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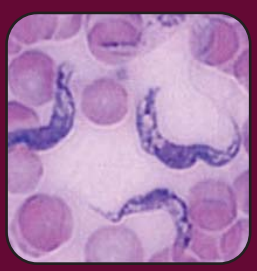

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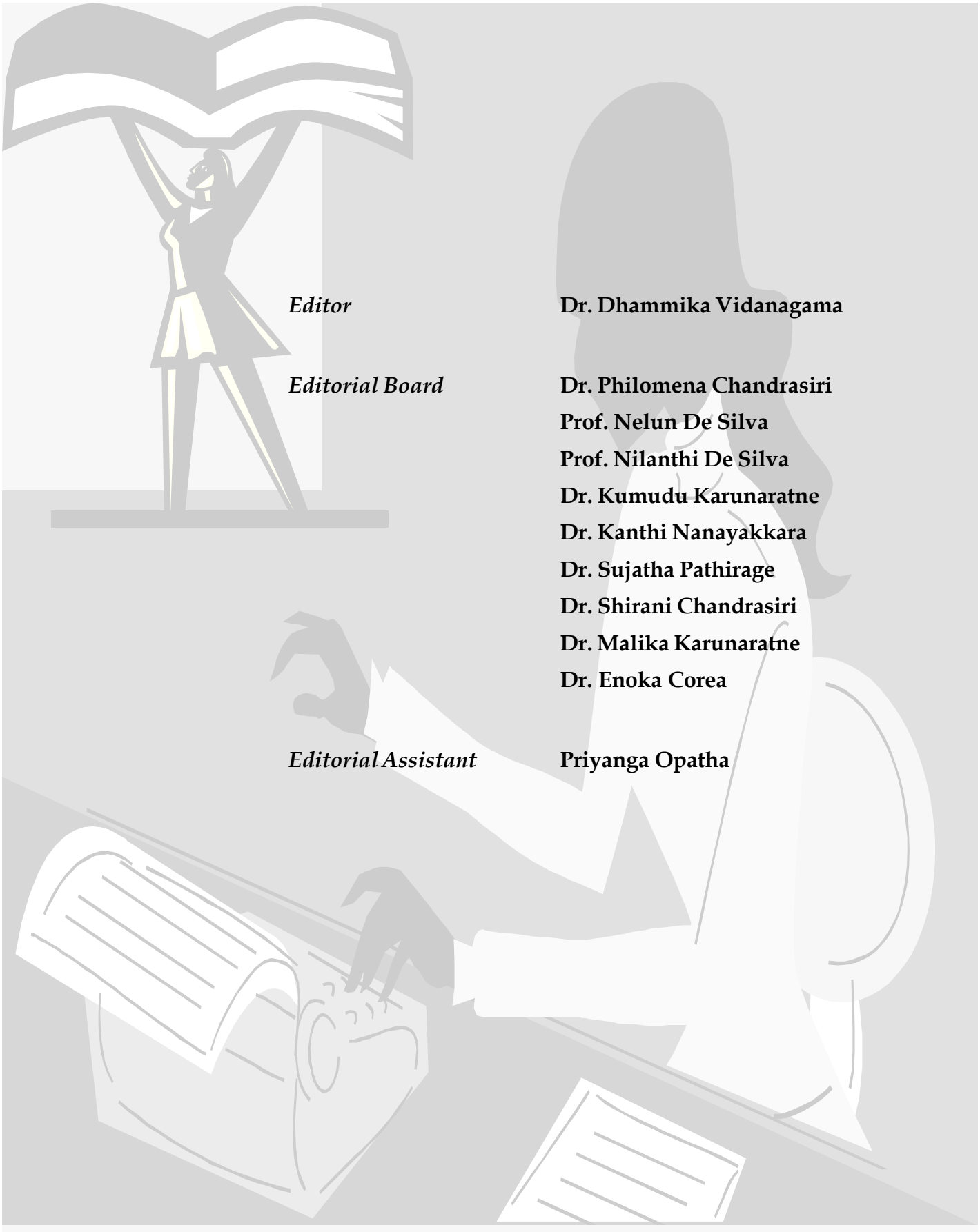

The Sri Lanka College of Microbiologists

Council 2011/2012

Seated (L-R) : Dr. Geethani Galagoda (Hony. Treasurer), Dr. Kumudu Karunaratne (Vice President), Dr. Shirani Chandrasiri (Hony. Secretary), Dr. Philomena Chandrasiri (President), Dr. Malika Karunaratne (Hony Secretary), Dr. Sunethra Gunasena (President Elect), Dr. Dhammika Vidanagama (Editor)

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MESSAGE FROM THE PRESIDENT

It is with great pleasure that I address you through the columns of this Bulletin to mark the Annual Scientific Sessions of the Sri Lanka College of Microbiologists. I warmly welcome all participants, resource personnel and researchers who are presenting their valuable work at our 21st Annual Academic Sessions. I am proud to say that this year, we are fortunate to have six international speakers to grace the sessions.

This year, this event will see us combining two annual activities of our College – the inaugaration of Annual Academic Sessions and the Siri Wickremesinghe Oration.

In retrospect, I am happy to say that we have achieved many of the targets that we set ourselves, at the beginning of the academic year. We were able to work in step with our theme of the year – "Ensuring patients' safety through infection control".

Let me now dwell briefly on some of the highlights of our efforts during the past year.

Infection control and patients' safety have become important issues in health care systems around the world. The incidence of hospital acquired infections has become a major threat to patients' well being and safety. As a developing country with very limited resources, Sri Lanka also faces this problem and it has become a major economic burden for our healthcare system. During the past year, our College has attempted to address these issues through training and awareness imparted via several workshops conducted amongst different categories of staff in hospitals. The College was able to conduct several workshops for paramedical staff on infection control. In the same vein, infection control nurses are on the front lines of the war against infection in any hospital. Upgrading their knowledge is thus vital to improve infection control in hospitals. Addressing this fact, the College conducted a four day refresher course for infection control for nurses serving throughout the country, the cost of which we were able to meet with financial assistance from the World Health Organization.

The emergence of multi-drug resistant organisms is gradually increasing in our hospitals. Overuse and/or misuse of antibiotics have lead to the grave problem we are facing today. Lack of knowledge about resistance patterns and inadequacies in our policy governing the use of antibiotics have also contributed to this growing problem. Towards mitigating the issue, we were able to conduct several workshops in different provinces for medical officers on the rational use of antibiotics. Two of these workshops were organized in collaboration with the College of Anesthesiologists and the Kandy Society of Medicine. The College has taken the initiative to develop guidelines and a policy for Sri Lanka, to streamline the proper application and use of antibiotics nationwide. The Antibiotic Resistance Surveillance Project that had been started in 2008 is still ongoing and we were able to initiate the second stage of the project this year. I would like to extend my gratitude to M/s GlaxoSmithkline for the valuable financial assistance they extended towards this important activity. The first report on the project was handed over to the Ministry of Health whilst the first article on the subject was also submitted for publication.

Laboratory support is essential to obtain reliable data on the subject of antibiotic resistance. In this regard, we conducted several workshops for medical laboratory technologists in different provinces with the collaboration of the Ministry of Health.

The private sector hospitals in the country are large scale contributors to Sri Lanka's healthcare system. In order to improve the quality of their laboratory work we were able to conduct a 'hands on experience' workshop for MLTT in the private sector.

All these activities would not have been feasible if not for the untiring commitment of the members of the College. I take this opportunity to thank all members who helped me to carry out all these activities successfully.

The Infection Control Manual that has been published by the College in 2004 needs revision. We plan to commence its revision on conclusion of the Annual Academic Sessions.

The monthly CME activity is a popular event among our membership. This year we were able to conduct nine CME lectures to date. We were fortunate to be able to welcome a few speakers from overseas to deliver some of these lectures.

An event of this magnitude is not feasible without the support of our sponsors. We thank all our sponsors for their invaluable support and contribution. Special thanks go to Astra Zeneca Company for their valuable contribution.

I would like to express my gratitude to all the council members for the encouragement and support given to me during this academic year. I thank the two secretaries for the commitment and dedication they have shown towards all our activities. My sincere thanks go to the secretary of the college office for her excellent work capacity and commitment.

Together with my council, I wish to thank everyone who has participated and contributed in different ways to ensure the success of this event and also for gracing our sessions with your valuable presence.

Dr. Philomena Chandrasiri
President
Sri Lanka College of Microbiologists

21st Annual Scientific Sessions
and
International Conference on Infectious Diseases

The Sri Lanka College of Microbiologists

Inauguration

28th August 2012 at 6.15 pm

Cinnamon Lakeside Hotel

Colombo

Pre-congress workshop

"Ensuring patient safety through infection control"

28th August 2012 from 8.30 am to 12.30 pm

Scientific Programme

– Theme –

"Difficult to treat infections – moving beyond headlines to clinical solutions"

29th & 30th August 2012

Sri Lanka Foundation Institute, Colombo 7

INAUGURATION PROGRAMME

6.15 pm	Invitees take their seats
6.30 pm	Arrival of the Chief Guest Introduction of Members of the Council
6.35 pm	Ceremonial Procession
6.40 pm	National Anthem
6.45 pm	Traditional lighting of the oil lamp
6.50 pm	Welcome Address Dr. Shirani Chandrasiri *Hony. Joint Secretary*
6.55 pm	Address by the Chief Guest Prof. Sriyal Malik Peiris *Professor of Virology, University of Hong Kong*
7.15 pm	Address by the President Dr. Philomena Chandrasiri *Consultant Microbiologist, NHSL*
7.45 pm	Introduction of the Orator of Siri Wickremesinghe Memorial Oration *Dr. Philomena Chandrasiri, President, SLCM*
7.50 pm	**Siri Wickremesinghe Memorial Oration** Dr. Sujatha Mananwatte *Consultant Microbiologist, NSACP*
8.20 pm	Presentation of Siri Wickremesinghe Memorial Award
8.25 pm	Vote of Thanks Dr. Malika Karunaratna *Hony. Joint Secretary*
8.30 pm	Cultural show
8.45 pm	Ceremonial procession leaves
8.50 pm	Reception

21st Annual Scientific Sessions of

The Sri Lanka College of Microbiologists

28th August 2012

Sri Lanka Foundation Institute, Colombo 7.

- Theme -

"Ensuring Patient Safety Through Infection Control"

8.00 am - 8.30 am	**Registration**
8.30 am - 9.30 am	**Bundled strategies in infection control** Dr. W. H. Seto *Director, WHO Collaborating Centre for Infection Control, Hospital Authority, Hong Kong and Senior Advisor (Q&S) Queen Mary Hospital and Hong Kong West Cluster, Hospital Authority (HCWC), Hong Kong*
9.30 am - 10.10 am	**Aerosol transmission of infection and ventilation control in surgical theatres** Dr. Varuna Navaratne *Senior Lecturer, Faculty of Medicine, General Sir John Kothalawala Defence University, Kandawala Estate, Ratmalana*
10.10 am - 10.30 am	Tea
10.30 am - 11.30 am	**Comprehensive approach to infection control in a neonatal care unit** Prof. Alison Kesson *Department Head, Diagnostic Services / Infectious Diseases & Microbiology, Children's Hospital, Westmead, Australia*
11.30 am - 12.10 pm	**Challenges in the prevention of HAI in Sri Lanka** Dr. Kumudu Karunaratne *Consultant Microbiologist, Lady Ridgeway Hospital, Colombo 8*
12.10 am - 12.40 pm	**Best practices in sterilization assurance** Dr. Norman Lu, M.D. *Consultant Anaesthetist* *Singapore*

Chairpersons: Prof. Nelun de Silva and Dr. Pranitha Somaratne

21st Annual Scientific Sessions
and
International Conference on Infectious Diseases

The Sri Lanka College of Microbiologists

29th & 30th August 2012

Sri Lanka Foundation Institiute, Colombo 7.

- Theme -

"Difficult To Treat Infections – Moving Beyond Headlines To Clinical Solutions"

Day 1 - 29th August 2012

800-8.30 am **Registration**

8.30-9.00 am **Free Paper Session 1**

Chairpersons: Prof. Neluka Fernando and Dr. Renuka Fernando

OP 1 Evaluation of knowledge on infection control among health care workers at Base Hospital, Angoda
Wadanamby JMRWW, Caldera TSKRD, Kanchana IDKU
Base Hospital, Angoda

OP 2 Establishment of a molecular diagnostic method to determine the aetiology of meningo-encephalitis
Danthanarayana NS[1], Thevanesam V[2], Galagoda GC[1], Fernando L[3]
[1]Department of Virology, Medical Research Institute, Colombo, [2]Department of Microbiology, Faculty of Medicine, University of Peradeniya, [3]District General Hospital, Gampaha

9.00-9.45 am **Plenary 1**

Chairperson: Prof. Vasanthi Thevanesam

Emerging viral infections
Prof. Sriyal Malik Peiris
Director, Centre of Influenza Research, Professor, Chair in Virology, School of Public Health, The University of Hong Kong and Honorary Consultant Microbiologist, Queen Mary Hospital, Pokfulam, Hong Kong SAR.

9.45-10.00 am Tea

10.00-11.00 am **Free Paper Session 2**

Chairpersons: Dr. Ajith Nagahawatta and Dr. Sujatha Pathirage

OP 3 An outbreak caused by *Pantoea* species in a neonatal intensive care unit
Senanayake NP[1], Thevanesam V[2], Karunanayake L[1]
[1]Teaching Hospital, Kandy, [2] Faculty of Medicine, Peradeniya

OP 4 Group B *Streptococcal* colonization in pregnancy
Herath GC, Dissanayake BN, Gamage TM
Department of Microbiology, Faculty of Medicine, University of Peradeniya, Peradeniya

OP 5 Surveillance on antibiotic resistance and hospital acquired infections in Cardiothoracic Surgical Intensive Care Units at Lady Ridgeway Children's Hospital, Sri Lanka
Udugama SG, Karunaratne GKD, Azmy FH
Department of Microbiology, Lady Ridgeway Children's Hospital, Colombo

OP 6 Hospital based study of invasive *Haemophilus influenzae* disease over a four year period at the Lady Ridgeway Hospital
Karunaratne GKD[1], Kathriarachchi K[1], Corea EM[2]
[1]Lady Ridgeway Hospital, Colombo, [2]Faculty of Medicine, University of Colombo

11.00-12.00 noon **Symposium 1 – Difficult to treat infections**

Moderators: Prof. Nadeera Karunaweera and Dr. Geethika Patabendige

- The management of febrile neutropenia
  Prof. Christopher Kibbler
  Professor of Medical Microbiology, Centre for Clinical Microbiology, University College London and Department of Medical Microbiology, Royal Free Hampstead NHS Trust, Pond Street, London

- Parasitic infections – challenges in treatment
  Prof. Nilanthi De Silva
  Professor of Parasitology, Faculty of Medicine, University of Kelaniya

12.00-12.45 pm **Plenary 2**

Chairperson: Dr. Kumudu Karunaratne

Rational antibiotic use in paediatric patients
Prof. Alison Kesson
Department Head, Diagnostic Services/Infectious Diseases and Microbiology, Children's Hospital, Westmead, Australia

12.45-1.45 pm Lunch

1.45-2.45 pm **Symposium 2 – Respiratory tract infections "beyond the obvious"**

Moderators: Prof. Nilanthi De Silva and Dr. Sepali Gunawardena

- Respiratory manifestations in primary immune deficiency
  Dr. Rajiva de Silva
  Consultant Immunologist, Medical Research Institute, Colombo

- Management standards of pneumonia
  Dr. W. H. Seto
  Director, WHO Collaborating Centre for Infection Control, Hospital Authority, Hong Kong, Sr. Advisor (Q&S), Queen Mary Hospital and Hong Kong West Cluster, Hospital Authority (HCWC), Hong Kong

2.45-3.30pm **Plenary 3**

Chairperson: Dr. Preethi Perera

Fungal infection in the ICU: prevaricate, medicate or extrapolate?
Prof. Christopher Kibbler
Professor of Medical Microbiology, Centre for Clinical Microbiology, University College London and Department of Medical Microbiology, Royal Free Hampstead NHS Trust, Pond Street, London

3.30-4.15pm **Free Paper Session 3**

Chairpersons: Prof. N. P. Sunil Chandra and Dr. Mahen Kothalawala

OP 7 Phenotypic detection of plasmid mediated *AmpC* beta-lactamses in *Escherichia coli* using Boronic acid as a reversible inhibitor of *AmpC*
Fernando R[1], *AndresenD*[2], *Kesson A*[2]
[1]District General Hosptal, Chilaw, [2]The Children's Hospital, Westmead, Australia

OP 8 Molecular diagnosis as an early and rapid diagnostic method for leptospirosis
Mubarak FN[1], *Thevanesam V*[1], *Agampodi SB*[2]
[1]Department of Microbiology, Faculty of Medicine, University of Peradeniya, [2]Department of Community Medicine, Faculty of Medicine, University of Peradeniya

OP 9 A study to compare genus specific MAT with genus specific PCR and culture for diagnosis of leptospirosis in clinically suspected patients
Welikumbura RS[1], *Somaratne P*[2], *Perera R*[2], *Ramesh R*[2]
[1]Postgraduate Institute of Medicine, [2] Medical Research Institute.

4.15pm Tea

Day 2 – 30th August 2012

8.00-9.00 am **Free Paper Session 4**

Chairpersons: Dr. Kanthi Nanayakkara and Dr. Kushlani Jayathilleke

OP 10 Genotypes of hepatitis C virus in thalassaemic patients and patients who undergo long-term haemodialysis
Jayamaha CJS[1], *Senevirathna AMDB*[2], *Senanayake CP*[3]
[1]Medical Research Institute, Colombo, [2]Genetech Molecular Diagnostics and School of Gene Technology, [3]Department of Microbiology, Faculty of Medicine, University of Colombo

OP 11 Establishment of polymerase chain reaction assay to detect active cytomegalovirus replication in the blood of renal transplant recipients
Nadeeka JS, Wickramasinghe GA
Medical Research Institute, Colombo

OP 12 Seroprevalence of herpes simplex virus type 2 infection among female sex workers attending central Sexually Transmitted Diseases (STD) clinic at National STD and AIDS Control Program (NSACP), Colombo
Nakkawita WMID[1], Mananwatte S[2], Galagoda GCS[1], Kulatunga GGAK[3]
[1]Medical Research Institute, Colombo, [2]National STD and AIDS Control Program, Colombo, [3]Postgraduate Institute of Medicine, Colombo

OP 13 Molecular surveillance of dengue serotype – May 2010 to March 2012
Gunasena S[2], Jayawardana BDS[1], Perera ML[1], Fernando WCGJ[2], Nawarathna IDK[2], Rathnayake RMDTK[2], Ramesh R[1], Abeynayake JI[2], Paranawidana T[2]
[1]Department of Molecular Biology, Medical Research Institute, [2]Department of Virology, Medical Research Institute

9.00-9.45am **Plenary 4**
Chairperson: Dr. Malika Karunaratne

Outcomes and reduction in mortality among patients with community-acquired bacteraemic infections
Dr. Mark Melzer
Consultant in Infection, Department of Infection, Barts Health Care, Whitechapel, London

9.45-10.00am Tea

10.00-10.45 am **Free Paper Session 5**
Chairpersons: Dr. Ranjith Perera and Dr. Roshan Jayasuriya

OP 14 Aetiological agents of ophthalmic infections
Chandrasiri P[1], Mudannayake MVSK[2], Navaratne H[2], Perera PD[3]
[1]National Hospital of Sri Lanka, [2]Eye Hospital Colombo, [3]Medical Research Institute, Colombo

OP 15 Prevalence of enterobiasis among primary school children in Ragama MOH region
Gunawardena NK, Chandrasena TGAN, Senarathna BP, Silva GMKS, De Silva NR
Department of Parasitology, Faculty of Medicine, University of Kelaniya

OP 16 Incidence of sensorineural hearing loss due to congenital cytomegalovirus infection and CMV DNA in urine as a disease marker
Jayamaha J[1,2], Hall B[1,2], Wilkinson M[3], Palasanthiran P[3], Rawlinson W[1,2,4]
[1]Virology Division, Department of Microbiology, South Eastern Area Laboratory, [2]Virology Research Laboratories, University of New South Wales, Kensington, Sydney, Australia, [3]Sydney Children's Hospital, Randwick, Australia, [4]School of Medical Sciences, Faculty of Medicine, University of New South Wales, Sydney, Australia

10.45-11.30 am **Plenary 5**

Chairperson: Prof. Jennifer Perera

Management of infections in stem cell transplant recipients
Prof. P. H. Chadrasekerar
Department of Internal Medicine, Division of Infectious Diseases, Harper University Hospital, USA

11.30-12.30 pm **Symposium 3 – New trends in the management of infections**

Moderators: Dr. Sujatha Mananwatte and Dr. Geethani Galagoda

- Modern approaches in wound bed preparation – what microbiologists should know?
  Prof. Mandika Wijeratne
  Profesor of Surgery, Faculty of Medicine, University of Colombo

- Bio-film associated infections
  Dr. Shirani Chandrasiri
  Consultant Microbiologist, Colombo South Teaching Hospital, Kalubowila

12.30-1.30 pm Lunch

1.30-2.00 pm **Plenary 6**

Chairperson: Dr. Jayanthi Elwitigala

Diagnosis of TB – new developments
Dr. Mark Melzer
Consultant in Infection, Department of Infection, Barts Health Care, Whitechapel, London

2.00-2.45 pm **Plenary 7**

Chairperson: Dr. Omala Wimalaratne

New insight into infectious diseases in Sri Lanka
Prof. Vasanthi Thevanesam
Professor of Microbiology, Faculty of Medicine, University of Peradeniya

2.45- 3.15pm **Interactive Session**

Chairperson: Dr. Dhammika Vidanagama

Dr. Kushlani Jayathilleke
Consultant Microbiologist, Sri Jayawardenapura General Hospita, Nugegoda

Dr Malika Karunaratne
Consultant Microbiologist, Teaching Hospital, Anuradhapura

3.15-3.45pm **Award ceremony**

3.45pm Tea

LIST OF GUEST SPEAKERS

Prof. Sriyal Malik Peiris
Director, Centre of Influenza Research, Professor, Chair in Virology, School of Public Health, The University of Hong Kong and Honorary Consultant Microbiologist, Queen Mary Hospital, Pokfulam, Hong Kong SAR

Prof. Alison Kesson
Department Head, Diagnostic Services/ Infectious Diseases and Microbiology, Children's Hospital, Westmead, Australia

Dr. W. H. Seto
Director, WHO Collaborating Centre for Infection Control, Hospital Authority, Hong Kong and Sr. Advisor (Q&S), Queen Mary Hospital and Hong Kong West Cluster, Hospital Authority (HCWC), Hong Kong

Prof. Christopher Kibbler
Professor of Medical Microbiology, Centre for Clinical Microbiology, University College London and Department of Medical Microbiology, Royal Free Hampstead NHS Trust, Pond Street, London

Prof. P. H. Chadrasekerar
Department of Internal Medicine, Division of Infectious Diseases, Harper University Hospital, USA

Dr. Mark Melzer
Consultant in Infection, Department of Infection, Barts Health Care, Whitechapel, London

Dr. Norman Lu, M.D.
Consultant Anaesthetist, Singapore

Prof. Mandika Wijeratne
Professor of Surgery, Faculty of Medicine, University of Colombo

Prof. V. Thevanesam
Professor of Microbiology, Faculty of Medicine, University of Peradeniya

Prof. Nilanthi De Silva
Professor of Parasitology, Faculty of Medicine, University of Kelaniya

Dr. Kumudu Karunaratne
Consultant Microbiologist, Lady Ridgeway Hospital, Colombo 8

Dr. Rajiva de Silva
Consultant Immunologist, Medical Research Institute, Colombo 8

Dr Kushlani Jayathilleke
Consultant Microbiologist, Sri Jayawardenapura General Hospital, Nugegoda

Dr. Varuna Navaratne
Senior Lecturer, Faculty of Medicine, General Sir John Kothalawalawa Defence University, Kandawala Estate, Ratmalana

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

Dr. Malika Karunaratne
Consultant Microbiologist, Teaching Hospital, Anuradhapura

OP1

Evaluation of knowledge on infection control among health care workers at Base Hospital, Angoda

Wadanamby JMRWW[1], Caldera TSKRD[1], Kanchana IDKU[1]

[1]Base Hospital, Angoda

Objective

To assess knowledge on infection control in health care workers at Base Hospital, Angoda and to assess the availability of resources for hand washing.

Design, setting and methods

All categories of workers who directly interact with patients or contaminated material were included in the study. A self administered structured questionnaire was given. Knowledge on infection control measures was assessed and given a score out of 100. Perception on adequacy of knowledge and availability of resources for hand washing was also assessed. The actual availability of resources for hand washing in the hospital in the meantime was assessed by the infection control nurse.

