

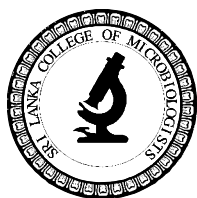


The Bulletin of the Sri Lanka College of Microbiologists

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The Sri Lanka College of Microbiologists

Council 2014 / 2015

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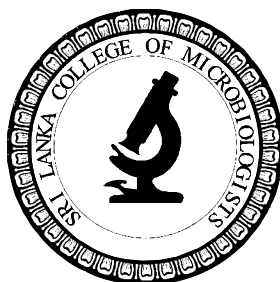
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Cover page designed by Dr Roshan Jayasuriya

24th Annual Scientific Sessions



The Sri Lanka College of Microbiologists

Inauguration Ceremony

12th August 2015 at 6.00 pm
Sri Lanka Foundation
Colombo 7

Pre-Congress Workshop

Theme:

“Food Microbiology”

12th August 2015

Scientific Programme

Theme:

“Neglected Tropical Diseases in Sri Lanka: towards elimination”

13th & 14th August 2015
Sri Lanka Foundation
Colombo 7

MESSAGE FROM THE CHIEF GUEST



It gives me great pleasure in sending a congratulatory message on the occasion of the 24th Annual Scientific Sessions of the Sri Lanka College of Microbiologists.

Sri Lanka College of Microbiologists has progressed immensely during the past two decades in growing to its present state to become one of the leading subspecialties in the country. The highly dedicated efforts of the members, contributed immensely towards strengthening of the microbiological services in Sri Lanka by expanding its role in the diagnosis, management, prevention and control of infectious diseases, education and research.

The theme chosen this year 'Neglected Tropical Diseases in Sri Lanka: towards elimination' is very timely and appropriate in keeping with the vision of the Ministry of Health.

With extreme gratitude while thanking the membership for taking timely efforts to enlighten the stakeholders on addressing the global health challenges, I wish the 24th Annual Scientific Sessions of the Sri Lanka College of Microbiologists every success and would like to pledge my fullest support for the future activities of the College.

Dr. Palitha Mahipala

Director General of Health Services

Ministry of Health & Indigenous Medicine

MESSAGE FROM THE PRESIDENT

The Annual Scientific Sessions and publication of the annual *Bulletin of the Sri Lanka College of Microbiologists* represents the high point in the activities of the Sri Lanka College of Microbiologists. This year, as we prepare for the 24th Annual Scientific Sessions, it gives me great pleasure to write a short message for publication in the *Bulletin*.



Our College brings together several specialities within the broader field of infectious diseases: clinical microbiology, medical parasitology, medical virology, and medical mycology. For the sessions this year, we have adopted the theme of Neglected Tropical Diseases, which cuts across all these disciplines since they include viral, bacterial and parasitic infections. Our country has six infections that come under the broad umbrella of Neglected Tropical Diseases. Over the course of the Sessions, we will hear from all those who are engaged in control and elimination of these infections in Sri Lanka, as well as from eminent speakers who are engaged in fighting the NTDs on a global scale.

While the sessions focus on infections that will, hopefully, be eliminated in the near future, the pre-congress workshop will focus on Food Microbiology, an area that needs a great deal of strengthening in Sri Lanka. We are greatly privileged to have with us two speakers who are eminent experts working with WHO and FAO on different aspects of food borne disease.

During the course of the past year, members of the Antibiotic Guidelines Committee of our College have spent an enormous amount of time and energy in working with other professional colleges to draft national antibiotic guidelines on empirical therapy. We will hear from other guest speakers from overseas and from Sri Lanka, regarding the importance of such guidelines in preventing the emergence of anti-microbial resistance, which is a major problem all around the world.

So, all in all, we have a very diverse, interesting, and exciting programme lined up for this year's Annual Scientific Sessions. I take this opportunity to thank all our guest speakers, both local and foreign, for having accepted our invitations so graciously, and taking time out from their busy schedules to share their knowledge and expertise with us.

The Council and many other members of the College have worked throughout the year on several different activities initiated by the College. They are too numerous to mention here, but I thank them all for their whole-hearted support, which takes the College from strength to strength.

I would like to extend a special word of thanks to the two joint honorary secretaries of the College, Dr. Rohini Wadanamby and Dr. Lakmini Wijesooriya, and Ms. Priyanga Opatha, our Office Secretary, who have supported me throughout the year in all our College activities.

Finally, on behalf of the Sri Lanka College of Microbiologists, let me welcome you to the 24th Annual Scientific Sessions. I hope you will find it both educational and enjoyable.

Prof Nilanthi de Silva

President

Sri Lanka College of Microbiologists

INAUGURATION PROGRAMME

12 - 08 - 2015 - Sri Lanka Foundation, Colombo 7

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	Lighting of the Traditional Oil Lamp
6.50 pm	Welcome Address Dr. Rohini Wadanamby Hony. Joint Secretary
6.55 pm	Address by the Chief Guest Dr. Palitha Mahipala Director General of Health Services, Ministry of Health
7.10 pm	Address by the President Prof. Nilanthi de Silva Senior Professor of Parasitology and Dean, Faculty of Medicine, University of Kelaniya
7.40 pm	Award of SLCM Fellowships
8.20 pm	Vote of Thanks Dr. Lakmini Wijesooriya Hony. Joint Secretary
8.25 pm	Ceremonial Procession leaves
8.30 pm	Cultural Show and Reception



24th Annual Scientific Sessions
The Sri Lanka College of Microbiologists

Pre congress workshop

Theme:

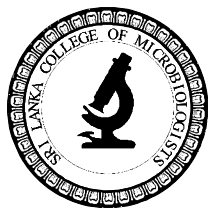
“Food Microbiology”

12th August 2015

**Sri Lanka Foundation
Colombo 7**

Chairpersons: Dr. Lilani Karunanayake and Dr. Roshan Jayasuriya

8.30 am - 9.00 am	Registration
9.00 am - 9.10 am	Welcome address Prof. Nilanthi de Silva <i>President, SLCM</i>
9.10 am - 9.40 am	Introduction to microbiological criteria applied to food safety Prof. Arie Havelaar <i>Emerging Pathogens Institute, University of Florida.</i>
9.40 am - 10.10 am	Introduction to Codex standards related to food safety Ms. Shashi Sareen <i>Senior Food Safety and Nutrition Officer, FAO Regional Office for Asia and the Pacific, Bangkok.</i>
10.10 am - 10.40 am	Microbiological analysis of foods Dr. Sujatha Pathirage <i>Consultant Microbiologist, Medical Research Institute, Colombo 8.</i>
10.40 am - 11.00 am	Tea
11.00 am - 11.30 am	Microbial risk assessment and risk management Prof. Arie Havelaar <i>Emerging Pathogens Institute, University of Florida.</i>
11.30 am - 12.00 pm	Role of GMP and HACCP in ensuring food safety Ms. Shashi Sareen <i>Senior Food Safety and Nutrition Officer, FAO Regional Office for Asia and the Pacific, Bangkok.</i>
12.00 pm - 12.30 pm	General discussion



24th Annual Scientific Sessions
The Sri Lanka College of Microbiologists

Scientific Programme

Theme:

“Neglected Tropical Diseases in Sri Lanka: towards elimination”

13th & 14th August 2015

**Sri Lanka Foundation
Colombo 7**

Day 1 – Thursday 13th August 2015

8.15 am - 8.45 am

Registration

8.45 am - 9.30 am

Free paper session I

Chairpersons: Dr. Sagarika Samarasinghe and Dr. Samanmalee Gunasekera

OP 1

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

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

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

OP 2

Is *Leishmania donovani* causing cutaneous leishmaniasis in Sri Lanka essentially dermatropic?

Kariyawasam KKGDUL, Siriwardana HVYD, Karunaweera ND
Faculty of Medicine, University of Colombo.

OP 3

Culture positive melioidosis in Sri Lanka in 2014

Corea EM¹, Kothalawala M², Patabendige G³, Fernando R⁴, Dassanayake M⁵, Karunaratne GKD⁶, Jayatilleke K⁷, Masakorala J¹, Edirisinghe A¹, Thevanesam V⁸

¹Department of Microbiology, Faculty of Medicine, University of Colombo, ²Department of Microbiology, Teaching Hospital, Kandy, ³Department of Microbiology, National Hospital of Sri Lanka, ⁴Department of Microbiology, General Hospital, Chilaw, ⁵Department of Microbiology, North Colombo Teaching Hospital, ⁶Department of Microbiology, Lady Ridgeway Hospital for Children, ⁷Department of Microbiology, Sri Jayawardanepura General Hospital, ⁸Department of Microbiology, Faculty of Medicine, University of Peradeniya.

SCIENTIFIC PROGRAMME

- 9.30 am - 10.15 am **Plenary I**
- WHO Perspective on NTD Control and Elimination**
Dr. Dirk Engels
 Director, Department for the Control of Neglected Tropical Diseases, WHO Headquarters.
Chairperson: Prof. Mirani Weerasooriya
- 10.15 am - 10.30 am **Tea**
- 10.30 am - 12.00 pm **Symposium I – Elimination of NTDs caused by helminths and protozoa: the Sri Lankan perspective**
- Chairpersons: Prof. Manel Wijesundara and Dr. Sharmini Gunawardena**
- Lymphatic filariasis**
Dr. Dilhani Samarasekera
 Consultant Community Physician, Anti-Filariasis Campaign.
- Soil-transmitted helminths**
Dr. Ayesha Lokubalasooriya
 Consultant Community Physician, Family Health Bureau.
- Leishmaniasis**
Prof. Nadira Karunaweera
 Professor of Parasitology, Faculty of Medicine, University of Colombo.
- 12.00 pm - 12.45 pm **Free paper session II**
- Chairpersons: Dr. Geethani Galagoda and Dr. Rajiva de Silva**
- OP 4 **An outbreak of respiratory syncytial virus infection from June to August 2014 in Sri Lanka**
CJS Jayamaha, MDA Sanjeeva, P Ekanayake, GDWS Gunathilaka, S Ranpatabendi, SU Wellmillage
 National Influenza Centre, Department of Virology, Medical Research Institute, Colombo.
- OP 5 **Human metapneumovirus (hMPV) infection in a selected group of children with severe acute respiratory symptoms**
Noordeen F¹, Pitchai FNN¹, Jayawardana PMGA¹, Kudagammana ST², Abeykoon AMSB¹
¹Department of Microbiology, Faculty of Medicine, University of Peradeniya, Sri Lanka,
²Department of Paediatrics, Faculty of Medicine, University of Peradeniya, Sri Lanka.
- OP 6 **Sensitivity of influenza viruses to oseltamivir and zanamivir: A study conducted on representative influenza A and B viruses isolated in Sri Lanka in 2009-14**
Jayamaha CJS¹, Wickramasinghe GA¹, Hurt A², Leang L²
¹National Influenza Centre, Department of Virology, Medical Research Institute, Colombo, ²WHO Collaborating Centre for Reference and Research on Influenza, Melbourne, Australia.
- 12.45 pm - 1.45 pm **Lunch**

SCIENTIFIC PROGRAMME

1.45 pm - 2.30 pm	Free paper session III Chairpersons: Prof. Vasanthi Thevanesam and Prof. Jennifer Perera
OP 7	Audit on hand hygiene compliance at the Surgical Intensive Care Unit (SICU), National Hospital of Sri Lanka. <i>Nakkawita WMID, Patabendige CGUA</i> National Hospital of Sri Lanka, Colombo.
OP 8	Incidents of ventilator associated pneumonia in two intensive care units and a high dependency unit at National Hospital of Sri Lanka. <i>Nakkawita WMID, Patabendige CGUA</i> National Hospital of Sri Lanka, Colombo.
OP 9	Epidemiology of ventilator associated pneumonia caused by <i>Acinetobacter</i> species and their antibiotic susceptibility patterns in different intensive care units at National Hospital of Sri Lanka. <i>Abeydeera WPH, Patabendige CGUA</i> National Hospital of Sri Lanka, Colombo.
2.30 pm - 3.15 pm	Plenary II Global burden of foodborne diseases <i>Dr. Arie Havelaar</i> Emerging Pathogens Institute, University of Florida. Chairperson: Dr. Sujatha Pathirage
3.15 pm - 4.00 pm	Plenary III Regional perspective on Antimicrobial Resistance and development of national action plans on AMR <i>Dr. Aparna Singh Shah</i> Regional Advisor, Health Laboratory Services, WHO-SEARO, New Delhi. Chairperson: Dr. Kumudu Karunaratne
4.00 pm - 4.30 pm	Tea

Day 2 – Friday 14th August 2015

8.30 am - 9.15 am	Free paper session IV Chairpersons: Dr. Enoke Corea and Dr. Sunethra Gunasena
OP 10	Immunoglobulin G immune status and vaccination history of measles, mumps, rubella and varicella in post-graduate medical trainees <i>Jayamaha CJS, Yasandi BS</i> Department of Virology, Medical Research Institute, Colombo.
OP 11	Assessment of seroprevalence of Hepatitis B antibody and hepatitis B infection after routine immunization in 1- 5 year old children <i>Amarasekara J¹, Palihawadana P¹, Peiris S¹, Galagoda GCS²</i> ¹ Epidemiology Unit, Colombo 10, ² Medical Research Institute, Colombo 8.

SCIENTIFIC PROGRAMME

- OP 12 **HIV INNO-LIA HIV I/II assay: modified minimum criteria for HIV-1 diagnosis**
Janage SN, Sudhanva M, Zuckerman M
 South London Specialist Virology Centre, Kings College Hospital NHS Foundation Trust, Denmark Hill, London, United Kingdom.
- 9.15 am - 10.00 am **Plenary IV**
Atypical mycobacterial infections
Dr. Angela Houston
 Consultant Microbiologist, St George's Hospital, London.
Chairperson: Dr. Kanthi Nanayakkara
- 10.00 am -10.15 am **Tea**
- 10.15 am -11.45 am **Symposium II – Elimination of NTDs caused by bacteria and viruses: the Sri Lankan perspective**
Chairperson : Dr. Nalini Withana and Dr. Kushlani Jayatilleke
Leprosy
Dr. Kaushalya Kasturiaratchi
 Consultant Community Physician, Anti-Leprosy Campaign.
Rabies
Dr. P. A. L. Harischandra
 Director, Public Health Veterinary Service.
Dengue
Dr. Hasitha Tissera
 Director, Dengue Control Unit.
- 11.45 am -12.45 pm **Free paper session V**
Chairpersons: Dr. Lilani Karunanayake and Dr. Malika Karunaratne
- OP 13 **Incidence, risk factors and outcome of acute lower limb cellulitis in patients admitted to a tertiary care hospital**
Nakkawita WMID, Chandrasiri NS, Rajanthi R, Sutharson A, Ferosa MBF
 Colombo South Teaching Hospital, Kalubowila.
- OP 14 **Respiratory colonizers in patients with primary antibody deficiencies attending an immunology clinic in Sri Lanka**
Hapuarachchi CT¹, de Silva R¹, Corea E M², Karunaratne GKD³, Pathirana KG³
¹Department of Immunology, Medical Research Institute, Colombo 8, ²Department of Microbiology, Faculty of Medicine, University of Colombo, ³Lady Ridgeway Hospital, Colombo 8.
- OP 15 **Analysis of data of urine culture isolates of 2014 sent from seven laboratories of National Laboratory Based Surveillance of Sri Lanka College of Microbiologists**
Jayatilleke SK¹, Patabendige G², Karunaratne GKD³, Perera J⁴, Perera RRDP⁵, Wijesooriya WRPLI⁵, Sunil-Chandra NP⁵, Kottahachchi J⁶, Athukorala D⁶, Dissanayake T⁶, Dasanayake M⁷
¹Sri Jayewardenapura General Hospital, Nugegoda, ²National Hospital of Sri Lanka, Colombo, ³Lady Ridgeway Childrens' Hospital, Colombo, ⁴Department of Microbiology, Faculty of Medicine, Colombo, ⁵Department of Microbiology, Faculty of Medicine, Ragama, ⁶Department of Microbiology, Faculty of Medicine, Sri Jayewardenapura, ⁷Colombo North Teaching Hospital, Ragama.

SCIENTIFIC PROGRAMME

OP 16	Comparison of bacterial characteristics (MICs) of Gram negative bacteria isolated from patients with neutropenic sepsis pre and post-levofloxacin prophylaxis <i>Abeywardena HMW, Perera DN</i> Leicester Royal Infirmary Hospital, Infirmary Square, Leicester, United Kingdom.
12.45 pm - 1.45 pm	Lunch
1.45 pm - 2.45 pm	Symposium III - Antimicrobial Resistance Chairpersons: Dr. Shirani Chandrasiri and Dr. Geethika Patabendige AMR in the South Asian region <i>Prof. R Ravikumar</i> National Institute of Mental Health and Neurosciences, Bangalore. Surveillance for AMR in Sri Lanka <i>Dr. Jayanthi Elwitigala</i> Consultant Microbiologist, National AIDS / STD Control Programme.
2.45 pm - 3.30 pm	Plenary V HIV and TB co-morbidity <i>Dr. Angela Houston</i> Consultant Microbiologist, St George's Hospital, London. Chairperson: Dr. Dhammika Vidanagama
3.30 pm - 3.45 pm	Award ceremony
3.45 pm - 4.15 pm	Tea

LIST OF GUEST SPEAKERS

FOREIGN FACULTY



Prof. Arie H. Havelaar

Emerging Pathogens Institute, Institute for Sustainable Food Systems and Animal Sciences Department, University of Florida, Gainesville FL, USA



Dr. Dirk Engels

Director, Department of Control of Neglected Tropical Diseases, World Health Organization, Geneva, Switzerland



Dr. Angela Houston

Consultant Microbiologist, St George's Hospital, London, UK



Dr. Aparna Singh Shah

Regional Adviser Health Laboratory Services and Regional Focal Point for Antimicrobial resistance, South East Asia Regional Office - World Health Organization



Prof. R Ravikumar

National Institute of Mental Health and Neurosciences, Bangalore, India

Ms. Shashi Sareen

Senior Food Safety and Nutrition Officer, FAO Regional Office for Asia and the Pacific, Bangkok, Indonesia

LIST OF GUEST SPEAKERS

LOCAL FACULTY



Dr. Dilhani Samarasekera

Consultant Community Physician, Anti-Filariasis Campaign



Dr. Ayesha Lokubalasooriya

Consultant Community Physician, Family Health Bureau



Prof. Nadira D. Karunaweera

Senior Professor of Parasitology, Faculty of Medicine, University of Colombo



Dr. Kaushalya Kasturiaratchi

Consultant Community Physician, Anti-Leprosy Campaign



Dr. P. A. L. Harischandra

Director, Public Health Veterinary Service

LIST OF GUEST SPEAKERS

LOCAL FACULTY



Dr. Hasitha Tissera
Director, Dengue Control Unit



Dr. Jayanthi Elwitigala
Consultant Microbiologist, National AIDS / STD Control Programme



Dr. Sujatha Pathirage
Consultant Microbiologist, Medical Research Institute, Colombo 8

TOWARDS ELIMINATION OF NEGLECTED TROPICAL DISEASES FROM SRI LANKA

Prof. Nilanthi de Silva

President, Sri Lanka College of Microbiologists

The NTDs are a very diverse group of infections with the commonality that all of them are strongly associated with poverty and social exclusion, and are often neglected in public health interventions. They consist of seventeen communicable diseases now slated for global eradication, elimination or control by WHO. Sri Lanka has to deal with only six of them: dengue, rabies, leprosy, leishmaniasis, lymphatic filariasis and the soil-transmitted helminthiasis. The WHO has targetted all except dengue, for elimination as a public health problem by 2020 in the WHO South East Asia Region, which includes Sri Lanka.

Rabies is a vaccine-preventable disease, for which Sri Lanka has set itself a more ambitious target than WHO, i.e., to eliminate human rabies from the country by 2016. The Public Health Veterinary Services (PHVS) is primarily responsible for preventing human and animal rabies. Its control strategy has resulted in a steady decline in the annual number of human rabies deaths, during the period 2009 - 2013.

Following a powerful social mobilization campaign in the early 1990s, the national prevalence of **leprosy** dropped rapidly to below 1 per 10,000 population in 1995, thus meeting the WHO definition for elimination as a public health problem. Leprosy services were integrated into the general health services in 2001. However, the number of new cases has stubbornly remained at around 2000 cases a year for well over a decade, and the elimination target has yet to be achieved at sub-national level in districts such as Polonnaruwa.

The targets for eliminating the **leishmaniases** in Asia, as set out in the NTD Roadmap, are to reduce the incidence of Visceral Leishmaniasis (VL) to <1 case / 10,000 population per year and to detect and manage 85% of Cutaneous Leishmaniasis (CL) cases. The vast majority of cases of leishmaniasis that are diagnosed in Sri Lanka are cutaneous, although the causative agent has been identified as *Leishmania donovani*, which causes VL in the rest of the Indian subcontinent. Thus Sri Lanka really does not have a problem with VL, and that leaves only the latter target of detecting and managing CL. Although it is now a notifiable disease, there is no separate entity within the Ministry of Health with remit to monitor leishmaniasis or implement control

measures. Indeed, there has been no definitive incrimination of the vector or the reservoir host(s) to date. In this vacuum, there is a danger that leishmaniasis may become much more of a problem than it is at present.

In 2002, Sri Lanka became one of the earliest countries to join the Global Programme for Elimination of **Lymphatic Filariasis**. The Anti-Filariasis Campaign successfully spearheaded the completion of five rounds of Mass Drug Administration in 2006. Transmission Assessment Surveys for post-MDA surveillance were carried out in 2008, 2009 and 2010 confirming that the microfilaraemia rate had dropped to <1%, the WHO elimination target. In 2011, Sri Lanka qualified for commencement of the WHO verification process for certification of elimination. The country dossier has now been submitted to the WHO for certification, but questions still remain regarding continued transmission in 'hotspots'.

The WHO target for **soil-transmitted helminthiasis** (STH) is that 50% of pre-school and school-aged children in need of treatment should be regularly treated in all countries by 2015, this figure reaching 75% by 2020. The STH infections have long been recognized as a problem in Sri Lanka, but unlike all of the other NTDs, they are neither notifiable, nor is there a specific entity to monitor their control. In 2012, the Ministry of Health issued a new directive with guidelines on de-worming children and pregnant women in the community setting but we have no national strategic plan that identifies explicit targets, milestones or performance indicators. This is an unfortunate situation, because given the current prevalence, it is very likely that Sri Lanka can eliminate STH as a public health problem in the near future.

Globally, there has been increasing recognition of the role played by NTDs as drivers and indicators of poverty. The goals set out in the WHO NTD Roadmap appear well within reach in our country. Achieving them will require sustained focus and commitment on the part of the Ministry of Health and all other stakeholders, but it will serve to reinforce Sri Lanka's image as a country with a health system that works for the poor and vulnerable as much as for the wealthy and affluent.

Plenary II

Estimating the burden of foodborne disease

Prof. Arie H. Havelaar

Summary metrics of public health are increasingly used to support priority setting and resource allocation in public health. Disability Adjusted Life Years (DALYs) have been introduced by the World Health Organization and the World Bank in the Global Burden of Disease project in the mid nineteen-nineties, and are currently used as the key metric to describe the health of the global population. DALYs are the sum of the number of life years lost (YLL) due to premature mortality, and the number of years lived with disability (YLD) due to diseases, weighted for the severity of the disease. Global and national burden studies also allocate the burden to a set of risk factors to further inform decisions about public health interventions.

Foodborne diseases have not yet been included as a risk factor in such studies. Foodborne diseases are highly complex, being caused by a large number of different hazards of microbial, parasitic or chemical nature. The health outcomes of foodborne diseases may range from mild gastrointestinal symptoms to cancer and death. This presentation will describe approaches to assessing the burden of foodborne disease by microbial and parasitic agents and will discuss the activities of the WHO Foodborne Disease Burden Epidemiology Reference GROUP (FERG). Examples how this information can support food safety decision making will be provided.

Plenary V

HIV-TB: The deadly combination

Dr. Angela Houston

Despite our best efforts, HIV-associated tuberculosis (TB) still remains the most deadly infectious disease of our times and is a huge challenge to public health. Early detection and rapid diagnosis of both HIV and TB remains the cornerstone to controlling this pandemic. The combination of early antiretroviral therapy, isoniazid preventative therapy and diagnostic technologies such as Xpert MTB/RIF are helping to reduce clinical mortality but more needs to be done to combat this deadly combination. Could point of care testing, whole genome sequencing and new and novel treatments offer hope in the future?

Symposium I

Lymphatic filariasis

Dr. Dilhani Samarasekera

Lymphatic filariasis (LF) is one of the leading causes of permanent and long-term disability and most disfiguring diseases in the world. The Anti Filariasis Campaign (AFC) is the National Programme of the Ministry of Health, which is responsible for control and prevention of filariasis in Sri Lanka. Filariasis is endemic in Western, Southern and North Western provinces and there are 7 Regional Anti Filariasis Units (RAFUs).

Wuchereria bancrofti and *Brugia malayi* parasites have been reported from Sri Lanka. A majority of microfilaria (mf) positive cases is due to *Wuchereria bancrofti*.

After the Mass Drug Administration (MDA) programme in 2002-2006, AFC has conducted post MDA surveillances and results showed very low transmission of LF in endemic districts.

Special surveys conducted in Galle District showed some areas having mf rate of >1%. MDA was conducted in selected Medical Officer of Health areas in Galle in 2014 and planning to repeat in 2015. In 2014 mf rate in Sri Lanka was 0.05% (177 mf positives, 121 positives from Galle), mosquito infection rate was 0.55% and 1027 lymphoedema patients were newly registered. AFC has detected few mf/antigen positives from some non endemic districts.

AFC has submitted a draft dossier to WHO to consider for certification on elimination.

