes that occupy surfaces of the human body (human microbiome). Hundreds of previously known and newly discovered microbial species (bacteria, fungi and viruses) living in distinct communities on the human body have been sequenced. The human microbiome is increasingly thought to be necessary for normal human development, physiology, immunity and nutrition. Pathologic perturbation of the microbial diversity of the

human microbiome is increasingly recognized as an important correlate of infectious and non-infectious diseases.

The human mycobiome, which refers to the fungal biota, is an important component of the human microbiome. By interfacing with other biomes, as well as with the host, the mycobiome appears to play a critical role in health and disease.

An overview of the methodology used to study the human mycobiome, it's composition and it's role in health and disease will be presented.

Plenary presentation 4

Viral Infections in the Immunocompromised

Dr. Mark Atkins

The effects of viruses on human civilisations have been documented for over 3000 years. We have known of the ability of small, filterable infectious agents to cause disease for about 100 years but the greatest expansion of our knowledge of viruses has come over the past 30 years.

Recently, our ability to manipulate the immune system has allowed the development of transplantation and the management of a plethora of immune mediated diseases. With these developments we have seen an increasing number of challenges due to viral infections. Initially this was mostly due to herpes viruses, principally HSV, VZV and CMV. The discovery of acyclic nucleoside analogues as effective antivirals against herpes viruses coupled with the development of molecular diagnostics, has revolutionised the management of these infections. Not only can we treat these infections more effectively but we are able to predict their emergence and prevent them occurring.

The emergence of HIV as a major cause of immunosuppression has given us many new challenges with respect to viral infections. We have had to develop treatments for HIV itself and also deal with new challenges from infections such as CMV, HSV, JCV and EBV. We have also discovered a number of new agents such as HHV8 as a consequence. HIV has taught us much about the workings of the immune system and its limitations.

The intimate relationship between the immune system and viruses and the pathology they induce has given us many challenges. However, it has allowed us to investigate not just how viruses behave and cause disease but has also increased our knowledge of our immune systems immensely. Continuing development of immunosuppressive treatments for an ever widening range of diseases will continue to challenge our ability to diagnose and treat the virus infections that emerge. It is important that we remain alert to the possibility of viruses causing disease in unfamiliar settings and that we continue to invest in antiviral research, diagnostic facilities and training of virologists.

Symposium 1

Combating Antimicrobial Resistance

National Strategic Plan for combating AMR

Dr. Kumudu Karunaratne

Since the introduction of antibiotics in the 1940s antimicrobial medicines such as antibiotics, antivirals and antifungals have significantly reduced morbidity and mortality due to infectious diseases. Since its discovery, its scope has expanded from human health to veterinary and agriculture sectors as well. Currently antimicrobials have taken a central place in advancement of modern medicine. However the development of resistance to antimicrobials has put this advancement at risk.

World Health Organisation (WHO) has identified antimicrobial resistance (AMR) as a key global health issue. It is a public health risk which can cross borders to affect the population worldwide. Though no region of the world is spared of this public health risk the major burden of this is borne by developing countries. Therefore in 2011 the member states of WHO South East Asia region including Sri Lanka became signatories to Jaipur Declaration, which agrees to regulate use of antimicrobial agents. Recognising the gravity of the problem, in May 2015 the World Health Assembly endorsed a global action plan to combat AMR, which called all countries to develop national strategies within two years. In response, a meeting was held in the WHO country office with the officials of the Ministry of Health (MoH) and representatives of Sri Lanka College of Microbiologists (SLCM), and the task of developing a draft national action plan for discussion was allocated to SLCM.

The global action plan was under 'One Health' concept which recognises that human, animal and ecosystem health are inextricably linked. Therefore a multi-sectoral coordinating group representing members from human health sector, veterinary sector, fisheries and agriculture played a key role in development of the National Strategic Plan for combating AMR. This was developed in line with the global action plan with five strategic objectives and several strategic interventions under each strategic objective. This activity was supported by the WHO. The National Strategic plan for combating antimicrobial resistance in Sri Lanka (2017-2022) was launched in May 2017 where the Hon. Minister of Health, Nutrition and Indigenous Medicine graced the occasion as the chief guest highlighting the political commitment.

Behavioural Aspects of Antimicrobial Stewardship

Dr. Carolyn Tarrant

Antimicrobial resistance is one of the largest and most widely acknowledged problems in 21st century medicine; tackling it requires work to optimise antibiotic prescribing and reduce over-use of antibiotics. Attempts to change the ways antibiotics are prescribed will be more effective if they take into account the way prescribing decisions are made by healthcare staff, and the social and contextual factors that impact on these decisions. This presentation will describe how psychological models of individual and social behaviour can help us better understand the barriers to optimal antibiotic use. The presentation will outline two ongoing studies in this areas: a study that aims to use behaviour change models to optimise prescribing across the healthcare economy in the UK, and a study that draws on theories of social dilemmas to gain a better understanding of the use of broad spectrum antibiotics in hospitalised acute medical patients, in England, Sri Lanka, and South Africa. The use of theory to design interventions will be discussed.

Detection of Carbapenemases in the Clinical Microbiology Laboratory

Prof. Balaji Veeraraghavan

Carbapenemase-mediated carbapenem resistance is a major concern across the world. Rapid identification of carbapenemase producing organisms is of great importance for timely detection, treatment and implementation of control measures to prevent the spread. The Modified Hodge Test (MHT) and Carba NP test is recommended by CLSI for the detection of carbapenemases in Enterobacteriaceae. However, MHT may give false positive results or fail to detect metallo β-lactamases (MBLs). In the US, MHT is the most widely used test for detection of carbapenemases and has been found to have a sensitivity and specificity of >90% for bla KPC producers. However, in many countries, the prevalence of bla NDM is higher than bla KPC producers. Therefore, it is advisable to evaluate an assay for better laboratory diagnosis at respective regions.

It is essential to have a test with good sensitivity and specificity. We compared the performance of RAPIDEC® CARBA NP and modified carbapenem inactivation method (mCIM) recommended by Clinical and Laboratory Standards Institute guideline 2017. A total of ninety carbapenem resistant *Escherichia coli* and *Klebsiella pneumoniae* have been tested. The presence of various carbapenemases was screened by conventional multiplex polymerase chain reaction. RAPIDEC® CARBA NP detected 90%, whereas mCIM detected 99% of the study isolates tested. Although RAPIDEC® CARBA NP is a rapid test, the sensitivity is reduced for blaOxa-48 Like detection; while mCIM could pick up blaOxa-48 Like enzymes with excellent sensitivity. Further, organisms producing low carbapenemase activity enzymes, thickness of the inoculum and the disc potency are likely to influence the test results of mCIM with an overnight delay.

Symposium 2

Advances in Combating Sepsis

PHANTASi Trial: (Pre) Hospital Management of Sepsis: Opportunities and Challenges

Dr. Prabath Nanayakkara

Despite the fact that the mortality of sepsis is ten times higher than myocardial infarction and to five times higher than stroke, relatively little attention is given to sepsis. In recent years successful clinical care management pathways have been developed for patients suffering from a myocardial infarction, stroke or a trauma. Even though there is strong evidence in scientific literature to support the need for a series of time-dependent actions, for sepsis this is still not the case. Prompt recognition and treatment are extremely important for improving survival, while patients who survive sepsis can still continue to suffer from physical or psychological symptoms. While mortality rates have fallen in the last few decades, the total deaths from sepsis has increased. This is due to an increase in the incidence of sepsis, which may have several causes. Firstly due to a growing number of patients who have a high susceptibility of infection such as the elderly, patients with chronic illnesses as AIDS, chronic obstructive pulmonary disease, transplant patients and those suffering from malignancies. Secondly due to an increased use of immunotherapy, cytotoxic agents, and invasive medical devices rendering patients with an inadequate immune system and more prone for an infection.

Patients with severe sepsis or septic shock can also benefit from early pre-hospital care. Pre-hospital care is the initial medical care, which is given by general practitioners (GP's) or emergency medical service (EMS) personnel once they reach the patient. Since time plays a crucial role in the treatment of sepsis, early recognition and initiation of treatment by the GP's and EMS personnel may help to reduce mortality. In addition, the provision of pre-hospital care is associated with a shorter start-up time of initiation of therapy in the hospital. Therefore, it can be expected that by the administration of broad-spectrum antibiotics in the ambulance, the survival of sepsis can be improved by greatly reducing the time to the administration of the necessary antibiotics. During this talk we will discuss the rationale behind the PHANTASI (Pre-hospital antibiotics against sepsis) trial, the largest pre-hospital sepsis trial to date and present the preliminary results.

Diagnostic Stewardship

Prof. Balaji Veeraraghavan

Traditional microbiological methods remain sub optimal in providing rapid identification and susceptibility testing. The need for rapid result is evident and current rapid molecular identification methods can provide results within minutes to few hours.

Rapid diagnostic testing (RDT), contributes to a reduction in antibiotic use. RDT allows for rapid identification of group A streptococcus, methicillin-resistant *Staphylococcus aureus* (MRSA), *Clostridium difficile*, and extended spectrum beta-lactamase (ESBL) and carbapenemase-producing organisms. Poor diagnosis may result in nosocomial outbreak of multi-drug resistant infection and spread of resistant pathogens. Molecular microbiology assists in rapid identification of pathogen and resistance mechanism without conventional culture. Many rapid diagnostic tools are now FDA cleared for clinical use.

In 2016, IDSA guidelines for ASP recommend rapid diagnostic testing in addition to conventional microbiology methods for blood culture. Improved clinical outcome would be appreciated with the earliest tailoring of therapy, reconcile conflicts associated with empiric therapy and avoid antibiotic agents causing collateral damage. Rapid diagnostic tests are “game changing” for patient care and provide new opportunity for stewardship program. Enhance function of clinical microbiology laboratories. The use of rapid diagnostic tools on patient care is an area of great interest; they can be best applied to stewardship effect. This includes the several commercially available rapid molecular assays for organism detection:

- PCR for identification and AMR
- Nano particle pro technology (Verigene blood culture system)
- Fluorescent in-situ hybridization (peptide nucleic acid)
- Matrix assisted laser desorption/ionization – time of flight mass spectrometry
- Procalcitonin as a sepsis biomarker

Antimicrobial Susceptibility Profiling; Slow or FAST?

Prof. Tim Inglis

The expanding global distribution of antibiotic resistance of clinical significance threatens to make common bacterial infections untreatable, and already places a heavy infection control burden on our hospitals. The scale of antimicrobial resistance (AMR) is so large that it is now considered one of the main threats to global health. Unfortunately our current systems for detection, surveillance and detailed analysis of AMR rely heavily on bacterial culture which introduces a delay into the laboratory process. Physicians who prescribe antibiotics need better tools to support their choice of antimicrobial agent at the point of care. The current standards for qualitative (S/R) and quantitative (MIC) antimicrobial susceptibility testing deliver their results 1-2 days after specimen collection because they rely on at least one culture step. These delays force antibiotic prescribers to make a presumptive, educated guess at the time of preliminary diagnosis. Current slow antimicrobial susceptibility profiling only informs the definitive choice of antimicrobial agent, or de-escalation. Emerging methods for detection of specific common resistance types include targeted PCR assays, substrate utilisation tests and gene sequencing. These all require at least preliminary culture and do not measure susceptibility. Flow cytometry assisted susceptibility tests (FAST) are under development for real time susceptibility testing. At present, these are technically demanding research methods that require detailed validation for each antibiotic-bacteria-colour dye (ABCD) combination.

Symposium 3

Blood Borne Viruses

Dilemmas in the Diagnosis of Blood Borne Viral Infections

Dr. Mark Atkins

Our ability to diagnose common blood borne virus infections has improved dramatically over the past two decades. We now have ready access to a range of reliable and rapid antibody, antigen and nucleic acid detection systems that have unprecedented sensitivity and specificity. This is particularly true for HIV and HBV assays. However, there are still scenarios that prove a challenge to even the best diagnostic services. I will attempt to describe how we can optimize the use of these tests, interpret the results and how to avoid being misled by them. There are still many unanswered questions regarding the use of some of the more recently introduced molecular assays. I will discuss some of the uses of these molecular tests, interpretation of the results and their limitations.

Clinical Dilemmas in Managing HIV Patients

Dr. Ananda Wijewickrema

Prevalence of HIV is low in Sri Lanka with a prevalence rate of <0.1%. However, the worrying fact is the gradual rise, though slow, in the incidence of HIV in Sri Lanka in contrast to many other countries. Still many patients are being diagnosed as HIV when they present with opportunistic infections (OI), i.e. in the stage of AIDS. As clinicians, we are keen in starting them on ARV as soon as possible as most of them have very low CD-4 cell counts. However, presence of multiple and recurrent OIs will delay the initiating of ARV. Tuberculosis, one of the commonest infections in HIV causes most dilemmas. TB/HIV patients are more prone to get liver toxicity with anti-TB drugs even when they are not on ARV. Drug interactions of ARV and anti-TB drugs are another common issue. Immune reconstitution syndrome is common especially in patients with low CD-4 cell count. This puts clinicians in to dilemma on whether to stop ARV when IRIS occurs. Whether to give steroids is another question we face in such patients.

Now that HIV has become a chronic illness, age related and ARV related other diseases like diabetes and hypercholesteralaemia is becoming a common problem in HIV patients. Changing ARV in a low resource setting like Sri Lanka is another dilemma.

On the other hand, refusal to take ARV or poor compliance on ARV will put the clinician in a dilemma as this may run counter to the public health interest in reducing the viral load of all patients as a way of reducing the likelihood of transmission. With more experience gaining on ARV, treating HIV is becoming more and more challenging.

OP 1

An audit on antimicrobial consumption in the special care baby unit (SCBU) at Colombo South Teaching Hospital (CSTH) during 2015 and 2016

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Colombo South Teaching Hospital

Introduction

An antibiotic guideline for the SCBU was decided by all stakeholders after an outbreak of carbapenem resistant *Acinetobacter* spp. An annual analysis of antibiotic consumption was also started as a part of it in 2015.

Objectives

- Reduction of carbapenem resistant isolates in the SCBU at CSTH by antibiotic stewardship
- Increase the awareness of pathogens and their sensitivity pattern to the clinicians
- Detection of trends in antibiotic consumption and resistant pattern

Method

After a guideline on antibiotic usage was issued to the SCBU, carbapenem resistant rate of the year 2015 was calculated and presented to the staff of SCBU along with advice against unnecessary carbapenem usage. At the end of 2016 antibiotic consumption data of 2016 was analyzed. The carbapenem resistant rate of 2015 was compared with the 2016 findings as well. The sensitivity of significant blood culture isolates was analyzed. The results were presented at the perinatal audit meeting and infection control committee meeting to make the clinicians aware. Antibiotic use is not presented in standard daily doses since data on duration of use was not collected. Speciation of Gram negative isolates was not done due to limited resources.

Results

In both years Gram positive isolates were sensitive to first line antibiotics and were community acquired infections. In Gram negative isolates carbapenem resistance was noted both in coliforms and *Acinetobacter* spp.

On comparison of usage of Meropenem (500 mg), 823 vials were used in 2015 and only 459 vials in 2016. The carbapenem usage was reduced by 364 vials. When comparing the carbapenem resistance rates, 11 out of 15 Gram negative isolates (92%) were carbapenem resistant in 2015 but only 4 out of 11 Gram negative isolates (36%) were carbapenem resistant in 2016. There was also an increase in use of cefoperazone sulbactam in 2016.

Conclusion

Carbapenem usage as well as carbapenem resistance has been reduced in the SCBU. The adherence to antibiotic guidelines and adequate infection control measures contributed to the reduction of carbapenem resistant isolates.

OP 2

The microbiology of bacterial peritonitis due to appendicitis

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Introduction

Common cause of secondary peritonitis is perforated appendix and intra-abdominal abscess arising from acute appendicitis. Secondary peritonitis is usually community acquired.

Objective

To determine the aerobic bacterial pathogens causing secondary bacterial peritonitis due to appendicitis and to find out the appropriateness of current antibiotic practice.

Method

A retrospective study was carried out in a District General Hospital in Sri Lanka from January to December 2016. Pus or peritoneal fluid samples from patients presenting with secondary bacterial peritonitis due to appendicitis diagnosed by the surgical team with clinical and radiological evidence were considered. In the microbiology laboratory, standard isolation and identification procedures were carried out using, the laboratory manual in microbiology. Antibiotic susceptibility testing was carried out using Stokes disk diffusion method.

Results

During the study period, 34 samples were received from ruptured appendix or appendicular abscess. Microbial growth was seen in 24 samples (71%). Ten (29%) samples showed no growth. Poly microbial growth was identified in 5 (15%) samples.

Escherichia coli was the most common organism (83%) causing bacterial peritonitis due to appendicitis. Forty percent (40%) of *Escherichia coli* were ESBL producers (8/20). *Klebsiella spp* was the other pathogen isolated (17%). *Pseudomonas aeruginosa* (3) and *Proteus* spp (2) were organisms seen in mixed growths.

Commonly affected age group was 10 to 20 yrs. The median age was 16 (6-71) years. The male and female distribution was 59% and 41% respectively.

Non ESBL producing *Escherichia coli* susceptibility to coamoxiclav, cefuroxime and gentamicin was 92%, 88% and 83% respectively. ESBL producing *Escherichia coli* showed 92% sensitive to gentamicin but 100% resistant to cefuroxime and coamoxyclav. Both Non ESBL and ESBL producing *Escherichia coli* were 100% susceptible to amikacin and meropenem. *Klebsiella spp* susceptibility was comparable to non ESBL *Escherichia coli* susceptability.

Conclusion

Escherichia coli was the most common organism causing bacterial peritonitis due to appendicitis. Community acquired ESBL producing *Escherichia coli* rate was alarmingly high. Antibiotics currently used by surgical teams for appendicitis are adequate. Addition of aminoglycosides in ESBL positive cases is justifiable. Periodic evaluation of pathogens and susceptibility using a larger sample size should be highly recommended.

OP 3

Typing of Gram negative bacteria obtained from respiratory specimens in an Intensive Care Unit

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Introduction

Infections with multi drug resistant (MDR) organisms are a major problem in intensive care units and lack of typing based surveillance systems is a draw back in detailed characterization of such organisms.

Objectives

To evaluate the feasibility of using random amplification of polymorphic DNA (RAPD) based typing and clinical data to monitor the spread of resistant Gram negatives.

Methods

A retrospective study was conducted on consecutive Gram negative isolates obtained from respiratory specimens from patients at the general Intensive Care Unit, Teaching Hospital, Peradeniya over a six month period from March 2015. Antibiotic sensitivity patterns and RAPD based typing was performed on the isolates. Selected genetic determinants of resistance were detected using PCR on the *Escherichia coli* and *Klebsiella pneumoniae* isolates. Clustering was performed using an open source software called GelJ using Unweighted Pair Group Method with Arithmetic Mean (UPMGA) based clustering.

Results

Of the seventy isolates included in the study, seven were *Escherichia coli* (10%), 14 were *Klebsiella pneumoniae* (20%), 15 were *Pseudomonas aeruginosa* (21.4%), 30 were *Acinetobacter* spp (42.8%) and the rest belonged to enterobacteriaceae. All *E.coli* were MDRs as defined by being resistant to three or more classes of antibiotics and Extended Spectrum β-actamse (ESBL) producers carrying bla_{CTX-M}. Fourteen isolates were *K. pneumoniae*, and all fourteen were MDRs and ESBL producers. All 14 *K. pneumoniae* harboured bla_{SHV} while 13 harboured bla_{CTX-M}. The MDR rate among *P. aeruginosa* was 13% while all acinetobacters (n=30) were MDRs.

Predominant clusters were identified within all types of Gram negatives using RAPD and the ICU stay of patients who carried MDR organisms were found to overlap in many instance, indicating possible intra-unit spread of organisms. Elaborating on the RAPD results, 71.4% of *E.coli,* 78.6% of *K. pneumoniae*, 53.3% of *P. aeruginosa* and 73.3% of acinetobacters represented predominant clusters for each group at >75% similarity.

Conclusions

We demonstrated that it is possible to conduct molecular typing methods with basic molecular facilities such as a PCR machine and gel doc and be analyzed using open source software.

Disclaimer: Work presented in this study has been since accepted and published in BMC Infectious Diseases DOI: 10.1186/sl2879-017-2590-7

OP 4

Use of antibiotics for acute respiratory tract infections in children: practice, irrational use and contribution to the emergence of antimicrobial resistance

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Introduction

Most of the acute respiratory tract infections (ARTIs) in children are caused by viruses. Clinical differentiation of bacterial and viral ARTIs is difficult and requires viral diagnostic tests. In Sri Lanka, ARTIs are managed by general practitioners (GP) and children with moderate to severe ARTI seek inward care.

Objective

The current study was undertaken to evaluate the use of antibiotics in outpatient department and inward patients for viral ARTIs.

Methods

Nasopharyngeal aspirates of inward patients (1 month - 5 years) with ARTIs were collected in Teaching Hospital, Anuradhapura from March 2013 – August 2014 to identify the viral aetiology using immunofluorescence assay. Use of antibiotics for the infectious diseases in the past 6 months, for the current episode and follow up for 6 months were assessed using clinical records and telephone interviews. The descriptive statistics was used to analyze the data.

Results

Of the 413 with ARTI, viral aetiology was detected in 165 (40%) children. Of these, 99 (60%) children were treated with antibiotics. Prior to seeking inward care, 200 (91%) of the 220 children treated by the GPs were on antibiotics, 40 (20%) had respiratory viral infections by viral antigen detection, 100 children were on β-lactams and β-lactamase inhibitor combination, 58 children were on cephalosporin and 42 children were on macrolides. Most children presented to the GPs on day 1 of the illness. Mean ± SD hospital stay for viral ARTIs with and without antibiotics was 4 ± 1.6 and 4.1 ± 1.8 days and the difference in the hospital stay was not statistically different ($p>0.05$). An average cost ± SD for antibiotics per day was LKR132 ± 28.50. Eighty children had 2 episodes of ARTI in past 6 months from the current episode and in every occasion, they were treated with antibiotics. Thirty children had urinary tract infections (UTI) within 6 months following the current ARTI episode: 22 had culture positive UTI with 16 having extended spectrum of beta lactamase producers.

Conclusion

Identifying the viral aetiology will reduce the irrational use of antibiotics, contributing to reduce the health cost and prevent the emergence of resistant bacteria.

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OP 5

Vancomycin resistant enterococcal colonization in Intensive Care Unit of a tertiary care hospital

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Introduction

Vancomycin resistance among enterococci is a current concern. It is being identified as a nosocomial pathogen globally.

Objective

To determine the epidemiology and the risk factors associated with vancomycin resistant enterococcal (VRE) colonization in the Intensive Care Unit (ICU) of a tertiary care hospital.

Study design, setting and methods

Study population comprised of 103 adult patients who were admitted to ICU. Two rectal swabs were collected from each patient within 24 hours of admission to ICU and further rectal swabs were collected within 5 day intervals. Swabs from each patient were processed in VRE selective agar and VRE broth. Presumptive colonies were identified as enterococci by routine biochemical tests. Vancomycin Minimum Inhibitory Concentrations (MIC) of identified enterococcal isolates was tested by agar dilution method with Muller Hinton agar according to CLSI methodology. Speciation of enterococci was done by sugar fermentation method. VanA was detected by (5'-ATGAATAGAATAAAAGTTGCAATAC) and (5'-CCCCTTTAACGCTAATACGAT) primers by a polymerase chain reaction. A known *Van*A positive enterococcus isolate was used as a positive control.

Results

Enterococci were isolated from 65 rectal swabs. Out of 65 enterococcal isolates, 32 were identified as resistant to vancomycin (MIC of ≥32μg/ml). Those 32 VRE isolates were recovered from 17(16.5%) patients as it included repeated isolates from a single patient. The colonization of VRE in the study population was 16.5%. Thirty (93.7%)

isolates were *E. faecium* and two (6.2%) were *E. faecalis*.14.6% of patients were found to be colonized with VRE at the time of admission to ICU and only 1.9% acquired it during ICU stay. *VanA* gene was identified in 34.4% of isolates. Use of meropenem and recent surgeries were identified as risk factors associated with VRE colonization.

Conclusions

A significant number of patients were found to be colonized with VRE even at the time of ICU admission. V*an*A gene was found only in about one third of isolates and other mechanisms of resistance need to be sought.

OP 6

Risk factors and outcome of infections caused by *Acinetobacter* spp among critically ill patients in a tertiary care hospital in Sri Lanka

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Introduction

Acinetobacter spp. are ubiquitous and often multi-drug resistant pathogens causing severe hospital acquired infections in intensive care units (ICU).

Objective

To study the significance of associated risk factors and the outcome of *Acinetobacter* infections.

Method

A prospective descriptive study was done including all patients who were staying for more than 48 hours in 3 ICUSs in a tertiary care hospital over a period of 4 months.

Screening cultures of urine, respiratory, and skin swabs were performed at the time of admission and repeated every 4th day and with new signs of infection.

Results

From 113 patients 78 (69%) were males. Age ranged from 2 months to 83 years with mean age of 67 years. Twenty eight point three percent (28.3%) of ICU admissions were due to sepsis.

Incidence of colonization and infection with *Acinetobacter* were 35 and 24 cases per 1000 patient days respectively. Incidence of bacteremia, ventilator associated pneumonia, central venous line (CVL) related bacteraemia, and shunt infection with *Acinetobacter* were 24, 10, and 11 per 1000 device days respectively.

Colonization rates varied among respiratory (39.8%), skin (18.6%), CVL tips (4.4%), drains (1.8%), and urine (1.8%) specimens.

Endotracheal tube and CVL in situ more than 12 days had 5 and 4 times risk of developing *Acinetobacter* infections respectively. Presence of Haemodialysis line had 3 times of risk. Presence of 3 or more such invasive devices in situ had 9 times of risk. ICU stay more than 12 days had 6 times risk (p=0.000). Colonization index >0.33 carried 4 times risk (p=0.000, RR=4.053, 95%C.I. =1.949-8.431). Neurological illness, cardiopulmonary resuscitation, and use of inotropes were other risk factors. Mortality rate (OR=3.43, 95% C.I= 1.188-6.797) and prolonged hospital stay (OR=15.109, 95% C.I. =5.658-40.350) of infected patients were higher than that of non-infected patients.

Conclusions

Intubation and CVL in-situ more than 12 days, more than 3 invasive devices in situ, and colonization index >0.33 were the most significant risk factors for the development of *Acinetobacter* infections. Patients with *Acinetobacter* infections had higher rates of mortality and hospital stays than non-infected patients.

OP 7

A multicenter study to determine the prevalence and the associated factors of New Delhi Metallo-β lactamase-1 (NDM-1) strains among Gram negative bacilli in clinical isolates

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Introduction

Carbapenem resistance is an issue in all carbapenemase producers, not specific to NDM-1 producers. *K. pneumoniae* carbapenemases (KPC) and Metallo-Beta-Lactamases are the most common mechanisms among carbapenemase resistance.

Objectives

To determine the rate of New Delhi Metallo β lactamase producing (NDM-1) strains among Gram negative bacilli in clinical isolates in three centers.

Method

A total of 145 Gram negative isolates were obtained from 3 centers (TH Jaffna, NHSL and MRI) and identified using Rapid identification system (RapID commercial kit- Remel) and ABST according to CLSI disk diffusion method. The modified Hodge test, IPM/EDTA only and IPM/IPM plus EDTA were used as screening for carbapenemases and metallo-betalactamases respectively and confirmed by conventional PCR for bla_{NDM-1} gene. All laboratory tests were performed at Bacteriology Laboratory, MRI, Colombo.

Results

Among the total isolates, 28.96% (42/145) were NDM-1 positive by PCR. Among the NDM-1 producing organisms *Klebsiella pneumoniae* was the commonest.

Sensitivity and specificity of modified Hodge test was 55.46%, 61.99%, EDTA inhibition tests (IPM/EDTA only) 68.55%, 63.26% and IPM/IPM plus EDTA 57.35%, 67.64% respectively.

Of the 42 PCR positive isolates, 32 (76.20%) NDM-1 producers were positive for the modified Hodge test. Twenty four isolates (57.14%) were identified by EDTA inhibition test (IPM/EDTA only) and 34 (80.95%) by IPM/IPM plus EDTA as 'MBL producers'.

Conclusions

A high prevalence of bla_{NDM-1} strains was noted in MDRGNB. Screening for carbapenemase production and identification of genetic determinants of resistance should be considered in hospitals considering the availability of resources. Implementation and strengthening of infection control strategies is an urgent necessity in Sri Lanka.

OP 8

Methicillin-resistant *Staphylococcus aureus*: high prevalence of suspected nosocomial colonization in the surgical, medical and orthopaedic wards of Teaching Hospital Karapitiya

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Introduction

Methicillin-resistant Staphylococcus aureus (MRSA) causes a substantial burden of infection. Colonization with MRSA is a recognized risk factor for infection. This study was designed to identify the prevalence and in-hospital acquisition of MRSA colonization in the surgical, medical and orthopaedic units of Teaching Hospital Karapitya.

Objectives

To describe the prevalence and in-hospital acquisition of MRSA colonization at admission and discharge.

Methods

Consecutive admissions to orthopaedic and every fifth admission to medical and surgical wards were enrolled from September 2016 to March 2017. A nasal swab was collected from the anterior nares within 24 hours of admission and in the 48 hours prior to discharge. Standard microbiological procedures were performed. Clinical and demographic data were collected and analyzed using STATA version 13.

Results

A total of 502 patients were enrolled, including 152 medical, 201 surgical, and 150 orthopaedic patients. Median age was 45 years and 57% were male. Median hospitalization duration was 3 days. At admission, 31 (6.2%) were colonized with MRSA; colonization was higher in orthopaedic (12.0%) compared to medical (4.0%) and general surgical (3.5%) patients, p=0.002. Patients colonized with MRSA were younger (median 38 vs 46 years, p=0.03) and more likely male (74.2% vs 56.3%, p=0.05). At discharge, 24 (6.7%) patients were colonized with MRSA (12.8% orthopaedic, 8.4% medical, and 1.3% surgical patients, p=0.001). Of the 24 patients, 10 (41.7%) were not colonized with MRSA at admission. MRSA isolates showed low susceptibility to oral antibiotics (tetracycline 64.5%, ciprofloxain 45%, clindamycin 19.4%, trimethoprim/ sulfamethoxazole 0%).

Conclusions

MRSA colonization was highest in orthopaedic patients. Almost half of patients with MRSA at discharge were not colonized at admission, raising alarm for nosocomial acquisition. Molecular typing (SCCmec and MLST) is being done to identify transmission patterns. These results may inform infection control and decolonization efforts.

OP 9

Prominent T helper (Th)-1 response to *Leishmania donovani*-induced cutaneous leishmaniasis in Sri Lanka

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Introduction

Leishmaniasis is a newly established vector-borne disease in Sri Lanka with cutaneous leishmaniasis (CL) as the main clinical presentation. The aetiological agent is *Leishmania donovani*-MON 37, known to cause visceral leishmaniasis in other countries. Localized host immune response at the site of infection may play a role in disease outcome. Leishmaniasis is well known for Th1/ Th2 paradigm of disease cure versus progression in animal models, though this is not quite obvious in human infections.