Results

Twenty doctors [response rate (RR)=64.5 %], 40 nurses (RR=54.0%), 13 paramedics (RR= 92.8%), and 45 assisting staff members (RR=39.8%) participated. All the doctors, 39 nursing officers (97.5%),12 paramedics (92.3%) and 38 assisting staff members (84.4%) perceived that they had average or better knowledge. When knowledge on infection control was assessed doctors had a mean score of 81.6% (SD=16.5), nurses 80.4%(SD=12.1), paramedics 75.5% (SD=13.1) and assisting staff 68.7% (SD=13.6). The proper techniques of hand washing was not known by 60% of doctors, 52.5% of nurses, 84.6% of paramedics and 53.3% of assisting staff members. Seventy percent of doctors, 55% of nurses, 38.5% of paramedics and 24.4% of assisting staff members stated that hand washing resources were not always available in the work place. When the actual availability of resources for hand washing was assessed, all the wards and other units in the hospital had at least 1 sink. Five out of the 10 wards (50%) had 2 sinks while in the ICU there were 5. Soap was available in all units. Hand towels were available in 10 out of the 15 units (66.7%) and bins for disposal of towels were available in 14 out of 15 units (93.3%). When the need for further knowledge was assessed, 40% of doctors thought they had inadequate knowledge on disinfectants/autoclaving and 35% on MRSA. Out of the nurses 35% needed further learning on disinfectants and autoclaving and 30% on immunisation. Fourty seven percent and 42% of assisting staff members thought they needed further learning on communicable diseases and waste management.

Conclusion

Knowledge on infection control needs to be improved in health care workers of all categories in the hospital. Proper hand washing technique should be taught and resources for hand washing made available in all areas of the hospital constantly. Training programmes need to emphasize the practice of disinfection and sterilization, immunisation, waste management and control of MRSA and other communicable diseases.

OP 2

Establishment of a molecular diagnostic method to determine the aetiology of meningo-encephalitis

Danthanarayana NS[1], Thevanesam V[2], Galagoda GC[1], Fernando L[3]

[1]Department of Virology, Medical Research Institute, Colombo 8, [2]Department of Microbiology, Faculty of Medicine, University of Peradeniya, [3]District General Hospital, Gampaha

Introduction

Meningo-encephalitis is a potentially life threatening inflammation of the brain. Published data on the prevalence or incidence of individual encephalitides and studies done using molecular diagnostic methods in Sri Lanka are scarce.

Objectives

- Establish a molecular diagnostic method for the aetiological diagnosis of meningo-encephalitis among patients in selected hospitals in the Colombo District.
- Determine the proportion of Herpes simplex virus (HSV), *Haemophilus influenzae*, *Streptococcus pneumoniae* and *Neisseria meningitidis* in the study population.

Design, setting and methods

A hospital based cross sectional descriptive study was carried out in selected wards at National Hospital Sri Lanka (NHSL), Lady Ridgeway Children's Hospital (LRH), Colombo and District General Hospital, Gampaha (DGHG).

51 CSF samples (21 adult and 30 paediatric) were collected from patients with fever and at least one of the signs of cerebral involvement with or without signs of meningitis between 1st of January 2010 and 30th of April 2010.

0.5 ml CSF was collected in sterile containers within 7 days of onset and stored at -70°C until tested. All samples were subjected to a nested PCR to detect HSV and 16 samples with a bacterial count in the CSF full report were subjected to a multiplex bacterial PCR to detect *S. pneumoniae, H. influenzae* and *N. meningitidis.*

Results

Nested PCR for HSV for 51 CSF samples did not show any positive results. In the multiplex bacterial PCR for S*. pneumoniae*, *H. influenzae* and *N. meningitidis* for the 16 CSF samples, *H. influenzae* was detected in a single paediatric patient.

Conclusions

False negative results would have occurred due to low CSF volume, multiple freeze-thaw cycles for CSF and economical but less sensitive nucleic acid extraction methods. Extensive studies representing the entire country to determine the aetiology and epidemiology of meningo-encephalitis in Sri Lanka are urgently needed.

Acknowledgement

Ministry of Health Care and Nutrition is acknowledged for the financial assistance.

OP 3

An outbreak caused by *Pantoea* species in a neonatal intensive care unit

Senanayake NP[1], Thevanesam V[2], Karunanayake L[1]

[1]Teaching Hospital Kandy, [2]Faculty of Medicine, University of Peradeniya

Introduction

Pantoea species are Gram negative opportunistic pathogens isolated from faeculent material, plants and soil, where they can be either pathogens or commensals. We report the world's largest known outbreak of blood stream infection (BSI) caused by *Pantoea* species in a neonatal intensive care unit (NICU) at a tertiary care hospital.

Objective

To investigate an outbreak of BSI in a NICU.

Design, setting and methods

An outbreak of BSI at the NICU was investigated during the period 5th to 30th March 2010.

Blood cultures were taken on admission to the NICU and 2-3 days later on the clinical suspicion of BSI. The isolates were identified by Gram staining, colony characteristics and the findings were confirmed using the API 20E system. Antibiotic susceptibility tests were carried out using the Clinical Laboratory Standards Institute (CLSI) guidelines. Environmental screening cultures were carried out to identify the source of the outbreak. The environmental samples included samples from intravenous fluids, intravenous drugs, cleansing solutions, oxygen humidifiers, sterile water, incubators, cots, mattresses, ventilation masks, water taps, sinks, door handles and other instruments.

Results

The neonatal intensive care unit (NICU) experienced an outbreak of 14 cases of BSIs during this period. Of the 14 neonates with BSIs 12 were preterm and two were term babies. Although the initial blood cultures were negative, the subsequent samples were positive for *Pantoea* species. All the isolates were susceptible to cefotaxime, ceftazidime, ceftriaxone, ciprofloxacin, gentamicin, amikacin, netilmicin, imipenem and meropenem. None of the environmental samples were positive for *Pantoea* species.

Nine of the 14 patients responded to treatment with appropriate antibiotics while five neonates died due to sepsis.

Conclusion

Pantoea species are unusual pathogens in the aetiology of neonatal sepsis. There are few reports of systemic infection with this organism in preterm neonates.

We conclude that *Pantoea* species was responsible for the outbreak, although the source was not established.

OP 4

Group B Streptococcal colonization in pregnancy

Herath GC[1]*, Dissanayake BN*[1]*, Gamage TM*[1]

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

Introduction

Group B Streptococci (GBS) can colonize the female genital tract and it can spread to the neonate causing early onset and late onset infections. Identification of the GBS colonization is important in prevention of infections. No recent studies have been published in Sri Lanka on GBS colonization in pregnant women.

Objectives

1. To find out the prevalence of Group B Streptococcus colonization in pregnant women.
2. To compare the GBS vaginal colonization rate with the rectal colonization, in pregnancy.

Method

This study was carried out from August to November 2011. Lower vaginal and rectal swabs were collected separately from pregnant women with gestational age of 35 to 37 weeks who were attending the Obstetric Clinics at Teaching Hospital Peradeniya. Swabs were enriched separately using Todd Hewitt broth supplemented with antibiotics and incubated at 35-37°C for 18-24 hours. Enriched broth culture was plated on blood agar and suspected colonies were identified with Gram stain, catalase and Lancefield's grouping.

Result

GBS colonization in pregnancy in the study samples was 30%. From 20 out of 100 pregnant mothers GBS were isolated from both vaginal and rectal swabs. In 4 mothers GBS were isolated only from vaginal swabs and from 6 mothers only from rectal swabs. Rectal GBS colonization was 26% and it was higher than the vaginal colonization rate, which was 24%.

Conclusion

This implies the need of routine GBS screening in pregnancy in the study population. It is important to collect both vaginal and rectal swabs for GBS screening in pregnancy.

OP 5

Surveillance on antibiotic resistance and hospital acquired infections in cardio-thoracic surgical intensive care units at Lady Ridgeway Children's Hospital, Sri Lanka

Udugama SG[1]*, Karunaratne GKD*[1]*, Azmy FH*[1]

[1]Department of Microbiology, Lady Ridgeway Children's Hospital, Colombo

Objectives

1. To detect the incidence of hospital acquired infections (HAI), the device associated infections (DAI) and device utilization ratios.
2. To determine the microbial colonization rates and the antibiotic sensitivity patterns of the bacterial isolates.

Methodology

A prospective descriptive study was conducted at cardiothoracic surgical intensive care units (ICU) at LRH from November 2011 to March 2012. Patients with ICU stay beyond 48 hours were included and followed up postoperatively for one month for surgical site infections (SSI).

Results

207 patients were studied for 1525 patient-days in the ICU. 84 HAIs and 26 DAIs, with an overall rate of 40.6% and 55.1 HAI per 1000 ICU days. Out of all 198 patients underwent surgeries, 24 patients had post-operative open chest and the majority underwent secondary suturing by day 3 following surgery. 82 (38%) cases were hospitalized for beyond five days before the ICU admission.

21 (10.1%) direct blood stream infections (BSI), 15 (7.2%) catheter related blood stream infections (CRBSI), 8 (3.9%) ventilator associated pneumonia (VAP), 32 (15.5%) health care associated pneumonia (HCAP), 3 (1.5%) catheter associated urinary tract infections (CA-UTI) and 5 (2.4%) SSIs were diagnosed during the ICU stay. 6 (2.9%) SSIs were detected at the follow up. CRBSI and central-vascular catheter colonization rates per 1000 central-vascular catheter days were 7.6 and 14.8 respectively. VAP rate per 1000 mechanical ventilator days was 10.8 and 5.3 per 1000 ICU days. CA-UTI rate per 1000 urinary catheter days was 4.2 and 1.9 per 1000 ICU days. Central-vascular catheter utilization ratio was 1.3. Mechanical ventilator and urinary catheter utilization ratios were 0.46 each.

Of 74 positive blood culture isolates, 40 were coliforms (60% ESBL). From 305 respiratory isolates 162 coliforms (28% ESBL), 38 *Acinetobacter* and 37 *Pseudomonas* species were identified. Overall, Coliforms and *Pseudomonas* species showed higher sensitivity for carbapenems and amikacin, while *Acinetobacte*r showed higher resistance for carbapenems. Coliforms and *Acinetobacter* species were resistant to most β-Lactams.

Conclusions

High rates of HCAP, blood stream infections, intravascular catheter and respiratory tract colonization were observed. High percentage of ESBLs increased the requirement of carbapenems.

OP 6

Hospital based study of invasive *Haemophilus influenzae* disease over a four year period at the Lady Ridgeway Hospital

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Objective

To describe clinical, microbiological and demographic features of invasive *Haemophilus influenzae* (Hib) disease.

Method

A prospective study of microbiological culture confirmed patients with invasive *Haemophilus influenzae* disease admitted to the Lady Ridgeway Hospital of Colombo from 2008 to 2011 was carried out.

Data on clinical diagnosis, culture results of blood, cerebrospinal and fluid from other sterile sites, antibiotic susceptibility pattern, disease outcome and other demographic data were obtained.

Results

A total number of 58 culture positive were identified in 54 patients during the period of 4 years from 2008. 33 (61.1%) were male and 21 (38.9%) were females.

Number of isolates in each year from 2008 was 16, 29, 06 and 07 respectively. 54 (93.1%) were isolated from blood cultures, 3 (5.2%) from CSF and 1 (1.7%) from a joint aspirate. 21 patients (38.8%) were infants and 24 (44.5%) were between >1-5 years.

Thirty three patients (61.1%) were clinically diagnosed as meningitis, 15 (27.7%) had sepsis, 3 (5.6%) had septic arthritis and 3 (5.6%) had pneumonia. Out of them 51 (94.4%) were not vaccinated and 2 had taken 1 dose and 1 had taken 3 doses of vaccine. Complications were noted among 10 (18.5%) patients and mortality was 1 (1.8%).

CSF was tested in all meningitis patients and 3 had positive cultures. Lumbar puncture (LP) was done prior to antibiotic therapy only in these 3 patients. All culture negatives were on antibiotics. Out of culture negatives, antigens were tested in 24. In 17(70.8%) Hib antigen was positive. Amongst the antigen positives, longest duration of LP done was after 4 days of antibiotics.

Sensitivity of isolates for cefotaxime was 94.6% (53/56), chloramphenicol 87.0% (47/54) and ciprofloxacin 100% (51/51). 40.8% (20/49) were beta-lactamase producers.

Conclusion

A reduction in microbiologically confirmed cases was noted after the introduction of Hib vaccine in 2008 in national immunization schedule and restarting in February 2010 after a brief suspension. 98.1% of patients who had disease were not fully vaccinated. Meningitis is the commonest clinical presentation. Prior antibiotic therapy has a major effect in CSF culture isolation. Ceftriaxone or cefotaxime can be recommended for empirical therapy.

OP 7

Phenotypic detection of plasmid mediated *AmpC* beta-lactamases in *Escherichia coli* using Boronic acid as a reversible inhibitor of *AmpC*

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Background

Plasmid mediated *AmpC* beta-lactamases are most commonly produced by *Escherichia coli* and *Klebsiella species*. They confer resistance to beta-lactam antibiotics except to cefepime and carbapenems. Reduced susceptibility to cefoxitin but retained susceptibility to cefepime may be an indicator of *AmpC* beta-lactamases. Boronic acid (BA) compounds are reversible inhibitors of *AmpC* enzymes which may be useful for confirmation analogous to inhibition by clavulanate for ESBL confirmation. Currently no recommended confirmatory detection

method is available. Therefore failure in accurate detection of *AmpC* producing organisms has contributed to their uncontrolled spread and even to therapeutic failures.

Objective

To find a simple, sensitive, easy to perform test for phenotypic detection of plasmid mediated *AmpC* in a routine microbiology laboratory.

Method

This study was carried out at the Children's Hospital, Westmead, Australia for 3 months from September in 2008. Sequential clinical isolates of *E. coli* which showed resistance to cefoxitin and susceptibility to cefepime by VITEK2 were included in the study. Cefotetan, cefotaxime, ceftazidime were used as substrates and 340 µg of 3-aminophenyl boronic acid (APBA) was used as the *AmpC* inhibitor. The inoculum, disk strength, was as for the standard CDS (Calibrated Dichotomous Sensitivity) disc diffusion method. Each of the three antibiotic substrates was used with and without BA, and a 3 mm or more increase in the inhibitory zone diameter of any antibiotic with APBA was considered as a positive phenotypic result for *AmpC* production. Positive isolates were sent to a Reference Laboratory to detect plasmid mediated *AmpC* genes using a Multiplex PCR assay.

Results

During the study period 17 suspected *AmpC* producing *E. coli* isolates were identified. Of those, 14 (82%) were phenotypically positive for *AmpC* beta-lactamases using APBA inhibition. Multiplex PCR assay detected an *AmpC* gene in 10 of the 14 phenotypically positive isolates (0.71, with 95% CI of 0.42-0.92), this being the positive predictive value (PPV) of the assay. Assay sensitivity is 1.0 with 95% CI of 0.69-1.0. But specificity is 0.43 with 95% CI of 0.10-0.82.

Conclusion

This study shows that APBA used as an inhibitor with multiple antibiotic substrates could be used in the routine laboratory to detect *AmpC* production. This may help in interpretation of ESBL negative, resistant sensitivity results with accuracy. This will also help to uncover the ESBL resistance in situation when both resistance mechanisms are coexisting. Resistance identification would help in better management of antibiotics among patients. Similar to ESBL, *Amp*C resistance transmission should be prevented through proper infection control practices to preserve useful antibiotics for the future.

OP 8

Molecular diagnosis as an early and rapid diagnostic method for leptospirosis

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Introduction

Leptospirosis epidemics have occurred with increasing magnitude over the past few decades in Sri Lanka. 2008 recorded the largest outbreak with the highest number of deaths. Differential diagnosis is difficult as many endemic tropical diseases have similar clinical presentations. As of 2008 the only confirmatory diagnostic method available in Sri Lanka was the microscopic agglutination test (MAT). Serological tests such as the MAT and enzyme linked immuno-sorbent assay (ELISA) cannot provide an early diagnosis and have limitations and drawbacks. Molecular diagnosis using the polymerase chain reaction (PCR) has been described as a rapid, simple, sensitive and specific method for early diagnosis of leptospirosis.

Objectives

To evaluate the feasibility of using PCR for early diagnosis of leptospirosis in Sri Lanka. The PCR performance, the optimal blood component for the test and the Bioanalyzer method for amplicon resolution were evaluated along with the utility of a field-deployable molecular diagnostic laboratory that will be useful in an outbreak situation.

Materials and methods

Blood was collected from 44 patients with a clinical diagnosis of leptospirosis in the first week of illness. Conventional PCR using primers specific for pathogenic leptospira was performed using the Bioanalyzer method for amplicon resolution. Results were compared with MAT and PCR results of another study which were performed on duplicate samples taken from the same patient.

Results

Leptospira DNA was detected in 18/44 (40.9%) samples. Fever duration did not significantly affect the detection of DNA by PCR (P=0.73). The PCR performed well with 5 of 6 samples tested in duplicate giving similar results. Results using different blood components were inconclusive. Compared with Gold Standard MAT, the sensitivity, specificity, positive and

negative predictive value of the PCR was 66.7%, 75%, 85.7% and 50% respectively.

Conclusions

The PCR performed satisfactorily and the field-deployable molecular laboratory was set up and functioned successfully. The initial setting up cost of the Bioanalyzer method was high compared to the conventional gel method. However, difference in the cost per test was relatively small. The main limitation was the limited number of samples tested.

OP 9

A study to compare genus specific MAT with genus specific PCR and culture for diagnosis of leptospirosis in clinically suspected patients

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Introduction

Leptospirosis is endemic in Sri Lanka with outbreaks occurring frequently. The disease is potentially serious, but treatable. Clinical illness varies from asymptomatic to severe multi-system disease with high mortality. Owing to its varied clinical presentations and its mimicry to other diseases, laboratory diagnosis is essential for confirmation of disease.

Objectives

- To compare genus specific Microscopic Agglutination Test (MAT) with a genus specific Polymerase Chain Reaction (PCR) and Culture for diagnosis of leptospirosis.
- To recommend an appropriate test depending on duration of fever.
- To compare the cost of MAT, PCR, and Culture.
- To determine the prevailing sero types.

Methods

A prospective study carried over a four month period from January 2011, involved 100 patients, from four base hospitals and a teaching hospital who were clinically suspected as having leptospirosis. Following an interviewer based questionnaire, paired blood samples (5 ml initially for culture, PCR and MAT, followed by 3 ml for MAT 10days later) were collected. Bedside inoculation of culture and typing isolates, PCR with primers G1/G2 and MAT with Patoc 1 strain were performed.

Results

The study consisted 92% males, majority (75%) being regular farmers of age range 20-60 years. Among them, 85% gave a contact history with paddy fields or muddy water. Mean day of presentation was fifth day of fever. At least one of the three tests was positive in 28% of the study group. Positive results were PCR 8%, culture 7% and MAT 25% confirmed with paired sera. PCR and culture were positive only in patients presenting within 5days of fever and MAT was positive later. Cultures were identified by WHO Reference Centre as belonging to three serogoups Autumnalis, Pyrogens and Icterohaemorrhagiae. Each PCR costs Rs. 3252.00 while a MAT test was Rs. 505.00 and the cost of culture was Rs. 1,672.00.

Conclusions

Strains identified are prevalent in buffaloes and rats, hence awareness on exposure should be increased. Duration of illness should be considered when requesting a test for diagnosis of leptospirosis. PCR is useful to confirm diagnosis in early illness especially during outbreaks of fever in the community.

OP 10

Genotypes of hepatitis C virus in thalassaemic patients and patients who undergo long-term haemodialysis

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Background

Hepatitis C virus (HCV) displays considerable nucleotide variations and has many genotypes. HCV genotypes are related to regional distribution, clinical manifestations, response to treatment and prognosis of HCV infection. Genotype of HCV is important in the management of infected patients. Genotypes of HCV are not known in multitransfused patients in Sri Lanka.

Objective

To determine the genotype/s of HCV prevalent among thalassaemic patients and patients who undergo long-term haemodialysis in Sri Lanka.

Methodology

Study group consisted of 228 thalassaemic patients and 147 patients who undergo long-term haemodialysis. Three ml of blood was collected in dry sterile bottles, serum separated and stored in aliquots at –70°C. Sera were tested for HCV antibody with an Enzyme Immuno Assay (Abbott Murex – third generation). Repeatedly reactive samples were subjected to reverse transcriptase-polymerase chain reaction (RT-PCR) for the detection of HCV RNA. Positive sera were further tested to identify the genotypes by semi-nested RT-PCR using type specific primers from previously published Asian studies.

Results

Eleven patients were repeatedly reactive (twice) for anti-HCV antibodies. HCV RNA was detected in five patients. Genotype II/1b (n=2) and type V/3a (n=1) were reported. Two strains were not typable using the type specific primers.

Conclusions

Genotype II and V were identified in thalassaemic patients and patients who undergo long-term haemodialysis. Results were compatible with other studies conducted in Asia. Non typable isolates should be gene sequenced to identify other subtype/s or mutations.

Acknowledgements

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OP 11

Establishment of polymerase chain reaction assay to detect active cytomegalovirus replication in the blood of renal transplant recipients

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Introduction

Incidence of cytomegalovirus reactivation and disease are high during the first 6 months after renal transplant surgery. CMV PCR and antigenemia assays are considered to be useful in CMV diagnosis to demarcate between CMV latency, incidental viral replication and CMV disease in immunocompromised people.

General objective

To establish the plasma CMV PCR assay and to compare its results with peripheral blood leukocyte (PBL) CMV PCR and CMV antigenemia assay results between two groups of renal transplant recipients; within and after the first six months of transplant surgery.

Design, setting and method

This was a descriptive cross sectional study taking convenient samples from renal transplant recipients of nephrology units at National Hospital, Sri Lanka and General Hospital, Sri Jayawardenapura.

Fourteen (14) out of 48 recipients were within the first 6 months of transplant surgery (Group A), while the other 34 were beyond that period (Group B).

Both groups were tested using CMV PCR assays and CMV antigenemia assay.

Pre-transplant CMV serostatus were obtained from recipients' medical records.

Results

All recipients were CMV seropositive before the surgery and remained asymptomatic during the study period. Plasma CMV PCR positivity rate was 35.7% in group A, while it was only 5.8% in group B. The positivity rates of PBL CMV PCR assay in group A and B were 35.7% and 23.5%, respectively. There was a statistically significant difference in plasma CMV PCR positivity rates between group A and B, while such a difference did not exist between two groups in PBL PCR positivity rates. All plasma CMV PCR positive recipients became negative for the repeat test two to three months later.

None of the 48 recipients became positive for CMV antigenemia assay.

Conclusions

Plasma CMV PCR results correlate well with the incidence of active CMV infection in blood without being affected by CMV latency unlike the results of PBL CMV PCR assay.

Renal transplant recipients are at a higher risk to develop CMV infection during first six months after surgery.

CMV antigenemia assay is less sensitive in detecting active cytomegaloviral infection in comparison to plasma CMV PCR assay.

OP 12

Seroprevalence of herpes simplex virus type 2 infection among female sex workers attending the Sexually Transmitted Diseases (STD) Clinic at National STD and AIDS Control Program (NSACP), Colombo

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Introduction

Herpes simplex virus type 2 (HSV2) is a cause of genital and neonatal infection. Genital herpes is one of the commonest sexually transmitted infections (STIs) and there is a rising prevalence worldwide. The majority of genital HSV2 infections are not recognized clinically. Serological testing is important in diagnosing asymptomatic infection. Female sex workers (FSW) are a population at high risk for acquiring genital herpes and studies on this group are useful for understanding the trends of the infection.

Objectives

1. To describe the seroprevalence of HSV-2 infection among FSW attending central STD clinic.
2. To compare the seroprevalence with that of antenatal mothers attending antenatal clinic at DMH, Colombo.

Design, setting and methods

This is a descriptive cross sectional study. 136 FSWs attending the central STD clinic and similar number of antenatal mothers attending antenatal clinic were assessed using an interviewer administrated questionnaire followed by serum sampling. Serum samples were tested for HSV2 IgG using an enzyme immuno assay specific for glycoprotein gG2 of HSV2. Cross tabulations were done with the dependent variable with the independent variables and the significance was tested with the chi square test using SPSS.

Results

HSV2 seroprevalence of FSW and pregnant mothers were 66.9% and 6.5% respectively. Of FSWs who were seropositive, only 14.3% reported a history of genital herpes infection (p=0.03). HSV2 sero-prevalence increased significantly with age (p=0.04), those with less education (p=0.002), increased number of sexual partners (p<0.05), duration of sex work (p=0.001), and in the street and brothel based sex workers (p<0.05).

Conclusion

Seroprevalence of HSV2 is very high among Sri Lankan FSW and it is very much less in antenatal mothers. Majority of FSW who had the disease did not have clinical genital infection, but they can shed the virus intermittently and act as potential carriers. Serological testing of all high risk populations will be helpful in reducing the transmission of genital infection with HSV2.

