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Plasmid-mediated verotoxin producing non-O157 *Escherichia coli* isolates from Malaysia

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Background: Verocytotoxin producing *Escherichia coli* (VTEC) is not only the most frequent cause of diarrhea but also life-threatening complications. A significant increase of verotoxin producing non-O157 *E. coli* (VTEC) infections has been represented as a serious public health concern due to its association with severe foodborne illness and outbreaks worldwide. These situations could be worsening through the dissemination of plasmid-mediated non-O157 VTEC worldwide. The aim of this study was to investigate the presence of plasmid-mediated verotoxin gene in non-O157 *E. coli*.

Methods and materials: Forty-nine clinically significant verotoxins producing non-O157 *E. coli* were isolated from 137 stool specimens at Hospital Tengku Ampuan Afzan, Kuantan, Malaysia. All the non-O157 VTEC isolates were identified using standard microbiological assays and confirmed by Polymerase Chain Reaction (PCR). Plasmids were isolated by Perfectprep Plasmid Mini preparation kit and subjected to disk diffusion assay, plasmid curing and molecular cloning in pGEM-T, and transformed into recombinant *E. coli*. The presence of plasmid-mediated verotoxin gene (VT1 and VT2) in recombinant *E. coli* was confirmed by colony PCR and Sanger sequencing.

Results: Among the 49 clinical isolates of non-O157 VTEC, twenty-six (26) isolates were multi-drug resistant and possessed single sized plasmid while others had multiple plasmids with a different size varied from 1.2 kb to 7.5 kb. Of the total 26 plasmid harboring isolates, 11 isolates showed variation in susceptibility patterns while 3 isolates completely lost plasmids after post plasmid curing treatment. This observation was considered encouraging as it suggested that the presence of the verotoxin gene could be plasmid-mediated. Polymerase chain reaction (PCR) derived DNA fragment containing the verotoxin gene of non-O157 *E. coli* was cloned into an expression vector. However, colony PCR and Sanger sequencing characterized 3 plasmids mediated VT1 (348 bp) and VT2 (584 bp) genes producing non-O157 *E. coli*.

Conclusion: The occurrence of plasmid-mediated verotoxin gene producing non-O157 *E. coli* signify a serious public health issues on plasmid-mediated transmission of virulence genes. Public health surveillance and timely diagnosis of the emergence of plasmid-mediated verotoxin gene should be prioritized to avoid the dissemination of plasmid-mediated virulence gene transfer amongst the same bacteria or other species.

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Pyogenic liver abscess: A 14-year retrospective analysis of the incidence trend and clinical outcomes at a Malaysian tertiary referral centre

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Background: Pyogenic liver abscess (PLA) is a potentially life-threatening disease. However, the last published local epidemiology data was more than two decades ago. We aimed to determine the temporal trends in incidence and clinical outcomes of PLA at our centre.

Methods and materials: Retrospective case mix study was performed at Universiti Kebangsaan Malaysia Medical Centre. Clinical data of all PLA patients admitted from January 2004 till December 2017 were gathered from medical records and the hospital database computer system.

Results: A total of 206 patients were diagnosed with PLA. Prior to 2010, the annual incidence rates of PLA were low (0.18 to 0.95 per 100,000 person-years). However, there was a sharp increase in the incidence rate from 2009 to 2010 (0.18 to 1.43 per 100,000 person-years) and the incidence rates had been maintained above 1 per 100,000 person-years until 2017. When stratified according to the ethnics group, Indian relatively had the lowest incidence throughout the study period (highest rate: 1.29 per 100,000 person-years) with Malays and Chinese had a comparable incidence rates from 2010 onwards (highest rates: 1.94 and 1.82 per 100,000 person-years respectively). Main source of infection for PLA was cryptogenic (44.7%) followed by biliary tract (38.3%). Only 33.5% of the blood cultures and 63.6% of the pus cultures were positive with *Klebsiella* spp. being the commonest isolated microorganism.

The abscess was predominantly right-sided of the liver (61.2%), multi-loculated (54.4%) with an average size of 7.18 cm (1 cm–21 cm). Percutaneous catheter drainage (53.7%) and 3rd generation of cephalosporin with metronidazole (26.7%) was the most common mode of treatment. Sepsis (26.3%) is the most common extrahepatic complication. Mean drainage, hospitalization and antibiotic duration were 12 days, 16 days and 45 days respectively. Over this study period, abscess resolution was achieved in 66.9% with 5.5% of relapse rates and 4.8% of mortality rate.

Conclusion: PLA showed a rising trend over the years and predominantly originating from biliary tract with *Klebsiella* spp. as the main bacterial species isolated. Pus culture gave a higher diagnostic yield of microorganism as compared to blood culture. Combined antibiotic with percutaneous intervention gave good abscess resolution.

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