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Title: Non-Helicobacter pylori Helicobacter species as a cause of refractory chronic Cellulitis in X-linked agammaglobulinemia

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Library preparation and metagenomic sequencing

DNA library was prepared by automatic nucleic acid extraction, enzymatic fragmentation, end repair, terminal adenylation and adaptor ligation. Finished libraries were quantified by real-time PCR (KAPA) and pooled. Shotgun sequencing was carried out on illumina Nextseq. Approximately 20 million of 50bp single-end reads were generated for each library. Bioinformatic analysis was conducted as described in a previous report. Briefly, sequences of human origin were filtered (GRCh38.p13) and the remaining reads were aligned to a reference database (NCBI nt, GenBank and in-house curated genomic database) to identify the microbial species and read count. For each sequencing run, a negative control (culture medium containing 104 Jurkat cells/ml) was included.

mNGS reporting criteria

Microbial reads identified from a library were reported if: 1) the sequencing data passed quality control filters (library concentration > 50 pM, Q20 > 85%, Q30 > 80%); 2) negative control (NC) in the same sequencing run does not contain the species or the RPM (sample) / RPM (NC) $\geq$ 5, which was determined empirically according to previous studies as a cutoff for discriminating true-positives from background contaminations