Acknowledgement

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OP 13

Molecular surveillance of dengue serotype – May 2010 to March 2012

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Introduction

Dengue has been an important public health problem and leading cause of hospitalization in Sri Lanka for the last two decades. Cyclical epidemics correlated with the monsoonal rain and spread to rural areas are seen through this period. Although all four serotypes of dengue circulate, one or two serotypes tend to predominate at a given period. It is important to monitor the circulating dengue serotypes, as this will help to predict the pending outbreak / epidemics.

Department of Virology, Medical Research Institute started the molecular surveillance with a biennium grant from World Health Organization. We are presenting the data from this surveillance for the period May 2010 to March 2012.

Method

The Out Patient Department, Lady Ridgway Children's Hospital was identified as the study centre. Peripheral venous blood sample was collected from each patient clinically diagnosed as having dengue and who were referred for haematological investigations and dengue laboratory diagnosis. Samples collected from patients with fever of less than 4 days duration were selected for this study. Reverse transcription-polymerase chain

reaction (RT-PCR) followed by nested typing PCR was carried out together with dengue IgM ELISA for all samples. Results of the assays were issued to the patients.

Results

1069 samples were tested by RT-PCR. 75 samples positive by RT-PCR, were typed by nested PCR. 71 were positive for dengue virus type 1 (DENV1), 2 were positive for Dengue virus type 4 (DENV4) and 1 was a mixture of DENV1 and DENV4. 3 isolates were not typed. DENV1 were also detected from samples from outbreaks and from hospitalized patients. 33 of the PCR positive samples were also positive for dengue IgM.

Conclusion

Surveillance data showed that DENV1 was the predominant serotype during the study period. Detection of DENV1 from other samples from outbreaks and from hospitalized patients also supports this conclusion. Appearance of DENV4 towards the later part of 2011 may be of importance and need to be clarified with continuation of the surveillance.

OP14

Aetiological agents of ophthalmic infections

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Introduction

Ocular infections are caused by bacteria, viruses and fungi. Many of these infections cause severe damage to eye sight if not treated adequately. Severe sight threatening complications can be avoided if the aetiological agents are identified and treated appropriately.

Objectives

1. To identify bacterial and fungal agents causing ophthalmic infections.
2. To describe the antimicrobial sensitivity pattern of the bacterial agents.

Methodology

All ocular specimens received at the Eye Hospital Laboratory in Colombo from 1st January 2010 up to December 2011 were analysed.

The conjuctival, corneal and vitreous specimens for bacterial pathogens were processed on blood, chocolate and MacConkey agar using standard microbiological procedures.

Antibiotic sensitivity testing was done according to the Joan Stokes method. Direct microscopy with KOH was done to identify the fugal filaments. Fungal cultures were performed on Sabouraud's dextrose agar using standard procedures.

Results

The total number of specimens processed for bacteriology was 2166. Of them 1566, 291 and 309 were conjunctival, corneal and vitreous specimens with 35.7%, 44.0% and 12.9% of positive culture rates respectively.

The common isolates in corneal and conjunctival specimens were *Staphylococcus aureus* (24.9%), Coagulase negative *Staphylococcus* (CONS) (18.7%), *Pseudomonas* spp (13.6%) and *Streptococcus pneumoniae* (10.3%). Coliforms, *Streptococcus* species, *H. influenzae* and *Morexella* spp accounted for 9.0%, 7.9%, 7.9% and 5.5% respectively.

84.6% of *S. aureus* and 64.7% CONS isolates were sensitive to methicillin. Chloramphenicol sensitivity of *S. aureus* and CONS was 88.9% and 84.3% respectively. Out of Coliforms 56.4% and 37.0% of *Pseudomonas* spp were sensitive to gentamicin. Chloramphenicol sensitivity among coliforms was 83.5%. Sensitivity of *S. pneumoniae* to both penicillin and chloramphenicol was 96.5% whereas 97.3% and 96.0% of *H. influenzae* were sensitive to cefotaxime and chloramphenicol respectively. *Streptococcus pneumoniae* was the commonest isolate in vitreal specimens (23.0%).

Hundred and eighty four corneal specimens and 58 vitreal specimens were examined for fungi with 26.8% and 21.4% positivity for direct microscopy and culture respectively.The two common fungal isolates were *Fusarium* spp (56.0%) and *Aspergillus* spp (36.0%). Other fungal species includes *Curvalaria lunata, Paecillomyces, Pseudoallescheria boydii.*

Conclusion

The commonest isolate in conjunctival and corneal specimens was *S. aureus* whereas *Streptococcus pneumoniae* was the commonest in vitreous specimens. Chloramphenicol sensitivity among Gram positive and negative organisms was above 83%.

Chloramphenicol can be recommended as the first line treatment for corneal and conjunctival infections. Direct microscopy can be used satisfactorily to detect fungi when culture facilities are not available.

OP 15

Prevalence of enterobiasis among primary school children in Ragama MOH region

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Introduction

Although frequently diagnosed clinically, there is only one previously reported survey of *Enterobius vermicularis* infection among Sri Lankan children, conducted in the 1980s.

Objectives

To determine the prevalence of enterobiasis among Year One school children in Ragama MOH area and investigate association between infection and risk factors.

Method

A cross sectional descriptive study was performed among Year One children in seven randomly selected schools. Infection was diagnosed using adhesive cellophane peri-anal swabs obtained on two consecutive days. A pre-tested, self-administered questionnaire completed by the mother, identified risk factors associated with enterobiasis. Initial univariate analysis was followed by multivariate analysis with logistic regression.

Results

Of 322 children, 276 (male: female ratio 1.1:1, mean age 6 years) returned the swabs and questionnaires (compliance 85.7%). Prevalence of infection by double and single swab examination was 37.5% and 20.1% respectively. Prevalence was significantly lower among children of more educated mothers (34.2%), fathers with permanent employment (28.9%) and those dewormed in the last 30 days (22%) compared to children of less educated mothers (46.5%), fathers with casual (46.9%) or no employment (36.4%) and those not dewormed recently (41.6%)($P<0.05$). Infected children belonged to households with significantly more members, siblings and persons sleeping with index child ($P<0.05$). Multivariate analysis showed that more children in a household and recent deworming were the only significant determinants of enterobiasis.

Conclusions

Over one-third of primary school children in the Ragama MOH area have enterobiasis. The risk of infection is higher in families with more children and is reduced by regular deworming.

OP 16

Incidence of sensorineural hearing loss due to congenital cytomegalovirus infection and CMV DNA in urine as a disease marker

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Background

Congenital cytomegalovirus infection is the leading cause of sensorineural hearing loss (SNHL) in developed world. Currently, newborns identified with sensorineural hearing loss are not routinely tested for CMV in Australia. However, testing for CMV within the first 3 weeks of life in these newborns permits the early identification of congenitally CMV infected infants. This allows for possible administration of timely antiviral therapy, the implementation of appropriate interventions at critical stages of language development, and regular clinical monitoring. Congenital CMV infection is commonly detected via testing Guthrie cards (dried blood spots) for CMV DNA.

Objectives

To determine the incidence of congenital CMV infection in infants identified with sensorineural hearing loss and the usefulness of urine as a screening tool for congenital cytomegalovirus infection.

Design, setting and methods

Between October 2009 to April 2011, urine samples were collected within 3 weeks of age from newborns who failed the newborn hearing screening test at Sydney Children Hospital. Other causes (known inherited metabolic disorders etc.) of SNHL were excluded. Informed written consent from parents was obtained after social worker and research nurse explained the benefits of the study. Urine was tested for CMV DNA by realtime PCR (Roche, Germany). Extraction of CMV DNA was performed by using MagNA Pure semi-automated machine (Roche, Germany). Internal controls were placed with every sample as urine has many PCR inhibitors.

Results

Only 34 samples were available from 40 neonates for testing. Nine percent (3/34) of the urine samples were

positive for CMV DNA. None of the mothers of infected newborns gave a history of CMV infection in pregnancy.

Conclusions/recommendation

Our current data shows that 1 in 10 newborns with sensorineural hearing loss have congenital CMV indicating that timely CMV testing in newborns with sensorineural hearing loss is important. Urine can be used to offer a non invasive way of screening congenital cytomegalovirus infection.

PP 1

An effort to understand microbiological properties of Sri Lankan bee honey

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Objective

To study the microbiological properties of Sri Lankan bee honey.

Design, setting and methods

Eighteen samples of bee honey (well mixed, 1 loopfull) were cultured in blood agar media to confirm sterility. Blotting paper discs (diameter; 8 mm) impregnated with 50µl and 100µl of bee honey was used in sensitivity testing for MRSA, MSSA and *Pseudomonas*. After 24 hours incubation the zone of inhibition was checked.

Results

Nine out of 18 samples were sterile when cultured. Following growths were observed in other samples; spore bearers only (7), spore bearers and Candida (1), spore bearers and Gram positive cocci (1). Growth of MSSA up to the disc (resistance) was observed in 12 samples (67%) when 50µl of bee honey was used and in 2 samples (11%) when 100µl was used. When 100µl was used the zone of inhibition ranged from 10mm to 18mm (mean=13.3 mm) in 15 samples. Lack of inhibition of MRSA by 50 µl and 100 µl of bee honey was observed in 7 (39%) and 1 (5%) sample respectively. The zone of inhibition ranged from 10 to 18 (mean=13.1 mm) in 17 samples when 100µl was used. Resistance of *Pseudomonas* to the above volumes of bee honey was 15 (83%) and 5 (28%) respectively. The zone of inhibition when 100 µl was used ranged from 10 mm to 12 mm (mean=11) in 13 samples.

Conclusion

Antibacterial properties were observed in some samples of bee honey for MSSA, MRSA and *Pseudomonas*. However, testing for sterility of bee honey before medical use is important as some samples were not sterile. There are no global standards discussed in laboratory testing of bee honey against bacteria even though some studies have shown MIC values against individual bacteria. Further evaluation on many samples with regards to microbiological and biochemical properties would give a better idea on Sri Lankan bee honey which would enable us to use it as a cost effective antiseptic agent.

PP 2

Evaluation of newly introduced steps to improve hand hygiene in a base hospital

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Introduction

Base hospital Mulleriyawa consists of 285 beds and serves about 0.7 million population. It consists of Medical, Surgical, Gynaecology and Obstetrics, Paediatrics, Microbiology, Rheumatology, Dental, X ray, Emergency Treatment Unit, Out Patient Department and Blood Bank services. The need for improvement of hand washing was stressed following a survey carried out on 230 health care workers.

Objectives

1. To evaluate liquid soap hand wash and 70% alcohol + glycerin hand rub.
2. To find out cost effectiveness of this project over previously used solid soap.

Methods

A self administered questionnaire with 10 questions was used to assess attitude and requirement to improve hospital infection control. As a result liquid hand wash and 70% alcohol + glycerine hand rub were introduced in a new dispenser. Eighty units of dispensers were distributed among all the units. Approximately 75l of liquid hand wash per month and 5l of hand rub per month were utilised. After 2 months we carried out a self administered questionnaire to evaluate the project. Twenty five randomly chosen persons were given the questionnaire while covering all the units. Nine questions were designed to get yes/no answers and the 10th question was on individual opinion of the project.

Results

Twenty four out of 25 (96%) gave yes answers to 7 out of 10 questions indicating positive response while only 1 had a negative comment.

Cost of liquid soap per month – Rs 6,000/-
Cost of hand rub per month – Rs.1,385/-
Previous solid soap cost per month – Rs 10,000/-

Conclusion

Liquid soap and 70% alcohol + glycerine hand rub were well received, user friendly and are cost effective methods of hand hygeine. Further evaluation should be done along with infection rates before and after introduction of hand wash and hand rub to get firm conclusions.

PP 3

Isolation of *Neisseria meningitidis* from cadaveric blood

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Introduction

We describe a case of sudden death of a 2½ year old boy with a history of fever and rash for one day. Child was admitted to the Paediatric Casualty Ward at Colombo South Teaching Hospital at 10.35 pm. On admission the child was found to be in circulatory collapse. There was a purpuric rash all over the body involving face, trunk and limbs.

Immediate resuscitation was started and penicillin 500 mg was given intramuscularly as attempts to obtain IV access failed. The child arrested within minutes of admission. Advanced resuscitation carried out for 45 minutes was unsuccessful. Death was confirmed at 11.55 pm. Attending consultant paediatrician made a probable diagnosis of meningococcaemia.

Clinical history

Child was well until the morning of that same day, when mother detected a mild fever and vomiting around 9.00 am. Mother gave him a dose of paracetamol following which the symptoms subsided. However, at about 5.00 pm, child developed high fever and a few purpuric patches in the face. He was taken to a general practitioner. He was given oral domperidone, ibuprofen, paracetamol and diclofenac sodium 12.5 mg suppository with tepid sponging. Child was brought home and parents noticed the purplish patches increasing in size around the chin but were not-itchy. These patches evolved into an extensive skin rash involving the whole body and child developed difficulty in breathing around 10.15 pm by which time parents decided to bring him to the hospital.

Further inquiries revealed that an aunt of the child had returned from Bahrain two weeks back and the child's father drives a van which transports tourists including those from Middle-East.

The probable cause of death was given as meningococcal septicaemia, but there was a query about a possible anaphylactic drug reaction following diclofenac sodium suppository. Parents consented for a post-mortem examination and it was carried out on the following day.

Postmortem findings

These included bilateral adrenal haemorrhages and haemorrhages in brain and lungs. There were no features of meningitis or laryngeal oedema. Cardiac blood and cerebro-spinal fluid was collected aseptically for microbiological investigations. Cerebro-spinal fluid was blood stained.

Microbiological investigations

Direct Gram stain of CSF failed to reveal organisms and this might be due to blood cells in the samples making it difficult to detect organisms.

Both samples were cultured according to the standard bacteriological methods. After 24 hours of incubation, chocolate agar yielded a growth of grey, glistening colonies of 0.5 mm in size. Gram stain showed Gram negative diplococci. The organism gave a strongly positive oxidase reaction. Carbohydrate utilization test using serum sugars was carried out and gave typical reaction pattern of *Neisseria meningitidis* (1). Thus the isolate was confirmed as *Neisseria meningitidis*. This isolate was from a cadaver kept for 14 hours in the freezer but the organism survived the low temperature to which it is usually susceptible.

PP 4

Identification of non lactose fermenting urinary isolates and reporting the antibiotic susceptibility pattern in a clinically effective manner

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Introduction

Incorrect identification of non-lactose fermenting (NLF) urinary isolates such as *Proteus* and *Pseudomonas* species will lead to treatment failures as *in vitro* antibiotic susceptibility of these organisms does not correlate with *in vivo* antibiotic activity.

Objectives

1. To identify NLF urinary isolates and report the ABST in a clinically effective manner using three simple tests namely, oxidase, urease and KIA tests.
2. To determine the percentage of urinary isolates of *Proteus* spp. with *in vitro* susceptibility to nitrofurantoin.

Design, setting and methods

Urine samples received from 1st April 2011 to 31st March 2012 were processed according to the standard procedure in our laboratory and results were analyzed. Urine samples were plated on CLED medium. Colony characteristics and lactose fermentation or non fermentation were recorded. The NLF isolates were further identified by oxidase (*Pseudomonas* spp.), urease (*Proteus* spp.), KIA (*Acinetobacter* spp.). The remaining NLF isolates were identified as coliforms. Antibiotic susceptibility tests were carried out using Stokes method.

Results

A total of 11960 urine samples were received in the laboratory during this period. Of these urine samples pure growths, mixed growths and no growths accounted for 2895 (24.41%), 2597(21.71%) and 6468 (54.08%) respectively. Of the pure growths, lactose fermenting (LF) coliforms constituted 1384 (47.18%) and NLF isolates accounted for 862 (29.77%). Of these NLF isolates there were 647 (75.07%) coliforms, 95 (11.02%) *Pseudomonas* spp., 41 (4.75%) *Acinetobacter* spp. and 79 (9.16%) *Proteus* species. Nitrofurantoin sensitivity of the LF coliforms, NLF coliforms and *Proteus* spp. were 1197 (86.48%), 533 (82.38%) and 36 (45.56%) respectively.

Conclusion

Identification of NLF urinary isolates and the reporting of the ABST in a clinically effective manner can be done by the use of three simple tests namely oxidase, urease and KIA tests, which can be easily performed. The percentage of urinary isolates of *Proteus* spp. with *in vitro* susceptibility to nitrofurantoin was approximately 45% and could have resulted in treatment failure if these isolates were not identified as *Proteus* species.

Recommendation

Three simple tests namely, urease, oxidase and KIA should be used in all clinical laboratories, which do not use an alternative method to identify NLF urinary isolates.

PP 5

An audit to determine the hepatitis B immune status of health care workers in a district general hospital

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Background and objectives

Healthcare workers (HCWs) have a high risk of occupational exposure to many blood-borne diseases including hepatitis B infections. Hepatitis B is not only the most transmissible infection, but also the only one that is preventable by vaccination. The purpose of this audit was to determine the immune status of HCWs against hepatitis B (anti-HBs) and to correlate the vaccination histories of them at a district general hospital.

Methods

During September 2011, 213 HCWs (74 medical officers, 100 nurses, 11 laboratory staff and 28 minor staff) were included in the audit. Their blood samples were tested by Passive Haemagglutination Assay for detection of anti-HBs. Vaccination histories were taken.

Results

Majority (61.68%) of HCWs were positive for anti-HBs. The positive rate was highest for nurses (68%), then for medical officers (60%) and laboratory staff (50%) and lowest for minor staff (46%). Nearly three-quarter (73%) said that they had taken 3 or more doses of Hep B vaccine while the rest were unable to remember the number of doses they had taken. Out of those 156 who said that they have taken 3 or more doses, 82% could not recall the year of last dose of vaccine and 20% gave negative results to anti-HBs. Only 12 HCWs knew their immune status prior to this audit.

Discussion and conclusion

A significant number of HCWs showed no antibodies to HBs antigen. Majority of the negative HCWs are working in very high risk units. Therefore revaccination was suggested. Negative anti-HBs among vaccinated could be due to the detection level of the testing kit or unreliability of the HCWs memory due to lack of personnel vaccination record. Therefore, infection control unit took actions to provide a hepatitis B vaccination record to HCWs. Revaccination rates of nurses, medical officers, laboratory staff and minor staff were 45%, 34%,100% and 57% respectively. Revaccinated HCWs were suggested to re-check their immune status following vaccination. It is surprising to note the lack of concern of HCWs in spite of the availability of a safe vaccine.

PP 6

Pediculosis among school children in Western province schools

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Introduction

Infestation of the scalp and head hair caused by *Pediculus humanus capitis* is a common health problem especially among school children. It is mostly a harmless nuisance but may result in physical and psychosocial problems.

Objectives

The aim was to determine the proportion of pediculosis among school children in Western province (WP) and control measures adopted by them.

Method

A descriptive cross sectional survey was conducted enrolling students from year 1-10 using randomized stratified cluster sampling method (1 class room = 1 cluster). The selected schools included 3 mixed and 1 girls' school. A self administered questionnaire was used to collect data from the parents/guardians.

Results

Parents/guardians of 598 children aged 5-17 years (mean age=9.5 years, SD=2.7) participated. Approximately 10% had visualized head lice during the last 30 days. Almost all positives were females (98.3%) and majority had medium length (45%) or long (41.7%) hair, whereas 50% had wavy and 46.7% had straight hair. Among the positives, habits of sharing of bed (75%), comb (28%), lying on common bed/ couch/pillow (65%) were observed. All had reported that the infestation affected school attendance and 80% reported that it affected studies. Eighty-five percent of children were embarrassed of having pediculosis whereas 25% reported being stigmatized. Measures used for control included manual removal (82%), combing (47%) and treatment (58.3%). Of those who sought treatment, 45% used western pediculocides including the recently banned product lindane (3.3%). Ayurvedic products (AP) had been used by 18%. A range of plant extracts used by participants included godapara, lime, guvava, margosa (kohomba), Ceylon olive (Veralu), camphor oil, fenugreek (uluhaal), aloevera (komarika). A larger proportion of those using AP reported cure (54.5%) compared to those using allopathic medicine (22%, $p<0.05$).

Conclusion

The pediculosis seems to be a continuing health problem among school children in WP. Therefore, measures should be incorporated into school health educational activities to draw parental attention to control this menace. Lindane had been popular and thus attention should be paid to remove the left over products in the market to prevent inadvertent usage. Reported higher cure rates of AP needs to be investigated.

PP 7

Epidemiology of a measles outbreak and the utility of different laboratory markers in a low incidence population in Australia

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Introduction

Prompt and accurate laboratory diagnosis of measles is essential for case detection, outbreak management and ongoing surveillance in low incidence countries. Serology (measles IgM) alone will have a low predictive value in a low incidence population.

Objectives

To define the epidemiology of a measles outbreak and to compare utility of different laboratory markers of infection.

Study design

Clinical and epidemiological data from suspected measles cases that were notified in the east coast of New South Wales during February to May 2011 were obtained. Sera for measles IgM and IgG, respiratory and urine specimens for antigen detection by indirect fluorescent assay (IFA) and for viral RNA detection by real-time RT-PCR (rt -PCR) were also collected.

Results

Thirty four suspected cases were investigated for measles. Sixteen cases were confirmed by several disease markers (see below). The mean age for confirmed cases was 22.4±12.39 (SD) years (range 1-35 years). A quarter (4/16) of confirmed cases were less than 11 years; the remainder were young adults. Fifteen were not vaccinated in an age-appropriate manner. Some adults developed complications, i.e. encephalitis, pneumonia, conjunctivitis and hepatitis which warranted in patient care. Many first contact physicians did not initially suspect measles leading to extensive contact tracing. The rt-PCRs were negative in all excluded cases where tested, whilst three respiratory swabs and two urine IFAs were considered false positive based on other clinical, epidemiological and laboratory features.

Table. Percentage of positive samples of different laboratory markers for confirmed measles cases

Disease marker	*Positive samples %*	
Swab PCR	100	(13/13)
Serum IgM	100	(15/15)
Urine PCR	90.9	(10/11)
Serum PCR	66.6	(6/9)
Urine IFA	54.5	(6/11)
Swab IFA	46.2	(6/13)

Discussion/conclusions

Measles should be considered in the differential diagnosis of a presentation with fever and rash, even in countries in the elimination phase of measles control. Improving vaccination coverage remains essential, particularly in young adults and travelers. Employing several laboratory markers helps to confirm or exclude suspected measles cases.

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PP 8

Longitudinal study of toxoplasma seroprevalence in East Sydney, Australia

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Background

Interest in toxoplasmosis and its public-health implications has been increasing in recent years. Horizontal age-stratified serological studies which measure the levels of specific antibody prevalence serve as a good basis for assessing the risk of infection in a community. In addition, longitudinal sero-epidemiological studies can yield information of temporal change, the findings of which may subsequently aid the interpretation of horizontal surveys.

Objective

To determine the seroprevalence of toxoplasmosis in patients who presented to South-East Sydney Health Area from year 2000 to 2010.

Study design

Serostatus of toxoplasma specific IgG from sera (more than one years old) available at South-East Sydney Health Area Laboratory from year 2000 to 2010 were analysed using Abbott (USA) chemiluminescent immunoassay. Duplicate entries, sample data from the same residential address and those without names and residential addresses were excluded. Each cross-sectional sample (2000 to 2010) was carried out on data grouped into 5-year age-classes (up to 70 years and >71 yrs). Time-dependent changes in seroprevalence by age- class and sex was analysed using a chi-square test.

Results

8934 sera were analysed. Even though there was a decrease in seroprevalence in every age group, statistically significant changes were observed only in age groups of 11-15, 21-25, 26-30 and 31-35 (chi-square trend statistic and p value were 8.26/ <0.01, 19.95/<0.001, 14.79/<0.001 and 5.04/ 0.025 respectively). There was no difference in sex dependant variation in seroprevalence.

Conclusions/recommendation

Declining levels of *Toxoplasma gondii* seroprevalence due to declining rates of exposure would be of significance in assessing the likelihood of reactivation of toxoplasma tissue cysts inmmunocompromised patients. Furthermore, a temporal decline in the rate of toxoplasma infection would have a critical bearing on the estimation of rates of infection in women of childbearing age.

PP 9

Comparison of cattle blood with sheep, rabbit and human blood in blood supplemented media

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Introduction

Culture media enriched with expired human blood are widely used in developing countries to isolate bacterial pathogens as recommended types of animal blood such as sheep and horse blood are not freely available. Human blood is associated with the risk of blood born pathogens and human blood agar shows poor haemolysis pattern causing difficulty in identification. An alternative source of animal blood is therefore required to overcome above difficulties.

Objective

To determine the suitability of cattle blood as an enrichment substance in blood supplemented media in microbiology.

