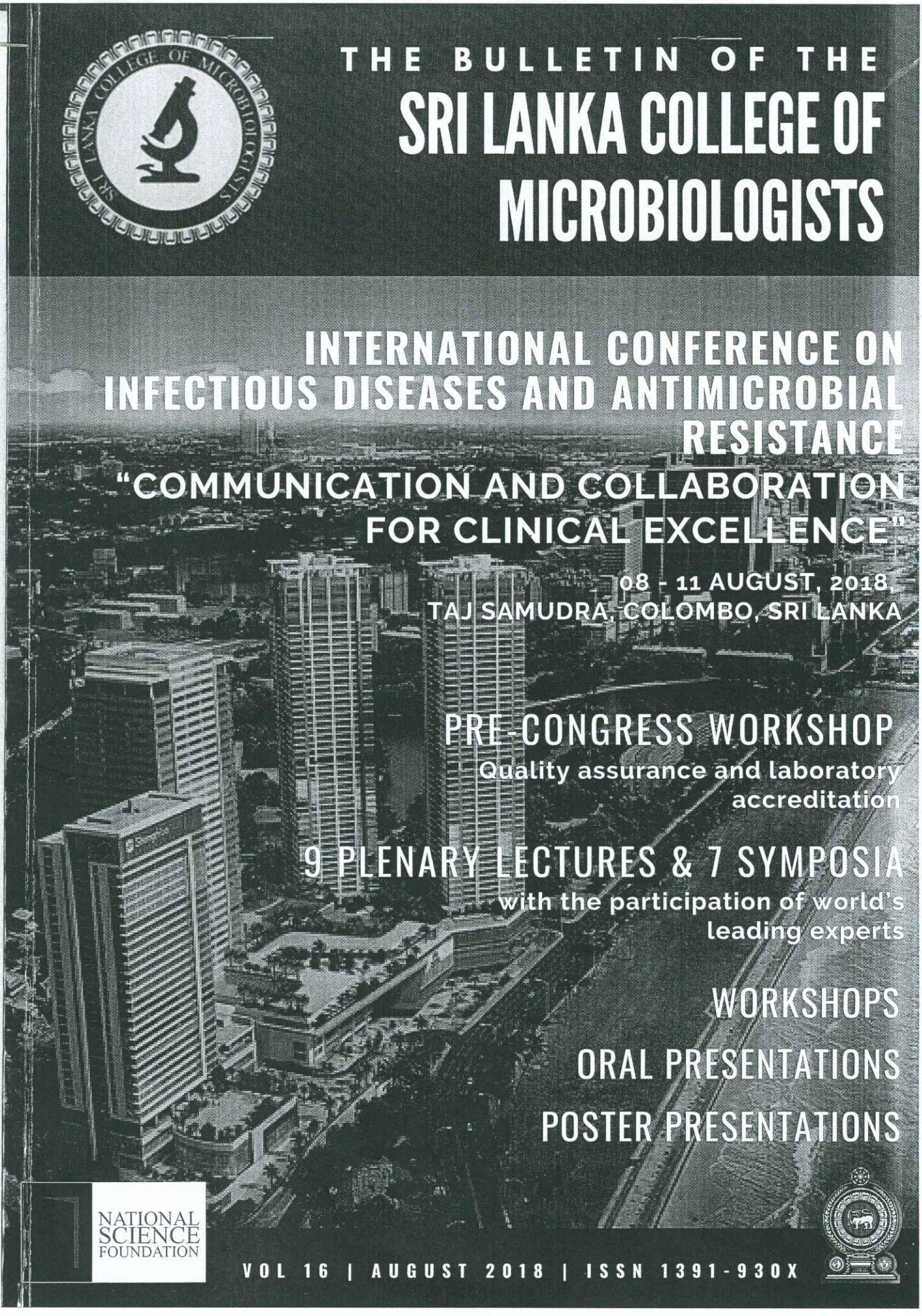

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OP 12

Isolation of bacteriophages against Listeria monocytogenes

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Introduction

The emergence of developing multidrug resistance in microorganisms has become an alarming public health crisis. Bacteriophages can kill pathogenic bacteria and even the multidrug-resistant bacteria without affecting the normal microflora. For this reason, bacteriophages are a potential alternative to conventional antibiotics.

Objectives

This study aimed to isolate and characterise bacteriophages infecting Listeria monocytogenes recovered from food and wastewater samples collected from Serdang, Selangor, Malaysia.

Methods

A total of 122 different food samples including chicken, beef, shrimp, cockles, clam, vegetables and wastewater samples were used to isolate bacteriophages using *L. monocytogenes* strains as host. Phages were enriched from the samples and plaques were obtained by double layer agar assay. The titer and host range of the isolated bacteriophages were determined through spot plate method, while morphology of the isolated bacteriophages observed through the transmission electron microscopy (TEM).

Results

A total of six bacteriophages effective against *L. monocytogenes* were isolated. The titers of the isolated bacteriophages were found within the range of 10^2-10^8 PFU/mL. Morphological characteristics observed through the TEM revealed that the isolated bacteriophages belonged to family Myoviridae family. One isolated phage demonstrated broad host range with infecting six strains of *L. monocytogenes* while the others were able to infect only one strain.