Leishmaniasis in Sri Lanka: paving the way for its control and elimination

Prof. Nadira D. Karunaweera

Leishmaniasis is the latest vector-borne disease established in Sri Lanka. Causative agent is *L. donovani* the most virulent and usually visceralizing species of the genus. According to the database maintained in the department, over 3000 cutaneous leishmaniasis (CL) patients have been reported during the past decade. CL per se is relatively uncomplicated though it could result in scar formation and disfigurement depending on the

affected site. However, other more debilitating clinical forms such as mucosal (MCL) and visceral (VL) have also been reported in the recent past. Though the volume of such complicated cases, as far as it is known, is low (<20), considering the potential of *L.donovani* to visceralize, notable numbers of imported VL cases also reported during the past few years and the high levels of morbidity and mortality of such patients warrant urgent attention of all stake holders in order to make genuine efforts towards its control.

The recent outbreak of leishmaniasis started in year 2001 in Northern/North-Central parts of Sri Lanka; with the majority affected being young adult male soldiers. However, by the year 2008 case distribution spread to all 9 provinces with most patients being civilians based in Southern and North-Central provinces. Latest figures indicate that the proportion of males is only slightly higher than females with the disease affecting all age groups. Most lesions are single, reported within 3 months of onset, with majority being ulcers of less than 1cm in diameter. Mostly affected sites are head, face and upper limbs. Bi-annual seasonal variation is seen in case presentations with 2 peaks in April-June and August-October. Prevalence figures obtained through active case detection programs range from less than 1% to over 7% with evidence of peridomestic transmission in the south and likely zoonotic transmission in the northern areas. *Ph. argentipes* was identified as the vector of parasite transmission, however the role of animal reservoirs in the transmission cycle still remains unproven.

Leishmaniasis caused by *L.donovani*, the most virulent species within the *Leishmania* complex is now widely prevalent in Sri Lanka. Team work with active participation of clinicians, epidemiologists, laboratory personnel and the researchers would ensure successful disease control paving the way for its elimination.

Symposium II

Elimination of leprosy from Sri Lanka

Dr. S. Kaushalya Kasturiaratchi

Leprosy is a chronic infectious disease caused by *Mycobacterium leprae* and it affects primarily the skin and the nerves. Transmission of leprosy is by droplet infection. Disease is transmitted from human to human and there is no proven reservoir or a vector. Leprosy is

easily diagnosable, treatable and curable. Diagnosis is made mainly on clinical features. Major complications associated with leprosy is the development of disabilities leading to loss of productivity and social stigma. These disabilities are due to reactions and could occur at any stage of the disease, even after completion of treatment and could be prevented by early detection and treatment.

Sri Lanka has a long history in leprosy. The first leprosy hospital was built in 1708 during the Dutch period and the Lepers Ordinance enacted in 1901 during the British period. Anti-Leprosy Campaign has been established in 1954 as a vertical programme. In 1989, the most successful social marketing campaign against leprosy was launched and it enhanced lots of self-reporting among patients. In 1995, Sri Lanka achieved the WHO elimination target as a country. In 2001, leprosy services were integrated in to the general health services and currently patients are treated in Dermatology clinics under Consultant Dermatologists.

During the last decade, annually around 2000 new patients are detected in Sri Lanka. In the year 2014, 2157 new patients were reported from all 25 districts in the country. Out of the total patients, 41% were reported from Western Province. There are 13 high endemic districts where new case detection rates are more than 10 per 100,000 population. Sri Lanka compared to other South East Asian countries has a high percentage (7.5%) of patients with visible deformities at the time of diagnosis. Percentage of affected children who are less than 15 years of age is 9.5% indicating active transmission of the disease. More than 55% of new patients are diagnosed late (more than 6 months after appearance of symptoms) indicating partly lack of awareness among the public. Major challenges faced by the national leprosy control programme includes unabated active transmission of the disease with a yearly reporting of around 2000 new cases, delayed presentation and defaulting from treatment at clinics, clinical management aspects including case detection, inadequate reaction management, inadequate services for the prevention of disability and rehabilitation and inadequate availability of trained human resources at the central and district levels of leprosy control. Some of the recent approaches for leprosy control in Sri Lanka that are put in place include establishing satellite clinics in all districts to improve accessibility and new case detection, house to house community screenings in high endemic pockets and Leprosy Post Exposure Chemoprophylaxis pilot implementation study.

Given that the elimination levels were achieved in the 1995, the challenge of leprosy is far from being over. More vigorous and attentive action based on evidence is required to control this disease.

Elimination of rabies from Sri Lanka: Are we on track?

Dr. P. A. L. Harischandra

Humane rabies control measures launched during last decade have had a significant effect on the incidence of human rabies. Dog is responsible for 94% of human rabies deaths. Dog accounts for 81% of animal rabies confirmed. Estimated dog population is 2.8 million. Rabies elimination required to achieve 70% coverage, and need to sustain over 40% at least for 5 years. From 2008 the Humane strategies for elimination of rabies were mass dog vaccination, mass sterilization and rabies post exposure treatment (PET) for animal bite victims. Using these strategies the number of annual human rabies deaths remained at 50 with pulsed nature fluctuations, as in previous decades. The Human PET annually consumed an average of 325,000 vials of one ml human anti-rabies vaccine ampoules with 100,000 equine rabies immunoglobulin. After 2010 habitat control was introduced as an addition to above strategies. Together these combined strategies contributed largely for achievement of human rabies incidence as low as 0.01 per 100,000 populations showing a continuous downward trend without fluctuations. Due to the pulsed nature of the dog rabies vaccination campaigns and the birth of susceptible dogs, rabies incidence among dogs remains static with an average of 600 rabies positive dogs reported in central and regional laboratories. During the year 2014, a total of 1,533,032 dogs were vaccinated which included 150,278 stray dogs. In addition 134,943 female dogs were surgically sterilized. As annual dog vaccination coverage is 48% and due to high turnover and death rate of 27%, achieved vaccination coverage vain within few months. This leads to a decrease in the anti-rabies coverage below the herd immunity required to maintain downward trend of dog rabies. Therefore annual mass dog vaccination campaigns must be supplemented with mid-year mop-up campaigns in order to achieve rabies elimination. Through strengthening the combined strategy with mass dog mop up rabies vaccination campaigns will keep Sri Lanka on the path of rabies elimination.

Symposium III

Antimicrobial resistance in South Asian region

Dr. R. Ravikumar

Antimicrobial resistance (AMR) is the resistance of a microbe to antimicrobial agents, which are used to treat or prevent infection. Today, acquired resistance in bacteria

to beta-lactams is mainly mediated by the ESBLs and metallo beta-lactamases like the ones involved the recent infections with New Delhi metallo β -lactamase-1-positive strains. In addition AMR is also commonly known to pose problems in treatment of diseases like TB, malaria, HIV etc. *Mycobacterium tuberculosis* was first seen resistant to drugs isoniazid and rifampicin. Mutation in *inhA* gene, *katG* gene and *ahpC* gene has been reported to cause resistance against isoniazid. Whereas mutation in *rpoB* gene and *gyrA* gene have been found in posing resistant to rifampicin and fluoroquinolones. Drug resistance has been described in *P. vivax* and *P. falciparum*, and has been implicated in the spread and re-emergence of malaria. The most commonly observed chloroquine resistance is through efflux pumps. Antiviral drug resistance is an increasing concern in immuno-compromised patients, where ongoing viral replication and prolonged drug exposure lead to the selection of resistant strains. A good and strictly followed antimicrobial policy and public health education can help in curbing the issue of AMR to a great extent.

Pre-Congress Workshop

Microbiological analysis of food

Dr. Sujatha Pathirage

Microorganisms are the primary cause of food spoilage and foodborne illness. The ability to detect microorganisms including pathogenic organisms and their toxins serves as a cornerstone to ensure the safety and quality of our food supplies.

Microbiological analysis of food is challenging due to the complexities of food matrices, heterogeneous level of distribution of low levels of pathogens, high levels of normal bacterial flora, presence of ingredients that can interfere with assays and bacterial stress injury occur during food processing.

Microbiological analysis of food include detection, enumeration and to identify microorganisms.

Different test methods are being used in food microbiology. Conventional culture based methods are considered as the gold standard. It is a lengthy process that includes multiple procedural steps. This approach produce accurate results to be used in public health regulatory and enforcement purposes.

While traditional microbiological methods have worked well for many years to detect and stop pathogen contamination in food, the speed of modern food

production practices requires dramatic improvements in speed and accuracy of microbiological methods to assure the same safety standards.

Rapid methods, including convenience-based, antibody based, and nucleic acid-based assays, have improved the detection methodology for microorganisms and their toxins in food. Convenience-based assays include hydratable media gel cards such as Petrifilm (3M Microbiology). In addition chromogenic media are available to detect coliforms and *E. coli*, as well as for detection of

specific foodborne pathogens such as *E. coli* O157:H7, *Salmonella* and *Listeria monocytogenes*.

Nucleic acid-based assays consist of two main types, hybridization using probes and amplification by PCR and related techniques. PCR based methods are available for most of food borne pathogens.

Although convenient, the application of rapid methods to food testing is not without its complications including validation and regulatory implications.

OP 1

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

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Objective

Long-lasting impregnated bed nets (LLINs) are one of the main methods used for malaria control in Sri Lanka. Despite the extensive coverage, the effectiveness of LLINs depends on utilisation and maintenance at the household level. A cross-sectional study was performed to examine the patterns and predictive factors of LLIN maintenance and use in Anuradhapura District.

Design, setting, methods

A community based cross sectional study where a representative sample of the district was selected by a multi-stage cluster sampling method. Interviewer administered data collection was done in 530 LLIN-owning households.

Results

A greater proportion (84%) was aware of the specific benefits of using LLINs such as insecticidal benefits, durability and greater ventilation compared to plain nets. The mean number of LLINs in the household was 1.34 ± 0.62 . Approximately 95% households had at least one LLIN but the majority of individual households (92%) did not have enough LLINs to cover the number of people. Seventy-five percent of households had used all LLINs the previous night, while 83% had used at least one. The commonest reasons for non-use of a LLIN the previous night were excessive heat and lack of mosquitoes during the dry season. Only 3% were maintaining the LLINs in such a way as to maximise the insecticidal efficacy of the net. If the shape was conical, the odds of LLIN use was 5.6 times than if the shape was square,

Conclusions

Majority of individual households did not have enough LLINs to cover the number of people as recommended by the WHO, which is one LLINs per 1.8 people. LLIN

distribution programmes should take into account recipient preferences, and emphasise the significance of proper net maintenance. With the changing epidemiology of malaria in Sri Lanka and the country's effort in preventing reintroduction due to imported infections, LLINs continue to remain a key strategy in maintaining Sri Lanka malaria free so as to enable the country to obtain the WHO certificate for malaria elimination by 2016.

Acknowledgements

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OP 2

Is *Leishmania donovani* causing cutaneous leishmaniasis in Sri Lanka essentially dermatotropic?

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Introduction and objective

Cutaneous leishmaniasis (CL) in Sri Lanka is caused by a genetic variant of *Leishmania donovani*, a usually visceralizing parasite in the rest of the world. The visceralization potential of *L. donovani* in Sri Lanka is not yet fully understood. Animal models are recognized as very useful tools for detailed studies of parasites. The current study was aimed at establishing an animal model that could be used at local level for parasite studies.

Methodology

Five patients with single large ulcerated lesions (>4cm) of laboratory confirmed CL were studied after informed consent. Lesion aspirates were grown in artificial media and parasites were harvested. Six female BALB/c mice were infected through the tail vein with 10×10^6 metacyclic promastigotes from each isolate with two control BALB/c mice similarly treated with normal saline. Mice were euthanized in 2 batches (n=3 each) at 4-6 weeks and at 10-12 weeks after inoculation. Both spleen and liver were removed and cultured. Parasite loads were determined using Dab smear.

Results

None of the mice euthanized at 4-6 weeks showed any signs of infection. However, visceralization was evident in 9 out of 15 infected mice, euthanized between 10-12 weeks (60%, n=9/15). All infected mice showed spleen infection (9/9) and 1/9 (11%) had liver infection.

Conclusion

Local strain of *L. donovani* has the capacity to establish infection in BALB /c mice, inducing visceral disease. Therefore BALB /c mice could be considered as a laboratory model for the study of *L. donovani* that causes CL in Sri Lanka. This study also may imply that the local strain, though predominantly dermatotropic in humans, might have or acquire the ability to visceralyze over time. The use of this model is being pursued for detailed investigation of this parasite.

OP 3

Culture positive melioidosis in Sri Lanka in 2014

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Introduction

Melioidosis is increasing in Sri Lanka.

Objectives

To describe the microbiology of melioidosis in Sri Lanka.

Design, setting and methods

All isolates of *Burkholderia pseudomallei* were included. Demographic and clinical data of patients were collected. Isolates were cultured on routine media and described. Antibody titres were measured using the indirect haemagglutination (IHA) test.

Results

B. pseudomallei was isolated from 23 patients in 2014. Sixteen patients were male and three were children. Clinical presentations included septic shock, pneumonia, liver, splenic, lymph node, skin and soft tissue, submandibular, cerebral and intramuscular abscesses, septic arthritis and nodular skin rash. Only two died, one prior to diagnosis. One was lost to follow up and one had many relapses. *B. pseudomallei* was isolated from blood (n=11), pus (n=10), knee joint aspirate (n=2), thoracotomy drainage (n=1) and sputum (n=1). Two patients were positive from multiple sites. Pus was obtained from liver

abscess (n=3), submandibular abscess (n=2) and one each of cerebral, intramuscular, chest wall, cervical lymph node and back abscesses. All isolates exhibited similar colonial and microscopic morphology. They grew with ease on routine culture media and colonies were pin point in size on blood and MacConkey agar at 24 hours. After 48 hours colonies on blood agar were 2mm, white and moist with a characteristic earthy odour. Over a week they became umbonate, developed a shiny metallic sheen and darkening of blood agar was noted. Colonies on MacConkey agar became bright pink on the second day. None of the isolates showed wrinkling. All isolates were resistant to gentamicin, polymyxin and colistin and sensitive to co-amoxycylav, oxidase positive and had a characteristic safety pin appearance on Gram stain. Identification was confirmed by PCR to amplify the *lpxO* gene. Antibody testing by the IHA test was done on 19 patients and all were positive with titres ranging from 1:80 to >1:10240.

Conclusions

Raising awareness of melioidosis among clinicians and capacity building of laboratory personnel to identify this unusual bacterium has led to a significant increase in the diagnosis of melioidosis in Sri Lanka. Antibody titres vary widely in culture positive patients.

OP 4

An outbreak of respiratory syncytial virus infection from June to August 2014 in Sri Lanka

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Background

Respiratory syncytial virus (RSV) causes significant morbidity and mortality among infants and young children. Direct fluorescent test (DFT) and real-time PCR are employed to diagnose RSV infection in many countries.

Objectives

To report an outbreak of respiratory syncytial virus (RSV) infection among young children.

Methods

Nasopharyngeal aspirate (NPA), nasopharyngeal and oropharyngeal swabs (NPS/OPS) obtained from children suspected with bronchiolitis and respiratory distress were sent to National Influenza Laboratory in viral transport media in ice. NPA were obtained using a mucus extractor with suction. Samples were tested for RSV and Adenovirus using Imagen (Oxiod) commercial fluorescent antibody assay. Realtime-PCR for influenza A and B was also performed. Demographic, clinical and disease

outcome data were obtained using request forms and by visiting two hospitals.

Results

Of 89 respiratory samples tested, RSV was positive in 26 children, Adenovirus (1), Influenza A(H3N2) (1), Influenza B (1) and both for RSV and Influenza A (1). In RSV cohort, male: female ratio was 1.2:1. Age range was 1 month to 54 months (mode 6 months). Highest positivity rate of RSV (61% (16/26)) was observed in age group of day 01 to 6 months, while 7 months to 1 year 19% (5/26), (1 year to 2 years 7.6% (2/26), 2 years to 6 years 7.6% (2/26). Presenting features of RSV infection were fever (84% 22/26), Cough (88% 23/26) and shortness of breath (84% 22/26). Mean duration of illness was 7.8 days. Lung crepitations were seen in 11 children and pneumonia was observed in 6. One infant was ventilated. Seven children needed ICU care. Two infants had cardiac anomalies and one had a chromosomal abnormality.

Three deaths were reported (age 1.5, 3 and 39 months). Oseltamivir was started in 14 children at the time of collecting samples. Others did not give a history of antiviral therapy.

Twelve infants were from Nuwara Eliya followed by Galle (4), Children's Hospital, Colombo (4).

Conclusions

RSV caused significant morbidity and mortality in young children in this outbreak.

OP 5

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

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Introduction

Respiratory viruses including human metapneumovirus (hMPV) (family: *Paramyxoviridae*) cause acute respiratory illness ranging from mild upper respiratory symptoms to severe life threatening lower respiratory tract infection (LRTI), particularly in children.

Objective

To detect the viral aetiology of a selected group of children who presented with severe acute respiratory symptoms to the Teaching Hospital, Peradeniya (THP).

Design, setting and methods

Twenty one children (age = 1-4 years) admitted to the THP, over three months from July to October 2014 were investigated. Patients presented with severe acute respiratory symptoms ranging from bronchopneumonia, LRTI, exacerbations of asthma and wheezing. Nasopharyngeal aspirates (NPA) obtained from all subjects were screened using a direct IFA (DAKO IMAGEN[®]) to test for the presence of any of the seven respiratory viruses (influenza A and B, parainfluenza 1, 2 and 3, adenovirus and RSV); screening positive NPA were then typed using indirect IFA (DAKO IMAGEN[®]). RNA was extracted from all samples using QIAmp Viral RNA Mini Kit and was subjected to a one step RT-PCR (QIAGEN OneStep RT-PCR) for the detection of hMPV using a set of specific primers (L6 and L7) that target the L gene (171bp) of the hMPV genome.

Results

Of the 21 NPA typed using IFA, two were positive for RSV (2/21), one for influenza A (1/21) and one had adenovirus and parainfluenza 2 as a co-infection (1/21). Of the 21 NPA tested using RT-PCR, 18 (18/21) showed 171bp band suggesting the presence of hMPV. These 18 samples included the 2 positives for RSV and the 1 positive for influenza A by IFA. Two samples did not have any of the tested viruses.

Conclusion

RSV, influenza A, parainfluenza 2, adenovirus and hMPV were found to be associated with severe acute respiratory symptoms in a selected group of children. hMPV was identified by RT-PCR in the majority of these children as a primary infecting agent or as a co-infection with RSV or influenza A. Sequencing studies are in progress to further characterise the hMPV.

Funding

We acknowledge University of Peradeniya (RG/AF/2013/38/M) for funding.

OP 6

Sensitivity of influenza viruses to oseltamivir and zanamivir: A study conducted on representative influenza A and B viruses isolated in Sri Lanka in 2009-14

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Background

Neuraminidase inhibitors are used in treating influenza

virus infection. The need for surveillance of antiviral susceptibility of influenza viruses has grown in recent years due to development of resistance to these antiviral agents. Fluorescence-based assays of neuraminidase inhibition (NA) assays are commonly used for determining influenza susceptibility to NA inhibitors.

Objectives

To detect sensitivity to oseltamivir and zanamivir of influenza A and B viruses isolated in Sri Lanka in 2009-14.

Methods

A representative clinical samples and tissue culture isolates (selected on according to temporal distribution, geographic distribution, age distribution, influenza types and subtypes) were sent to WHO Collaborating Centre, Melbourne, Australia for Neuraminidase inhibitor susceptibility testing from 2009 to 2014. Susceptibility testing was performed using fluorescence-based enzyme-inhibition assay utilising the substrate 2'-(4-methylumbelliferyl)- α -D-N-acetylneuraminic acid (MUNANA). The median inhibitor concentration (IC₅₀) required to inhibit 50% of the neuraminidase enzyme activity for each type and subtype of influenza isolates was calculated, according to the WHO antiviral susceptibility testing guidelines, viz. normal, reduced and highly reduced inhibition patterns.. Three categories of inhibition were determined: normal inhibition, reduced inhibition and highly reduced inhibition according to WHO antiviral susceptibility testing guidelines.

Results

Eighty six (86/422; 20.5%) influenza isolates were tested for antiviral sensitivity (11 isolated in 2009, 7 in 2010, 9 in 2011, 31 in 2012, 18 in 2013 and 10 in 2014). Two seasonal influenza A H₁N₁ viruses isolated in 2009 showed highly reduced inhibition to oseltamivir with sensitive to zanamivir. Remaining influenza A H₁N₁ pdm 2009 (19), H₃N₂ (38) and all influenza B (29) viruses displayed normal inhibition pattern to oseltamivir and zanamivir.

Conclusions

Except two Influenza A seasonal H₁N₁ (in 2009), other selected strains were sensitive to oseltamivir and zanamivir from 2009 to 2014

OP 7

Audit on hand hygiene compliance at the Surgical Intensive Care Unit (SICU), National Hospital of Sri Lanka.

Nakkawita WMID, Patabendige CGUA
National Hospital of Sri Lanka, Colombo.

Introduction

WHO Guidelines on 'Hand Hygiene in Health Care'

provides evidence based recommendations to practice five moments of hand hygiene in healthcare settings to reduce transmission of pathogenic microorganisms. As hospital acquired infections especially ventilator associated infections are found to be common in this ICU, it was decided by the Consultant Clinical Microbiologist and the team to conduct an audit on hand hygiene compliance.

Objective

To describe the hand hygiene compliance rates of different categories of health care staff at SICU of NHSL before and after an awareness program.

Design, setting and method

Audit was carried out in two stages at SICU, NHSL from 01/04/2014 to 08/05/2014 and 01/06/2014 to 03/07/2014. An awareness program was conducted in between audits to teach on five moments and correct technique of hand hygiene. Standard data collection form was used to collect data. Total of 526 and 452 different moments of hand hygiene were observed and recorded by the auditor. Data was analyzed using Microsoft Office Excel 2007.

Results

Overall compliance rates were 31.94% (168/526) and 46.28% (209/452) in first and second audit respectively and the difference is statistically significant ($P < 0.05$). Out of hand rub users, female consultants had the highest compliance rate of 60% and 100% in audit 1 and 2. Hand washing compliance was highest among female nurses (25.4%) and lowest among female doctors (1.7%). Significant improvement in hand rub use was observed from 9.43% to 65% among physiotherapists in audit 1 and 2 ($P < 0.001$). Compliance rate was 0% in radiographers, cardiographers and outside doctors in both audits. Highest compliance rates of 55.55% and 68.75% were seen in moment 2 (before aseptic procedure) in audit 1 and 2.

Conclusion

Statistically significant improvement in hand hygiene compliance rate was observed after the intervention program. Further improvement in hand hygiene compliance can be achieved with time to time awareness programs especially for categories with 0% compliance rate, audits at regular intervals and giving feedback of these audit results with the intension to improve on problematic areas.

OP 8

Incidents of ventilator associated pneumonia in two intensive care units and a high dependency unit at National Hospital of Sri Lanka

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Introduction

Ventilator Associated Pneumonia (VAP) is one of the commonest healthcare associated infections. Identification of VAP rates and associated factors in ICUs will be of great value to implement and monitor infection control activities to minimize the spread of multidrug resistant organisms.

Objectives

- To identify the VAP rates in Medical Intensive Care Unit (MICU), Accident Service Intensive Care Unit (ASICU) and Accident Service High Dependency Unit (ASHDU)
- To describe associated factors, organisms responsible and their ABST patterns

Design, setting and method

Descriptive cross sectional study was carried out at MICU, ASICU and ASHDU at National Hospital of Sri Lanka from 13/06/2014 to 11/08/2014. VAP was diagnosed according to clinical and radiological criteria, CDC 2014. Interviewer administered questionnaire was used to collect data. Endo tracheal aspirations were collected from all VAP patients. Sample processing and ABST were done according to standard operating procedures and CLSI standards. VAP rates were calculated for 1000 ventilator days. Associated factors for VAP were analysed using Microsoft Excel.

Results

Overall VAP rate in 3 units were 37.57 per 1000 ventilator days with 75, 40.61 and 23.53 per 1000 ventilator days in ASICU, ASHDU and MICU respectively. Mean age of VAP patients were 54, 38 and 31 in MICU, ASICU and ASHDU respectively. Ninety five percent of VAP cases were diagnosed 5 days after intubation and 70% of all admissions were transfers from another hospital. *Coliforms*, *Acinetobacter* and *Pseudomonas* spp were the isolates from respiratory specimens and out of them commonest isolate was *Acinetobacter* spp (45%). Almost all *Acinetobacter* isolates were multidrug resistant, whereas 60% of *pseudomonas* isolates were multidrug sensitive. *Coliform* spp were 100% resistant to cephalosporins out of which 25% were multidrug resistant.

Conclusion

VAP rates in 3 ICUs at NHSL were very high and it is well above the NHCN benchmarks. Ongoing surveillance of VAP rate with time to time correction of modifiable risk factors is important to minimize the VAP rates in these ICUs.

OP 9

Epidemiology of ventilator associated pneumonia caused by *Acinetobacter* species and their antibiotic susceptibility patterns in different intensive care units at National Hospital of Sri Lanka

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Introduction

Ventilator associated pneumonia (VAP) is the most common nosocomial infection in patients receiving mechanical ventilation and contributes for about half of all antibiotics used in the intensive care units.

VAP can occur 48 hours or more after endotracheal (ET) intubation and mechanical ventilation. It remains an important cause of morbidity and mortality despite advances in antimicrobial therapy. Prevalence data for VAP in the country are limited.

General Objective

To describe the epidemiology of VAP caused by *Acinetobacter* spp. at different intensive care units (ICUs) and their antibiotic susceptibility patterns.

Specific Objectives

- To determine the incidence of *Acinetobacter* spp. in different ICUs.
- To identify the common *Acinetobacter* spp. causing VAP in different ICUs and their antibiotic susceptibility patterns.
- To identify the associated factors for VAP due to *Acinetobacter* spp.
- To describe the distribution of VAP caused by *Acinetobacter* spp. and their antibiotic susceptibility patterns in different ICUs.

Design, setting and method

A hospital based descriptive cross sectional study was carried out involving all the ICUs at the National Hospital of Sri Lanka (NHSL). VAP was diagnosed clinically according to the HELICS criteria. Two hundred ET aspirates were obtained from clinically diagnosed VAP cases and were processed. The phenotypic identification of *Acinetobacter* spp., speciation using API 20NE and antibiotic susceptibility testing according to CLSI standards were done at Microbiology laboratory, NHSL.

