Crohn's Disease Patient Infected With Multiple Co-occurring Nontuberculous Mycobacteria

To the Editors,

Inflammatory bowel disease (IBD), defined as chronic inflammation of the gastrointestinal tract, is a condition that encompasses Crohn's disease (CD) and ulcerative colitis.[1] The diagnostic procedure for IBD always involves a challenging infection assessment.[1] Identification of the microbes present and their pathogenic potential are essential for choosing a treatment plan. Gastrointestinal infections caused by nontuberculous mycobacteria (gNTM) are challenging to diagnose based on only disease symptoms. Diagnosis is especially difficult in the case of IBD patients suspected of having CD. Diagnostic studies that investigated symptom similarities have suggested the potential need for investigating mycobacteria-related infections in IBD cases.[2, 3] Both CD and mycobacterial infections have been associated with the development of granuloma and branching fistula. However, conventional methods are likely insufficient for the identification of NTM associated with CD.[4] Furthermore, the diagnosis and treatment of mycobacterial infection in an IBD patient could depend on the type of mycobacteria and the presence of certain chromosomally encoded virulence factors, such as the toxin-antitoxin (TA) elements *parD*, *hipB*, *mazEF*, and *VapB*.[5] The TA loci have been implicated in mediating bacterial adaptive responses, resulting in the development of latent infections and virulence.[5] Nontuberculous mycobacteria infections are exacerbated by their intrinsic drug resistance, their TA mechanisms, and the lack of adequate or timely identification.[5]

In 2018, a 42-year-old Saudi male (resident of Western Province, Saudi Arabia) presented to the emergency room at King Abdulaziz Medical City (KAMC) Hospital in Jeddah with hypoglycaemia, hypovolemia, and a decreased level of consciousness. He was admitted for 3 months and had a history of vomiting, diarrhea, chest tightness, and abdominal pain for 2 weeks along with joint pain, decreased oral intake, weight loss, subjective fever, and night sweats for 3 months. In addition to computed tomography (CT) of the abdomen and pelvis, magnetic resonance imaging (MRI) of the perianal area was performed. The patient was tested for mycobacteria using an interferon-gamma release assay (IGRA; QuantiFERON) and acid-fast bacillus (AFB) smear and culture. Eventually, the patient was subjected to an ileo-colonoscopy procedure based on a suspicion of IBD. The biopsy from the terminal ileum was subjected to shotgun metagenomic sequencing on a HiSeq 4000 platform (Illumina, San Diego, CA, USA) followed by metagenomic assembly, binning, and analyses (see Methods in online supplementary material).

The MRI of the perianal area showed complex branching perianal fistulae with fluid collections (Supplementary Fig. S1). There was mild inflammation with cobblestoning at the terminal ileum, suggesting CD. Histopathological examination of the biopsy revealed acute ileitis but was negative for microorganisms and granuloma. A repeat of the AFB smear along with culturing from the extracted biopsies presented negative results. The metagenomic sequencing results revealed a unique co-occurrence of 3 different NTM species: *Mycobacterium smegmatis* (32.8x average sequencing coverage), *M. riyadhense* (29.7x), and *M. kansasii* (12.4x; Fig. 1 and Supplementary Table S1). Antibiotic resistance genes were detected in the *M. smegmatis* metagenome-assembled genome (MAG; Bin 1), whereas no resistance genes were found in the other 2 MAGs (Fig. 1 and Supplementary Table S1). The distribution of the TA profiles of the NTMs indicated that they were highly similar to human-infecting NTMs that were sequenced previously (Supplementary Fig. S2). The comparison of the TA systems of clinically relevant infective strains and that of our isolates illustrated similarities in the presence of virulence factors, thereby suggesting pathogenicity.

The detection of 3 co-occurring and actively replicating pathogenic mycobacteria within an immunocompetent CD patient is particularly unique and requires further investigation. Metagenomic analysis allowed us to identify the first case of *M. riyadhense* colonizing the gastrointestinal tract. We recommend the shotgun metagenomic sequencing approach as a valuable diagnostic tool for the accurate identification of any infectious agent(s) in suspected IBD patients.

The raw reads and MAGs from this study are publicly available at the European Nucleotide Archive (ENA) under study accession number PRJEB30883. The project was approved by the Institutional Biosafety and Bioethics Committee (IBEC) KAUST (17IBEC38_Pain), and informed patient consent was obtained.

