Metagenome of SARS-Cov2 from a patient in Brazil shows a wide range of bacterial species - *Lautropia*, *Prevotella*, *Haemophilus* - overshadowing viral reads, which does not even add up to a full genome, explaining false negatives

Sandeep Chakraborty

Letter

Here, I analyze the metagenome from the nasopharyngeal swab of a suspected case of local transmission of Covid-19, in Brazil (Accid:PRJNA613951,nreads=115933).

Very little viral load, not covering the entire genome

There are just 152 reads (out of 115933 reads) matching to SARS-Cov2 [1] (SIbrazil/SARS-Cov2.reads.fa), which adds up a total of 21190 bp, much lesser than the 30000bp SARS-Cov2 genome. This is a very plausible cause for false negatives, there is just not enough virus to detect.

For eg, if the RT-PCR test was looking for a genomic fragment in the spike protein (3879bp) from 369–1174, it would not find a match. Thus, it is important to use multiple primers spread across the genome, something which the CDC test does not do.

Much more bacterial load - *Lautropia*, *Prevotella*, *Haemophilus* dominating

There are a wide range of bacteria (SIbrazil/allbact.list.txt,n=117) - *Lautropia*, *Prevotella*, *Haemophilus* dominating (Table 1). These are the same bacteria found in China [2] and San Diego county [3]. There are 10851 reads matching to bacteria (SIbrazil/allbactsequences.fa). These bacterial co-infections form the basis of hydroxychloroquine and azithromycin working in clinical trials [4].

Table 1: **Bacterial reads in a patient from Brazil** GN=Gram-negative, GP=GP, FAC-ANE=facultatively anaerobic (aerobic, but capable of switching to fermentation if oxygen is absent). Is there a link to anaerobic coinfection (like *Prevotella*, which is again present here)? While *Haemophilus*, *Lautropia* and *Prevotella* are common to other metagenomes, *Cellvibrio* and *Massilia* are two unique species found in the Brazilian patient.

NReads	Bacteria	Type	Diseases
1747	<i>Haemophilus</i>	GN FAC-ANE	pneumonia, meningitis and bloodstream infection oral cavities of HIV-infected children [5] aspiration pneumonia, lung abscess, pulmonary empyema, etc
1634	<i>Lautropia</i>	GN FAC-ANE	
1094	<i>Prevotella</i>	GN anaerobic	
726	<i>Escherichia</i>	GN FAC-ANE	
714	<i>Schaalia</i>	GP aerobic	robust capacity for plant polysaccharide degradation pharyngitis, pneumonia, sepsis, and endocarditis chronic blepharitis and endophthalmitis, Lymphadenopathy [6]
589	<i>Cellvibrio</i>	GN aerobic	
561	<i>Streptococcus</i>	GP aerobic	
446	<i>Cutibacterium</i>	GP anaerobic	
434	<i>Massilia</i>	GN ?	
368	<i>Pseudomonas</i>	GN FAC-ANE	

Prevotella, which had taken over in a Chinese patient [7] is also known to increase IL-6 in plasma [8–10], cause ground glass opacity in lungs [11], and associated with cardiac injury [12] - all symptoms associated with Covid19. In 2003 SARS-Cov1 outbreak, secondary infection and IL-6 was highly correlated with hospitalization and deaths [13].

References

1. Perlman S (2020). Another decade, another coronavirus.
2. Chakraborty S (2020). Metagenome of sars-cov2 patients in shenzhen with travel to wuhan shows a wide range of species - lautropia, cutibacterium, haemophilus being most abundant - and campylobacter explaining diarrhea. doi:10.31219/osf.io/jegwq. URL osf.io/jegwq.
3. Chakraborty S (2020). San Diego county Nanopore SARS-Cov2 sequencing data shows metagenomic Prevotella, Haemophilus parainfluenzae, a lot of unknown species and chimeric reads. doi: 10.31219/osf.io/cvbqf. URL osf.io/cvbqf.
4. Gautret P, Lagier JC, Parola P, Meddeb L, Mailhe M, et al. (2020) Hydroxychloroquine and azithromycin as a treatment of covid-19: results of an open-label non-randomized clinical trial. International Journal of Antimicrobial Agents : 105949.
5. Rossmann SN, Wilson PH, Hicks J, Carter B, Cron SG, et al. (1998) Isolation of Lautropia mirabilis from oral cavities of human immunodeficiency virus-infected children. Journal of clinical microbiology 36: 1756–1760.
6. Van Craenenbroeck AH, Camps K, Zachée P, Wu KL (2011) Massilia timonae infection presenting as generalized lymphadenopathy in a man returning to belgium from nigeria. Journal of clinical microbiology 49: 2763–2765.
7. Chakraborty S (2020). The 2019 Wuhan outbreak could be caused by the bacteria Prevotella, which is aided by the coronavirus, possibly to adhere to epithelial cells - prevotella is present in huge amounts in patients from both China and Hong Kong. doi:10.31219/osf.io/usztn. URL osf.io/usztn.
8. Leite AZ, Rodrigues NdC, Gonzaga MI, Paiolo JCC, de Souza CA, et al. (2017) Detection of increased plasma interleukin-6 levels and prevalence of Prevotella copri and Bacteroides vulgatus in the feces of type 2 diabetes patients. Frontiers in immunology 8: 1107.
9. Larsen JM (2017) The immune response to Prevotella bacteria in chronic inflammatory disease. Immunology 151: 363–374.
10. Choi EY, Jin JY, Choi JI, Choi IS, Kim SJ (2014) Effect of azithromycin on Prevotella intermedia lipopolysaccharide-induced production of interleukin-6 in murine macrophages. European journal of pharmacology 729: 10–16.
11. Berardino ADM, Inchingolo R, Smargiassi A, Re A, Torelli R, et al. (2014) Empyema caused by prevotella bivia complicating an unusual case of spontaneous chylothorax. Journal of clinical microbiology 52: 1284–1286.
12. Dorn BR, Dunn WA, Progulske-Fox A (1999) Invasion of human coronary artery cells by periodontal pathogens. Infection and immunity 67: 5792–5798.
13. Jiang Y, Xu J, Zhou C, Wu Z, Zhong S, et al. (2005) Characterization of cytokine/chemokine profiles of severe acute respiratory syndrome. American journal of respiratory and critical care medicine 171: 850–857.