Results

Sixty seven *Acinetobacter* spp. were isolated from 200 VAP cases (33.5%). All the isolates were *Acinetobacter baumannii/calcoaceticus* (100%). Most number of cases was from MICU followed by RU, ASICU & NTICUs. Most were associated with neurosurgical procedures (32.8%).

A slight female predominance was noted (53.7%). Most of the isolates were multidrug resistant and cefoperazone-sulbactam had the least resistance.

Conclusions

Acinetobacter baumannii/calcoaceticus was the only species isolated during this study, which were multidrug resistant and predominated in the MICU. Significant association with neurosurgical procedures was noted.

- Financial assistance by MRI from research grant 42/2013 acknowledged.

OP 10

Immunoglobulin G immune status and vaccination history of measles, mumps, rubella and varicella in post-graduate medical trainees

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Background

Medical officers are at higher risk of acquiring and transmitting infectious diseases in hospitals. Knowing immunity status and vaccination history against such infections can prevent transmission.

Objectives

To report IgG immune status of measles, mumps, rubella and varicella of postgraduate medical officers and their vaccination and past contact history.

Methods

Immunoglobulin G antibody of mumps, measles, rubella and varicella of trainees who were expected to follow post-MD overseas training was performed using VirolImmune and Human (both, Germany) E.L.I.S.A. assays. Their demographic details, specialty, past contact and vaccination history were analyzed.

Results

Complete data set was only available in 201 out of 251 doctors who requested immune status. Average age was 37.9 yrs SD± 3.49. Male:Female ratio was 1.35: 1. Ninety six doctors gave a past history for mumps, 86 for measles and 70 for varicella. Vaccination history against varicella n=32 (15%), MMR n=52 (25%), rubella n=67 (33%) were recorded. IgG was positive and negative in 140, 45 (mumps), 190, 11 (measles), 33, 8 (rubella) and 118, 28 (varicella, two equivocal results) individuals respectively (some ELISA assays were not available in certain months, hence not tested in all). IgG was negative for one disease (n=58), two (n=15), three diseases (n=1) and none for all 4 diseases. Those who gave a past history of mumps

(13/96) and varicella (6/70) and those who were vaccinated against mumps (33), measles (3), varicella (8) and rubella (6) were not having detectable IgG levels by the used commercial assays.

Conclusions/Recommendation

Significant number of doctors in postgraduate training were not aware nor had records of vaccination and were not immune to mumps, measles, rubella and/or varicella infections. This possesses a risk of transmission of above infectious diseases to patients and to health care workers. Immune status should be screened when they enter medical practice or postgraduate training and records should be maintained.

OP 11

Assessment of seroprevalence of Hepatitis B antibody and hepatitis B infection after routine immunization in 1- 5 year old children

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Introduction

Hepatitis B infection is a major public health problem globally causing 600,000 deaths each year. Studies in Sri Lanka have shown rates of chronic hepatitis B infection ranging from 0.27% to 2.5%. Routine immunization of infants against hepatitis B was recommended by the WHO in 1991. Hepatitis B vaccine was introduced to the Expanded Programme of Immunization (EPI) in Sri Lanka for all infants at 2, 4 and 6 months of age in 2003. This study was conducted to find out the impact of the vaccination programme.

Objectives

- To assess the sero-prevalence of current Hepatitis B infection among children in the 1 - 5 year age group in a district in Sri Lanka.
- To assess the immunity among children to Hepatitis B infection in the 1 - 5 year age group in a district in Sri Lanka.

Methods

This was a cross-sectional sero-epidemiological survey conducted from June to September 2014 in the Kalutara district. 407 children of 1 - 5 years of age were tested for Hepatitis B surface antigen (HBsAg) and hepatitis B surface antibody (HBsAb) using a commercial enzyme immunoassay method. An antibody level of ≥10 mIU/mL was taken as the protective level.

Results

All (n=407) the study participants had been vaccinated

with 3 doses of hepatitis B vaccine. 310 (76.2%) of the participants had protective antibody while 97/407 (23.8%) did not have protective antibody. The mean antibody titer was 247 mIU/mL. A gradual decrease in percentage protected was seen with increasing age (79.4%, 78.6%, 76.4% and 67.9% among 1-2, 2-3, 3-4 and 4-5 year age groups respectively). All participants were negative for current Hepatitis B infection.

Conclusions

The infant vaccination program is successful in Sri Lanka as 100% vaccination was observed among the study participants. A majority of the children have protective levels of humoral immunity against Hepatitis B. Immunity levels decrease gradually with increasing age which is comparable with global literature. A larger study with a larger sample size should be conducted to detect low hepatitis B infection levels Sri Lanka as we are a low endemic country.

OP 12

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

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Introduction

INNO-LIA™ HIV Score is an established line blot assay with manufacturer defined minimum criteria for HIV confirmation.

Objective

To define locally, a set of minimum HIV band pattern in INNO-LIA™ HIV Score in confirmation of HIV serology.

Methods

INNO-LIA™ HIV Score assay results of HIV screening ELISA positive samples were analysed over 3 months (June to August 2014). Additional HIV markers were assessed on first and follow-up samples for HIV-1 p24 antigen (ELISA), HIV-1 RNA and HIV-1 proviral DNA (PCR).

Results

A total of 105 INNO-LIA™ HIV Score blot results were included in this study. Ninety four samples were positive for both HIV-1 envelope bands (sgp 120 and gp41) and at least one other HIV-1 band (p31, p24 or p17). All were confirmed to be HIV-1 positive in additional assays. Two samples were positive exclusively for HIV-1 envelope bands with no other bands. One was a known HIV-1 positive patient and the other was clarified as HIV negative. Seven samples were positive for one HIV-1 envelope band, with or without additional HIV-1 band. Of these, 2 were

confirmed to be HIV positive including a HIV-1 seroconversion and the other 5 were confirmed to be HIV-1 negative. Two of these 5 negative samples had p24 band in addition to the single envelope band. Two samples did not have any band on INNO-LIA™ HIV Score blot and these turned out to be HIV-1 seroconversion.

Conclusion

Using manufacturer's minimum interpretation criteria of two positive bands as HIV-1 confirmation, we found three patients to be false positive for HIV. But, none of the samples with two envelope HIV-1 bands with at least one additional band was confirmed as HIV negative following additional tests. We suggest using two envelope bands with one more additional band as minimum criteria for HIV type 1 diagnosis instead of the manufacturer's minimum criteria of two positive bands.

Established HIV-1 positive patients on treatment or HIV-1 seroconverting patients may need final interpretation of HIV status based on HIV-1 p24 antigen EIA, HIV-1 RNA and HIV-1 proviral DNA results.

OP 13

Incidence, risk factors and outcome of acute lower limb cellulitis in patients admitted to a tertiary care hospital

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Introduction

Lower limb cellulitis is a common condition in patients seeking admission to hospitals. Better understanding of incidence, risk factors and outcome of cellulitis patients will help to improve management in a more cost effective way.

Objectives

To describe the incidence, risk factors and outcome of patients with acute lower limb cellulitis and to identify the significance of *Staphylococcus aureus* nasal carriage in these patients.

Design, Setting and Method

Prospective case control study was carried out at all surgical and medical wards of CSTH from 01/10/2014 to 31/12/2014. All clinically diagnosed acute lower limb cellulitis patients and age and sex matched control group were included for the study. Demographic data and data related to cellulitis were collected using an interviewer administered questionnaire. Nasal swabs were collected from all patients. All Cellulitis patients were followed up for 30 days. Data was analyzed using SPSS 20. Ethical clearance was obtained from the Ethical Review Committee at CSTH.

Results

Incidence rates of lower limb cellulitis in patients admitted to all wards and surgical wards were 4.65 and 9.5 per 1000 admissions respectively. BMI >25 ($p<0.001$), diabetes mellitus ($p<0.01$), lower limb oedema ($p<0.001$), lower limb ulcer ($p<0.001$), penetrating trauma ($p<0.001$), toe web problems ($p<0.001$) and past history of cellulitis ($p<0.001$) were statistically significant risk factors. Nasal carriage of *Staphylococcus aureus* was not a significant risk factor. Correct first line antibiotics were started in 86.7% of patients and the doses were correct only in 50%. Antibiotic regime was changed in 41.1% of patients during stay. Of these 74.4% were discharged without complications and 18.9% were hospitalized for >10 days. During 30 days 21.2% of cellulitis patients had recurrence and 15.6% needed readmission.

Conclusion

Risk factors for lower limb cellulitis in hospitalized patients include several predisposing factors and presence of entry sites in lower limbs for pathogens. Considerable number of patients require readmission due to cellulitis of the same limb. Correction of modifiable risk factors, administration of proper antibiotics in correct doses will reduce hospital stay and readmissions.

OP 14

Respiratory colonizers in patients with primary antibody deficiencies attending an immunology clinic in Sri Lanka

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Although primary antibody deficiencies (PADs) are associated with recurrent respiratory infections leading to bronchiectasis, longitudinal data on respiratory pathogens in this population is limited. In cystic fibrosis an orderly progression of pathogens colonize the lungs. The objectives were to determine whether a similar progression is seen in PADs and to identify the common respiratory pathogens and their antibiotic susceptibility profiles.

Methodology

A prospective study of 28 patients with PADs attending the MRI Immunology Clinic was performed. Those with current respiratory infections/exacerbations or on antibiotics were excluded. Sputum was collected from chronic sputum producers and an oral rinse from others. Specimens were repeated after 4.48(±0.284) months. Specimens were processed using quantitative culture and

potentially pathogenic microorganisms isolated from each patient were identified as persistent or intermittent colonizers and their antibiotic susceptibility patterns were determined.

Results

H. influenzae, *S. pneumoniae* and *M. catarrhalis* were isolated from 43%, 21% and 7% of patients on initial assessment and 61%, 32% and 14% on follow up review. *P. aeruginosa*, *Acinetobacter* spp., Group C and Group G *Streptococcus* spp. were isolated from 1-2 patients each. Thirteen (46%) were persistently colonized with one or more pathogens and 10 (36%) were persistently colonized with *H. influenzae*.

A trend towards a reduction in intermittent *H. influenzae* colonization and increase in persistent *H. influenzae* and *S. pneumoniae* colonization with disease duration was seen. Some patients with persistent *H. influenzae* colonization showed differing antibiotic susceptibility patterns in the follow-up isolate. Most *H. influenzae* and *M. catarrhalis* were sensitive to amoxycillin-clavulanate, cefuroxime and cefotaxime. Trimethoprim-sulfamethoxazole resistance was common. *H. influenzae* resistant to levofloxacin was recorded.

Conclusions

Respiratory colonizers among patients with PADs are mainly *H. influenzae*, *M. catarrhalis* and *S. pneumoniae*. Empirical antibiotic therapy with coamoxiclav or cefuroxime may be adequate in patients with exacerbations, who do not have baseline respiratory cultures. Antibiotic cover for Gram negative non-fermenters may not be necessary in the absence of colonization/infection.

It is probable that initial *H. influenzae* colonization is intermittent, with subsequent persistence.

OP 15

Analysis of data of urine culture isolates of 2014 sent from seven laboratories of National Laboratory Based Surveillance of Sri Lanka College of Microbiologists

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Objectives

1. To determine the aetiological agents of midstream urine cultures with a colony count of $>10^5$ CFU/ml.
2. To analyse the antimicrobial susceptibility patterns of urine culture isolates of 2014.

Method

The National Laboratory Based surveillance on antimicrobial resistance is a collaborative project of the Ministry of Health and the Sri Lanka College of Microbiologists. In this project midstream urine cultures with a colony count of $\geq 10^5$ CFU/ml were analysed. The specimens were processed according to the standard protocol specified in the laboratory manual in microbiology. Antibiotic susceptibility tests were performed according to the method established in the centre which is either by CLSI method or by Stoke's comparative disk diffusion method. Data of 2014 sent by the participating laboratories were analysed using WHONET 5.6 software.

Results

The data was received from seven centres. They were The National Hospital of Sri Lanka, Sri Jayewardenapura General Hospital, Lady Ridgeway Childrens' Hospital, Faculty of Medicine, Colombo, Faculty of Medicine, Ragama, Faculty of Medicine, Sri Jayewardenapura and North Colombo Teaching Hospital, Ragama.

A total of 4441 significant isolates were analysed. The majority were Gram negative enteric organisms, commonly known as coliforms, with 3975/4979 (79.8%) isolates. The others were *Candida* species 408, *Enterococcus* species 254, *Pseudomonas* species 194, coagulase negative *Staphylococcus* species 59, *Staphylococcus aureus* 36, *Acinetobacter* species 35 and Group B beta-haemolytic *Streptococcus* 18.

The coliforms from adults who were attending outpatient clinics had 55.2% (112/203) susceptibility to cephalexin and cephadrine, 54% (161/298) to amoxycillin/clavulanic acid, 65.1% (278/427) to nitrofurantoin, 48.3% (144/298) to norfloxacin, 63.4% (189/298) to cefotaxime, 97.4% (113/116) to imipenem and 100% (90/90) to meropenem. The adult inward patients had 39.5% (519/1313) susceptibility to cefotaxime, 87.9% (445/506) to meropenem, 62.6% (812/1298) to gentamicin and 31.9% (405/1281) to ciprofloxacin. The coliforms isolated from paediatric outpatients had 58.5% (69/118) susceptibility to cephalexin and cephadrine, 58.5% (76/130) to amoxycillin/clavulanic acid, 80% (16/20) to nitrofurantoin, 85% (17/20) to cefotaxime and 89.7% (26/29) to meropenem. The paediatric inward patients had 64.6% (53/82) susceptibility to cefotaxime, 90.5% (19/ 21) to meropenem and 80.2% (65/81) to gentamicin.

Conclusion

Coliforms, the commonest organism causing urinary tract infections (UTI), had high resistance rate in in-ward

patients but the resistance was less in outpatients, especially in the paediatric age group.

OP 16

Comparison of bacterial characteristics (MICs) of Gram negative bacteria isolated from patients with neutropenic sepsis pre and post-levofloxacin prophylaxis

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Febrile neutropenia is a life-threatening complication, that occurs frequently during chemotherapy with associated high mortality. Antibacterial prophylaxis is an established strategy to prevent this. Fluoroquinolone prophylaxis has been considered for high-risk patients with prolonged and profound neutropenia ($ANC < 1000 \text{ mm}^3$), but risk of emergence of resistance has been a concern.

Levofloxacin was used as prophylaxis during the neutropenic period in chemotherapy-induced neutropenic patients at Leicester Royal Infirmary Hospital (LRIH), United Kingdom since 2010.

Objectives

Compare number of blood culture positivity in pre (2007-2010) and post (2010-2012) levofloxacin prophylaxis periods and compare sensitivity of ciprofloxacin and meropenem in Gram negative isolates from neutropenic patients of above periods in LRIH.

Design, setting and methods

1. Retrospective data collection done using haematology Gram negative bacteraemia data base, and relevant clinical and laboratory data were retrieved from case notes and computer based data system.
2. VITEC-MIC and E-strip MIC for ciprofloxacin and meropenem were performed on the Gram negatives retrieved from saved beads.
3. From 210 total blood culture positives of pre-levofloxacin period, for 45 isolates ciprofloxacin MIC and 44 isolates for meropenem MIC were performed.
4. From 88 total blood culture positives of post-levofloxacin period, for 79 for ciprofloxacin MIC and for 78 isolates for meropenem MIC were performed.

Results

Number of blood culture positivity has reduced from 210 to 88 with prophylaxis. Both MIC methods (VITEK & E-strip) gave similar sensitivities for tested Gram negative isolates. The numbers of resistant isolates identified with

both MIC methods (VITEK and E-strip) were the same. Resistant rate for meropenem was 4.5% (2/44) in pre-prophylaxis period and 11.5% (9/78) in post-prophylactic period. Resistance to ciprofloxacin was 17.7% (8/45) in the pre-prophylaxis period and 25% (20/79) in the post prophylaxis period. Both differences were not statistically significant at a p value of 0.05 (Fisher's exact test)

Conclusion

The use of levofloxacin as a prophylactic agent did not result in a statistically significant increase of antibiotic resistance among the common Gram negative pathogens. However, close monitoring is warranted as a trend towards increase in the proportion of resistance was noted.

PP 1

Analysis of accidental occupational exposure injuries among health care workers in a tertiary care hospital

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Introduction

Accidental needle stick injuries, cuts, bites or splashes into mucous membrane (exposure injuries) in the hospital carries the risk of infection by hepatitis B virus (HBV), hepatitis C virus (HCV) and HIV. Incident should be reported immediately to the infection control team (ICT) for proper post exposure management (PEM).

Objectives

To analyse: 1. The types of exposure injuries (EI) among health care workers (HCW), 2. The occurrence of injuries among different categories of the HCW, 3. The status of PEM

Method and setting

We analysed all reported EI among HCW in our hospital since January to December 2014. Thorough risk assessment including nature of the injury, source, circumstances and victims immunity for HBV was done in each incident and managed according to the standard protocols. Post exposure prophylaxis (PEP) for HIV was considered for severe injuries with unknown source at high risk set up.

Results

There were 105 reported cases of different types of EI during 2014 among HCW. Nurses reported the highest number of incidents (44/105, 42%). Number of doctors, minor employees and other categories was 25 (24%), 13 (12%) and 23 (22%) respectively. Most incidents were following incorrect sharps discard (36, 34%). Cut injuries during surgery, capillary blood sugar testing, and blood drawing caused EI to 20 (19%), 15 (14%), and 12 (11%) respectively. Two doctors, one nurse and a medical student reported EI following recapping of the needle (4/105, 4%). Only 74 (70%) were fully vaccinated against HBV. Ten (9%) were never vaccinated. Forty four (42%) had checked the HBV antibody status. Post exposure HBV vaccine was given to 31 (29%) HCW and PEP for HIV was started in 08 HCW.

Conclusion

Among the HCW, nurses report the highest number of

EI. Even after continuous education on prevention of EI, incorrect sharp discard and recapping still causes a significant number of EI. Importance of proper immunization against HBV has to be emphasized to all categories of HCW. Prompt reporting of the EI to the ICT allows standard and effective PEM of EI.

PP 2

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

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Introduction and objectives

A dermatropic variant of *L. donovani* causes cutaneous leishmaniasis (CL) in Sri Lanka. Current standard treatment includes painful and costly sodium stibogluconate (SSG) injections and cryotherapy, both of which require multiple hospital visits.

There are increasing numbers of patients who do not respond to regular therapy. A need exists to test for effective alternatives. Thermotherapy has been tested for management of CL caused by *L. tropica* and *L. major*. Efficacy, safety and cost-benefit of thermotherapy vs standard intra-lesional (IL) SSG were assessed for the first time, as a mode of treatment for *L. donovani*-induced CL.

Method

Laboratory-confirmed CL patients (males 162, females 51) with single lesions were randomly assigned to (i) test group (n=98; received a single session of radio-frequency induced heat therapy (RFHT) at 50°C for 30 seconds using a radiofrequency generator - ThermoMed 1.8 Thermosurgery USA) and (ii) control group (n=115; received weekly IL-SSG until cure or 10 doses). Patients were followed-up fortnightly for 12 weeks to assess clinical response and adverse events. Chi squared test and logistic regression analysis were done. Cost of treatment was assessed using scenario building technique.

Results

The cure rates by 8, 10 and 12 weeks in the thermotherapy group were 46.5%, 56.5% and 65.9% as opposed to 28.0%, 40.8% and 59.4% in the IL SSG group. The cure rate by thermotherapy was significantly higher ($p=0.009$) at 8 weeks and 10 weeks ($p=0.035$) while they were comparable thereafter. At 8 weeks, the response to thermotherapy was significantly higher in females [OR 1.93 (95% CI 0.997-3.738)], papular lesions [OR 2.73 (95% CI 1.29-5.77)] and in lesions $<2\text{cm}$ [OR 1.95 (95% CI 0.98-3.87)] when compared to those treated with IL SSG ($p = 0.05$, $p=0.009$ and $p = 0.05$ respectively). No major adverse events were recorded in either arms of treatment. It was 8.8 times cheaper to use thermotherapy (including capital expenditure) (Rs 164.00/patient) than IL SSG (Rs 1453.00/patient).

Conclusion

A single application of thermotherapy was found to be safe, cost-effective and patient-friendly as compared to multiple doses of IL SSG in the treatment of *L. donovani* CL. Submitted to SLMA 2015.

PP 3

Usefulness of cattle blood as an enrichment substance in blood supplemented culture media, in the clinical microbiology laboratory

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Objectives

To determine the ability of cattle blood as an enrichment substance in blood supplemented media in microbiology, to assess

- growth of common bacterial pathogens in clinical specimens
- an indicator medium showing different haemolytic patterns
- basic identification tests
- performance of Antibiotic Sensitivity Testing
- the rate of isolation from the clinical specimens

Method

303 clinical samples were processed during the study period on cattle, human and sheep blood containing medium. Isolation rates, colony appearance, pattern of haemolysis, relevant identification tests and antimicrobial susceptibility were compared qualitatively with the pattern on sheep blood.

Results

Human blood supplemented chocolate agar could isolate 100% while cattle and sheep blood supplemented media

could only isolate 75% and 50% of *Haemophilus* species respectively. All the other organisms grew on all the media. Colony sizes in human blood supplemented blood and chocolate agar is obviously smaller than colony sizes in cattle and sheep blood supplemented blood and chocolate agar. All the Gram Negative bacteria had the same colony size and morphology in all three media. Except in *Haemophilus* species the colony size was largest in human chocolate agar than sheep and cattle agar media. Zones of haemolysis of Streptococcal species were smaller in human blood supplemented agar but almost equal in cattle and sheep blood supplemented agar. CAMP test showed positive results for Group B Streptococcus and negative results in negative control with sheep as well as cattle blood but not with human blood supplemented media. Optochin and bacitracin sensitivity and ABST results were similar in all three media.

Conclusions

Human blood supplemented chocolate agar is better than cattle and sheep blood supplemented chocolate agar for isolation of *Haemophilus* species from clinical specimens. Cattle blood supplemented media has almost all qualities of sheep blood agar which are useful in identification of bacteria eg. Colony morphology, Haemolysis, CAMP test and optochin sensitivity. ABST done according to CLSI method also gave similar zone diameters with all 3 types of media.

PP 4

Persistent colonization with levofloxacin and moxifloxacin resistant *Haemophilus influenzae* in a patient with common variable immunodeficiency (CVID)

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Introduction

Fluoroquinolones remain important antibiotics for the treatment of respiratory tract infections and resistance to them amongst *H. influenzae* is rare. We report a case of persistent respiratory tract colonization with an *H. influenzae* strain resistant to ciprofloxacin, levofloxacin and moxifloxacin.

Case report

The patient was a 41 year old lady with CVID. In the 7 years preceding diagnosis, she had repeated lower respiratory tract infections leading to central bronchiectasis and was a chronic sputum producer. She had been on monthly intravenous immunoglobulin (IVIG)

replacement for 7 months following diagnosis, but had developed anaphylaxis to IVIG necessitating discontinuation. She had been treated with multiple oral antibiotics by several general practitioners over the years. Although documented evidence of previous fluoroquinolone therapy in this patient was not available, given its widespread empirical use in lower respiratory tract infections by doctors in Sri Lanka, it is quite likely that a past exposure to quinolones was present.

Two quantitative sputum cultures obtained 4.5 months apart, isolated *H. influenzae* in colony counts in excess of 10^7 CFU/ ml of sputum. During this time the patient was not on any antibiotic and was free of respiratory tract infections or exacerbations. The isolates were identified by Gram stain appearance, characteristic odour, satellitism around *S. aureus* and requirement for Factor X and V. Speciation was confirmed using MALDI-TOF. Antibiotic susceptibility testing was done by disk diffusion according to the Clinical Laboratory Standards Institute recommendations and MICs were determined using the E-test. Beta lactamase testing was done using the acidometric and biological methods. Fluoroquinolone resistance was determined using both the zone diameters and MICs.

The antibiotic susceptibility profiles of both isolates were similar. They were sensitive to ampicillin, amoxycillin-clavulanate, cefuroxime, cefotaxime, ceftriaxone, chloramphenicol, tetracycline and azithromycin. They were resistant to trimethoprim-sulfamethoxazole, ciprofloxacin, levofloxacin and moxifloxacin and were beta lactamase negative.

Discussion

Although quinolone resistant *H. influenzae* has been sporadically reported, its incidence remains rare. The emergence of such strains present microbiological and clinical challenges with important implications for the treatment of community acquired respiratory infections.

PP 5

Combining PCR and IgM will be useful for detecting dengue virus infection at an early phase

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Introduction

Selecting a suitable test during the evolution of dengue virus (DENV) infection in a patient is crucial.

Objective

Thus the objective of the current study was to investigate the clinical profiles of suspected dengue fever (DF) /

dengue haemorrhagic fever (DHF) patients admitted to the General Hospital, Kandy (GHK), together with DENV IgM and two types of conventional DENV genus specific reverse transcription PCR (RT-PCR).

Design, setting and methods

The study was carried out from July 2011 to January 2012 and the ethical clearance was obtained from Ethical Review Committees of Faculty of Medicine University of Peradeniya and GHK. Blood samples were collected from 292 patients between fever days 1-5 based on the WHO criteria for the clinical diagnosis of DF/DHF. Clinical and non-specific laboratory data were collected using a detailed questionnaire. RT-PCR was performed with 2 sets of primers targeting the capsid and envelope regions of the DENV genome. DENV IgM was tested using an ELISA (SD Diagnostics). Data were statistically analyzed using Minitab, Version 17.

Results

The study cohort had 167 males and 125 females. Myalgia was the commonest complaint experienced by 65% (190/292) of the patients followed by headache in 55% (161/292) of the patients and arthralgia in 42% (122/292) of the patients. PCV was >45% in 27% (79/292) of the patients; 42.12% (123/292) of the patients had reduced platelets and 62.67% (183/292) of the patients had reduced WBC. RT-PCR of 292 patients that had fever ≤ 5 days showed 15% (43/292) positivity whereas DENV IgM of 289 patients that had fever ≥ 3 days revealed 62% (181/292) positivity. Thirty eight and 6 patients were positive for the capsid gene and the envelop gene RT-PCR, respectively. Altogether 43 patients were viraemic as detected by the RT-PCR; 181 patients were positive for DENV IgM.