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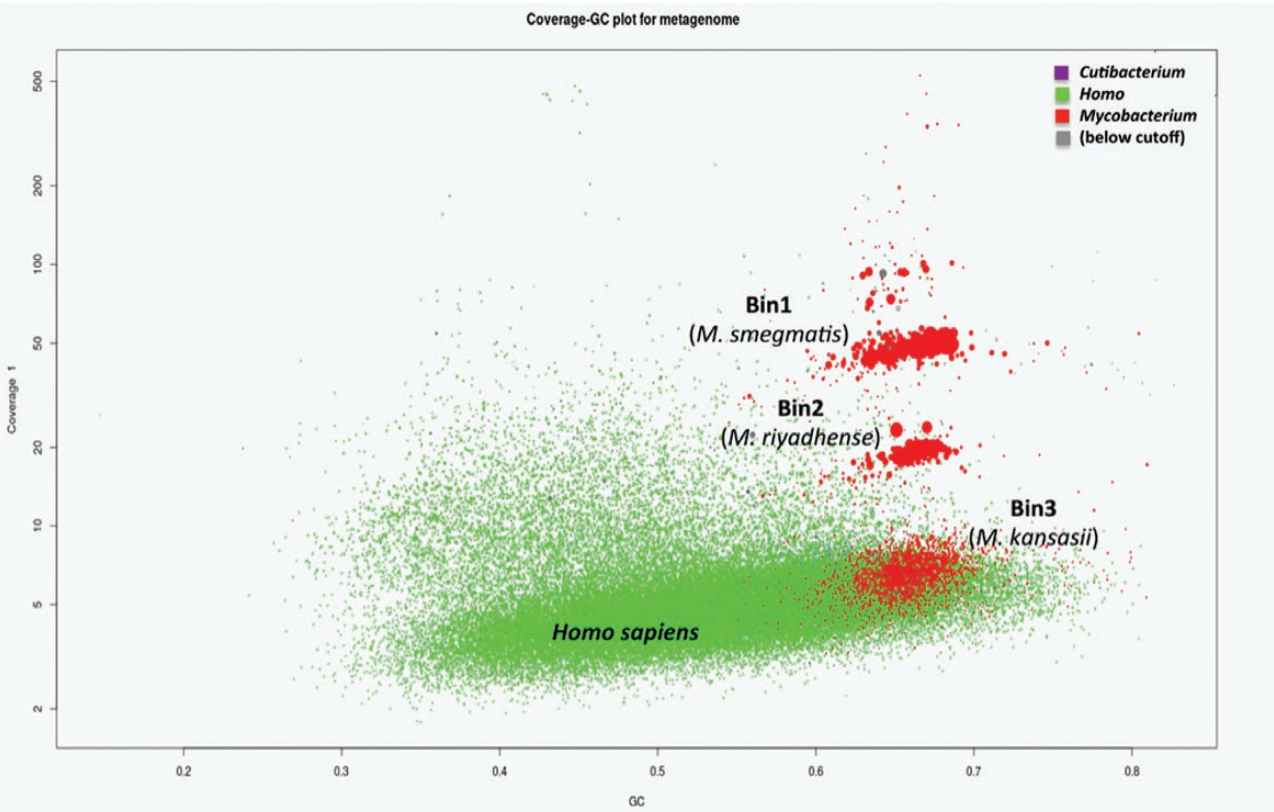

MAG (Size;%GC)	Completeness[a] (%)	Contamination[a] (%)	Relative abundance[a] (%)	Replication rate[b]	Taxonomic identity (% ANI[c];TCS Z-score[d])
Bin1 (6.11 Mb; 67.74)	97.01	0.38	43.07	1.24	*M. smegmatis* (99.01%/0.99)
Bin2 (6.15 Mb; 65.12)	98.47	0.53	40.64	1.32	*M. riyadhense* (99.45%/0.99)
Bin3 (6.15 Mb; 66.28)	99.04	0.38	16.29	1.30	*M. kansasii* (99.97%/0.99)

[a]Estimated on CheckM
[b]Estimated on iRep
[c]% Average nucleotide identity (ANI) determined using GTDB-Tk
[d]Tetra correlation search (TCS) Z-score determined on JSpeciesWS

FIGURE 1. Metagenome binning plot (total number of read pairs in shotgun library, 27.3 Mio) and characteristics of bacterial MAGs. Each dot represents a contig, and all contigs are colored according to their taxonomic identity.

SUPPLEMENTARY DATA

Supplementary data is available at *Inflammatory Bowel Diseases* online.

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Author Contribution: SH, AbA, and AsA collected and interpreted the clinical data. SH and MA performed the biochemical experiments. SH performed the laboratory characterization experiments. AlA analyzed the medical imaging data. CPA and QG performed the bioinformatics analysis. SH, AbA, AsA, and AP conceived the study. SH, CPA, AlA, QG, and AP prepared the figures and wrote or revised the manuscript.

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