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Metagenomics Reveals Impact of Geography and Acute Diarrhoeal Disease on the Central Indian Human Gut Microbiome

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Abstract

Background: The Central Indian gut microbiome remains grossly understudied. Herein, we sought to investigate the burden of antimicrobial resistance and diarrhoeal diseases, particularly *Clostridioides difficile*, in rural-agricultural and urban populations in Central India, where there is widespread unregulated antibiotic use. We utilised shotgun metagenomics to comprehensively characterise the bacterial and viral fractions of the gut microbiome and their encoded functions in 105 participants. **Results:** We observed distinct rural-urban differences in bacterial and viral populations, with geography exhibiting a greater influence than diarrhoeal status. *Clostridioides difficile* disease was more commonly observed in urban subjects, and their microbiomes were enriched in metabolic pathways relating to the metabolism of industrial compounds and genes encoding resistance to 3rd

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generation cephalosporins and carbapenems. By linking phages present in the microbiome to their bacterial hosts through CRISPR spacers, phage variation could be directly related to shifts in bacterial populations, with the auxiliary metabolic potential of rural-associated phages enriched for carbon and amino acid energy metabolism. Conclusions: We report distinct differences in antimicrobial resistance gene profiles, enrichment of metabolic pathways and phage composition between rural and urban populations, as well as a higher burden of *Clostridioides difficile* disease in the urban population. Our results reveal that geography is the key driver of variation in urban and rural Indian microbiomes, with acute diarrhoeal disease, including *C. difficile* disease exerting a lesser impact. Future studies will be required to understand the potential role of dietary, cultural and genetic factors in contributing to microbiome differences between rural and urban populations.

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