Objective

To determine the localized host immune response at the site of infection in CL in Sri Lanka.

Methods

Skin punch biopsies obtained from 58 patients with parasitologically confirmed CL and 25 healthy controls were quantified for localized cytokine gene expression of Th1 type cytokines interferon (IFN)-γ, interleukin (IL)-12A and tumor necrosis factor (TNF)-α and Th2 cytokines, IL-4 and IL-10 by real-time RT- PCR. Relative copy numbers for each specimen was calculated using the 2-$^{\Delta\Delta Ct}$ method. Forty four patients were followed up to assess the duration of treatment. Non parametric Mann-Whitney U test and Spearman's correlation test were used for statistical analysis.

Results

Study group consisted of 37 (63.8%) males and 21 (36.2%) females with a mean age of 35.0 years (SD= 12.1, range= 18-66). Study and control groups were comparable with no significant difference in their age (p=0.19) and gender (p=0.72) distribution. Lesions had a mean duration of 6.75 ±9.1 months (range: 1-48) and a mean size of 176.59 ±185.76 mm^2 (range: 12.6 - 908.3 mm^2). Significant up regulation of the Th1 cytokine IFN-γ (p<0.001) and down regulation of the Th2 cytokine IL-4 (p<0.001) was seen in patients compared to healthy controls. All patients followed up achieved complete healing and the time taken to heal correlated significantly with in situ expression level of IL-4 (Spearman's r =0.321, p=0.034).

Conclusion

A prominent Th1 response appears to support resolving of lesions, whereas a Th2 biased milieu tends to favor poor responsiveness to antimony and delayed lesion healing in *L. donovani* infections in Sri Lanka.

Acknowledgements

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Declaration

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OP 10

Genomic analysis of Sri Lankan cutaneous *Leishmania donovani* isolates from poor responders to Sodium stibogluconate

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Introduction

Sri Lanka is endemic for leishmaniasis caused by *Leishmania donovani*. The same parasite zymodeme, *L. donovani* MON-37 causes both cutaneous (CL-SL) and visceral (VL-SL) leishmaniasis in Sri Lanka. There is no satisfactory vaccine yet to prevent leishmaniasis and chemotherapy is the standard option for treatment and disease control. Pentavalent antimonials, despite their toxicity, have long been the main form of treatment but increased instances of drug resistance have been reported in endemic regions, which is largely attributed to the genomic diversity of clinical leishmanial populations.

Objective

We investigated resistance mechanisms to Sodium stibogluconate (SSG) in local *Leishmania* isolates using genomic sequencing data.

Method

Isolates were identified for the study based on clinical and epidemiological data of patients. DNA extraction, sample preparation, DNA quantification and library preparation were done according to standard protocols. Genomes of six CL-SL clinical isolates,including two with poor response to SSG, were sequenced by next generation methods. Reads were mapped to *L. donovani* BPK282A1 reference genome and gene copy numbers were inferred by read depth coverage using bioinformatics tools.

Results

Several genes associated with SSG resistance in other settings, such as ATP-binding cassette domains in ABC transporter gene family and heat shock protein *(HSP70)* genes had higher copy numbers among CL-SL poor responders compared to CL-SL wild type. A higher copy number of folate/biopterin transporter and nucleoside transporter genes were also observed, which have been associated with methotrexate resistance and tubercidin/ formycin B resistance in *Leishmania* respectively. Aquaglyceroporin genes *(AQP1)* which have previously been associated with antimonial resistance did not show a significant increase in copy number in the local isolates.

Conclusions

Our results suggest that some common resistance mechanisms of *Leishmania* parasites to SSG at a genomic level are also common to the local parasite population. The possible role of folate/biopterin transporter and nucleoside transporter genes in SSG resistance needs to be further investigated.

Acknowledgements

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OP 11

Brugian filariasis in Sri Lanka: a preliminary report on survey in Gampaha District

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Introduction

Sporadic cases of Brugian filariasis have been reported during surveillance for evaluation of the lymphatic filariasis (LF) elimination program.

Objectives

To study the epidemiology of Brugian filariasis in Gampaha district, identify the most vulnerable age groups affected and characterize the periodicity pattern of the microfilariae (mf).

Methodology

A community-based cross-sectional survey was conducted in selected areas of the Gampaha District in December 2016. Selection of study areas (SA) were based on distribution of past positive cases. Areas with two or more positive cases within a 500 m radius were defined as a SA. An age stratified population was selected. Criteria for enrolment of study population were residence in a SA for more than one year and age above one year. Thick night blood smears (NBS) and a rapid dipstick test (Brugia Rapid, Reszon Diagnostics International, Malaysia) were used for detection of mf and IgG4 antibodies to *Brugiamalayi* respectively. To study the periodicity of mf, counts were done periodically over a 24-hour period using Nuclepore Membrane Filtration. Ethical clearance for the study was obtained from the Ethics Review Committees of the Faculty of Medicine University of Kelaniya and Medical Research Institute.

Results

A total of 467 persons from 153 households in Pubudugama (Wattala MOH area) were screened. Two persons (0.4%) were antibody-positive but negative for mf (age groups, 5-10 and 10-20 years). Two mf positives were detected by NBS (0.4%). One person (age group 20-50 years) had a *Wuchereria bancrofti* infection, while the other (age group 10-20 years) had a *Brugia* species infection. Both mf positive individuals were negative for *B. malayi* antibodies. The *Brugia* mf counts in blood collected at 7.00 am, 9.00 am, 1.00 pm, 5.00 pm, 9.00 pm, 11.00 pm, 1.00 am, 3.00 am and 5.00 am were 75, 24, 14, 39, 159, 143, 136, 109 and 128 per ml respectively.

Conclusions

Low grade transmission of bancroftian and Brugian filariasis continue to occur in Gampaha District. The *Brugia* species exhibited nocturnal sub-periodicity indicating a probable zoonotic origin. Definitive species identification of the Brugian parasite by molecular methods is envisaged.

Acknowledgements

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OP 12

Do we have antifungal resistance in invasive candida isolates?

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Introduction

Invasive *Candida* infections are an important cause for increased morbidity and mortality in hospitalized patients. This study evaluates the demographic and mycological characteristics of invasive *Candida* infections and the change in antifungal susceptibility pattern over time.

Method

The data provided in specimen request forms, along with results of speciation and susceptibility testing of *Candida* isolates submitted from Microbiology departments island wide from 2009-2016 were analyzed. The speciation was via germ tube test, Candida Differential agar and an in-house rice agar (and API Candida system, sugar fermentation and carbon assimilation test as needed) and susceptibility testing was done using CLSI disc diffusion method for fluconazole and amphotericin B.

Results

One thousand two hundred and eighty-nine *Candida* isolates were received from 42 hospitals and two medical faculties islandwide.The isolates were from blood (86%), visceral abscesses, fluid collections, peritoneal dialysate fluid, bronchoalveolar lavage fluid etc. Samples obtained from superficial sites such as oral thrush and vagina were excluded. Overall, nine *Candida* species were isolated.

Males predominated (58%), and 41% samples were from those in the extremes of age.Fifty one percent of samples were from non-ICU patients. The predominant species isolated was *Candida tropicalis* (61.1%).

Non albicans *Candida* species predominated throughout, with 81.4% (n=79) of isolates sent in 2009, and 80.5% (n=273) isolates in 2016, accounting for same.

When comparing 2009 with 2016, the percentage of isolates sensitive to fluconazole, reduced from 100% (n=18) to 47% (n=66) for *C. albicans*, and from 94.2% (n=52) to 82.7% (n=127) for *C. tropicalis*. It was also noted that the mean zone diameters for fluconazole for *C. tropicalis*, *C. albicans* and *C. parapsilosis* decreased over time. Amphotericin B resistance was not detected.

Conclusion

Sri Lanka has high rates of fluconazole resistance among *Candida* species. All *Candida* isolates should be subjected to susceptibility testing and continuous surveillance is needed to mitigate this situation.

OP 13

Epstein-Barr virus (EBV) infection and associated factors for Post-Transplant Lymphoproliferative Disorder (PTLD) in a group of renal transplant patients

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Background

EBV infection can lead to EBV disease/PTLD in post renal transplant patients. Early detection with pre-emptive measures can prevent disease progression. Guidelines recommend EBV screening in high risk recipients (recipient seronegative/donor seropositive) within first post-transplant year. In Sri Lanka magnitude of EBV infection/disease in post renal transplant population is unknown.

Objective

To describe EBV viraemia and some selected associated factors for PTLD in the study population.

Methods

Descriptive cross sectional study, recruiting all adult renal transplant recipients within the first post-transplant year, attending renal clinics in two transplant hospitals in Sri Lanka over four months. EDTA blood samples collected at the time of enrolment were tested for EBV viraemia using a commercially validated quantitative EBV real time PCR assay and data on selected associated factors were collected using an interviewer administered data extraction sheet. Statistical analysis was by SPSS.

Results

Total of 118 patients were enrolled. 73.7% were males. Median age was 44.5 years (IQR-17.25). Most were more than 6 months after transplantation (54.3%) and had received a live related kidney (90.8%). EBV viraemia was not detected in any of the participants. Analysis of selected associated factors for PTLD revealed that pre transplant EBV serology records were available only in 22.8% of recipients, out of which 75.6% were seronegative. Only 11.86% were more than 60 years of age and all were on triple therapy with a mean dose of 4.74 mg (SD-1.34) for tacrolimus, 1493.64 mg (SD-285.8)

for MMF and 13.83mg (SD-8.3) for prednisolone. None had received atg. Out of 81/118 who had available HLA reports 79.01% had at least one B locus mismatch and had negative HLA types like A1, A11, B35, B5 and DR7.

Conclusion

EBV viraemia was not detected in the selected population. All of the participants had at least one associated factor for PTLD which were observed during the study. However, prospective studies with frequent monitoring for EBV viraemia in high risk patients are indicated to get a broader picture of the situation.

OP 14

Prospective study to ascertain the relationship between rabies infection and anti-rabies immunization status of dogs and cats in Sri Lanka

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Introduction

Rabies, a fatal viral encephalo-myelitis, could be prevented by post exposure therapy (PET) of humans and immunization of animals. Animal immunization coverage should be maintained around 70% for herd immunity. Rabies PET depends on vaccination and health status of the bitten animal according to national guidelines.

Objectives

To determine:

- the anti rabies immunization status of dogs/cats submitted for rabies diagnosis
- the relationship between immunization status and rabies positivity
- the proportion of stray, community owned and domestic animals with rabies positivity

Methodology

Dog and cat specimens submitted to Medical Research Institute for rabies diagnosis from 01.07.2015 to 31.12.2016, with a reliable history were included. Direct smear and Fluorescent antibody test for confirmation of rabies were performed. Interviewer administered questionnaire was used to collect data with documented evidence of vaccination.

Results

Total of 642 samples including 426 (66%) dogs and 216 (34%) cats were analysed. Out of the total study population, 476 (74%) were unvaccinated which include 65% dogs and 91% cats.

Total of 344 (54%) were positive for rabies, of which 262 (76%) were unvaccinated. Among the positives, 281 (82%) were dogs and 63 (18%) were cats.

Out of 426 dogs, 281 (66%) were positive for rabies, of which 203 (72%) were unvaccinated and 78 (28%) were vaccinated. Of the vaccinated rabies positive dogs, 60 (77%) were once/twice vaccinated and 18 (23%) were more than twice vaccinated.

Out of 216 cats, 63 (29%) were positive for rabies of which 44 (70%) were unvaccinated.

Among the positives, 213 (62%) were domestic and 131 (38%) were either community owned or stray animals. Out of 213 rabies positive domestic animals, 79 (37%) were vaccinated.

Conclusions

Nearly three fourths of the study population was not vaccinated against rabies. Compared to dogs, a larger proportion of cats were unvaccinated.

Almost a quarter of rabies positivity was among the vaccinated group, where a considerable proportion was in animals vaccinated more than twice.

A higher proportion of rabies positivity was shown among domestic animals indicating irresponsible pet ownership. This study shows the importance of considering health status of the animals in addition to the vaccination status when recommending PET for patients.

OP 15

An outbreak of concurrent infections with dengue virus serotypes in Kinniya, Trincomalee Sri Lanka

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Introduction

Co-circulation of multiple dengue virus serotypes has been reported from many parts of the world including

Sri Lanka. However, concurrent infection with more than one serotype of dengue viruses in the same individual is rarely documented. An outbreak of dengue hemorrhagic fever/dengue shock syndrome occurs in most parts of the country in 2017, though significant mortality reported only from Kinniya, in Trincomalee District, during the month of March. We investigated this dengue out break with serological and molecular test methods.

Objective

To identify the acute dengue infection and describe the dengue virus serotypes in the Kinniya outbreak, in 2017.

Methodology

The study analyzed a total of 22 serum samples which were sent to Medical Research Institute, for routine testing for dengue virus infection, during third week of March, 2017. Samples were from patients within 7 days of fever, presented to Base Hospital Kinniya. The received samples were tested for dengue virus infection, using multiplex typing rRT-PCR assay (Waggoner et al 2012), NS1 rapid immunochro-matographic assay, and Dengue IgM ELISA (SD Korea). All the samples were tested with multiplex typing rRT-PCR assay, and Dengue IgM ELISA assay. Only 13 samples were tested with dengue NS1 immunochromatographic assay.

Results

All 22 (100%) samples gave evidence for positive dengue virus infection at least through one of the above mentioned assays. Of the tested samples, 19 (86%), 15 (68%) and 8 (38%) were positive with typing rRT-PCR, Dengue IgM ELISA and NS1 rapid immunochromatographic assay respectively. All rRT-PCR assay positive samples showed up DENV-2, followed by DENV-4 in 14 (73%) and DENV-1in 1 (5%) sample. Concurrent infection with multiple dengue serotypes were identified among 15 (79%) of the rRT-PCR assay positive samples. Fourteen (73%) samples were infected with both DENV-2 and DENV-4 and 1 sample (5%) co-infected with DENV-2 and DENV-1. Single serotype 2 infection was identified in 4 (21%) samples.

Conclusions

The results confirmed that the Kinniya outbreak is an acute dengue virus infection. It also revealed that DENV-2 dominated the outbreak whereas DENV-2 and DENV-4 lead to concurrent infection. DENV-1 is responsible only for a lesser extent for this outbreak.

OP 16

Human bocavirus infection in a selected group of children with severe acute respiratory symptoms: Preliminary survey

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Introduction

Human bocavirus (HBoV) of the family Parvoviridae causes acute respiratory illness that vary from mild upper respiratory illness to severe life threatening lower respiratory illness like pneumonia. Presence of HBoV in respiratory samples in Sri Lanka has not been reported.

Objective

To detect HBoV in respiratory samples collected from a selected group of children with severe respiratory illness.

Design, setting and methods

Nasopharyngeal swabs and aspirates from children with severe acute respiratory symptoms sent to the respiratory reference laboratory were investigated for Influenza A, B, RSV and HBoV. RNA was extracted using (QIAmp Viral RNA Mini-Kit) and subjected to Routine Real-time PCR for influenza A & B (CDC, USA) and a previously validated real-time PCR assay for RSV and HBoV (NIV, Pune). Demographic and available clinical data were analysed.

Results

Of the 82 children tested, 13 (15.85%) were positive for HBoV, five (7.3%) were positive for RSV, one influenza B (1.2%), influenza A (0). There were two (2.4%) co-infections of HBoV with RSV and Influenza B. Mean age of HBoV positives were 2.3 years (range 7 months to 6 years). Presenting symptoms of HBoV positives were fever (100%), cough/cold (61%), sore throat (23%), and shortness of breath (23%). Clinical data were available only in seven HBoV positive children, whom had lung signs of LRTI (one was positive for RSV also).

Conclusion/Recommendation

Human bocavirus was associated with a cohort of children with severe acute respiratory illness. Further studies needed to ascertain prevalence and epidemiology of HBoV.

Acknowledgements

We acknowledge real-time PCR reagents from NIV, Pune, India.

OP 17

Validation of multiplex PCR in the detection of pathogen causing acute bacterial meningitis

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Background

Lack of rapid, sensitive laboratory diagnosis remains a key issue in diagnosing infections in the central nervous system (CNS). Despite the advancement in technology, affordability prevents most developing countries using the newest of the techniques. However, basic PCR is currently becoming more affordable.

Objectives

To validate a multiplex PCR for the laboratory detection of common bacterial pathogens causing CNS infections.

Methods

CSF specimens were collected from patients with acute meningitis being admitted to Teaching Hospital, Peradeniya from December 2016 to March 2017. Clinical data were gathered using a data entry form. A multiplex PCR was developed using previously described primers to detect *Neisseria meningitidis*, S*treptococcus pneumoniae* and *Haemophilus influenzae.* The PCR was optimized using known controls, adjusting primer concentrations, $MgCl^2$ concentration and annealing temperature. External validation could not be conducted due to time and financial constraints.

Results

Eighty specimens were collected during the study period. The mean duration till sample collection was 4.78 (SD2.6) days from the onset of symptoms.

None of the samples yielded a bacterial growth by routine culture. CSF antigen detection was performed on 50 specimens and all were negative. Of the total samples, eight tested yielded positive PCR results. In 3 of the positives, the full report was normal, 2 were suggestive of viral aetiology and 3 were suggestive of bacterial aetiology. Out of the positive specimens, 3 were positive for *S.pneumoniae* and 5 for *H. influenzae.* None of the specimen tested were positive for *N. meningitidis.*

Conclusions

PCR could be utilized in the identification of bacterial aetiology of acute meningitis even if CSF culture and antigen detection is negative suggesting the importance of equipping hospital laboratories in molecular diagnostic methods along with suitable quality assurance programmes.

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OP 18

Comparison of real time PCR with the culture method for detecting *Salmonella* spp in raw chicken

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Introduction

Non-typhoidal *Salmonella* has lead to significant morbidity and mortality. Chicken provides the main route of transmission. Culture is labour intensive and time consuming when comparing with real time PCR(rt- PCR).

Objectives

1. To compare the sensitivity, specificity, and rapidity of rt- PCR with the culture method in artificially contaminated samples
2. To find out the detection rate of *Salmonella* in raw chicken samples
3. To determine the serotypes isolated from the chicken samples

Method

For the comparison study artificially contaminated, known positive and negative samples that included 35 samples of *Salmonella*, 12 samples of *Shigella*, 12 samples of *Escherichia coli* and 11 samples of Buffered peptone water were processed by culture as well as by rt- PCR. To detect isolation rate, 130 random samples of chicken were collected from 10 wet markets (n=88) and six supermarkets (n=42) in selected MOH areas to process by culture and rt- PCR.

Results

The sensitivity and specificity of rt- PCR in the comparison study was 100%. The random sample positivity rate was 26% (34/130) by culture and 35% (46/130) by rt- PCR which was not statistically significant (P=0.106). Out of all the samples collected from wet markets *Salmonella* was isolated in 39% (34/88) by culture and 49% (43/88) by rt- PCR. Out of the samples collected from supermarkets 0% (0/42) was isolated by culture and 7% (3/42) by rt- PCR. There is a statistically significant difference between *Salmonella* positivity in supermarkets and wet markets when tested by PCR (P<0.01) and by culture (p<0.01). The commonest serotype was *Salmonella* Agona (16/34), then *Salmonella* Corvalis (10/34), and lastly *Salmonella* Kentucky (4/34) and *Salmonella* Newport (4/34). The cost analysis showed an equal amount for both methods if infrastructure is available.

Conclusion

The rapidity and reduction in labor makes this highly sensitive and specific rt- PCR an excellent alternative to culture in maintaining food safety. Rapid negative results are also helpful to reduce storage time of perishable food for industry and business operators.

OP 19

Comparison of direct fluorescent test with real-time PCR for diagnosis of respiratory virus infections in children

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Introduction

Accurate detection of respiratory viruses is important to guide antiviral therapy and to provide data for surveillance. Direct fluorescent test (DFT) is used in many medical laboratories for routing diagnosis. However real-time PCR (rt-PCR) is gaining popularity and acceptance.

Objective

To compare results and cost of DFT and rt-PCR in detection of influenza A (FluA), B (FluB), parainfluenza (PIV) 1,2,3,4 and respiratory syncytial virus (RSV) A, B in a selected group of children admitted with severe acute respiratory symptoms.

Methods

Nasopharyngeal aspirates/swabs were obtained from children admitted to Lady Ridgeway Hospital from May to July, 2014. Cell pellets processed according to a standard protocol were subjected to DFT (DAKO/ Imagen). RNA was extracted using QIAmpRNA kit. Elutes were subjected to three different real-time commercial multiplex PCR assay (IVD and CE approved) for the detection of influenza A/B, parainfluenza 1/2/3/4 and RSV A/B.

Results

Of 75 specimens from children (age =3.8±7.04 years), at least one virus was detected in 6 (8%) specimens by DFT and in 57 (76%) specimens by PCR (P<0.05). Specimen quality was poor for DFT in 14 (18.6%) specimens; 4 (28%, 4/14) of these were positive by PCR. One FluB positive specimen by DFT was negative for FluB but positive for RSVB when analyzed by PCR. The average cost for one sample by DFT was LKR 4020/=, whereas LKR 9312/= by rtPCR.

Virus	DFT positive (%)	Real-time PCR positive (%)
Influenza A	0 (0%)	6 (8.0%)
Influenza B	1 (1.3%)	0 (0%)
Parainfluenza 1/3	1 (1.3%)	11 (14.6%)
Parainfluenza 2/4		1 (1.3%)
Respiratory syncytial virus A	4 (5.35%)	32 (42.6%)
Respiratory syncytial virus B		7 (9.3%)

Conclusion

The rtPCR described here gives more significant results than DFT for the detection of respiratory viruses in clinical specimens. PCR can be employed with good results when cells are less in a sample for DFT.

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OP 20

Detection and genotyping of Human Papillomavirus in patients with oral and oropharyngeal carcinomas

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Introduction

Human Papillomavirus (HPV) causes oral carcinomas (OC) and oropharyngeal carcinomas (OPC). These cancers have different clinical presentation, good response to de-escalated treatment with better prognosis. Although there has been a steady rise in the incidence of high-risk HPV (HR-HPV) infection associated OC and OPC globally, data and knowledge in this regard is very limited in Sri Lanka.

Objectives

To determine the prevalence, genotypes of HPV infection and risk factors of OC and OPC patients.

Methodology

This was a comparative cross sectional study from 15th of December 2015 to 15th of April 2016. Swabs were taken from 127 of OC and OPC patients from Cancer Institute, Maharagama and similar number of age and

sex matched non cancer controls. Commercial extraction kits and commercially validated genotyping real time polymerase chain reaction kits were used to extract, detect and genotype the HPV. Interviewer based questionnaire were used to gather information on socio-demographic and behavioral factors. Data was analyzed using SPSS. Univariate and multivariate analyses were performed to analyze the risk factors of OC/OPC patients with comparison to control group and alpha set at 0.05.

Results

Total of 127 cancer patients were comprised of 63 (50%) OC and 64 (50%) OPC patients. Of the total, 19 (14.3%) cancer patients were infected with HR-HPV genotypes of 16, 39, 52, 58, 59. Seven (11%) of OC and 12 (19%) of OPC were infected with HR-HPV. Predominant genotype was 16, and 18 was not detected. Four (3.1%) of the control group were infected with HR – HPV types of 52, 59, but not with 16 and 18. Alcohol consumption (OR= 1293.8 95% CI 1078.34-1789.61), smoking (OR= 1.56 95% CI 1.13-16.65), betel chewing (OR= 1.21 95% CI 1.10-19.67) and HPV infection (OR= 1.27 95% CI 1.08- 27.87) showed significant association with OC/ OPC patients.

Conclusion

HR-HPV infection with 16, 39, 52, 58, 59, betel chewing, smoking and alcohol consumption can be considered as risk factors for oral and oropharyngeal carcinomas. Findings of this study convince the importance of screening OC and OPC patients for HR-HPV before starting treatment.

PP 1

Validation of Gram stain guided testing of tube coagulase, bile aesculin and germ tube tests from the positive automated blood culture broths

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Introduction

Laboratory diagnosis of bacteremia and candidaemia depends on blood cultures, and the timing of results can significantly influence the therapy and final outcome of the patient. Gram stain guided direct tests can be used to identify the pathogen sooner when compared to the conventional methods.

Objectives

To validate the Gram stain (GS) guided direct testing of tube coagulase, bile aesculin, and germ tube tests, from the positive automated blood culture broths, for identification of *Staphylococcus aureus*, *Enterococcus*, group D streptococcus spp and *Candida albicans* respectively.

Method

A prospective descriptive study was carried out at a tertiary care center for 5 weeks from January to February 2017, using 75 positive blood cultures with Gram positive cocci (GPC) in clusters, 25 positive blood cultures with GPC in chains and 19 positive blood cultures with yeasts to perform GS guided direct tube coagulase test, GS guided direct bile aesculin test and GS guided direct germ tube test respectively. Tests results were compared with the outcome of the conventional standard operating procedures on above samples.

Results

Of the 75 positive blood cultures with GPC in clusters in direct GS, the GS guided direct coagulase test had 100% for sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV). Of the 25 positive blood cultures with GPC in chains in direct GS, the GS guided direct bile aesculin test had sensitivity of 100%, specificity of 92.8%, PPV 91.6% and NPV 100%. GS guided direct germ tube test had sensitivity of 50%, specificity of 100%, PPV 100% and NPV 81.25%.

Conclusions

The study validates GS guided direct tube coagulase test and GS guided direct bile aesculin test and shows that they can be used routinely to give faster results in probable identification of *Staphylococcus aureus* and Group D streptococci along with the culture based conventional methods. GS guided direct germ tube testing was not found as equal with the standard tests and needs modification to increase the sensitivity.

PP 2

Chromoblastomycosis: a five year retrospective analysis of clinical and mycological data from the National Mycology Reference Laboratory, Sri Lanka

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Introduction

Chromoblastomycosis is a chronic cutaneous and subcutaneous infection, occurring after traumatic inoculation of dematiaceous fungi. It is prevalent in tropical and subtropical regions, including Sri Lanka.

Method

We retrospectively analyzed available data (from patients with mycologically proven chromoblastomycosis) at the Department of Mycology, Medical Research Institute from 2012-2016. The laboratory results, demographic data (age, sex and occupation), clinical presentation (duration to diagnosis, presence of trauma, type of lesion, anatomical location) and mycological diagnosis were analyzed.

Results

Out of 172 skin biopsy samples, 74 were proven as chromoblastomycosis (43%), through observation of sclerotic cells by direct smear or isolation of causative organisms by culture. Among these, 71.6% were males. Majority of males (n=15, 30%) and females (n=9, 42.8%) belonged to 51-60-year age group at presentation. Nearly 40% (n=16) of patients had lesions for less than one year. The commonest site/s of the lesion/s was in the lower limbs in both males (n=20, 66.7%) and females (n=16, 72.7%). Out of the patients' whose occupations were mentioned, 45.4% (n=10) belonged to the agricultural community, and had an average age of presentation of 55.1 years (standard deviation 8.4).

Most of the lesions were of the nodular type (n=18, 43.9%). Sclerotic bodies were seen in 58.1% samples (n=43), while culture was diagnostic in 87.8% (n=64). Nearly 45% were positive in both direct smear and microscopy (n=33). The commonest aetiological agent was *Fonsacaea pedrosoi* (n=30, 46.2%), followed by *Cladophialophora* species (n=20, 30.8%).

Conclusion

Chromoblastomycosis is prevalent in Sri Lanka, with mainly males in the agricultural community at risk. Mycological diagnosis is essential to reduce unnecessary use of antifungal drugs for other alternative diagnosis.

PP 3

Filarial parasites in selected cats and dogs in Madampe, Sri Lanka and their public health implications

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Introduction

Brugian filariasis has been reported from several districts of Sri Lanka in the last decade. Cats and dogs are known to harbour a zoonotic strain of *Brugiamalayi* in the Southeast Asian region, but the role of reservoir hosts in the recent cases of Brugian filariasis in Sri Lanka has not been established to date.

Objectives

To study the filarial parasites infecting cats and dogs in an area with transmission of Brugianfilariasis and to identify the possible reservoir/s for on-going infection.

Methodology

Ethical clearance was obtained from the Ethics Review Committees of the Faculty of Medicine University of Kelaniya and Medical Research Institute. Surveillance was done in a study area (SA) spanning a radius of 350m (mean flight range of vector mosquito, *Mansonia* species) from the residence of an index case of microfilaria (mf) positive Brugianfilariasis, in Madampe (Puttalam district). All domestic cats and dogs and stray dogs residing within the SA were taken as study population and screened for mf in January 2017 between 8.00 am and 8.00 pm. Screening was done by thick blood smears (TBS) prepared following an ear-lobe prick. Two TBS were prepared and stained with Giemsa. The TBSs were examined microscopically for presence of mf independently by two experts in the field.

Results

Of 77 dogs (74 domestic and 3 strays) and 52 domestic cats screened, 63 dogs (82%) and 39 cats (75%) were mf positive; 39 dogs (50.6%) and 17 cats (32.7%) were co-infected with *Dirofilaria repens* and *Brugiaspecies*. Mono-infections with *Brugiaspecies* and *D. repens* were seen in 14 (18%) and 10 (13%) other dogs and 12 (23%) and 10 (19%) other cats, respectively.

Conclusions

There is a very high prevalence of *Brugia* species and *D. repens* among cats and dogs in Madampe. Species identification of canine and feline *Brugia* parasites and their association with human infections requires further investigation by molecular methods.

Acknowledgements

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PP 4

Epidemiology and causative organisms of deep-seated abscesses

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Introduction

Deep seated abscesses of lungs, kidneys, liver, brain and other organs are often causing increased mortality and morbidity with a significant contribution by the associated comorbidities.

Objectives

To describe the epidemiology, associated comorbidities and the aerobic organisms causing deep seated abscesses.

Method

A retrospective analysis of the records of the patients with deep seated abscesses who were referred to the microbiology unit of a teaching hospital was carried out from January 2014 to February 2017. Cases of skin and soft tissue abscesses were excluded.

Results

There were 100 patients with deep abscesses during the study period. 69% (69/100) were males. 40% were aged between 40 and 60 years while 35% were above 60 years.

There were 34, 28, 17 and 10 patients with abscesses in lungs, kidneys, brain and liver respectively.

80% of the patients had identifiable comorbidities such as diabetes (37%), chronic kidney disease (10%), shunts

and stents (9%), and recent surgery (8%). Twenty percent (20%) had multiple associated factors. Type specific risk factors such as renal calculi (3%), chronic pulmonary diseases (3%) and shunts and extra-ventricular drains (3%) were also recorded.

Treatment of 55 cases was guided by positive aerobic cultures such as pus (21), and blood (18). There were respiratory samples with significant isolates from 9 lung abscesses and positive urine cultures from 7 renal abscesses. Most frequent isolates from all samples were *Klebsiella* species(18/55, 33%) followed by *Escherichia* coli (14/55, 25%) and *Staphylococcus aureus* (9/55, 16%). Fifty sic percent (56%) of coliforms were extended spectrum beta lactamase producers while 45% of *S. aureus* were methicillin resistant. There were pathogens like *Burkholderia pseudomallei* (7 of lung and liver abscesses) and *Chromobacterium violaceum* (1 liver abscess).