Methods

A selected group of bacteria was tested for their growth, identification, isolation from clinical specimens and antibiotic sensitivity on cattle, sheep, rabbit and human blood enriched media. Citrated and defibrinated forms of animal blood and citrated form of human blood were used in the study. The performance of cattle blood enriched media was compared with sheep, rabbit and human blood enriched media.

Results

Defibrinated and citrated forms of cattle blood gave similar results for growth, identification, isolation of bacteria from clinical specimens and antibiotic sensitivity testing. Synergistic haemolysis in CAMP test was given by cattle blood. Sheep and cattle blood produced similar pattern and degree of haemolysis. Haemolysis given by rabbit and human blood was poor compared to sheep and cattle blood. Results for the disk diffusion antibiotic sensitivity testing were similar on sheep and cattle blood enriched Mueller Hinton agar for the strains tested.

Conclusions

The performance of cattle blood in blood enriched bacterial culture media is superior to rabbit and human blood. Defibrinated or citrated forms of cattle blood can be used to enrich bacterial culture media for growth, identification and isolation of bacteria from clinical specimens as the performance of cattle blood is similar to sheep blood. Further studies with a wider range of bacterial species are necessary before recommending cattle blood as an enrichment substance in antibiotic sensitivity testing.

PP 10

A study of peritonitis in chronic peritoneal dialysis patients, University Hospitals of Leicester (UHL), UK

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Introduction

Approximately 60 patients a year undergo peritoneal dialysis (PD) in UHL. The most frequent and serious complication amongst these patients is peritonitis. PD peritonitis is clinically suspected when patients present with fever, abdominal pain and vomiting with or without a cloudy dialysate and PD fluid is sent for cellular analysis and culture.

Objectives

1. To study the causative organisms of PD peritonitis.
2. To analyse the treatment modalities and outcome.
3. To assess the efficacy of the standard practice on management.

Methods

All patients who had a PD fluid sent to the laboratory from August 2011 to March 2012 were included in the study. Each PD fluid sample was considered as a single episode. Samples sent from the same patient within a month were excluded.

Results

A total of 35 patients with 50 PD peritonitis episodes were recorded. In 44 episodes the patients had a cloudy PD dialysate whilst only 14 had clinical signs and symptoms. Ten patients had more than one episode of PD peritonitis.

Thirty six episodes had documented white cells >100/mm^3. Thrity seven samples were culture positive. Commonest isolate was coagulase negative *Staphylococcus* (CNS, 30%) followed by Coliforms, *Corynebacterium* spp, and *Staphylococcus aureus* (16%, 13%, and 11% respectively). 4 episodes had mixed cultures.

Empirical treatment was given in 43 episodes. The patients were started with either intraperitoneal (IP) vancomycin and oral ciprofloxacin, or IP vancomycin and IP gentamicin regimes. IP vancomycin alone was given to 5 patients. Overall 36 episodes were treated as per the standard protocol. Following result of culture, the treatment regime was adjusted in 33 episodes.

Twenty culture positive episodes and 7 culture negative episodes had a documented recovery of symptoms with a clear dialysate whilst 23 failed to respond to treatment. Eight patients required changing to haemodialysis. Two patients died due to peritonitis. Peritonitis episodes caused by *Enterococcus* spp., mixed organisms, *Pseudomonas* spp. and *Coryne-bacterium* spp. had a poor outcome.

Conclusion

The commonest organisms causing PD peritonitis were Gram positive organisms (62%). The best outcome was noted with CNS (73% recovery). Thirty six (73%) episodes were managed as per the standard protocol with 58% documented recovery in that category.

PP 11

Comparison of a rapid dengue test with ELISA for the detection of IgM in dengue suspected patients at the Teaching Hospital, Peradeniya

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Introduction

Dengue is an arthropod-borne virus infection. Sri Lanka (SL) is in a region with high endemicity of dengue fever (DF).The reported incidence of DF for the last year was 26,130 with 172 deaths. Several outbreaks have been reported in the recent years. On the other hand, laboratory diagnosis of DF in SL is not complete or validated to assist the clinical management, surveillance and outbreak investigation. In many laboratories detection of IgM and IgG by rapid assays have been used due to the high cost and lack of technical competence and infrastructure for enzyme linked immunosorbent assay (ELISA).

Objective

In the current study we compared the accuracy indices of a rapid immunochromatographic (ICT) dengue IgM assay with the IgM capture ELISA for the detection of IgM in clinically suspected DF patients.

Design, setting and methods

Blood samples (n=119) were collected between September 2011 to January 2012 from DF suspected patients from Teaching Hospital, Peradeniya (THP). The blood samples were analyzed for the presence of dengue IgM using the rapid ICT dengue IgM assay (Hexagon dengue, Human) in the Microbiology Laboratory, THP and the IgM capture ELISA (Pan Bio Diagnostics Inc, Australia) in the Department of Microbiology, Faculty of Medicine, University of Peradeniya. Both assays were performed following the manufacturer's instructions.

Results

ELISA readings were analyzed to compile the results, sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV). IgM detection by the rapid assay was calculated using ELISA as the standard. Sensitivity of the rapid assay for IgM detection was 79% while the specificity was 94.7%. PPV of the rapid assay for IgM detection was 98.75% while NPV was 46.15%.

Conclusion

Sensitivity and the NPV for IgM detection by rapid assay was comparatively lower than that of ELISA, thus the rapid assay might not detect true negatives under a given setting, whereas the higher specificity and PPV indicated the capability of rapid assay in detecting the true positives. Hence, negatives of the rapid test should be tested by a standard ELISA before making a final conclusion. In other words, the rapid assays of dengue IgM detection should be used in conjunction with the other tests such as a standard ELISA for the serological diagnosis of DF.

PP 12

A case of bronchiectasis with invasive aspergillosis due to *Aspergillus fijiensis* n. sp: the first report

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Introduction

Aspergillus species are frequently involved in human broncho-pulmonary diseases, especially in immuno-compromised patients. Although *A. fumigatus* is the

predominant species causing these diseases, *A. niger, A. flavus, A. nidulans, A. oryzae,* and *A. terreus* have occasionally been responsible.

Objective

This report documents the first-known instance of the pathogenicity for humans of the recently-identified species *Aspergillus fijiensis*, in the world's literature.

Case report

We report a case of invasive aspergillosis in a 60-year old male with a productive cough of 6 months' duration, and with a history of previous pulmonary tuberculosis. The patient also had haemoptysis, shortness of breath, loss of weight, and 'night-sweats'. A pleural effusion was present. An earlier chest X-ray had showed a mass in the superior lobe of the lung with a provisional diagnosis of "aspergilloma"; the mass was removed at thoracotomy. Subsequent X-ray of the chest showed bronchiectasis.

Results

Microbiology

Microscopy Branched filaments compatible with septate, fungal mycelia, in the absence of other pathogens, were detected in the bronchial washings. Culture – The pure growth from the bronchial washings cultured on Sabouraud agar (RT 28°C ambient, 10 days) resembled that of *A. japonicus*, with a dark brown-black surface, yellow pigmentation on the reverse.

Microscopy of culture

Septate mycelia bearing globose vesicles, uniseriate oval sterigmata and spherical echinulate spores were identified.

Pathology

A bronchial biopsy-specimen of the affected lobe showed focal ulceration of the lining epithelium and focal squamous metaplasia, the stroma showed infiltration with lymphocytes and plasma cells with old and fresh haemorrhages. Blood vessels showed endarteritis. Diagnosis – appearances are those of an aspergilloma within a cystically-dilated bronchus with bronchiectasis.

Molecular biology

Multilocus sequence analysis of *benA, CaM* partial genes allowed us to identify the culture as *A. fijiensis*. This species was recently identified as a new uniseriate species within *Aspergillus* Sect. *Nigri* isolated from the soil of Fiji islands.

Treatment

The patient responded to 3-months treatment with itraconazole.

PP 13

A study of Gram stain findings of centrifuged and uncentrifuged cerebrospinal fluid samples

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Introduction

Cerebrospinal fluid Gram stain is important to detect organisms causing central nervous system infections before the culture results are available in order to initiate appropriate treatment

Objective

1. To compare the Gram stain findings between centrifuged and uncentrifuged but concentrated, samples of cerebrospinal fluid.
2. To compare the Gram stain findings with culture results.

Methodology

A prospective study was conducted during 25th of February to 25th of April 2012 on 300 CSF samples received at the NHSL Microbiology Laboratory. Gram stained smears were prepared on all samples with uncentrifuged specimens which are concentrated, by placing 3 drops of CSF on clean glass slides. Uncentrifuged samples were plated on blood agar, MacConkey agar and chocolate agar and incubated for 48 hours at 35°C. Chocolate agar plates were incubated in CO_2 jar. Centrifugation of all CSF samples (nearly 0.5 ml) performed under 3000 g for 15 minutes in sterilized glass tubes. Centrifuged deposits were used to prepare the Gram smear on clean glass slides (all these were performed inside a safety cabinet).

Results

Out of 300 samples 28 specimens were culture positive. Gram stain smears of all 28 samples were similar in both centrifuged and uncentrifuged samples with regard to organisms. Increased number of pus cells was seen in the centrifuged samples. Out of 28 culture positive samples, 10 specimens were positive for Gram positive cocci including one each of *Streptococcus pneumoniae, Staphylococcus aureus* and coagulase negative *Staphylococcus* spp. Three of the Gram stains of centrifuged samples showed Gram positive cocci in addition to Gram negative bacilli which were seen in the uncentrifuged specimen. However, all three samples yielded only Coliform spp. on culture. Out of 272 culture negative samples, except for two samples, all the others did not show any organisms both in the centrifuged and

uncentrifuged samples. Those two samples showed increased numbers of pus cells and Gram positive cocci which looked like *Staphylococcus* spp.

Conclusion

No significant difference between the Gram stain findings of centrifuged and uncentrifuged CSF specimens was found with regard to identification of the causative organism. Uncentrifuged CSF Gram stain by using three drops is an alternative to centrifugation in a resource poor setting.

PP 14

The epidemiology of hepatitis A virus (HAV) infection based on samples analysed at Medical Research Institute in 2011

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Introduction

The epidemiology of hepatitis A infection is associated with socio-economic and hygienic status of the population. In the past, HAV infection was acquired during early childhood. In 1976, 88.7% of population less than 19 years of age had sero-positivity and nearly half of the population was infected by 9 years of age. A study conducted in Colombo in 2001-2002 found a much lower sero-prevalance in paediatric age group, suggestive of changes in epidemiology. Progressive increase in the number of adult cases was noted during the last several years. Hepatitis Laboratory, Medical Research Institute (MRI) is the only government sector laboratory which provides HAV IgM diagnostic service to the whole country.

Objectives

1. To describe the epidemiology of HAV infection in samples analysed at MRI in 2011.
2. To compare the epidemiology of HAV infection with past and regional data.

Methodology

The laboratory records of HAV IgM results of the years 2008 and 2011 at the Hepatitis Laboratory, MRI were analyzed. Socio-demographic data were obtained of all HAV specific IgM positive patients from the request forms. Records of the patients with incomplete data and repeated or duplicated samples were excluded.

Results

In 2011, 466 were positive for HAV IgM. Of them, 420 were included in the study. Male to female ratio was 1.02: 1. Majority of patients (48.71%) belonged to 20-39 years age group. 41.94% of the patients were less than 19 years and 17.18%, less than 9 years of age. A similar age specific epidemiology pattern was observed in 2008.

Conclusion

A shift of epidemiology of HAV infection towards adult age group has been noted. This trend is similar to developed countries and differs from that of other South Asian countries where infection is acquired in childhood (WHO, 2009). Improvement of sanitation, personal hygiene and change in socio-economic status may contribute to this change.

As a consequence, there is an increase in the pool of susceptible adult population which can and has resulted in explosive outbreaks. Therefore updated sero-epidemiological data is needed to identify those susceptible to infection for implementation of preventive measures.

PP 15

Microbiological evaluation of the efficacy of different hand-rub products used at a tertiary care hospital in Sri Lanka

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Introduction

Hand hygiene is important to prevent hospital acquired infections. Although commercial hand-rubs are available in certain units of National Hospital of Sri Lanka most of the need is met by an in-house preparation containing alcohol and glycerol. Microbiological efficacy of these has not been assessed at NHSL to date.

Methodology

Twenty participants representing all staff categories from two selected ICU were enrolled. Fingertip imprints were taken on blood-agar plates before and after hand hygiene procedure using commercial and in-house hand-rubs on separate occasions. Use of 2-3 ml of hand-rub, covering all surfaces of hands and rubbing together till dry was considered satisfactory procedure. Organisms were identified with Gram stain and coagulase test.

Results

Two sets of plates were excluded due to unsatisfactory technique. 39 (97.5%) plates taken before procedure showed organisms. Coagulase negative *Staphylococci* (CoNS), spore bearers, diphtheroids, *S. aureus* (SA) and coliforms were isolated. Latter two were considered potentially pathogenic flora.

Reduction of colonies was observed in the majority. Reduction with commercial preparation was 99.6% for CoNS and 100% for both coliforms and SA. Although there was a 95.6% reduction of spore bearer colony count, count did not decrease in four instances.

With in-house preparation reduction in CoNS was 88.2%. In 3 instances for spore bearers and 2 instances for coliforms, counts did not decrease. Reduction in diphtheroids was 100% and SA 75%.

Discussion

Sample size was restricted due to limited availability of blood agar plates. Reasons for non reduction of colonies could be, less stringent criteria used to define satisfactory hand rubbing, poor efficacy of hand-rub, inconsistency of the surface of fingers placed on blood agar and possible contamination of hand rub containers. Spore bearers are generally resistant to alcohol.

Conclusions and recommendations

Superior efficacy was observed in commercial preparation. Reason for relatively poor performance of in-house preparation was difficult to ascertain as batch details was not available. Quality control of in-house hand-rub should be done and reevaluated with several batches before using in high risk units. Continuous education for staff on hand hygiene is recommended.

PP 16

Drinking water bacteriology in Central province of Sri Lanka and application of solar disinfection for drinking water

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Waterborne diseases are still common in developing countries as drinking water sources are contaminated and feasible and reliable methods of disinfection are not available. Approximately 78 percent of the population in Sri Lanka is rural, where chlorinated pipe borne water is not available.

Objective

To test drinking water sources in the Central Province for contamination with *Escherichia coli* and determine the effectiveness of solar energy as a cheap method of disinfection of drinking water.

Design, setting and methods

Previously autoclaved natural drinking water was spiked with *E. coli*, exposed to solar energy for 6 hours with control samples kept indoors and retested by *E. coli* count at the end of this period.

64 samples of drinking water samples collected from natural drinking water sources from selected places in Kandy and Matale Districts were tested for *E. coli* contamination. A portion of each sample was exposed to solar energy for 12 hours from 9 am to 3 pm on two consecutive days and a portion kept indoors as control.

E. coli counting was done using Most Probable Number (MPN) method at 0 hours, 6 hours and 12 hours.

Results

Previously autoclaved natural drinking water spiked with *E. coli*, following 6 hours of sun exposure, did not contain *E. coli*, whereas the control samples kept indoors had the same count or increased count at 6 hours.

Sixty four samples of drinking water samples collected from natural drinking water sources showed 100% contamination with *E. coli* with counts of >5/100ml of water.

Forty one samples out of 64 (64%) reached drinking water standards after 6 hours, whereas 16 (25%) samples reached of drinking water standards after 12 hours. Seven (11%) remained unsuitable for drinking even after 12 hours of sun exposure.

Conclusion

Although *E. coli* in spiked water was eliminated, solar energy was not uniformly effective at all sites. The consistent lack of effect of sunlight requires further study if this method is to be advocated to be used for disinfection of drinking water.

Acknowledgement

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PP 17

Group B *Streptococcus* isolation over a period of one year in a maternal tertiary care hospital

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Introduction

Group B *Streptococcus* (GBS) is responsible for severe morbidity and mortality in early-onset neonatal sepsis (EONS). Despite prevention strategies, disease burden remains substantially high in the USA. 6% carriers remain undetected due to inadequate culture techniques. Optimal management strategy remains unresolved. Some countries recommend universal antenatal screening. Others do not recommend either bacteriological or risk-based routine screening. Intrapartum chemoprophylaxis is recommended, but selection of women 'at risk' remains controversial. Sri Lanka does not have a national guideline for the prevention of EONS due to GBS. A local study done by Samarasekara et al (2005) reported 32.7% colonization using both low vaginal and rectal swabs for screening; the rate of EONS was only 0.42%.

Objectives

To assess the rates of isolation of GBS in HVS specimens received for culture from patients admitted to a maternal tertiary care hospital.

Materials and methods

The total number of GBS isolates in high vaginal swabs (HVS) recovered over a period of one year were determined retrospectively. Specimens taken for various indications from antepartum, postpartum and gynaecology patients, were processed according to standard microbiological techniques. GBS was isolated on sheep blood agar and identified by CAMP test and Lancefield grouping.

Results

GBS was isolated in 13.42% (216/1610) HVS in both obstetric and gynaecological patients.

Conclusions

Though not directly related to EONS, we analyzed HVS's of all patients to estimate the presence of GBS among this group of patients. High rates of colonization (~20%) favours a screening-based approach compared to a risk based approach at 35-37 weeks' gestation. Costs of screening and the costs and disadvantages of intrapartum antibiotic prophylaxis should be considered. The optimal strategy depends on the incidence of GBS-sepsis and the prevalence of anogenital GBS colonization. We recommend further prospective studies to determine the GBS related disease burden to help formulate policies for the prevention of EONS in Sri Lanka. Further development of laboratory facilities for GBS detection is also recommended.

PP 18

Identification of *Acinetobacter* spp. isolated in a tertiary care hospital in Sri Lanka by phenotypic tests and study their anti-microbial susceptibility status

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Objective

To identify the *Acinetobacter* spp. isolated in Sri Lanka by phenotypic tests and to study antimicrobial susceptibility status of those isolates.

Methodology

This study was a hospital based descriptive study. The study sample consisted of *Acinetobacter* spp. positive, clinically significant culture samples received at the 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 as described by Gerner-Smidt, Tjernberg et al. (1991) and Bouvet, Grimont et al (1989). The assimilation tests were performed as described by Stainer, Palleroni, and Doudoroff (1966). Antimicrobial susceptibility determined by disc diffusion tests following Clinical and Laboratory Standards Institute (CLSI) guidelines.

Results

From the *Acinetobater* isolates found, during the period of 3 months 30 were defined as clinically significant. 7% of the *Acinetobacter* isolates were from the wards, 26.7% were from paediatric intensive care unit, 10% were from premature baby unit and 46.7% were from 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 is 70%. When considering aminoglycosides, amikacin showed the best sensitivity (47%). Levofloxacin showed a better sensitivity (40%) than ciprofloxacin (27%). Cotrimoxazole showed 77% resistance. 53% of the isolates were cefoperazone sulbactam resistant but only 20% showed complete resistance. Tigecycline (90%) and colistin (100%) are the only drugs which gave 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%).

PP 19

An audit to determine the device associated infection rate in the ICU at Colombo South Teaching Hospital

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Introduction

Healthcare-associated infections are defined as infections not present and without evidence of incubation at the time of admission to a healthcare setting. Within hours after admission, a patient's flora begins to acquire characteristics of the surrounding bacterial pool. Most infections that become clinically evident after 48 hours of hospitalization are considered hospital-acquired. The high morbidity, mortality, and economic cost justify the need to have continued surveillance, with sound infection control programme.

Objective

To determine the device associated nosocomial infection rates in ICUs.

Methods

A prospective surveillance of device associated infections was done at SICU (8 beds) and MICU (5 beds) of CSTH from 16th February to 16th March 2012.The number of patients and their devices were counted on daily basis at a particular time and type of Hospital Associated Infections (HAI) and causative organisms were noted. CDC-NNIS (National Nosocomial Infections Surveillance) system definitions for all device associated infections were used and rates calculated per 1000 device days.

Device associated infection (DAI) rate = Total number of specific DAI divided by the Total number of specific device days and multiplying the result by 1000.

Device utilization ratio = Total number of device days for each type of device, divided by the total number of patient days.

Results

85 patients admitted to ICU, (62 to SICU and 23 to MICU) represented 367 patient days. The overall device associated nosocomial infection rate was 3.52% (3/85) or 8.17 (3/367) DAI per 1000 patient days. The overall ventilator associated pneumonia rate was 4.87 infections per 1000 ventilator days. Central venous catheter blood stream infection (CVCBSI) rate was 18.51 per 1000 central venous catheter days and catheter associated urinary tract infection rate was 3.50 per 1000 catheter days. Device utilization ratio for central line, ventilator and urinary catheter was 0.14, 0.55 and 0.77 respectively. Non fermenters, *Acinetobacter* and *Pseudomonas* spp. were the most common pathogens. Candiduria and bacteriuria was frequently seen among the ICU patients.

Conclusions

Device associated infection rate observed in the ICU, for CVCBSI is high. Regular calculation of device days allows establishing base line infection rates and would be a monitoring tool for continuous surveillance to support strict infection control practices.

PP 20

Genotyping of measles virus strains provide a powerful molecular-epidemiological tool to assist measles control in low incidence countries

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Background

Measles virus can be divided in to several genotypes by sequencing the COOH terminus of the viral nucleo-protein gene. Molecular epidemiology studies have made significant contributions to the control of measles virus infection through the identification of source and transmission pathways of the virus.

Objectives

To define the molecular epidemiology of a measles outbreak that occurred in the east coast of New South Wales and to determine the usefulness of measles genotype in epidemiologically, in a country where measles transmission has reached the elimination phase.

Design, setting and methods

Respiratory (nasopharyngeal aspirate or throat/nasal swabs (n=14), urine (n=12) and serum (n=6) were obtained from 16 confirmed patients during a measles outbreak in 2011. Following RNA extraction, RT-PCR was performed using a commercial kit (Liferiver, China). PCR products were purified using a QIAquick PCR purifcation kit (QIAGEN) and were sequenced in the forward and reverse directions using a cycle sequencing reaction with an ABI Prism Big Dye Terminator Cycle Sequencing Kit (ABi systems). The carboxy-terminal 456 nt of the N gene were sequenced using the hemi-nested PCR primers MVF2 and MVB1. The reaction products were analysed using an ABI Prism 377 automatic DNA sequencer. Nucleotide sequences were analysed with the SeqEd programme, version 1.0.3. Sequence alignments were performed using Multalin. Phylograms were created with PHYLIP, version 3.5c using DNAdist (maximum likelihood) followed by neighbour-joining. A database of WHO-designated reference sequences for each genotype was obtained from GenBank. The assignment of each MV strain to a particular clade and genotype was based on the PHYLIP analysis.

Results

The genotypes detected (from respiratory and urine specimens) during the investigation were D4 (2), D8 (1) and D9 (11) which were distributed in 5 clusters. There were two imported cases; one from the Philippines (genotype D9) and Italy (genotype D4). The sequences of these viruses were close to the strains circulating in these countries. Genotype D4 was seen in cluster 1, while genotype D9 was observed in clusters 2 to 5.

Conclusions

Molecular analysis confirmed clustering based on epidemiological findings in all five clusters. Importations can be confirmed by genotyping the virus strains. Genotyping is a powerful molecular-epidemiological tool to assist low incidence countries towards eradication goals.

PP 21

Congenital toxoplasmosis over 10 years in South Eastern Sydney, Australia

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Introduction

Vertical transmission *Toxoplasma gondii* can produce significant morbidity and mortality in the fetus and newborn, although it is rare in Australia. Lack of recent studies on congenital toxoplasmosis, prompted us to estimate the incidence of congenital toxoplasmosis.

Objectives

To determine the temporal incidence of congenital toxoplasmosis on serological investigations at a large serological laboratory in New South Wales.

Design, setting and methods

Sera of 1684 infants (mean age ± SD = 1+1.08 months) for investigation of suspected intrauterine infection at South Eastern Sydney Area Laboratory Services (SEALS) area serology laboratory were reviewed between 2000 to 2010. Multiple entries were excluded. A diagnosis of congenital toxoplasmosis was established when IgM (Dia Sorin, Italy) and/or IgA (Platella, BioRad, USA) were positive, as well as an IgM immunofluorescent assay-IFA (bioMérieux, France) was positive for confirmation of presence of toxoplasma IgM. The same quality assurance and calibration and control methods were used over the 10 year period.

Results

There were no diagnoses from 2000-2009 and one case was diagnosed in each year of 2009-2010. The clinical history and findings in each diagnosed case were consistent with congenital toxoplasmosis. In one there was a documented history of maternal infection confirmed by histological examination of the placenta and in the other case there was no history of infection

in pregnancy but subsequently toxoplasma IgM was detected in mother's serum at time of birth. During the 10 year period the laboratory provided a diagnostic service to obstetric facilities south eastern Sydney and other areas, having a minimum of 120,000 live births (12,000 live births per year). Therefore the incidence of two cases in 10 years in this population was estimated as no more than 0.017 per 1000 live births.

