Type: Poster Presentation

Final Abstract Number: 45.015
Session: Bacterial Infections
Date: Friday, June 15, 2012
Time: 12:45-14:15
Room: Poster & Exhibition Area

Demonstration of primary and asymptomatic DNAaemia in participants challenged with *Salmonella* Typhi (Quailes strain) during the development of a human model of typhoid infection

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Background: Typhoid infection remains a major cause of global morbidity. Effective vaccination programmes and new diagnostic tests are urgently needed but are hindered by incomplete understanding of *S.*Typhi pathogenesis, in part due to insufficiently sensitive methods for detecting bacteria in the peripheral circulation of those encountering infection. Here, new insights into *S.*Typhi pathogenesis gained using a culture-PCR methodology during a human typhoid challenge model are described.

Methods: 40 healthy adult participants were challenged with *S.*Typhi (Quailes strain) at doses of 1-5 × 103 or 1-5 × 104 colony-forming units. During the 2-weeks after challenge, participants were reviewed daily with clinical data and specimen collection, including blood drawn for 'routine' microbiological culture and a novel culture-PCR assay. For this, 5 mL venous whole-blood was collected into heparin prior to 5-hour culture in 5% ox-bile tryptone soya broth. Centrifugation was performed to collect the blood pellet/bacteria and DNA was extracted using a commercial bloodspin kit. PCR using primers to amplify the *fliC-d* gene was performed.

Results: Bacterial DNA was detected in the peripheral circulation in 57/684 (8.3%) culture-PCR and 53/674 (7.9%) routine blood culture samples. Positive culture-PCR results were detected from 12 hours after oral challenge; 10/40 participants had positive culture-PCR results (but negative routine cultures) within 5 days of challenge. Seven of these participants went on to develop typhoid infection during the 2-week challenge period (typhoid diagnosis defined by development of bacteraemia or persistent fever >38 °C for >12-hours). DNA was detected in the peripheral circulation of 5/40 participants who were not diagnosed with typhoid infection during the challenge period. Several of these participants had mild symptoms or elevation of inflammatory markers (including C-reactive protein) only.

Conclusion: These data suggest that a culture-PCR methodology targeting the *fliC-d* gene may be used to detect DNA in peripheral blood of those challenged with*S.*Typhi. Aside from unique confirmation that the mechanism of typhoid infection includes primary dissemination of bacteria in the peripheral circulation, we also demonstrate that asymptomatic infection/circulation of bacteria maybe more common than previously anticipated. Sensitive detection of *S.*Typhi DNA in peripheral blood samples may represent a useful additional endpoint in the evaluation of typhoid vaccines.

http://dx.doi.org/10.1016/j.ijid.2012.05.807

Type: Poster Presentation

Final Abstract Number: 45.016
Session: Bacterial Infections
Date: Friday, June 15, 2012
Time: 12:45-14:15
Room: Poster & Exhibition Area

First report of a natural reservoir of emetic *Bacillus weihenstephanensis*

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Background: *B. weihenstephanensis* has been described as psychrotolerant *B. cereus* strain producing emetic and diarrhoeal type enterotoxins. This new species grow at 4-7oC but not at 43oC and this new species can be identified by 16SrDNA and *cspA* targeted PCRs. This study was designed to explore earthworms as a possible natural habitat of this bacteria.

Methods: Two litter-dwelling earthworm species commonly used for vermicomposting, e.g. *Eisenia foetida* (exotic) and *Perionyx excavatus* and two earthworms commonly found in Indian soils, *Metaphire posthuma* and *Lampito mauritii* were selected for the study. Microorganisms from the gut of these earthworms were isolated and identified by standard techniques. This was followed by isolation of their genomic DNA, amplifications by PCR and bacterial typing-comparison with database. Sequence data was aligned and analyzed for finding the closest homologs for the microbe from National Center for Biotechnology Information (NCBI GenBank) and The Ribosomal Database Project (RDP database).

Results: Based on nucleotides homology and phylogenetic analysis the microbe obtained from the gut of *P. excavatus* was detected repeatedly to be *Bacillus weihenstephanensis.* (GenBank Accession Number: DQ345791).

Conclusion: This study described the gut of the earthworm *P. excavatus* as the natural habitat of *Bacillus weihenstephanensis.*

http://dx.doi.org/10.1016/j.ijid.2012.05.808

Type: Poster Presentation

Final Abstract Number: 45.017
Session: Bacterial Infections
Date: Friday, June 15, 2012
Time: 12:45-14:15
Room: Poster & Exhibition Area

Bacteriological profile and susceptibility pattern of neonatal blood stream infections

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Background: Neonatal blood stream infection is a major cause of neonatal mortality and morbidity. Due to the low sensitivity and reporting delay of blood cultures, presumptive treatment usually starts with a broad spectrum antimicrobial agents. It is therefore necessary to periodically review and analyze the bacteriological profiles and susceptibility pattern of common isolates to help the local physicians in designing management strategies.