Most frequently used antibiotics in treatment were carbapenems (55%), broad spectrum penicillins (23%) 3rd generation cephalosporines (22%) and glycopeptides (16%).

Conclusions

There is a male predominance in deep seated abscesses and the middle aged and elderly are the mostly affected. Diabetes is the main associated comorbidity. Treatment should be guided by the cultures of relevant samples due to the antibiotic resistance and emergence of rare pathogens.

PP 5

Neonatal sepsis following maternal carriage of *Streptococcus pneumoniae*

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Introduction

Streptococcus pneumonia can be present in the respiratory tracts of about 50% of normal population. It is not a common organism to colonize the female vagina but when present, it can lead to early onset neonatal sepsis with severe morbidity and mortality.

Case report

One day old baby boy transferred from a peripheral hospital to the Special Care Baby Unit (SCBU) of a tertiary care hospital due to respiratory distress following preterm normal vaginal delivery at 32 weeks. This delivery was not associated with prolonged rupture of membranes or history of maternal fever. After birth, baby became tachypnoeic, had poor sucking and remained inactive. After admitting to the SCBU, IV penicillin and IV gentamicin were started after septic screening. Patient was ventilated and on the second day antibiotics were changed to IV vancomycin and IV meropenem.

Baby's total white cell count was $7.8x10^9$/L and C- reactive protein was 96mg/L. Chest X-ray revealed right upper zone consolidation. Blood culture became positive for a highly mucoid *Streptococcus pneumonia* after seven hours of incubation. Patients' deep ear swab culture and the later requested maternal high vaginal swab culture yielded similar mucoid pneumococci. All 3 isolates had the same antibiotic sensitivity pattern with sensitivity to penicillin, cefotaxime, erythromycin, clindamycin and vancomycin.

Patient had expired by the time blood culture became positive.

Discussion

Neonatal sepsis due to *Streptococcus pneumoniae* acquired from maternal birth canal is rare and only very few proven cases are reported worldwide.

This case was confirmed because not only the blood culture but also the deep ear swab of the neonate became positive for the same organism with the same antibiotic sensitivity pattern which was similar to that isolated from the maternal high vaginal swab. Prematurity being a risk factor could have worsened the condition. The organism could have been highly virulent due to its mucoid nature showing capsular formation. Though it is very rare to have maternal carriage of *Streptococcus pneumoniae*, if found in the ante-natal screening, it should be considered in possible neonatal sepsis for adequate empirical treatment.

PP 6

In-vitro evaluation of antimicrobial effects of Sri Lankan bee honey against microorganisms causing chronic wounds – A preliminary study

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Introduction

Bee honey is an ancient remedy for natural healing of chronic wounds, especially in indigenous medical treatment. But these practices have not been properly evaluated and documented in Sri Lankan literature. In this preliminary study we are evaluating the effectiveness of different preparations of bee honey from different parts of the country as an alternative antimicrobial agent for organisms causing chronic wounds.

Objective

To evaluate the in-vitro efficacy of Sri Lankan bee honey as an antimicrobial agent against pathogens causing chronic wounds.

Methods

Standard strains of 4 bacterial and 4 fungal species and 14 bacterial isolates from chronic wounds (including MRSA, ESBL, MDR *Acinetobacter*, and MDR *Pseudomonas*) were tested against twelve honey types belonging to seven Agro Ecological Regions (AERs) of Sri Lanka. Antibacterial activity was determined by agar well diffusion, phenol equivalent methods and MIC by agar dilution given as % (w/vol).

Results

6/12, 5/12, 11/12 and 11/12 honeys gave inhibitory zone diameters ranging 12.5-19.5 mm for *Staphylococcus aureus* (ATCC-25923), *Escherichia coli* (ATCC-25922), *Pseudomonas aeruginosa* (ATCC-27853) and *Klebsiella pneumoniae* (ATCC-700603) respectively.

Depending on results of standard strains, six honey samples were chosen for further testing. Four out of these originated from the lower country indicating higher efficacy compared to other regions.

Out of six selected honeys, four types gave therapeutic level activity for all tested clinical isolates except for *Pseudomonas aeruginosa*.

MIC of 10-20% (w/v) was reported for all ATCC strains. Honey originated from low country regions showed superior activity against multidrug resistant bacteria. Commercially available honey reported lowest antibacterial activity.

No inhibitory zones were observed for fungal species (*C. albicans* ATCC10231, *C. glabrata* ATCC90030, *C. parapsilosis* ATCC13803, *C. tropicalis* ATCC13803) and MIC was >40% (w/v) for all types of honey.

Conclusions

Sri Lankan bee honey exhibits significant antibacterial activity against both Gram positive and negative bacteria including multidrug resistant organisms in-vitro. This finding needs to be confirmed by further large scale studies.

Antibacterial potency varies in different types of honey from different AERs. Low country honey was superior to others while antibacterial activity of commercially available honey was negligible.

None of the honeys had antifungal activity against *Candida* species.

PP 7

Extended-spectrum beta-lactamase (ESBL)-producing *Escherichia coli* and *Klebsiella* species causing urinary tract infection (UTI) in children

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Introduction

ESBL-producing *Escherichia coli* and *Klebsiella* species causing UTI among children is an increasing problem worldwide.

Objective

To determine ESBL production among uropathogenic *Escherichia coli* and *Klebsiella* spp. in children and evaluate current antibiotic resistance patterns.

Method

This retrospective study was carried out in a district general hospital in Sri Lanka from January to December 2016. Total of 1904 urinary specimens from children (<13years) with suspected UTI were processed for isolation of bacterial pathogens and to determine their antimicrobial susceptibility. Standard isolation and identification procedures were carried out using the Laboratory Manual in Microbiology. UTI chromogenic agar was used for identification. Antibiotic susceptibility testing was done by Stokes disk diffusion method. Isolates were screened for ESBL production using double-disk diffusion method and confirmed by combination disk diffusion test.

Results

Of 124 positive isolates, 107 (84%) were coliforms. *Escherichia coli* n=71 (66%) was the predominant isolate followed by *Klebsiella* spp. n=21(20%) and *Proteus* spp. n=15 (14%). ESBL production rate in *Klebsiella* spp. 7/21 (33%) was higher than in *E. coli* 20/71 (28%). ESBL-producing *E.coli* were more common among females whereas ESBL producing *Klebsiella* spp. were higher in males.

Age group 1-5 years had the highest number of coliform positive UTIs (n47). Children with ESBL-positive *Klebsiella* spp. had clinical histories of recurrent UTIs and were frequently on cephalexin prophylaxis.

ESBL-positive *E.coli* and *Klebsiella* spp. showed similar susceptibility to amikacin, meropenem and nitrofurantoin (100%). ESBL-producing *E.coli* showed greater resistance compared to *Klebsiella* spp. for gentamicin and ciprofloxacin/norfloxacin (77% and 25%, and 77% and 50% respectively). Non-ESBL-producing *E. coli* showed 90% sensitivity to co-amoxiclav and cefuroxime

and 100% sensitivity to gentamicin. Non-ESBL-producing *Klebsiella* spp. showed 50% sensitivity to co-amoxiclav and cefuroxime and 85% sensitivity to gentamicin. Both groups showed 100% sensitivity to cefotaxime.

Conclusions

An alarming rate of ESBL-producing *E. coli* and *Klebsiella* spp. among paediatric uropathogens was observed. Greater resistance was seen in non-ESBL-producing *Klebsiella* spp. compared to non-ESBL-producing *E.coli*. It is very important to perform routine urine culture and sensitivity testing prior to starting antibiotics in paediatric UTI for effective treatment.

PP 8

Identification of aerobic and anaerobic bacteria in chronic diabetic wounds

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Introduction

Diabetic chronic wounds consist of a diverse microbial community. Majority of chronic wound microbes may be unculturable. Since diabetic wounds are a growing problem in the country, culture identification of both aerobes and anaerobes in diabetic chronic wounds is very important.

Objective

The study was aimed in identification of aerobic and anaerobic bacteria in chronic diabetic wounds.

Design, setting and methods

A prospective study was carried out at Department of Microbiology, Faculty of Medical Sciences, University of Sri Jayewardenepura. Specimens were collected from 34 patients having diabetic chronic wounds, undergoing routine debridement, at Colombo South Teaching Hospital. Two pieces of wound debrides specimens were collected from each patient at the time of surgical debridement. One specimen was collected in to a sterile Eppendorf tube and the other in to sterile cooked meat medium for aerobic and anaerobic culture respectively. Gram stain of the collected specimens were carried out. Specimens were microbiologically processed for aerobic and anaerobic cultures following the standard operating procedure. The isolates were presumptively identified based on the morphological characteristics, Grams' stain and biochemical tests. Aero-tolerance test and Rapid ANA II panel was used for the identification of strict anaerobes.

Results

All the 34 wound debrides specimens carried >25 pus cells/LPF. Aerobic and facultative anaerobic bacteria were isolated from all 34 specimens. Of them strictly anaerobic species had grown in nine specimens (26%). Among aerobically grown bacterial isolates, a total of 29 specimens (85%) yielded coliforms. *Pseudomonas* spp. were isolated from 20 specimens (59%). *Staphylococcus* spp. and *Streptococcus* spp. were isolated from 14 (41%) and 16 specimens (47%) respectively whereas 07 specimens (21%) carried *Corynebacterium* spp. Thirty three specimens (97%) carried more than two type of species with different combinations of microbes whilst only one specimen (3%) carried only one type of single species.

Among strict anaerobes, *Peptostreptococcus species* (7/9) was most predominant followed by *Prevotella bivia* (1/9) and *Bacteroides fragilis* (1/9).

Conclusions

Coliform and *Pseudomonas* spp. were the frequently isolated aerobically grown bacterial species in chronic diabetic wounds. *Peptostreptococcus* species was the most predominantly identified strict anaerobe.

PP 9

A study on waste management in a tertiary care hospital in Sri Lanka

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Introduction

Waste management is extremely important and an integral function of a health care institution. The infectious and hazardous nature of hospital waste especially the bio-medical waste poses a threat not only to health care worker but also to public in general as well as to the environment. Further new regulations were introduced by the Colombo Municipal Council regarding waste segregation and disposal.

Objective

Objective is to evaluate the present waste management practices at Lady Ridgeway Hospital for Children, Colombo.

Methods

To evaluate the various levels of waste management including segregation according to colour code of the

bin and the bag, waste bin condition, sharp management, waste-transportation, awareness of staff on waste management and storage and disposal. A check list consisting of 62 parameters was prepared and observations were made by visiting 39 selected units in the hospital during 3-month period from 1st of January to 31st of March 2017.

Results

Simple statistical analysis done to evaluate results. The mean percentage of overall waste managed according to the recommended practices of the hospital was 82%. The percentages of individual five components, the waste segregation, waste bin condition, sharp management, waste transportation and awareness of staff were 85%, 94%, 81%, 92% 57% respectively.

Overall waste management percentages according to the recommended practices in the areas under consideration were, medical wards (83%), surgical wards (89%), ICUs (80%), laboratories and blood bank as one group (60%) and OPD, accident service and clinics analyzed as one group (89%).

Final waste storage area had appropriate storage spaces for infectious waste, sharps and paper waste, plastic and polythene. However, the space for general waste and for food waste had access to animals.

Conclusion

Majority of the waste was managed according to the recommended practices. More emphasis should be given to further improve the awareness of health care workers as well as the bystanders of the patients regarding the correct procedures. Frequent training sessions should be conducted to improve the knowledge.

PP 10

Unusual case of multiple hepatosplenic abscesses

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Introduction

Combined liver and splenic abscesses are very rare in people without underlying diseases or immune-deficiencies, and can be fatal. Clinical presentation may not be straight forward.

Case report

A 19 year old male was admitted to Emergency Unit, with 2 days fever, left-sided loin pain and vomiting. After examination, patient was sent to medical ward with presumptive diagnosis of urinary tract infection. Physical examination revealed signs of acute abdomen and the patient was referred to the surgeon. On further history, a fall from a tree with blunt trauma to abdomen was reported. Splenic rupture was suspected and confirmed by ultra sound scan abdomen, and a laparotomy was performed.

Spleen was lacerated in several places with peri-splenic haematoma formation. Splenectomy was performed. Multiple small abscesses like lesions wrer observed on the liver, biopsies were taken and sent for microbiology and histo-pathalogy. Post surgically, meropenem and clindamycin were started.

Primary culture of liver tissue had no growth in 24hrs. But the enriched specimen showed a pure growth of *Staphylococcus aureus* sensitive to cloxacillin.

Treatment was changed to intravenous cloxacillin and ciprofloxacin. Patient was kept under strict observation. Histopathology report revealed colonies of Gram positive cocci in the biopsies taken from the liver and spleen.

Treatment continued and condition was monitored. Size of the lesions on liver initially increased up-to 1cm and then gradually reduced. Inflammatory markers (CRP, FBC) initially increased and then gradually normalized. Patient was fever free by 5th day.

On the 25th day of cloxacillin, a skin rash appeared which was treated by a consultant dermatologist without omitting cloxacillin.

Computed tomography (CT) chest, abdomen and head were done to exclude abscesses in other organs and 2 D-Echocardiogram done to exclude endocarditis.

Patient was treated with IV cloxacillin for 6weeks and discharged on oral cloxacillin for 3weeks after giving vaccines, indicated for asplenism.

Discussion

Atypical presentation of hepatosplenic abscesses may lead to misdiagnosis. Vigilance and open-mindedness for unusual manifestation of hepatosplenic abscesses on the differential diagnosis, even in patients without any risk factors, may improve the prognosis.

(This was presented as a poster at annual sessions of SSM held on 21st October 2016).

PP 11

Acinetobacter outbreak in an Intensive Care Unit: Refilling disinfectant bottles may increase the spread of microorganisms

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Background

Acinetobacter is a water-tropic bacterium, which has high potential to develop antibiotic resistance particularly in ICU patients. Increased number of *Acinetobacter* infections were reported from ICU, Base Hospital, Wathupitiwala during the last two weeks of February 2017.

Objective

Identify the point source of the *Acinetobacter* outbreak in the ICU.

Methodology

Samples were obtained from various sites including swabs from bedrails, bench-tops, tap and door handles, sink, ventilator equipment, wiping towels and fluids including humidifier water in wall oxygen, disinfectants used for endoscopes (peracetic acid), floor surfaces (trichloroacetic acid); bedrails, table-tops, door and tap handles, telephones, ECG monitors and alcohol hand rub (surgical spirit). Swabs and liquid samples were plated on blood and MacConkey agar and organisms were identified using standard laboratory procedures. Efficacy of disinfectants was tested against methicillin resistant *Staphylococcus aureus*, extended spectrum beta lactamase producing *Escherichia coli*, and multi-resistant *Acinetobacter* spp. Thorough cleaning with approved disinfection procedures of ICU were done. Infection control measures were reinforced in the unit. Sampling was repeated from sites positive *for Acinetobacter.*

Results

Heavy growth of *Acinetobacter* which had the same antibiogram to the outbreak isolate was identified from the sink. A scanty growth of the same was identified from bedrail, tap handle and interior handle of entrance door to ICU. Heavy growth of *Acinetobacter* and *Klebsiella* spp. were isolated from surgical spirit used to clean surfaces of ICU. Surface disinfectant was in a plastic bottle which was refilled regularly for long periods. This disinfectant bottle was identified as the point source of infection. Disinfectant in the original stock bottle remained uninfected. Other solutions were found to be sterile. Repeat sampling revealed all swabs and fresh surgical spirit from stock bottle were free of bacteria.

Conclusion

Acinetobacter has extraordinary potential to reside in liquids as evidenced in this outbreak. The point source was disinfectant solution with heavy bacterial load due to repeated refilling which allowed organisms to multiply, resulting in reduced disinfectant activity. This report highlights that refilling of small disinfectant bottles has a risk of spreading infections leading to outbreaks.

PP 12

Study on public knowledge, attitudes and practices of antibiotic usage in a cohort of adults in Sri Lanka

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Introduction

Antibiotic resistance has emerged rapidly during the past few years, threatening health systems globally and locally, resulting in limited range of antibiotics for effective use. The rapid emergence of resistant organisms has been disproportionate to the discovery or development of novel antibiotics. To help with minimizing antibiotic resistance (ABR), patients play a major role as end user.

Objective

To assess the knowledge, attitudes and practices of the public in antibiotic use.

Methods

A cross sectional study was conducted involving randomly selected, 285 individuals who attended the Medical Exhibition, Faculty of Medicine, Ragama, Sri Lanka, 2016. Data including demographic characteristics, knowledge and attitudes on antibiotic usage were collected before participants visited the exhibition, using a self-administered questionnaire.

Results

Age distribution of the study group was 8.1% (23/285) 15-18 years, 36.1% (103/285) 19-25 years, 19.3%(55/285) 26-40 years, 20% (57/285) 41-60 years, 16.5%(47/285) >60 years with 68.4% (195/285) females and 31.6% (90/285) males. The education level was 28.4% (81/285) graduates and 40.4% (115/285) Advanced Level and 27% (77/285) Ordinary Level respectively. There were 37.5% (107/285) employees.

Though 90.2% (257/285) were aware of antibiotics, only 56.8% (162/285) knew antibiotics are for bacterial infections. There were 43.9% (125/285) who were aware of ABR, only 38.2% (109/285) knew that ABR is due to inappropriate use of antibiotics. At least one common side effect of antibiotics was known by 73.0% (208/285) viz. allergy (43.15%), rash (31.9%), vomiting (16.1%), nausea (15.4%) and (14.03%) diarrhoea.

With questions on how antibiotics should be taken; 83.5% (238/285) indicated consulting a doctor, while 9.5% (27/285) directly from pharmacists. How to obtain antibiotic information 61.4% (175/285) indicated from a

doctor, 27.4% (78/285) from information on drug container and 15.4% (44/285) from pharmacists. Only 43.5% (124/285) believed antibiotics should be taken for the prescribed period. Reason for early cessation of antibiotics; 43.2% (123/285) believed that they had recovered from the illness.

Conclusion

Although 90.2% of the public was aware of antibiotics, there were important deficiencies in knowledge on appropriate antibiotic usage. These indicate that public health awareness and education need to be improved in order to minimize the emergence of antibiotic resistance in the community.

PP 13

Case report: Mite infestation of external ear with cholesteatoma

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Introduction

Mites are a diverse group of ubiquitous acarines occupying many ecological niches on plants and animals. A small number is of medical importance being ectoparasitic, vectors of diseases or allergens to humans. We report a case of mite infestation in the external ear of a patient who presented with ear discharge for one year.

Case report

An 18 year old female presented to the ENT ward of Base Hospital, Wathupitiwala, with a history of ear discharge for one year. She had cholesteatoma and surgery but the ear discharge continued with a tympanic membrane perforation communicating mastoid cavity.

Her neighbor kept birds for commercial purposes with grains stored as bird food. During the past year,cultures of the ear discharge yielded several species of bacterial pathogens but no resolution was achieved despite appropriate antibiotic regimens. The patient was referred to the Department of Medical Microbiology, University of Kelaniya. Culture of ear discharge yielded *Pseudomonas* spp. Direct smear of the ear discharge showed a number of adult mites with nymphs. Morphologically, they were food storage mites belonging to the Genus *Tyrophagus*. Treatment with topical crotomiton cream for three weeks was unsuccessful. She was managed with topical benzyl benzoate (BB) cream and antibacterials, applied twice daily for two months along with regular ear washings. Mites were then successfully eradicated.

Discussion

Tyrophagus mites infest various stored organic materials viz. grain, dried fruits and dried egg. Occasionally, mites colonize body cavities such as external ear, feeding on accumulated dead cellular debris. A cholesteatoma presents the perfect environment for infection due to limited or poor drainage. This allows the potential for mite infestation and secondary bacterial infections. Although the BB cream application was effective, this required long duration of application together with regular ear washings to eradicate the mite infestation. It is noted that the cream may not penetrate all parts of the infected area. Topical crotomiton cream was not effective. Oral ivermectin helps eradication of mite colonisation/ infestation but this medication is not freely available for human use in Sri Lanka.

PP 14

Knowledge on antibiotic use, misuse and resistance among participants who visited Medical Exhibition 2016, Faculty of Medicine, Ragama

Perera PI, Pathiraja PPMCK, Sagarika SL, Sandakumari SGCNM, Badanasinghe CN

Faculty of Medicine, University of Kelaniya

Introduction and background

The knowledge on public regarding antibiotic use is valuable to minimize antibiotic abuse and to minimize the development of antibiotic resistance. We evaluated the knowledge on antibiotic use, misuse and antibiotic resistance among participants who visited Medical Exhibition 2016, Faculty of Medicine, Ragama.

Methodology

Awareness of public on antibiotic use was evaluated using a self administered questionnaire, at the Medical Exhibition held at Faculty of Medicine, Ragama in September 2016. People over 16 years of age were randomly selected. The questionnaire contained 8 closed ended questions; 2 on knowledge of proper antibiotic use, 3 on antibiotic misuse and 3 on antibiotic resistance.

Results

Out of 2442 participants, 92.89% had responded for all the questions and 958 (39.23%) had responded correctly to all the questions. 2217 (90.79%) responded that antibiotics were used for treatment of bacterial infections and 2294 (93.94%) responded that medical advice should be strictly followed when using antibiotics. 211

(8.64%) responded that antibiotics can be taken without medical advice for a common cold. 92 (3.77%) responded that it is suitable to take antibiotics prescribed for another person with similar symptoms and 137 (5.61%) responded that antibiotics prescribed for previous episodes of same disease condition can be taken without seeking medical advice. 1969 (80.63%) were aware that overuse and misuse of antibiotics lead to the development of antibiotic resistance and 1871 (76.61%) responded that infections due to antibiotic resistant bacteria can be fatal. 1585 (64.91%) of participants were aware that use of antibiotics in livestock farming is a leading cause of antibiotic resistance.

Conclusion

Only 39.23% of the participants were able to respond correctly to all the questions. Above 90% of the study population was aware of the proper use of antibiotics. The commonest cause for antibiotic misuse was use of antibiotics without medical advice for common cold (8.64%). Awareness on antibiotic resistance and its consequences was satisfactory among the study population.

PP 15

A study on postoperative infections among patients undergoing neurosurgical interventions at a tertiary care hospital in Southern Sri Lanka

Gamage TSH[1], *Vidanagama DS*[1], *Nagahawatte A*[2], *Perera B*[2]

[1]Teaching Hospital, Karapitiya, [2]Faculty of Medicine, University of Ruhuna

Introduction

A descriptive study was carried out in the Neurosurgical Unit at Teaching Hospital Karapitiya for 4 months to study the postoperative infections among patients undergoing neurosurgical interventions.

Objectives

1. To determine the incidence of postoperative infections following neurosurgical interventions.
2. To assess the device utilization ratios of urinary catheters, endotracheal tubes, central venous catheters, external ventricular drains and ventriculoperitoneal shunts.
3. To identify the causative organisms of the postoperative infections and their antibiotic sensitivity patterns.

Method

One hundred and seventy patients were included in the study and were followed up for 30 days postoperatively to detect postoperative infections. Relevant specimens for microbiological cultures were collected accordingly.

Results

There were 127 (74.7%) elective and 43 (25.3%) emergency surgeries in the study. Twenty patients (11.8%) developed postoperative infections and total episodes of postoperative infections were 26 (15.3%).

Incidence of surgical site infections (SSIs) was 7.65% followed by catheter associated urinary tract infections (CAUTI) 3.53%, ventilator associated pneumonia (VAP) 1.76%, catheter related blood stream infections (CRBSI) 1.18% and ventriculoperitoneal shunt infections (VP shunt) 1.18%.

Pseudomonass spp were the commonest pathogens isolated in SSIs (45.46%) followed by *Acinetobacter* spp (27.27%). One ESBL producing coliform strain (9.09%) was isolated. The *Acinetobacter* isolates were resistant to all the antibiotics tested. The *Staphylococcus aureus* strain (9.09%) isolated was a MSSA.

Two *Pseudomonas* spp, 1 non ESBL producing coliform and 3 *Candida* species were isolated in CAUTI.

Two ESBL producing coliforms,1 *Pseudomonas* species and 1 *Acinetobacter* spp. were isolated in VAP.

One *Acinetobacter* spp. and 1 *Enterococcus* spp. were isolated in CRBSI.

One *Pseudomonas* species and 1 carbapenem resistant coliform were isolated in VP shunt infections.

Conclusions

Surgical site infections were the commonest postoperative infection followed by CAUTI, VAP, CRBSI and VP shunt infections. Gram negative organisms were the predominant pathogens in all types of postoperative infections including SSIs. Majority of SSIs were superficial incisional SSIs. Carbapenems and amikacin were the most effective empirical treatment. Carbapenem resistance among *Acinetobacter* spp. causing postoperative infections reflects a significant problem.

PP 16

Sensitivity and specificity of litmus milk decolourization test as a rapid identification method of *Enterococcus* causing urinary tract infection

Wijayaratne WMDGB, Dissanayake MND

Faculty of Medicine, University of Ruhuna

Introduction

Enterococcus species are conventionally identified by combination of bile aesculin and heat survival tests which takes minimum of 24 hours. Litmus milk decolorization (LMD) test has been used as a single test to identify *Enterococcus* in only 4 hours. Early identification of the pathogen is helpful to produce a proper sensitivity test on time.

Objectives

The study evaluate the sensitivity and specificity of LMD test in compared with the combined use of bile aesculin test and heat survival test to identify *Enterococcus* causing urinary tract infection.

Method

Significant (>10^5 CFU/ml) urine culture isolates that are catalase negative, Gram positive cocci in chains were collected from the Microbiology Laboratory of Teaching Hospital Karapitiya. Isolates were subcultured on blood agar and reconfirmed by repeating the above tests. Bile aesculin test and the heat survival test were used to confirm as *Enterococci* according to the conventional method. The LMD test was performed on all the culture isolates in parallel. Litmus milk medium was inoculated with test organism and incubated at 35°C for 4 hours. An *Enterococcus* is suggestive by change of normal blue mauve colour into white or pale yellow-pink colour where no colour change probably excludes *Enterococcus*.

Results

Out of 44 samples, 72.7% (n=32) were bile aesculin test positive. Out of the bile aesculin test positive isolates (n=32), 81.3% (n=26) were heat survival test positive. Out of bile aesculin test positive and heat survival test positive samples (n=26), 92.3% (n=24) were LMD test positive. All bile aesculin test positive but heat survival test negative samples (n=6) and all bile aesculin test negative samples (n=12) were LMD test negative.

Sensitivity, specificity, positive predictive value and negative predictive value of LMD test were 92.31%, 100%, 100% and 90.91% respectively when compared with the conventional method.

Conclusion

A positive result in the litmus milk decolourization test can be used with high specificity as a single test to identify *Enterococcus* causing urinary tract infection when compared to conventional method of combined bile aesculin and heat survival test.

PP 17

Case of invasive neonatal *Haemophilus influenzae* infection

Abeywardena HMW, Mohottala LPW, Priyankara HDR

District General Hospital Nuwara Eliya, Nuwara Eliya

Introduction

Early-onset sepsis remains a common and serious problem for neonates, especially preterm infants. Invasive disease is defined as isolation of the organism from a normally sterile site such as blood or cerebrospinal fluid.

Causative organisms of early-onset neonatal sepsis are usually colonizers of the maternal genitourinary tract such as Group B streptococcus and *Escherichia coli*, leading to contamination of the amniotic fluid, placenta, and the birth canal. The pathogen can ascend when the amniotic membranes rupture or at the onset of labour, causing chorioamnionitis and as a result, the infant may acquire the pathogen either in utero or during the process of labour.

Haemophilus influenzae not being a common cause of early onset of neonatal sepsis and being fastidious can be missed during the laboratory process.

Case report

A case of neonatal *Haemophilus influenzae* was diagnosed in the laboratory when a pure growth of *Haemophilus influenzae* was isolated from a blood culture of a neonate in 24 hours of incubation, using conventional blood culture system. Blood culture was taken from a premature infant of 28 weeks, just after the delivery, whose mother had mild pregnancy induced hypertension but had no signs of any infection or premature rupture of membranes.

The neonate had not cried after delivery, been resuscitated and sent to the premature baby care unit for management and blood culture was taken as a routine procedure. Baby was treated for respiratory distress with crystalline penicillin and gentamicin. Chest Xray showed signs of reduced air entry. White blood cell counts remained normal. In spite of the efforts taken by the resuscitation team, the neonate died after 9 hours.

Identification of *Haemophilus influenzae* was done using satellitism, apple plate and X,V and XV factors. Further confirmation was done at the Medical Research Institute. Organism was sensitive to ampicillin, amoxicillin-cavulanic acid, cefuroxime, cefotaxime, clarithromycin and ciprofloxacin.

Discussion

This case highlights the need to be vigilant for uncommon pathogens and the importance of “automated blood culture system” where positive results can be obtained within hours, in contrast to conventional culture system which takes a minimum duration of 2 days.

FELLOWSHIPS OF THE SRI LANKA COLLEGE OF MICROBIOLOGISTS

Fellowships of the Sri Lanka College of Microbiologists were awarded to Prof. S. N. Arseculeratne, Dr. S. D. Atukorala, Prof. Jennifer Perera and Prof. Nelun De Silva on 10th August 2016 at the Sri Lanka Foundation, Colombo 7.

Professor S. N. Arseculeratne
MBBS (Ceylon), Dip. Bact. (Manchester),
D. Phil. (Oxford), D. Sc. (Hon. Ruhuna)

Sarath Nanda Arseculeratne, MBBS (Ceylon), Dip. Bact. (Manchester), D. Phil. (Oxford), D. Sc. (Hon. Ruhuna) is an Emeritus Professor of Microbiology of the University of Peradeniya. He held the post of Professor of Microbiology from 1971-1982, and 1989-1995. He was Professor of Medical Microbiology at the Faculty of Medicine, University of Malaya in KL, Visiting Professor of Microbiology at the University Sains Malaysia in Kelantan, Malaysia, and was a DANIDA, Wellcome, Commonwealth and Fulbright Research Fellow at universities in Denmark, Thailand and at Harvard University in Boston, Mass., USA.

He supervised the first Ph. D. in Sri Lanka's Faculties of Medicine, a D. M., two M. Phil's and a second Ph. D. in the Faculty of Medicine. He has served as an external examiner for the Ph. D. in an Indian university.

He was in the first batch of recipients of the National Award for Scientific Achievement from the Government of Sri Lanka.

In the administration of science, he was a Council member of Sri Lanka's National Science Council, and Chairman of its Science and Technology Policy Research Committee that drafted the first National Science and Technology Policy of Sri Lanka in 1978.

He has served as the President of Section A of the Sri Lanka Association for the Advancement of Science, and of the Kandy Society of Medicine.

He served on the Expert Advisory Panel on Bacterial Diseases of the WHO in Geneva. He participated in conferences on education and science in Czechoslovakia, Thailand, India, Nepal, Cambridge UK, and China.

In microbiology, he established the first bacterial type culture collection in Sri Lanka No. 9, World Federation of Type Cultures. His main research contributions began with Aflatoxins. He obtained a patent on his collaborative invention of an apparatus for the solar detoxification of aflatoxin contaminated coconut oil, a project of economic importance to Sri Lanka.