Conclusions/recommendation

Incidence of congenital toxoplasmosis is lower than previous prevalence estimates in Australia (no congenital toxoplasmosis in Tasmania, 0.16 per 1000 births in Victoria and 2/1000 births in Western Australia). Diagnosing congenital toxoplasmosis is important for prognostic and therapeutic reasons, and serology remains central to diagnosis, as most patients present after acute infection.

PP 22

Age stratified seroprevalence to hepatitis E virus infection in samples received at MRI

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Introduction

Hepatitis E virus infection is caused by a faeco-orally transmitted hepatitis virus. It causes acute icteric hepatitis which is clinically indistinguishable from hepatitis A infection. In countries like India, Nepal and Egypt, the prevalence of infection is 7-14%, 16-31% and 80% respectively.

Objectives

1. To find out the prevalence of hepatitis E infection in samples received at MRI.
2. To describe the demographical data of patients who are positive for hepatitis E infection.

Methods

The serum samples from patients with infections other than hepatitis which were received at Department of Virology were tested for hepatitis E IgG using Bioelisa, Biokit, Spain.

Results

164 serum samples received at the Department of Virology, MRI were tested for presence of hepatitis E IgG. 4/164 (2.43%) were positive for hepatitis E IgG denoting past infection. The positive samples were in the 5-14 year group. All the patients were male.

Conclusion

Hepatitis E infection is not common in Sri Lanka despite the mode of transmission. This is in direct contrast to the neighbouring countries like India and Nepal.

PP 23

Comparison of the efficacy of pediculocides available in Sri Lanka

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Introduction

Infestation of the scalp and head hair by *Pediculus humanus capitis* is endemic worldwide. Treatment includes manual removal, use of plant extracts and chemical pediculocides. Pediculocides include both allopathic and ayurvedic preparations which are easily reached as over the counter products.

Objectives

In Sri Lanka, there is a gap in the knowledge about the effectiveness of the available 'pediculocides', thus we conducted this study to determine the efficacy of five 'pediculocides' used by Sri Lankans.

Method

Live head lice were collected by combing the hair by the parents/guardian pooled and tested within 2 hours of capture. Permethrin (0.5%), lindane, ayurvedic product (AP) and two types of plant extracts (godapara and veralu/ceylon olive) were tested for the efficacy. Water was the control. Fifteen lice were exposed to each pediculocide and examined at regular intervals for death of lice. Death was confirmed microscopically when all movement and peristalsis of the gut had ceased. Each test was repeated 6 times.

Results

Godapara and veralu killed lice fast with mortality of 62% and 63% respectively at 5 minutes. Slowest action was observed for lindane (40% at 3 hours). At 20 minutes all except lindane had >50% effectiveness with AP and plant extracts having >70%. At 20 minutes lindane (19%) and permethrin (56%) were less effective compared to the AP ($p<0.001$), godapara ($p<0.01$) and velaru ($p<0.05$). Effectiveness of permethrin was 63% after 1 hour (recommended) and 58% at 2 hours. At 1 hour, lindane showed the least (28%) and AP the highest effectiveness (89%, $p<0.001$). All the lice

subjected to the AP were dead by 3 hours (100%) followed by godapara (78.9%), veralu (74.4%), permethrin (72.5%) and lindane (40.4%, $p>0.05$).

Conclusion

AP was the most effective pediculocide but the constituents had not been given by the manufacturer. Therefore the safety of this product to use on humans (especially on children) is doubtful. Exceeding the duration of application does not lead to higher effectiveness for permethrin. Home remedies had a quick and higher effectiveness than permethrin and lindane and studies should be conducted to explore the mechanism of action.

Emerging viral infections

Prof. Sriyal Malik Peiris

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Mankind's triumph over infectious disease may be short lived – the "germs" are now striking back. New or drug-resistant infections continue to emerge through microbes exploiting new niches created by the ecological and environmental impact of human activity, including the abuse of antimicrobials. Many newly emerging infections are caused by RNA viruses and they have a zoonotic origin (1). Arboviruses such as Japanese encephalitis (JE), dengue and chikungunya, nipahvirus, SARS, avian flu H5N1 and the pandemic "swine" influenza H1N1 are examples (2). SARS arose through the adaptation of a previously unrecognized bat-coronavirus to transmit efficiently in humans (3). Increasing economic prosperity and the demand for exotic foods in Guangdong Province, China, led to the development of large "markets" wherein a diversity of live exotic game-animals were housed and sold for human consumption. These provided the milieu for the adaptation and amplification of the SARS-precursor virus in small mammals such as bats and civet-cats and provided the opportunity for repeated exposure of the human population allowing the virus the opportunity to adapt to efficient human transmission. Global air travel rapidly disseminated the SARS coronavirus to affect over 25 countries in 5 continents. The current pandemic H1N1 swine influenza virus emerged through genetic reassortment of swine influenza viruses (4). It emerged in Mexico in early 2009 and spread rapidly with >40% of children in Hong Kong being infected by September 2009. Fortunately, the virus was relatively mild, especially so in young children. This experience highlights the challenge that would be posed by a more virulent pandemic virus in the future. New approaches for the early detection and identification of novel pathogens and for rapid assessment of disease severity are needed. Defining the viral genetic basis for trans-missibility in humans may help detect and stop animal viruses of potential threat to human health before they become pandemic. Avian influenza H5N1 remains endemic in poultry across many Asian countries. Although this virus infects humans relatively rarely at present, when it occurs, human disease is associated with high (>30%) mortality (5). If such a virus was to become pandemic, its impact could be catastrophic. Understanding the viral and host determinants that prevent or permit efficient transmission of animal viruses in humans is crucial (6). Recent studies demonstrate that only a few viral genetic changes are required to allow this avian virus to transmit efficiently in mammals (7). Understanding the mechanisms underlying the severity of avian flu H5N1 disease may help to devise novel therapeutic options (8). Novel "universal" vaccine strategies that provide broad cross protection against multiple influenza virus subtypes are needed and are now an area for intense research. Confronting emerging infectious disease threats requires a broad ecological perspective and a multi-disciplinary effort involving those in the human health sector, veterinary medicine, wild-life conservation, environmental sciences – what is now increasingly recognized as "One Health".

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Parasitic infections – challenges in treatment

Prof. Nilanthi de Silva

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The term 'parasitic infections' includes infection by uni-cellular protozoan organisms as well as multi-cellular helminths. Many of these organisms have complex life-cycles with several morphological forms

and sophisticated mechanisms to evade the host's immune response.

Challenges in the treatment of parasitic infections may arise due to a variety of different reasons. It may be an infection where it is difficult to make a conclusive diagnosis in the first place. Examples include toxoplasmosis and hydatidosis, infections that are confined to the tissues, and a conclusive laboratory diagnosis is notoriously difficult to establish. Other parasitic infection are difficult to treat simply because of the lack of effective drugs (e.g. cryptosporidiosis) or because the available drugs are difficult to administer and have toxic adverse effects (e.g. visceral leishmaniasis, human African trypanosomiasis). There are yet other infections that are challenging to treat because the pathogen has a complex life cycle that involves auto-infection. Strongyloidiasis and enterobiasis are examples of such infections where recurrent re-infection resulting from auto-infection may make it necessary to repeat treatment several times over. Finally, the emergence of drug resistance could also mean that the infection may be a challenge to treat. *Falciparum malaria* is a prime example of an infection that can be very difficult to treat because the pathogen is resistant to many different drugs.

The presentation will focus on two intestinal parasitic infections that can be particularly challenging to treat: strongyloidiasis and cryptosporidiosis. Both infections are briefly reviewed under the following aspects: life cycle and morphology; epidemiology; clinical manifestations; pathogenesis and immunity; diagnosis; treatment and prognosis.

Outcomes and reductions in mortality among patients with community-acquired bacteraemic infections

Dr. Mark Melzer

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There is lack of outcome data in patients admitted to hospital with severe community-acquired infections. In a UK cohort of consecutive patients with community-acquired bacteraemic infections (September 2007-August 2008) at Barking, Havering and Redbridge University Trust (BHRUT), 30-day mortality was 170/681 (25.0%). Non-modifiable factors significantly associated with 30-day mortality were age, Pitt score, Charlson comorbidity index and undefined site of infection. The only modifiable factor significantly associated with 30-day mortality was delay in administering appropriate antimicrobial treatment.

A quality improvement programme of continuous surveillance, teaching and training, competency testing and modification to the antibiotic policy was implemented. From October 2010 to July 2011, 30-day mortality fell significantly, 118/668 (17.7%). BHRUT is one of 3 centres to have published data demonstrating a reduction in mortality through a continuous cycle of regular bacteraemia surveillance and implementation of a quality improvement programme.

Diagnosis of TB – new developments

Dr. Mark Melzer

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An estimated one third of the world's population is latently infected with TB and the incidence of TB and drug resistant TB continue to rise. The mainstay of control is active case finding and effective treatment which requires the detection of patients with smear positive sputa and initiation of effective treatment.

This talk will focus upon the advantages of the contained real time PCR platform, Xpert MTB/RIF, and its role in both case and rifampicin resistance detection. It remains to be seen if this platform can be used in settings outside reference laboratories and whether the price of testing by PCR is reduced, particularly for those in resource poor countries where the burden of drug resistant TB is greatest.

Respiratory infections in primary immunodeficiency

Dr. Rajiva de Silva

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Primary immunodeficiencies (PIDD) are due to innate defects of the immune system. 150 diseases have been identified thus far, with an incidence around 1: 2000 live births in the US. PIDD have been classified in to 8 groups, depending on the component of the immune system affected as well as on clinical features. These include predominantly T cell, B cell, neutrophil, complement and innate immune defects. Patients with PIDD present with severe, prolonged or recurrent infections or with opportunistic infections. The clinical features and the type of infectious agent depend on the underlying immune defect.

Respiratory infections are the commonest manifestation of PIDD. Left untreated, these infections lead to bronchiectasis.

Patients with predominantly T cell defects are prone to infections with microbes that reside within cells, such as viruses, fungi and mycobacteria. Antibody

and complement deficiencies lead to infections with capsulate bacteria such as *Pneumococci* and *H. influenzae*, whereas patients with neutrophil dysfunction suffer from infections with *S. aureus*, Serratia and fungal infections (candida and aspergillus). The group of diseases, termed MSMD (Mendelian Susceptibility to Mycobacterial Disease), are noted for infections with mycobacteria, specially non tuberculous.

Early diagnosis and appropriate treatment is necessary to prevent severe lung damage and even death. This presentation gives an overview of the respiratory infections associated with PIDD.

Aerosol transmission of infection and ventilation control in surgical theatres

Dr. Varuna Navaratne

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Surgical site infections (SSIs) are common health care associated infections (HAIs) and cause considerable morbidity and mortality. Airborne infections in a surgical theatre can result from exposure to clinically significant microorganisms by susceptible hosts. They may be brought in by many ways (e.g. people, air, water, materials, and equipment). These organisms can establish and proliferate in various indoor niches and serve as a source for airborne HAIs. Ventilation control systems are used to control the level of airborne contaminants, which can effectively reduce the post-operative infection risk.

Airborne infection can occur as a result of generation of small particles carrying microorganisms or by spores which can be carried by air currents. Many of these microorganisms contaminated particles originate from within the facility such as aerosolized respiratory secretions, shedding of skin scales, various procedures which may generate aerosolized particles or from external environment.

Building construction and renovation may produce dust contaminated with airborne fungal spores. The fungal spores are able to resist desiccation and can remain airborne indefinitely in air currents and travel far from their source. Aspergillosis is a common opportunistic fungal infection associated with HAI.

Gram positive bacteria, especially *Staphylococcus aureus* are important pathogens causing HAI. They can persist in the environment and on surfaces for extended periods. Gram-negative bacteria are rarely associated with airborne transmission because they generally require moist environments for persistence and growth. *Mycobacterium tuberculosis* is associated with airborne transmission. Droplet nuclei generated by pulmonary TB patients are carried in air currents for prolonged periods and can spread throughout a building.

Very few viruses are consistently transmitted via airborne route. Airborne transmission of VZV and measles has been documented in health care facilities. There is some evidence to supports airborne transmission of influenza viruses, some respiratory viruses and enteric viruses.

Heating, ventilation, and air conditioning (HVAC) systems in health care facilities are designed to facilitate air-handling requirements to protect from airborne pathogens and minimize the risk for transmission of airborne pathogens from infected patients. Various ventilation systems that have been designed and installed in operating theatres are largely classified into categories based on their respective operating principles.

In conventional or plenum ventilation, the air is supplied through an air diffusion system located on the ceiling. Many modern OTs have conventional plenum ventilation with filters which can remove particles greater than 5μm. The air flow is in this system is not uniform and is unstable. Therefore, the risk of air borne contaminants around the operating table will be higher. Operating theatres maintain approximately 15-20 air changes per hour, of which at least 3 should be fresh air from outside.

Laminar Flow Ventilation (Ultra Clean Ventilation) operates on the principle that air supply is conducted parallel and uni-directional through the room. It is usually combined with high efficiency particulate air (HEPA) filters. HEPA filters remove particles >0.3 micron in diameter with an efficiency of 99.97%. Ultra clean air can reduce the incidence of SSIs especially for orthopaedic implant operations. Laminar flow operating theatres may have greater than of 300 air changes per hour.

Wall mounted air conditioners are installed in some countries more for comfort than for clean air delivery and should not be considered as a ventilation system. The operating table does not receive any significant air changes and the bacterial counts remain unaffected.

In conventionally ventilated theatres where engineering parameters are satisfactory and regularly monitored,

routine microbiological air sampling need not be done unless there is a specific requirement. However, in a UCV theatre it is recommended that air velocity assessment and bacteriological air sampling in a working theatre are done annually.

Further reading

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Comprehensive approach to infection control in the neonatal care unit

Professor Alison M Kesson

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Neonates, especially premature neonates, requiring intensive care constitute a highly vulnerable population at extreme risk for nosocomial health care associated infections. Nosocomial infections in neonates carry high attendant morbidity and mortality and health care costs. Control over birth weight and prematurity, which are the most significant predictors of nosocomial infection risk, is limited so correct NICU customs, environment and procedures can reduce this risk.

The development of a system for infection control practices and processes to prevent or manage transmission of infections or multi-resistant organisms between patients, staff, visitors and students can be divided into three parts.

1. Individual protections are the most fundamental of the requirements. They protect the individual and include:
    a. Implementation of:
        i. Hand hygiene
        ii. Standard and transmission based precautions
        iii. Use of personal protective equipment
        iv. Staff vaccinations
        v. Management of sharps
2. Environmental measures according to local priorities or resources include:
    a. Cleaning strategies
    b. Sterilisation and reprocessing of equipment and devices
    c. Food services
    d. Antimicrobial stewardship to decrease MRO prevalence
    e. Investigation of outbreaks
    f. Surveillance
3. Structure and governance of infection control should be the responsibility of a multi-disciplinary infection control committee with members who are interested and knowledgeable in infection and infection control and represent areas of the hospital where infection is either a potential problem or involved in controlling of these infections. The infection control committee must have the delegated authority of the hospital executive to efficiently and tactfully implement actions to control infection.
    a. Establishing a multidisciplinary infection control committee which resides within the hospital's patient safety governance structure with membership including:
        i. Medical microbiologist and virologist,
        ii. Infection control practitioner
        iii. Infectious disease physician
        iv. Personnel from medical and nursing and administration, pharmacy, operating theatres, house-keeping and engineering and maintenance.
        v. Ideally the chair of the committee should have an expertise in hospital epidemiology and infection control.

b. Hospital management should provide dedicated resources for:

   i. Infection control activities,

   ii. Education and training of staff, patients, visitors and students.

   iii. Measuring and monitoring hospital acquired infection rates.

   iv. Measuring and monitoring infection control activities.

Understanding the epidemiology of nosocomial infections in neonates and methods for their prevention and control is critical to minimising poor outcomes.

Rational antibiotic use in paediatric patients

Professor Alison M Kesson

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Antimicrobial stewardship is defined as processes to assist and support clinicians with decisions regarding the optimal selection, dose and duration of an antimicrobial agent. The objectives are to ensure the best clinical outcome in terms of the treatment or prevention of infection, with minimal toxicity to the patient and minimal impact on subsequent resistance development. Cost-savings are often observed with successful stewardship programmes, but are not their primary purpose.

The three main goals are:

1. Improved patient care (maximize efficacy, minimize toxicity) by provision of education and decision support for antibiotic prescribers.
2. Decreased pressure for the development of multi-resistant organisms.
3. Decreased drug acquisition costs.

The development of a system for antimicrobial stewardship can be divided into three parts.

1. **Essential or core strategies,** are the most fundamental of the requirements. They include:

   a. Implementation of clinical prescribing guidelines which are consistent with an acceptable basis for management of patients with specific infections or infectious syndromes and which take into account local microbiology and antimicrobial susceptibility patterns.

   b. Establishment of an antimicrobial formulary with restriction and approval system.

   c. The active review of antimicrobial prescribing with intervention and direct feedback to the prescriber.

   d. Monitoring performance of prescribing.

   e. Susceptibility reporting of microbiology results must be selective.

2. **Ancillary strategies,** or stewardship activities according to local priorities or resources include:

   a. Educating prescribers, pharmacists and nurses about good prescribing practices and antimicrobial resistance.

   b. Other interventions, including streamlining or de-escalation of therapy, dose optimisation or parenteral to oral conversion.

   c. Electronic prescribing with clinical decision support or online approval systems if available.

   d. Annually publishing facility-specific anti-microbial susceptibility data.

3. **Structure and governance:** the overall responsibility for antimicrobial management control lies with the hospital administration. They are responsible for ensuring an antimicrobial management programme is developed and implemented, and outcomes are evaluated. Hospital management support includes:

   a. Providing dedicated resources for stewardship activities, education and measuring and monitoring antimicrobial use.

   b. Establishing a multidisciplinary Antimicrobial Stewardship Committee (AmSC).

   c. Ensuring that the AmSC resides within the hospital's patient safety governance structure.

PRESIDENTIAL ADDRESS — 2011

Presidential address delivered at the inauguration of the Annual Scientific Sessions of the Sri Lanka College of Microbiologists on 14th September 2011

by **Dr. Pranitha Somaratne**
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Head, Department of Bacteriology, Medical Research Institute, Colombo.

Chief Guest, Professor Uditha Liyanage, Director, Postgraduate Institute of Management, Colombo, Guest of Honour, Professor K. Lily Therese, Professor of Microbiology, Vision Research Institute, Sankara Nethralaya, Chennai, India, other foreign guests, officials of the Ministry of Health, past Presidents, Council Members, Members of the Sri Lanka College of Microbiologists, distinguished guests, ladies and gentlemen, I warmly welcome all of you.

When you accepted our invitation for the Inauguration of the Annual Scientific Sessions 2011 of the Sri Lanka College of Microbiologists, you have already sensed that you have to bear with at least a small dose of 'microbes' during this evening and I am destined to carry it out! In my profession as a microbiologist, I have opted to recognize, understand, appreciate and make space for microbes. So, I will be focusing on some aspects of microbes as they have been understood over the years.

Microbes though unicellular organisms, are no simple beings. Man, with all his advanced technology and craftsmanship of today is yet to fully grasp the even more intricate advances achieved by these microscopic companions. They are found everywhere; on living beings, in the environment, in flora and fauna, mostly in a symbiotic relationship. The skin bacteria also called normal flora of the skin does more to protect and maintain a healthy skin than all commercial soaps and creams. The normal flora can do away the probable disease causing pathogenic microbes by the production of various acids and by successfully competing for essential nutrients. These are microbial armaments used for getting rid of the enforcing enemies and thus contribute to maintaining the ecological balance.

The human alimentary canal from mouth through to large bowel, harbour billions of bacteria. Thus the colonizing microbial flora protects the gastrointestinal tract from pathogens such as Salmonella species by their fermentation products as acetic and butyric acids. Other sites which are inhabited by normal bacterial flora are mucous membranes of the oropharynx and genitalia, protecting them from invading microbes. The soil microbes help keep the environment clean by processing organic matter in the waste.

If microbes always behaved such we would not have this event or plan to commemorate them annually. But infectious diseases caused by microbes go far back into history. Most of these were a result of a breach of some protective barrier which was demarcating boundaries for man and microbe. In the past when existence of microbes was not clearly understood, many misconceptions deceived reality. Man blamed unseen devils, gods and stars for sickness. Containment of infections was hard to come. Life span was short. Many famous personnel were brilliant within their very short lives. Some notable examples are the great scholar John Harvard, instrumental in founding the Harvard University College who died of tuberculosis at the age of 31. The three famous novelist Bronte sisters of 'Jane Eyre', 'Wuthering Heights' and 'Emma' fame had died tragically at the tender ages of 29, 30 and 39 either due to tuberculosis or typhoid complicated by tuberculosis. Black Death, the outbreak of plague caused by the bacterium *Yersinia pestis* is estimated to have killed 30-60 percent of Europe's population. The influenza pandemic of 1918-1919 had apparently killed more people than the World War I; recorded at a figure of 20 to 40 million people. It has been cited as the most devastating epidemic in recorded world history.

The singular honour of discovery of microbes as the cause of infectious diseases goes to the French chemist Louis Pasteur (1822-1895). He and his wife had five children, only two of whom survived to adulthood, while the other three died of typhoid fever. These personal tragedies inspired Pasteur to try to find cures for diseases such as typhoid.

Ignaz Philipp Semmelweis (1818-1865) was a Hungarian physician now considered as the 'Father

of Hand Washing and Infection Control'. His observations on transmission of infections contributed to confirm the 'germ theory' sadly only after his death.

Now, almost two centuries later, today, we are still struggling to understand these 'germs' better. Epidemics and outbreaks are still causing havoc globally and more so in the developing world. Infectious outbreaks occur both in the hospital settings and in the community.

The Department of Bacteriology of the Medical Research Institute have contributed to contain many outbreaks in the State sector hospitals, some examples of which will be quoted here.

In January 2006 District Hospital, Nawalapitiya reported an outbreak of septicaemia in the Premature Baby Unit. Blood cultures from sick babies yielded *Pseudomonas* species. Several samples were taken for the investigation from the Premature Baby Unit, Operating Theatre and the Labour Room. Samples of humidifier water, suction tubes and suction bottles grew *Pseudomonas* species and Coliform organisms. The Base Hospital, Nikaweratiya in March 2006, reported a large outbreak of infection in the special care baby unit (SCBU) where blood cultures became positive for a *Pseudomonas* species. *Pseudomonas* was also isolated from operating theatre resuscitation bed, sucker tubes, labour room beds, sucker bottles and incubators. The cleaning, disinfecting and sterilizing methods and hand hygiene adopted by these hospitals had to be monitored and strengthened to contain these outbreaks.

In June 2006 Base Hospital, Puttalam recorded an infection outbreak involving several neonatal deaths. The hospital was visited on the instructions of the Director General of Health Services. Several probable problems leading to the ongoing outbreak came to light. In this instance also *Pseudomonas* species and other Coliform bacteria were isolated from several samples obtained from the unit including humidifier water and multi-dose injection vials of dextrose, vitamin K and even from antibiotic vials of gentamicin. After adopting proper cleaning measures and infection control practices, the outbreak was contained. Several interventions had to be taken. Single-use injection vials were introduced replacing all multi-dose vials. The use of surgical hand-rubs were encouraged through the ICN while advising to purchase liquid hand soap as *Pseudomonas* even grew on cakes of soap and certain disinfectants. Yet due to lack of funds further instructions were given to use small pieces of soap per each day and to refrain from re-using them on a subsequent occasions. Staff training on infection control practices was carried with the assistance of the Regional Director of Health Services.

Several structural changes with regard to the building, such as introduction of more hand washing facilities with elbow taps, tiling of walls and floors to make them more amenable to cleaning and disinfection, changing existent direct access doorway from labour rooms to corridor had to be done to prevent further similar outbreaks in this hospital.

In April 2010 Base Hospital Avissawella had a large outbreak of *Pseudomonas* sepsis in both the adult ICU and Premature Baby Unit with resultant neonatal and adult deaths. Laboratory investigations revealed the presence of *Pseudomonas* species in several samples obtained from these areas. Infection control practices adopted by the staff were assessed by visiting the hospital. Most mothers in the baby unit breast fed the babies under supervision of the staff. The consultants and nursing staff were very committed towards maintaining standard precautions and other infection control practices. Thorough cleaning and disinfection of the units were done. Infection persisted in-spite of the steps taken. Laboratory investigations continued to yield the *Pseudomonas* species with the same antibiogram.

At this juncture we suggested getting the hospital tap water tested. Water samples tested at the Medical Research Institute yielded the identical *Pseudomonas* species. Hospital water storage tanks were cleaned, and the sealed water system was chlorinated double strength, which at last contained the infection outbreak.

Pseudomonas is an organism found in soil and environmental water sources. It can contaminate and grow in a building's water system in a retrograde manner, probably contaminating hand washing sinks, if in a hospital setting. This warrants the system to be chlorinated on a regular basis as part of an annual maintenance regime. Regular checks should ensure correct amount of chemical biocide and absence of contaminating pathogenic bacteria.

