Title

Characterization and SARS-CoV-2 Testing of the Clinical Laboratory Microbiome

Author

Kovalick, Grace Ellen

Publication Date

2021

Peer reviewed|Thesis/dissertation

UNIVERSITY OF CALIFORNIA SAN DIEGO

Characterization and SARS-CoV-2 Testing of the Clinical Laboratory Microbiome

A thesis submitted in partial satisfaction of the requirements for the degree Master of Science

in

Biology

by

Grace Ellen Kovalick

Committee in charge:

Professor David Pride, Chair
Professor Justin Meyer, Co-chair
Professor Rachel Dutton
Professor Jack Gilbert

2021

The thesis of Grace Ellen Kovalick is approved, and it is acceptable in quality and form for publication on microfilm and electronically.

University of California San Diego
2021

TABLE OF CONTENTS

LIST OF FIGURES

ACKNOWLEDGEMENTS

I would like to acknowledge Dr. David Pride, Dr. Govind Sah, Dr. Jessica Chopyk, Peiting Kuo, Lina Huang, Dr. Susan Realegeno for their contributions to this project.

Experimental methods were developed by Dr. David Pride, Dr. Jessica Chopyk, Dr. Govind Sah, Peiting Kuo, and Grace Kovalick. Methods were conducted by Dr. Govind Sah, Peiting Kuo, Dr. Jessica Chopyk, Dr. Susan Realegeno, Lina Huang, and Grace Kovalick.

Figures from this paper are coauthored with Dr. Govind Sah and are currently being prepared for submission for publication.

ABSTRACT OF THE THESIS

Characterization and SARS-CoV-2 Testing of the Clinical Laboratory Microbiome

by

Grace Ellen Kovalick

Master of Science in Biology

University of California San Diego, 2021

Professor David Pride, Chair
Professor Justin Meyer, Co-Chair

Clinical microbiology laboratories perform testing of patient samples, including bacterial and fungal cultures, and nucleic acid diagnostics for bacteria and viruses such as SARS-CoV-2. Even though such laboratories have been performing testing for many decades, it is unknown what microbes inhabit these laboratory surfaces. Particularly, in the setting of the COVID-19 pandemic, it is important to understand whether laboratory surfaces harbor pathogens such as SARS-CoV-2. Therefore, we characterized the microbiota present on different surfaces of the clinical microbiology laboratory using 16S rRNA amplicon sequencing to understand whether there were unique microbes associated with different surfaces that technicians commonly come in contact with. We also deciphered whether SARS-CoV-2 might also be harbored on these surfaces to further assess the potential risk for occupational exposures. We analyzed laboratory surfaces, including workbenches, floors, and sinks within the main bacteriology, molecular microbiology, and newly-established COVID-19 testing laboratory. We performed this study during the early COVID-19 pandemic time period from July to October 2020. RT-PCR testing found that SARS-CoV-2 was found primarily on the floors, which suggests that it may have been brought in on the shoes of laboratory employees or was the result of the aerosolization of the virus from processing the high numbers of SARS-CoV-2 PCR tests in the microbiology laboratory. Characterization of the bacteria on the clinical lab surfaces revealed distinct workbench, floor, and sink communities, colonized primarily with human-associated microbes. These data provide much needed insights into the complex microbial communities that make up the clinical microbiology laboratory.

Introduction

A clinical laboratory, also known as a medical laboratory, is a place where diagnostic testing is performed on clinical specimens from patients. These laboratories are typically either part of a hospital system or private/commercial entity. One subset of clinical laboratories are clinical microbiology laboratories, which perform cultures of bacteria and fungi, and run nucleic acid diagnostics for bacteria and viruses including tests for SARS-CoV-2.[1] Some clinical microbiology facilities also test for antigens from pathogens and antibody responses to pathogens, and are more specifically referred to as serology laboratories. Mechanical technology and automation is increasingly being used in clinical laboratories to increase testing speed, efficiency, and volume.[2]

The first case of COVID-19 was reported in December 2019 in Wuhan, China, and the novel coronavirus SARS-CoV-2, was discovered to be the cause.[3] From there the disease spread worldwide, and on March 11th, 2020, COVID-19 was declared a pandemic, which prompted countries to close borders, businesses and schools to close, and social distancing guidelines to be enacted.[4] As of November 16th, 2021, there have been 254,256,432 confirmed COVID-19 cases, and 5,112,461 deaths attributed to the disease worldwide[5], but there are estimates that the true infection rate is 5-20 times greater than the number of confirmed cases,[6,7] so the true number of cases is unknown and may be much higher than previously thought.