Conclusions

Only 15% (43/292) of the patients were viraemic as detected by the PCR. When PCR and IgM (181/292) results were combined, 77.51% (224/292) of the patients had evidence for DENV infection. Thus combining PCR and IgM will be useful for detecting DENV infection in the laboratory in patients with fever days ≤ 5 .

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PP 6

Toxoplasmosis awareness, sero-prevalence and risk behavior among pregnant women in the Gampaha district, Sri Lanka

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Objectives

To determine the prevalence and awareness of toxoplasmosis and to identify risk factors and possible routes of infection among pregnant women in the Gampaha district, Sri Lanka.

Design, setting and methods

Pregnant women attending obstetric clinics at the Colombo North Teaching Hospital (CNTH) in February – June 2014 were systematically selected and tested for *T. gondii* specific IgG and IgM antibodies using the *OnSite* Toxo IgG/ IgM Rapid Test-Dip Strip®. Socio-demographic details and information regarding disease awareness and risk behavior patterns of the participants were collected.

Results

The mean age of the 293 women tested was 27 years (SD, +/-5.92). Thirty eight percent were primigravidae with a mean gestational age of 16.2 weeks (SD 7). The prevalence of anti-*T. gondii* IgG antibodies was 12.3% (n=36). All participants were sero-negative for anti-*T. gondii* IgM antibodies. Prevalence of anti-*T. gondii* IgG antibodies was significantly higher among those eating commercially prepared meals, thrice a week or more (17.3%) compared to once a month or not at all (9.3%) (p<0.05). No significant relationships were observed with other risk factors (cat-ownership, handling cats, consumption of meat, consumption of unwashed raw vegetables and fruits, handling soil and not washing hands after handling soil). Awareness of toxoplasmosis was 4.4% (n=13); health personnel (46.2, n=6) and media (53.8%, n=7) being sources of information.

Conclusions

The presence of a large population of non-immune women of child bearing age (88%) with low disease awareness, indicates the necessity of an educational program targeted at this high risk group to avoid exposure to toxoplasmosis during pregnancy. The importance of consuming hygienically prepared meals during pregnancy needs to be emphasized.

PP 7

***Bordetella pertussis* specific Immunoglobulin G antibody levels among asymptomatic individuals aged 4-24 years admitted to two selected hospitals in Sri Lanka**

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Introduction

Pertussis continues to circulate in the community and cases among adolescents and adults have been increasing. Waning of pertussis-specific immunity following natural infection or immunisation may contribute to the persistent circulation. Even though it is not included in the extended programme of immunization in Sri Lanka, the booster doses including the adolescent booster dose of dTap, (acellular pertussis) are included into the list of recommended immunizations in several countries. Even though the protective titre yet not established, information on immunity to pertussis in this age group is needed before any vaccination policy can be considered.

Objectives

To determine the antibody levels against pertussis toxin to determine the need and the optimal age for booster immunization.

Methods

The quantitative determination of specific IgG antibodies to *Bordetella pertussis* toxin was done by the ELISA using sera of 385 asymptomatic individuals aged 4 - 24 years admitted to surgical units of Lady Ridgeway Hospital, Colombo and Colombo South Teaching Hospital, Kalubowila. Mann-Whitney U test and Kruskal-Wallis test were used in analysis and p≤0.05 was taken as significant.

Results

Median age was 12 years (IQR 8-19) with 212 (55.1%) females. The median (IQR) anti PT antibody level was 3.31 IU/ml (0.73-15.12) and 352 (91%) had anti PT level <55 IU/ml. Median (IQR) anti PT levels were 3.18 IU/ml (0.591-8.00) for 4-7 years, 1.43 IU/ml (0.336-6.27) for 8-11 years, 4.28 IU/ml (0.978-13.39) for 12-15 years, 6.14 IU/ml (1.44-63.25) for 16-19 years and 4.89 IU/ml (1.11-16.78) for 20-24 years and all of these difference were statistically significant (Spearman Correlation Coefficient P=0.0121). Females (p<0.003) and those having a sibling above 12 years (p=0.017) had significantly higher anti PT levels.

Conclusion

The majority of the study population, especially 8 to 11 years age group had very low anti PT IgG levels. The infection may occur in early adolescents. A booster dose of acellular pertussis vaccine could be considered.

PP 8

A retrospective analysis of fungi causing onychomycoses in Sri Lanka

Kudavidanage S, Sigera LSM, Jayasekera PI, Perera PD

Department of Mycology, Medical Research Institute, Colombo 8.

Introduction

Accurate diagnosis of onychomycosis (fungal infection of nails) depends on direct microscopy and culture. Onychomycosis is difficult to treat because of slow growth rate of the nail and requirement of longer course of antifungals with considerable side effects. Dermatophytes account for most (90%) of the toe nails and at least 50% of the finger nail infections. However, non dermatophytes also cause infections, including *Candida* spp, *Fusarium* spp, *Scytalidium* spp, *Curvularia* spp, *Cladosporium* spp, *Acremonium* spp, *Penicillium* spp and *Paecilomyces* spp.

Objectives

- To determine the species of fungi, isolated from nail samples (scrapings and clippings) from 2001-2014 at Department of Mycology, MRI in Sri Lanka
- To determine the changing pattern of species during the 14 years

Methodology

Nine thousand eight hundred and eighty two samples were analyzed. Specimens were processed using 10% KOH for direct microscopy and cultured on SDA supplemented with antibiotics +/- cycloheximide and incubated at 26°C. Fungal species identified, morphologically and biochemically, were plotted against time.

Results

Out of 9882 samples, 5903 (59.73%) were positive for direct microscopy. Among them, fungi grew in 5220 (moulds-2430 and yeasts 3194) samples. The majority (61.18%) were *Candida* spp. followed by *Fusarium* spp. (22.56%) and *Aspergillus niger* (20.19%) etc. Dermatophytes accounted for 0.93% (47 isolates), among them *Trichophyton rubrum* was the commonest. To compare toe nails with finger nails, data is not available.

Numbers of all the above isolates, *Candida* spp., *Fusarium* spp. and *Aspergillus niger* have increased steadily over the years, all three isolates have shown high peaks in 2013. But number of candida isolates was four times more compared to *Fusarium* spp. and *Aspergillus niger* throughout the study period, whereas the other two isolates were showing same pattern of distribution. Results of this study cannot be compared with earlier data as they are not available.

Conclusion

During the past 14 years *Candida* spp. was the commonest isolated fungi from onychomycosis patients in Sri Lanka. *Candida* spp., *Fusarium* spp. and *Aspergillus niger* have increased steadily over the years. Dermatophytes have no significant role in onychomycosis in Sri Lankan patients.

PP 9

Seroprevalence of measles, mumps and rubella antibodies in infants in Colombo district

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

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

Introduction

The placental transfer and waning-off rate of maternal IgG antibodies in infants varies widely among different nationalities. This emphasized on the significance of determining the age for vaccination.

Objective

To determine the degree of maternal antibody transfer to the newborns and to determine the anti-measles, -mumps and rubella maternal antibody levels in 6-12 month old infants.

Methods

This is a cross sectional descriptive study. A total of 480 serum samples; including 280 samples from infants of 6 to 12 months of age and 200 samples from mothers and their newborns of 100 mother-infant pairs were tested for Immunoglobulin G (IgG) by commercial ELISA kits. Antibody titers were compared using student T- test.

Results

There was a high degree of transfer of maternal antibodies from mothers to their newborns. The prevalence of seropositivity against measles, mumps and rubella in infants at birth was 96.5%, 88.5% and 98.5% and at 6 months of age was 4.3%, 2.8% and 1.4% respectively. In the 8 month old infants recruited, 91.6% were negative for antibodies against measles and mumps viruses, and 98% of infants were sero-negative for rubella. One hundred percent sero-negativity was observed by the completion of nine months for measles, ten months for mumps and eleven months for rubella.

Conclusions

There is a high degree of maternal transfer of IgG antibodies to the newborns against measles mumps and rubella viruses and it has given rise to protective levels of

maternal IgG levels in newborns. Regardless of the high degree of transfer of maternal antibody and high levels of antibodies at the early infancy, concentrations of passively acquired antibodies decreased rapidly within 6 to 9 months of life. Prevalence of IgG antibodies in infants between six to twelve months of age in Colombo District were low.

Acknowledgements

Medical Research Institute (48/2011) and National Science Foundation (NSF/SCH/ 2013/07).

A part of this work was presented at the 7th Biennial sessions of AISSL and Institute of Biology, Sri Lanka.

PP 10

Microbiological diagnosis of a rare surgical case

Asanthi MAI, Patabendige CGUA

National Hospital of Sri Lanka, Colombo 10.

Introduction

Microbiological culture not only helps to determine correct antibiotic therapy but with good overall clinical knowledge will be able to assist in providing clues in early diagnosis of rare cases.

Case history

A forty eight year old man presented with a 6 hour history of chest pain, difficulty in breathing and vomiting after having alcohol and meat. He was discharged after myocardial infarction was excluded. He got readmitted next day and streptokinase was given as electrocardiogram showed ST elevations in leads I, II, III and AVF and was managed for atrial fibrillation too. Troponin I was negative. Chest x-ray and echocardiogram on day 3 revealed pneumo-mediastinum, pericardium and bilateral pleural effusions. Patient developed fever and was started on cefotaxime and clarythromycin. His CRP was 96 and blood culture was negative. He was transferred to the tertiary care hospital. Culture of 200ml of pericardial aspirate revealed mix growth of enterococci, staphylococci, lactose fermenting and non-fermenting coliforms, *Acinetobacter* spp. and candida. Antibiotics were changed. The repeat aspirate of 480ml grew similar species and the possible oesophageal/bowel perforation was suggested. Surgical team ordered oral contrast study as oesophageal perforation was not apparent clinically. On day 21 a perforation of lower third of oesophagus was detected with fistula to pericardial sac and stenting was done. He passed away on day 26.

Discussion

Spontaneous non penetrating (Boerhaave's) or penetrating perforation of oesophagus is rare. Contents are sucked in to pleura/ mediastinum once perforated by negative thoracic pressure leading to chemical and septic

mediastinitis. The mortality rate is high and survival depends on early diagnosis which needs high degree of clinical suspicion and appropriate intervention. The microbiome of the distal oesophagus differs in healthy people (78% belongs to streptococci) and in oesophagitis (inflamed mucosa hospitable for micro-aerophilic/ anaerobic bacteria, mostly Gram negative taxa).

Though our patient had classical symptoms diagnosis was delayed. The presence of enteric organisms in pericardial fluid with negative blood culture raised the suspicion of oesophageal perforation. As the patient was a daily drinker, recurrent hospitalization may have led to the alteration of orodigestive tract flora.

PP 11

A case report on a patient with multiple abscesses and septic arthritis

Priyarangani WKAP, Piyasiri DLB, Palangasinghe S, Thabrew H, LewkeBandara RH

Teaching Hospital Karapitiya, Galle.

Introduction

Melioidosis is a systemic infection of humans and animals caused by non-fermenting Gram negative bacilli *Burkholderia pseudomallei*. Melioidosis is endemic in Southeast Asia and Northern Australia and it is now recognized as an emerging infectious disease in Sri Lanka. Here we report a case of melioidosis who presented with multiple soft tissue abscesses and septic arthritis.

Case presentation

A 46 year old house wife from Bussa, was admitted to the medical ward with a history of fever and swelling, redness and pain of the right thigh for one week associated with anorexia, nausea and recent weight loss. She was on prednisolone for systemic lupus erythematosus for last 10 years and on warfarin for deep vein thrombosis. She sustained bilateral femur fracture following an accident 8 months back and became wheelchair bound since then. They are living in a chena cultivation.

On admission she was febrile (100 °F), had signs of an abscess over the right thigh. Incision and drainage was done and started on oral co-amoxiclav which was later changed to intravenous metronidazole and cloxacillin. After fever free 6 days, new abscess appeared on the forearm with high fever and left knee joint septic arthritis. This time pus, aspirated joint fluid and blood were sent for culture. Due to clinical deterioration, intravenous imipenem and vancomycin were started.

Within the next few days all pus, joint fluid and blood cultures were positive for an oxidase positive non-lactose fermenting, gentamicin and polymyxin resistant, co-amoxycylav sensitive Gram negative bacillus with safety pin appearance which was identified as *Burkholderia*

pseudomallei by API 20NE. Antibody to meliodi by indirect haemoagglutination test was positive. Oral cotrimoxazole was added due to poor response while vancomycin was discontinued. Patient gradually improved over the following weeks and was discharged on long term eradication therapy.

Discussion

B. pseudomallei has been isolated from soil and surface water in endemic region. It is mainly transmitted by percutaneous-inoculation, ingestion and inhalation, while few laboratory acquired cases were also reported. *B. pseudomallei* culture is often misinterpreted as a coliform or a *Pseudomonas* due to its colony morphology and oxidase positivity. However, the clinical history and the typical antibiotic sensitivity pattern will guide a careful microbiologist to the correct identification.

PP 12

A retrospective study of methicillin-resistant *Staphylococcus aureus* (MRSA) infections in a private hospital in Sri Lanka

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¹Dept. of Microbiology, Faculty of Medicine, South Asian Institute of Technology and Medicine (SAITM), ²Neville Fernando Teaching Hospital (NFTH), Malabe.

Objectives

To characterize MRSA strains isolated in NFTH; their susceptibility patterns, types of infections, and outcomes in terms of antibiotic therapy.

Methodology

A retrospective study was done to identify MRSA infections, in the OPD and inward setting at NFTH from April 2013 to November 2014. Patients' age, gender, medical record numbers, date(s) and sites of isolation of MRSA were obtained from the laboratory database. WHONET data system was accessed for antibiograms of MRSA cultures. BHTs of patients, hospitalized with MRSA infections were perused to obtain clinical history and outcome. Descriptive analysis was used to determine prevalence, appropriateness of antibiotic therapy and outcome of MRSA infections. Ethical clearance was obtained from the Ethics Review Committee of the Faculty of Medicine, SAITM.

Results

Of 366 *S. aureus* isolates from clinical specimens of inward and OPD patients, 42% (156/366) were MRSA. Of these, 27.5% (43/156) were from children and 72.5% (113/156) were from adults (54% females and 46% males). Of the isolates 70% of isolates were from patients 50 years or older. Majority of clinical specimens were pus and wound swabs (93, 59%). The majority of MRSA

isolates were CA-MRSA since they were susceptible to non beta lactams such as ciprofloxacin (93/150; 62%), tetracycline (139/141; 99%), gentamicin (113/153; 74%) and cotrimoxazole (68/86; 79%). However, erythromycin susceptibility was low (32/150; 21%). Susceptibility for clindamycin, netilmicin, and vancomycin were (79/151; 52%), (136/138; 99%), (141/141; 100%) respectively. Susceptibility rates of MRSA isolates from OPD and inward patients, to all antibiotics tested were very similar. The outcome of 47 inward patients with MRSA skin and superficial infections was good. Of these, 23 (49%) were not given anti MRSA antibiotics systemically. However topical applications of fusidic acid and povidone iodine had been used.

Conclusion

The antibiogram of MRSA isolates indicate that the majority were CA-MRSA. Systemic antibiotic therapy for MRSA, may not be necessary for uncomplicated skin and superficial infections due to CA-MRSA. The limitation of the study was that OPD patients could not be followed up to determine the outcome of their infections.

PP 13

Evaluation of the association between use of medical devices and duration of hospitalization with health care associated infections in a tertiary care hospital

Dilrukshi KS, Marapana MBMRDT, Bandara MGSD, Weerasekara MM, Kottahachchi J

Microbiology Department, Faculty of Medical Sciences, University of Sri Jayewardenepura.

Objectives

1. To evaluate the association between the use of medical devices and duration of hospitalization which predispose to health care associated infections (HCAIs) in a tertiary care hospital in Sri Lanka.
2. To determine the proportion of health care associated urinary tract infections (UTIs), respiratory tract infections (RTIs) and surgical site infections (SSIs) in a tertiary care hospital in Sri Lanka.
3. To compare the 3 types of health care associated infections with the type of ward, age and sex of patients.

Methodology

Data extraction sheets (study instrument) were filled with information obtained from bed head tickets, and laboratory reports and confirmed by clinicians. Bed head tickets of 423 patients who had stayed more than 48 hours or had been readmitted to the hospital in surgical, medical, pediatric and gynecology wards were studied.

Results

There was a statistically significant relationship between the proportion of HCAs and usage of medical devices such as ventilators, nebulizers, urinary catheters and central intravenous lines, etc as the chi square test is highly significant ($\chi^2 = 34.3$, $df = 1$, $p = .000$). Further there was a statistical significant relationship between the length of stay in hospital and HCAs. Patients who had acquired HCAs in the health care institution had a mean duration of hospitalization for more than 12 days and patients who had not acquired HCAs had less than 5 days and the p value of the independent sample t test was <0.05 . Out of 423 patients, 71 (16.8%) had been diagnosed as health care associated infected patients by the clinicians. The predominant type of HCAs was RTIs (5.7%) followed by UTIs (5%), SSIs (4%), and other infections (4%). The highest percentage of UTIs were seen in surgical wards (38.1%) whereas RTIs were seen in medical wards (45.8%). Females had presented with higher percentages of SSIs, UTIs, RTIs than male patients. Elderly patients were the most susceptible age group (24.46%).

Conclusion

There is a significant association between use of medical devices and duration of hospitalisation with development of HCAs.

PP 14

Histopathological spectrum in acute and chronic cutaneous leishmaniasis in Sri Lanka

Manamperi NH¹, de Silva MVC², Fernando C², Pathirana KPN³, Abeyewickreme W¹, Karunaweera ND⁴

¹Department of Parasitology, Faculty of Medicine, University of Kelaniya, ²Department of Pathology, Faculty of Medicine, University of Colombo, ³Sri Lanka Army Medical Services, Colombo, ⁴Department of Parasitology, Faculty of Medicine, University of Colombo.

Introduction

Cutaneous leishmaniasis is a newly established parasitic disease in Sri Lanka. Histological spectrum of cutaneous leishmaniasis is wide and varied.

Objectives

To describe the histological spectrum of acute and chronic cutaneous leishmaniasis.

Method

Patients from Sri Lanka army were recruited by active and passive case detection methods and punch biopsies were obtained. Skin biopsies of 35 patients with smear positive for *Leishmania* amastigotes were processed routinely for histopathology, examined at a conference microscope and classified into 4 groups using modified Ridley criteria for Leishmaniasis as: I – parasitized macrophages with variable lymphocytes and plasma cells; II – parasitized macrophages with lymphocytes, plasma cells and ill formed histiocytic granulomata; III – a mixture of macrophages (with or without parasites), lymphocytes, plasma cells and epithelioid granulomata; IV – epithelioid granulomatous response with a few lymphocytes and plasma cells but no amastigotes. Lesions were categorized as acute (< 6 months) or chronic (≥ 6 months).

Results

Study group composed of males with a mean age of 32.6 years (range 22-47) and lesion duration of 5.6 months (range 1-24). Twenty nine (82.9%) were also positive by histopathology. Twenty two (62.9%) were acute and 13 (37.1%) chronic. Group I, II, III and IV patterns were seen in 14 (40%), 12 (34.3%), 5 (14.3%) and 4 (11.4%) respectively and 9 (40.9%), 9 (40.9%), 2 (9.1%) and 2 (9.1%) of acute lesions and 5 (38.5%), 3 (23.1%), 3 (23.1%) and 2 (15.4%) of chronic lesions respectively.

Conclusion

Histology of cutaneous leishmaniasis shows marked inflammatory cell infiltrate with or without granuloma formation. Majority of patients presenting with either acute or chronic cutaneous leishmaniasis belong to histological groups I or II.

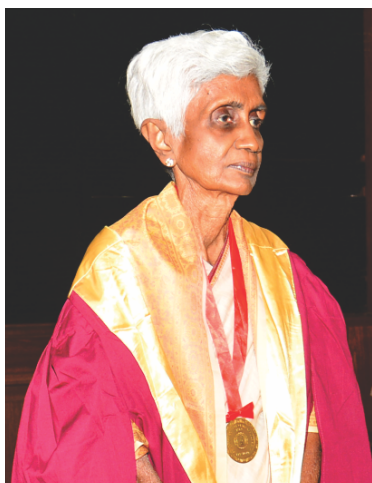
Acknowledgements

Financial assistance from the University Grants Commission, Sri Lanka (UGC/VC/DRIC/PG/2013/KLN/03) and University of Kelaniya (RP/03/04/06/01/2014) are acknowledged.

An abstract based on similar work was presented at the 128th Anniversary International Medical Congress of the Sri Lanka Medical Association, 5th to 8th July 2015.

FELLOWSHIPS OF THE SRI LANKA COLLEGE OF MICROBIOLOGISTS

Fellowships of the Sri Lanka College of Microbiologists were awarded to Professor Ivy de Fonseka, Professor M. M. Ismail, Professor Emil Wijewantha and Dr. Tissa Vitarana on 13th March 2015 at the Aldo Castellani Auditorium of Medical Research Institute, Colombo 8.



Professor Claribelle Ivy de Fonseka

MBBS (Cey), Dip.Bact (Lond.), Ph.D (Lond.)

Madam President, I am privileged to have been requested to introduce a dedicated teacher of Microbiology, Dr. Claribelle Ivy de Fonseka who has spent nearly forty years of her career as a teacher of medical microbiology in Universities in Sri Lanka and abroad.

Ivy Fonseka comes from a strong Methodist background. She had her education at Newstead College, Negombo, Girls High School, Kandy and Methodist College, Colombo, where she came under the tutelage of Methodist Missionaries who instilled into their pupils the values of discipline, dedication and integrity. She excelled in her studies while in school passing the senior school Certificate examination with a first division and entered the University of Ceylon in 1952 to read for the MBBS. While in medical school, she excelled in her studies and obtained a first class in the 3rd MB examination and an upper second in the Final MBBS examination and qualified as a medical graduate in 1958.

After graduation she served in the Department of Health services for six years working mainly in the field of Paediatrics. She joined the Department of Microbiology, Faculty of Medicine, University of Colombo in 1964 to embark on an academic career. She proceeded to the UK and Obtained the Diploma in Microbiology from the prestigious London School of Hygiene and Tropical Medicine in 1968. She joined the Bernhard Baron Medical Research Laboratory for her Postgraduate Research Studies and obtained the Degree of Doctor of Philosophy from the University of London in 1972.

On her return she was appointed Senior Lecturer in 1972 and became Associate Professor in 1977. She was

selected as the Chair in Microbiology in 1982. She served as Head of the Department of Microbiology from 1979 until 1987. Her main research interests were on Candida, Group B streptococcus and Chlamydia and has publications in national and international journals. She was awarded a number of fellowships from the World Health Organization and the Association of Commonwealth Universities to further her research interests.

The commencement of the first MD Microbiology course of the PGIM took place while she was the Chairperson of the Board of Study in Microbiology. She took early retirement from the University of Colombo in 1988 and joined the Faculty of Medicine and Medical Sciences at King Faizal University, Saudi Arabia as a Professor in Microbiology from 1985-1996 where she was held in high esteem. From 1997 to date she has been actively engaged in teaching at the Faculty of Medical Sciences of University of Sri Jayewardenepura as a visiting Professor in Microbiology.

During her tenure of office at the Faculty of Medicine Colombo I had the good fortune to be her undergraduate and postgraduate student. She is a dedicated teacher, a pillar of integrity, appreciated and loved by all those who have been associated with her.

Madam President it is my pleasure and privilege to present Pro. Claribelle Ivy de Fonseka for the award of the Honorary Fellowship of The Sri Lanka College of Microbiologists.

Citation read by Prof. Jennifer Perera
*Dean and Chair Professor of Microbiology,
Faculty of Medicine, University of Colombo.*



Professor M. Mohamed Mahroof Ismail

MBBS, DTM & H, MD, PhD, FNASSL

Mohamed Mahroof Ismail obtained his MBBS from the University of Ceylon, his PhD from McGill University, Canada, and spent a post doctoral year at the London School of Hygiene and Tropical Medicine, in the UK. He worked at the MRI for several years and became its Director in 1983. In the same year, he joined the Faculty of Medicine, University of Colombo as the Professor of Parasitology and later served as Dean of the Faculty from 1994 to 1996. He was a founder member and president of the Sri Lanka College of Microbiologists in 1987.

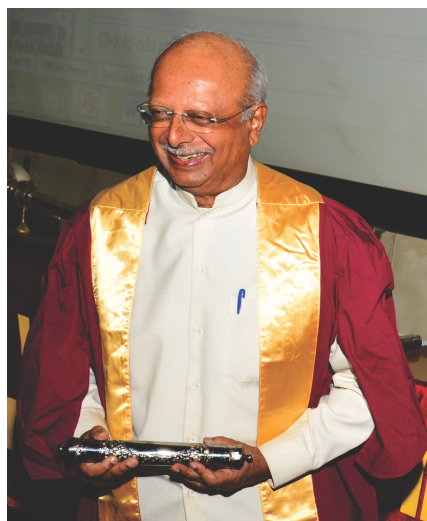
He has been a member of the University Grants Commission; Chairman, Board of Management of the Post-Graduate Institute of Medicine; and external examiner in Parasitology of the University of Malaya as well as the National University of Malaysia.

He has served the WHO in many different capacities: these include Chairman of the WHO Expert Committee on Soil-Transmitted Helminthiases; a member of the WHO Expert Committee on Lymphatic Filariasis; and the WHO Technical Advisory Group for Lymphatic Filariasis; WHO Consultant to Egypt and Bangladesh to revise their

National Filariasis Control Programmes; and Chairman of the South East Asian Programme Review Group for the elimination of lymphatic filariasis from 2002 until 2006. Professor Ismail's research was mainly on soil-transmitted helminthiases and lymphatic filariasis, with over 80 publications to his credit. His group determined for the first time that albendazole combined with DEC or ivermectin has a pronounced and sustained effect of reducing microfilaraemia for over 2 years. This combination is currently being successfully used by the WHO and the Ministries of Health in 83 endemic countries as part of the global strategy to eliminate filariasis. He has been the recipient of Presidential Awards for Excellence in Research from their inauguration in 1999, right up to 2007.