Pseudomonas infections in ICUs are difficult to contain and difficult to treat due to its many virulence factors and being a biofilm associated infection. *Pseudomonas* could effectively grow on moist soap or disinfection solutions. It can colonize on moist environmental surfaces and on hospitalized patients. The source of infection could be exogenous or endogenous from a patient's own site. Biofilm-state

protects the organism from environmental stresses, currently used antibiotics and from the host immune defences.

Antimicrobial agents in use have been developed with activity against free living bacteria existing in a non biofilm-state. Thus, infections caused by *Pseudomonas* fail to respond to many anti microbial agents, adding insult to injury of the on-going burden of *Pseudomonas* infections in the intensive care settings.

In July – August 2005 an outbreak of meningitis involving 14 patients was reported from four hospitals in the Colombo district and from two other hospitals outside the Western Province. Four out of these fourteen patients died. All patients had similar clinical presentation and appeared within a short period of time and were either exposed to spinal anaesthesia, lumber puncture or intrathecal drug administration. Wide publicity was given in mass media for this outbreak. Case histories and laboratory investigations carried out pointed at the possible sources of infection as contaminated needles, syringes and anaesthetic solutions. Samples of items obtained from many hospitals and from the stores of the Medical Supplies Division were tested at the Bacteriology Department of the Medical Research Institute. Aerobic spore-bearing bacteria, Coliform organisms, *Staphylococcus aureus, Pseudomonas* species and fungi were isolated from several samples while the Mycology Department isolated *Aspergillus* species from clinical samples as well as from anaesthetic items and hospital environment. WHO and CDC expertise was sought by the government of Sri Lanka and the outbreak was contained after high level political intervention together with all other stakeholders.

In the General Circular No. 1-18/2005 dated 17th August 2005, issued by the Director General of Health Services, reference for infection control measures were drawn from the Hospital Infection Control Manual compiled by the Sri Lanka College of Microbiologists, 2005. Epidemiology Unit in its report on the outbreak concluded that infections might be hospital acquired due to contaminated products or material used for spinal anaesthesia. It also stated that unsatisfactory storage conditions as reason for bacterial and fungal contamination of patient-care items. Recommendations were drawn accordingly to avoid similar future incidences and the Minister of Health publicly wowed to take necessary steps to prevent similar happenings in the future.

Outbreaks of infections are not uncommon in the community. Methicillin resistant *Staphylococcus aureus* (MRSA), termed 'Super Bug' later, was first described in 1961 largely as a hospital pathogen. MRSA was incriminated in many lethal hospital outbreaks globally. Almost two decades later epidemic community-associated MRSA or CA-MRSA, was reported in Australia in early 1990's and has since spread globally. Lethal infections were reported in the US in children. Patients affected were previously healthy.

Antimicrobial treatment was the cornerstone of therapy for infectious diseases. The dramatic rise of average life expectancy in the 20th Century is attributed to antibiotics. The first antibiotic penicillin discovered by Sir Alexander Fleming in 1929 had a huge impact on public health, yet few years into its use saw emergence of resistance. A resistant gene pool has probably existed in nature even before antibiotics were produced. Bacteria acquire resistant genes through transferable plasmids which are small pieces of genetic material coding for resistance to one or more antibiotics. Plasmids are exchanged between strains and species of bacteria rendering newly resistant microbes. Thus microbes are more innovative and adaptive to an environment of antibiotics than the rate of new antibiotic production by industry.

CA-MRSA has thus evolved, and is causing isolated infections and outbreaks in non-hospitalized communities. 90% are usually skin infections such as abscesses, boils and other pus filled lesions. Most of these infections are difficult to control, given the complex transmission dynamics, and the limited antimicrobials available for treatment.

The Centers for Disease Control and Prevention, Atlanta in their Guidelines for CA-MRSA emphasizes '4 C's' as risks for CA-MRSA transmission, i.e. **C**rowding, **C**ontact (skin to skin), **C**ontaminated items such as towels, surfaces and (lack of) **C**leanliness.

In a resource poor country such as Sri Lanka it is obvious that elimination of all these 'risks' to achieve a satisfactory outcome will be a far cry from reality. The answer and responsibility lies with the public and healthcare workers alike. Unnecessary and over prescribing of antibiotics by doctors, self medication and adhering to incomplete antibiotic courses by public are some of the important causes leading to the development of resistant bacteria.

Lastly, I will touch on an infectious disease of the community of a different nature. Leptospirosis is a

zoonotic infection commonly known as '*mee una*' in Sri Lanka. It is transmitted to man through the urine of an infected animal which harbours the leptospirae in their renal tubules. Organisms from contaminated soil or water enter through breached skin or mucous membranes resulting in infections seen mainly among paddy farmers.

Any warm blooded animal may be a carrier of the infection, although rats and mice are better known as the infectious source; hence the term 'rat fever' or '*mee una*'. Knowledge about rats as a source of infection is accordingly well known, but knowledge is extremely limited regarding other domestic or farm animals as dogs and cattle. An increase in rodent populations is often directly related to human populations. Highly populated areas on the outskirts of urban centres may serve as amplification areas for disease risk.

Leptospirosis is a notifiable disease. There are more than 200 types (sero-vars) causing disease in man. Each of these types may have characteristic clinical features and a unique disease process. Each type can originate in a different reservoir animal. Thus the identity of the reservoir animal is necessary to understand the epidemiology of the disease for prevention and control measures.

Large outbreaks of leptospirosis occurred in Sri Lanka in the recent past, highest being in 2008 with more than 7400 cases and 204 deaths. According to reports from the Epidemiology Unit, so far 4,779 cases of leptospirosis have been reported in the country this year resulting in 60 deaths.

Kurunegala district has reported the highest number of cases with 1,386, resulting in 10 deaths during this year. From mid-February to early-March this year, during Yala season there was a large outbreak of leptospirosis in Kurunegala district with one death. Blood samples taken from patients were typed (for sero-vars and sero-groups) but the results do not direct to an incriminating animal source; as cross-reactions are common.

This reflects the difficulties encountered in controlling the disease. Hence emphasis of control programmes is mainly focusing on prevention of exposure to contaminated environments, though not an easy task in the developing world.

I would like to say that the time has come for Medical Microbiologists to work hand in hand with both the other health care workers and the general public, if we are to come to terms with the ever evolving microbes.

I will conclude my address with a quotation by Louis Pasteur. I quote, *"There does not exist a category of science to which one can give the name applied science. There are science and the application of science, bound together as the fruit of the tree which bears it"* un-quote.

Finally, I wish to pay my deep gratitude to my parents for nurturing me with love, devotion and guidance; my family for their tolerance and understanding; all my teachers, friends, colleagues and associates for their influence on my life, which moulded me into what I am today.

Thank you.

FOOD ALLERGY

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Food Allergy

Immune response is meant to be protective. However, under certain circumstances it can be harmful either due to excessive immune response (hypersensitivity) or inappropriate immune response (autoimmunity). When an immunologically mediated adverse clinical reaction results from a food protein it is known as food allergy. Immune mechanisms involved with food allergy are either IgE mediated or non-IgE mediated.

IgE mediated food allergy

Those who produce IgE instead of IgG, are known as atopic individuals and they are prone to develop allergy. The main mediator of allergic reaction is histamine produced by mast cells. In addition to IgE, activation of mast cells slowly produces thromboxane and leukotrine from cell wall phospholipids. All these substances together contribute to allergic manifestations involving almost all the organs of the body.

Clinical manifestations vary from mild to fatal anaphylactic reaction. Common clinical features include itching, flushing and hives (urticaria) of the skin and difficulty in breathing due to bronchospasms. It is important to exclude non-allergic manifestions resembling food allergy due to food intolerance which could result from food additives such as sodium chromoglycate.

Non-IgE mediated food allergy

Protein causing allergy is taken up by antigen presenting cells and carry out cell mediated immunity with more delayed and prolonged clinical manifestations including enterocolitis, proctocolitis and enteropathy.

Mixed mechanisms

Both IgE-mediated and cell-mediated immunity collectively lead to harmful effects in certain patients with gastro-oesophageal reflux and atopic dermatitis.

Clinical spectrum of food allergy

IgE mediated conditions

Anaphylaxis
Urticaria and angio-oedema
Immediate gastrointestinal reaction (Eg: diarrhoea)
Immediate respiratory reaction (Eg: bronchospasm)

Non-IgE mediated conditions

Food protein induced enterocolitis syndrome
Allergic proctocolitis
Allergic enteropathy

Conditions resulting from mixed mechanism

Atopic dermatitis
Allergic eosinophilic gastroenterocolitis
Gastro-oesophageal reflux
Unexplained abdominal symptoms

Food items causing allergy

The type of food causing allergy vary in different age groups and different geographical locations.

In chidren, classical allergy march start from respiratory manifestations followed by gastrointestinal conditions and end up with skin allergies. However, throughout the life certain food items could be responsible for allergic manifestations.

Common allergens among children in Western countries include cow's milk, egg and peanuts, whereas, in adults they include shellfish, peanut, tree nuts, wheat, fish and eggs. Sri Lanka being a tropical country, we encounter a variety of fruits and it is common to find allergy at any age to fruits and in particular to unripe fruits such as mango.

Seventy-five percent of children who have allergy to milk products are able to tolerate baked-in milk products such as cookies and cakes.

Wide geographical variation has been found on red meat allergy even within developed countries. Although scientific data are not available regarding the picture in Sri Lanka, it is observed that we see more red meat allergy than in the west.

Natural history of food allergy

In the United States, food allergy affects as many as 5% of children less than 3 years of age and 3 to 4% of adults. About 50% of children with allergy to milk, egg, soy and wheat will outgrow their allergy by the age of 6 years. Those who are still allergic by the age of 12, have less than 8% chance of outgrowing allergy. Only 20% of peanut allergy and 9% of tree nut allergy outgrow as they get old.

The true prevalence of food allergy in Asia is uncertain. Estimates from Chinese studies range from 4.9% to 16.4%. The latter percentage represented those diagnosed by skin prick test and therefore reflects sensitisation rather than true clinical allergy.

Diagnostic testing

For IgE mediated food allergy

1. History

IgE mediated allergic manifestations usually occur within two hours of taking an offending food item. Therefore carefully taken history and a food diary play a crucial role in finding out the responsible allergen.

2. Food challenge

Introduction and elimination of food items help in identification of the offending allergen. However, introduction of food item may sometime induce a severe reaction. Therefore it involves careful monitoring in a place with facilities for rescucitation.

3. Serum food-specific IgE antibody

It has over 95% positive predictive value and is useful in eliminating the offending food. However, it is important to note that measurement of total IgE plays a very little role in the diagnosis as it would simply indicate the tendency to develop allergy. It is also important to note that the commercially available kits developed for allergens in western countries are not directly applicable in our settings.

4. Toral serum IgE estimation

The measurement of total serum IgE has very little role to play in the diagnosis of allergic diseases except in difficult cases of distinguishing food intolerance from food allergy. Moreover, we have shown that the total IgE is higher in our setting compared to the west due to the higher prevalence of helminthic infestations.

5. Skin prick test

Each allergen from a panel is placed over the volar aspect of the forearm and a prick is made with a lancet. 0.9% NaCl and histamine are used as negative and positive control respectively. The diameter of the wheal is read in 15 minutes and the test is considered to be positive if the difference in the diameter between the test and the negative control is more than 3 millimeters. The subject is advised to refrain from taking antihistamine for a period of 3 days prior to the test. If the positive control is negative the results of the test is considered invalid. One can do the skin-prick test as a bed side test with fruits and it is found to be a safe procedure to be carried out in the clinic.

6. Allergen epitope recognition

Peptide microarray technology is used to detect the exact epitope of the allergen which is useful in deciding appropriate immunotherapy for essential food items.

7. Challenge with recombinant allergen

The risk of food challenge is drastically reduced by challenging with recombinant allergens. Although the specificity of the challenge increases, the sensitivity declines with such a procedure.

For non-IgE mediated food allergy

1. Patch testing

A panel of allergens is placed over the upper back and look for skin reaction in 72 hours. It is important to note that patch test is not done for the detection of allergens responsible for type 1 hypersensitivity.

2. Measurement of cytokine production by T lymphocytes following stimulation with food allergens

Pro-allergic cytokines are targeted using in vitro testing such as ELISA and RT/PCR.

3. Measurement of eosinophilic markers and cytokines in stools

The presence of eosinophilic markers in stool points to allergy and IL-5 acts as a chemotactic cytokine for eosinophils.

4. Endoscopy and biopsy

In addition to the detection of infiltraction of cells such as mast cell, basophils, eosinophils and Th_2 lymphocytes, mucosal biopsy helps in the exclusion of inIfammatory bowel diseases and neoplastic diseases.

Treatment

The mainstay of the treatment is avoidance of foods that have been identified as allergens. For people who are extremely sensitive, this may involve the total avoidance of any exposure with the allergen, including touching or inhaling the problematic food.

With the accidental exposure of the food, the treatment has to be decided depending on the severity of the symptoms. Mild reaction such as itching or hives of the skin could be managed with either local or systemic antihistamine preparation. Short acting antihistamine such as chlorpeniramine would be sufficient in such a situation. If drowsiness is a problem, non sedative longer acting antihistamine such as loratadine, cetrizine or fexafenadeine is preferred. However, in the case of severe reactions such as anaphylactic reaction or those not responding to initial antihistamine therapy, may need urgent treatment with adrenaline and hydrocortisones at the closest medical institution. It is advisable to carry self injectable adrenaline pens (Epipen) by those who are liable to get anaphylactic reactions. However, it is not practical in Sri Lankan settings due to social and economical reasons. It is not socailly acceptable by the people in our part of the world to administer an injection by one self. Moreover, it is not economical as the epipen expires within one year and it is not cost-effective.

Commonly used drugs in allergy

Antihistamines:

Chlorpheniramine 4 mg twice a day

Cetrizine 10 mg daily

Loratadine 10 mg daily

Desloratadine 5 mg dailty

Fexafenadeine 180 or 120 mg daily

Mast cell stabilizers:

Ketotifen 1 mg daily

H_2 receptor blockers

Ranitidine 150 mg twice a day

Steroids:

Dexamethasone 0.5 mg daily

Prevention

There is evidence that breastfeeding for at least 4 months, compared with feeding infants formula made with intact cow's protein, prevents or delays the occurrence of atopic dermatitis, cow's milk allergy, and wheezing in early childhood. In order to avoid an allergic reaction, a strict diet can be followed. It is difficult to determine the amount of allergenic food required to elicit a reaction, so complete avoidance should be attempted unless otherwise suggested by a qualified medical professional. In some cases hypersensitive reaction can be triggered through skin contact and inhalation. It is also advised to avoid exercise and alcohol just after a meal as is linked with higher risk of allergy.

When avoiding certain foods in order to lessen the risk of reaction, it is important to note that they may develop nutritional deficiencies and one should take necessary precautions accordingly.

Although desensitization is widely practiced for aeroallergens such as pollen and house dust mite in the case of asthma, the same process in principle can be used in the cure of food allergy in precisely identified food allergies.

In conclusion, education of health care professionals and the public on the expanded spectrum of food allergic disorders together with proper preventive and management strategies reduces considerable morbidity and mortality linked with food allergy.

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CHALLENGES OF NOSOCOMIAL CANDIDAEMIA IN CRITICALLY ILL PATIENTS – PAST, PRESENT AND THE FUTURE

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Introduction

Invasive fungal infections are a major public health burden worldwide, especially among immunocompromised patients and those hospitalized with serious disease. A more recent multicentre surveillance programme found that *Candida* species caused over 70% of invasive fungal infections in hospilalized patients. Between 1995 and 2002, the frequency of nosocomial candidaemia rose significantly from 8% to 12% of all reported nosocomial blood stream infections (BSI) (1). Candidaemia is associated with significant morbidity and mortality and remains the most frequent life-threatening fungal disease. It is associated with a prolonged hospital stay and a resulting rise in cost. Risk factors for candidaemia are well established and are primarily related to immunosuppression, mucosal disruption, colonization due to exposure to broad-spectrum antimicrobial agents, pathogen exposure and direct vascular access to pathogens. Patients with haematological malignancies and neutropenia have an increased risk of candidaemia, and non-neutropenic patients who are hospitalized in the surgical ICU are recognized as an important at risk group for this infection (2, 3). Total parenteral nutrition, burns, assisted ventilation and intravenous drug use are some of the other risk factors identified (1). Candidaemia leads to infective metastatic foci in different organs if not managed appropriately following approved guidelines with the help of clinical, microbiological and radiological parameters with recommended antifungal agents for a recommended duration of time and close follow up of patients (4,5,6).

Epidemiology in Europe, USA and Australia

Incidence

Candidaemia is a worldwide concern with rising incidence rates and high mortality. The incidence of systemic candida infection has increased over the past few decades. In 1996, the incidence rate of invasive candidiasis in the US was 23 per 100,000 population and by 2003 this had risen to 29 per 100,000 population. Data from the US also suggest that mortality associated with candida infection remains high, with reported crude mortality rates of 47.1% (1,3). In Europe and Australia, population-based surveillance data suggest that the incidence of candidaemia is lower than that seen in the US but also increasing. The countries participating in the ECMM (European Confederation of Medical Mycology) survey reported rates of candidaemia (0.02-0.38 per 1000 admissions and 0.31-0.44 per 10,000 patient-days) comparable with those reported in other European surveys but lower than rates reported in the USA (1.5 per 10,000 patient days) (2).

Risk factors

It had been noted that larger the hospital, the broader the patient population requiring invasive diagnostic and therapeutic interventions are associated with a greater risk for developing candida infection. In the ECMM survey similar to other reports, candida BSI predominates in males (60%). Infants younger than 1 year of age accounted for 8% of cases and patients older than 70 years for 28%. Candidaemia was often associated with a surgical intervention (48%), including solid organ transplantation and with intensive care treatment (40%). Patients with solid tumour or haematological malignancy accounted for a total of 35% of episodes. In identifying predictors of candidaemia associated mortality, an Australian study found: age $\geq$ 65 years, long duration of ICU stay, corticosteroid therapy and haemodialysis, to play a significant role (3).

Prevalent species

Over 90% of invasive candida infections are attributed to five species namely *Candida albicans*, *Candida glabrata, Candida parapsilosis, Candida tropicalis* and *Candida krusei*. *Candida albicans* is the most common causative species of invasive candidiasis. It remains the most significant cause of candidaemia in North America, Europe and Asia Pacific, accounting for 51%, 60% and 56% of clinical candidaemia isolates in these regions, respectively (1,3). However, the frequency of *Candida albicans* in clinical candidaemia isolates appears to be decreasing worldwide, whilst *Candida glabrata* and *Candida parapsilosis* are becoming increasingly common. *Candida tropicalis* is an important pathogen in patients with neutropenia and haematological malignancies and an increasing trend in prevalence of this organism has also been observed, especially in Asia Pacific (3).The ECMM survey showed that *Candida albicans* was responsible for more than one-half of the cases in all the patient populations, except in patients with haematological malignancies (2). In these patients, *Candida albicans* was isolated in 35% of the cases – in 17% and other *Candida species* in 24%, with *Candida krusei* involved

in one-half of these cases (12%) (2). The same pattern of species distribution was observed in the surveillance study performed by the European Organization for Research and Treatment of Cancer (EORTC). *Candida glabrata* was the most frequent non-albicans isolate in surgical (16%) and solid tumour (26%) patients, whilst *Candida parapsilosis* was isolated with the highest frequency in premature neonates (29%) (2). *Candida glabrata*, causing 145 of the episodes, was the second most common species recovered in all the countries in ECMM survey except in Italy and Spain where it ranked number 3 and 4 respectively. *Candida tropicalis* occurred in 7% of cases, ranging from 2 to 10%.

Antifungal sensitivity

Although *Candida albicans* is typically susceptible to most antifungal agents, resistance to fluconazole is increasing, and fluconazole is less effective against some non-albicans *Candida* spp., such as *Candida glabrata* and *Candida krusei*. *In vitro* antifungal resistance is rarely detected in European multi-institutional surveys. In ECMM survey, less than 2% of *Candida albicans* isolates were resistant both to flucytosine and fluconazole, and all were inhibited by amphotericin B. Among non-albicans *Candida isolates*, 5.32% and 10.29% were resistant to flucytosine and fluconazole, respectively. Old and new azoles appeared to be active against majority of blood stream isolates. As previously reported by them, fluconazole resistance is not always associated with resistance to the other azoles. In some cases only an increase in the MIC endpoint was observed. In addition, intrinsically fluconazole-resistant *Candida* spp. (*Candida krusei*, *Candida norvegensis* and *Candida inconspicua*) showed no cross resistance with other azoles (2,7). This low proportion of antifungal resistance detected in European survey is consistent with the data reported in US surveillance programme (2).

Treatment options for candidaemia

Triazoles, polyene and echinocandin antifungals comprise the treatment armamentarium against *Candida* spp. Treatment choice is controversial and depend on the clinical status of the patient, knowledge of infecting species or antifungal susceptibility of isolate, prior antifungal exposure and relative drug toxicity (6). The Infectious Diseases Society of America (IDSA) guidelines for the treatment of candidiasis recommend IV or oral fluconazole, IV amphotericin B, or an echinocandin namely caspofungin, micafungin or anidulafungin, as first-line therapies for candidaemia. Australian Guidelines for the Treatment of Invasive Candidiasis also recommend fluconazole as first-line therapy in non-neutropenic patients where the infecting organism is known to be fluconazole-susceptible. In neutropenic patients, or where the infecting organism is known to be fluconazole-resistant, an echinocandin is recommended. New antifungal agents are available to counter the rise of antifungal resistance and changing trends in candidaemia epidemiology (3,5). Extended-spectrum azole antifungals such as voriconazole are effective against *Candida albicans* and non-albicans *Candida* spp., and voriconazole also exhibits activity against fluconazole resistant pathogens (7). Voricinazole is indicated in Europe for the treatment of fluconazole-resistant invasive candida infections. Echinocandins are particularly effective against *Candida* spp. and the development of newer echinocandins such as anidulafungin provided further opportunity to improve treatment response and to decrease mortality rates in patients with candidaemia.

The increasing incidence of non-albicans candidaemia highlights the need to identify the infecting isolate and ensure appropriate treatment using an agent that is effective against that isolate (7). In ECMM survey, 84.5% of patients received antifungal treatment and others did not receive antifungals mainly because candidaemia was associated with terminal stage of the underlying disease. Fluconazole was used as the initial antifungal agent in the majority of cases (59%). The approach to antifungal therapy differed according to different countries in the survey. Intravascular lines were removed in 61.4% of the episodes. Candidaemia associated with intravenous lines and /or bioprosthetic devices is problematic since these devices act as substrate for biofilm. A total of 23 of 59 (39%) *Candida albicans* blood isolates from the ECMM study tested for biofilm production were good producers. No relationship between biofilm production and a specific pattern of antifungal susceptibility was detected (1,2).

Sri Lankan situation

Though antifungal agents are being used widely for the management of patients with candidaemia at different health care settings in the country, there is no well established surveillance system involving multiple centres in Sri Lanka at present. Quite a few surveys which have been done in Sri Lanka are limited to single institutions or specific patient populations over a limited period of time.

A prospective study was carried out for five years from January 2007 at the National Cancer Institute of Sri Lanka (NCISL) which is the final referral centre for malignancies. Fungal blood cultures collected from suspected candidaemia in patients with haematological malignancies, neutropenia and post surgery were processed. Speciation of isolates and antifungal sensitivities were performed at the Medical Research Institute (MRI).

Incidence of candidaemia was 1.28 per 1000 admissions in first three years and 0.99 and 0.56 per 1000 admissions in 2010 and 2011 respectively. In the five years, *Candida tropicalis* 19/515 (3.68%), 31/828 (3.74%), 32/825 (3.88%), 41/461 (8.89%) and 21/355 (5.92%), *Candida glabrata* 20/515 (3.88%), 21/828 (2.53%), 20/825 (2.42%), 02/461 (0.43%) and 01/355 (0.28%),*Candida albicans* 01/515 (0.19%), 03/828 (0.36%), 05/825 (0.60%), 07/461 (1.52%) and 03/355 (0.85%), *Candida parapsilosis* 01/515 (0.19%), 03/828 (0.36%), 02/825 (0.24%),00/461 (0%) and 01/355 (0.28%) were isolated in samples taken and one isolate of *Candida guilliermondii* in 2008 which is the first blood culture isolation of this *Candida* spp. in Sri Lanka. There were one each of *Candida famata* and *Candida haemulonii* in 2010. The most prevalent species was *Candida tropicalis* 31/59 (52.54%), 32/59 (54.23%), 41/52 (78.85%) and 21/26 (80.77%) from 2008 to 2011. In 2007, the most prevalent species was *Candida glabrata* 20/41 (48.78%) followed by *Candida tropicalis* 19/41 (46.34%). *Candida glabrata* was the second most prevalent species 21/59 (35.59%) and 20/59 (33.89%) in 2008 and 2009. Candida albicans was the second prevalent spp. 07/52 (13.46%) and 03/16(11.54%) in 2010 and 2011. Both *Candida albicans* and *Candida parapsilosis* were in the third position 01/41(2.44%) and 03/59 (5.08%) in 2007 and 2008.*Candida krusei* had not been isolated at NCISL though it had been isolated in 12% of patients with haematological malignancies in Europe. Majority of isolates were sensitive to commonly used antifungal agents locally namely fluconazole and amphotericin B (8). Though voriconazole is being used for the management of candidaemias in some patients the sensitivity of voriconazole is not performed routinely at MRI. Echinocandin, anidulafungin too is now available in Sri Lanka to be used on needy patients and its antifungal sensitivity too is not routinely performed at present.

