Supplement Material and Methods

1. Processing of specimens

Two independent slide readers conducted malaria microscopy on Giemsa stained thick and thin smears. In case of discrepancies a third decisive reading by an additional reader was performed (see supplement material and methods for more details). From CSF, a direct cell count was done using a Fuchs-Rosenthal counting chamber. Urine was tested for the presence of leucocytes and nitrite using dipsticks (Urine Control Stripes, Diagnostik Nord, Germany). Alanine transaminase (ALT) and aspartate transaminase (AST) concentrations were measured in all serum samples on a Biolyzer 100 instrument (Analyticon, Biotechnologies, Lichtenfels, Germany). Patients with ALT or AST concentrations above 150 U/L were screened for hepatitis A, B and E virus (see **supplement table S1**).

Bacterial cultures were performed on standard media from positive-flagged blood culture bottles (BACTEC Peds Plus/F, Becton Dickinson, USA), from CSF, urine and stool specimens (all Oxoid, Basingstoke, UK). Environmental bacteria and bacteria belonging to the skin flora (e.g. coagulase negative staphylococci, *Corynebacterium* spp. and *Bacillus* spp.) were considered as contaminants. Bacterial isolates were identified biochemically with the VITEK 2 system (bioMérieux, Marcy L'Etoile, France) and MALDI-TOF MS (Bruker Daltonics, Bremen, Germany). *Salmonella* isolates were serotyped by a multiplex PCR for *S.* Typhi, *S.* Typhimurium, *S.* Enteritidis and *S.* Dublin as described before [1]. PCR non-typable *Salmonella* strains were serotyped following the Kauffmann-White-Le Minor scheme [2].

DNA was extracted from EDTA blood with the GenoType DNA extraction Kit (Hain

Lifescience, Nehren, Germany) and from stool using the QIAamp DNA Stool Mini Kit (Qiagen, Hilden, Germany) including six freeze-thaw cycles. DNA and RNA was extracted from all serum, CSF and throat swabs samples using the RTP Pathogen Kit (Stratec biomedical, Birkenfeld, Germany). A panel of PCRs for respiratory, gastrointestinal and systemic bacterial, viral and parasitic pathogens was applied as detailed in the **supplement table S3**.

A screening and confirmatory HIV rapid diagnostic test was offered to all study participants above 18 months of age after separate informed consent was signed. 16S rRNA gene-based identification of bacterial pathogens and next-generation sequencing (NGS) for viruses were performed from CSF samples (see section **3.** and **4.**).

2. Malaria Diagnostics

Thick and thin blood smears were made from venous samples and stained with 10% filtered Giemsa. *Plasmodium* species were differentiated and parasitaemia was estimated by counting the number of asexual forms of *Plasmodium* corresponding to 200/500 leukocytes in the thick blood film, and 5000 erythrocytes in the thin blood film according to parasitaemia level. A slide was declared negative when no asexual parasites were detected against 100 fields examined on a thick film. Each slide was examined by two independent expert microscopists. A slide was read by a third microscopist in case of at least one of the following discrepancies: i) in the species, ii) in the positivity of the smears, iii) in the parasitaemia by a factor of ≥ 2 fold, iv) discordant results in case of parasitaemia < 2000 parasite/µL or by a factor of ≥ 4 fold, or v) discordant results in case of parasitemia > 2000 parasite/µL. For a slide reading to be considered valid, a minimum of two concordant readings by two

different expert microscopists was necessary. The parasitaemia was calculated by the mean of the two closest values for each slide.

3. 16S RNA/DNA gene sequencing for CSF patient samples

Microbial DNA was extracted out of CSF patient samples using the RTP® Pathogen Kit (Stratec biomedical, Birkenfeld, Germany) according to the manufacturers instructions. 16S rRNA gene sequencing was performed based on the principle established by Weisburg et al., 1991 [3]. Two different primer pairs were used to amplify the rRNA gene (see table 1) using Phusion High Fidelity DNA Polymerase (Thermo Scientific, Waltham, US) with 3% DMSO for 35-40 cycles with the following reaction conditions and then sent for sequencing (SANGER):

98°C 5min

98°C 10sec

55°C 30sec

72°C 45sec

72°C 7min

Table 1. Primer pairs used to amplify the rRNA gene

Primer name	Name (Alm et al. 1996[4])	Sequence
F27*		5'- AGA GTT TGA TC**A** TGG CTC AG -3'
1492R	S-D-Bact-1492-a-A-22	5'- TAC GGY TAC CTT GTT ACG ACT T -3'
GM3F	S-D-Bact-008-a-S-16	5'- AGA GTT TGA TCM TGG C -3'
GM4R	S-D-Bact-1492-a-A-16	5'- TAC CTT GTT ACG ACT T -3'

4. Next Generation Sequencing – Material and methods

The CSF samples were filtered through a 0.45-µm filter (Millipore, Darmstadt, Germany) to remove larger debris, bacteria and digested with a mixture of nucleases (Turbo DNase, Ambion, Carlsbad, CA, USA; Baseline-ZERO, Epicenter, Madison, WI, USA; Benzonase, Novagen, San Diego, CA, USA; RNAse One, Promega, Fitchburg, WI, USA) to digest unprotected nucleic acids including host DNA/RNA. Enriched viral particles were then extracted, fragmented, reverse-transcribed, ends repaired, dA-tailed, adaptor ligated and purified. Library preparation was performed using the Nextera XT library prep Kit (Illumina, San Diego, CA, USA). Next generation sequencing was performed on the MiSeq Illumina platform (2 x 300 bp paired-end sequencing). Resulting raw reads were trimmed, de-duplicated and de novo assembled using a customized NGS pipeline. The assembled contigs and unassembled singlets were compared with a viral proteome database using BLASTx using E-value cutoff 0.01.

References

1. Al-Emran HM, Hahn A, Baum J, et al. Diagnosing Salmonella enterica Serovar Typhi Infections by Polymerase Chain Reaction Using EDTA Blood Samples of Febrile Patients From Burkina Faso. Clin Infect Dis **2016**; 62 Suppl 1: S37-41.
2. Grimont P, Weill F. Antigenic Formulae of the Salmonella Serovars. WHO Collaborating Centre for Reference and Research on Salmonella Vol. 9th Edition: World Health Organization, **2007**:1-166.
3. Weisburg WG, Barns SM, Pelletier DA, Lane DJ. 16S ribosomal DNA amplification for phylogenetic study. J Bacteriol **1991**; 173(2): 697-703.
4. Alm EW, Oerther DB, Larsen N, Stahl DA, Raskin L. The oligonucleotide probe database. Appl Environ Microbiol **1996**; 62(10): 3557-9.