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Background: Identification of pathogens that cause febrile neutropenia (FN) remains extremely difficult, as the diagnostic performance of blood cultures is very poor. Enomoto et al. reported that a new *in situ* hybridization (ISH) method that detects global bacterial DNA in leukocytes may uncover bacterial infection in spontaneous bacterial peritonitis ascites (J Hepatol. 2012;56:85-94). The utility of this method in FN is however unknown.

Methods: We prospectively evaluated the utility of the ISH method in patients with hematological disorders who developed FN during chemotherapy between March 2012 and April 2013. Neutropenia was defined as a neutrophil count of less than 500/μl or less than 1,000/μl with an expected decline to less than 500/μl. Fever was defined as an axillary temperature ≥ 37.5 °C based on a single measurement. Blood samples for cultures and the ISH test were collected simultaneously, at the onset of fever. Serum concentrations of procalcitonin (PCT) were also measured, with the cut-off value set at 0.5 ng/ml. In addition, we performed the ISH test at the time of neutrophil recovery and completion of antibiotics. Where the last follow-up test remained a positive result, an additional ISH test was performed. Patients with FN were classified into three groups: local infection, bacteremia or fever of unknown origin (FUO).

Results: A total of 28 patients aged 20 - 70 (median 35) years were enrolled and evaluable. The underlying disease included acute leukemia (68.0%), MDS (3.5%), NHL (25.0%) and others (3.5%). The median neutrophil count at study enrollment was 50/μl (range 0 to 636). In four patients found to have bacteremia, all had a positive ISH [100%] and two had a positive PCT test [50%]. In 8 patients diagnosed as local infection, one had a positive ISH [12%] and two had a positive PCT [25%]. In 16 patients with FUO, five had a positive ISH [31%] and all had a negative PCT [0%]. In 24 patients with negative blood cultures, six had a positive ISH [25%] and two had a positive PCT [8%]. In nine patients whose ISH test was positive at the onset of fever, four became negative with neutrophil recovery [44%] and other two finally became negative on resolution of the fever [22%]

Conclusion: The new ISH method may be more sensitive than blood cultures in detecting bacterial etiologies of FN.

275. Genotypic Characterization of Streptococcus canis Isolated from Distinct Hosts with Special Emphasis on Multilocus Sequence Typing

Part of Session: 49. Diagnostic Microbiology; Novel Molecular Methods

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Background: The animal pathogen *Streptococcus canis* is increasingly being noticed in human infections. Our aim was to develop a new multilocus sequence typing (MLST) scheme for *Streptococcus canis* and to compare isolates recovered from house pets and humans, in order to define the clonal structure of the *S. canis* population and explore the zoonotic potential of distinct *S. canis* genetic lineages.

Methods: Eighty-five *S. canis* isolates recovered from infections in animals (n = 78, recovered from 2000 to 2010 in three European countries, mainly from house pets) and humans (n = 7, recovered from 2006 to 2010 in Portugal) were studied. Isolates were identified by API 20 Strep, 23S rRNA gene targeted PCR and 16S rRNA gene sequencing, and characterized by MLST, pulsed-field gel electrophoresis (PFGE) and *emm* typing.

Results: All isolates were successfully typed with the proposed MLST scheme, indicating its applicability to *S. canis* from distinct sources. The MLST analysis showed a polyclonal structure of the *S. canis* population, where the same genetic lineages are found infecting house pets and humans and are disseminated in distinct geographic locations. PFGE confirmed the MLST findings, as it identified the same prevailing lineages and further strengthened the similarity between animal and human isolates. Phylogenetic analysis conducted with the 16S rRNA and MLST loci sequence data indicated that *S. canis* was a divergent taxon of the sister species *Streptococcus pyogenes* and *Streptococcus dysgalactiae* subsp. *equisimilis,* and found evidence of acquisition of genetic material by *S. canis* from the latter species. The presence of *emm*-like genes was restricted to a few isolates and correlated with MLST defined genetic lineages.

Conclusion: Our data shows that *S. canis* isolated from house pets and humans are a single population, and demonstrates that isolates belonging to the main genetic lineages identified are able to infect the human host, providing strong evidence for the zoonotic nature of *S. canis* infection in humans. A MLST database for *S. canis* was established at http://pubmlst.org/scanis/ (hosted by the Department of Zoology, University of Oxford, United Kingdom), constituting a valuable tool for future studies on the molecular epidemiology of this pathogen.