This study concluded that the incidence is higher compared to the reported general incidence in Europe. The most prevalent species was *Candida tropicalis*. *Candida albicans* causing candidaemia in NCISL has gradually increased during the past two years. Currently used antifungals can be continued for the management unless there is a special need for other antifungal agents. This study revealed uncommon *Candida species* such as *Candida guilliermondii* one isolate in 2008 and *Candida famata* and *Candida haemulonii* one isolate each in 2010. It is reported that less frequently encountered non-albicans *Candida* spp. appear to be increasing and certain species for instance *Candida guilliermondii* has been reported to be less susceptible or resistant to antifungal agents thus local epidemiological trends have important implications for clinical management (11).

A study was carried out in paediatric patients with candidaemia at the Lady Ridgeway Hospital (LRH) which is the referral centre for children, for a period of 6 months from November 2012 to analyze the epidemiological data of patients with candidaemia and to speciate the isolates of *Candida* spp. Species identification was done at MRI.

It revealed that of 22 patients with positive blood cultures, 17 were clinically significant (73.9%). Six patients were neonates while 9/17 were in the age group of 1 month to 1 year. Male to female ratio was 10.7. Six (35.2%) were from ICU while others were inward patients. Patients had a variety of clinical conditions but candidaemia was more common among patients with congenital heart disease (6/17), lower respiratory tract disease (4/17) or sepsis (3/17). *Candida albicans* was isolated in only 2 (11.7%) patients while other isolates were non-albicans *Candida* spp. Of 8 germ tube negative isolates tested, one was *Candida albicans*, one was *Candida parapsilosis* and the other 6 were *Candida tropicalis*. It was concluded that non-albicans *Candida* is more common than *Candida albicans* among candidaemia patients at LRH (9). Though *Candida parapsilosis* is of special concern in critically ill neonates, causing more than one-quarter of all invasive fungal infections in low birth weight infants in the UK and up to one-third of neonatal candida blood stream infections in North America, this study had only one isolate of *Candida parapsilosis* probably due to the low sample size (12).

A ten year retrospective study was carried out at the Department of Mycology, MRI from 2001 – 2010 to determine the *Candida* spp. isolated in blood cultures received from different hospitals in the country and also to determine the changing pattern of *Candida* spp. during the past 10 years.

Out of 3137 samples fungal aetiological agents were identified in 392 samples (12.49% isolation rate). Among the 392 positive samples 366 samples yielded *Candida* spp. (93.6%). *Candida tropicalis* was the commonest species isolated (187/366 – 51.09%). Hundred and twenty four samples yielded *Candida glabrata* (33.87%). There were *Candida albicans* (40), *Candida parapsilosis* (08), *Candida guilliermondii* (03), *Candida famata* (01), *Candida luscitaniae* (01) and speciation not done (02) isolates. It was concluded that *Candida tropicalis* and *Candida glabrata* show higher isolation rates than *Candida albicans* in contrast to the past few decades. Uncommon species such as *Candida guilliermondii*, *Candida famata* and *Candida luscitaniae* were isolated in this study. In a prospective surveillance of all episodes of candidaemia within Australia undertaken during the period of August 2001 and July 2004, it was found that immunocompromised patients with haemato-logical malignancy were at particular risk of *Candida luscitaniae* or *Candida guilliermondii* candidaemia.

Candida luscitaniae infection has also been reported to be associated with prior antifungal treatment (11).

Conclusion and recommendation

The incidence of candidaemia has increased over the past decades and there has been a shift towards non -albicans *Candida* spp. as the cause of it. Fluconazole remains the primary therapy chosen for candidaemia but increasing resistance and the rise of non-albicans *Candida* spp. limit its efficacy. Extended-spectrum azoles play a role in treating invasive candidiasis, and echinocandins provide a therapeutic alternative to polyene and other azole agents.

In order to monitor the epidemiological, clinical and antifungal susceptibility data in different risk groups managed in different health care settings in the country, a well planned surveillance system is needed. A prospective epidemiological surveillance using a common database and methodologies can monitor trends in incidence and changes in species distribution and identify new groups of patients who are at risk for candidaemia. Evaluation of the impact of the introduction into the market of new antifungal agents could also be carried out with the introduction of an appropriate surveillance system. Monitoring epidemiological parameters with timely interventions together with rational use of antifungal agents will enable us to win the challenges of nosocomial candidaemia in the future.

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AN UPDATE ON ENTERIC FEVER

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Aetiological agents

The causative agents of enteric fever are *Salmonella typhi*, *Salmonella paratyphi* A,B and C. They belong to the family Enterobacteriacae and genus *Salmonella* (1). The genus S*almonella* are divided in to two species *Salmonella enterica* and *Salmonella bongori*. *Salmonella enterica* contains six subspecies (1,11,111a,111b,1V and V1). *S. enterica* subspecies 1 contains most of the human pathogens and the enteric fever causing serotypes belong to this subspecies 1.

They are Gram negative, non spore forming, facultatively anaerobic bacilli. They are oxidase negative motile, non lactose fermenters. They ferment glucose, reduce nitrates similar to other members of the family Enterobacteriacae. *S. paratyphi* B has two phenotypically different *Salmonellae*. *S. paratyphi* B strain which is lysine +ve and tartrate +ve is known as S*almonella java* and it is generally associated with gastroenteritis. The classical tartrate negative phenotype is generally invasive and causes enteric fever.

Epidemiology

Enteric fever has a worldwide distribution but is more common in developing countries. Unlike other *Salmonella* species these serotypes are exclusively human pathogens. The organism is transmitted by faeco-oral route. Most commonly the transmission occurs via either food borne or water borne route mainly from asymptomatic carriers. Although direct contact transmission is rare *S. typhi* can be transmitted by sexual contacts by anal and oral sex. Infective dose is small which varies from 1000-1,000,000 bacilli. The risks of infection include consumption of contaminated water or ice, food and drinks prepared by street vendors, raw fruits and vegetables, flooding, poor hygienic habits and close contact with an ill person or asymptomatic carrier.

The global burden of enteric fever is considered to be 22 million cases with 200,000-600,000 deaths and 6 million cases of paratyphoid fever annually (1,2,3). The incidence ranges from 25-1000 cases /100,000-population in endemic region (1). In Indonesia the annual incidence is 900,000 cases with 20,000 deaths (4). Regions with high incidence of typhoid fever (>100/ 100000/year) include South Central Asia and Southeast Asia. Regions with medium incidence (10- cases / 100000 / year) includes the rest of Asia, Africa, Latin America, Caribbean and Oceania excluding Australia and New Zealand. Europe, North America and the rest of the developed world has a low incidence (<10 cases/100000/ year). Although the overall ratio of disease caused by *S. typhi* to that caused by *S. paratyphi* is about 10 to 1, the proportion of *S. paratyphi* infections is increasing in some parts of the world (3).

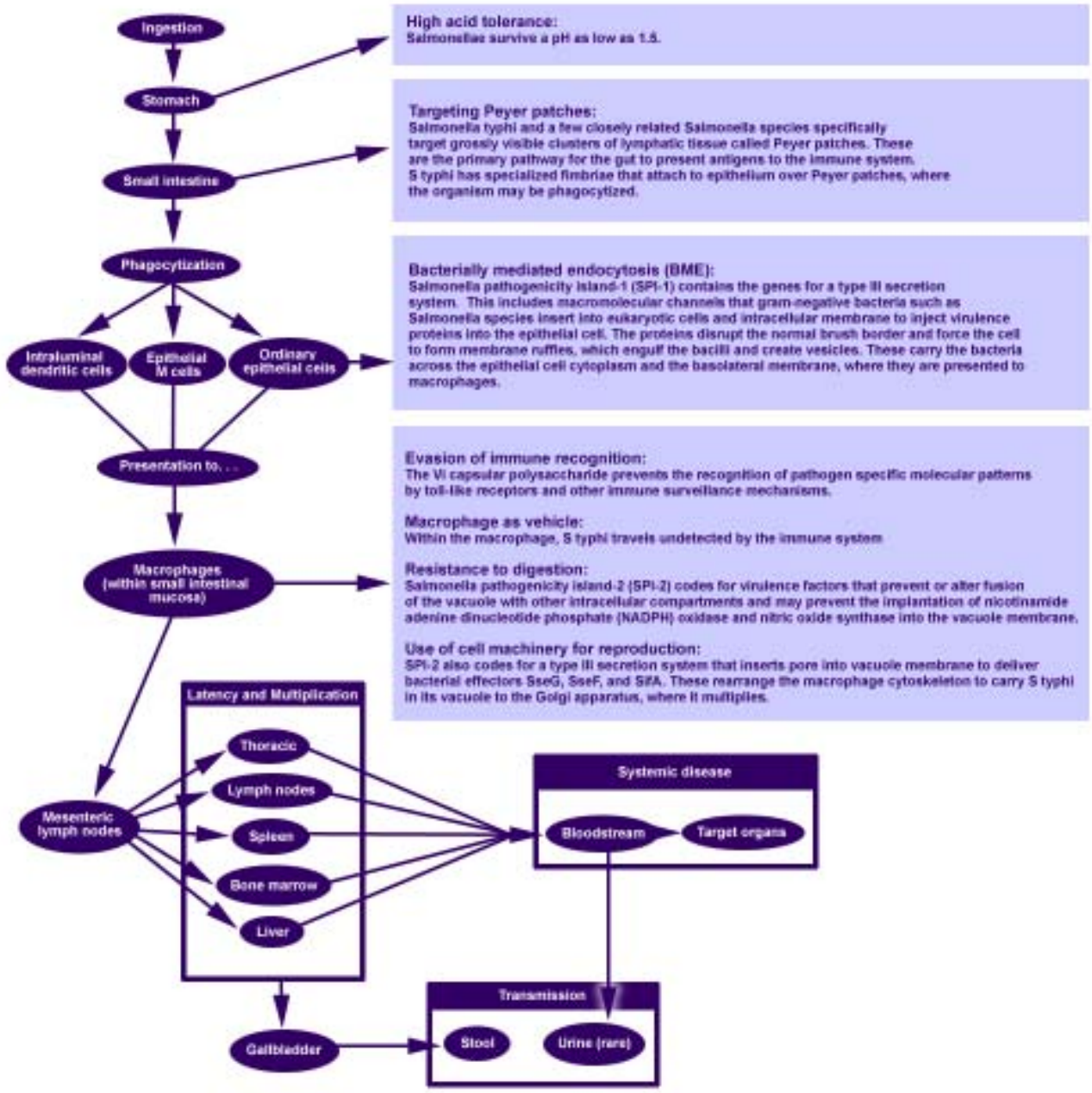
Pathogenesis

The incubation period varies from 5-21 days. Once the *Salmonellae* enters orally it bypasses the acid barrier of the stomach and other secretory products of the intestine to cross the mucus layer of the intestine. They enter the enterocytes and M cells by a process called “bacterially-mediated endocytosis”. Subsequently the organism interact with the macrophages and lymphocytes in Peyer’s patches. Dissemination of organism to blood stream occurs through the thoracic duct. Eventually the organisms are taken up by tissue macrophages in the bone marrow, liver, spleen and payers patches. The salmonellae are capable to survive within macrophages and this ability helps the organism to spread the organism to the systemic circulation.

Clinical features

Enteric fever is a systemic infection. The disease is characterized by fever. Non specific symptoms such as chills, dull frontal headache, anorexia, weakness, cough, sore throat, dizziness may present before the onset of fever. The presentation of enteric fever may be altered by co-morbidities and early administration of antibiotics. Initially fever is low grade and rises during the second week of illness. Fever may continue up to 4 weeks and some may resolve without antibiotic treatment. Constipation is present in 10 -38% of cases. Abdominal pain is present in 30-40% of patients.

On examination patients may appear acutely ill. 20-30% will have hepatosplenomegaly. 50% of patients may have relative bradycardia which is neither a specific nor a sensitive sign of typhoid fever. About 30% of patients may have rose spots on the trunk by the end of first week of illness which resolves after 2-5 days. Some may develop cervical lymphadenopathy (1).



Fugure. Pathogenesis of typhoid fever – Adopted from Medscape 2011 (5).

10-15% may have a severe form of the disease depending on host factors such as immuno-suppression and antacid therapy, virulence and infective dose of the organism. Gastro intestinal bleeding and intestinal perforations are the common complications of enteric fever which occurs during the third or fourth week of the illness. Gastrointestinal bleeding occurs up to 10% of patients. In the majority of patients bleeding is minimum but in 2% of patients bleeding is clinically significant and rapidly fatal if a large vessel is involved. Intestinal perforation occurs in 1-3% of patients (6).

Encephalopathy often accompanied by shock is associated with high mortality. It is another serious complication of typhoid fever. The rare complications include disseminated intravascular coagulation, endocarditis, pericarditis, myocarditis, hepatic and splenic abscesses, hepatitis, parotitis, pneumonia, arthritis and osteomyelitis. Typhoid fever during pregnancy may be complicated by miscarriage, although antimicrobial treatment has made this outcome less common. Vertical intrauterine transmission from an infected mother may lead to neonatal typhoid, a rare but severe and life-threatening illness in neonates.

Relapse occurs in 5 to 10 percent of patients, usually two to three weeks after the resolution of fever. The relapse is usually milder than the original attack, and the *S. typhi* isolate from a patient in relapse usually has the same antibiotic-susceptibility pattern as the isolate obtained from the patient during the original episode.

Diagnosis

Isolation of *S. typhi* or *S. paratyphi* A, B and C from blood, bone marrow, stool or urine will give a definitive diagnosis of typhoid or paratyphoid fever. The organism can also be cultured from rose spots or other sterile fluids. The sensitivity of blood culture varies from 40-80%. Clot culture, culture of buffy coat and lysis centrifugation methods have variably improved the sensitivity and reduction of time needed for isolation of the organisms from blood (1). Sensitivity of bone marrow culture is 55-90%. The duodenal string test will have a sensitivity around 58%. Stool culture are positive in 30% of patients during the third week of illness (6). Combine culture of blood or bone marrow together with stool culture will improve the culture positivity in about 90% patients.

A number of serological tests are available to assist the diagnosis of enteric fever. The Widal test that is used over 100 years is still being used by many clinicians. The sensitivity and specificity of this test is 47-77%, and 50-92% respectively (1). This test can be negative in 30% of culture proven typhoid patients (4). False positives can occur in infections with none typhoidal *Salmonellae* and other *Enterobacteriacae* species and also in patients with malaria, typhus fever and cirrhosis.

New tests to detect IgM are available for rapid diagnosis. Molecular methods such as DNA probe and PCR are also helpful in the rapid diagnosis of enteric fever.

Other non specific laboratory abnormalities include leucopenia, anemia and elevated liver functions tests.

Chronic carrier state

Up to 10 percent of convalescing patients with untreated typhoid excrete *S. typhi* in the feces for up to three months. Approximately 1-4% of people becomes chronic carriers and are responsible for dissemination of infection to others. Chronic carrier is defined as the person who excretes the organism in feces for more than a year. The incidence is higher among women, in persons with biliary tract abnormalities, gallstones, carcinoma of the gallbladder and other gastrointestinal malignancies. It is also common in patients older than 50 years and patients with schistosomiasis. These carriers have high antibody titre against Vi antigen of Widal test and is useful in diagnosis of chronic carrier state. Up to 25% of these carriers do not give a history of typhoid fever (6).

Eradication of carrier state can be done by giving ampicillin or amoxicillin 100 mg/kg/day with probenecid 1gPO for six weeks or cotrimoxazole 160-800 mg twice a day for six weeks (4). These two regime will eradicate 60% of carriers. Ciprofloxacin 750 mg given over 28 days will eradicate 80% of carrier state (7). Surgery may be needed for those who have anatomical abnormalities in order to eradicate the carrier state.

Antibiotic resistance

In 1948 chloramphenicol became the standard antibiotic for treating typhoid fever. Although resistance emerged within two years after its introduction, chloramphenicol-resistant typhoid fever became a major problem only in 1972. Chloramphenicol resistance was associated with high-molecular-weight, self-transferable, *Inc*HI plasmids. Toward the end of the 1980s and the 1990s, *S. typhi* developed resistance simultaneously to all the drugs that were then used as first-line treatment (chloramphenicol, trimethoprim-sulfamethoxazole and ampicillin). Outbreaks of infections with these strains occurred in India, Pakistan, Bangladesh, Vietnam, the Middle East and Africa.

There have been sporadic reports of high-level resistance to ceftriaxone (minimal inhibitory concentration [MIC], 64 mg per liter) in *S. typhi* and *S. paratyphi* A.

S. typhi strains with reduced susceptibility to fluoro-quinolones have become a major problem in Asia. An outbreak of typhoid with such strains in Tajikistan in 1997 sickened 8000 people in a six-month period and caused 150 deaths (6). Quinolone resistance is frequently mediated by single point mutations in the quinolone-resistance-determining region of the gyrA gene.

The strains that are resistant to nalidixic acid but susceptible to fluoroquinolones according to disk diffusion-testing should be considered as resistant to quinolones. Although resistance to nalidixic acid (minimum inhibitory concentration > 256 mg/l) remains an important marker for failure of fluoroquinolone treatment in typhoid fever, some authors have found several isolates that showed reduced susceptibility to fluoroquinolone while remaining susceptible to nalidixic acid (8). These findings highlights the importance of estimating the minimum inhibitory concentration of the agent selected for treatment.

Treatment

Early diagnosis and appropriate antibiotic therapy are necessary to prevent complication and reduce the fatality rates. The choice of antibiotic should be according to the susceptibility patterns of isolated organism or depending on the local susceptibility data. If susceptible floroquinolones are effective with a cure rate of 98%. However, quinolone resistance is increasing in Asian countries making this ineffective agent against enteric fever. Overuse and misuse of quinolnes by practitioners and the over the counter

availability of drug has lead to increase incidence of quinalone resistance. In the laboratory nalidixic acid sensitivity should be used to determine the sensitivity for quinalones. If the infection is due to a strain which is resistant to quinalones the patient should be treated with either ceftriaxone 2 g daily or cefixime 200 mg twice daily for 10-14 days days. Azithromycin 500 mg daily for 7 days can be used to treat uncomplicated typhoid fever (3). Compared with ceftriaxone, azithromycin significantly reduced relapse but not other outcome measures. Few adverse events were reported, and most were mild and self limiting (9).

Vaccines

Ttyphoid vaccine can be offered for travelers to areas where there is an increased risk of exposure to *S. typhi*. The typhoid vaccines do not protect against *S. paratyphi* infection. Three types of typhoid vaccines are available. Protection against typhoid fever by these vaccines varies from 50-80% of recipients.

1) Oral live, attenuated vaccine -Ty21a strain of *S. typhi*
2) Vi capsular polysaccharide vaccine (ViCPS) for intramuscular use
3) Killed whole cell vaccine

Primary vaccination with oral Ty21a vaccine consists of 4 capsules, 1 taken every other day. The capsules should be kept refrigerated (not frozen), and all 4 doses must be taken to achieve maximum efficacy. Each capsule should be taken with cool liquid no warmer than 98.6°F (37°C), approximately 1 hour before a meal. This regimen should be completed 1 week before potential exposure. The vaccine manufacturer recommends that Ty21a not be administered to infants or children aged <6 years.

Primary vaccination with ViCPS consists of one 0.5 ml (25 mg) dose administered intramu ≥2 weeks before expected exposure. The manufacturer does not recommend the vaccine for infants and children aged <2 years.

Adverse reactions to Ty21a vaccine are rare and mainly consist of abdominal discomfort, nausea, vomiting, and rash. ViCPS vaccine is most often associated with headache (16-20%) and injection-site reactions (7%). No information is available on the safety of these vaccines in pregnancy. Vaccination with Ty21a should be delayed for >72 hours after the administration of any antibacterial agent.

The killed whole cell vaccine is not currently in use due to many adverse effects. These adverse effects are due to the LPS of the cell wall.

Dosage and schedule for typhoid fever vaccination

Vaccination	Age (y)	Dose/mode of administration	Number of doses	Dosing interval	Boosting interval
Oral, live, attenuated Ty21a vaccine[1]					
Primary series	≥6	1 capsule,[2] oral	4	48 hours	Not applicable
Booster	≥6	1 capsule,[2] oral	4	48 hours	Every 5 years
Vi capsular polysaccharide vaccine					
Primary series	≥2	0.50 ml, intramuscular	1	Not applicable	Not applicable
Booster	≥2	0.50 ml, intramuscular	1	Not applicable	Every 2 years

[1]The vaccine must be kept refrigerated (35.6°F-46.4°F, 2°C-8°C).
[2]Administer with cool liquid no warmer than 98.6°F (37°C).

Adopted from CDC yellow book 2012

Enteric fever situation in Sri Lanka

Since 2007 it was noted that the prevalence of *S. paratyphi* A infection is rising in Colombo district and suburbs. The number of cases was gradually increasing over the last few years. During the period of 2007–2010, 293 culture positive *S. paratyphi* and 54 *S. typhi* cases were reported from National Hospital Colombo, North Colombo Teaching Hospital and Sri Jayewardenepura Hospital (10). Colombo South Teaching Hospital had reported 95 culture proven *S. paratyphi* and 39 *S. typhi* cases during 2006-2009 period (11). During the year 2006 CSTH had only 3 cases of *S. paratyphi* and this shows that the increase in incidence of paratyphoid fever in Colombo had started since 2007.

According to the antimicrobial surveillance project that has been commenced by the Sri Lanka College of Microbiologists during the year 2009 out of the total number of enteric fever pathogens 86% of isolates from adults were *S. paratyphi* A whereas *S. typhi* was the predominant isolate among children (85.7%). Five hospitals scattered around the country participated in this study (unpublished data).

According to the studies done in Colombo district a male preponderance was noted ranging from 64.5-67% (10,12). Adults were more commonly affected than children (10). Among the patients presented to the National Hospital of Sri Lanka, Colombo all the patients presented with fever. 50% of them had fever for more than 7 days whereas 31% had fever for more than 2 weeks. 37% of patients complained of abdominal pain and 24.1% patients had loose stools and vomiting. Only 13.7% had the classical head ache described for typhoid fever (12).

All the above studies have reported high resistant rates for ciprofloxacin in *S. paratyphi* ranging from 98-100% and 50% in *S. typhi*. Sensitivity of ceftriaxone varied from 89-100% in *S. paratyphi* and was 100% in *S. typhi*. According to the antimicrobial surveillance project data sensitivity to chloramphenicol and cotrimoxazole among *S. paratyphi* was 100% where as *S. typhi* showed 63% and 72.2% sensitivity for cotrimoxazole and chloramphenicol respectively. Wide use of ciprofloxacin use in the community may have lead to the high incidence of quinalone resistance of these organisms.

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RESEARCH ARTICLE

SYNOPSIS OF FINDINGS FROM A STUDY OF LEPTOSPIROSIS IN SOUTHERN SRI LANKA

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Introduction

Leptospirosis is perhaps the most common zoonosis in the tropics, where the pathogenic spirochete *Leptospira interrogans* is able to readily persist in the environment (1). Furthermore, continuous exposure is frequent in poor persons living among infected animals that excrete leptospires in their urine.

Sri Lanka has an estimated annual incidence of leptospirosis of 5.4 per 100,000, the sixth highest in the world (2). Many (>25%) members of Sri Lanka's workforce are agricultural workers, and the incidence of leptospirosis appears to coincide with rain and harvest cycles. Historically, the annual incidence has been ~200 cases per million, mostly from the hyperendemic South and North-central regions (2). However, rates are rough estimates, because leptospirosis mimics other causes of fever and few cases are laboratory-confirmed (1). In the recent past, clinical cases have been increasingly recognized, including >7000 cases in 2008 (3).

To determine the burden of and features associated with leptospirosis in Southern Sri Lanka, we obtained epidemiologic and clinical data and acute and convalescent (paired) sera prospectively from children and adults presenting with acute fever. We herein summarize findings detailed elsewhere (4).