Madam President, I present Professor Ismail for Honorary Fellowship of the Sri Lanka College of Microbiologists.

Citation read by Dr. Sharmini Gunawardena
*Senior Lecturer, Department of Parasitology,
Faculty of Medicine, University of Colombo.*



Dr. Tissa Vitarana

MBBS, MD, Dip.Bact (Lond.), PhD (Lond.)

Upali Tissa Vitarana graduated from the University of Ceylon in 1959, and obtained his MD in Clinical Medicine in 1964. He gave up a career in clinical medicine to focus on research into local health problems, by joining the Medical Research Institute. He obtained a Diploma in Bacteriology and a PhD in Virology from the University of London.

He served the MRI as Head of Virology, and during his last 10 years there, as Director. With Japanese support, he made the MRI a modern, fully equipped laboratory of international standards, with trained staff and support for research and testing. He retired from the MRI to become the Founder Professor of Microbiology at the Faculty of Medical Sciences, Sri Jayewardenepura. His original research on dengue, viral hepatitis, Japanese encephalitis, hantavirus infections and rubella received international recognition, and he has over 80 publications and book chapters to his credit. He has been an advisor to the WHO for many years, serving on the Dengue Task Force and the Global Forum on Health Research.

He was a founder member of the Sri Lanka College of Microbiologists. During his term as President in 1991, proper academic sessions were held for the first time with pre-congress lectures, an arbovirus workshop and an international seminar on dengue. The College logo, flag, pennant, banner and proceedings booklet were also designed during this period. Dr Vitarana has been

President of SLAAS Section A, founder president of the Allergy and Immunology Society of Sri Lanka and is a Fellow of the National Academy of Sciences of Sri Lanka. He has delivered the SLMA Sir Marcus Fernando Oration as well as the inaugural Aldo Castellani Oration, and several other orations and lectures in foreign countries. He has served as the Acting Director of the Regional Virus Laboratory in Edinburgh and as the Deputy Director of the WHO Reference Laboratory on Viral Diseases in Melbourne, Australia.

These achievements are those of a person who felt for the health needs of our people, and who is unassuming and approachable by all. While being a dedicated scientist and researcher, he enjoyed life to the full as a sportsman, a lover of music, and an admirer of nature and wild life, but most of all, he is a person who loves his fellow human beings both as a doctor and a politician. Today when opinion on politicians is at a very low ebb, he is undoubtedly an exception.

Madam President, I present Dr. Tissa Vitarana for Honorary Fellowship of the Sri Lanka College of Microbiologists.

Citation read by Dr. Nalini Withana

Former Consultant Virologist,
Medical Research Institute,
Colombo 8.



Professor Emil Arthur Wijewantha

BVSc (Cey), PhD (Lond)

Emil Arthur Wijewantha was educated at Richmond College, Galle and later at St. Peters College Colombo where he excelled in studies as well as in sports. He captained the Peterite Tennis Team in 1948 and was a member of the 440 Yards relay team. In 1948, he entered the University of Ceylon and graduated with BVSc (Hon), obtaining distinctions in Bacteriology, Parasitology and Pathology. He was appointed a Lecturer in Veterinary Bacteriology and Pathology in 1955.

After proceeding to the UK in 1958, he conducted research on pathogenic bacteria and obtained his PhD from the University of London. His research was highly commended by Professor C.L. Oakley, FRS Dean, School of Medicine University of Leeds.

He was appointed Professor of Veterinary Microbiology in the Faculty of Medical, Dental and Veterinary Science of the University of Sri Lanka in December 1975 which post he held until his retirement in 1999. He served as the Head of the Department of Veterinary Paraclinical

Studies from 1975 to 1994. In addition, Professor Wijewantha has held postdoctoral fellowships in many universities in UK and other countries. I had the good fortune to be a student of Professor Wijewantha whose lectures were extremely interesting. He is a fluent orator, wrote English verse for which he has received many prizes, and is a composer of songs.

Professor Wijewantha was a Founder Member of the Ceylon Association of Bacteriologists and the President of the Sri Lanka College of Microbiologists in 1992. He was a member of the Board of Study in Microbiology of the PGIM University of Colombo during 1995 - 1998.

Madam President, it is with great pleasure I present Professor Emil Arthur Wijewantha for Honorary Fellowship of the Sri Lanka College of Microbiologists.

Citation read by Dr. Ranjith Perera

Senior Lecturer, Department of Medical Microbiology, Faculty of Medicine, University of Kelaniya.

WINNERS OF PRESIDENT'S AWARDS FOR SCIENTIFIC PUBLICATIONS

President's Awards for Scientific Publications

The President's Awards for Scientific Publication were started in 2001 by the National Research Council to recognize scientists whose work reached international standards and to increase national scientific production. The scheme covers all the "hard science" journal titles (excluding the social sciences), with a Sri Lankan institutional affiliation against at least one Sri Lankan author.

The scheme was initially based on publications in journals listed in the Science Citation Index (SCI). Since there have been a steadily increasing number of awards over the years, the criteria have been changed from 2010 onwards, with the introduction of a two-tier system. This system includes a measure of journal quality based on Impact Factor also taking into consideration that different disciplines have widely differing Impact Factors for their journals, and the quantum of research published by a scientist.

Tier 1: President's Awards for Scientific Publication

The criteria for selection:

- 1) A publication in the top 10% of Science Citation Index Expanded journals, ranked on their Impact Factor under each journal category, based on SCI categorization of journals
- OR
- 2) Two or more publications in Science Citation Index Expanded journals with an Impact Factor of 1 or more in the given year.

Tier 2: National Research Council Merit Awards for Scientific Publication

The criteria for selection:

- 1) A publication in a Science Citation Index Expanded journal with an Impact Factor of 1 or more, in the given year.

A list of members of the Sri Lanka College of Microbiologists who won President's Awards for Scientific Publication for the years 2007 to 2012 is given below.

➤ President's Awards for Scientific Publication in 2007, 2008 and 2009, awarded in January 2014

1. Prof. Nilmini Chandrasena, University of Kelaniya.
2. Prof. Nilanthi de Silva, University of Kelaniya.
3. Prof. Sirimali Fernando, University of Sri Jayewardenepura.
4. Prof. Deepika Fernando, University of Colombo.
5. Dr. Geethani Galagoda, Medical Research Institute.
6. Dr. Sunethra Gunasena, Medical Research Institute.
7. Prof. Mahroof Ismail, University of Colombo.
8. Dr. Kumudu Karunaratne, Lady Ridgeway Children's Hospital.
9. Prof. Nadira Karunaweera, University of Colombo.
10. Dr. Sujatha Mananwatte, National STD / AIDS Control Programme.
11. Dr. Rohitha Muthugala, Ministry of Health.
12. Prof. Jennifer Perera, University of Colombo.
13. Dr. Harsha Perera, University of Kelaniya.
14. Dr. Preethi Perera, Medical Research Institute.
15. Dr. Shalindra Ranasinghe, University of Sri Jayewardenepura.
16. Prof. Vasantha Thevanesam, University of Peradeniya.
17. Dr. Ruchika Wijesinghe, University of Sri Jayewardenepura.
18. Dr. Omala Wimalaratne, Medical Research Institute.

➤ **President's Awards for Scientific Publication, 2010, 2011 and 2012, awarded in October 2014**

1. Prof. Nilmini Chandrasena, University of Kelaniya.
2. Prof. Nilanthi de Silva, University of Kelaniya.
3. Prof. Deepika Fernando, University of Colombo.
4. Dr. Sunethra Gunasena, Medical Research Institute.
5. Dr. Sharmini Gunawardana, University of Colombo.
6. Dr. Kushlani Jayatilleke, Sri Jayawardenepura General Hospital.
7. Prof. Nadira Karunaweera, University of Colombo.
8. Dr. Ajith Nagahawatte, University of Ruhuna.
9. Dr. Susilakanthi Nanayakkara, Medical Research Institute.
10. Dr. Harsha Perera, University of Kelaniya.
11. Prof. Jennifer Perera, University of Colombo.
12. Prof. Vasanthi Thevanesam, University of Peradeniya.
13. Prof. Mirani Weerasooriya, University of Ruhuna.
14. Dr. Omala Wimalaratne, Medical Research Institute.

PRIZE WINNERS AT THE 23RD ANNUAL SCIENTIFIC SESSIONS 2014

Following presentations were awarded first, second and third places at the 23rd Annual Scientific Sessions of the Sri Lanka College of Microbiologists held on 14th and 15th August 2014.

Oral presentaions

1st prize

- OP 7 - **Rapid detection of rifampicin and isoniazid resistance in *Mycobacterium tuberculosis* culture isolates: an evaluation of a line probe assay.**

Francis VR¹, Elwitigala JP¹, De Silva AD²

¹National Tuberculosis Reference Laboratory.

²Gene-Tech Research Institute.

2nd prize

- OP 12 - **Evaluation of bactericidal effect of three antiseptics on bacteria isolated from wounds**

Kottahachchi J¹, Kumara DUA², Dissanayake DMBT¹, Athukorala GIDDAD¹, Chandrasiri NS³, Damayanthi KWN¹, Hemarathne MHSL¹, Fernando SSN¹, Pieris H⁴, Pathirana AA²

¹Department of Microbiology and ⁴Department of Biochemistry, Faculty of Medical Sciences, University of Sri Jayewardenepura.

²Professorial Surgical Unit, Colombo South Teaching Hospital.

³Department of Microbiology, Colombo South Teaching Hospital.

3rd prize

- OP 18 - **Occurrence of KPC producing *K. pneumoniae* and associated factors in a selected hospital in the Colombo District**

Suranadee YWS¹, Perera AJ¹, Gamage S¹, Pathirage SC²

¹Department of Microbiology, Faculty of Medicine, University of Colombo.

²Medical Research Institute, Colombo 8.

Poster presentaions

1st prize

- PP 11 - ***Salmonella enterica* serotype Paratyphi A with an unusual biochemical pattern**

Wickramasinghe D¹, Pathirage SC², Vidanagama DS¹, Corea E³

¹Department of Microbiology, Teaching Hospital Karapitiya, Galle.

²Medical Research Institute, Colombo.

³Faculty of Medicine, University of Colombo.

2nd prize

- PP 3 - **Three attacks of *Salmonella* Typhi bacteraemia in an adult returned from India**

Mendis KHC, Dassanayake KMMP, Premawansa G

Colombo North Teaching Hospital, Ragama.

3rd prize

- PP 14 - **Fungal keratitis - Sri Lankan picture**

Jayasekera PI¹, Sadara K¹, Perera PD

Department of Mycology, Medical Research Institute, Colombo 8.

Presidential address delivered at the inauguration of the 23rd Annual Scientific Sessions of the Sri Lanka College of Microbiologists on 13th of August 2014



Dr. Kumudu Karunaratne

Consultant Microbiologist, Lady Ridgeway Hospital for Children

Paediatric infections – *Sharing my Experience*

The chief guest Hon. S. Palitha Fernando, President's Counsel and Attorney General of Sri Lanka, Guest of Honour Dr. Christopher Coulter, Director Queensland Mycobacterium Reference Laboratory and Infectious Diseases Physician and Clinical Microbiologist, The Prince Charles Hospital, Australia, other foreign guests, Officials of the Ministry of Health, past Presidents, Members of the Council, Members of the Sri Lanka College of Microbiologists, distinguished invitees, ladies and gentlemen, today is the inauguration of the 23rd annual academic sessions of the Sri Lanka College of Microbiologists.

At the outset let me brief you about our College. Our membership comprises of medical microbiologists holding post graduate degrees from Diploma in Medical Microbiology through PhD and MD in Microbiology.

The parent organisation of the College was founded in 1969 as the Ceylon Association of Microbiologists with 16 founder members. In 1974 the name of the association was changed to the Sri Lanka Association of Microbiologists. However five years later in 1979 a unanimous decision was taken that the Association should evolve into the Sri Lanka College of Microbiologists and a new constitution was drafted. It is with pleasure and respect that I would like to note that out of 16 founder members Prof. Emil Wijewantha and Prof. S. N. Arsecularatne are present to witness how the College grew and progressed from 1969 to date. In keeping with the objectives of the College, members had been working over the past years under the able leaderships and

councils to promote the advancement of medical microbiology to help in diagnosis and management of patients and to emphasize the importance of medical microbiology in Sri Lanka in relation to prevention and control of infections both in the hospitals as well as in the community.

I am very pleased to say that the Ministry of Health has identified the expertise of the College of Microbiologists and has worked collaboratively in upgrading the microbiology services to provide quality patient care. Dr. Ajith Mendis in his capacity both as the former Director General of Health services and the former Deputy Director General of Laboratory Services worked diligently and very closely with the college to improve microbiology services in the country. Similarly Dr. P. G. Mahipala present Director General of Health services too continued to allocate a time in his calendar monthly, to meet the members at the meetings of Task Force in Microbiology.

Currently there are only about 15 consultant microbiologists attached to MoH serving on full time basis in government hospitals in Sri Lanka. Provision of offsite consultancy services by the consultants of the College attached to institutes, campaigns and universities, on a voluntary basis to cover many other hospitals was one such important outcome of these meetings.

Representation of the College had been requested by the MoH in many important decision making committees like National Advisory Committee on Infection Control, developing quality indicators in health related infections,

streamlining and organising of laboratory items in the field of Microbiology in the medical supplies division, technical evaluations and developing specifications and in many other forums which requires input from the field of Microbiology.

Many members of the College had contributed in different capacities over the years in the College activities to improve microbiology services both diagnostic as well as clinical and in infection control activities. To upgrade the knowledge of different categories of health care staff which includes nurses and medical laboratory technologists, and to provide standard infection control and microbiological services by them to the patients, the college published the infection control manual for hospitals and laboratory manual in microbiology both in which I had the opportunity to provide my services as the chair person in the subcommittees. Presently the College is at the final stage of revision of the previous edition of the biosafety manual for laboratories too.

Currently the College is in the process of developing national antibiotic guidelines for empirical therapy of infections in collaboration with all relevant professional colleges, a daunting task which is expected to be completed before the end of this year. Until all the guidelines are compiled as a booklet, at present, under the instruction of the Director General of Health Services, each guideline is issued as a circular to the government hospitals as and when it is ready.

A revision of the nurse training curriculum in the modules of Microbiology and infection control was done by members in the task force in Microbiology and the revised curriculum was handed over to the Deputy Director General (Education, Teaching and Research) to incorporate it in the training programme.

During this year in collaboration with the MoH we were able to conduct in-service training in Microbiology to medical laboratory technologists at Teaching Hospital Batticaloa for participants from Eastern province and in Family Health Bureau for laboratory technologists in the Western province. These two workshops were the last two in the series we had, to cover all parts of Sri Lanka. The private sector too provides medical care to a large population in the country. In June this year a workshop was held for private sector nurses and doctors in infection control which had good participation. It too was well rewarded. Not only updating of other staff we had programmes for our members too. Continuous medical education programme was a regular event in our calendar to update the knowledge of our general membership. Both local and foreign speakers shared their knowledge with our members.

With this brief overview of our College I would like to move on to a topic which I have been enjoying for quite some time in my career – Paediatric infections – *Sharing my Experience*. Being a microbiologist, more a bacteriologist, I thought to share some of my experience of infections in

childhood. Almost all the data were generated at the Lady Ridgeway Hospital.

Infections are a cause of significant mortality and morbidity in children. They affect virtually any organ or tissue in the body. Some of these infections are acquired in the community and some of course are hospital associated infections.

Meningitis is infection of meninges, which occurs within the confines of a skull which is a rigid structure with hardly any space to expand. Because of high intracranial pressure this disease entity is associated with very high morbidity and mortality. Acute bacterial meningitis occurs in all age groups. Literature reveals that meningeal pathogens, *Haemophilus influenzae*, *Neisseria meningitidis* and *Streptococcus pneumoniae* account for more than 80% of childhood meningitis in patients after the neonatal period. Specimens of cerebrospinal fluid are sent to the microbiology laboratory for culture in patients suspected of having meningitis.

A retrospective analysis was done on cerebrospinal fluid culture results in year 2006 and 2007, processed at the Lady Ridgeway Hospital. A total of 2247 specimens were processed during those two years. 37 revealed an aetiological agent giving an isolation rate of 1.6%. The commonest organism isolated at that time was *Haemophilus influenzae*, being 16 out of 37 bacteria isolated from these specimens. The next most common organisms isolated were *Streptococcus pneumoniae* and Group B streptococcus. Patients who had uncommon organisms like Group G streptococcus, Methicillin Resistant *Staphylococcus aureus* (MRSA) and *Pseudomonas* species too were within this study group.

These specimens were tested for antigen detection as well. Antigen detection was 4% compared to 1.6% culture isolation rates. Though the gold standard of microbiological confirmation of bacterial meningitis is by culture, as was shown isolation rate is poor. Organisms get inhibited due to antibiotic treatment prior to collection of specimens.

In another prospective study carried out during a 3 month period from January to March 2012 at the LRH, CSF samples and blood cultures from confirmed, probable and suspected cases of meningitis were analysed. This study was done primarily to analyse bacterial aetiological agents in acute childhood meningitis but the results showed that detection of an aetiological agent either by CSF culture or by CSF antigen detection test was rather poor. However this study revealed evidence of the primary contributory factor leading to poor detection rate of an aetiological agent.

Almost 88% of CSF samples were taken after antibiotic therapy. About 46% of blood cultures too were taken subsequent to administration of antibiotics, majority of which had been intravenous antibiotics administered following hospitalisation. This highlights the importance

of collecting specimens for culture prior to antibiotics unless the patient's condition does not deem feasible to do so.

Not only the prior antibiotic therapy but the volume of blood inoculated to the blood culture bottle too has an effect in the final outcome of detecting bacterial pathogens. Certainly it is not an easy task to take blood from little children.

A prospective study was done in January 2007 at the Lady Ridgeway Hospital to determine the volume of blood inoculated by medical officers into the blood culture medium provided. A total of 300 specimens were analysed. The study revealed that only 9.7% of specimens had an adequate blood volume. As seen here culture isolation rate was 13.8% in bottles with adequate blood volumes compared to 8.1% in specimens with a lower volume. Therefore, not only prior antibiotic therapy but low volume of blood also has a negative impact on detection of bacterial pathogens from specimens submitted to the microbiology laboratory.

As I indicated at the outset a common bacterial pathogen causing acute bacterial meningitis in children is *Haemophilus influenzae*. Its most common invasive manifestation is meningitis but it also causes other invasive diseases like epiglottitis, septic arthritis, osteomyelitis, pneumonia, cellulitis and bacteraemia without a clearly defined focus of infection.

A retrospective study was done to analyse invasive *Haemophilus influenzae* disease among children admitted to the Lady Ridgeway Hospital during January 2005-March 2006. The study population comprised of all bacteriologically confirmed patients with *Haemophilus influenzae*.

Results revealed that 91.2% of patients comprised of a clinical diagnosis of meningitis. In them subdural effusion was found in 16.1% of patients, 6.5% were also diagnosed to have hydrocephalus. Seizures occurred in 48.4% patients. Cerebral oedema was established in 9.7% and hearing loss was confirmed in 9.7% patients prior to discharge. Other complications developed in these patients were septic arthritis, pericardial effusion and cranial nerve palsies. Not only morbidity, 5.9% patients succumbed to their illness as well.

Hence, this is an organism which causes significant morbidity and mortality. At this time *Haemophilus influenzae* type b conjugate vaccine was not introduced to the national immunization programme in Sri Lanka and none of these patients had received even a single dose of the *Haemophilus influenzae* vaccine.

It is interesting to follow the isolation rate of this organism in the laboratory before and after the introduction of conjugate *Haemophilus influenzae* type b vaccine into the national immunisation programme.

The effect of the introduction of conjugate *Haemophilus influenzae* vaccine into the national immunisation programme in 2007/2008 is reaping benefits with an observable reduction in the number of cases due to *Haemophilus influenzae* invasive disease.

Now I would like to bring to your attention, another important organism causing infections in children. The World Health Organisation estimates that about 1 million deaths among children annually are due to pneumococcal infection and most of these deaths occur in developing countries. In addition to causing acute respiratory tract infections *Streptococcus pneumoniae* also causes invasive pneumococcal diseases like meningitis and septicaemia.

Lady Ridgeway Hospital serves as the sentinel site in Sri Lanka for a multicentre international surveillance on invasive pneumococcal disease which was initiated as South Asian Pneumococcal Surveillance Network in 2005. The study group consists of children aged 2 months to 5 years who are clinically diagnosed with meningitis, pneumonia or sepsis. Blood and CSF cultures were analysed in the microbiology laboratory and pneumococcal isolates were couriered for further studies to Christian Medical College, Vellore, India which serves as the regional reference centre for this international surveillance. It is an honour that Prof. Balaji Veeraghavan, Head of the Department of Microbiology at the regional reference centre is present in the audience today and sharing the current data at our sessions.

Though many cultures have been isolated up to now, I am presenting data on a publication in *Clinical Infectious Diseases* 2009 on Sri Lankan isolates. Study of 23 isolates analysed from January 2005 to March 2007 revealed that most common serotypes in Sri Lanka were 19F, 14, 23F and 6B. Out of the serotypes found on analysis of these samples, it was made to be known that about 61% are covered by, then available, 7 valent conjugate pneumococcal vaccine which can be given to the children of this age group to prevent occurrence of severe invasive disease. With current data and with 10 valent vaccine available now the vaccine coverage is about 70-75%. These findings initiated recommendation and justification for the availability of this vaccine in Sri Lanka to reduce morbidity of the disease.

Another remarkable outcome of this study was the antibiotic susceptibility pattern of these pneumococcal isolates. At the regional reference centre in India antibiotic susceptibility was performed by determining the MIC values. The findings revealed that 91.3% of the Sri Lankan isolates were resistant to penicillin in patients with meningitis. This was quite different from data from the other countries like India, Nepal and Bangladesh. This made a change to the empirical therapy recommended for childhood meningitis by some paediatricians at that time from penicillin to cefotaxime.

Prior to my appointment at the Lady Ridgeway Hospital I had the fortune to work and establish microbiology services at the National Cancer Institute, Maharagama, a pleasant experience which I cherish all my life. There wasn't a microbiology laboratory at cancer hospital at that time. The consultant pathologist Dr. Kamini Amerasinghe did ground work with me to purchase equipment to initiate microbiology services there while I was working at the Medical Research Institute. I was able to establish microbiology services in the cancer hospital from scratch, within two months of my appointment there, due to unstinted support given by then Director, consultant oncologist Dr. Yasantha Ariyaratne. On the second day of my appointment to the hospital a consultant oncologist looking after the paediatric oncology ward told me about the noticeably high incidence of hepatitis B infection in children who are undergoing treatment in the hospital, a fact which was known by then to many of us.

A study was carried out in asymptomatic paediatric patients selected randomly from wards and outpatient clinics of the National Cancer Institute to assess prevalence of Hepatitis B viral markers. The study was done for a period of 4 months in 2003.

The study revealed an alarming prevalence of 33.0% of Hepatitis B surface Ag among asymptomatic children taking treatment from the Cancer Hospital, Maharagama. That is every third child is infected with hepatitis B. This is quite in contrast to the prevalence in the general community in Sri Lanka which is about 1.8%. On investigation it was found that one of the major contributory factors was inadvertent sharing of syringes when administering parenteral therapy among patients who had intravenous cannulae in place. The unintentional practice by the staff was immediately corrected with the help of hospital administration and then Director General of Health Services with provision of large stocks of disposable syringes immediately. In addition many other measures have been introduced even till today by my successors, since I left the hospital to take up the appointment at the Lady Ridgeway Hospital for Children. This highlights the importance of infection control activities in a hospital to curtail health care associated infections. There is much progression of modern medicine in the world under the umbrella of antibiotics. Regardless of the resources available in developing countries like ours, high risk and sophisticated procedures have been introduced in management of patients to provide a quality life to them.

Paediatric cardiac surgery was established in the Lady Ridgeway Hospital in January 2007. Presently the hospital has a cardiac intensive care unit and a cardiac high dependency unit which can accommodate together up to about 17 patients at a time. Many of the children with multiple complex heart diseases require intensive care for a longer period of time when compared to adults undergoing cardiac surgery. Longer ICU stay, compromise

the patient's normal defences of skin and mucous membranes by various devices such as intravascular catheters, urinary catheters and endotracheal tubes etc. will place the patient at a high risk of developing healthcare associated infections.

A prospective descriptive study was conducted at Cardiothoracic Surgical Intensive Care Units (ICU/HDU) at LRH from November 2011 to March 2012. All patients who were admitted following surgery and stayed for more than 48 hours in these units were included in the study. 204 patients were studied for 1501 patient-days in the ICU. All patients had surgical antibiotic prophylaxis.

10% developed primary blood stream infections, 7% had catheter related blood stream infections, 3.9% were diagnosed with ventilator associated pneumonia, 1.5% had catheter associated urinary tract infections and 2.5% were complicated with surgical site infections during the ICU stay.

Many measures were taken by all staff attached to these units in the following years. A subsequently conducted audit by infection control unit in 2013 revealed 6.6% primary blood stream infections with 2% catheter related blood stream infections indicating a reduction in blood stream infections. Early removal of central venous lines was an important contributory factor in reduction of catheter related blood stream infections.

Moving now to a common infection encountered in children, acute lower respiratory tract infections. It is one of the commonest causes of hospitalisation in children. Identifying the possible pathogenic organisms in these children is important both in management and in implementing preventive strategies. A hospital based prospective analytical study was done in March 2013 in a paediatric ward at the Lady Ridgeway Hospital for over a 3 month period where, 82 children who presented with acute lower respiratory tract infections were enrolled randomly. Nasopharyngeal aspirates were obtained for bacterial cultures and viral antigen detection. Blood was taken for culture and mycoplasma antibodies. 43% of children were less than 2 years. None were vaccinated with special vaccines like influenza or pneumococcal.