His personal approach to teaching of medical microbiology included a stress on the stimulation of originality (creativity) and a WHO consultant who examined the teaching methods in 13 Asia-Pacific medical schools commented: "The question directed to 'creativity' rather than conformity to correct answers, with Microbiology in Peradeniya was a radical departure from current practice. I hope the idea stimulates other examiners to consider what they would like to encourage in their students……."

He was joint author of a chapter on Rhinosporidiosis in the Topley and Wilson's Microbiology and Microbial Infections: Medical Mycology, 10th ed, 2005, and a chapter on Rhinosporidium, to Molecular detection of fungal pathogens (Australia) and has authored a monograph on "Rhinosporidiosis in Humans and animals; Rhinosporidium seeberi".

His publications and interests are not limited to microbiology. He has authored 4 books, Sinhalese Immigrants in Malaysia and Singapore, 2 books on humour in science with Sidney Harris (USA), and a compilation of 88 essays in "I think therefore I am – Rene Descartes". His general essays included, Thoughts on Darwinian Evolution, Descartes' Cogito, ergo sum, and Parapsychology on which he has researched extensively.

This compilation illustrates the diversity of his academic and general interests that range from tertiary education, the structure of modern science, creativity, scientific research, medical ethics, traditional medicine and Parapsychology.

His recent university lecture, Knowledge, its scope and its acquisition, was commended on by the Chairman of the Indian Council for Philosophical Research in New Delhi: "It is amazing how you continue to be so vigorously pursuing your intellectual interests which are, in a conventional sense, outside the scope of your profession. There is much that the younger generation can learn from you".

In sports, he was the Founder-President of the University Rowing Club and the staff representative on the Amateur Rowing Association of Sri Lanka. He played cricket for the University of Ceylon, and the Staff Recreation Club of the University of Oxford. He constructed and flew model aircraft in Malaysia and in Sri Lanka, and did sailing in Lankan lakes and the sea coast.

Madam President, it is my honour and privilege to present Prof. Sarath Nanda Arseculeratne for award of the Honorary Fellowship of the Sri Lanka College of Microbiologists.

Citation read by **Dr. Geethani Galagoda**
Consultant Virologist

FELLOWSHIPS OF THE SRI LANKA COLLEGE OF MICROBIOLOGISTS

Dr. S. D. Atukorala

MBBS, MD (Micro), Dip Bact. (Manchester),
FACP, FRCPath, FSLCM, FCPathSL

Madam President, I am privileged to have been requested to introduce a renowned consultant in the specialty of clinical microbiology Dr. S. D. Atukorala.

Srilal Dharmadasa Atukorala had his primary and secondary education at Royal College Colombo where he excelled in studies as well as in sports. He captained the Royal College cricket 'A' team and was also a member of Royal College athletics, swimming and basket ball teams. He was the vice captain of the champion athletic team that carried away all trophies at public schools athletics meet in 1963. He was a co-holder of 4 x 110 yards public schools junior relay record.

He obtained his MBBS from the University of Ceylon, Colombo in 1972. While at the University he rowed for the University participating in local and international boat races and captained the Medical College Cricket team. In 1975 he was appointed as a medical officer at the then anti VD campaign Colombo. He obtained Diploma in Bacteriology from the University of Manchester in 1978. His post graduate training in Clinical Microbiology was at the Royal Free Hospital in London from 1979 - 1981. The Clinical Pathology training was at the National Hospital for Nervous Diseases at Queens Square, London.

He was awarded the membership of the Royal College of Pathologists of the United Kingdom in 1980. On his return to Sri Lanka he was appointed Assistant Bacteriologist at the then anti-VD campaign Colombo. He was appointed as Consultant Clinical Bacteriologist at the National Hospital of Sri Lanka in 1981 where he served in this position for 23 years. He also served as Head of the Department of Pathology for 12 years until his retirement from government service. During his tenure at the National Hospital of Sri Lanka I had the good fortune to be his post graduate trainee in medical microbiology as a registrar and later as a senior registrar. He obtained his MD Microbiology from the University of Colombo in 1988. He was awarded the Fellowship of the American College of Physicians in 1990 and the Fellowship of the Royal College of Pathologists, UK in 1992. He was also awarded a Fellowship of the Sri Lanka College of Pathologists in 2013.

After retirement, he served as the National Advisor in laboratory services of the Ministry of Health for five years. He also served as the advisor to the Microbiology Section of the Military and Navy Hospitals in Colombo.

He has been a Visiting Scientist of the World Health Organization at the Columbia Presbyterian Medical Centre, New York City and at Hospitals affiliated to the Harvard Medical School in Boston, USA. He served as a Consultant to the WHO on numerous occasions and is a co- author of the two WHO guidelines on infection control for South East Asia. He has also authored several

booklets on infection control in healthcare settings and also on rational use of antibiotics published by the Ministry of Health, Sri Lanka. He has over twenty publications in local and international journals and has delivered many orations and guest lectures in Sri Lanka and abroad. He has been a Temporary Advisor to the WHO on numerous occasions and helped the Ministries of Health in Bhutan, Bangladesh, Maldives and Nepal in Infection control and rational use of antibiotics. He served as the co-ordinator and a member of the Board of study in Pathology and was also a member of the Board of study in Microbiology of the Post Graduate Institute of Medicine (PGIM) of the University of Colombo. He served as an examiner for the Diploma in Pathology and also been an examiner at the MD examination in Microbiology. At present he is the Visiting Consultant Clinical Microbiologist at the Asiri group of Hospitals and New Delmon Hospital in Colombo.

Madam President it is my pleasure and privilege to present Dr. Srilal Dharmadasa Atukorala for the award of the Honorary Fellowship of the Sri Lanka College of Microbiolgists.

Citation read by **Dr. Geethika Patabendige**
Consultant Clinical Microbiologist

FELLOWSHIPS OF THE SRI LANKA COLLEGE OF MICROBIOLOGISTS

Professor Aurelia Jennifer Perera
MBBS, MD Micro (Col), PG Dip Women Studies,
Dip Med Edu (Dundee), MBA (Wales)
Dean and Chair and Senior Professor of Microbiology

Professor Aurelia Jennifer Perera is no stranger to the medical and academic world. Her career spanning over three decades has been spent in teaching and guiding medical students at the Faculty of Medicine, Colombo. Born in April 1955, she underwent her primary and secondary education at Holy Family Convent Bambalapitiya and subsequently at Holy Cross College, Gampaha.

In 1974, she entered the Faculty of Medicine, University of Colombo for her undergraduate studies in medicine and successfully completed the MBBS degree with second class honors. Following completion of her internship she joined the Department of Microbiology at the Faculty of Medicine, Colombo, as a young Lecturer in 1980. Successful completion of her postgraduate studies would pave the way for her to be trained at the prestigious Clinical Research Centre in Middlesex in the United Kingdom. Having been board certified as a Consultant Microbiologist by the Postgraduate Institute of Medicine, in 1986, she assumed duties as a Senior Lecturer in the Department of Microbiology.

Ladies and gentleman from here on her rise was meteoric! Being promoted to Associate Professor and subsequently to Professor of Microbiology in 1995 and 2001 respectively, she was appointed to the prestigious Chair of the Department of Microbiology and the Senior Professorship in 2009, the tenured posts that she continues to hold to date. Presently she functions as the Dean of the Colombo Medical Faculty a position which has truly tested her tenacity in being both elected and continuity.

Her contribution as a teacher in the faculty of medicine is invaluable and immeasurable. She has taught microbiology to medical and science undergraduates, para-medical personnel, post graduate students of the Diploma and MD Microbiology courses as well as other post graduate courses conducted by the Post Graduate Institute of Medicine. She has supervised many a post-graduate student reading for research degrees and guided them to successful completion of their masters' and doctoral degrees. She has been an examiner in Medical Microbiology for medical undergraduates of Faculties of Medicine across Sri Lanka. She has also functioned as an examiner for both post graduate students of the PGIM as well as for students reading for research degrees from other universities in Sri Lanka.

Professor Jeniffer Perera's contribution towards curricular reforms undertaken by the Faculty of Medicine, Colombo, has been outstanding. As an active member of the Curriculum Development and Evaluation Committee of the faculty, she was behind some of the innovative approaches in medical teaching that were incorporated into the curriculum in the late nineties.

She has been the Warden of the women's hostel as well as a Senior Student Counsellor of the Faculty of Medicine

for many years and continues to maintain that excellent relationship with students as the Dean of the Faculty. On a personal note I find one quality of hers that contributes a great deal towards this harmony with students; and that is the ease in which students approach her and delivery of a practical solution to a frustrating issue.

Professor Perera has held the posts of President of the Faculty of Medicine Teachers Association, She has been a member of the Board of Study in Microbiology of the PGIM since 1990 which she headed from September 2011 to 2014. She was the President of Section A of the Sri Lanka Association for the Advancement of Science in 2000, the Sri Lanka College of Microbiologists in 2002 and the Vaccine Forum of Sri Lanka in 2010 and the president of the SLMA in 2015.

She has been invited to deliver many lectures pertaining to microbiology and infectious diseases at various forums both internationally as well as locally including the Dr Siri Wickremesinghe oration of the Sri Lanka College of Microbiologists in April 2010.

Her research interests vary widely as evidenced by the vast number of publications appearing in peer reviewed journals. However, research on tuberculosis has particularly been of special interest to her. She has been a pioneer in analyzing the molecular diversity as well as patterns of drug resistance of *Mycobacterium tuberculosis* isolated from patients in Sri Lanka. Research in this direction has led to the development of new techniques for rapid detection of rifampicin resistant *M. tuberculosis* strains in low resource settings.

Furthermore, she is the country representative for the Asian Network for Surveillance of Resistant Pathogens (ANSORP) study, which evaluated the prevalence, spread and clinical outcomes of drug resistant *Streptococcus pneumoniae* as well as the antimicrobial susceptibility of *Shigella* and *Salmonella Typhi* in Asian countries.

She has also been actively involved in the immunization programme in Sri Lanka and has supervised research on strategies for immunization against rubella as well as hepatitis B vaccination. She was a key contributor to the book on "Guidelines and Information on Vaccines" as well as "Guidelines for the Use of Non-EPI vaccines" published by the SLMA.

She has authored many chapters for books on topics such as healthcare waste management, *Varicella zoster* virus, congenital syphilis, antibiotic guidelines, standard precautions and hand hygiene to name a few! She has presented her work at 25 international conferences and over 125 conferences held in Sri Lanka.

Her interests have not been limited to microbiology alone. Her ability to learn is continuous and aptly demonstrated by the successful completion of a Post Graduate Diploma in Gender Studies from the University of Colombo which she achieved with distinction in April 2002 and thereafter the Diploma in Medical Education from the University of Dundee, United Kingdom in April 2010 as well as a Masters in Business Administration from the University of Wales in 2013.

She has received many academic distinctions, scholarships, awards and prizes during her distinguished career. Commonwealth Fellowship award to United Kingdom in 1986, Visiting scientist grant from WHO to the Royal Tropical Institute, Netherlands and Pasteur Institute, France; WHO Fellowship on Medical Education to Singapore and Malaysia; Awards and prizes for research papers from the SLMA alone include the Daphne Attygalle award in 1995, HKT Fernando award in 1998, Professor Rajasuriya award in 1998, S E Seneviratne Award in 2001, Glaxo Welcome Research Award in 2005, Sir Frank Gunasekera Prize in 2008, Prize for best poster in 2011 and the CNAPT award in 2013.

She has also received awards and prizes for her research work from the Sri Lanka College of Microbiologists (1995, 1996, 2003, 2008 and 2010); the Allergy and Immunology Society of Sri Lanka in 2010. Association of Pulmonologists, Sri Lanka (2010, 2014); and at the International Medical Education Conference (IMEC in 2008 and 2010) in Malaysia. She is also the recipient of the prestigious Presidents Award for Research (1999, 2001,2006, 2007,2008, 2010, 2011, 2012, 2013, 2014) and was selected as one of thirty strong women in Sri Lanka in commemoration of International Womens' day by the Ministry of Child Development and Womens' affairs with the award being presented by His Excellency the President of Sri Lanka in 2012.

She has faced many daunting challenges with utmost determination and courage. She is truly a dedicated teacher, doctor, wife and a mother. I have had the privilege of being a student, colleague and a dear friend to her during both good and bad times. I truly believe that she has earned this award.

Citation read by **Dr. Channa Senanayake**
Specialist Virologist and Senior Lecturer,
Faculty of Medicine,
University of Colombo.

FELLOWSHIPS OF THE SRI LANKA COLLEGE OF MICROBIOLOGISTS

Professor Nelun De Silva

MBBS (Patna); MD Medical Microbiology (Colombo)

Nelun De Silva received her primary and secondary education from St. Joseph's School, Nugegoda and Holy Family Convent, Bambalapitiya. Being a recipient of an Indian Cultural Scholarship she subsequently travelled to India to study medicine where she obtained her MBBS in 1972. Having joined the very first batch of Postgraduate trainees in Medical Microbiology of the PGIM she obtained her Diploma in Medical Microbiology in 1988 and MD Medical Microbiology in 1993. She is a Board Certified Specialist in Medical Microbiology and an Accredited Senior Teacher in Higher Education (ASTHE) from the University of Colombo with accreditation from Staff and Educational Development Authority (SEDA) UK in 2004.

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.

She served the Department of Microbiology, Faculty of Medicine, University of Colombo from 1990 to 1997 before joining the Department of Microbiology, Faculty of Medicine, University of Ruhuna in August 1997 as Senior Lecturer and Head Department of Microbiology and became the first cadre Chair in 2005. During her tenure at Ruhuna her contribution towards Medical Education and Staff Development and the planning and the implementation of the IRQUE QUF project of the Faculty of Medicine needs special mention.

She also served the Faculty of Medicine and Allied Sciences Rajarata University as Visiting Professor on assignment basis from 2009 to 2010. Having retired from Ruhuna in 2011 she continues with her enthusiasm and commitment to teach and currently serves as the Professor in Microbiology at Faculty of Medicine, SAITM.

She was a member of the Board of Study in Microbiology since 1997 and the Chairperson from 2006 to 2011. She has also been an examiner for the Diploma & MD Medical Microbiology examinations since 2000. Her contribution to improve the Diploma and MD Medical Microbiology training has been immense.

In spite of her busy academic career she has never lost her clinical touch and has been the Honorary Consultant Microbiologist at the Teaching Hospital Karapitiya from 1998-2006, Consultant Microbiologist at Sri Jayewardenepura Teaching Hospital in 1997 and the Consultant Microbiologist at Neville Fernando Teaching Hospital since 2012.

As a researcher she has published widely and received many awards. She is a member of the Sri Lanka Association for the Advancement of Science since 1997 and served as its Section A President in 1999.

She has been a member of the Sri Lanka College of Microbiologists since 1990 and served the College as Secretary, Editor and Vice President before being the President of the College in 2002/3.

Her dedication, integrity, courage, perseverance, positive attitude and generosity are worthy of emulation.

Madam President, I present Professor Nelun De Silva to receive an Honorary Fellowship of the Sri Lanka College of Microbiologists.

Citation read by **Dr. Ajith Nagahawatte**
Specialist Microbiologist and Senior Lecturer, Faculty of Medicine, Galle.

PRIZE WINNERS AT THE 25TH ANNUAL SCIENTIFIC SESSIONS 2016

Following presentations were awarded first, second and third places at the 25th Annual Scientific Sessions of the Sri Lanka College of Microbiologists held on 11th & 12th August 2016.

Oral presentations

1st prize

OP 10

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

Premathilake MIP[1,2], *Jayamaha CJS*[1]

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

2nd prize

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

3rd prize

OP 4

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

Premaratne KKMK[1], *Corea E*[2], *Karunanayake L*[2]

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

PRIZE WINNERS AT THE 25TH ANNUAL SCIENTIFIC SESSIONS 2016

Poster presentations

1st prize

PP 18

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

Mahagamage MGYL[1], *Pathirage MVSC*[2], *Pathmalal M Manage*[1]

[1]Department of Zoology, University of Sri Jayewardenepura, Gangodawila, Nugegoda, [2]Food and Water laboratory, Medical Research Institute.

2nd prize

PP 9

Epidemiology of melioidosis in Sri Lanka 2006 – 2016

Corea E[1]*, Patabendige G*[2]*, Kothalawala M*[3]*, Francis V*[4]*, Dassanayake M*[5]*, Vidanagama D*[6]*, Nanayakkara G*[7]*, Fernando R*[8]*, Abeykoon M*[9]*, Elvitigala JP*[10]*, Piyasiri DLB*[11]*, Jayatilleke SK*[12]*, Dissanayake N*[13]*, Chandrasiri S*[14]*, Namalie KD*[11]*, Karunaratne GKD*[15]*, Wadanamby JMRWW*[16]*, Jayasuriya R*[17]*, Mubarak FN*[18]*, Udagama S*[8]*, Wijayaratne WMDGB*[19]*, Pathirage S*[20]*, Masakorala J*[1]*, de Silva AD*[21]*, Sathkumara H*[21]*, Krishnananthasivam S*[21]*, Thevanesam V*[13]

[1]Faculty of Medicine, University of Colombo, [2]National Hospital of Sri Lanka, [3]Teaching Hospital, Kandy, [4]Faculty of Health-Care Sciences, Eastern University of Sri Lanka, [5]Teaching Hospital, North Colombo, [6]National Hospital for Respiratory Diseases, Welisara, [7]Teaching Hospital, Kurunegala, [8]General Hospital, Chilaw, [9]District General Hospital, Polonnaruwa, [10]National STD/AIDS Control Programme, [11]Teaching Hospital, Karapitiya, [12]Sri Jayewardenapura General Hospital, [13]Faculty of Medicine, University of Peradeniya, [14]Teaching Hospital, Colombo South, [15]Lady Ridgeway Hospital for Children, [16]National Institute of Infectious Diseases, Angoda, [17]General Hospital, Kalutara, [18]Teaching Hospital, Jaffna, [19]Faculty of Medicine, University of Ruhuna, [20]The Central Hospital, Colombo, [21]Genetech Research Institute, Colombo

3rd prize

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

Presidential address delivered at the inauguration of the 25th Annual Scientific Sessions of the Sri Lanka College of Microbiologists on 10th of August 2016

The importance of Laboratory Confirmation of Rabies Sri Lankan Experience

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Good evening, ladies and gentlemen. Chief guest – Professor Mohan de Silva, Chairman, University Grants Commission, officials from the Ministry of Health, foreign and local guest speakers of the 25th Annual Scientific Sessions, the past presidents, Council and the members of the Sri Lanka College of Microbiologists, 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.

I am greatly honored and privileged to address you this evening, at the inauguration ceremony of the 25th Annual Scientific Sessions of the Sri Lanka College of Microbiologists.

At the beginning, let me briefly introduce you to our College, some of our activities and this years' annual scientific sessions.

The inception of our College was in 1969 as the Ceylon Association of Microbiologists with 16 founder members of whom, Prof. S N Arsecularatne and Prof. Emyl Wijewantha are with us today.

The College has evolved to the present Sri Lanka College of Microbiologists with a new constitution from 1979. Presently our membership has risen up to 220 members, holding postgraduate qualifications from Diploma in Medical Microbiology through MD and PhD in Medical Microbiology / Virology / Parasitology and related sub-specialties working for the Ministry of Health, Universities and the private sector.

Our College is actively involved in tasks like prevention and control of hospital acquired infections and combatting antimicrobial resistance which is a global issue, both in healthcare institutions and in the community. Our members represent the College at important decision making committees of the Ministry of Health such as the National Advisory Committee on Infection Control, National Advisory Committee on Communicable Diseases, Medicines Evaluation Committee of the National Medicines Regulatory Authority, Revision of the National Formulary of the Medical Supplies Division, and numerous Technical Evaluation Committees on antimicrobials, vaccines and laboratory items; to name a few.

Many of our members have contributed in different capacities over the years in our College activities to improve the microbiology services of the country; through diagnosis of infections, prevention and control of health care associated infections and control of antimicrobial resistance.

Teaching and training of medical and para-medical staff at undergraduate and postgraduate levels, is another important responsibility.

The College has published several manuals including the Laboratory Manual in Microbiology and Biosafety Manual for Medical laboratories which have been revised in 2011 and 2014 respectively, which are widely used by health care staff throughout the country. The Hospital Infection Control Manual which has been published about 10 years ago, is currently being revised.

The SLCM together with other professional colleges and associations developed 21 National Guidelines for Empirical and Prophylactic use of Antimicrobials. This activity was done during the past three years with the financial assistance by the World Health Organization. These guidelines were finalized and uploaded to our College website for easy accessibility and currently is in the process, of being printed as a booklet.

The most recent activity undertaken by our College this year, is taking the lead in preparation of the National Action Plan for Combating Antimicrobial Resistance in Sri Lanka. This activity is performed with a multi-sectoral approach by the Ministry of Health, together with the Veterinary and Agricultural sectors, with the assistance from the WHO.

The Annual Scientific Sessions of the College began in 1991 during Prof. Tissa Vitharana's presidency and had continued without an interruption.

This year we are organizing the 25th Annual Scientific Sessions with the theme "Microbial infections: Facing Challenges and Exploring possibilities".

Today, in the morning, we successfully concluded the Pre-congress workshop on "Acute Encephalitis – a clinical dilemma" which is an important, interesting and a timely topic where four, foreign and local guest speakers shared their knowledge and expertise with our members. This year's scientific sessions, will be held in this same venue tomorrow and the day after. There will be five free paper sessions and presentation of posters on research activities performed by our members. There will be five plenary lectures and three symposia with the participation of local and foreign resource personnel. I am sure, the topics we have selected for this year will be of great interest to our members, especially the trainees, and we expect high participation and interaction by members and non-members on both days.

With this brief overview of our College and its activities, now I will move on to a topic which has been close to my heart for nearly two decades.

The importance of Laboratory Confirmation of Rabies – Sri Lankan Experience.

The history of rabies goes as far back as the ancient Greek and Roman times. Rabies has been described in the writings of Democritus, Aristotle, Hippocrates and Celsus.

Hippocrates had described rabies as "that persons in a frenzy drinks very little, are disturbed and frightened, trembles at the least noise or are seized with convulsions" which could even be accepted today as a typical description of a clinical presentation of rabies.

In 100 AD the physician – Celsus described human rabies and has used the term hydrophobia which means "fear of water". Celsus recognized that the saliva of the biting animal contained the poisonous agent, which caused this disease. At that time it was not known that this fatal disease was caused by a virus.

One of the earliest vaccines developed in the world was against rabies. The vaccine was developed by Louis Pasteur, who discovered the germ theory of infection. His first paper on rabies was published in 1881 when he experimentally transmitted the rabies virus by inoculating CNS material of a rabid animal in to the brains of healthy animals.

The first rabies vaccine was produced in 1885, using desiccated spinal cords of rabbits inoculated with rabies virus and successfully immunized the first patient, a 9 year old boy Joseph Meister, who survived multiple bites from a rabid dog.

Since then, rabies has been endemic in many parts of the world and only few countries are considered as being free from this dreaded disease.

With that short history, I would like to introduce you to this fatal disease – rabies.

Rabies is caused by an enveloped, bullet shaped RNA virus, which is in the family *Rhabdoviridae* in the genus *Lissaviridae*. Any warm blooded animal, usually the mammals are susceptible to rabies, which is often fatal to most species.

Transmission is usually via the saliva of an infectious animal – through a bite and to a lesser extent through scratches or contamination of mucous membranes or abraded or unhealthy skin with saliva.

Incubation period can be very variable, usually 1 to 3 months, but periods as short as 1 week and as long as several years have been described.

Furious form and the paralytic or the dumb form, are the two extremes of clinical presentation, but often present with some degree of overlapping symptoms. In whichever form, the disease is invariably fatal.

The clinical diagnosis of rabies is easy with a typical history and symptoms, but these symptoms could be mimicked by conditions like tetanus and other forms of viral meningo-encephalitis and Guillain-Barre Syndrome. Hence, laboratory tests are needed to confirm the diagnosis.

Several tests, both ante-mortem and post-mortem are available for diagnosis.

There are tests, which are routinely used in diagnostic laboratories and also special techniques which are performed only for doubtful cases and for research.

Diagnosis of rabies in Sri Lanka – The preliminary test performed on postmortem fresh brain specimens, is the direct smear test stained with Seller's stain for Negri bodies. This test while being specific, has a poor sensitivity around 40 - 50%. However, we still do this test as the first test, to make rabies diagnosis cost-effective.

The fluorescent antibody test (FAT) which is the gold standard test for rabies diagnosis with high specificity and sensitivity around 98 - 99% is done for all samples which are inconclusive with the direct smear test.

Though expensive, all rabies diagnostic tests are done free of charge at the MRI.

Other available back-up tests include

- Immunochromatography – rapid strip test, which is not yet validated for routine use
- Mouse Inoculation Test
- Conventional RT- PCR
- And the most recently established real time RT-PCR

Why is it important to confirm or exclude rabies – in humans and animals?

People sometimes take animal bite exposures lightly without suspecting rabies and may not seek treatment. We have observed that >95% of human rabies deaths in Sri Lanka are due to this reason. Therefore, laboratory confirmation is important

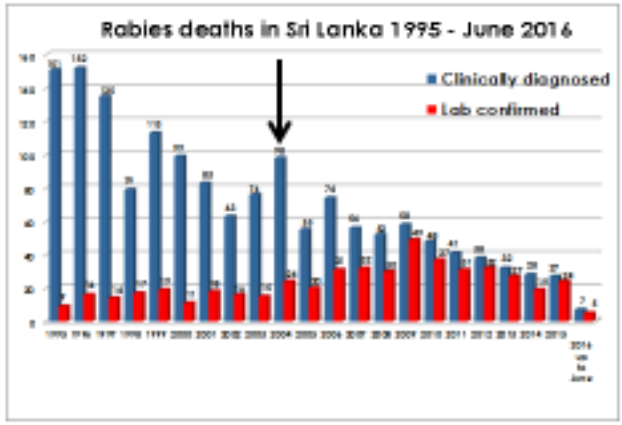
- For appropriate post exposure therapy to be offered for patients (Both under-treatment or over-treatment could be avoided).
- To prevent unnecessary treatment for exposures to animals free of rabies, thus reducing the financial burden to the government.
- To provide data for disease epidemiology and surveillance (the types of animals, geographical distribution, changing trends of disease etc. would be noted).
- To assist the rabies control programme – in directing the animal vaccination and sterilization programmes.

  and
- For rabies research.

To support the rabies elimination goals, such as “zero human rabies deaths by the year 2020”, laboratory confirmation of all suspected human rabies deaths, is a must.

The number of human rabies deaths in Sri Lanka have declined during the past 50 years, from a peak of 377 deaths in 1973, to single digits during the first six months of this year.

This slide shows. The Human rabies deaths in Sri Lanka from 1995 to June 2016 –- the clinically suspected / lab confirmed cases.



- Most rabies deaths were only clinically diagnosed, before 2004.
- A change of this trend occurred with the issue of a DGHS circular in 2004 – on mandatory post-mortem and laboratory confirmation of all suspected rabies deaths in hospitals.
- Since then, majority of these deaths are confirmed by laboratory testing.

I will now present two recent case scenarios, to show the importance of laboratory confirmation.

The first case, shows the advantage of having a battery of tests for confirmation.

A 48 year old male – living close to a district hospital, sustained a major bite on the forearm by his own dog, which was behaving suspiciously, and died 2 days later.

- Wound management was done at the local hospital and was then referred to a General hospital and then to a Teaching Hospital for post exposure therapy (PET).
- Appropriate treatment with ARS / ARV was started. But, there was a delay of 48 hours. The delay was due to the patient needing to visit 3 hospitals before the PET was initiated
- This patient developed fever, chest pain, palpitations and muscle twitching, three weeks after the bite, which were not typical symptoms of rabies.
- He died within 3 days of hospitalization.
- The brain was sent to MRI for confirmation after the post-mortem.

In our laboratory

- The Direct Smear was inconclusive
- FAT was done twice – No rabies antigen was observed

- Immuno-chromatography test – gave a faint band (but the ICT is not highly recommended for human brains as it could give false positive results, which we have published)
- Mouse inoculation test was done
- During this time, we were establishing the real time RT - PCR in our laboratory, and this sample was also included in the test
- The Real time RT - PCR gave a positive result within few hours
- And the MIT also became positive, 14 days after inoculation

This is a good example to show the importance of having, several confirmatory tests for diagnosis of rabies in a reference laboratory.

The second case, shows the disadvantage of not confirming the diagnosis.

A 52 year old male was bitten by a wild animal, possibly a fishing cat.

- He presented with multiple, deep bites on the thigh and hands.
- He was given appropriate treatment at a Teaching Hospital without delay. That is, ARS and ARV within 8-10 hours of the bite.
- However, he developed typical symptoms of rabies, 4 days after the Day 30 ARV dose, which is the last dose of the treatment course.
- Two serum samples were received at MRI – which showed a 4 fold rise of rabies Abs – which is suggestive of rabies.
- He was ventilated and managed in the ICU for 19 days and died.

The postmortem was not performed. Therefore, the brain was not sent for confirmatory tests.
Hence,

- The diagnosis was not confirmed and it was only reported as a probable case of rabies
- This is a situation where a patient was treated appropriately and without delay, but succumbed to a disease, most likely to be rabies

Since the postmortem was not done – We have several unanswered questions in this case.

- The brain tissue was not available to confirm rabies and to do the genotyping of the virus
- Could this be a more virulent form of a rabies virus? Or
- A different virus strain, where the vaccine was not effective?

These questions could have been only answered, if the post mortem brain was sent to a rabies reference laboratory for confirmation and further testing.

Now I will present, data on rabies surveillance in Sri Lanka. Majority of samples received at MRI are from animals and on average around 1500 to 1800 samples are received per year.

We have analyzed and published the trends in rabies in Sri Lanka during the past decade with the available data.

- When considering the total number of samples received, the number positive and the % positivity – There is no significant difference in the numbers received or % positivity during the past 10 years.
- Distribution of animal samples – dogs, cats, squirrels and wild animal specimens received at MRI during the past 10 years.
  - The number of dog specimens received has decreased with no significant reduction in % positivity.
  - The number of cat specimens are gradually increasing with no significant change in % positivity.
  - The number of squirrel samples received has gradually increased up to 2012 and then declined, but the % positivity is increasing.
  - Wild animal samples received, are gradually decreasing, however the % positivity is Increasing.
  - Up to now, no rat sample has become positive for rabies in Sri Lanka.

The specimens submitted for confirmation at MRI during 2006, 2010 and 2015, shows that there is a reduction in the relative proportion of dog samples received, compared to an increase in the proportion of cats and other mammal samples. This proportion has more than doubled, during the last decade.

This indicates an increase in awareness among the public, regarding possibility of rabies, in animals other than dogs.

Now, I will discuss the – availability of rabies diagnostic facilities in Sri Lanka – our strengths and weaknesses.

The Rabies Reference Laboratory of Sri Lanka is at the MRI, where I work, which is adequately equipped, has well trained, responsible and enthusiastic staff.

