SUPPLEMENTAL METHODS

16S RIBOSOMAL DNA ANALYSIS

DNA extraction. For each cerebrospiral fluid (CSF) sample, DNA of two aliquots (200 μL each) was extracted in separate experiments using the Ultraclean DNA blood spin kit (MO Bio Laboratory Inc., Carlsbad, CA) according to manufacturer's instructions. For each batch of extraction, a negative control (polymerase chain reaction [PCR] grade water) was processed.

PCR and sequencing primers. The primer pair used for amplification and sequencing consisted of fD1 (5-AGAGTTT GATCCTGGCTCAG-3) and rP2 (5-ACGGCTACCTTGT TACGACTT-3), which produce an ~1,500-bp fragment and is considered to be universal for most eubacteria.

16 rDNA PCR amplification. PCR was performed in duplicate in a blinded manner.

The final PCR mixture (50 μL) contained 1.5 μM of MgCl2, 1× of PCR buffer, 0.5 μg/μL of bovine serum albumin, 0.2 mM of deoxynucleoside triphosphate (dNTPs), 0.5 μM of each primer and 1 U of native Taq polymerase. The thermal cycle profile used was as follows: 5 min at 95°C, followed by 40 cycles of 45 sec at 95°C, 45 sec at 57°C, 1.5 min at 72°C, and finally followed by 5 min at 72°C. Amplicon detection was carried out by agarose gel electrophoresis of 10 μL of amplification product through 1.5% agarose gel containing ethidium bromide (EtBr) 0.5 μg/mL of agarose in 1× of Tris-Acetate EDTA (TAE) buffer. Amplicons were visualized under UV irradiation.

The presence of amplifiable DNA was assessed by 1) using qualitative PCR to detect NRAS gene (human housekeeping gene) in each DNA eluate[2] and 2) spiking two of the culture negative CSF samples with *Escherichia coli* before DNA extraction. The DNA eluates from CSF sample known to have negative culture and negative 16S rDNA PCR were spiked with 1,380 pg of purified *E. coli* DNA to assess for PCR inhibitor. The detection level of the assay was determined by plate counting of 10-fold serial dilution of *E. coli* laboratory strain HB-101. Detection level of this PCR assay was 600 cfu/mL.

Sequencing. Crude PCR product was sent to Functional Biosciences, Inc., Madison, WI for sequencing. The sequence was compared with the sequences stored in GenBank by using Basic Local Alignment Search Tool (BLAST) analysis (http://blast.ncbi.nlm.nih.gov/Blast.cgi).