An aetiological agent was detected in 66% of children. Majority of patients had a bacterial aetiology and 30% were of a viral aetiology. Some had a mixed aetiology of viral and bacterial. 3% had mycoplasma. Among the bacterial aetiologies, *Streptococcus pneumoniae* was the commonest followed by *Moraxella catarrhalis*. Among viruses, Influenza A and B (80%) were the commonest, followed by RSV. Viruses were isolated only in children below 6 years and Mycoplasma was seen only in more than 6 year age group. Chest X-ray changes were significantly higher in children with a bacterial aetiology, than viral. Similarly, duration of hospital stay was also significantly higher in children infected with bacterial compared to viral organism.

Before concluding my presentation let me share with you two interesting pathogens causing childhood infections which I saw at the Lady Ridgeway Hospital.

An 8 year old girl residing in a tsunami camp at Devinuwara was admitted to General Hospital, Matara in September 2005 with a history of fever, pain in shoulder joint and vomiting of 1 day duration. The following day she developed high fever, swelling of the shoulder joint, headache and drowsiness. Child was transferred to the Lady Ridgeway Hospital for secondary orthopaedic opinion for focal cellulitis of the upper right deltoid. On examination, the right deltoid swollen and had a small necrotic area. The same day child's skin became dark and blisters developed. She had low urine output and pulmonary oedema eventually requiring mechanical ventilation. The child died the following day. Two blood cultures taken on admission to Lady Ridgeway Hospital grew *Chromobacter violaceum* after overnight incubation.

This Gram negative bacillus rarely infects humans. In most cases, the main portal of entry appears to be broken skin exposed to the organism through contaminated soil and water. It commonly occurs as a pigmented strain which gives the colonies their distinctive purple colour. Though it rarely infects humans, when it does, it may be fatal also as in this case.

And finally I would like to present you a rare presentation of an organism which we are quite familiar with. A one year old little boy was admitted to BH Awissawella in 2009 with fever, loose stools and vomiting. Urine culture done there had isolated a coliform with a significant colony count and the patient was treated and discharged on prophylactic antibiotics. 13 days later the child was admitted to Lady Ridgeway Hospital with high fever and vomiting and examination revealed a drowsy child with bulging fontanelles. Ultrasound examination revealed

ventricular dilatations, mild subdural effusion and mild to moderate hydrocephalus. We received blood and CSF specimens on the day of admission of the patient to the ward. CSF full report analysed in the laboratory revealed evidence of bacterial meningitis. CSF glucose was 0.0 mmol/L. CSF antigens were negative to the meningitis causing bacteria.

To our surprise patient's CSF culture grew *Salmonella typhi* on the following day. The blood culture which was sent in an in-house media bottle, took 7 days to detect the same organism. As many of you are aware this organism is the causative agent of typhoid fever and meningitis is a very rare presentation. Unlike the previous patient, outcome here was good. Following appropriate treatment baby went home well.

With this I would like to conclude my presentation and I acknowledge all who contributed in generation of these data including my present and past laboratory staff.

Since I joined the Sri Lanka College of Microbiologists 21 years ago I have represented many councils and have provided my contribution to the best of my capacity for the advancement of medical microbiology. The related work for advancement of the speciality gave me a sense of fulfilment. Serving as the President of the College is a milestone of one's professional career. Therefore foremost I remember with deep gratitude my mother and my late father for nurturing me for what I am today. I remember in appreciation of all my teachers for their influence on my life. I owe a very special thank you to my husband Kanishka, for being a strength to me all the time and my son and daughter, Kushan and Rangana for bringing joy to my life and letting me have enough time and space to work on my interests.

Finally, thank you very much all of you for patient hearing and gracing this occasion.



Dr. Ranjith Perera

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The Late Dr. Rakkitha Sirimal Bandara Wickremesinghe, or Dr. Siri Wickremesinghe as we called him, was born on 28th November 1937 to Dr. Artie and Helen Wickremesinghe. He had his education at Royal College Colombo and obtained his MBBS in 1963 from the Faculty of Medicine, Colombo.

He started his medical career in the dermatology unit at Kandy and thereafter joined the Medical Research Institute (MRI) to work in the department of Microbiology. He obtained the Diploma and Master of Science in Microbiology from the University of Manchester, and MD with Board Certification in Microbiology from the Postgraduate Institute of Medicine, University of Colombo.

He continued to work at MRI as the Consultant Microbiologist until he left to Australia with his family. Having worked as a successful microbiologist in the Fairfield Hospital in Melbourne, he came back to the MRI and continued as the Consultant Microbiologist in charge of the Bacteriology division until his retirement. He was

the Director of MRI from 1996 to 1998. After retirement he worked as the Resident Pathologist and Laboratory Manager at Durdans Hospital, Colombo.

He was a past President of the Sri Lanka College of Microbiologists and the Secretary to the Board of Study in Microbiology at the Postgraduate Institute of Medicine at the very early stages, during a difficult time when the board was established.

He passed away on 08th April 2003.

Dr. Siri Wickremesinghe was a well-read person who had a vast knowledge in many other subjects, in addition to Microbiology. His knowledge in history, especially on Mahavamsa, was amazing.

Therefore, as a tribute to his multi-faceted knowledge, I thought that it would be most appropriate to select a topic so close to his heart, and to mine, to deliver the Siri Wickremesinghe memorial oration – 2014.

NOTES OF A MEDICAL MICROBIOLOGIST

A JOURNEY THROUGH HISTORY, RELIGION, MYTHOLOGY AND EVOLUTION

King Seethawaka Rajasinghe and the cause of his death

King Seethawaka Rajasinghe, born in 1544, is considered as a great warrior. He came to the battle field at the age of 16, and fought against the Portuguese invaders. Although he was a fearless fighter who faced Portuguese with great courage, he used brutal methods to punish people. He suspected everyone and showed no compassion towards anyone and even killed his own father Mayadunne.

In later years, he became very close to Aritta Kee-Vendu, an Indian emigrant, and destroyed Buddhist temples and killed Buddhist monks. Gradually he became very unpopular. His last battle was with the King Wimaladharmasurya in Kandy. King Seethawaka Rajasinghe lost the battle and, while retreating, came to Pethangoda Uyana, which is situated on the Anguruwella – Warakapola Road in Kegalle district. There he fell down from the horse and sustained an injury. A bamboo prick pierced his leg and after a few days he died. Thus a legendary life came to an end.

Dr. Philip G Veerasingam, FRCS Ed, MA analyses the reported signs and symptoms that preceded the death of King Rajasinghe of Sithawaka and draws the conclusion that he died of tetanus (1).

Dr. Ruwan M. Jayatunge, analyzing the behavior of Seethawaka Rajasinghe, came to the conclusion that he was suffering from combat related Post Traumatic Stress Disorder (PTSD) (2).

Black Death

“They ate their lunch with friends and dinner with their ancestors in paradise”

– Giovanni Boccaccio

The Black Death was one of the most devastating pandemics in human history, resulting in the deaths of an estimated 75 to 200 million people and peaking in Europe in the years 1346 – 53. Although there were several competing theories as to the aetiology of the Black Death, analysis of DNA from victims in northern and southern Europe published in 2010 and 2011 indicates that the pathogen responsible was the *Yersinia pestis* (3).

Plague was introduced into Europe from Asia in the 13th century. After the last major epidemic in England in 1665, plague disappeared spectacularly from Europe. This may be due to the displacement of the black rat, *Rattus rattus*

by the brown rat, *Rattus norvegicus*. Although this brown rat is susceptible to plague, it does not commonly frequent human dwellings. Improvement in housing may also have played an important role in the elimination of plague from Europe.

In Sri Lanka, the first documented outbreak of plague was reported by Aldo Castellani in Colombo, in 1908. The last recorded outbreak was in Colombo and Kandy in 1932. Since fumigation of food cargoes was commenced in the port in early 1940s, there have been no cases of plague reported in the country (4).

In 1994, an outbreak of plague occurred in Surat in Gujarat state, India. This was linked to the earthquake that devastated Gujarat that year, and the changes in the environment led the wild rodents to enter villages and cities. Strict anti-rodent measures have reduced the incidence of urban plague. However, endemic foci of wild rodent plague continue to exist in many rural parts of the world. In July 2014, it was reported that a Chinese city has been sealed off and 151 people placed in quarantine after a man died of bubonic plague (5).

Sweating sickness

This was a mysterious and highly virulent disease that struck England, and later continental Europe, in a series of epidemics beginning in 1485. The last outbreak occurred in 1551, after which the disease apparently vanished. The onset of symptoms was dramatic and sudden, with death often occurring within hours. Anne Boleyn, the second wife of King Henry VIII and the mother of Queen Elizabeth I, is thought to have suffered from sweating sickness. Later she was executed by Henry. William Carey, the husband of “The Other Boleyn Girl”, Mary, is among those died of sweating sickness. Interestingly, sweating sickness seemed to be more virulent among the higher classes in contrast to plague.

Though its cause remains unknown, it has been suggested that sweating sickness is an infection with Hantavirus which can progress to Hantavirus pulmonary syndrome, which can be fatal (6).

Roman fever

At its height, the Roman Empire stretched from Scotland in the northern hemisphere to the deserts of Africa in the south. The empire lasted for over 500 years, although its eastern part lasted for several more centuries. Roman fever during the fifth century AD may have contributed to the fall of the Roman Empire.

While there are several mentions of a disease sounding very similar to malaria in historical documents from Roman times, there has never been any hard evidence of its presence. In 2011, BBC reported that, for the first time, a British scientist proved conclusively that the most dangerous type of malaria was a killer in imperial Rome. The malarial DNA from a Roman site, dating from around AD 450, is the oldest definite evidence of malaria in history. The finding of malaria was a remarkable and complicated piece of detective work, which spanned the last several years (7).

Thun Biya (the three fears) in Visala Maha Nuwara

About 2570 years ago, during the time of Lord Buddha, residents of Visala Maha Nuwara (The Great City of Visala) faced three fears, namely, famine, diseases and demons. Their sufferings were relieved after Lord Buddha visited Visala Maha Nuwara and chanted Rathana sutra.

Girimananda sutra describes different ailments that can affect a human being, and it gives the clue that the diseases that spread in Visala Maha Nuwara are most probably the infections like smallpox, chickenpox, measles and diarrhoeal diseases (8).

Christ and Leprosy

Leprosy patients in Christ's time were required to live outside the city and yell "leper" anytime people came near, so they would not get close. They were left to live on their own and to die a slow death.

Jesus was compassionate to those who suffered from leprosy and showed that he cared about even the people that were considered the lowest on the social scale. He cared about them physically and spiritually. Jesus cleansing a leprosy patient is one of the miracles of Jesus in the Gospels, namely in Matthew 8:1-4, Mark 1:40-45 and Luke 5:12-16. Patron saints are chosen by different leprosy hospitals. In England it appears to be St. Mary Magdalene. This is due to her status as an outcast, who realized her sin and cleansed by Jesus Christ.

2001 Anthrax attacks in the US (9)

The 2001 anthrax attacks in the United States, also known as Amerithrax from its Federal Bureau of Investigation (FBI) case name, occurred over the course of several weeks beginning on Tuesday, September 18, 2001, one week after the September 11 attacks. Letters containing anthrax spores were mailed to several news media offices and two Democratic U.S. Senators, killing five people and infecting 17 others. According to the FBI, the ensuing investigation became "one of the largest and most complex in the history of law enforcement". Continuous investigations were carried out and, at the end, a bio defense expert named Bruce Edwards Ivins was suspected of providing the spores of *Bacillus anthracis*. Later, in July 2008, he committed suicide. A review report

published by the National Academy of Science cast doubts over the government investigation. For this observation, the response of the FBI was that a combination of factors led them to their conclusion.

Bacillus anthracis, the causative agent of anthrax, is a Gram positive rod. On sheep blood agar, it produces 2-5 mm, non-haemolytic colonies with wavy edges, known as "Medusa head" colonies. Who is this Medusa? According to Greek mythology, Medusa was originally a ravishingly beautiful maiden, "the jealous aspiration of many suitors," but because she made love with Poseidon in Athena's temple, the enraged Athena transformed Medusa's beautiful hair to serpents (resembles the wavy edges of *B. anthracis* colonies) and made her face so terrible to behold that the mere sight of it would turn onlookers to stone. The hero, Perseus, was sent to fetch her head by King Polydectes of Seriphus because Polydectes wanted to marry his mother. The gods were well aware of this, and Perseus received help. He received a mirrored shield from Athena, gold, winged sandals from Hermes, a sword from Hephaestus and Hades's helm of invisibility. Perseus was able to slay her while looking at the reflection from the mirrored shield he received from Athena.

On his way back with Medusa's head, in northwest Africa, Perseus flew past the Titan Atlas, who stood holding the sky, and transformed him into stone when he tried to attack him. In a similar manner, the corals of the Red Sea were said to have been formed of Medusa's blood spilled onto seaweed when Perseus laid down the petrifying head beside the shore during his short stay in Ethiopia where he saved and wed his future wife, the lovely princess Andromeda. Furthermore, the poisonous vipers of the Sahara were said to have grown from spilt drops of her blood.

Perseus then flew to Seriphos, where his mother was about to be forced into marriage with the king. King Polydectes was turned into stone by the gaze of Medusa's head. Then Perseus gave the Medusa's head to Athena, who placed it on her shield (10).

Buruli ulcer and continental drift

Buruli ulcer (also known as the Bairnsdale ulcer, Searls ulcer, or Daintree ulcer) is an infectious disease caused by *Mycobacterium ulcerans*. The disease was so named after Buruli County in Uganda (now called Nakasongola District), because of the many cases that occurred there. This infection has been reported from at least 32 countries around the world, mostly in tropical areas (11).

In Sri Lanka, skin ulcers consistent with *M. ulcerans* infection were found among Australian troops in 1942. The ulcers occurred in only one battalion of the regiment, which was stationed along the shores of Lake Koggala near Galle in the south of the country. Similarly,

M. ulcerans infection was first reported in Australia in 1948 in a paper describing 6 patients from Victoria, 5 of whom came from the Bairnsdale district in south-east Australia (12).

In December 1986, Dr. Siri Wickremesinghe came back to Sri Lanka from Melbourne, Australia, and joined the Medical Research Institute once again. By that time, we also had come to the MRI as students of the first batch of Diploma in Medical Microbiology programme of the PGIM. In a personal conversation, Dr. Wickremesinghe, referring to the reported cases of Buruli ulcer in Koggala, and Victoria, suggested the possibility that, a long, long time ago, at the beginning of the evolution of the earth, Sri Lanka and Australia were together, later they were separated due to continental drift.

What is continental drift? Continental drift is the movement of the Earth's continents relative to each other by appearing to drift across the ocean bed. The important question is that, could there be a link between Koggala in Sri Lanka and Melbourne in Australia? If it is so, could it be due to continental drift? To come to a conclusion, more research has to be done.

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Do we know enough of our own backyard ? Influenza surveillances in Sri Lanka over the last decade

The President and the Council of Sri Lanka College of Microbiologists, ladies and gentlemen, my dear colleagues. It is a great honor to have been invited to deliver this year's Sri Wickramasinghe oration.

The Late Dr. Rakkitha Sirimal Bandara Wickremesinghe, or Dr. Siri Wickremesinghe as we called him, was born on 28th November 1937 to Dr. Artie and Helen Wickremesinghe. He obtained his education at prestigious Royal College Colombo, and graduated MBBS in 1963 from the Faculty of Medicine, Colombo.

He started his medical career in the Dermatology Unit, General Hospital Kandy. He later joined the Medical Research Institute (MRI) and began his career in the Department of Bacteriology. He travelled to the University of Manchester to obtain his Diploma and Master of Science in Microbiology. Subsequently, he obtained MD in Microbiology from the Postgraduate Institute of Medicine, University of Colombo and was Board Certified in Microbiology in 1983. He continued to work at the MRI as the Consultant Microbiologist until he left to Australia with his family. Having worked in the Fairfield Hospital in Melbourne, he returned to the MRI and continued his service as the Consultant Microbiologist in charge of the bacteriology division until his retirement. He held the Director post in the MRI from 1996 to 1998.

He was a past President of the Sri Lanka College of Microbiologists and provided yeoman service as Secretary of the Board of Study in Microbiology at the Postgraduate Institute of Medicine. He was an exemplary microbiologist and a versatile teacher. I was one of the later generation microbiologists who immensely benefited

from his oceanic knowledge in the field of bacteriology. Yet, he was a humble personality and I have travelled in a number of occasions in his ash "doctor" sunny from Ragama to MRI, when I was reading for the diploma in microbiology.

After retirement he worked as the Resident Pathologist and Laboratory Manager at Durdans Hospital, Colombo. At the age of sixty-six, on 08th April 2003, our beloved Dr. Siri Wickremesinghe departed from this world, leaving behind a legacy that to date stands unchallenged and unparalleled in the field of microbiology in Sri Lanka.

Background

Imbalance of the host pathogen homeostasis has led to various novel infectious diseases among humans. Infectious pathogens are arguably among the strongest selective forces that act on human populations. Most of these infections have been transmitted from wild or domesticated animals and co-evolved with human ancestors. Unarguably, hunter gatherers may have had no major infectious threats. However, cultural, agricultural and industrial revaluations narrowed the animal-human interface, which not only permitted spillover of pathogens, but also facilitated sustained transmission of infectious agents in communities leading to epidemics. Mass migrations exposed geographically confined novel pathogens.

Documented historical sources, "influenza like" outbreaks have appeared at intervals since ancient times. Hippocrates described a disease, which resembled influenza as early as 412 BC. A number of outbreaks

suggestive of influenza have also been documented in medieval Europe.

Influenza infections are reported globally with an annual attack rate estimated at 5% – 10% and 20% – 30% in adults and children respectively, leading to about 3 – 5 million cases of severe illness and about 250,000 to 500,000 deaths annually.

Introduction

Influenza viruses are enveloped, negative stranded segmented RNA viruses belonging to the Family *Orthomyxoviridae* and Genus *Orthomyxovirus* that in turn consists of five genera: *Influenza virus A-C*, *Isavirus* and *Thogotovirus*. Recent studies have identified a novel genus of influenza virus in cattle and dogs and have tentatively been named *Influenza virus D*. Of these, influenza virus types A and B cause regular epidemics, and type A occasionally causes pandemics. Influenza type C infects humans, but causes little or no disease. Influenza types A and B are grouped based on antigenic differences of their nucleocapsid and matrix proteins. Influenza A viruses are further subtyped based on the antigenic differences between two surface glycoproteins, the haemagglutinin (HA) and neuraminidase (NA). Eighteen (18) HA subtypes and 11 NA subtypes have been identified, and they are designated H1-H18 and N1-N11 respectively. Wild aquatic birds are considered to be the natural reservoir of influenza A viruses of subtypes H1-H16 and N1-N10. Recently, novel influenza A H17N10 and H18N11 viruses have been detected in bats. Nevertheless, limited number of influenza subtypes; 3 HA subtypes (H1-H3) and 2 NA subtypes (N1, N2) are so far known to have established themselves in the human population, although a number of other subtypes (e.g. H5-H7, H9, H10) have caused occasional zoonotic infections.

There are no known animal reservoirs of influenza B and influenza C viruses and these infections are mainly confined to humans, although infrequently isolated in non-human species. Only single subtypes of HA and NA are recognized in influenza B and C viruses. Influenza B has two designated virus lineages; Victoria and Yamagata that differ in their serological cross-protection.

The hallmark of influenza virus is its ability to evolve continuously through the mechanisms of antigenic drift and shift. Antigenic drift is a result of slow antigenic changes in the virus HA and NA genes through the accumulation of mutations in the error prone viral genome due to lack of “proof-reading” mechanism of the viral polymerase, under influence of positive selection pressure of the host immune system, which selects mutants that escape preexisting neutralizing antibodies. The emergence of antigenically drifted viral strains forces to change the constituents of influenza virus vaccine strains annually.

Influenza A virus occasionally undergoes swift changes of the antigenic properties of the HA and NA. These unpredictable events lead to dramatic antigenic changes in the major immunogenic surface proteins of the influenza A virus through genetic reassortment between a human and non-human influenza virus and is known as antigenic shift. If the reassortant virus has gained efficiency and sustains human-to-human transmission, it could lead to unprecedented spread in the immunologically naïve human population leading to a pandemic. Four such human pandemics have occurred in the past 100 years: in 1918 (H1N1), 1957 (H2N2), 1968 (H3N2) and 2009 (H1N1).

The severity of annual seasonal epidemics is determined by serotype of the virus and immune status in the general population. The peak activities of influenza vary depending on the prevailing climate in a given region. For example, in Sri Lanka, influenza virus activity peaks correspond to a peak in rainfall. In general influenza viruses in tropical and subtropical regions could be detected at low levels outside the peak periods of viral activity, indicating possible circulation throughout the year with peaks during rainy seasons. In contrast, in temperate climates influenza shows a seasonal pattern with high incidence in winter months. Although the seasonality of influenza differs in the tropical and temperate regions, the overall disease burden and mortality remains comparable.

Influenza is an acute respiratory disease with incubation period ranging from 1-4 days that spreads by large droplet, small-particle aerosols or through contact with fomites. Peak virus shedding occurs from 1 day before onset of symptoms to 2-3 days after disease onset. Children younger than 2 years and the elderly population >65 years of age, and those with co-morbidities (respiratory or cardiac disease, diabetes and renal failure) have highest hospitalization rates. Acute influenza symptoms start abruptly with fever, headache, muscle ache, malaise and fatigue symptoms, which are caused by a number of inflammatory cytokines released during the early stages of the illness. These are followed by respiratory symptoms such as cough, sore throat and coryza. The clinical spectrum of infection could range from asymptomatic infection to primary viral pneumonia. In general, acute illness lasts for a week or so, although malaise and dry cough may continue for 2-3 weeks or much longer. Known pre-existing medical conditions (respiratory or cardiac disease, diabetes and renal failure), pregnancy and smoking could worsen the clinical outcome. Secondary bacterial pneumonia and exacerbation of underlying chronic health conditions are known complications of influenza. Myositis, myocarditis, toxic-shock syndrome and Reye's syndrome in children have also been infrequently reported.

Antigenic properties of influenza HA are the key determinant of virus tropism and host range as well as pathogenesis of influenza virus. The HA molecules of influenza viruses that have been isolated in avian species

and horses showed preference for binding to sialic acids with $\alpha 2, 3$ configurations. In contrast, HAs of influenza viruses from humans and other mammalian species show enhanced binding to $\alpha 2, 6$ -linked sialic acids configuration. Additional human studies have shown that $\alpha 2, 6$ receptors are predominant on respiratory epithelial cells in the nasal mucosa, paranasal sinuses, pharynx, trachea and bronchi and $\alpha 2, 3$ receptors are abundantly found on non-ciliated cuboidal bronchial cells at the junction between the respiratory bronchial and alveolus and on type II cells in the alveolar walls. For influenza virus to be efficiently transmitted among humans, they need to infect the upper human airways, which predominantly contain $\alpha 2, 6$ receptors. This explains, that avian viruses that bind to $\alpha 2, 3$ receptors unlikely to replicate in the upper human airways and fail to be transmitted efficiently between humans. Thus if a highly pathogenic avian influenza (HPAI) H5N1 viruses were to gain access to the human respiratory tract, it could potentially infect and replicate in the lower respiratory tract possibly leading to severe life threatening pneumonia, but shows limited human-to-human transmission.

In contrast, pig trachea contains dual receptors ($\alpha 2, 3$ and $\alpha 2, 6$) and has been recognized as a host where co-infection of avian and human influenza viruses may occur. Thus facilitates genetic reassortment and subsequent adaptation of novel influenza virus that leading to pandemics.

Despite the common belief that influenza viruses exhibit tight species barrier, zoonotic infection of HPAI have been reported; H5N1 in Asia, H7N7 in the Netherlands and H7N9 in China. A number of low pathogenic avian influenza viruses such as H9N2, H6N1, H10N8 and H5N2 have also been sporadically isolated from humans in Asia. Adaptation of HPAI H5N1, emergence of H1N1pdm09 and a novel swine-origin human A H3N2 variant viruses [A(H3N2)v] in the USA have raised pandemic concern.

A number of different antivirals have been used against human influenza infections. The adamantanes (amantadine and rimantadine) act by blocking influenza A virus uncoating. However, many contemporary influenza virus strains are resistant to these group antivirals. Neuraminidase inhibitors (oseltamivir and zanamivir) block the activity of the virus neuraminidase in releasing the virus after replication in infected cells and provide clinical benefit when used within the first 48 hours after onset of disease. Current seasonal and zoonotic influenza viruses are generally sensitive to neuraminidase inhibitors. Occasional resistance to oseltamivir has been reported in individual patients but such resistance is not currently widespread. Early treatment with antivirals could reduce the duration of illness, antibiotic usage and lower the risk of complications.

Vaccine remains the cornerstone for prevention of seasonal influenza. Such vaccines contain two influenza A subtypes H1N1 and H3N2 and one influenza B virus lineage, viz the one assessed to be the lineage that is

likely to become globally dominant. Since the assessment of the influenza B virus lineage that may become globally dominant in the year ahead is difficult, more recently, quadrivalent vaccines have been produced. Thus, the emergence of the unexpected influenza B lineage or the antigenically drifted can occur at repeated intervals. Currently available vaccines against influenza do not induce long-lasting immunity and provide protection only against strains closely related to the vaccine strains. Thus new vaccines are required to be reformulated for every flu season, based on global human influenza surveillance data. The subtype of future outbreaks or pandemic influenza strains is also unpredictable. Hence, development of a successful "universal" vaccination strategy is urgently needed and a number of research teams are currently involved in such vaccine design. A systematic approach of interdisciplinary research, newer approaches of vaccination, increasing use of vaccines and rational usage of antiviral drugs to combat annual influenza outbreaks are essential to reduce the global toll of epidemic and pandemic influenza.

Influenza surveillance in Sri Lanka over the last decade

Human influenza

During the period July 2003 – August 2004, 300 nasopharyngeal aspirate (NPA) samples were obtained from patients reported to the Out Patients Department, Colombo North Teaching Hospital, Ragama, with ≤ 4 days history of acute respiratory tract infection (ARTI). Influenza virus was isolated in monolayers of Madin Darby Canine Kidney (MDCK) cells. Isolates were identified by influenza A and B FITC monoclonal antibodies and characterized by haemagglutination inhibition assay. RT-PCR was carried out on all human influenza A isolates. Genetic sequencing and phylogenetic analysis of the haemagglutinin gene of representative isolates were carried out and phylogenetic tree was constructed.