In addition, we only have two more laboratories – at Teaching Hospital Karapitiya and at the Veterinary Faculty, University of Peradeniya.

The geographical distribution of the samples received at MRI shows that the majority (around 60-70% of samples) are from the western province due to the difficulty in transport of specimens from faraway places.

Nearly 15-20% of samples are from the Southern Province, due to having a rabies laboratory in Karapitiya, and the rest is from all other provinces of the country.

Therefore, we are unable to give accurate surveillance data for the country, since most animal deaths from the peripheries of Sri Lanka are not investigated.

Ideally, at least one diagnostic laboratory should be available for each province – to make it easy for people throughout the country to submit samples, which will also improve disease surveillance.

Why is it difficult to de-centralize rabies diagnosis?

- Recruitment of staff to handle rabies virus is not easy – due to the undue fear of contracting this disease
- Rabies pre-exposure therapy is mandatory for all staff handling this virus and some do not like receiving routine vaccination
- Difficulty in handling and disposal of animal carcasses are some of the other reasons.

Responsibilities of the Rabies Reference Laboratory include

- Providing rabies diagnostic services for the whole country
- Teaching and training of laboratory staff and medical officers on proper post exposure therapy
- Doing external quality control for the peripheral laboratories
- Offering rabies antibody testing for all staff handling rabies virus
- Supporting the rabies control activities of the Department of Public Health Veterinary Services by providing laboratory data
- Informing human rabies deaths to the Epidemiology Unit of the MoH, for further investigation
- Developing and revising guidelines for proper rabies PET – the latest guideline was issued as a DGHS circular in March 2016 and research activities

To provide a quality service, our Reference Laboratory undergoes external evaluation once every two years, conducted by the WHO Reference Centre – Nancy, France.

Advances in the Rabies Reference Laboratory include

- Rabies antibody testing in serum and CSF by tissue culture technique – RFFIT which was established, in 1996 following the training given by a visiting Japanese expert
- Conventional RT - PCR which was established in 2003/2004 after my post MD training in Centers for Disease Control and Prevention (CDC), Atlanta, USA
- Rabies immuno-chromatography test in 2010 in collaboration with Oita University, Japan
- The most recent was the establishment of real time RT - PCR in February 2016 – following Dr. Dulmini Kumarasinghe's training at NIMHANS, Bangalore, India.

These new techniques would minimize the requirement to do the Mouse Inoculation Test (MIT) for confirmation of doubtful samples in the future.

So, ladies and gentlemen, with this presentation, I hope I have achieved my task of addressing the importance of laboratory confirmation of rabies, which is necessary

- to offer the best possible post exposure management for our patients with correct and rational use of anti-rabies serum and vaccines and prevention of wastage of these expensive biologicals, which are of limited supply
- to provide data for disease surveillance and help the animal rabies control programme
- and for research purposes.

I thank the following

I am indebted to my mentor and colleague, Dr Omala Wimalaratne, former Head of the Department of Rabies and Vaccine QC, MRI – for bringing international recognition to this department and providing the guidance for my professional career. She pioneered the intra-dermal anti rabies vaccination program in Sri Lanka, saving millions of rupees for the country.

I am grateful to the present and past members of my department, especially, Ms Devika Perera, Senior Research Officer, and Dr. Dulmini Kumarasinghe, Consultant Virologist who joined recently and is enhancing the potential of our unit.

Mr. Nuwan Udara for analyzing our data.

My good friends Geethani and Rajiva, for helping me with this presentation.

I wish to thank the council of the SLCM and a very big thank you for my two secretaries Primali and Pavithri, for being such a strength for me in all College activities during my term Mrs. Priyanga Opatha, our College Secretary, whose services are invaluable, thank you Priyanga.

All my teachers who helped me during my school days, university and post graduate education, which has contributed to my professional development

My late parents, Dr. D. S. Dharmage and Mrs. Dorothy Dharmage, for nurturing me for what I am today.

My three sisters, Devi, Mali and Rani and my family, Tissa, Sachini and Nisal for being there for me at all times …..

Thank you.

Prof. Faseeha Noordeen
Professor in Microbiology
Faculty of Medicine, University of Peradeniya

Dengue in Sri Lanka – past and the present trends

President and the council, members of the College, members of the family of the late Dr Siri Wickremesinghe including Mrs Ranganie Wickremesinghe, friends, Ladies and Gentlemen.

The 2017 Dr Siri Wickremesinghe oration compiles the dengue story in the country from the 1960s to the present times including changes taking place in different aspects of dengue interactions including the disease burden, vector dynamics, changes in the socio-geo-climatic factors and the evolution of dengue viruses (DENV) in Sri Lanka. Before getting deeper into the dengue story, we recollect and reflect on the story of Dr Siri Wickremesinghe, one of the great microbiologists of his time in the country and an extraordinary human being!

Dr Rakkhita Sirimal Bandara Wickremesinghe was born on 28th November 1937 to Dr Artie and Helen Wickremesinghes. He received his school education at Royal College Colombo, obtained his MBBS degree in 1963 from the Faculty of Medicine, University of Colombo, Diploma and Master of Science degree in Microbiology from the University of Manchester, United Kingdom and MD with Board Certification in Microbiology from the University of Colombo.

He joined the Medical Research Institute (MRI), Colombo in 1968. On completion of his postgraduate studies and one year training in the UK, he was appointed as the Consultant Microbiologist, MRI and was in charge of the General Bacteriology Division until his retirement from the MRI. He also held the post of Director at MRI from 1996-1998. After his retirement, he served at the Durdans Hospital, Colombo as the Resident Pathologist and Laboratory Manager.

Dr Wickremesinghe has been admired by many of his trainees, juniors and colleagues. He worked to inculcate enthusiasm and commitment in his followers in Microbiology. His say had been "We have to learn to do our best – not to be satisfied just being a diamond but try to be a brilliant". He was an excellent teacher/trainer in Microbiology and was happy to share his wealth of knowledge and expertise with his trainees, juniors and colleagues.

He was interested in several research and development activities including work on haemolytic streptococci and he established the streptococcal grouping protocols at the MRI. He also introduced the use of a single plate with blood and McConkey agar for urine culture and antibiotic sensitivity testing to facilitate fast reporting of microbiological data. He then moved on to work on more complex bacteria like *Corynebacterium haemolyticum*, *Legionella pneumophila* and *Bacillus anthracis*, the aetiological agent of anthrax. He published his work in many peer reviewed journals. His non-academic/non-microbiological interests included literature, history, geography, wild life, endangered species of turtle conservation and cricket.

He was a kind and compassionate human being, who embodied a calm composure. He practiced patience and tolerance in difficult situations and this trait made him an exemplary leader! I do not belong to his generation and thus I did not have any direct association with this exemplary human being! However, I started to admire the qualities of this extraordinary microbiologist of Sri Lanka form the stories I heard from those that received his training and had work associations with him. He passed away more than a decade ago but his legacy will stay with us – the admiration and gratitude we have for Dr Siri Wickremesinghe will continue.

Introduction

Dengue was considered as an 'urban' disease in the past and the epidemics occurred mainly in densely populated urban areas of the tropical world. At present in Sri Lanka and our most close neighbor India, this pattern has changed and the disease is now spreading to rural areas [1]. During the 19th century dengue was considered as a sporadic disease, causing epidemics at longer intervals. However, dramatic changes in the epidemic pattern has made dengue as the most important mosquito borne viral disease in the world [2] and dengue is now one of the leading causes of hospitalization and death among children and adults in most of the tropical countries [3]. The rapid increase in dengue virus (DENV) activity in India and Sri Lanka from 1999 to 2003 suggests its potential to cause even more severe epidemics in the future [2,3]. Although vector densities are higher in urban areas when compared to rural areas, vector densities in the latter are now seen to be increasing [4,5]. Thus the 'threat' of major dengue outbreaks in rural areas is imminent in the tropical dengue endemic regions including Sri Lanka.

DENV are mosquito-borne flaviviruses that have plagued humans for centuries. Urbanization and population growth in the tropical regions of the world have produced favorable conditions for DENV transmission [6]. *Aedes aegypti* and *Aedes albopictus* transmit DENV among humans [7]. Dengue has been endemic in Sri Lanka since the mid 1960s and the disease was serologically confirmed in the island in 1962 [8]. The presence of dengue in all of the major towns situated below 1200 m elevation was confirmed in 1966 and in 1976-1978 [9]. In the country, although the highest incidence is seen in the Western Province in recent times (Figure 1), there has been an increase in the disease incidence in all other provinces.

Dengue burden and dynamics in Sri Lanka

An estimated population of 2.5 billion people around the world are living in dengue endemic countries with a risk of contracting dengue. Nearly 75% of the current global disease burden is from the Southeast Asia region together with Western Pacific region. Cases occurred in epidemic proportions for the first time in Sri Lanka during 1965-1966 with sporadic cases of dengue haemorrhagic fever (DHF). Initially, the disease was mainly spread in the western coastal belt, but it was later found in other suburbs. In 1965, there was a dengue outbreak throughout the country with 51 cases and 15 deaths. After that, epidemics occurred in many parts of the country fairly regularly, in 1966, 1967, 1968, 1972, 1973 and 1976 and the number of cases reached a peak in 1988/1989 [8,9]. In 2002, Dengue/DHF was the third most common notifiable disease in Sri Lanka (1st and 2nd were malaria and tuberculosis) [10]. Sri Lanka obtained the malaria elimination in mid 2016. The availability specific anti-malarial drugs and relatively less mutations in the malaria parasites when compared to the RNA bound DENV and the malaria vectors, Anopheles mosquitoes being night biters collectively contributed to the effectiveness of malaria control programmes leading to the malaria elimination in the country [11].

On the other hand, dengue/DHF has become more established due to the absence of antiviral drug against DENV, lack of an effective vaccine despite many ongoing clinical trials, relatively less stable DENV, availability of susceptible populations to new DENV types/strains and the day biting DENV vector dynamics [11]. These factors collectively contributed to the establishment of DENV in the country, causing regular outbreaks [11]. At present, dengue/DHF are prevalent in many urban and semi-urban areas of Sri Lanka with seasonal and periodic epidemics occurring regularly in the island [12]. In recent decades a higher incidence of dengue occurs in the districts of Colombo, Gampaha, Kalutara, Kurunegala, Kegalle, Ratnapura, and Kandy [13]. From 2000 to 2008, the reported number of suspected and serologically positive dengue/DHF cases varied from 4749 to 15643, involving 25-88 deaths, with a major epidemic in 2004 [14]. Since the 30-year ethnic conflict in the country came to an end in 2009, dengue/DHF has become endemic in northern and eastern Sri Lanka [15] due to the open movement of people in both directions from the south to the north and east and vice versa; this open movement of people for trade and other purposes was absent or very limited prior to 2009 [16].

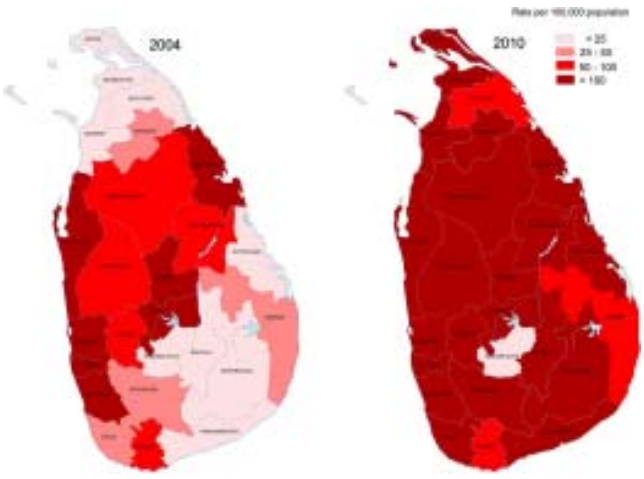


Figure 1. Comparison of temporal and spatial distribution of dengue cases in 2004 and 2010 [19].

Another important aspect of the epidemiology of dengue/DHF in Sri Lanka is the shift in the affected age group from children to adults; also, in many age groups, males have predominantly been affected. According to a regional study done by the WHO in Sri Lanka based on reported cases from 1996 to 2005, there were consistently and significantly larger proportions of males with dengue/DHF in those aged 15 years. This male preponderance was reported in many provinces of the country [11]. Among those aged 1-4 and 5-14 years, there were significantly fewer male cases than expected, although there was some annual variation [17]. The highest incidence occurred in the 5-9 years age group [8]. Before 2000, one large peak of dengue/DHF cases was observed in children and a few cases were observed in adults. After 2000, two reported dengue/DHF peaks were observed in children and young adults. Moreover, the mean age of reported dengue/DHF cases have increased from 15 years in 1996 to 25 years in 2006 [18]. Dengue/DHF epidemiology of Sri Lanka underwent a marked change between 1996 and 2005 at the provincial level. The proportion of reported dengue/DHF cases from the Western Province, in which the country's largest city, Colombo, is situated, decreased from 84% in 1999 to 37% in 2003 [18]. The age distribution of reported cases shifted from children aged less than 15 years making up more than 60% of cases in 1996-1999 to less than 40% of cases in 2001-2005 [17].

Spread and expansion of dengue vectors in the island

The mosquito *A. aegypti* has been thought to be the main vector responsible for virtually all dengue epidemics. *A. albopictus* has been considered as a vector in which the DENV is maintained but does not contribute to the viral transmission in epidemics. *Aedes* mosquitoes are primarily container breeders and they thrive in both clean and organically rich water in both natural and artificial containers [12]. An entomological study showed the presence and abundance of *A. albopictus* in many locations in all districts. Conversely, 100% of the DENV-positive *A. albopictus* pools highlight the importance of this vector in the transmission of DENV. The ability of *A. albopictus* to be infected with low virus loads and to permit replication has an impact on the maintenance and the transmission of DENV in the long run [12]. Co-circulation of two or more DENV serotypes in a single pool or in different pools of Aedes mosquitoes is suggestive of hyper-endemic transmission of DENV [20]. Thus there is evidence for the capability of *A. albopictus* as a vector in transmitting DENV in the absence of *A. aegypti*. *A. albopictus* is underrated in the transmission of DENV, especially during the peak transmission periods in the island [20]. Dengue cases have been encountered in areas where there is no *A. aegypti* breeding, but *A. albopictus* breeding is prevalent [21]. In Kandy, four species of Aedes larvae were collected from water storage tanks, with the majority being *A. albopictus* (41.05%) and *A. aegypti* (13.43%); the remaining tanks contained larvae of *A. macdougalli* (39.55%) and A. vittatus (5.97%) [14]. The greater susceptibility of *A. albopictus* to DENV infection is said to have led to greater DENV adaptation [20, 22].

A. aegypti and *A. albopictus* are widely adapted to urban and suburban environments in Sri Lanka [23]. Studies show the brackish water adaptation of *A. aegypti* and *A. albopictus* and their larvae and pupae survive to emerge as adults in brackish water collections. Brackish water habitats have been identified with Aedes larvae in the peri-urban areas of the country. The brackish water larval sites identified are located in popular beaches and in coastal areas closer to densely populated residential areas showing the potential role of brackish water adapted Aedes mosquitoes in DENV transmission [24]. The Batticaloa district situated in the dry zone of Sri Lanka is one of the districts badly affected by dengue in recent years. A seasonal shift in the density of the two Aedes species has been noted in Batticaloa. *A. aegypti* tends to predominate during the pre-monsoon season and *A. albopictus* during the monsoon season. Since the monsoon rains fall in the Batticaloa district from October to December, the density of *Aedes* increases during this period and a positive association between rainfall and *Aedes* density exists [25].

The application of vector control methods solely in freshwater habitats in the urban environment may select for genetic changes in the DENV favoring the development of *A. aegypti* and *A. albopictus* in artificial collections of brackish water in coastal urban areas may lead to vector adaptation to brackish water habitats in the future. Such changes could have serious consequences for the health of millions of people in many parts of the world, through a higher incidence of dengue, chikungunya urban yellow fever and now Zika fever, as these are transmitted through Aedes mosquitoes [26,27]. The shift in various aspects of epidemiology of has been seen in dengue endemic countries in the world including Sri Lanka.

Geo-socio-climatic factors on the spread and expansion of dengue

The Colombo District from the Western Province has been experiencing a very high dengue incidence and the highest number of cases reported from 2009-2016 was from this District. The major reason behind the high number of reported cases in the Colombo District appears to be due to the high population density [28,29]. A previous study [28] suggests an increase in the reported cases from Jaffna (NP) and Batticaloa (EP) and the total number of cases from these Districts might have dominated even the total reported cases from Colombo in 2010. One of the reasons for the increase in the cases from Jaffna and Batticaloa might also be increased population density in these districts [28]. Taken together these findings, an increased population density contributes to an increase in dengue incidence as noted in several highly populated Districts of Sri Lanka such as Colombo, Kandy, Jaffna and Batticaloa [29,30].

On the other hand, the dengue incidence is very low in the Nuwara Eliya District despite this being one of the highly populated District in the wet zone. High altitude seems to play a pivotal role in limiting the distribution of *A. aegypti* in the Nuwara Eliya District which is situated at 1880 m above the sea level. Badulla District is situated in the next highest elevation of 670 m and has a lower population density than Nuwara Eliya, yet reported high dengue incidence in the last 5 years. In India, *A. aegypti* breeding sites range from the sea level to 1000 m above the sea level. Lower elevations (<500 m) have moderate to heavy mosquito populations, while mountainous areas (>500 m) have low mosquito populations [31] supporting the inverse association between higher elevation and the vector activity and thus less dengue incidence as seen in Nuwara Eliya.

Tropical climate (temperature, rainfall, and humidity) favours the vector breeding and abundance. In addition climate change due to global warming expands the geographical range of vector mosquitoes, extend the disease transmission season, shorten the gonotrophic cycle and reduce the time taken for ingested viruses to develop to infective stages in mosquitoes, thereby increasing the propagation rates of arboviral diseases transmitted by *A. aegypti* and *A. albopictus* [27, 32-34].

There is a strong positive correlation between dengue/DHF outbreaks and the rainfall pattern, which increases the number of breeding habitats of Aedes vectors. In certain regions of the tropics where there are two annual rainy seasons, a positive correlation has been observed in only one season [35]. The impact of rainfall on adult vector density is not the same for all vector species. *A. aegypti* prefers indoor habitats, hence, it is less affected by rainfall than *A. albopictus* and other vectors that have outdoor larval habitats [6]. Two dengue/DHF peaks occur annually in association with the monsoon rains, when the densities of two mosquito vectors (*A. aegypti* and *A. albopictus*) are high in Sri Lanka. Generally, the first peak occurs in June/July, coinciding with the south-western monsoon that commences in late April. The second peak, comparatively a smaller one, usually occurs at the end of the year and is associated with the north-eastern monsoon rains that prevail from October to December [8,30,36].

Temperature is another important factor controlling the seasonality of dengue/DHF outbreaks in sub-tropical or temperate regions. It influences vector distribution, the blood feeding activity of the vector, the extrinsic incubation period and adult longevity. *A. aegypti* has been shown to transmit DENV when the temperature is above 20°C but not less than 16°C [11]. A positive correlation has been shown between the temperature and the female vector abundance. In addition, high temperatures may increase the frequency of blood feeding due to a rapid reduction in energy reserves [11]. It is expected that global warming may further facilitate the expanded distribution of DENV mosquito vectors to new areas and regions [11]. This concern has become serious with the expanding distribution of *A. albopictus* [11,30,37].

High humidity results from high rainfall combined with high temperatures. High humidity is associated with increased feeding activity, survival and development of eggs in *A. aegypti*. Moreover, the daily minimum temperature and an increase in the rainfall from the previous month were associated with increase in the larval abundance [38]. Thus there is a collective contribution of temperature and humidity on dengue outbreaks triggered by the feeding activity, survival and development of vectors [39].

Expansion and the evolution of DENV in Sri Lanka

The origin of DENV is reported to be African, with the distribution of DENV around the world due to the slave trade [40,41]. Currently scientists think that DENV probably had an Asian origin, which is supported by serosurveys conducted in rural communities of Malaysia in the early 1950s [41]. Biologically, DENV are highly adapted to their mosquito host and are maintained in the mosquito species responsible for forest cycles, with periodic amplification in lower primates [42]. In the past, due to the clearing of forests and the development of human settlements has contributed to the spread and expansion of DENV. On the other hand, migration of people and commerce have ultimately moved the DENV into the villages, towns and cities of tropical Asia including Sri Lanka [6].

The four distinct serotypes of DENV have been co-circulating in Sri Lanka for more than four decades and their distribution has not changed drastically in the last 30 years. Although the Sri Lankan population had been exposed to DENV for a long time, the severe forms of DENV infection (DHF/dengue shock syndrome (DSS)) were rare before 1989. Studies have shown the existence of more than one DENV serotype in many parts of the country. There was an island-wide dengue epidemic associated with DENV serotypes 1 and 2 from 1965 to 1968. This epidemic caused 51 DHF cases and 15 deaths [8]. DENV-1 and DENV-2 were isolated from the outbreaks in 1965 and 1966 [43]. A study conducted using mosquito pools in the Western and North-Western provinces of Sri Lanka, including the districts of Colombo, Gampaha, and Kurunegala, has indicated the circulation of multiple DENV serotypes within close proximity to each other [21]. Mosquito pools from Kurunegala district were positive for both DENV-2 and DENV-4, while mosquito pools from Gampaha and Colombo districts had DENV-2 and DENV-4. Higher numbers of positive pools of DENV-1 and DENV-4 have been reported in Kurunegala [21].

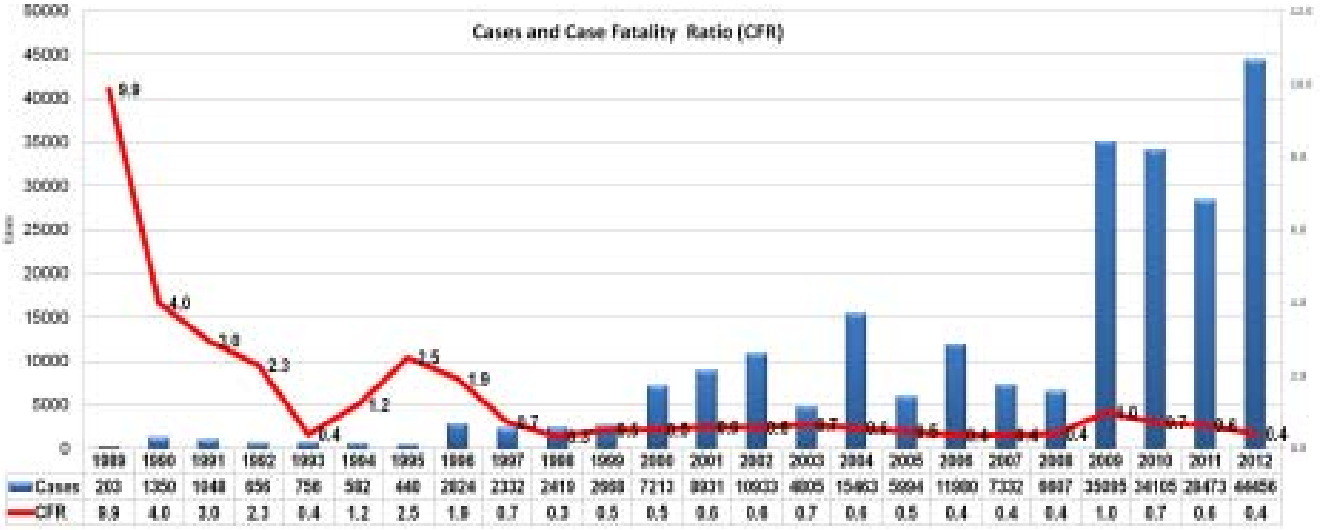


Figure 2. Number of dengue cases reported to the Epidemiology Unit of Sri Lanka from 1989 to 2012 with case fatality ratio indicated by the red line [46].

Another study performed between 2003 and 2006, indicate the circulation of the DENV-1 serotype in the Colombo district of Sri Lanka [18]. However, this study showed a change in the genetic characteristics of the DENV-1 serotype during the study period. The two isolates of DENV-1 serotype from Sri Lanka obtained in 1983 and 1984 belonged to the South Pacific genotype and it is believed that some time during the period 1984-1997, the Africa/America genotype of the DENV-1 serotype became established in Sri Lanka; this new genotype of the DENV-1 serotype continued to circulate through 2004. Moreover, the South Pacific genotype of the DENV-1 serotype has not been detected during the past decade in Sri Lanka [18].

In 2009, the largest epidemic of dengue/DHF occurred in Sri Lanka (35095 reported cases, 170 cases/100000 population, and 346 deaths) (Figure 2) and that outbreak was found to have been caused by a new strain of the DENV-1 serotype [44]. Results from DENV nucleic acid detection by reverse transcription (RT)-PCR in patients with DHF from August to December 2010 showed the predominance of the DENV-1 which accounted for more than 95% of dengue/DHF cases in the Western Province of Sri Lanka [45] in agreement with the observations of the Epidemiology Unit of Sri Lanka during that period. Hence, it appears that the serotype shift may have contributed in some way to the larger dengue/DHF outbreaks in the last 5 years in the country.

All DENV-2 isolates from Sri Lanka are closely related and belong to the Indian subcontinent/Malaysia genotype. There is also no evidence of any recent introduction of a DENV-2 strain from outside the island, because the DENV-2 strains from Sri Lanka are more closely related to one another than to any other DENV-2 strain [18].

DENV-3 strains from Sri Lanka isolated in the 1980s and 1990s belong to the Indian subcontinent genotype (III) [47,48]. Genotype III of the DENV-3 strains from Sri Lanka are divided into two distinct clades linked to mild (IIIA) and severe (IIIB) disease epidemics in the island [47]. Moreover, the DENV-3 strains from Sri Lanka isolated in 2003 and 2004 form a new distinct clade that is closely related but different from the DENV-3 clade IIIB viruses that were isolated in the 1990s. This new 2003/2004 clade includes an isolate from 1993, which strongly suggests that the clade is derived from strains that have been circulating on the island for some time [18]. Unlike group A viruses, the Sri Lankan group B viruses may be associated with severe disease, as the group B viruses are inherently more virulent. Alternatively, the ability of pre-existing antibodies against DENV to neutralize group A viruses but enhance group B viruses may account for the severe disease in group B DENV-3 infections but mild disease in group A DENV-3 re-infections. DENV-2 and DENV-3 are the common serotypes reported in many parts of Sri Lanka. Individuals with previous primary DENV-2 infections have been shown to neutralize the DENV-3 group A viruses better than the DENV-3 group B viruses, and this might be contributing to the severe disease in group B DENV-3 infections [49].

In Sri Lanka, regular epidemics of dengue/DHF have been observed only since 1989 (Table 1; Figure 2) and DENV-3 was responsible for many of the infections that progressed to DHF [50,51]. DENV-3 isolates obtained before and after the emergence of DHF are very closely related and belong to subtype III, indicating that the emergence of DHF in the island was not due to the introduction of a new subtype of DENV-3 from outside. During DENV surveillance studies in 1997, only DENV-

3 was isolated from hospitalized dengue patients whereas DENV-1, DENV-2 and DENV-3 were isolated from patients visiting outpatient clinics [52] suggesting the role of DENV-3 in severe dengue in the country.

The DENV-4 strain isolated in Sri Lanka in 1978 and in 2003/2004 was the Southeast Asian genotype indicating the circulation of this genotype in the island for decades. Two DENV-4 isolates from 1992 belong to the Indonesian genotype suggesting the transient introduction of this genotype to the island [18]

In 2003, DENV-1 and DENV-4 showed a genotype switch, which is not observed in the phylogeny of DENV-2 and DENV-3 [18]. Instead, new clades of DENV-3 genotype III viruses have replaced older clades, and DENV-2 also showed a similar trend. The emergence of new clades of DENV-3 in 1989 and 2000 coincided with an abrupt increase in the numbers of reported dengue cases [18] suggesting the contribution of this serotype in severe epidemics (Table 1).

Table 1. Reported number of dengue cases in Sri Lanka from 1965-2016 [53].

Year	Reported number of cases
1965-1989	A few hundred cases
1990-2001	A few thousand cases
2002	8931
2003	4672
2004	15463
2005	5994
2006	11980
2007	7332
2008	6607
2009	35095
2010	34188
2011	28473
2012	44461
2013	32063
2014	47502
2015	29777
2016	54945

Overall, the new clades of DENV-3 genotype III replaced older clades and the emergence these new clades in 1993/1996 coincided with an abrupt increase in the number of dengue/DHF cases. The new genotype of DENV-1 replaced an old genotype in 2004/2005, contributing to the increased dengue burden until late 2015. In 2016, Sri Lanka has experienced an unprecedented dengue outbreak affecting mainly the Western, Central and the Northern Provinces. In the first half of the year the country almost had the total number cases reported last year (Table 1). Interestingly, 2016 outbreak has been dominated by infections caused by DENV-2 and DENV-3 eliminating the dominance of the well established new genotype of DENV-1 which dominated the infections in the country for a decade.

Improvements in the diagnosis of dengue in the island

In the early 1960s, dengue cases were confirmed in Sri Lanka by antibody detection, either by anti-DENV IgM detection in a single serum sample or by demonstrating an increase in the anti-DENV antibody levels in paired sera.

At present dengue NS1 antigen and anti-DENV IgM detection are used in some state and many private laboratories for the diagnosis of DENV infection with a strong clinical suspicion. In many laboratories, rapid immuno-chromatography assays have been used to detect dengue NS1 antigen and anti-DENV IgM due to their simplicity and low cost. According to a study performed in the Sri Lankan population, the sensitivity and specificity of the NS1 antigen detection using the rapid immunochromatography assay ranged from 49 to 59% and from 93 to 99%, respectively [54].

The sensitivity and specificity of the rapid immuno-chromatography assay for detecting anti-DENV IgM antibody using the same study sample ranged from 71 to 80% and from 46 to 90%, respectively [54]. More recently, another Sri Lankan study [55] that compared a CDC approved ELISA and a different rapid immuno-chromatography assay also reported findings similar to that of [54] indicating the need for a central validation of the rapid immunochromatography assays used in the country [19].

A very few state and many big private laboratories use ELISA due to the availability of expertise and infra-structure in those laboratories. Big laboratories test large numbers of samples during the outbreaks and thus they are able to offer ELISA based testing.