Methods

We enrolled patients presenting to the Emergency Department, clinics and medical wards of Galle's Teaching Hospital Karapitiya between March and October 2007. Febrile (38°C, tympanic) patients ≥2 years old without antecedent (within 7 days) trauma or hospitalization were eligible. Using a consent form and protocol approved by the institutional review boards of Ruhuna University, Johns Hopkins University, and Duke University Medical Center, we obtained written informed consent from patients (≥18 years) or parents (<18 years) and assent if 12 -17 years. We recorded epidemiologic and clinical data, and obtained blood for serology at the acute visit; thereafter (2-4 weeks later), we obtained a second serum sample.

We assayed acute and convalescent sera for the presence of specific anti-leptospiral IgM antibodies by ELISA (Institut Viron Serion GmgH, Warburg, Germany) per the manufacturer's instructions. Rheumatoid factor (RF)-absorbent was first diluted 1:4 in buffer. Sera from patients and controls were then diluted (1:100) in RF-absorbent buffer to accomplish removal of IgM RF, transferred to antigen-coated microtest wells, and incubated at 37°C for 60 minutes. After washing with phosphate buffered saline, anti-human IgM conjugated to alkaline phosphatase and para-nitrophenylphosphate was added. After 20 minute incubation, NaOH was added to each well to stop the reaction and the absorbance at 405 nm measured.

The ELISA yielded qualitative results – positive, negative and equivocal. The optical density (OD) measurements, adjusted for plate-to-plate variation with a correction factor, gave quantitative results that correlated with titres by using a standard curve and evaluation table provided with the kit (5).

Patients with seroconversion (negative acute phase to positive convalescent phase sera) or the equivalent of a 4-fold rise in IgM titre were determined to have acute leptospirosis and those with stable or decreasing IgM titres past leptospirosis.

Statistical analyses were done with Stata IC 11.0 (StataCorp, College Station, TX).

Results

Acute and convalescent sera were available for 889/1079 (82.4%) patients enrolled. Of these, 116 had inconclusive serologic results; therefore, a diagnosis of acute leptospirosis could be confirmed or rejected for 773/889 (87.0%). Patients who returned for follow up were similar to those who did not. The median age of the 773 patients was 30.1 years (IQR, 19-47). Reported illnesses were short (median reported duration of fever and of illness 3 days [IQR, 2-5 and IQR, 2-7, respectively]); convalescent samples were obtained at a median of 21 days (IQR, 15-33).

One hundred and twenty patients had acute leptospirosis. Few were clinically suspected (sensitivity and specificity of clinical impression 22.9% [95% CI, 15.4-32.0] and 91.7% [95% CI, 89.2-93.8], respectively). Most of the 279 patients who were leptospiroisis IgM-positive at enrollment had past leptospirosis rather than acute leptospirosis. Hence, the sensitivity and specificity of acute-phase IgM for acute leptospirosis was poor (sensitivity 17.5%, 95% CI, 11.2-25.5) and specificity 69.2%, 95% CI, 65.5-72.7).

Headache was the symptom reported most often (~80%), but was equally common in those with and without acute leptospirosis (p=0.63). Muscle pain and joint pain were also common (present in >50%) and more frequent (p<0.005 and <0.01, respectively) in those with leptospirosis. Lethargy and cough were reported less frequently in those with acute leptospirosis, whereas oliguria, dysuria, and muscle and joint pain more often. Conjunctival suffusion (RR 2.4; 95% CI, 1.7-3.4; (p <0.0001) was associated with acute leptospisosis, whereas pharyngeal exudates were not. Jaundice, splenomegaly, arthritis, rash, and meningismus were uncommon in both groups. Patients with and without acute leptospirosis had similar leukocyte counts (7800 and 7900 per μL, respectively), slightly lower hemoglobin concentrations (12.3 and 12.6 g/dL, respectively) and platelet (200 vs 231 x 1000 per μL, respectively) counts and lower absolute lymphocyte counts (1638 vs 2140 per μL, respectively) than did other patients.

No one with confirmed acute leptospirosis died, but most (11 of 12) deaths occurred before convalescent follow up. Among those who died, the acute-phase serum was IgM-negative in 8, positive in 2, and equivocal in 1.

Those with IgM evidence of leptospirosis were older than others (median 32 vs 27 years, respectively). Exposure to paddy fields (relative risk [RR] 1.9; 95% CI, 1.6-2.1; p<0.0001) and working as a farmer (RR 1.9, 95% CI, 1.6-2.3; p=0.0001) were strongly associated with both acute and past leptospirosis. Absence of fresh water exposure and boiling drinking water were associated with lower risk of acute leptospirosis. Patients enrolled in July, August, September, and October were more likely to have acute leptospirosis. Those <10 years of age were much less likely than older persons to have leptospirosis IgM antibody.

Discussion

Leptospirosis was a common (13.5%) but unsuspected cause of acute febrile illness in our Southern Sri Lankan cohort. Testing acute sera only for IgM would have been misleading, since more often associated with past rather than acute leptospirosis. The diagnostic standard for acute leptospirosis is a definitive rise in titre between paired sera (1), since isolation of *Leptospira* requires special media, prolonged incubation (up to 13 weeks) and has low sensitivity (1).

Microscopic agglutination test (MAT) has historically been the standard serologic method. However, MAT may be less sensitive than IgM ELISA, even in convalescent specimens, since it detects both IgM and IgG (1) and IgG is more variably produced than IgM (12). Relative to isolation of *Leptospira*, the sensitivity of MAT for acute, late acute, and convalescent sera has been reported as 30, 63, and 76%, respectively, and of IgM ELISA 52, 89, and 93%, respectively in one study in Barbados (6); in another study, single acute-phase MAT and IgM ELISA were comparable (49%) (7). With MAT, sera are reacted with live antigen suspensions of different leptospiral serovars and, after incubation, the serum-antigen mixtures are examined and titres determined. MAT requires estimation of the highest dilution of serum at which 50% agglutination occurs, which is labor-intensive and requires judgement (1). Sensitivity is compromised if all locally-relevant serovars are not represented. Laboratory-acquired infections have occurred (1). Further, MAT requires a dark-field microscope, which is unavailable in most laboratories. We tested paired sera by IgM ELISA, which requires only an inexpensive plate reader, is relatively easy to perform, and provides objective, reproducible results (7). Further, IgM ELISA on paired sera from patients from varied geographic regions has compared well (sensitivity 86.5%, specificity 97.0%) with MAT (8). We assayed paired IgM instead of IgG, since the kinetics of IgG are more variable (9). Additionally, some patients with culture and MAT-confirmed leptospirosis never develop IgG (9). However, those with fulminant illness may still die before seroconversion occurs.

We chose a commercially-available IgM ELISA that is likely to detect serovars present in Southern Sri Lanka. In central Sri Lanka, reported predominant serovars include Mednensis and Hardjo, but also Australis, Ballum, Canicola, Celledoni, Cynopteri, Pomona, Robinsoni (10), and Icterhaemorrhagiae (11). The assay has reliably reacted with serovars Icterohaemorrhagiae, Canicola, Grippotyphosa, Bataviae, Pomona, Tarassovi, Copenhageni, Bratislava, Hebdomadis, Sejroe, Australis, Panama, Pyrogenes, Patoc, Hardjo, and Cynopteri (5, 12).

The use of acute-phase IgM to identify acute leptospirosis in endemic areas is complicated by the existence of pre-existing antibody. Although some have suggested using a higher cut-off (e.g., 800) to differentiate between acute and past infection (1), data to support this strategy are lacking.

A different diagnostic standard may have yielded different results. The pitfalls of culture have been discussed. If a wider array of serovars is circulating in Southern Sri Lanka than are detected by the ELISA used, we may have underestimated burden; however, no other available commercial assay would be expected to have performed better.

We conclude that leptospirosis is an important cause of fever in Southern Sri Lanka. Identification of acute-phase IgM is a poor tool for the diagnosis of acute leptospirosis, since both non-specific and insensitive. Clinical identification of leptospirosis is difficult. Hence, better tests for rapid diagnosis are needed.

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CASE REPORTS

VRE: A SUPER BUG INFECTION FOLLOWING COLONIZATION

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Introduction

A 28-year old lady was transferred from a local hospital to the Intensive Care Unit (ICU) of Princess Alexandra Hospital, Australia. On admission she was febrile with a temperature of 39 °C, her Glasgow coma scale was 6, respiratory rate was 50 per minute, pulse rate was 131 per minute, and oxygen saturation was 67% hence the diagnosis was type 1 respiratory failure. Chest X ray showed bilateral lung infiltrates while influenza A H1N1 DNA detected from nasopharyngeal aspirate.

The patient had to be managed connected to extracorporeal membranous oxygen (ECMO) providing instrument for more than two weeks and by that time she had been on intra venous ceftriaxone, azithromycin and oseltamivir which she was started initially.

Despite of antimicrobial treatment the patient was febrile continuously. Routine screening carried out for multi resistant organisms was positive for vancomycin resistant enterococci (VRE). As the patient was confirmed colonized with VRE, the clinical team in charge changed the treatment to linezolid and gentamicin and isolated the patient. Although septic screening done culturing specimens from different sites in order to isolate the probable causative organisms was negative initially, later VRE organisms were isolated from blood and urine specimens.

Both isolates from the clinical samples had minimal inhibitory concentrations (MIC) of 32 μg/ml and 2 μg/ml for vancomycin and teicoplanin respectively. All VRE organisms isolated from the patient were of Van B phenotype.

At the time of discharge, the patient had spent 40 days in ICU, had received long term expensive anti-biotics and was nursed isolated with VRE control precautions.

Discussion

The super bug vancomycin-resistant enterococci (VRE) was first reported in France and Europe in 1988 (1). Out of all enterococcal species, most of the human infections are caused by *Enterococcus faecalis* (80-90%) and *Enterococcus faecium* (5-10%).

Although multiple factors predispose to infection with VRE, colonization precedes most. In the United States, nosocomial transmission of VRE from patient to patient had been reported (2). A study confirmed that, during the routine examination of patients colonized with VRE, 67% of examiner's gowns, gloves, and stethoscopes became contaminated with the organism (3).

To minimize the colonization and subsequent infections with VRE, all the patients at potential risk are screened in Australian health care setting. Rectal, perineal and groin swabs or faeces (with a special request) are collected and transported to the laboratory. In the laboratory the swabs are added to VRE (Enterococcosel) broth containing 6 mg/L of vancomycin, azide and esculin. After 24 hours of incubation VRE broths which have been changed in to black colour only are subculture on Colombia colistin nalidixic acid (CNA) agar plates. All the preliminary tests are carried out for the isolated colonies. VRE are Gram positive, catalase positive, L-pyrrolidonyl-beta-napthylamide (PYR) test positive organisms. The suspected VRE are identified by VITEK 2 automated machine which provides a definitive identification of organisms. MIC for vancomycin and teicoplanin is detected by epsilometer test (E test). The isolates reporting MIC for vancomycin more than 4 μg/ml are sent to the central laboratory for genotyping which confirms VRE.

Enterococci acquire resistance to several classes of antibiotics either by mutation or by receipt of foreign genetic material through the transfer of plasmids and transposons (4). Six different types of vancomycin resistance are shown by VRE, namely Van-A, Van-B, Van-C, Van-D, Van-E and Van-F. Of these, only Van-A, Van-B and Van-C have been seen in clinical practice. The importance is that Van-A VRE is resistant to both vancomycin and teicoplanin, Van-B VRE is resistant to vancomycin but sensitive to teicoplanin, and Van-C is only partly resistant to vancomycin, and sensitive to teicoplanin.

Active surveillance of patients on ICU admission would be expected to reduce levels of VRE colonization and could greatly reduce the number of VRE outbreaks (5). It is often considered that nosocomial VRE infections cannot be controlled. But a continuous study of VRE for 5 years at a tertiary care medical centre documented that not only can it be controlled initially, but this control can be sustained for long periods by preventing transmission (6). Further the study concluded the primary reservoir for the spread of VRE within a health care facility consists of symptomatically colonized patients.

The increasing rate of VRE has been associated with higher treatment costs, prolonged morbidity, and greater mortality. Screening for colonization of these organisms allows early detection and reduce the risk of patient to patient spread and can avert outbreaks. Health care personnel may pay extra attention on infection control practices if the VRE status is known among our patients.

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HAEMOPHAGOCYTIC SYNDROME

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Introduction

We describe a case of haemophagocytic syndrome (HPS) in a 29-year old male presented with continuous fever following chicken pox. Two weeks after the onset of chicken pox he was admitted to another hospital with continuing fever. According to the health record card the chickenpox rash has settled by the time of his first presentation. Since admission he was extensively investigated for pyrexia of unknown origin. Lymphadenopathy (cervical, supraclavicular, right epitrochlear) was the abnormal clinical finding. CT scans of the abdomen and chest showed multiple enlarged para-aortic lymph nodes. The histology of the cervical lymph node biopsy revealed granuloma formation and necrosis. It stated that the possibility of *Mycobacterium tuberculosis* (TB) cannot be excluded. Bone marrow biopsy was normal except for increased macrophage activity. Blood cultures were sterile. The patient was started on tuberculosis treatment (Rifampicin, Isoniazid, Pyrazinamide and Ethambutol) empirically as the fever was persisting. He was treated with a course of intravenous meropenem as well.

He was transferred to our hospital for further evaluation while on the 10th day of antituberculosis treatment.

On admission he was having fever for more than one month, dry cough and vomiting. He also complained of bilateral ankle joint pain. On examination he was ill looking, febrile, not icteric and not pale. Ankle joint examination was normal. No skin rashes were noted except for few healed chickenpox lesions. Liver was enlarged 2 cm below the costal margin. It was non tender. Cardiovascular and respiratory systems were normal. As the patient developed a reaction and elevation of liver enzymes presumably to anti TB drugs,

his drugs were changed to Streptomycin, Ofloxacin and Ethambutol.

Patient's condition deteriorated on the third day of admission. He became dyspnoic with diffuse crepitations in both lung fields. Chest X ray showed changes compatible with acute respiratory distress syndrome. He was transferred to the Intensive Care Unit.

Anti TB medications were stopped and he was started on Meropenem 500 mg tds with IV Hydrocortisone 100 mg 6 h. On the same day he had a generalized tonic clonic fit. He was paralysed and ventilated. Full blood count on Day 3 showed pancytopaenia (Hb – 3.6g/dl, WBC – 2300/ml, platelet – 57,000/ml). In the 2D ECHO global hypokinesia was reported. CT Brain was normal. Cerebrospinal fluid analysis was normal and CSF culture was sterile. Patient was started on IV Acyclovir. Repeat CXR showed progressive alveolar shadows.

CT scan of the chest showed bilateral pneumonia. The other investigations revealed ESR of 105 mm, Serum ferritin of 5690 µg/l, CRP of 6.5 (normal <6.0) and low total cholesterol with high triglycerides. Plasma fibrinogen was normal.

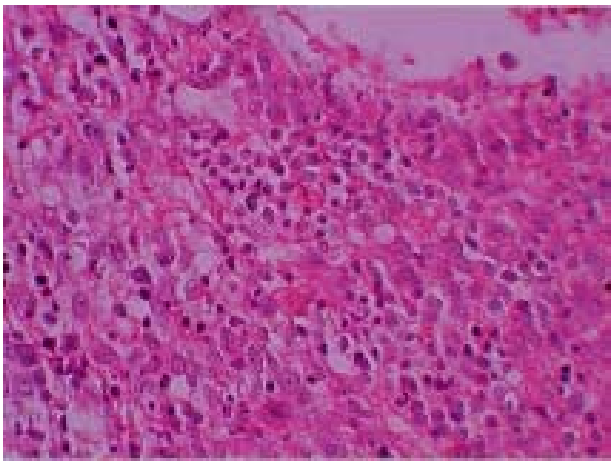

Figure. Bone marrow showing >15% of phagocytes engulfing haemopoietic elements.

Intravenous vancomycin was added as there was no improvement. Bone marrow biopsy was repeated as there was no response to the treatment. It showed features suggestive of haemophagocytic syndrome. Fourth day in the ICU the patient's condition further deteriorated with acute renal failure (potassium – 6.5 mmol/L). Blood pressure was low and inotrophs were commenced. Blood culture grew a probable *Acinetobacter spp*., isolated after 48 hours of incubation. He was started on cefeperazone-sulbactam. All antibiotics were adjusted to his renal condition and vancomycin was omitted with blood culture results. Haemofiltration was planned but withheld due to unstable haemodynamic status of the patient. Peritoneal dialysis was started while the patient was on four ionotrophs. Serum creatinine was rising (241.2µmol/L) with potassium 7.3 mmol/L. Packed RBC transfusions were given as the haemoglobin was low. Intravenous immunoglobulin and IV Dexamethasone were started with antibiotics. By fifth ICU day blood counts were rising (total WBC count-12 260/ml). Fever settled.

On sixth ICU day there was no fever and WBC count was 15000/ml. Platelets also started to rise but Potassium level was persistently high. Inotrophs could not be tailed off as the blood pressure was low. Patient rapidly deteriorated on day five and he sustained a cardiac arrest and resuscitation failed.

We rarely come across haemophagocytic syndrome patients but this condition is entertained in the differential diagnosis of patients with decreased cell counts in full blood report. Heamophagocytic syndrome or haemophagocytic lymphohistiocytosis (HLH) is an uncommon life threatening hyperinflammatory syndrome caused by severe hypercytokinaemia due to a highly stimulated but ineffective immune response. This immune dysregulatory disorder is prominently associated with cytopaenias and a unique combination of clinical signs and symptoms of extreme inflammation (1).

The incidence was reported as 1.2 cases per million individuals per year but there is a possibility of false low incidence as this condition is difficult to diagnose (2).

Haemophagocytosis is phagocytosis by macrophages of erythrocytes, leucocytes, platelets and their precursors in bone marrow and other tissues. This is a non specific condition found in conditions like haemolytic anaemia, malignant diseases, infections and haemophagocytic syndrome.

This is not a single disease entity but a clinical syndrome that can be associated with variety of underlying conditions leading to the same characteristic hyperinflammatory phenotype with hypercytokinaemia and excessive activation of lymphocytes and macrophages. It can be primary (genetic) or secondary (acquired).

Genetic haemophagocytic syndrome can be familial (due to unknown or known gene defects) or a part of immune deficiency syndrome like Chediac Higashi syndrome, Gricelli syndrome or X-linked lympho-proliferative syndrome. This can present at any age from in utero presenting as hydrops faetalis to as old as 70 years. Median survival is less than 2 months if untreated (2).

Most primary HLH episodes are triggered by an infection. The first insight of genetic abnormality was when perforin mutations in some affected patients were discovered in 1999 (3).

The familial form of HLH occurs in young children as a genetic disorder with autosomal recessive inheritance; possible loci for a responsible gene or genes have recently been mapped to the long arms of chromosomes 9 and 10.

Secondary HPS is associated with infections, rhumatological disorders, malignancies, some metabolic diseases, autoimmune conditions, organ transplant and immunosuppression. Autoimmune HPS is also known as macrophage activation syndrome.

In literature EB virus is the commonest cause for secondary HPS due to infections. In addition some deaths associated with avian flu and severe acute respiratory syndrome was due to HPS (2). Cytomegalovirus (CMV) infection, viral hepatitis and acute HIV seroconversion also reported to have caused secondary HPS. Bacteria like TB, enteric fever, brucella, sprocheates and leishmaniasis can cause HPS. Some cases are due to fungi (4).

The pathogenesis is due to proliferation, ectopic migration and infiltration of organs by CD 8 T lymphocytes and macrophages resulting in progressively high hypercytokinaemia leading to organ failure.

When immune system is triggered in a healthy person, histiocytes, natural killer (NK) cells, and cytotoxic T lymphocytes (CTL) are all activated which then mutually stimulate each other by receptor interaction as well as by secretion of inflammatory cytokines and chemokines. In healthy individuals this leads to killing of infected cells, removal of antigen and then termination of the immune response. In HLH, there is an inherited or acquired defect of the NK and CTL cells, so they are unable to cope effectively with the infectious agent or antigen. This results in accumulation of activated T-lymphocytes and activated histiocytes with increasingly high levels of cytokines. Key cytokines found at extremely high levels in the plasma of patients with HLH include interferon gamma, tumor necrosis factor-alpha, interleukins IL-6, IL-8, IL-10, IL-12, IL-18 and soluble IL-2 receptor (CD25). In short these patients are unable to terminate their immune response once activated.

The reason for this may be due to inadequate killing function which is mediated by a secretory pathway involving the activation, polarization, and release of cytotoxic granules into the immunological synapse.

This process is blocked in HLH. This results in accumulation of activated T-lymphocytes and activated histiocytes with increasingly high levels of cytokines. In secondary HPS exact mechanism is not clear but there are some hypotheses.

Viruses may interfere with CTL function. High levels of cytokines may impair NK cells and CTLs. Genetic polymorphisms for CD45, leukocyte common antigen, have been described in several HLH cases. Perhaps certain individuals are more likely to deliver a HLH response to certain underlying conditions.

Common signs and symptoms

- Prolonged fever (60- 100%)
- Hepatosplenomegaly
- Cytopaenia

At least two cell lines are affected. Anaemia and thrombocytopenia are more common. Pancytopaenia can occur.

- Of central nervous system manifestations, encephalopathy, meningismus and seizures are the most commonly reported but cranial nerve palsies can occur.

- CSF in more than half of patients have elevated cell count and moderately increased protein.

- Imaging can include diffuse abnormalities, focal lesions, and parenchymal calcifications.

Less common signs are bleeding, lymphadenopathy, rash (maculopapular commonly but nodular eruptions also reported) and jaundice.

Characteristic laboratory results

- Elevated ferritin
  Likely secreted by activated macrophages.
- Elevated tryglycerides
  Increased levels of TNF-alpha suppress activity of lipoprotein lipase.
- Elevated lactate dehydragenase
- Depressed fibrinogen
  Increased levels of plasminogen activator secreted by activated macrophages.
- Impaired NK cell activity
- Elevated soluble IL-2 receptor (sCD25)
- Transaminitis
- Hypoalbuminemia
- Hyponatremia

Diagnosis

Diagnostic criteria are used in diagnosis of HPS by Histiocytosis Society since there is no specific test for diagnosis. The diagnosis is made if molecular diagnosis consistent with HPS is made or 5 out of 8 criteria for diagnosis are fulfilled (2004 criteria).

These include fever, splenomegaly, decrease of two or more cell lines in peripheral blood, hypertriglyceridaemia and /or hypofibrinogenaemia, haemophagocytosis in bone marrow ,spleen or lymph nodes, low or absent NK cell activity and elevated ferritin (>500mg/ml), soluble CD 25 >2400 U/ml. The NK cell activity and measurement of soluble CD25 is not possible in Sri Lanka.

Ferritin levels >10 000 g/dL were highly sensitive and specific for the diagnosis of HLH in an institutional series (5). However, the results of newer clinical laboratory studies evaluating expression of HLH-associated proteins (perforin, SLAM-associated protein, or X-linked inhibitor of apoptosis protein) or measurement of surface CD107a exposure (indicative of genetic abnormalities affecting degranulation) are now available with results rapid enough to assist in identifying immunologic defects and diagnosing HLH in other countries (1).

Treatment

There are trials which used immunosuppressive therapy and chemotherapy. Effective early therapy reduced the mortality from 95% to 30-35% (94 trial). In this trial high dose dexamethasone was used with etoposide, a proappoptotic chemotherapy drug and cyclosporine A. Etoposide brought sustainable remission. In 2004 trial same drugs were used (6). For patients with central nervous system HPS whom remission was not achieved after 2 weeks of dexamethasone, intrathecal methotrexate was added. There are other trials with prednisolone, antithymocyte globulin and cyclosporine A.

Epstein-Barr virus-associated HLH is almost universally fatal if untreated, with death usually resulting from haemorrhage, infection or resulting from multiorgan failure. These patients should be treated with combination of chemotherapy and immunosuppressive therapy.

HLH associated with viral infection may be difficult to distinguish from familial HLH triggered by a viral infection. Familial HLH should be considered more likely in infants even in the absence of a positive family history. The distinction is important, as allogenic bone marrow transplantation is the therapy of choice in patients with familial HLH who attain remission (7). In patients without a clear diagnosis of familial HLH, bone marrow transplantation should be considered if remission is not attained by 8 weeks of chemotherapy and immunotherapy. Patients in remission without a clear diagnosis of familial HLH should be monitored closely for signs of relapse (8). Place of intravenous immunoglobulin is doubtful. In EBV associated HPS patients, acyclovir was not useful. Secondary HPS patients due to bacterial causes usually respond well for treatment of underlying disease.

In a patient diagnosed with HPS, investigations to exclude underlying infective agents, genetic assessment as well as exclusion of lymphomas need to be done. Especially early bone marrow may give normal results as in our patient so repeat testing is helpful.

Further understanding of this clinical entity may be achieved with improvements in our knowledge in immunological mechanisms of infection and its control.

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