It is revealed that both serotypes of influenza viruses (A and B) circulate at different times of the year and was the aetiological agent causing 11% of all ARTI. The largest peak of influenza A virus activity detected during May-June 2004, corresponded to peak in rainfall in the country. However, the peaks of influenza A did not correlate with that of influenza B activities. Influenza B virus activity occurred from September 2003 to December 2003 at low levels without a major peak of virus activity. About sixty percent (60.6%) of influenza viruses (influenza A and B) were isolated from patients less than 15 years of age, suggesting that influenza virus infection is more prevalent among this age group, at different times of the year. It was revealed that RT-PCR was more sensitive for the detection of influenza A than culture and 14% of influenza A positives would have been missed if only virus culture was relied upon. As we did not perform RT-PCR for influenza B, we probably may have missed a proportion of influenza B viruses as well. Our data were based only

on a single year and data from several years are needed before conclusions can be drawn on the seasonality of influenza virus activity in Sri Lanka. Such information is important before the timing of influenza vaccination for Sri Lanka is determined. Identification of prototype influenza virus for the annual influenza vaccination programme heavily depends on systematic surveillance of these viruses. Thus it is important to strengthen the influenza surveillance programme in the country.

In 2006, a sporadic respiratory outbreak was reported in the remand prison Mahara. A/H1N1/2000/99-like influenza virus was isolated from NPA samples obtained from inmates by inoculating into MDCK cells. Early aetiological detection enabled the Epidemiology Unit to start rimantadine, which controlled the outbreak that affected more than 100 prisoners and reflects the importance of early detection of the aetiological agent in public health perspectives. However, two prisoners succumbed due to secondary pneumococcal pneumonia.

Nasopharyngeal aspirate or nasal swab samples that were tested positive for H1N1pdm09 by RT-qPCR were obtained from the Medical Research Institute, Borella, Colombo, Sri Lanka. These samples had been collected in 2009 ($n=263$), 2010 ($n=100$) and 2011 ($n=100$). Twenty six (26/463) human H1N1pdm09 viruses were isolated on MDCK inoculations. These samples had been sourced from hospitals located in different administrative districts of the country. Poor cold-chain maintenance and repeated freeze-thaws may be responsible for the low success rate for virus isolation from RT-PCR positive specimens. In addition, 9 Sri Lankan human H1N1pdm09 viruses isolated in 2009-2012; three viruses in 2009, one virus in 2010, two viruses in 2011 and three viruses in 2012; were obtained from World Health Organization, Influenza Collaborating Centre, Melbourne, Australia. Except in two Sri Lanka human H1N1pdm 2009 isolates, A/SLK/24206/2009 and A/SLK/24558/2009, which had D222E substitution in HA gene, the other Sri Lankan humans isolate had conserved the consensus D222 in HA gene. Both Sri Lankan patients detected with D222E mutant H1N1pdm 2009 viruses had fatal infections. A mutant with a D222G or D222E substitution (D225G or D225E in the H3 numbering system) in the receptor-binding site of the virus haemagglutinin of H1N1pdm09 virus has caused a substantial number of severe and fatal infections. Although detected sporadically, the D222G substitution has been observed to correlate with cases of severe or fatal disease.

The H275Y and N295S substitutions in the NA gene, which are associated with oseltamivir resistance, were not present in Sri Lankan human isolates. However, these findings do not reflect the broader molecular picture of the human H1N1pdm 2009 viruses isolated in the country as only 35 human viruses were included in the molecular characterization.

Swine surveillance

H1N1, H3N2 and H1N2 subtypes of influenza A viruses have been widely reported in pigs. Influenza A infections

in swine causes respiratory disease in pigs, can lead to significant economic impact and plays paramount importance in generation of pandemic influenza in humans. Endemic swine influenza viruses have derived either directly from avian or human influenza viruses or by reassortant between them. The concept of pigs as a “mixing vessel” for influenza A viruses was proposed. The presence of both Sia α 2-6 and Sia α 2-3 in the swine trachea enables it to play a unique role in the epidemiology of influenza A infection. Concurrent infection of a single porcine host cell by distinct subtypes of influenza A viruses could lead to reshuffling of the gene segments leading to the generation of an influenza virus strain with a novel gene constellation. It is believed that most human pandemic viruses of 1957 and 1968 were generated in this manner.

The H1N1pdm09 virus probably emerged from swine into humans though reassortment between the recent North American triple reassortant H1N2 swine viruses and Eurasian avian-like swine viruses. The double (either avian and human or human and swine), and triple (human, avian and swine) reassortant influenza A viruses isolated in pigs in North America in the 1990's period provide good examples of the “mixing vessel” theory. Isolation of wholly avian influenza viruses in European swine in 1979, a novel wholly avian H1N1 influenza in pigs in China, wholly avian influenza viruses (H4N6, H3N3 and H1N1) in Canadian pigs, sero epidemiological evidence of infection of H4, H5 or H9 avian influenza viruses in Asian swine herds, H9N2 avian influenza viruses isolation from pigs in several provinces in China and Hong Kong and isolation of highly pathogenic avian influenza H5N1 in pigs in some Asian countries supports the concept that pigs are permissible to influenza viruses of avian origin. Experimental settings documented that pigs are susceptible to H1-H13 subtypes of avian influenza viruses and may be susceptible to H14-H16 subtypes as well. These observations and experimental findings strongly support the fact that swine could serve as both direct and intermediate hosts to different subtypes of avian influenza viruses particularly highly pathogenic avian influenza of the H5 and H7 subtypes. However, more recent studies suggest that swine may not be as readily permissive to a wide range of avian influenza viruses. For example there is low susceptibility of highly pathogenic avian influenza H5N1 infection to domestic pigs in nature and under laboratory conditions.

The landscape of the epidemiology of swine influenza viruses has been dramatically altered by the isolation of H1N1pdm09 in swine herds leading to emergence of a range of novel reassortants viruses containing one or more gene segments of the H1N1pdm09 virus. Therefore, susceptibility to both avian and human influenza A viruses in pigs will continue to provide opportunities for the introduction of new influenza viruses, some of which may have capacity for interspecies transmission. Such reassortments are facilitated by regular close contact of swine with humans or birds.

Since ancient times, Sri Lankans had engaged in hunting and consuming wild boars (*Sus scrofa*). Accurate data on the introduction of domestic pig rearing in Sri Lanka is not available. However, it is highly likely that during eighteenth century the British planters introduced the practice of pig farming into the country simply to meet with their own pork demand. Initially, swine husbandry was based on smallholder backyard farming. In more recent years, backyard swine farming had reduced and modern livestock practices have been incorporated into the pig production system. The livestock statistics show that the swine population in Sri Lanka is around 80,000 and pork contributes to 1% to the livestock component of the Gross Domestic Product (GDP) of the country. Swine farms are predominately (~ 61%) located in the Western coastal belt spanning Puttalam, Gampaha, Colombo and Kalutara administrative districts of the country, identified as the “pig belt” of Sri Lanka.

Currently, several different pig production systems are in operation in the country. This is based on the herd size, breeds, feeding systems, and market channels. Sixty percent (60%) of the farms are of small scale (<50 animals), 25% are of medium scale (51-100 animals) while only 15% are of large-scale.

The small-scale farmers mainly raise indigenous animals and cross breeds deriving from them. The animals are mainly fed with swill, rice bran kitchen refuse and non-human edible chicken refuse. Exotic breeds such as Land Race, Large White are backcrossed with Duroc breed. These animals are descendants of two main nucleus herds from the state-owned National Development Board (NLDB), Sri Lanka swine breeding farms located in Welisara and Horekelle. Medium scale farmers practice semi intensive farming system and raise indigenous pigs and their crosses.

The Government Slaughterhouse located at Dematagoda, Colombo operates for 6 days per week and slaughters around 20 pigs per day or more depending on the available number of pigs on a given day. The Dematagoda swine abattoir is under the direct purview of a veterinary surgeon in-charge of the site, which comes under the authority of chief veterinary surgeon of the Colombo Municipal Council (Dr. Colin Perera, personal communication). More than 90% of the animals that are slaughtered at the Dematagoda Government Swine Slaughterhouse receive pigs from the swine farms located in the Puttalam, Gampaha, Colombo and Kalutara administrative districts.

The pig population densities in the Puttalam, Gampaha, Colombo and Kalutara districts in 2010 were 7, 15, 12, and 1 animal per km², respectively. In 2001 (latest available human census data) for these districts, the human population densities were 246, 1,539, 3,330, and 677 persons per km², respectively.

Pigs are not imported on a regular basis to the country. Only on three occasions during last three decades have

such imported consignments been documented. It is recorded that 30 live pigs were imported from Australia in 1987, and 32 and 26 animals from USA in 1995 and 1998, respectively (Dr. Pushpa Wijewantha, personal communications). Hence, the breeding program of the country is heavily dependent on artificial insemination. However, the pig-breeding farm located at NLDB Welisara maintains Land Race and Large White animals and surplus piglings are sold to farmers for breeding.

Although epidemiological and virological studies on influenza in humans have been carried out there is only one other study on influenza virus circulating among pigs in the country. Therefore, this study was carried to determine the ecology and evolution of swine influenza viruses in Sri Lanka.

The information that is generated from this study is not only important for monitoring the emergence of new influenza pandemics but would provide the opportunity to understand genetic diversity of the influenza viruses circulating among pigs in a South Asian country.

2004-05 period

Paired tracheal and nasal swabs, and blood samples were collected from freshly slaughtered pigs (n=300) brought to Government slaughterhouse, Dematagoda, Colombo in 2004-05. Samples were inoculated into monolayers of Madin Darby Canine Kidney (MDCK) cells and embryonated chicken eggs.

One influenza A virus, A/swine/Colombo/48/2004(H3N2), was isolated in MDCK cells from a tracheal swab sample collected in 2004 – 2005. All genes of this virus were closely related to human influenza (H3N2) virus isolate A/Ragama/190/2003 from Sri Lanka and to other subtype H3N2 influenza viruses isolated worldwide at this time. During January 2004 – March 2005, a total of 185 (61.6%) of 300 serum samples tested were positive for A/swine/Colombo/48/2004(H3N2); indicating that this human-like influenza (H3N2) virus was widespread in the swine population.

2009-13 period

Recommencement of our systematic virological and serological surveillance in swine abattoirs in Sri Lanka, during 2009 – 2013 detected H1N1pdm09 like virus in local swine herds. Infection in pigs followed each of the H1N1pdm09 outbreaks in humans; October 2009 – January 2010, October 2010 – February 2011 and November 2012 – March 2013, respectively. Genetic, phylogenetic, and epidemiologic analysis of the human, and swine influenza viruses indicated spillover events of H1N1pdm09 from humans into pigs, with self-limited transmission and extinction within pig herds. The data also indicated that although H1N1pdm09 was able to spill over from humans to swine, it is not ideally adapted to establish sustained transmission among swine in the absence of further reassortment with other swine influenza virus lineages.

These findings might reflect characteristics of swine husbandry in Sri Lanka, which has a low density pig population and remains isolated from global swine influenza viruses because of the absence of regular cross-border and cross-continental movements of swine. In contrast to some other parts of the world, we failed to isolate established lineages of swine influenza viruses, viz. Classical, North American triple reassortant and European Avian lineages. Seroprevalence to these endemic swine viruses was largely absent in local swine herds. Serum samples collected from swine during 2009-2012 were also mostly seronegative to this and to more contemporary human influenza (H3N2) viruses. To clarify transmission patterns between affected swine farms in Sri Lanka, we obtained contact patterns by interviewing pig farmers using a structured questionnaire with approval from the Ethics Review Committee, Faculty of Medicine, Ragama, Sri Lanka. There was no evidence of movement of persons or fomites between farms. However, during the peak demand period (November-December) of each year that surveillance was performed, a common truck owned by one farm, and driven by a single driver and an assistant, provided transportation from multiple farms to the abattoir, including from affected farms. On some occasions, animals taken to the abattoir for slaughter were returned to the farm.

This extended swine influenza study demonstrates natural independent spillover events of H1N1pdm09 influenza viruses from humans to swine. H1N1pdm09 viruses appear to be spread by multiple, discrete introductions to swine, after which clonal expansion occurs within the swine. The spread of such virus lineages across multiple farms is consistent with virus dispersal by breaches of external biosecurity measures, including the manner of swine transportation, although this remains unproven given the small sample size. Unlike classical swine influenza, North American triple reassortant, and European avian swine viruses that have persistently circulated among swine for several decades in other countries, H1N1pdm09 does not appear to establish long-term lineages in swine in the absence of further reassortment. This observation requires confirmation in other geographic settings.

Avian Surveillance

Avian influenza: 2003-05

We conducted surveillance of avian influenza viruses in the live poultry markets, backyard flocks and small-scale poultry farms in Western Province in the country during 2003-2005. In total, 750 birds were sampled, and a tracheal and cloacal swab and blood sample were collected from each bird. Tracheal and cloacal swab samples were inoculated into embryonated chicken eggs and serum samples were tested by haemagglutination inhibition assay.

Ninety six (96, 12.8%) birds were seroconverted for A/quail/HK 1721-30/99 (H6N1) and 39 birds (5.2%) were seropositive for A/duck/ Hong Kong /Y280 (H9N2) – like viruses. A total of 28 out of 750 (3.7%) bird sera tested

were seroconverted for both A/quail/HK 1721-30/99 (H6N1) and A/Duck/ Hong Kong /Y280 (H9N2) – like viruses indicating dual infection. Higher number of layers showed seroconversion for both subtypes of viruses to that of broiler birds. However, we failed to isolate avian influenza virus.

Avian influenza: 2009-2013

We sampled 3650 domesticated birds (chicken $n=2500$, duck $n=600$, turkey $n=250$, quails $n=300$) Like in the 2004-05 period, a tracheal and cloacal swab and blood sample were collected from each bird. Serological studies revealed that H6 subtype of avian viruses largely replaced by H9 in 2009-13. Interestingly, seroprevalence of both serotypes of avian influenza viruses; H9 and H6 have dropped to 4.3% and 6%, respectively. Over 1500 migratory birds droppings and 500 bat rectal swab samples tested negative for influenza virus by RT-PCR method.

We failed to detect avian influenza viruses by embryonated chicken eggs or molecular methods in both domesticated and wild birds. However, NDV virus was frequently isolated in avian swab samples that tested negative for avian influenza viruses. However, seroepidemiological studies indicates that prevalence of avian influenza viruses is low in comparison to neighboring countries.

Avian astrovirus

We extended our studies to identify novel avian astroviruses. From our surveillance of astroviruses in poultry, chicken astroviruses (9.6%, 27/282) were detected from chicken samples collected in Sri Lanka, while Turkey Astroviruses 1 (TAsTV1) was detected from 3 cloacal swab samples collected from apparently healthy chickens in poultry in Sri Lanka. This is the first report of the detection of TAsTV1-like virus in chickens anywhere. The chicken farm where these chicken samples were collected did not house turkeys. The source from which chickens acquired infection of these viruses was unknown. No avian astrovirus were detected in cloacal swabs collected from quails, ducks, and geese. The sample sizes of the minor poultry were smaller.

Novel corona and astro viruses in bats

A project on identification of novel corona and astrovirus in bats was commenced in early 2012. We collected 290 droppings, 250 rectal swabs and 220 oral swabs from 15 different bat species from different locations in the country. A novel coronavirus lineage was detected in Sri Lankan flying foxes (*Pteropus giganteus*). A species of bat viruses that related to BtCoV-HKU-3 and SARS corona virus was detected in *Rousettus leschenaultii*. Another species of bat corona virus that is related to BtCoV-HKU5-1 was detected in *Rhinolophus rouxii*.

History has proven that influenza is and will remain as a grave medical threat to humans and many livestock. A

systematic approach to interdisciplinary research and efficient networks with regular close communication between infectious diseases, scientific and veterinary professionals is crucial in order to assess and early detection of novel influenza viruses, specifically subtypes which could potentially lead to future influenza pandemics.

Therefore, a systematic approach of interdisciplinary research, newer approaches of vaccination, increasing use of vaccines and rational usage of antiviral drugs to combat annual influenza outbreaks are essential to reduce the global toll of epidemic and pandemic influenza.

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Invited article:

THE MICROBIOLOGY OF MYCOBACTERIA – PAST AND PRESENT

(Summary of a presentation made at the Symposium on Tuberculosis organized by the Sri Lanka College of Pulmonologists in March 2015)

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Dr. Carl Sagan (1934-1996) commented 'You have to know the past to understand the present'. Does having an increased knowledge of the 'past' of tuberculosis help us in any way to understand the current trends and improve planning for the control of tuberculosis?

Tuberculosis remains a scourge of modern life, just as it has been in past centuries. The advent of anti-tuberculous drugs and improved standards of living including better housing and nutrition were believed to have eliminated the disease. However, the fallacy of this thinking was revealed with the advent of HIV infection in the 1980s. The incidence and prevalence of tuberculosis continues to rise with 1/3rd of the world's population believed to be infected with *Mycobacterium tuberculosis*, the majority of whom (≈ 1.8 billion) being asymptomatic, 16 million with active symptomatic tuberculosis and 1.7 million deaths annually. Multidrug resistance and XDR-TB compound the problems caused by this organism.

Where and when did this organism emerge? Modern techniques have allowed the mapping of *M. tuberculosis* from ancient times¹. Consensus opinion is that *M. tuberculosis* is an ancient organism, and has co-evolved with man. Dating for its emergence ranges from 40,000-70,000 years ago from what is believed to be the cradle of modern man – North Eastern Africa and the Mesopotamian region.

Tuberculosis is characterized by two phases of infectious diseases.

As is well known, most people infected with *M. tuberculosis* remain well and show no symptoms or signs of the infection for most or even all of their life. This phase of infection – known as the 'latent phase', the bugbear of control programmes, allows slow progression to clinical disease. It is suggested that this was the main form of infection in the 'hunter-gatherer' era and possibly reflects adaptation to low host population densities by allowing repletion of the reservoir of susceptible individuals.

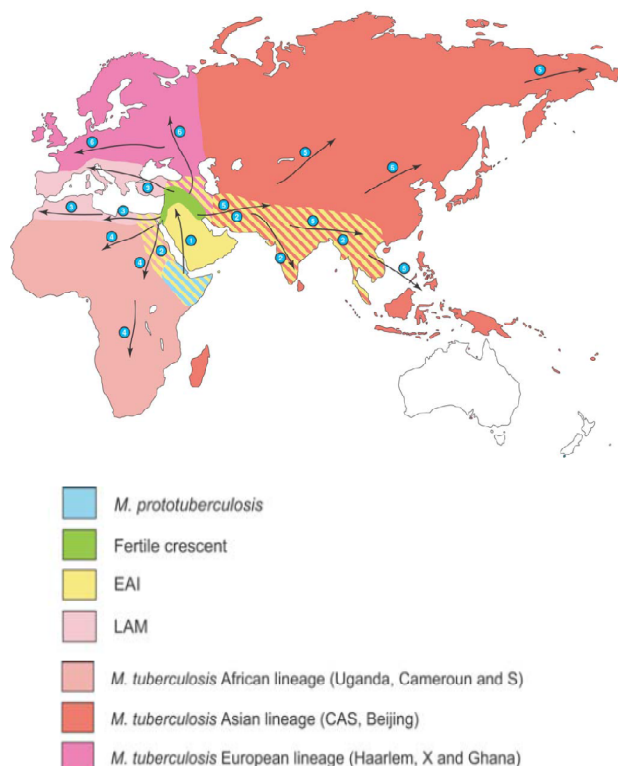


Figure 1. Origin, spread and demography of MTb complex (1)

However, tuberculosis is also known to cause explosive clinical illness with rapid deterioration and resulting fatal outcome. This is typical of 'Crowd disease' where a highly virulent organism spreads rapidly in a population. Crowd disease is dependent on high host population densities which maximize pathogen transmission and reduce the risk of pathogen extinction through exhaustion of susceptible hosts. Tuberculosis is reminiscent of a typical crowd disease in that it kills up to 50% of untreated individuals and has evolved a mode of aerosol transmission promoted by high host densities. It has been postulated that the change from a classically latent

disease to a crowd disease occurred in the Neolithic Demographic Transition (NDT) approximately 10,000 years ago characterized by animal domestication, agricultural settlements and resulting density of human population. What is fascinating about this is that *M. tuberculosis* seems to have co-migrated with the human population as shown by studies on the biographical structure of strains recovered over the vast period of time as depicted in Figure 2.

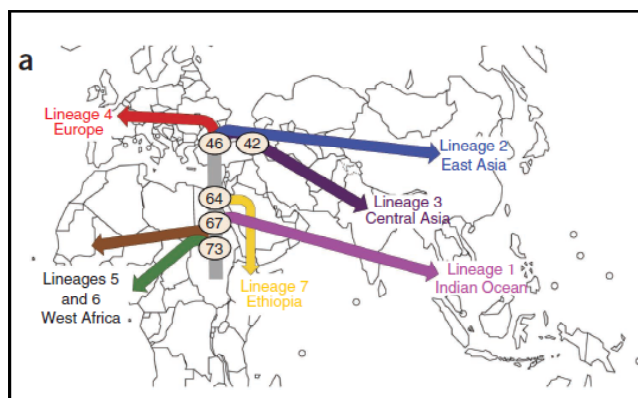


Figure 2. Out of Africa and Neolithic expansion of MTBC (2)

The emergence and spread of the Beijing strain of *M. tuberculosis* in modern times (3) appears to corroborate

these hypotheses and bring to the forefront the difficulties in control of this disease.

M. tuberculosis is a constant companion to the human population and appears to have the ability to adapt to changing human populations. Disease characteristics might also change over time with selection of different MTBC populations in different human societies.

Challenges to the control of tuberculosis include the identification of asymptomatic infection, adequate treatment of those infected with sensitive as well as resistant organisms, lack of an effective vaccine, an ever moving human population and an increasing population of people with risk of reactivation. It is difficult to be optimistic about the worldwide elimination of this extremely well adapted and successful microorganism!

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Review article:

BITING BACK AGAINST RABIES: RECENT DEVELOPMENTS IN A SRI LANKAN PERSPECTIVE

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Rabies is a zoonotic disease that remains an important public health problem worldwide and causes more than 55,000 human deaths each year (1, 2, 14). In Sri Lanka, presently around 20-30 people die of rabies annually and it is mainly due to exposure to rabid dogs (1, 3). Rabies control measures launched in Sri Lanka since 1975 have had a tremendous effect (4) in controlling this deadly disease in human.

Aetiology

Rabies virus (RABV) is bullet shaped; 75nm × 200nm in size and belongs to the genus *Lyssavirus*, one of the

seven genera which are included in the family *Rabdoviridae* within the order *Mononegavirale*. Genera *Lyssavirus* contains 12 species as of 2013 (5, 6). Lyssaviruses have a 12-kb non segmented RNA genome that encodes five viral proteins including "G" protein which encodes the main antigenic site of the virus. Nucleoprotein occupies a major part of RABV structure and it is the target protein of all rabies diagnostic techniques.

Transmission

Human infection usually occurs following a transdermal bite or scratch by an infected animal. Transmission may

also occur when infectious material, usually saliva, comes into contact with the victim's mucous membranes or with fresh skin wounds or ingestion of raw milk of a rabid cow/goat. Human to human transmission of rabies, has never been reported other than in very few cases of solid organ or corneal transplant. Ingestion of raw meat or other tissues from a rabid animal is not a known source of infection.

Epidemiology

Rabies is primarily a disease of non-human mammals. The information on rabies infection in dogs and wild carnivores has gone back to the history of animals written in the 4th century BC, Aristotle and the Indian *Susrutasamhita* of the 1st century AD. South America had first reported bats as possible vectors of the rabies virus (7).

The disease is classified into two main epidemiological cycles; urban and sylvatic rabies. Dogs are mainly responsible for the transmission and maintenance of urban rabies whereas sylvatic rabies is maintained by wild mammals such as mongooses and civets. In developed countries, mass vaccination along with effective control of stray dog population has successfully controlled urban rabies, showing that the disease can be eliminated in dogs and thereby in humans. However, infected dogs remain the primary source of human exposures in Asia, Africa and Latin America (8).

Rabies is endemic in Sri Lanka. It affects older individuals more than children, which is different to most of the other Asian countries. Phylogenetic analysis of the rabies N and G gene revealed that Sri Lankan rabies viruses are distinct and appeared to be originated from a single clone (6). Further analysis of highly diverse G-L noncoding region showed that the circulating Sri Lankan rabies viruses belong to seven clades. Viruses belonging to some clades are unique to a specific geographic region whereas others occur at multiple locations (6). This may indicate that the movement of dogs, is restricted in some areas, but porous in others (6). Since bat *lyssavirus* specific surveillance is limited, no information on the presence of *lyssaviruses* in bats is available in Sri Lanka.

Human rabies is a notifiable disease in Sri Lanka. All suspected rabies deaths should be investigated and laboratory confirmed following a post mortem. The number of deaths due to rabies has dramatically reduced from 55 in 2008, to 19 in 2014 (9).

Dogs continue to be the major reservoir of rabies virus in Sri Lanka. In the meantime, cats are taking the second place. Cows, pigs and goats also contribute to the positivity of domestic rabid animals. Among the wild animals – mongoose, jackals, monkeys, pole cats, bandicoots and rock squirrels have been proved positive for rabies (9).

Importantly, animal surveillance statistics at the Medical Research Institute (MRI) have shown a gradual increase in the positivity rate of dog specimens received from 2010 to 2014 (unpublished data).

Prevention of rabies

Sri Lanka has set a target to eradicate human rabies by 2020. A multi pronged approach has been launched by Ministry of Health, Public Health Veterinary Services, Department of Animal Production and Health and other nongovernmental organizations to achieve the target through the “one health” programme (10).

Prevention of rabies in animals

Elimination of stray dogs had been the major strategy of controlling viruses in Sri Lanka under the rabies ordinance introduced in 1894 (11). In 2006, Ministry of Health revised its strategy to be more humane towards dogs by promoting canine birth control measures and mass immunization of dogs (3, 4, 17).