Conclusion

Bacteriophages have the ability to lyse *L. monocytogenes* and potentially can be used as an alternative for antimicrobials.

OP 13

Polymicrobial etiology of infected chronic diabetic wounds

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Introduction

Majority of chronic diabetic wounds are infected and polymicrobial in etiology. Accurate identification of the pathogens are important for optimal wound care.

Objective(s)

The study aimed to identify aerobic and anaerobic bacterial pathogens in chronic diabetic wounds using culture and 16S-rRNA gene sequencing.

Design, setting and methods

A prospective study was carried out at Department of Microbiology, University of Sri Jayewardenepura. Fifty patients with chronic diabetic wounds were included in the study. Two wound debridement specimens were collected from each patient during surgical debridement and subjected to aerobic and anaerobic culture.

Specimens were processed according to the standard Microbiology Operating Procedure (SOP). Colony morphology, Gram stain and the battery of biochemical tests were used for presumptive identification. Identification of anaerobes was performed using Rapid ANA II panel and confirmed by 16S-rRNA gene sequencing.

Results

The patient age ranged between 30-84 years and wounds were located on their lower limbs. Their C-reactive protein levels ranged from 10-393 mg/L, white blood cells counts were between 9.2-28.1×10^3/µL, neutrophil counts were 15.1-22.8 ×10^3/µL and haemoglobin levels ranged from 5.3-14g/dL. Direct Gram stain revealed >25 pus cells/LPF (Low Power Field) and presence of organisms which indicated infection. Aerobes and facultative anaerobes were isolated from all fifty specimens. Fourteen (28%; N=50) were positive with obligate anaerobes. *Finegoldia magna* (12%) was the predominant followed by *Peptoniphilus harei* (4%), *Anaerococcus* spp. (4%) *Peptostreptococcus russelli* (2%), *Peptostreptococcus anaerobius* (2%), *Streptococcus intermedius* (2%), *Propionibacterium acnes* (2%), *Veribaculum cambience* (2%), Bacteroides spp. (2%), *Prevotella bivia* (2%) and *Prevotella buccalis* (2%). Anaerobes coexisted with common aerobes and/or facultative anaerobes in 14 specimens.

Among aerobes/facultative anaerobes, *P. aeruginosa* (58%) was the commonest followed by beta-hemolytic *Streptococci* (22%) and methicillin-resistant *Staphylococcus aureus* (MRSA) (18%). MRSA were isolated together with one or more other bacterial species including species of Streptococci, *Pseudomonas,* Enterococci, *Acinetobacter*, Corynebacteriae, *Veribaculum* and family Enterobacteriaceae.

Out of total fifty specimens 47 had more than one species indicating polymicrobial etiology.

Conclusions

Majority of chronic diabetic ulcers were infected with multiple pathogens. Commonest anaerobe was *Finegoldia magna* followed by aerobes/facultative anaerobes: *Pseudomonas aeruginosa*, beta-hemolytic Streptococci and MRSA.

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Financial assistance by University of Sri Jayewardenepura (ASP/RE/MED/2016/50) and Medical Research Institute (67/2015) are acknowledged.

OP 14

Genotypic characterization of extended spectrum beta lactamase producing genes blaTEM, blaSHV, bla CTX-M among Uropathogenic *Escherichia coli*

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Introduction

Escherichia coli accounts up to 80% of urinary tract infection. Extended spectrum beta lactamases (ESBLs) production among UPEC (Uropathogenic *E. coli*) strains are increasing nowadays which reduces the treatment options to limited number of antibiotics making the clinical management of UTI deleterious. Some bacteria may show variations in their phenotypic and genotypic expressions. blaTEM, blaSHV and CTX-M genes are the common β-lactamase producers which were studied with specific oligonucleotide primers using PCR.

Aim

To characterize ESBL producing uropathogenic *Escherichia coli* using Polymerase Chain Reaction.

Materials and Methods

A total of 208 *E. coli* strains isolated from urine samples were confirmed by conventional culture and biochemical methods and antibiotic susceptibility test was done using Kirby Bauer disc diffusion method as per CLSI guidelines. ESBLs were screened for isolates which showed resistance to third generation cephalosporins (Ceftriaxone and Ceftazidime) using Cefazidime and Ceftazidime plus clavulanic acid and further confirmed by Cefotaxime and Cefotaxime plus clavulanic acid. DNA extraction was done by boiling lysis method. Genotypic characterization was done for all 208 isolates to detect the presence of TEM, SHV and CTX-M genes by conventional PCR.

Results

A total number of 208 *E. coli* were isolated of which 38% were screened and confirmed as ESBL producers using double disc approximation test. 40.8% and 37.9% were resistant to Ceftazidime and Ceftriaxone respectively. Genotypically, 39% were ESBLs with CTX-M 60.2%, TEM 54.2% and SHV 10.8%. Association of these three genes