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Evaluation of three PCR schemes for detection of Bartonella bacilliformis in blood samples: sensitivity, specificity and applicability

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Background: *Bartonella bacilliformis* is the etiological agent of Carrion's disease, a neglected illness with a febrile lethal stage and a warty benign phase, being the human the only known reservoir. The diagnostic by microscopy in endemic areas is several times erroneous. Furthermore, the culture of this bacterium is time-consuming, being the diagnostic by PCR the easiest way to perform a correct diagnostic. The objective of this study was to evaluate the detection limit of three PCR schemes, designed to detect *B.bacilliformis*, both in blood and filter papers to test their potential use for transferring samples from endemic areas to reference centers. Moreover, the specificity was also observed as well as the applicability of the technique with clinical samples from different stages of the disease.

Methods & Materials: Fragments of *16SrRNA* and *fla* genes were amplified as well as the variable-intergenic region (*its*). The detection limit was determined by bacterial quantification with flow cytometry and performing dilutions (10^6cfu/ml-10cfu/ml) both in blood and filter papers. DNA was extracted and PCRs were performed. Specificity was tested by processing other bacteraemia microorganisms. Clinical samples, 12 from febrile patients, 13 from warty and 71 from healthy asymptomatic individuals living in endemic area(Mandinga-Cajamarca) were also processed.

Results: The *16SrRNA* PCR scheme showed the lower detection limit (5 cfu from blood and filter paper) being the PCR scheme chosen to be tested in clinical samples. All febrile patients' samples were positive, whereas in warty individuals only 3(23%) faint bands were obtained. No amplification was obtained in samples from healthy people. Fainter bands were always obtained when PCRs were made of filter papers. All PCRs were specific for *B.bacilliformis.*

Conclusion: The *16SrRNA* PCR seems to be the best technique to detect feverish patients. However, the applicability to identify asymptomatic carriers was undetermined. Filter paper may be an alternative for easy transportation of samples but is need to consider the decreasing sensitivity of the results. It is critical to develop rapid, sensitive and specific technique capable of being applied in endemic rural areas, to avoid misdiagnosis and facilitate the detection of asymptomatic carriers that will allow progress towards the eradication of this disease.

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Use of 16S rRNA gene sequencing on direct culture-negative clinical specimens for the diagnosis of bacterial infections, including 2 case reports

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Background: Molecular techniques, such as 16S rRNA sequencing are useful for identification of an unidentified bacterial isolate as well as culture-negative clinical specimens. Where indicated, our laboratory performs 16S rRNA sequencing on culture-negative specimens. Two case studies are presented where the causative pathogen was successfully identified by performing sequencing on CSF and tissue which were culture-negative. As 16S is an expensive and time-consuming investigation, specimen choice is critical. Analysis of all specimens was undertaken and recommendations are given.

Methods & Materials: Analysis of laboratory 16S data is presented.

Case study 1: A 44 year old female patient with AML presented with symptoms of ankle pain. The pain progressed to both knee joints and the left wrist. After six weeks, discharge from the left wrist was noted. Wash out of both knees and left wrist was performed. Samples remained culture negative. 16S rRNA PCR on tissue from both knees detected *Ureaplasma urealyticum*.

Case study 2: A 5 year old boy presented with otitis media, seizures and meningitis. Microscopy on the ear swab showed Gram-variable bacilli. The swab remained culture-negative. Microscopy of the CSF showed an increased WBC and RBC count and Gram-negative bacteria. No causative organism was identified. 16S rRNA sequencing on the CSF identified the pathogen as *Fusobacterium necrophorum*.

Results: 16S provided a useful diagnosis in both patients who were then placed on appropriate antibiotic therapy. Since 2006, >500 specimens have been referred to our laboratory for 16S rRNA PCR. 90 were clinical isolates sent for confirmation of bacterial identification. The highest yield came from cardiac valve tissue with 48% having a significant pathogen identified. Tissues from other sites had a yield of 23%. CSF, brain tissue or subdural abscess pus showed the presence of a pathogen in 17.5% samples. Specimen quality and quantity is critical to detect the presence of the causative organism. Factors that affect the sensitivity of the assay are discussed.

Conclusion: 16S rRNA although an expensive and time laborious technique does provide important information in cases where routine culture techniques fail. The use of 16S rRNA should be limited to clinically indicated cases where an appropriate specimen is submitted.

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