More than 95% of human rabies deaths in Sri Lanka are following bites by unvaccinated dogs [9]. A dog ecology study conducted during 2009-2010 in Polonnaruwa, Kegalle, Gampaha and Matara districts showed that human: dog population ratio is 6:1 (10). In 2014, over 80 percent of animal rabies positivity was reported among dogs out of all animal head specimens received at MRI. Of the dog specimens received at MRI, rabies was confirmed in 67.3% [unpublished data]. Low vaccination coverage in the resident dog population and ineffective management of stray animals are the most likely reasons for the program's lack of success, but the abundant wild fauna in Sri Lanka could also provide an unrecognized reservoir of rabies virus (9).

Current strategies for dog rabies control are awareness campaigns on responsible dog/pet ownership, vaccination of different groups of dogs, animal birth control programme for stray dogs, habitat control, continuous monitoring and evaluation and mass campaigns for rabies vaccination of household dogs conducted on a regular basis. In Sri Lanka it is recommended to vaccinate dogs against rabies at 6 weeks, 14 weeks, and booster vaccinations annually. Vaccination record should be kept securely with the owner and in case of an animal bite, owner should submit it to the hospital where the victim is treated (10).

If we are to eradicate dog rabies, we need to vaccinate at least 70% of the dog population in Sri Lanka with a safe and quality vaccine for three consecutive years. This will create herd immunity. A quality vaccine is one that should be able to maintain protective immunity for a minimum of three years. Community support is very important to achieve 70% of the dog population vaccinated (1). If everyone vaccinates their own dogs and those they look after or feed in their neighbourhood, we will be well

on our way to achieve herd immunity and finally eradicate rabies.

Sri Lanka is reputed to have a large population of roaming dogs. Most of them live in packs, either fed by owners or scavenging in the neighborhood. The occupier of any house or premises where any dog is kept to live or remain shall be liable to pay a registration fee for such dogs and in default of such payment shall be liable to penalties under the Registration of Dog Act. 1961 (11).

Although occasional cases of rabies have been reported in wild animals in Sri Lanka, abundant wild fauna could provide an unrecognized reservoir for rabies virus (19). It is unclear whether wild life rabies is independent of the dog rabies transmission cycles. An oral vaccination strategy is used successfully in other countries to control wild animal rabies. House rat rabies has not been reported so far from any part of the world, neither as primary host nor as a role in epidemiology or transmission (1).

Prevention of human rabies

Rabies is an invariably fatal disease. Therefore prevention of an exposure to suspected rabid animals would be the best way of avoiding this lethal disease. As this virus is highly neurotropic and it stays at the local site for some time before entering the peripheral nerves, infiltrating rabies specific immunoglobulin would neutralise the rabies virus present at the site of injury (12). Subsequent administration of a course of anti-rabies vaccine (ARV) will ensure a lasting immunity against rabies.

Prompt thorough flushing and washing of the wound with soap and water is an important first aid step in management of post exposure prophylaxis (PEP). If soap or any antiseptic solution is not available the wound should be thoroughly and extensively washed with running water. Thus after suspected or proven exposure to rabies virus, prompt use of anti-rabies vaccine with proper wound management and simultaneous administration of rabies immunoglobulin is almost invariably effective in preventing rabies, even after a severe exposure (1, 18).

PEP should be given when indicated by assessing the nature of the exposure, health and vaccination status of the animal. As rabies is hundred percent fatal, there are no contraindications for PEP. Infants, pregnant women or immunocompromised including HIV/AIDS should be considered if indicated (1). PEP should be initiated and expert opinion should be sought in complex cases of potential exposures.

Vaccines and rabies immunoglobulin for humans

Sri Lanka had been producing nerve tissue anti rabies vaccine until 1995, and abandoned the production and use of it for PEP in humans (13). Since then, safe and effective anti-rabies cell culture vaccines (20) are being used for pre and post exposure rabies prophylaxis. Since 1997, Sri Lanka has shifted to the use of intradermal

route of anti-rabies vaccine delivery which was approved by the WHO in 1992 (13). This led to a significant reduction in the cost and volume of vaccine consumption to one tenth of the conventional intramuscular route.

Anti-rabies serum/rabies immunoglobulin provides passive immunity in the form of ready made anti-rabies antibody to tide over the initial phase of the infection. Anti-rabies antibody has the property of binding with the rabies virus, thereby resulting in the loss of infectivity of the virus. Therefore equine rabies immunoglobulin (ERIG) and human rabies immunoglobulin (HRIG) are available and recommended for use.

Presently, 17 major government hospitals in the country have been identified as centers for administration of HRIG. A study done on the consumption of HRIG in these hospitals in Sri Lanka has shown an increase from 2010 to 2012 with an increasing trend of unnecessary administration. Majority (58-60%) of HRIG administration was for exposures to stray animals (15). Pre-exposure prophylaxis is recommended for any person who is at continual, frequent or increased risk of exposure to the rabies virus as a result of their residence or occupation, such as laboratory workers, veterinarians and animal handlers (3). In Sri Lanka, such people should be immunized according to the pre-exposure schedule of 1 vial of anti-rabies vaccine administered intramuscularly, on days 0, 7, 28 and a booster 1 year later. Additional boosters are recommended once every five years. Facilities for routine serological monitoring of the immune response following pre-exposure schedule is still not available in Sri Lanka. MRI provides this facility to assess rabies neutralising antibody titer for persons whose lives are at risk of rabies, by prior appointment. Protective rabies virus neutralising antibody titer is $>0.5\text{IU/ml}$ according to WHO. In the near future, Sri Lanka is planning to change the pre-exposure anti rabies vaccine schedule from intramuscular (IM) to intradermal (ID) in consistent with WHO recommendations. A preliminary study done on veterinary students of Peradeniya University using ID pre-exposure prophylaxis schedule has shown satisfactory immune response after a primary course of rabies vaccine (unpublished data). Therefore, shifting from IM to ID ARV pre-exposure management will be implemented in Sri Lanka to further reduce cost and volume of ARV consumption.

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Research article:

IDENTIFICATION OF ACINETOBACTER SPP. ISOLATED IN TEACHING HOSPITAL ANURADHAPURA IN SRI LANKA BY PHENOTYPIC TESTS AND THEIR ANTIMICROBIAL SUSCEPTIBILITY STATUS

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Abstract

Objective: To identify the *Acinetobacter* spp. isolated in Teaching Hospital Anuradhapura, Sri Lanka by phenotypic tests and to study antimicrobial susceptibility status of those isolates.

Methodology: This hospital based descriptive study consisted of *Acinetobacter* spp. positive, clinically significant culture samples received at a microbiology laboratory of a tertiary care hospital in Sri Lanka from 1st of August to 31st October 2009. All *Acinetobacter* isolates were confirmed using simplified phenotypic tests described by Gerner-

Smidt, Tjernberg et al. (1991) and Bouvet, Grimont et al. (1989). Assimilation tests were performed as described by Stainer, Palleroni and Doudoroff (1966). Antimicrobial susceptibility determined following Clinical and Laboratory Standards Institute (CLSI) guidelines 2008.

Results: Of the clinically significant *Acinetobacter* isolates, 7% were from wards, 26.7% were from the paediatric intensive care unit, 10% were from the premature baby unit and 46.7% were from the intensive care unit. *Acinetobacter* spp. were isolated from respiratory tract secretions (63.3%), blood (26.7%), urine (3.3%) and wound exudates (6.7%).

With the simplified phenotypic identification testing, the 30 isolates in the study population belonged to only 5 phenotypes (phenotype numbers 1, 2, 5, 12, 13). Phenotype 2 (*A. baumannii*) is the predominant species (56.7%) followed by Phenotype 1 (*A. calcoaceticus*) 23.3%, Phenotype 5 (*A. junii*) 10%, Phenotype 13 (*Acinetobacter* genomosp.13) 6.7% and Phenotype 12 (*A. radioresistens*) 3.3%. Resistance to cephalosporins (>73%) was very high. Resistance to imipenem was 70%. Of the aminoglycosides, amikacin showed the best sensitivity (47%). Levofloxacin had a better sensitivity (40%) than ciprofloxacin (27%). Co-trimoxazole showed 77% resistance. Of the isolates 53% were cefoperazone - sulbactam resistant but only 20% showed complete resistance. Tigecycline (90%) and colistin (100%) were the only drugs which showed good sensitivities.

Conclusion: A simple identification scheme is useful for laboratories with limited resources for typing *Acinetobacter* isolates. Majority of the isolates were multi-drug resistant (70%).

Introduction

Acinetobacter spp. was first described in 1911 as *Micrococcus calco-aceticus* (1). The genus *Acinetobacter* is classified under the family Moraxellaceae and comprises strictly aerobic, gram-negative, non motile, non-lactose-fermenting, oxidase negative, catalase positive, coccobacillus. *Acinetobacter* spp. as a human pathogen has been reported sporadically. Currently, at least 32 different DNA groups have been reported. They are ubiquitous, free-living and fairly stable in the environment. They are commonly isolated in water, soil, foods, arthropods etc (2). In humans, *Acinetobacter* can colonize skin, wounds, and the respiratory and gastrointestinal tracts. They are natural reservoirs of and account for up to 35-45% of bacteria isolated from human skin (3).

Acinetobacter speciation by a simplified phenotypic identification has not been done in Sri Lanka. In the present study an attempt was made to type the *Acinetobacter* isolates obtained from clinical samples by a simplified phenotypic identification scheme with a numerical approach and also to determine their antimicrobial susceptibility.

Methodology

This study was a hospital based descriptive study. The study sample consisted of *Acinetobacter* spp. positive, clinically significant culture samples received at a microbiology laboratory, Teaching Hospital Anuradhapura, Sri Lanka from 1st of August to 31st October 2009. Confirmed all *Acinetobacter* isolates, subjecting to simplified phenotypic tests as described by Gerner-Smidt, Tjernberg et al. (1991) (5) and Bouvet, Grimont et al (1989)

(6). The assimilation tests were performed as described by Stainer, Palleroni and Doudoroff (1966) (7).

Determination of antibiotic susceptibility (AST) was done using the standard disk diffusion method recommended by the Clinical and Laboratory Standards Institute and zone diameter interpretation was done according to the zone diameter interpretive standards for the CLSI guidelines 2008. Reading of zone diameter of Cefoperazone-Sulbactam was done according to the Sulperazone manual for the Microbiologist Pfizer pharmaceutical division, Pfizer infection control, USA. October 1988 pp 25-27, 31-33. The research protocol was approved by the Ethical Review Committee of the Colombo South Teaching Hospital.

Results

From the isolates, during the period of 3 months 2.7% (30/1117) were found as clinically significant *Acinetobacter* isolates. 16.7% (5/30) of the *Acinetobacter* isolates were from the wards, 26.7% (8/30) were from paediatric intensive care unit, 10% (3/30) were from premature baby unit, and 46.7% (14/30) were from intensive care unit (Figure 1.)

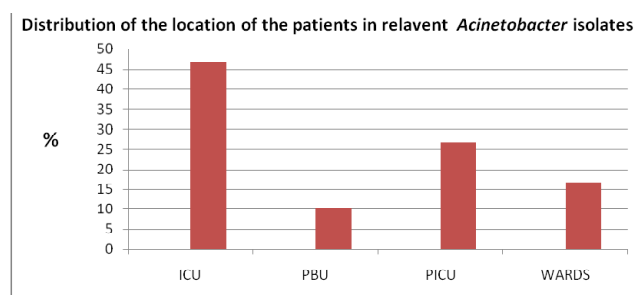


Figure 1.

Acinetobacter spp. were isolated from respiratory tract secretions (63.3% (19/30)), blood (26.7% (8/30)), urine (3.3% (1/30)) and wound exudates (6.7% (2/30)). With the simplified phenotypic identification testing, 30 isolates in the study population belonged to only 5 phenotypes (phenotype numbers 1, 2, 5, 12, 13). Phenotype 2 (*A. baumannii*) is the predominant species 56.7% (17/30) followed by Phenotype 1 (*A. calcoaceticus*) 23.3% (7/30), Phenotype 5 (*A. junii*) 10% (3/30), Phenotype 13 (*Acinetobacter* genomosp.13) 6.7% (2/30) and Phenotype 12 (*A. radioresistens*) 3.3% (1/30) (Table 1).

Majority of the isolates (70% (21/30)) were multi-drug resistant (MDR), showing resistance to two or more antimicrobial agents. From all *Acinetobacter baumannii* isolates 94.11% (16/17) were MDR. Tigecycline shows 85.7% (18/21) and Colistin 100% (21/21) sensitivity against MDR *Acinetobacter* isolates. Resistance to cephalosporins (>73%) were very high. Resistance to

Table 1. Species differentiation of the isolates and their distribution in clinical specimens

Acinetobacter spp.	Geno species	Blood	Sample numbers			Number
			Respiratory	Urine	Pus	
<i>Acinetobacter calcoaceticus</i>	1	2	3, 5, 18, 19, 20, 30			7 (23.33%)
<i>Acinetobacter baumannii</i>	2	1, 17, 25,27	6, 7,8, 10, 11,13,14,15, 16,23,24, 28	9		17 (56.66%)
<i>Acinetobacter junii</i>	5	21,26			12	3 (10%)
<i>Acinetobacter radioresitens</i>	12				29	1 (3.33%)
<i>Acinetobacter genomsp.13</i>	13	4	22			2 (6.66%)

imipenem was 70% (21/30). Of the aminoglycosides, amikacin showed the best sensitivity (47% (14/30)). Levofloxacin had a better sensitivity (40% (12/30)) than ciprofloxacin (27% (8/30)). Cotrimoxazole showed 77% (23/30) resistance. Of the isolates 53% (16/30) were Cefoperazone Sulbactam resistant but only 20% (6/30) showed complete resistance. Tigecycline 90% (27/30) and colistin 100% (30/30) are the only drugs which gave good sensitivities (Table 2).

Table 2. Antimicrobial susceptibilities of *Acinetobacter* isolates

Antibiotic	% resistant/ intermediate
Cefotaxime	90
Coamoxiclav	73.3
Ceftazidime	73.3
Imipenem	70
Amikacin	53.4
Gentamicin	73.4
Ciprofloxacin	73.4
Cefoperazone - sulbactam	53.4
Tigecycline	10
Piperacillin - tazobactam	70
Polymyxin E (Colistin)	0
Levofloxacin	60
Cefepime	73.3
Co-trimoxazole	76.6

Discussion

Acinetobacter species has emerged as an important nosocomial pathogen that is often MDR and associated with life-threatening infections (4). The phenotypic identification of *Acinetobacter* to the species or DNA group level by Bouvet and Grimont (1987) seems to be an appropriate but large panel of phenotypic tests that is tedious and time consuming to perform (4). Simple identification schemes such as those of Gerner-Smidt, Tjernberg and Ursing (1991) (5); Tjernberg (1990) (9) alone with antimicrobial susceptibility testing may be useful for laboratories with limited resources and can be adapted for typing isolates, thus avoiding the need for expensive molecular methodology. Identification of *Acinetobacter* species based upon growth at 44°C, 41°C and 37°C in BHI, penicillin G (10µg) and chloramphenicol (30µg) sensitivity and acid production from glucose could be very useful. In addition, only 5 assimilation tests were used to differentiate isolates to species level (5). Apart from that isolates were tested for haemolysis on sheep and human blood (5%) agar and gelatin liquefaction. According to the literature if there are haemolytic isolates we have to do additional carbon source assimilation tests to differentiate those isolates to species level (9). Simple identification scheme with few phenotypic tests would be a better alternative method for the resource poor laboratories like in Sri Lanka.

Phenotypic characteristics of *Acb*-complex (DNA groups 1, 2, 3, 13) were helpful in differentiating the species within the complex. Most studies have discussed the problems in separating DNA groups 1, 2, 3, and 13 by phenotypic tests. The responses for most of the assimilation tests were very similar for DNA groups 1, 2, and 13. This similarity is in accordance with the observations that these four groups are genotypically more closely related to each other than to other DNA groups (10).

However, there were no unequivocal tests separating DNA group 2 from DNA group 13, which was not recognized by Bouvet and Grimont (8). But in this study susceptibility to both penicillin (10µg) chloramphenicol (30µg) were used to separate those 2 groups as described by Tjernberg (9).

According to the literature hemolytic strains were found in DNA groups 4, 5, 6, and 14 (8). For all of these groups except group 5, hemolysis of human and sheep blood correlated with a positive gelatinase test. Even though the isolates were tested for haemolysis on sheep and human blood (5%) agar and gelatin liquefaction, there were no positives in this study. So, additional carbon source assimilation tests were not performed.

The tests for identification of *Acinetobacter* selected by Bouvet and Grimont (1987) (8) seem appropriate; for the DNA groups, which were isolated in this study.

As further continuation of this study, genotypic identification was done for 24 isolates out of 30 and they tally with the phenotypic identification results.

The literature search demonstrates that genomic species Gs-2 (*A. baumannii*), together with Gs-1 (*A. calcoaceticus*), Gs-3 and Gs-13TU, is predominantly involved in infection and is often collectively known as the *Acinetobacter calcoaceticus* - *A. baumannii* (Acb) - complex (Gerner-Smidt 1991) (5). This holds true in our study too and 86.66% of the isolates belonged to *Acinetobacter calcoaceticus* - *A. baumannii* (Acb) - complex.

Majority of the species isolated in this study (Teaching hospital, Anuradhapura) were *A. baumannii* (56.66%). According to past studies this is the main species responsible for nosocomial infection in other parts of the world too (11). In this study majority of the isolates were multi-drug resistant (70%), showing resistance to two or more antimicrobial agents. From all *Acinetobacter baumannii* isolates 94.11% (16) were multi-drug resistant.

Tigecycline shows 85.7% and colistin (100%) sensitivity against MDR *Acinetobacter* isolates while cephalosporins and Carbapenems 100% resistant and all the other antibiotics showing >80% resistance. Tigecycline resistance was noted only in *Acinetobacter baumannii*.

A high efficiency of ampicillin/sulbactam is worth considering. Sulbactam is known to be much more efficient against *Acinetobacter* than clavulanic acid which is closely related to its affinity to PBP (12). It has to be pointed out that the opinions about the efficacy of beta-lactamase inhibitor tazobactam rather differ (13). As ampicillin/sulbactam disks were not available during the study period, sensitivities were checked only for cefoperazone sulbactam. In this study cefoperazone sulbactam shows fairly good sensitivity comparing to other group of drugs (sensitivity 46.6%, intermediate sensitive 33.3%, resistant 20%).

The resistance of *Acinetobacter* to beta-lactam antibiotics can be due to basic mechanisms, namely enzyme destruction through beta-lactamases, alterations of target sites and reduced permeability of bacterial walls (14).

The development of resistance to antimicrobials in *Acinetobacter* appears to be unstoppable, when we compare the study reports published over time. Polymyxin B and colistin have demonstrated a reasonable success in controlling MDR *Acinetobacter* in the past, but were used only for selected cases of serious nature due to their higher toxicities. In our study colistin is the only drug that gave 100% sensitivity. Apart from colistin, tigecycline is the only alternative with 85.7% sensitivity against MDR *Acinetobacter* isolates.

Many institutions around the world are faced with the challenging issue of pandrug resistance. Concerns have been raised about the use of tigecycline for *A. baumannii* infection, particularly for bacteraemia, leaving colistin as the only therapeutic option for some.

Conclusion

With the simplified phenotypic identification testing, 30 isolates in the study population belonged to only 5 phenotypes (phenotype numbers 1, 2, 5, 12, 13). Phenotype 2 (*A. baumannii*) is the predominant species (56.66%) followed by Phenotype 1 (*A. calcoaceticus*) 23.33%. A simple identification scheme is useful for laboratories with limited resources for typing *Acinetobacter* isolates. Majority of the isolates were multi-drug resistant (70%).

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CME lecture – 05th December 2014

Effect of silver in the control of infections

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Multidrug resistant pathogens are a global health challenge as few antibiotics have been developed over the last 30 years. The strong antimicrobial property of silver has provided an alternate product that can be used against multidrug resistant organisms. The effect of silver against microbes is diverse. Once silver nanoballs enter into bacteria, they inhibit cell wall synthesis and damage cytoplasmic membrane, resulting in increased permeability and cell death (1). Silver has broad antibacterial property against Methicillin resistant *Staphylococcus aureus*, *Escherichia coli* O157:H7, carbapenem resistant Enterobacteriaceae etc (2). Silver nanoparticles can also act as an antiviral agent by binding with the virus and preventing attachment. Such a broad-spectrum virucidal agent can be also used against HIV (3). Nano-silver can also be an antifungal agent, disrupting cellular membrane and inhibiting the budding process for *Candida* spp, Dermatophytes, *Aspergillus* etc (4). Of benefit is the anti-inflammatory agent of nano-silver whereby altering expression of proinflammatory cytokines can facilitate healing process.

The use of silver in healthcare is extensive. Nanosilver coated medical prosthesis for instance; artificial joints, heart valves, pacemakers, Teflon sleeves and catheters prevent biofilm formation and thereby infection (1). Nanocomposite with nanosilver particles as a surface coating for heart valves and stents has shown anti-thrombogenic and antibacterial properties. Nanosilver incorporated bone cement and endodontic fillings have significant anti-bactericidal effect. Silver impregnated

wound dressings reduce healing time through accelerated reepithelialization. Incorporation of ionizable silver onto hospital curtains, reduce risk of transmission of nosocomial infections (5).

Silver exhibits low toxicity with limited side effects and rare occurrence of microbial resistance.

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BK virus nephropathy: A challenge in renal transplant and laboratory role in patient management

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Background

The clinical syndrome, Polyomavirus - Associated nephropathy (BKVN) in solid organ transplant was first described, in 1995, USA, after nephropathy developed following renal transplant (1). Degree of immunosuppression is a key determinant acquiring disease in transplant population, numerous risk factors; donor/graft determinants, recipient/patient determinants, and post-transplant period factors (2). Viruria detected early post-transplant, precedes BK viremia by a median of 4 weeks. Viremia precedes BKVN by a median of 12 weeks, if no intervention (3).

Laboratory role and patient management

Laboratory goal is screening, early diagnosis and monitoring of treatment response. Clinical intervention often based on the surrogate markers of viral replication, though various tools are available to facilitate screening and monitoring (4). Surveillance techniques and testing frequency is center specific and most protocols recommend pre-determined, periodic quantitative testing of urine and plasma for BK viral load in all patients with renal transplant, using more sensitive screening tool, quantitative real time PCR assay (sensitivity ~ 100%, specificity 75-95%) (2). Pre-determined serial measurements in urine and plasma viral load detects presumptive BKVN, identify patients at risk, before they develop

histologic changes and also useful for monitoring the course of BKVN².

The management focus to clear BK viremia and to stabilize renal function, preventing graft failure; achieved by early diagnosis (screening) and detection of viral clearance while treatment (monitoring) (5). Generally treatment strategies centered on; reduction or modification of immunosuppression, mainstay of treatment and adjunctive use of agents with antiviral activity, limited use and center specific (1,2).

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Use of IV antibiotics in nebulisation – How practical ?

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Hospital acquired pneumonia (HAP) remains the leading cause of death in intensive care units (ICU) with mortality of 30% - 65%. HAP is commonly associated with multi-resistant bacteria (*Acinetobacter* spp, ESBL and Carbapenamase producers, MRSA) with treatment failure, demanding alternative therapeutic options.

Nebulisation of antibiotics in serious respiratory tract infections ensures high antibiotic concentrations at the site of infection, well above the minimum inhibitory concentration (MICs) without causing systemic toxicity. Nebulised antibiotics have been used for the treatment of chronic infection with *Pseudomonas aeruginosa*, particularly in cystic fibrosis.

Aerosol delivery of antibiotics was first reported in the 1940s. Aerosolized antibiotics have been studied as alternative or adjunctive agents to intravenous antibiotics in patients with ventilator-associated pneumonia (VAP). It has shown favourable results as adjunctive treatment in treating resistant microorganisms.

Antibiotics currently marketed for inhalation include nebulised and dry powder forms of tobramycin, colistin and aztreonam.

But injectable formulations of gentamicin, tobramycin, amikacin, ceftazidime, meropenem, piperacillin-tazobactam, vancomycin, colistin and amphotericin are currently nebulized “off-label”.

Adverse effects of inhaled antibiotics fall into three categories:

- Local - with transient broncho-constriction due to osmolality and preservatives within some of the solutions

- Systemic - in the setting of diminished glomerular filtration rate resulting systemic drug accumulation
- Emergence of antibiotic resistant organisms

When first dose of a nebulised antimicrobial (test dose) given, broncho-constriction should be excluded by pre and post inhalation spirometry and chest auscultation.

Antibiotic nebulisation has been practiced in ICUs of Teaching Hospitals Kandy, Batticaloa and District General Hospital Nuwara-Eliya with very good outcome.

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The Bulletin of the Sri Lanka College of Microbiologists

The Bulletin of the Sri Lanka College of Microbiologists is the annual publication of the Sri Lanka College of Microbiologists issued along with the Annual Scientific Sessions of the College. The *Bulletin* includes the summaries of the speeches/lectures/symposia and abstracts of oral/ poster presentations to be made during the Annual Scientific Sessions in addition to review/ research articles and case reports relevant to microbiology and infectious diseases sent by the membership. The aims of the *Bulletin* are to encourage the membership to conduct and publish good quality research to support and improve the practice of microbiology in Sri Lanka and to share experiences to enrich and upgrade the professional standards.

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

TYPES OF CONTRIBUTIONS

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

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.

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These articles should be limited to 1500 words and 12 references. The *Bulletin* will give priority to articles dealing with topics of interest and importance in microbiology and infectious diseases in Sri Lanka.

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These should not exceed 750 words and 5 references and should be structured as Introduction, Case report and Discussion. Abstract is not required. Editorial board will be paying attention to the significance of the case report to the practice of microbiology in Sri Lanka.

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

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 - that the contents have not been published or accepted for publication elsewhere
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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:

1. Conceived and planned the work that led to the paper, or interpreted the evidence it presents, or both.

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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.

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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.

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