PCR is done by some private laboratories for diagnostic purposes and overall use of PCR for the diagnosis of DENV infection is <10% in Sri Lanka [19]. On the other hand, PCR needs expertise and infrastructure with a high cost for reagents and high tech machineries. Despite its high specificity and sensitivity, the suitability of its use in the diagnostic laboratories for dengue has to be considered seriously as most of the time patients present to the hospitals post-viraemic or at the time when less viral numbers are present in the peripheral blood

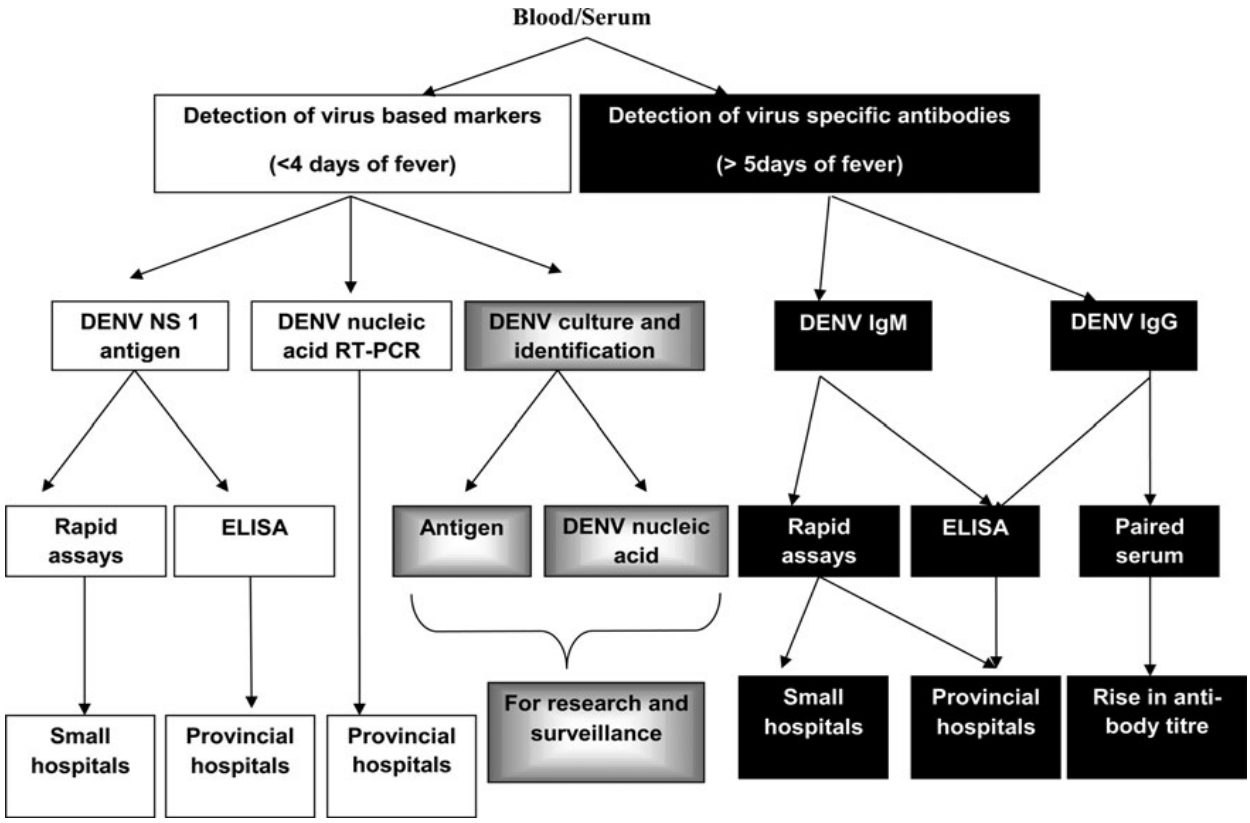


Figure 3. Laboratory diagnosis of DENV infection in patients with clinical diagnosis of dengue – a model algorithm proposed for Sri Lanka [19].

making the PCR based viral nucleic acid detection difficult [56]. In Sri Lanka, most of the time PCR is done using an already published protocol for research purposes. However, no data are available on the sensitivity and the specificity of PCR in the country for a diagnostic use. The absence of an internal positive control for DENV PCR for the island is a major drawback, however, not many diagnostic laboratories in the state sector are equipped to do PCR except the state funded Medical Research Institute (MRI) of the country which is based in the capital (Western Province) of Sri Lanka.

A recent review [19] proposes priority tests for a diagnostic algorithm (Figure 3) including the dengue NS1 antigen and anti-DENV IgM detection using the rapid immunochromatography assays for the smaller hospitals where laboratory facilities and infrastructure are relatively less than those in the provincial hospitals. For the latter, dengue NS1 antigen and anti-DENV IgM detection can be done using ELISA due to better laboratory facilities and infrastructure as well as the demand for testing.

DENV culture and identification and PCR for research institutes and universities for the surveillance and research. In the meantime if provincial hospitals become equipped with better laboratory facilities and infrastructure for performing PCR those laboratories can also contribute to DENV surveillance and research.

Prospects and new strategies in dengue control in the island

In Sri Lanka, dengue control efforts have been targeted at the disease and vector (Figure 4), including laboratory surveillance for DENV infections in patients and vectors, vector control, social mobilization, clinical management of dengue/DHF patients and the emergency response during outbreaks in terms of accelerated vector control and public awareness through the media. A national-level multidisciplinary task force on dengue/DHF has been established to govern the dengue/DHF control activities [30]. Furthermore, there are provincial and district level dengue/DHF control activities in place.

Training clinicians on clinical management has been carried out continually in an attempt to bring the dengue/DHF mortality to zero, or to a minimum level. Moreover, initiatives have been taken to start the vaccine trials and the success of this vaccine trail needs many years from the completion of the vaccinations to evaluate the protection against different DENV infections in the vaccinees. It is hoped that with the implementation of collective control programmes (Figure 4) in collaboration with other governmental and non-governmental organizations, with maximum cooperation from the community, the morbidity and mortality of dengue/DHF will be reduced in the near future.

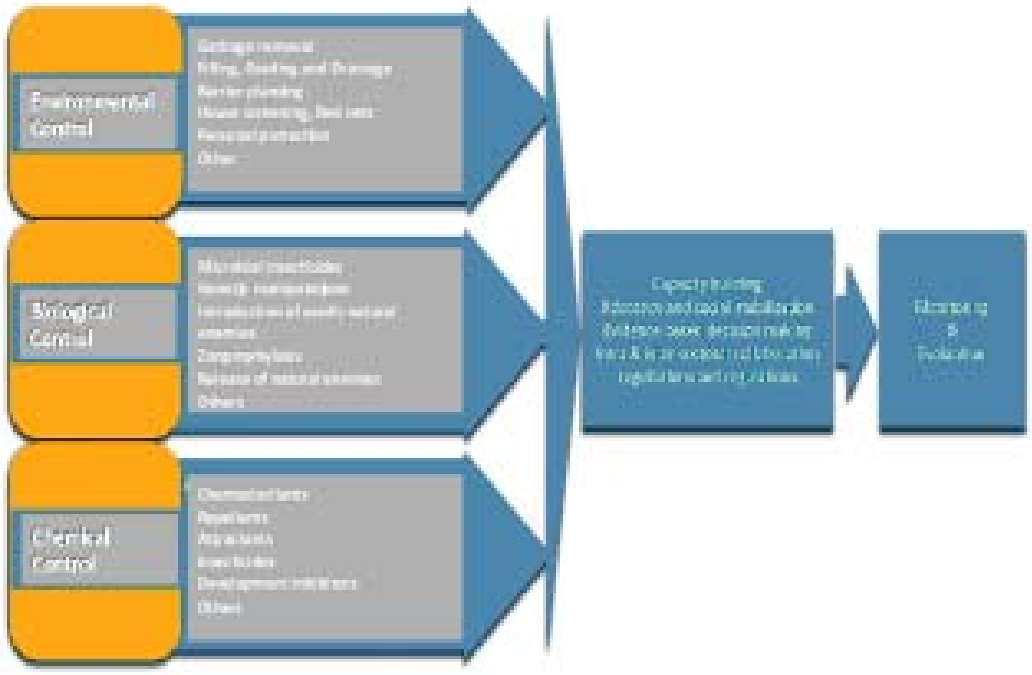


Figure 4. A proposed integrated vector control approach, involving different parties [30].

Conclusions

Despite the presence of dengue in Sri Lanka since the early 1960s, dengue has become a major public health issue causing high morbidity and mortality. *Aedes aegypti* and *Aedes albopictus* are the vectors responsible for the transmission of DENV in Sri Lanka. The four DENV serotypes have been co-circulating in Sri Lanka for more than 4 decades.

Dengue has become an established vector-borne infection in the island causing regular outbreaks with high incidence during the rainy months. The country has been taking vigorous vector control measures to reduce the dengue burden. However, the evolution of DENV in the island has been on the run despite the control measures due to population growth, developmental projects and movement of people to the cities contributing to the breeding and expansion of the vectors. Climatic factors and changes in those factors due to global warming are also accelerating the spread and expansion of the disease. The economic impact of dengue resulting from the expenditure on dengue critical care units and the cost of case management is also increasing and thus draining the country's developing economy.

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ALLERGIC BRONCHOPULMONARY ASPERGILLOSIS

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Summary

Allergic Bronchopulmonary Aspergillosis (ABPA) is non-invasive allergic response to *Aspergillus fumigatus*. The diagnosis is difficult, requiring a constellation of clinical, radiological and immunological criteria. Steroids and allergen avoidance is the mainstay of therapy. This entity should be suspected in any patient with difficult to control asthma or cystic fibrosis, as it is a reversible cause of bronchiectasis, if managed early in the disease process.

Introduction

Aspergillus is a ubiquitous organism, producing hardy, hydrophobic spores, which enables their wide dispersion, especially in dry climates. Therefore, many aspergillosis pulmonary syndromes exist, ranging from hypersensitivity syndromes to colonization of pulmonary cavities, to invasive disease. The host immune response plays a major role in determining the disease manifestation [1].

Allergic Bronchopulmonary Aspergillosis (ABPA) is caused by the body's immune response to sensitization by *Aspergillus fumigatus*. This is essentially a non-invasive hypersensitivity reaction, seen most commonly in patients with bronchial asthma and cystic fibrosis, with around 2% asthmatics and 1-15% with cystic fibrosis patients developing the disease [2]. The relentless distortion of lung architecture leads to deterioration of lung function. Clinically, this manifests as worsening of pre-existing symptoms, or development of new ones.

Immunology

ABPA manifests itself in patients with an atopic predisposition. The dendritic cells process the spores and germinated mycelia and present to the T-lymphocytes.

Out of the T-lymphocytes, the CD4 cells, specifically the type 2 helper T-cell play a major role in the pathogenesis of ABPA. Naïve T-cells may differentiate into distinct T cell subsets, the nature of which is determined by the inflammatory context induced by the pathogen. For example, early exposure of naïve T cells to IFN-γ and IL-12 at the time of priming will lead to differentiation to type 1 helper T-cells. These cells play a major role in the defence against intracellular bacteria. If naïve T cells are exposed to IL-4 at the time of priming, they will differentiate into type 2 helper T-cells. These type 2 helper T cells express many cytokines, which induce differentiation and antibody production of B-cells, increase mucous production and eosinophil recruitment. The type 2 helper T cell response predominates in atopic individuals, and is the main immune response seen ABPA [3].

Activated type 2 helper T cells release IL-4, which mediates B-cell class switching to IgE production. These antibodies, when bound to circulating *Aspergillus* antigen, form cross-links and release of inflammatory mediators from mast cells. In addition, IL-5 recruits eosinophils, which are found in large numbers in the mucous produced in the airways. The inflammatory mediators produced in the airways lead to bronchoconstriction and vasodilatation [2].

Due to the impaired clearance of *Aspergillus* spores from the airways, local inflammation occurs, resulting in increased mucous production. Hypersensitivity reactions, which may be type I or III, causes bronchospasm and bronchial wall oedema. The impacted mucous, together with fungal hyphae, occlude the airways, leading to segmental collapse. When this happens for a longer period, this may result in damage to bronchial wall, loss of muscle and cartilage, leading to saccular bronchiectasis, which is more pronounced in the upper lobes [2].

Cystic fibrosis

Cystic fibrosis (CF) occurs due to a single gene mutation, which is inherited in an autosomal recessive manner. It is mostly found in Caucasians, with incidence reaching 1:2500 in some populations. In this condition, increased sodium reabsorption and intracellular chloride retention leads to thick secretions. This impairs the proper clearance of respiratory secretions, causing chronic colonization by opportunistic pathogens, as well as persisting lung inflammation. For reasons which are still being elucidated, bronchial hyper-reactivity has been found in many CF patients. It has been hypothesized that CF patients may have heightened access to antigens due to enhanced permeability of respiratory mucosa, and defects in IgA secretion coupled with ciliary dysfunction. This may lead to increased and relentless IgE production. Additionally, due to thick secretions, inhaled fungal spores have opportunity to germinate. As hyphal elements

are significantly more allergenic than spores, this may contribute to sensitization. Finally, an atopic tendency increases the risk of ABPA in those with CF. It has been found that 22% of atopic individuals with CF developed ABPA, but only 2% of CF patients without a history of atopy developed the same [4].

Bronchial asthma

Current evidence suggests that a close association exists between fungal sensitization and the severity of asthma. Many fungal elements produced during germination of fungal spores may induce eosinophil-driven allergic airway disease. Others may damage the respiratory epithelium, allowing access of these allergens to sub-epithelial tissue. Therefore, they act in concert to enhance the host response and may prove detrimental in the long term [5].

Diagnostic criteria

The minimal diagnostic criteria are as follows [6].

1) The presence of asthma or CF, with deterioration of pulmonary function
2) Immediate reactivity in the *Aspergillus* skin test
3) Total serum IgE $\geq$ 1,000 IU/ml
4) Increased *Aspergillus* specific IgE and / or IgG antibodies
5) Infiltrates on chest radiographs

Essentially, diagnosis requires the demonstration of allergy to *A. fumigatus* antigen, such as positive *Aspergillus* skin test or *Aspergillus* specific IgE.

Sputum culture is nonspecific, as patients without ABPA may have a small number of spores present in their sputum. The presence of *Aspergillus* hyphae is more specific.

Apart from the above, other criteria are,

6) Peripheral blood eosinophilia
7) Positive *Aspergillus* precipitating antibodies
8) Central bronchiectasis
9) Mucous plugs containing *Aspergillus*

Radiological imaging must be undertaken in patients with possible ABPA. Initially, plain chest x-rays may be undertaken, which can demonstrate changes seen in asthma. Alternately, transient areas of patchy consolidation may be seen, which correspond to eosinophilic pneumonia. Later, bronchiectasis features may develop. Areas of mucous impaction may appear as sausage shaped or branching opacities (finger-in-glove appearance), which may eventually lead to areas of lung collapse. However, early in the disease, chest x-rays may appear completely normal. Additional features which may be noted in CT imaging can be bronchial wall thickening, cavitation and fibrosis [7].

Diagnosis of ABPA is difficult, more so due to the lack of a gold standard. Additionally, many serological tests used have been hampered by the lack of an optimum cut-off value.

The patient may complain of new onset or worsening cough or wheeze. There may be expectoration of increased volumes of sputum. He may produce viscid and tenacious mucus plugs, which may be light brown to brownish-black in colour. There may be haemoptysis, although coughing up frank blood is rare [2].

Chest roentgenography may reveal a myriad of signs. There may be fleeting parenchymal shadows, which may even be mistaken for infective pneumonia. They may be due to mucus plugs, lobar collapse, bronchoceles or atelectasis. In addition, there may be features to suggest bronchiectasis. The full spectrum of radiographic changes is best appreciated on high resolution CT imaging, which may show mucus plugging, atelectasis, fibrosis and bronchiectasis. The presence of multiple lobes with central bronchiectasis is highly suggestive of ABPA [2].

Problems faced in diagnosis

There are few studies estimating the prevalence of ABPA, and none in Sri Lanka thus far. The reasons are many-fold. The diagnosis of ABPA requires a combination of clinical, radiological and serological tests. In developing nations, these tests are often unavailable outside of reference centres, and even then, are unaffordable to the masses. In addition, diagnosis requires specialist referral, which may be delayed and inadequately followed through.

Despite these obstacles, early diagnosis and management of ABPA is crucial, to avert irreversible sequelae of lung fibrosis and bronchiectasis, and subsequent respiratory failure.

It has been estimated that the prevalence of asthma globally is 193 million. In the USA, it was found, that in 2009, out of the adult population, 8.2% suffered from asthma. In contrast, Scotland, with 16% of its adults suffering from asthma in 2003-4, has the largest percentage worldwide. Closer to home, in the same study, it has been estimated that India, Pakistan and Bangladesh have a prevalence of asthma of 1.8%, 2.3% and 2.2% respectively [8].

According to the above, it has been estimated that, globally, 4.8 million of its population suffered from ABPA. This data has been derived, assuming that 0.7% to 3.5% of asthmatics developed ABPA, with a mean of 2.5%.

Using the same model, a study in India in 2011 has estimated the prevalence of asthma as nearly 24 million out of the total population of 1.2 billion. ABPA was estimated to complicate asthma in nearly 600,000 patients [9].

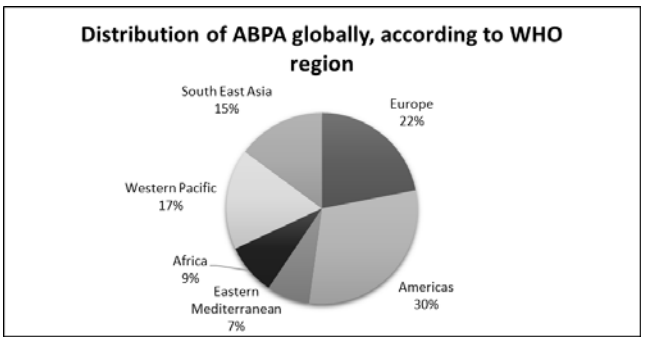

Figure 1. Global ABPA distribution [8].

The incidence of ABPA among patients with bronchiectasis was determined in a population based study in the UK, between 2004 to 2013. It was found that ABPA was present as a co-morbid condition in only 1.8% of the patients [10].

In contrast, a study in India has concluded that, the prevalence of ABPA among out-patients with bronchial asthma was 12.9%, and this increased to 39% in patients admitted to the respiratory ICU with a diagnosis of acute severe asthma [11].

In Sri Lanka, asthma is diagnosed in 2.75% of the adult population (nearly half a million adults). Using the international mean rate of 2.5% of asthmatics suffering from ABPA, this would mean that over 10,000 people should be having ABPA in Sri Lanka [12].

This highlights several issues with diagnosis of ABPA. Even though it is known that development of ABPA leads to worsening of asthma symptoms, there is a dearth of a standardized protocol to screen all asthma patients. In addition, many patients with ABPA may give positive results to some of the tests, but not to others, depending on the stage of their disease.

Management

The goals of therapy are to manage the disease process of asthma /CF, as well as environmental control, in order to minimize exposure to spores. Early and effective management can prevent disease progression and irreversible lung destruction. Specific management depends on the stage of disease at diagnosis [13].

Stage 1: acute stage

These patients are symptomatic, with cough, increased sputum production, fever or haemoptysis. There can be lung infiltrates in the upper or middle lobes and an elevated serum IgE. They should be treated with oral prednisolone, which should be tapered over 6-8 weeks. Monitoring for response should be by way of radiological imaging and serum IgE measurement.

Stage 2: remission

These patients are either asymptomatic, or have stable asthma for more than 6 months, after stopping steroids. Therapy at this point involves management of the underlying asthma / CF.

Stage 3: exacerbation

These patients have re-appearance of clinical symptoms, or a rise in serum IgE to more than 100% of baseline on routine monitoring. They are managed the same as acute stage of ABPA.

Stage 4: steroid dependant asthma

These patients cannot be weaned off steroids, due to exacerbation of asthma symptoms or persistence of increased serum IgE levels. They have to be prescribed daily oral steroids indefinitely.

Stage 5: end-stage fibrosis

These patients may be dyspnoeic or cyanotic, and have cavitatory, fibrotic or bullous lesions on imaging. They too have to be continued indefinitely on oral steroids.

Other management issues

Antifungal drugs are not considered the primary treatment modality, but can be used as a steroid sparing agent. The rationale is that, eliminating the offending agent from the airways may modify the hypersensitivity reaction to the fungus, and thus, the disease process. The azole antifungal drugs are effective against *A. fumigatus*, with itraconazole used most commonly, with its favourable adverse effects profile, availability and cost.

Environmental modification should be prioritized, along with patient counselling. Avoidance of areas with high bio-burden of *A. fumigatus* spores, such as dead and decaying organic matter should be encouraged. The patients' living quarters and workspace should be inspected for dampness which may encourage moulds.

Conclusion

ABPA is a difficult disease to diagnose. However, with increased awareness among practitioners, early diagnosis and effective therapy should lead to better therapeutic outcomes.

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Review article:

CONIDIOBOLOMYCOSIS – A REVIEW

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Introduction

Conidiobolomycosis is a chronic, localized subcutaneous infection [1,8]. This infection is caused by a fungus belonging to the genus *Conidiobolus* within the order Entomophthorales and in the class Zygomycetes [1]. The disease frequently presents as a painless hard swelling of the rhino-facial region of immunocompetent patients in tropical countries [8]. However the disseminated form of conidiobolomycosis has been reported rarely among both immunocompetent and immunocompromized patient populations [2, 8, 9]. A high degree of clinical suspicion is required for early diagnosis and treatment of the condition which is associated with severe facial disfigurement.

This paper reviews the mycological aspect, epidemiology, clinical climanifestations, diagnosis and treatment of conidiobolomycosis.

Mycology

The fungi of the class zygomycetes produce predominantly aseptate or pauci-septate, wide and irregularly branching ribbon like hyphe [3]. This class is divided in to two orders, Mucorales and Entomo-phthorales [1,3]. The fungi of the two orders have dramatically different clinical presentations [3,6].

When compared with the acute, fulminant, angio-invasive nature of infections in immunocompromized patients due to the order Mucorales, fungi of the order Entomophthorales results in chronic, indolent subcutaneous infections in immunocompetent patients [3,6].

The order Entomophthorales includes two histopathologically similar, but clinically and mycologicaly distinct genera namely, *conidiobolus* and *basidiobolus* [1,2]. Both conidiobolus and basidiobolus infections are associated with granulomatous inflammation [1,3]. However infections due to the genus *Basidiobolus* involve the trunk and limbs while genus *Conidiobolus* primarily affects the rhino-facial area [1,9].

Epidemiology

The first human case of conidiobolomycosis was reported from Indonesia in 1960 [1]. Since then it has been reported worldwide but mainly from tropical and subtropical countries like West Africa, Central Africa, America and countries in South East Asia including Sri Lanka [1-6, 8,10,11].

Conidiobolus spp. are saprobes distributed in decomposing plant matter, soil and rotting vegetation in moist warm climates [2,8,]. *Conidibolous* spp. has also been

isolated from the gastrointestinal tracts and faeces of lizards, frogs, reptiles and other animals [2, 7].

Of 27 identified species, *Conidiobolus coronatus*, *C. incongruus* and *C. lamprauges* are known to infect humans [2,7].

Adult males in the 20-50 year age group, who engage in outdoor activities or outdoor occupations are more affected [1,8]. This infection is uncommon among children in contrast to basidiobolomycosis [2].

Eventhough the exact mode of transmission has not been clearly established, the predilection of the organism to infect the head and face suggests that the inhalation of spores and introduction of them in to the nasal cavity by soiled hands could be the most probable routes of infection [1,2,8].

Although most infections have been reported from healthy patients, the disseminated form of the disease has been reported among the immunocompromised population eg. Patients with lymphoma, renal transplant recipients etc. [7].

It is also a zoonotic infection and it can parasitize certain insects, frogs, horses, dolphins, chimpanzees and sheep [2,7,8]. Especially, *C. lamprauges* has been identified as the causative agent of nasopharyngeal infection in horses and sheep [4].

Clinical manifestations

This rare subcutaneous mycosis is associated with chronic granulomatous inflammation of upper-respiratory mucosa and adjacent subcutaneous tissues [1].

Affected individuals are usually immunocompetent and otherwise healthy individuals [1].

Localized chronic rhino-facial mycosis is the most common clinical presentation of human infection with *Conidiobolus* species [1].

The disease begins from inferior turbinate, and spreads to all adjacent structures, including the paranasal sinus, and to the subcutaneous tissue of the forehead, periorbital region and upper lip [1,2,8]. It will present as a progressive swelling over the nose, perinasal tissue, eyelids, periorbital region, upper lip and over the malar regions of the face [6,7]. Affected patient may experience chronic symptoms of epistaxis, nasal polyposis, nasal block, nasal discharge and chronic sinusitis [1,6]. Firm, painless subcutaneous nodules appears as the infection spreads and these nodules are palpable through the skin [7, 8]. These lesions are firmly attached to the underlying tissue sparing the bone while overlying skin remains intact (7,8). Most of the long term untreated conidiobolomycosis patients may present with severe facial disfigurement [8].

Rare cases with involvement of nasolacrimal duct, orbit and contiguous lymph nodes have been reported [2,6]. Involvement of pharynx and larynx with extensive destruction, results in dysphagia and laryngeal obstruction. Chronic lymphoedema is another uncommon clinical presentation [7].

Distant dissemination is not a common feature of conidiobolomycosis, however disseminated infection have been reported rarely [1].

Disseminated cases with fatal outcomes are seen among both in immunocompetent and immunocompromised patients, such as patients with lymphocytic lymphoma and in renal transplant recipients [6]. Cases of disseminated *Conidiobolus* infections with endocarditis, pericarditis, vasculitis and pulmonary involvement have been reported [6, 7].

In such patients, granulomatous reactions have been detected in lymph nodes, esophagus, liver, jejunum, pericardiam, endocardium, lung, kidneys, skeletal muscles, and brain [6,7,8]. Disseminated patients with fatal outcomes have been clinically similar to mucormycosis due to their acute presentation and angio-invasiveness[7].

Diagnosis

With the characteristic clinical features of chronic subcutaneous rhino-facial mycoses present, biopsy of subcutaneous or sub mucosal tissue is required to establish the diagnosis [7].

The direct smear, and fungal culture and histopathologic demonstration of the etiologic agent, are required for the definitive diagnosis [8]. The direct smear with 10% potassium hydroxide of the biopsy tissue from the lesion reveals broad, non-septate or infrequently septate, thin-walled fungal filaments along with infiammatory cells [8]. The standard mycology medium, including Sabouraud's Dextrose, potato dextrose and cornmeal agars provide satisfactory growth of *Conidiobolus* spp. [7,8]. They have relatively fast growth and their growth is more rapid at 37°C. Colonies demonstrate a white obverse view and pale reverse view initially. However their surface will turn into beige to brown with time. *Conidiobolus* spp. demonstrates flat, waxy colonies with folds and become powdery with age. Forcibly discharged conidia can stick to the lid of the petri dish giving cloudy appearance in older cultures. These expelled spores may land on adjacent medium producing characteristic satellite colonies [4,7].

The microscopic appearance of the culture isolate with lactophenol cotton blue, demonstrates more or less septate broad, thin hyaline hyphae. The short, erect, unbranched conidiophores (sporangiophores) produce single-celled (one-spored) large conidia (25 to 45 µm in diameter). These conidia are round to pyriform in shape and have prominent papillae on the wall. A conidium of *C. coronatus* also produce hair like appendages called

villae. However, unlike *C. coronatus*, *C. incongruus* does not produce villous sporangioles [7].

The histopathologic examination of the tissue section stained with H&E will show broad thin walled hyphae encased in eosinophilic sleeves (Splendore-Hoeppli phenomenon) and fungal elements are well demonstrated than PAS or GMS stain [6,7,8]. Thin fungal hyphal fragments phagocytosed within the giant cells are seen along with chronic granulomatous inflammatory reaction [1,6,8]. However both acute and chronic tissue reactions are observed and the inflammatory infiltrate will contain eosinophils, histiocytes, neutrophils, lymphocytes, plasma cells and giant cells [6]. Histopathologic evidence of angio-invasiveness is observed very rarely with conidiobolomycosis [7].

The detection of β-D glucan in serum are often less useful in infections with zygomycetes and Cryptococcus species because these fungi have lower β-D glucan content [4].

However positive serum β-D glucan and its increased level, along with the burden of infection have been detected in certain reported cases [4].

Other than above mentioned diagnostic evidence, patient may show peripheral leucocytosis and eosinophilia [6].

Treatment

The delayed diagnosis of the conidiobolomycosis results in difficulties in the management [8].

The choice of the best therapeutic agent for conidiobolomycosis, its dosage and duration remain unclear [2].

Different antifungals like miconazole, clotrimazole, ketoconazole, itraconazole, amphotericin B and terbinafine are used in treatment with variable success [6,7].

According to recent antifungal susceptibility studies, multidrug resistant *Conidiobolus* spp. have been identified and these explain the therapeutic failure among the infected patients [4]. For example, the geometric mean MIC values for *Conidiobolus* spp. obtained by the susceptibility testing performed by Guarro et al on *Conidiobolus* spp. isolates are as follows: itraconazole, 11.3 l µg / ml; ketconazole, 20.7 l µg / ml; miconazole 11.3 l µg / ml; amphotericin B, 3.1 l µg / ml; fluconazole, 107.5 l µg / ml; and flucytosine, 234.6 l µg / ml [6]. None of the single antifungal agents have showed reliable consistent antifungal activity towards conidiobolomycosis and in-vitro susceptibility testing may be helpful in guiding therapy [6].

According to reports this condition could respond to oral itraconazole (200-400 mg/day), ketoconazole (200-400 mg/day), fluconazole (100-200 mg/day), amphotericin-B and clotrimazole [1,8].

Both itraconazole and potassium iodide seem reasonable first line choices for Conidiobolomycosis [6]. Itraconazole is an effective and relatively safe antifungal drug giving satisfactory results [8].

Although the exact mechanism of its action is not known, potassium iodide is traditionally used because of its ease of administration and low cost [7,8]. A good success rate of treatment with potassium iodide at 40 mg /kg / day has been reported in some cases [6].

Poor success rate has been observed with clotrimazole, amphotericin-B and amphotericin-B with flucytosine against disseminated infection [4]. Antifungal sensitivity for the amphotericin B has demonstrated both sensitive and resistant patterns [7]. Clinical failure has been reported with amphotericin B and it is usually not considered as the first choice of treatment [6,7]. Inhibition of the organism requires in vitro high doses of trimethoprim-sulfamethoxazole for the effective treatment limiting its use [7].

Favorable in vivo responses to combination therapy have been reported in the literature [7]. In the cases of failed single-drug therapy, the combination therapy of amphotericin B and terbinafine, sulfamethoxazole-trimethoprim and potassium iodide, ketoconazole and saturated potassium iodide and itraconazole and terbinafine have been reported to be effective [7].

Successful outcomes with the combination of itraconazole and terbinafine for the treatment of rhino-facial *C. coronatus* infection, have been reported even with in vitro susceptibility testing of the isolate revealing resistance to both drugs [4]. It is reported that the combination therapy with azoles and potassium iodide also give rapid and lasting results [8].

Since surgical resection may enhance the spread of infection it is not usually recommended [8]. However surgical debridement of paranasal sinuses followed by antifungal therapy has been proved to be effective in some case reports [1,7]. According to most case reports cryotherapy has shown little success [8].

There is no recommended duration for the antifungal treatment [8]. However treatment must be continued for long periods until a negative mycological examination and a good clinical response is achieved [6,7,8]. Some authors suggest that treatment should be continued for at least 1 month after the lesions have cleared [7]. Even after successful treatment, relapses are common [8].

Conclusion

Conidiobolomycosis is a chronic, localized rhino-facial subcutaneous mycosis affecting both immunocompetent and immunocompromized patients, due to fungi of genus *Conidiobolus*. Disseminated and fatal conidiobolomycosis

cases have been reported rarely. The therapy refractory nature of conidiobolomycosis often requires long term antifungal treatment. Awareness of the existence of this clinical entity is required for the early and accurate diagnosis, as early intervention will reduce the morbidity.

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AN ANALYSIS OF ACCIDENTAL EXPOSURES OF HEALTH CARE WORKERS TO BLOOD AND BLOOD STAINED BODY FLUIDS IN A TERTIARY CARE HOSPITAL IN SOUTHERN SRI LANKA

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Background

Needle stick injuries (NSI) pose a significant risk of occupational acquisition of blood borne pathogens such as human immunodeficiency virus (HIV), hepatitis B virus (HBV) and hepatitis C virus (HCV) to health care workers (HCWs) due to accidental exposure to infected blood and body fluids. NSI present the single greatest risk to medical personnel [1].

The first HIV seroconversion in a HCW was reported in 1984 [2]. EPInet data for 2003 reports a rate of approximately 27 NSIs per 100 beds in teaching hospitals [3]. It is estimated that approximately 3 million HCWs experience percutaneous exposure to blood borne viruses each year. This results in an estimated 16,000 hepatitis C, 66,000 hepatitis B and 200-5000 HIV infections annually [4].

This study was undertaken to review the epidemiology of NSIs and to determine the risk factors and the population at risk in a tertiary care teaching hospital, which has staff and trainees of varying levels of experience.

Materials and Methods

The study hospital is a 1680 bed tertiary care hospital that serves as the teaching hospital for colleges of medicine and nursing. The Staff Student Health Service has maintained a NSI register since 2008 and protocols for management and follow-up of NSIs have been established.

All Health Care Workers (HCW) were instructed to report any NSI to the infection control unit after performing basic first aid. We carefully documented the NSI and implemented any required post-exposure prophylaxis (PEP).

Serum samples were collected from all patients involved to identify sero status regarding HBV, HCV, and HIV and no positives were detected. If the HCW had completed hepatitis B immunization, blood samples were taken to check anti-HBsAb levels. If the source was HBsAg positive, HCWs were given a hepatitis B immunization booster. If the HCW was anti-HBsAb negative hepatitis B vaccine was administered. For HCWs who sustained injuries from HIV positive or highly suspicious sources, immediate post exposure therapy was started. Clinical follow-up was done after three and six months of exposure with serology in high risk cases.

Results

We report a two and a half year (2011.01.01 to 2013.05.31) retrospective surveillance study where 145 HCWs sustained NSI (Figure 1). Of these, 108 (74.4%) were reported from medical wards and 37 (25.5%) from surgical wards. Of 145 injuries, only 122 (84.13%) were from known sources.

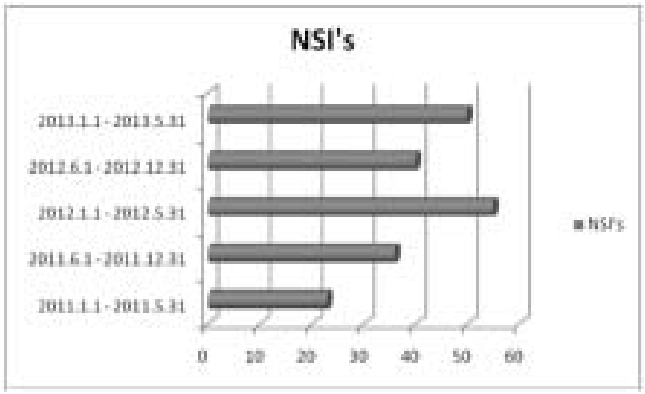

Figure 1. of needle stick injuries sustained by healthcare workers year wise.

The composition of the 145 HCWs reporting NSIs were 56 (38.6%) nurses, 20 (13.8%) doctors, 48 (33.1%) training students (medical and nursing), 17 (11.7%) minor staff, and 4 (2.7%) workers in the cleaning service.

Training students (nursing and medical trainees) comprised a significantly larger proportion of staff sustaining NSIs ($p < 0.001$).

Evaluation of the kind of activity during which the NSI occurred showed that most occurred during procedures 81 (55.7%). The most common procedure was blood

collection 70 (48.2%) followed by surgical procedures 11 (7.5%). A large proportion occurred because of incorrect handling of sharps such as improper disposal / overflowing containers which accounted for 43 (29.6%) of the NSI and recapping of needles 19 (13.1%).

Out of the 145 HCWs, 85 (58.6%) had completed 3 doses of HBs vaccine. Only 25 checked their anti-HBsAb status and of them 23 were anti-HBsAb positive and two were negative after 3 doses of vaccine. Subsequent 6-month follow-up for human immunodeficiency virus infection showed zero seroconversion in the 6 cases tested.

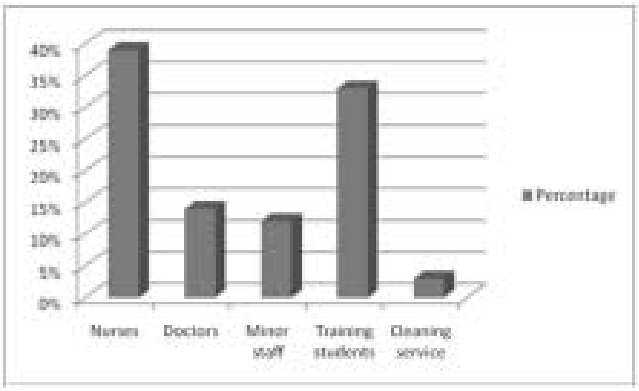

Figure 2. Percentage distribution of healthcare workers sustaining needle stick injuries.

Discussion

With the ongoing HIV epidemic, occupational exposures to HIV infection are a cause of concern to all HCWs, especially those in hospitals. The risk of HIV infection after single percutaneous exposure has been calculated to be 0.3% by Ippolito et al. [5] and CDC study 0.42% [6].

Trainees (nursing and medical) form a small proportion of the medical or nursing staff in a teaching hospital but accounted for a large proportion 48 (33.1%) of the NSI. This may be a reflection of the larger number of exposure-prone procedures conducted by these categories of staff or their inexperience.

This data emphasizes that improper handling and disposal of sharps and a lack of adherence to standard procedures are responsible for the majority of NSIs. This points to the need for increased and continuing education on the use of universal precautions and standard procedures in all categories of staff because most NSI occurred in staff who did not follow written protocols.

In the absence of active surveillance, it is not possible to define whether the perceived increase in NSI is a true finding or is due to increased reporting. It is also important to consider that there may be significant under-reporting of NSI, with reporting being more likely if the index patient is a known positive for blood borne virus infection, but less likely if the index patient is not known to be positive.

Six-month follow-up in HCWs offered PEP showed zero HIV seroconversion which is similar to the other studies in India [7,8].

Conclusion

No case of HIV seroconversion has taken place, so far, as a result of needle stick injuries at our hospital. It is important to provide guidance and measures to reduce accidental exposures while encouraging reporting of incidents.

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HOW TO ENSURE CORRECT THERAPY WITH GENTAMICIN? AN ANALYSIS OF THE PRESENT SITUATION, TO IMPLEMENT THE MODIFICATIONS

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Introduction

Gentamicin is a first line aminoglycoside which is widely used in the treatment of infections caused by Gram negative aerobes. Unique pharmacokinetic and pharmacodynamic properties of this group of antibiotics are responsible for most of the side effects associated with their use. Aminoglycosides are well known to cause ototoxicity, nephrotoxicity and neurotoxicity. They are also known to be frequently associated with prolonged treatment duration and incorrect drug level monitoring [1].

Due to the poor distribution of gentamicin in adipose tissues it can easily get overdosed, especially in obese patients. Therefore corrected body weight (CBW) should be used for dosing calculations for obese patients, rather than ideal (IBW) or actual body weight (ABW) [2].

CBW = IBW + 0.4 (ABW - IBW)

Narrow therapeutic window between effective therapeutic dose and toxic dose of gentamicin has also led to the low threshold for being overdosed. In the presence of already existing renal impairment and even in patients without pre-existing renal disease, haphazard therapy with gentamicin can end up in toxicity. Therefore prescription of gentamicin has to be done with extreme care. Safe and effective therapy with the antimicrobial gentamicin requires good practice in dose selection and monitoring of serum levels.

One important intervention to minimize medication errors due to high-risk drugs is using clinical decision support tools (CDS) [3]. A pre-post intervention study carried out in National Health Service teaching hospital in London has shown that implementing gentamicin dose calculator, a CDS tool has improved gentamicin dosing [4].

Monitoring of serum gentamicin levels is done using validated nomograms like Urban and Craig (UC) or Hartford nomogram. If once daily 5mg/kg dose is used, the 'Urban-Craig nomogram' can be applied to monitor and interpret gentamicin levels [5].

However in Lancashire Teaching Hospital Trust (LTHTR) where this study was carried out, gentamicin is prescribed as 5mg/kg per day and they were not using the online gentamicin dose calculator. Monitoring of drug levels was according to a nomogram that has been developed by the microbiology unit of the hospital, hoping to avoid the practical difficulties associated with the above mentioned standard curves.

Considering the importance of ensuring safe prescription practice with regard to gentamicin, this audit was carried out.

Objectives

- To assess the gentamicin dosing accuracy
- To compare the hospital nomogram with the Urban and Craig (UC) nomogram and to assess the practicality of implementing UC nomogram.

Methodology

Study design

A pre-intervention study was carried out in 897-bed LTHTR consisting 2 major hospitals; Royal Preston Hospital which is a tertiary-care center and the major Trauma Center in North West of England and Chorley District General Hospital.

Information regarding prescription of gentamicin was collected prospectively for five months from March, 2015. The study was categorized as a clinical audit by the hospital. Therefore ethical approval was not required. The study was registered with the Audit Department.

Inclusion / exclusion criteria

Adults on 5mg/kg once daily gentamicin dosing regimen were included.

Patients on different dosing regimens, paediatric and pregnant patients, patients in critical care unit and patients undergoing dialysis were excluded.

We picked patients, using the gentamicin level results from the biochemistry laboratory. Patient demographic data, information on gentamicin therapy and investigation results of renal function were obtained using electronic records and paper notes of patients. Date, time and dose of gentamicin given and date and time of collecting

blood sample for gentamicin level and the level of gentamicin were noted.

Correct doses of gentamicin were calculated using online gentamicin calculator, which calculates the gentamicin dose using patient's CBW. Any given gentamicin dose not within +/- 10% of the calculated dose, was considered incorrect.

Evaluation criteria for gentamicin levels

The investigators determined the safe, intermediate and toxic levels of gentamicin separately using the hospital nomogram and the UC nomogram depending on the number of post dose hours at which blood samples were collected for levels. According to the standard UC nomogram (Fig. 1) gentamicin levels have to be checked 6 to 14 hours after starting the gentamicin infusion. Hospital nomogram (Fig. 2) allows gentamicin levels to be checked in 12 to 24 hours post dose.

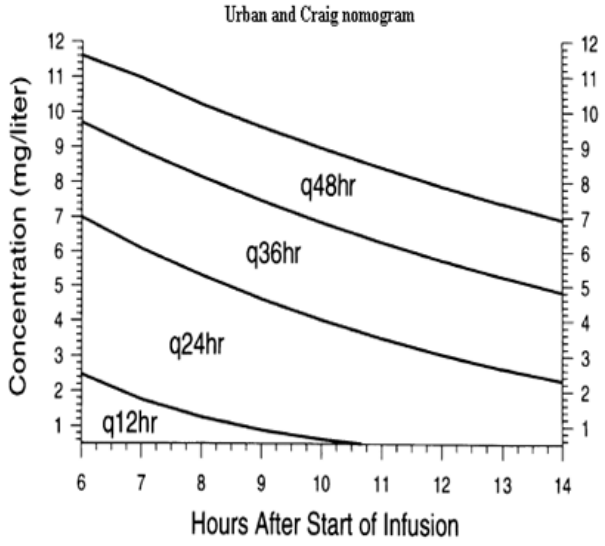

Figure 1. Urban and Craig nomogram.

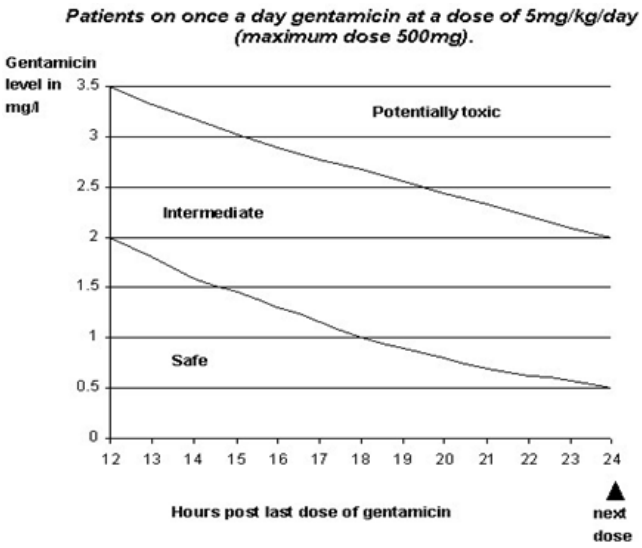

Figure 2. Hospital nomogram.

Episodes with toxic levels of gentamicin in blood were investigated further with estimated glomerular filtration rate (eGFR) after 7 days of dosing, to see whether renal function is deteriorated compared to that of pre-dose.

Results

Patient characteristics and description of observations

In total, 116 patients were studied including 59 males. The median age of the study group was 57 years. About 46% (n=53) of the population were above 65 years. Among 164 gentamicin doses studied, 200 episodes of gentamicin level monitoring were noted. Out of 200 observations, in 70% (n=139) timing of level, fit at least into one of the nomograms. While 91% (n=127) of the blood samples have been taken within 12 to 24 hours post dose, 36% (n=51) have been checked within 6 to 14 hours post dose. Less than 1/3 of (n=39) measurements are shared by both nomograms having being done between 12 to 14 hours. Almost half of the blood samples were drawn between 9 AM to 12 N, when the phlebotomists are on normal duty.

When the blood gentamicin levels were analyzed according to the hospital nomogram, 64.6% (n=82), 25.2% (n=32) and 10.2% (n=13) of the levels were within safe, intermediate and toxic range, whereas according to UC nomogram 86% (n=43) and 14% (n=7) of the levels were in safe and toxic range (Figure 3).

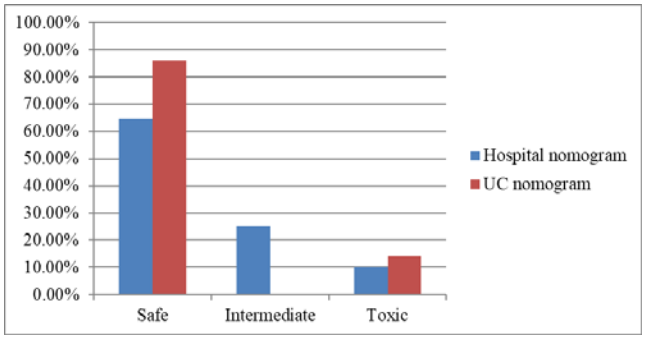

Figure 3. Analysis of blood levels of gentamicin using 'hospital nomogram' and the UC nomogram.

Analysis of dose selection of gentamicin

Stat doses of gentamicin was noted in 1/3 (n=34) patients. Others received as regular once daily dosing. When compared with the online calculator 56% (92/164) of gentamicin doses were incorrect; 41 underdosing and 51 overdosing. Incorrect dosing was high among obese patients; 55.43% (51/92).

Effect of overdosing and toxic gentamicin levels on eGFR and renal function

There were 16 occasions with toxic levels. However, only 37.5% (n=6) coincided with overdosing. However, none were associated with significant deterioration of eGFR, and hence not associated with renal toxicity.

Discussion and conclusions

Interventions like these help minimize prescription errors of gentamicin by addressing potentially amenable factors like dosing and monitoring. It was found that more than half of the gentamicin doses were incorrect. Therefore the value of implementing the online dose calculator in LTHTR, was re-emphasized in our study.

With the online calculator, we will have to treat our patients with a higher dose of gentamicin than at present, as evident by the present study. Therefore it was decided to monitor levels according to a standard nomogram, despite the fact that hospital nomogram was not found to be associated with any additional harm to the patients. However, we also proved the practical difficulty in sampling blood for gentamicin levels, out of normal duty hours of phlebotomists. Literature showed that other hospitals in National Health System of UK also follow one or combination of the following methods; UC nomogram, Hartford nomogram or monitoring trough level of gentamicin [6,7]. Therefore it was recommended to implement the standard UC nomogram, (measuring levels from 6 -14 hours after the start of the first infusion in the presence of normal renal functions) in combination with trough levels (1 hour before the next due dose) if 6 - 14 hour time period is missed.

Educating the nursing staff about the importance of proper documentation of patient details; especially height, current weight and gentamicin treatment details was recommended in order to improve the accuracy of gentamicin prescription and monitoring.

With regard to gentamicin related nephrotoxicity, we did not see patients developing acute kidney injury (AKI) following toxic levels of gentamicin. However, another study shows that gentamicin-associated AKI is a common and potentially serious clinical problem [8]. The number of occasions with toxic levels in our study is not large enough to come to any conclusion about the relationship. Re-audit, to assess the feasibility and compliance of the staff with the new recommendations and the effect on improvement on gentamicin dosing and effect on eGFR and thereby renal functions, was thought to be appropriate.

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BATTLE WITH THE BUGS; WINNING WITH NON-ANTIBACTERIAL APPROACHES

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Summary

Treatment of infections by multi-drug resistant bacteria has become a real challenge to the clinicians as the therapeutic options are very limited. The rate of production of new antibiotics is hardly noticeable when compared to the speed of the emergence of drug resistance. If we are to beat the bacteria effectively it is necessary to find ways for which that the bacteria cannot adapt or escape, or methods to avoid them and their virulence. There are several such innovative non-antibacterial methods available at present and also being developed with a promising future.

Introduction

The era of antibiotic resistance is a threat to the well-being of man-kind because of the scarcity of newer antibiotics. There are several attempts taken to handle the situation by inventing newer antibacterial products, developing antibacterial policies and following strict infection control measures. Still, we are far behind the pathogens in the race. Hence a change in the approach towards these infections is the need of the hour. Alternative non-antibacterial management will definitely play an important role in the future to win the war against multi drug resistant (MDR) pathogens. This management strategy consists of various methods such as impairing the ability of pathogen virulence factors, enhancing host tissue damage control, altering the microbiota to provide colonization resistance, immune resistance and disease tolerance against pathogens.

1) Quorum sensing inhibitors

Quorum sensing is a mechanism found in bacteria to regulate the expression of genes according to their population density by various signal molecules called auto inducers [1]. Behavioral changes of the bacterial population such as expression of virulence factors and phenotype switching are coordinated by this mechanism during the process of infections. There are several antagonizing molecules available to inhibit this mechanism. These are called quorum sensing inhibitors. Once the inter-communication of the bacterial community is lost, there is no way for them to find the size of the population and they might remain dormant or inactive without attacking our system. In long-stay intensive care patients with MDR colonizing flora, this approach might prevent subsequent infections.

2) Haemofiltration devices

Haemofiltration has been used as an adjunct therapy in the treatment of sepsis [2]. It is effective in removing cytokines such as TNF-α, IL-1β, IL-6 and IL-8 which play a major role in septic shock and multi organ failure. There are extracorporeal filters such as mannose binding lectins in the pipeline which can bind with blood stream pathogens and remove them from the blood by filtration [3]. Reduction in the bacterial load can also allow a competent immune system to clear the remaining pathogens, despite them being multi-resistant.

3) Lytic bacteriophages

Bacteriophages were introduced in the treatment for bacterial infections in 1920s and still in practice in some parts of Europe [3]. A selected lytic phage or phage cocktails can induce bacteriolysis by digestion of peptidoglycan cell wall. It can be administered both topically and intravenously. One of the important drawbacks to use bacteriophages for the treatment of bacterial infections is that they have an exceptional specificity towards bacterial strains; hence they cannot be administered empirically without knowing the identification of the pathogen. But their action is not dependent on concentration, they do not undergo any metabolism in the human body and they also have higher penetration into infected sites with lesser adverse effects [4].

4) Advanced immunotherapies

Therapeutic antibodies are so specific and do not modulate the normal flora. Using antibodies against molecular targets of pathogens is an innovative approach. They can be polyclonal or monoclonal.

Boosting humoral and cellular specific immune response with the help of immune adjuvants is another innovative therapy in development.

5) Alternative efforts to limit virulence

There are liposome based cyto-toxin inhibitors which have been tested experimentally for the use against exotoxin producing bacterial infections.

6) Non-immune tolerance to pathogens

It is another non-antibacterial approach to bacterial infections by MDR pathogens to make the host be able to compensate for the presence of infective agent till they are cleared by the immune system. Studies in mice found that mouse strains vary in their susceptibility to Ebola virus disease primarily due to the variation of expression of a gene known as *Tek* which is the human homologue for tyrosine kinase receptor for angiopoietin-1. High level of angiopoietin-1 promotes endothelial barrier protection which is important in Ebola virus infection as the virus specifically targets endothelium and kills endothelial cells. So mouse strains with high levels of *Tek* are more able to defend their endothelial surfaces till the immune cells come to the scene to clear the virus [3].

7) Probiotics

Probiotics are microorganisms which are identical or similar to the microorganisms found naturally in the human body (gut flora). They can regulate the microbiome of the human intestine, can replace the pathogenic microorganisms, can synthesize antibacterial substances and can enhance the immune system [4]. Probiotics are being used effectively to treat diarrhoea especially after food poisoning and there is research towards its potential use in treating sinusitis, bronchitis, and pneumonia as well.

8) Antimicrobial peptides

They are naturally present in all animals as a part of the innate immune defense. Most of them attack the cell membrane of bacteria directly. Damage to the membrane causes outward flow of bacterial essential molecules. They have wide therapeutic spectrum, fast antibacterial activity and low probability of appearance of resistance [4].

9) Faecal microbiota transplant (FMT)

Faecal microbiota transplant (FMT), also known as a stool transplant, is a new, simple and cost effective approach to treat drug resistant or severe recurrent *Clostridium difficile* diarrhoea. Healthy donor faecal samples are given by mouth or by enema to a patient and it results in restoring of natural microbial colonies. This method needs a lot of counselling to increase the patient acceptance of the treatment and further studies are being carried out to find more convenient ways of administration of donor faeces. It is not easy to donate faeces as strict criteria should be fulfilled such as devoid of any comorbidity, not having any recent antibiotic history, and free of any bacterial or parasitic infections especially gut associated, etc.

10) Vaccines

They reduce the incidence of infection and, hence, need for antibacterials. A potent vaccine is one of the most cost effective methods to tackle MDR pathogens. They can target not only community acquired infections but also hospital acquired infections such as *E.coli* and *C. difficile*. Vaccines against hospital acquired infections are still in developmental stage. Adjuvanted, multi-eptitope bacterial vaccines are also in the pipeline of active immunisation to tackle bacterial infections [5].

11) Vitamins and minerals

Vitamin D has a potential antimicrobial activity. It may reduce the risk of infection through multiple mechanisms. It boosts innate immunity by enhancing anti-microbial peptides production and cytokine response.

Copper and Zinc (Cu and Zn) are required for optimal innate and adaptive immune function [6] and metal and metal oxide nanoparticles of silver, copper, zinc, etc, have inherent antibacterial properties and can be used as antibacterial for treatment of bacterial infections [7]. An important advantage of using metal and metal oxide nanoparticles for treating bacterial infections is that it is difficult for bacteria to develop resistance to them as the nanoparticles have multiple modes of action. Their limited application as antimicrobial therapeutic agents may be partially due to safety concerns because of potential toxicity.

Conclusion

Currently there are several non-antibacterial therapeutic options available to treat the infections of MDR bacteria when conventional antibacterial therapy fails. Most of these methods are in developmental stage and still being studied extensively. However, these approaches, with the optimum infection control measures in place, give us hopes for a better future which is free of nightmares of therapeutic failures in infections due to drug resistant micro-organisms.

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SPOTTING THE PROTOZOAN TROUBLE MAKER PASSED ON DURING INTIMACY: LABORATORY DIAGNOSIS OF TRICHOMONIASIS

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Summary

Trichomoniasis is a sexually transmitted infection (STI) caused by the flagellated protozoan *Trichomonas vaginalis*. According to the World Health Organization (WHO), it accounts for 248 million new cases among the 15-49 year age group globally, with over 142 and 105 million cases occurring among males and females respectively. Vaginal trichomoniasis is linked with several adverse outcomes of pregnancy, reduction of fertility, cervical neoplasia and acquisition and transmission of HIV.

Microscopy of wet and stained smears, culture and nucleic acid amplification test (NAAT) are established as diagnostics. A rapid antigen test and nucleic acid probe test have also been approved for clinical use.

Introduction

Trichomoniasis is a sexually transmitted infection (STI) caused by the flagellated protozoan *Trichomonas vaginalis*. According to WHO, the estimated number of new cases globally has been projected at 276 million [1]. The prevalence in Sri Lanka varies from 4.4%-7.2% [2].

T. vaginalis inhabits the genitourinary tract of both females and males. *T. vaginalis* is non-invasive thus adherence, immune evasion mechanisms are critical in the pathogenesis [3]. The clinical spectrum varies from asymptomatic carrier state to vaginitis and/or urethritis. The majority are asymptomatic and detected only during routine screening for other STIs. An asymptomatic female may continue to have the infection for 18 months without being treated while the symptomatic female may suffer for about three months prior to clearance with treatment [4].

T. vaginalis, is a 'facilitator of HIV' [5] and a 'trouble maker during pregnancy' [6]. It is easily treatable yet it is greatly ignored by the medical professionals. This article recapitulates what is known about laboratory diagnosis of trichomoniasis infection.

Why do clinicians need laboratory support?

There are different approaches for the diagnosis of trichomoniasis (clinical, syndromic and aetiological), with each having its own pros and cons. Clinical diagnosis is based on the complaints made by the patient and the findings of the physical examination. In trichomoniasis, signs that can be elicited during physical examination are minimal or none in most patients. Trichomoniasis can coexist with other infections like candidiasis and bacterial vaginosis. All of these present with a vaginal discharge of varying descriptions which are often clinically indistinguishable from each other. Syndromic management for STIs was composed by WHO to suit the primary care providers who have limited access to diagnostic laboratories. In this approach, symptoms and signs are considered together to decide on the possible differential diagnosis and treatment. The ability to make the diagnosis and provide treatment at the same visit is the main advantage whilst the cost and side effects of over treatment remains a major disadvantage of syndromic management [7,8].

Which test is best for laboratory diagnosis of trichomoniasis?

Microscopy of wet and stained smear, culture, immuno-chromatographic dipstick assay for antigen detection and nucleic acid amplification tests (NAAT) are all established as diagnostic tests for *T. vaginalis* infection.

Direct microscopy of the saline wet mount prepared from vaginal swab or urine is the most commonly used method in clinical diagnostic services all over the world, including Sri Lanka, as it is easy to perform, inexpensive and available in almost all laboratories. In a wet mount, *T. vaginalis* can easily be identified by its pear shape, presence of flagella and its nervous twitching jerky movements and the size which resembles the size of a white blood cell. Since *T. vaginalis* is the only species of *Trichomonas* that inhabits the human urogenital system, no further studies are necessary to differentiate it from *Pentatrichomonas hominis* which lives in the intestine. Thus wet mount microscopy has a high degree of specificity yet the sensitivity is less than 60% when compared with the "gold standard" culture, even in the hands of trained skilled microscopists [9]. However, there are certain limitations to wet mount microscopy. Presence of white blood cells in the vaginal fluid may be helpful as an indicator of inflammation. Conversely, detection may be difficult if there is excessive inflammatory response concealing the parasite and especially if the parasite count is low. On rare occasions, ciliated bodies from epithelial cells of the genital tract may be mistakenly identified as parasites [10]. For best results, wet mount microscopy should be performed within 30 minutes of collection of the sample, as the organism loses its motility. There is research evidence that one fifth of wet mount

preparations initially positive for *T. vaginalis* become apparently negative within 10 minutes, whereas others have documented survival of *T. vaginalis* in Amies gel agar medium, swimming pool water, urine and semen up to 6-24 hours [11].

Microscopy of the stained preparations is another method used in laboratory diagnosis. Giemsa stain, Field's stain, Leishman stain, periodic acid Schiff and Acridine orange for fluorescent microscopy can be used as stains. In stained preparations, trichomonads are seen against a background of large numbers of polymorph nuclear leucocytes. However, in clinical diagnostic services in Sri Lanka and elsewhere, *T. vaginalis* is also reported as an accidental but rather common finding in cervical scrapings stained with Papanicolaou stain. Papanicolaou stained smears have a sensitivity of 60% and specificity of 96% for *T. vaginalis* [12]. However, when it is used as the sole criterion for diagnosis, there had been an error of 48.4% in the diagnosis due to false positives and false negatives owing to loss of typical morphology during fixation and staining [13].

Since a negative wet or dry mount result does not rule out infection with *T. vaginalis*, it is useful to perform the "gold standard" culture. There are a number of liquid culture media such as Cystein-Peptone-Liver-Maltose (CPLM), Trypticase-Yeast Extract-Maltose (TYM) that can be used to grow *T. vaginalis*. All these are easy to prepare and store and commercially available for a reasonable price. Culturing is not done as a routine diagnostic test in most clinical diagnostic services as it is laborious and frequently inaccessible compared with the wet mount. Yet the commercially available liquid medium in a clear pouch (InPouch TV system) has revolutionized the culturing of *T. vaginalis* as it comes readymade for sample collection, transport and culture and convenient in usage. The results of any type of culture will be available within 2-5 days time. An obstacle to the use of culture media is the difficulty in achieving an on-the-spot diagnosis at the outpatient clinic. As a solution, combined use of immediate wet mount and culture-based testing can be recommended. However the quality of wet mount and culture techniques is strongly dependent on the skills, experience of the microscopist and also on the quality of the sample, as organisms often die during transfer.

Direct detection of *T. vaginalis* antigens (the 'OSOM Trichomonas rapid test' from Genzyme Diagnostics, West Malling Kent, UK) offers a point-of-care method of diagnosis, avoiding the negative aspects of other tests. It does not require specific training or instruments to perform, is easy to run, gives results in 10 minutes, is easy to interpret, is unaffected by other common infections and can be stored at room temperature for 16 months. The specimens collected in the kit can be kept for 24 hours at room temperature and for 36 hours at 4°C or -20°C. According to the manufacturer and researchers, this rapid test has the sensitivity of 83% and specificity of 99% compared to 'culture and PCR combination' and 96% sensitivity and 95% specificity compared to wet mount [14]. In a study carried out in Sri Lanka to evaluate the usefulness of the 'OSOM Trichomonas rapid test', results indicated 100% sensitivity, 96% specificity, 60% positive predictive value and 100% negative predictive value compared to culture [15]. The higher sensitivity in this study could have been due to detection of products of *T. vaginalis* by the antigen test, whereas culture required live parasites to give positive results.

Nucleic acid amplification tests (NAAT) are now the preferred diagnostic test due to its 100% sensitivity, in places where the cost is not a concern. These include Affirm VPIII Microbial Identification Test (a nucleic acid probe test), APTIMA *Trichomonas vaginalis* Assay (a Transcription Mediated Amplification) and polymerase chain reaction.

Conclusion

Considering the risks and complications resulting from infection, and the ease of treatment with the prospect of a definite cure, trichomoniasis warrants better detection and treatment. Considering the cost, feasibility and time taken for test results, combining wet mount with culture and/or rapid dipstick test for trichomoniasis can be recommended for Sri Lanka.

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FOOD HANDLERS: A POTENTIAL THREAT IN FOOD SAFETY

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Introduction

Food borne illness has a large public health and economic burden worldwide. An estimated 600 million - almost 1 in 10 people in the world – fall ill after eating contaminated food and 420 000 die every year, resulting in the loss of 33 million healthy life years (DALYs). Often this has been underestimated due to under reporting and difficulty in establishing causal relationships between food contamination and outcome. The primary responsibility of food safety lies with the food handlers [1].

A food handler is a person who, in their routine work, comes into contact with uncovered food not intended for personal use. Such person may involve in production, manufacturing, processing, packaging, preparing, selling or serving of any food item including water and beverages.

The main method of transmission of these infections is via the contaminated hand of the food handler. They may transfer pathogens from their own flora, from another person or from the environment.

Certain infectious agents have the potential risk of transmission through food handlers. These infections include,

- infection of the eyes, eyelids or ears
- oral sepsis
- skin and soft tissue infections due to *Staphylococci* and/or *Streptococci* such as boils or open wounds
- recent gastrointestinal infection such as Salmonella food poisoning, cholera, dysentery and typhoid, hepatitis A and E, parasitic infestations
- suffering from viral upper respiratory tract infections

How to prevent food borne infections?

Food Hygiene Regulations have been developed by the Department of Health and Human Services of United States and by the Food Standards Agency, United Kingdom to ensure food safety [2,3]. These aims to check the overall health of food handlers using a detailed questionnaire, followed by medical checks of the mouth, ears, hands, nails, blood pressure and selected investigations. Most of the other countries have followed these guidelines to develop their own.

Food handlers check

The aim of surveillance of food handlers is to ensure that they are “free from illness” and not to pose a hazard to the public. According to the literature, there is no uniformity of selected tests that have been included in health check packages of food handlers. Some packages have even included screening for occupational diseases and certain other tests to ensure employee safety [4,5].

Among these wide array of tests being requested in the past following may be useful in the current context to ensure food safety,

- Stool full report
- Stool culture
- Screening for *Staphylococcus aureus*
- Widal test
- Hepatitis A virus antibodies

These screening tests can be performed before the enrolment into the employment and then conducted annually, or following a period of sickness. After this examination, they can be given a certificate of fitness [6].

Other measures to be taken

Medical examinations of food handlers are costly, therefore they must be effective to justify the cost. They do not guarantee the detection of more than a small portion of carriers of pathogenic organisms. Routine medical examinations of food handlers may also lead to a false sense of safety. Furthermore, there is no uniformity in the procedures adopted for the surveillance by authorities all over the world. So, there is a debate among health professionals and public health authorities on the benefits of health surveillance of food handlers by means of routine medical examinations.

Therefore, medical surveillance of food handlers should be expanded into a planned program of periodic examination of the food processing facility by a Range Public Health Inspector and its employees by the Medical Officer of Health of the respective Medical Officer of Health area. This can be supervised at the district level by the Environment and Occupational Health Medical Officer.

For this process the employer should commit to provide necessary infrastructure and the engineering control to create optimum hygienic working conditions. All employees must know and understand the basic principles of food safety and their own responsibility in this respect. Adequate opportunities should be made available for continuous education and training of employees, conducted by properly trained personnel. There should be a mechanism to respond to consumer complaints regarding hygiene and correct them and to take preventive actions.

It is important to recognize that food handling at work cannot be treated as same as that at home. Every person engaged in food handling needs to maintain a high degree of personal cleanliness while on duty. They need to wear appropriate protective clothing including head covering/caps, gloves, masks, aprons and foot wear. They should adhere to basic food handling practices and basic personal hygienic practices at all times. The most important component of this is hand washing. Hands should be washed with soap and water:

- Before food is handled
- After visiting the toilet
- After blowing the nose
- After smoking/eating
- Between handling raw /cooked food
- After handling any soiled objects – eg. dustbin.

Washed hands should be dried with a paper towel instead with a communal towel. Further they should adhere to the following when handling food:

- Finger nails kept short and clean
- Hands to be away from nose, ear, eye, hair during the time food is handled
- Not to lick fingers while preparing food
- Cuts and abrasions to be covered with a water-proof dressing
- Wear a clean, washable, pale color overall
- Hair to be kept covered to prevent falling into food
- Never cough or sneeze over food
- Not to smoke or chew tobacco/betel while preparing food
- Personal belongings such as jewellery, watches or even mobile phones to be kept away from food processing area
- A daily shower before commencing work

Both cooked and uncooked food need to be adequately protected from contamination during storage, transport or sale by keeping within the conditions fit for human consumption. In an event of non-compliance of the final product; the facilities should be available to trace the specific lot of the food item, rapidly recall them from the market and to store separately until they are destroyed.

As a part of this process medical examinations or health interviews can take place. This interview may include a detailed medical history (including recent travel overseas, family illness and access to pets and other animals), clinical examination and medical tests. If a food handler suffers from a medical condition which can be transmitted via a food handler, he/she temporarily disqualify from handling food until they are certified as having good health. The period of exclusion from work varies with the medical condition [7].

For example,

- A hepatitis A patient for six weeks from onset of jaundice
- Salmonella food poisoning, cholera and dysentery until two consecutive stool samples taken 24 hours apart become negative or until completely free from any symptoms for at least 48 hours
- Typhoid until three stool samples taken one month apart become negative [8]
- Parasitic conditions: until successfully treated

- Staphylococcal and Streptococcal infections: until successfully treated
- All other gastrointestinal illnesses: until symptom free

Also, particular attention should be given to encourage self-reporting of illness by food handlers to the management. The managers must be aware of the daily health status of their employees to take necessary actions.

Vaccination of food handlers against certain disease such as hepatitis A and Salmonella are recommended by some guidelines, where necessary to reduce the risk of contamination of the food, taking into account the epidemiological situation and/or the immune status of the local population. If feasible they also recommend to check the hepatitis A immune status of food handlers [9].

World situation

One of the earliest records of screening of food handlers dated back to 1969 where Dr Owen McGirr screened airline catering staff at London airport using stool examinations, nasal swabs and chest X-rays. About 4% of the catering staff had positive results in stool examination. The commonest parasite was *Giardia intestinalis* followed by *Entamoeba* and *Titrichura*. Salmonella and Shigella were also isolated in stool cultures. Routine nasal swabbing for *Staphylococcus aureus* (formerly *Staphylococcus pyogenes*) was positive in about 5% of staff [10].

A cross-sectional study done in 2009 in Turkey, have examined nasal swabs, throat cultures, and stool samples among a random sample of 299 food handlers. It reveals that 52.2% of food handlers carried intestinal parasites including *Giardia intestinalis* (26.8%), *Ascaris lumbricoides* (10.7%) and *Taenia saginata* (10.0%). *Staphylcoccus aureus* nasal carriage was 23.1%. None of the food handlers were positive for Salmonella sp and Shigella sp [11].

In another study in Sudan they have screened different types of food handlers including restaurant workers, store keepers, butchers and street venders. Of this cohort, a total of 30.1% were found to be carriers of pathogenic organisms. 24.4% of positive food handlers were restaurant workers; out of which 73.7% were found to be nasal carriers of *Staphylococcus aureus* and 21.1% were harboring *Giardia* [12].

Stool examination of food handlers in hotels in the Dead Sea area of Jordan in 2014, recovered five species of protozoa (*Blastocystis hominis*, *Giardia intestinalis, Entamoeba coli, Entamoeba histolytica*, and *Endolimax nana*), one helminth *(Hymenolepis nana)*, and one cylindrical worm *(Enterobius vermicularis)* with an overall infection rate of 3.7%. *Giardia intestinalis* was the most prevalent parasitic infection with infection rate of 2.44%. All samples were negative for both Salmonella and Shigella [13].

A recent study conducted in Malaysia found that the food handlers have adequate knowledge on food safety. But their perceived knowledge is not reflected in the microbiological assessment of their hands, in which 65% of the food handlers had a total aerobic count 20 CFU/ cm^2 and Salmonella was detected on 48% of the food handlers' hands [5].

A review conducted in Ghana state that the government recommends to receive a necessary training to equip the food handlers for their work and to conduct health screening to obtain a health certificate which is subject to renewal on a yearly basis. But it cite that this training is seriously neglected by the food industry in the country and that only 40% of sampled food handlers for that study had health certificates with no documented evidence of periodic screening in the capital city of the country [6].

Situation in Sri Lanka

The Food Act Number 26 was established in Sri Lanka in 1980 and this has been cited as the Food (Hygiene) Regulations in 2010 and was in operation since 2011. These regulations apply to all establishments dealing with processing, transporting, distributing, handling, storing or selling of food or any other material related to food establishments. It describes the design, facilities and cleaning and disinfection requirements of such a facility. According to this every person who works in the capacity of a food handler needs to be certified fit by a Medical Officer registered with the Sri Lanka Medical Council, prior to their employment. Also, it should be carried out periodically as determined by the Medical Officer of Health of the area and at other times when clinically or epidemiologically indicated [14].

In addition, the Manual of Public Health Inspectors in Sri Lanka has further elaborated these conditions including a categorization of food handling establishments [15]. Both these documents have been followed by the Sri Lanka Tourism Development Authority (SLTDA) when approving restaurants in the country [16] and by Ministry of Health when producing the guideline for healthy canteens in work places [17].

According to a recent study done among food handlers in a teaching hospital in Sri Lanka by Adikari et al (2016) indicate that the knowledge on food safety was inadequate despite having a satisfactory level of personal hygiene among them [18].

Recent analysis of test results of food handlers (n=438) produced by the hotels in the Southern Province for annual screening to a private sector laboratory in Galle, reveal that 19.41% of them were positive for *Staphy-*

lococcus aureus (SA) in nasal swabs. 27.06% of the food handlers that were positive for SA were resistant to Cefoxitin; indicating overall of 5.25% of all food handlers were harboring Methicillin Resistant *Staphylococcus aureus* (MRSA). None of the MRSA isolates were resistant to Mupirocin (5µg), and 3.23% of Methicillin sensitive SA isolates show low level resistance to Mupirocin (5µg). All the tested stool samples for culture (n=339) were negative for Salmonella and Shigella. Interestingly none of the tested stool samples were positive for amoebae, ova and cysts. None of the performed Widal tests had a significant level of antibody titers (All positive titers were below 80) [19].

Conclusion

The findings from these studies indicate the key role of food handlers in spreading and transmitting food and water borne communicable diseases. The available data confirms that the knowledge on food safety and hygiene were inadequate among food handlers. The nasal carriage of *Staphylococcus aureus* among food handlers has been increased while the majority of food handlers are negative for Salmonella and Shigella.

Frequent hand washing with soap and water before food handling and after use of toilets; is the single most important procedure to prevent disease transmission by food handlers. This should be done as a part of good hygienic food handling practices.

A periodic medical examination is of minimal use in the prevention of disease transmission by food handlers. But an annual checkup will at least remind them on the necessity of having good hygienic food handling practices. A medical examination in food handlers will also be useful during the time of acute infectious illness or in an outbreak.

Food handlers should be motivated to self-report illnesses and the management to take appropriate steps accordingly. Health education of food handlers would be far more beneficial, but is often neglected. This problem is compounded further by rapid staff turn-over in this field. Yet it is a timely need to address this issue of food safety globally!

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AN INFECTION ACQUIRED FROM A GLASS OF MILK

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Introduction

Brucellosis is a systemic zoonotic infection with multiple organ involvement. Four species of *Brucella* can cause human disease, *B. melitensis, B. abortus*, *B. suis* and *B. canis*. Most cases are caused by *B. melitensis*, though *B. abortus* is the most widespread species, mainly among animals. Ingestion of raw dairy products, consumption of infected meat and close contact with infected animals' secretions and carcasses can transmit the infection to human [1]. Brucellosis is an occupational disease in shepherds, abattoir workers, veterinarians, dairy-industry professionals, and personnel in microbiology laboratories.

Clinical manifestations vary from multi system involvement to asymptomatic infection [2]. Onset of disease is acute in about half the cases, while in others it is insidious, with signs and symptoms developing over a period of weeks to months from the infection. The clinical manifestations include fever, sweats, fatigue, malaise, anorexia, weight loss, headache, arthralgia and back pain. Commonly, symptoms worsen as the day progresses. If untreated, the pattern of the fever waxes and wanes over several days ("undulant fever").

It is an important human disease in many parts of the world especially in the Mediterranean countries of Europe, North and East Africa, Middle East, South and Central Asia and Central and South America.

Brucellosis is uncommon in Sri Lanka, but continues to be an important infection since several published sero-epidemiological surveys reveal that the presence of the disease in cattle and buffaloes is common in the country [3]. The most human cases in Sri Lanka were acquired from Middle East countries [4].

We describe a case of a young student, who acquired *Brucella abortus* from Saudi Arabia, who recovered following appropriate diagnosis and treatment.

Case report

A 16 year old school boy presented with 3 weeks of intermittent fever, anorexia, profuse night sweating, malaise, muscle pain and arthralgia. He was investigated for pyrexia of unknown origin in a local hospital. In ultra sound scan (USS) abdomen, there was mild splenomegaly with multiple hypoechoic lesions in the spleen. The largest one was 18mm×17mm and suggestive of abscess or infarction.

He was treated with several antibiotics including ciprofloxacin and vancomycin for a short period. Surgical intervention was not done. Blood cultures were not done in that period of illness.

Though fever had settled initially, he continued to have constitutional symptoms, until admitted to the National Hospital of Sri Lanka with high fever spikes.

On further inquiry, he gave a history of a visit to Saudi Arabia about 3 months prior to the onset of illness where he had consumed a glass of fresh camel milk without boiling. He had neither contact with animals nor laboratory exposure to any pathogen.

On admission his white cell count was 5.9×10^3 cells/mm^3 with 58% neutrophils 39% lymphocytes and 182×10^3 platelets. Blood picture revealed rouleaux formation due to inflammation or infection.

The repeat USS abdomen performed did not detect any lesion. *Mycoplasma* antibodies were positive with a titre of 640. Melioidosis antibodies were negative.

The blood culture was positive after 3 days of incubation in the automated blood culture system. The organism was grown in agar media after 48 hours of incubation. The isolate was identified as *Brucella abortus* at a reference laboratory. Antibodies for *Brucella abortus* also became positive with the titer of 1:320 in serum agglutination test. The second blood culture, which was obtained while on sulphamethoxazole – trimethoprim for 4 days, (which was started from the ward) became positive for the same organism. The repeat abdominal and cardiac imaging studies were normal.

He was treated with oral doxycycline 100 mg/BD and rifampicin 600mg daily for six weeks, combined with IV gentamicin 5mg/kg/day for initial 10 days [5]. After completing treatment, he recovered without any complication of the infection or treatment.

Discussion

Human brucellosis is not endemic in Sri Lanka. The possible mode of transmission of infection in this patient is consumption of unpasteurized camel milk while he was in Saudi Arabia, which is an endemic country for brucellosis.

He had classical symptoms of human brucellosis, including arthralgia and excessive sweating. Multiple splenic abscesses were detected in early stage of the presentation but it was not found in repeated scans. The disappearance may have been due to the treatment with several antibiotics or due to the normal healing process of the spleen. He was treated with ciprofloxacin for a short period, which is described as a treatment option of brucellosis [6]. But due to inadequate duration of treatment with ciprofloxacin, he presented with bacteraemia.

Requirement of prolonged incubation indicates the need for clinical vigilance to isolate the organism. The diagnosis was supported by the history and positive serology results. Positivity of *Mycoplasma* antibodies may indicate cross reactive antibodies

The essential element in the treatment of human brucellosis is the administration of effective antibiotics for an adequate length of time. He was treated according to the standard treatment recommendations. Patient completely recovered from the infection without any complication.

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ENDOCARDITIS DUE TO *CORYNEBACTERIUM DIPHTHERIAE* IN A CHILD

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Introduction

Corynebacterium diphtheriae has become increasingly rare in the world with widespread immunization. World literature has shown a few case series of endocarditis due to *C. diphtheriae*. There are no reported cases of *C. diphtheriae* endocarditis in Sri Lanka.

Case report

A 14 year old boy having Marfan syndrome and multiple cardiac valve problems presented with a fever of 102°C for 3 days with no history of respiratory symptoms. His childhood immunization was up to date. Examination revealed a thin, ill looking, febrile child with tachycardia (heart rate 120/min) and normal blood pressure. There were murmurs related to mitral regurgitation (MR) and aortic regurgitation (AR) with few bilateral crepitations. No splenomegaly or neurological involvement.

The transthoracic echocardiogram (TTE) showed thickened mitral and aortic valves with Grade I AR and Grade III MR with no apparent vegetations. Investigations revealed white cell count of 23000/mm^3 with 79%

neutrophils, C-reactive protein of 225mg/dL and ESR of 95 mm/ 1st hour. Blood picture was suggestive of severe bacterial infection. The three blood cultures taken more than 12 hours apart became positive with a growth of nonhaemolytic smooth opaque colonies on blood agar and Gram stain revealed Gram positive bacilli with Chinese letter arrangement. A preliminary report was issued as *Corynebacterium* species. Child was clinically diagnosed as having infective endocarditis. The culture isolate was sent to Medical Research Institute for further identification. Characteristic black colonies on blood tellurite agar with positive catalase and nitrate and negative gelatinase and urease activity were consistent with *Corynebacterium diphtheriae*. Identification was confirmed by the Automated BD Phoenix bacterial and fungal identification system with confidence value of 99%. Toxigenicity was not performed due to unavailability of resources. The isolate was susceptible to ampicillin, penicillin, cefotaxime, ciprofloxacin and vancomycin and was resistant to erythromycin, rifampicin and clarithromycin by Stokes disc diffusion method. The patient was treated with 4 hourly IV penicillin and 8 hourly IV gentamicin for 8 days with no response. Patient's renal function deteriorated. Antibiotics were changed over to IV imipenem, teicoplanin and linezolid. Since there was no response and renal functions improved, teicoplanin was replaced with vancomycin on the 16th day. Patient's fever responded. Imipenem and linezolid were omitted on day 26 and vancomycin continued. Patient was clinically well for one week before his valve regurgitation worsened along with arrhythmia and left ventricular dysfunction. Patient succumbed to cardiac failure.

Discussion

Out of about 120 species in the genus *Corynebacterium*, many are nonpathogenic and known to colonize the human skin and mucous membrane. As commonly present in skin flora, they are frequently ignored as contaminants in cultures. *Corynebacterium diphtheriae* is the most significant infective agent in the genus which causes diphtheria and has declined incidence after implementation of vaccination. Nontoxigenic *C. diphtheriae* seems to cause increasing number of cases of invasive disease [1]. This child belonged to the commonly affected age group of 7-14 years. In most of the studies there was male preponderance. Risk factors for nontoxigenic *Corynebacterium diphtheriae* endocarditis were preexisting cardiac disease, alcoholism, homelessness, intravenous drug use etc. The patient in this case report also had multiple cardiac valve defects.

Usually *Corynebacterium diphtheriae* causes valvular destruction and dysfunction with frequent embolic complications [1]. Vegetations are usually large. In this patient, only mitral valve thickening was detected in TTE. Transoesophageal echocardiography was not done because he had good echo windows.

Corynebacterium diphtheriae is usually susceptible to cell wall synthesis inhibitors like penicillin, cephalosporin, and glycopeptides. In many studies, patients were successfully treated with IV penicillin and aminoglycoside though this patient did not respond [2].

There are cases that have been successfully treated with vancomycin alone or in combination with imipenem which was evident in this child with response to vancomycin before his death due to arrhythmia and cardiac failure. Early surgical intervention is indicated for patients with abnormal valves while medical management under close supervision is offered to patients with normal valves where facility for immediate surgery is available [3]. However the clinical condition of this patient did not favour surgery. *C. diphtheriae* endocarditis has significant morbidity and mortality [1].

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MELIOIDOSIS: AN UNCOMMON CAUSE OF ABSCESS

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Introduction

Melioidosis is a disease that presents acutely, or as a chronic infection caused by an intracellular Gram negative bacterium *Burkholderia pseudomallei*. The disease is endemic in Southeast Asia and is an emerging infection in the Indian subcontinent.

The disease is acquired via inhalation, or inoculation of contaminated soil and water. Melioidosis has different presentations such as pneumonia, septicemia, endocarditis, abscesses and lymphadenitis. Diabetes mellitus is an important risk factor for Melioidosis.

The definitive diagnosis of Melioidosis requires the isolation of *B. pseudomallei* from clinical specimens. *B. pseudomallei* grows on routine laboratory media. The colonies have a metallic appearance and give a sweet earthy smell. A positive oxidase reaction, safety pin appearance on Gram's stain and resistance to colistin and gentamicin with sensitivity to co-amoxiclav are the key identification steps. There is no change on Kligler Iron Agar.

Case Report 1

A 63 year old male from Minuwangoda presented in March 2017 with a three week history of a lump in the anterior abdominal wall along with a history of fever. He was a diabetic on oral hypoglycaemic drugs. WBC was $14x10^9$/L. Pus culture following incision and drainage grew *B. pseudomallei*. Melioidosis antibody titer was >10240. Patient was discharged on co-amoxiclav and co-trimoxazole as eradication treatment and is currently being followed up at the Melioidosis clinic.

Case Report 2

A 24 year old non diabetic female from Katana presented in February 2017, with left sided cervical lymph node enlargement for which a cervical lymph node biopsy was done. Histology showed granulomatous lymphadenitis. Blood for melioidosis antibodies revealed high titers (>10240). WBC was $8.2x10^9$/L. Tissue culture grew *B. pseudomallei*. While on eradication treatment for two weeks, she developed high fever spikes and was readmitted with bilateral cervical lymph node enlargement. She developed severe leucopenia (lowest WBC: $1.66x10^9$/L) with an ESR of 28mm/h but blood culture showed no bacterial growth. She was started on meropenam 1g 8hly for 2 weeks and changed to ceftazidime 2g 8hly for 2 weeks. She was discharged on co-amoxyclav and doxycycline. On discharge, the enlarged cervical node on the right side had completely settled while the left sided cervical node was still present. Repeat scan showed lymph node tissue encircling the jugular vein. She is currently on doxycycline and co-amoxiclav as eradication treatment.

Case Report 3

A 35 year old female from Raddolugama, presented in June 2016 with a 4 day history of a painful lump in the right side of the neck along with fever. She was an uncontrolled diabetic on insulin. Scan revealed multiple, enlarged, submandibular deep cervical posterior lymph nodes of which the largest lymph node showed suppuration and early abscess formation. WBC was $13x10^9$/L and ESR: 98mm/h. Incision and drainage was done. Pus culture grew *B. pseudomallei*. Her Mantoux test was positive and histology report revealed chronic suppurative abscess. She was referred to Chest Hospital Welisara and thereafter could not be contacted, in order to start eradication treatment.

Case Report 4

A 52 year old male from Minuwangoda, presented in June 2016 with a 3 week history of lump in the right groin along with fever. He had a history of diabetes, hypercholesterolemia, and asthma. Fine needle aspiration cytology (FNAC) revealed a suppurative lesion. Following incision and drainage pus culture grew *B. pseudomallei*. He was given co-amoxiclav and co-trimoxazole for three months as eradication treatment for melioidosis.

Case Report 5

A 44 year old female from Minuwangoda, presented in June 2016, with a painful swelling over the left parotid region for 10 days along with fever. She was a diabetic on oral hypoglycemic drugs. Ultrasound scan revealed an enlarged parotid gland with parotid abscess surrounded by oedema along with a few reactive lymph nodes in the submandibular region. After aspiration of the abscess; pus culture grew *B. pseudomallei*. She defaulted on follow up after 2 months on eradication treatment of co-amoxiclav and doxycycline for melioidosis.

Discussion

Melioidosis is largely, an under-diagnosed disease due to unfamiliarity among clinicians.

It was noted that a majority of cases which presented to DGH Negombo were associated with the local floods in the Minuwangoda area. Minuwangoda is probably a geographical pocket with soil and water contaminated with *B. pseudomallei*. Therefore greater awareness among health care personnel is essential to minimize disease morbidity as well as mortality.

Sending pus for cultures is not routine for most clinicians. Tissue specimens from enlarged nodes are hardly received for cultures but are only sent for histology. Therefore, it is necessary to educate clinicians on the importance of requesting culture for pus and tissue specimens for correct diagnosis. An additional area of importance in identifying melioidosis cases is, communication with histopathologists to consider melioidosis in their differential diagnosis. Communication with the histopathologist helped to diagnose the 2nd case stated above.

Laboratory staff should also be made familiar with the identification of *B. pseudomallei*.

Melioidosis is associated with disease recurrence if eradication treatment is not given following acute stage treatment. It was noted that the default rate was high during eradication treatment of patients who presented with abscesses or lymphadenitis. Greater awareness amongst healthcare professionals and patients is vital to reduce the burden caused by this disease.

References

1. Corea E, Thevanesam V, Perera S, et al. Melioidosis in Sri Lanka: an emerging infection. *Sri Lanka Journal of Infectious Diseases* 2012; **1**(2): 2-8.
2. Wijekoon S, Prasath T, Enoka Corea M, et al. Melioidosis presenting as lymphadenitis: a case report. *BMC Res Notes* 2014; **7**: 364.

Instructions to Authors

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

Review articles

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.

Research (original) articles

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.

Case reports

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).

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Please see separate guidelines issued with the notice calling for abstracts.

SUBMITTING A MANUSCRIPT

- Manuscripts should be submitted with **a cover letter** stating:
  - that the contents have not been published or accepted for publication elsewhere.
  - that the paper has not been submitted simultaneously to another journal.
  - Cover letter should include a declaration signed by the principal author to certify.
  - the originality of the article and that each author has made a significant contribution to the work.
  - The name, full mailing address, e-mail address and telephone number of the corresponding author should also be included.

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 analysing 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:

1. Conceived and planned the work that led to the paper, or interpreted the evidence it presents, or both.
1. Wrote the paper or reviewed successive versions, and took part in revising them.
2. Approved the final version.
3. Each author should have contributed sufficiently to the work to take public responsibility for the content.

Collecting and assembling data reported in a paper and performing routine investigations are not, by themselves, criteria for authorship.

PREPARATION OF MANUSCRIPTS

All parts of the manuscript, including references, tables and figure legends should be typed with double-spacing and formatted in Times New Roman font (size 14 for the title and 12 for the rest of the article) for A4 sized paper. All pages of the manuscript should be numbered consecutively, starting with the title page.

The **title page** should contain the following:

1. Main title and subtitle (if any): capital letters should be used only for the first letter in the first word in the title and proper nouns. (Use Times New Roman font size 14, bold).
2. Name(s) of the author(s) should be given below the title. The author's surname should be preceded by the initial(s) or forename(s) but not by prefixes such as Mr. or Dr. or Prof. See above for guidelines regarding authorship. The name of the principal author should be stated first. Authors' names will be published in the order submitted by the principal author.
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Units/ abbreviations

Authors should follow the SI system of units (except for blood pressure which will continue to be expressed in mmHg). Abbreviations if used should be consistent throughout the text.

Photographs

Photographs will be published in black and white. If author wishes to publish a colour photograph he / she should bear the cost of publication. All photographs of the patients will be published with covered eyes. Photo-micrographs should have scale markers that indicate the degree of magnification.

Tables

All tables must be double-spaced and numbered with Arabic numerals in the order in which they are cited in the text. The title should describe the contents of the table briefly and concisely. Explain all abbreviations and symbols as footnotes to the table.

Acknowledgements

Acknowledge only persons / organizations who have contributed to the scientific content and provided financial or technical support.

References

These should conform to the Vancouver style. The reference in the text should be numbered consecutively in Arabic numerals in parenthesis in the same line of the text in the order in which they appear in the text. The first five authors should be listed. If there are more than five then the first three should be listed followed by *et al*. An example is given below.

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.

PROOF READING

- The manuscript must be proof read by the author prior to submission.
- The acceptable rates for spelling and grammatical errors are as follows.
    - Spelling mistake – 5% (e.g. in a 2000 word document up to 10 misspelled words will be allowed)
    - Grammatical errors – 5% (e.g. in a 2000 word document up to 10 grammatical errors will be allowed)
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Manuscripts should be submitted as two **hard copies**, along with the cover letter, to

The Editor, Sri Lanka College of Microbiologists,
No. 6, "Wijerama House", Wijerama Mawatha,
Colombo 7

An **electronic version** must be also submitted by email to slcmicrobio@gmail.com or slcmicrobio@sltnet.lk. Your email should be marked for the attention of the Editor, SLCM, and the manuscript should be attached to the email as a Microsoft Word document.

Guidelines for preparing abstracts

(A) Authors

- At least one of the authors of the paper should be a member of the SLCM.
- 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:
    - Conceived and planned the work that led to the paper, or interpreted the evidence it presents, or both.
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    - Approved the final version.
    - Each author should have contributed sufficiently to the work to take public responsibility for the content.

Collecting and assembling data reported in a paper and performing routine investigations are not, by themselves, criteria for authorship

- The principal author should sign the statement given in Form A to certify that each author has made a significant contribution to the work.

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- **Name(s)** of the author(s) and place(s) where research has been carried out with the title of the abstract should be given in the title page. Authors surname should be preceded by the initial(s) but not by prefixes such as Mr. or Dr. or Prof.
- The name of the principal author should be stated first. Authors' names will be published in the abstract book in the order submitted by the principal author.
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- The abstract must report the results of original research. If the work has been presented or published previously in whole or in part, form and the year of presentation or publication and the forum or journal should be stated in the abstract. This does not disqualify a paper. Work already presented/ published in Sri Lanka will only be considered for poster presentations.
- Abstract page should carry only the title and the text. *(It should not contain Name(s) of the author(s) and place(s) where research has been carried out)*
- The abstract (including the title) should not exceed 350 words.
- It should be structured as far as possible into the following
    - **(i) A brief introduction may indicate why the study was undertaken**
    - **(ii) Objective(s)**
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Prospective authors are requested to see the abstracts of research papers in a recent issue of the *CMJ* for further guidance on writing abstracts.

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- Where units are used, they should be in SI units, and abbreviation of units should follow standard practice.
- Tables: should be included only if absolutely essential.
- Diagrams / Chemical structures: should be included only if absolutely essential.
- The Abstract must not contain statements such as *"Results will be discussed".*
- Acknowledgements: Should be restricted to Agencies/ Institutions providing funding or sponsorship and should be in the form, "Financial assistance by …… for research grant (number) …….. is acknowledged".
- Abstracts will be reviewed by the Editorial Board, two reviewers and by a third reviewer in case of any arbitration.

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The titles of articles, names and affiliations of authors are published as it has been submitted to the Sri Lanka College of Microbiologists by the principal or corresponding authors. Editorial Board is not responsible for the typographical or any other errors.

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