Testing infrastructure had to be rapidly constructed across the world to accommodate demand. The primary test used is a SARS-CoV-2 RT-PCR test,[8] which is used to detect and quantify the presence of virus RNA. At the University of California, San Diego Health's Center for Advanced Laboratory Medicine (UCSD CALM), testing expanded from around 20 tests per day at the beginning of the pandemic, to up to 4,500 at its peak.[9]

In February of 2020, the World Health Organization (WHO) established that clinical laboratories should follow Level-2 (BSL-2) or Level-3 (BSL-3) protocols to handle SARS-CoV-2.[10] There were still many unknowns, including whether surfaces were becoming contaminated or if the virus could be transmitted via aerosols. In April of 2020, SARS-CoV-2 was shown to be viable on surfaces for up to 72 hours after its introduction,[11] although later reports suggest that the risk of transmission from surfaces is low.[12] In July 2020, the WHO officially acknowledged that COVID-19 could be airborne, while new case counts surged to unprecedented daily levels.[13] August 2020 saw the beginning of a surge in COVID-19 cases,[14] and by late September, the death toll from the virus reached 200,000 in the United States.[15]

SARS-CoV-2 testing of a hospital environment performed contemporaneously with this study displayed that the floors in and near patient rooms tested positive most often when there was a COVID-19 patient in the room (29-39%), but still had a significant positive rate without a COVID-19 patient in the room (17-27%). Surfaces in COVID-19 positive patient's rooms like bedrails (11%), door handles (3%), and

keyboards (4%) tested positive less often.[16] The infectivity of the virus on surfaces was not investigated.

Occasionally employees will become ill with laboratory-acquired infections, likely due to contamination or improper personal protective equipment (PPE) usage. Historically, there have been disease outbreaks emanating from clinical laboratories. There have been several outbreaks of the pathogenic bacteria *Salmonella* over the last decade from clinical and teaching laboratories.[17] Gastrointestinal illnesses such as Salmonellosis and Shigellosis are the most common lab-acquired infections, but Brucellosis, which causes flu-like symptoms, is also amongst the most common.[18] Laboratory associated infections have been decreasing in recent years,[19] likely due to engineering controls, proper training, and safety protocols. However, when considering the COVID-19 pandemic, which is caused by a novel virus, there are many unknowns as protocols are not based on years of rigorous testing, and the risk of infection from laboratory surfaces may be a concern.

In addition to negatively affecting employee health, cross-contamination can also affect the validity and confidence of test results between patients, and lead to false positives and misdiagnoses. Hands/gloves, coat cuffs, biosafety cabinets, testing devices, cartridges, and accessories may become contaminated through normal laboratory use.[20]

Considering the risk of cross-contamination and laboratory-acquired infections including bacterial and SARS-CoV-2 infections, it is of interest to know what microbial communities contribute to the environment of a clinical microbiology laboratory.

Additionally, laboratories of this type have been operating and performing tests for decades, and the makeup of microbes on their surfaces remains unknown. A 16S rRNA analysis would provide a more complete picture of what bacteria are in which environmental surfaces of a clinical microbiology laboratory. With this information, risks for laboratory acquired infections could be assessed, and improved sanitization guidelines could be put in place depending on a particular community makeup of certain locations. This could result in a safer environment for employees, and a lower risk of cross-contamination.

For these reasons, the goal of this study was to perform SARS-CoV-2 PCR testing and use 16S rRNA analysis to analyze the microbiome of different surfaces of several sections within a clinical laboratory during the early stages of the COVID-19 pandemic to (i) better understand the laboratory distribution of SARS-CoV-2, (ii) identify bacterial communities present and consider the risks they may pose to employees and community health, and (iii) assess differences in bacterial community compositions amongst different surfaces.

Methods

Sample Collection

Samples were collected between July 20th, 2020 and October 30th, 2020 from the University of California, San Diego's Center for Advanced Laboratory Medicine (UCSD CALM) in La Jolla, CA. Sterile swab tubes (BD Falcon Swube Screw

Cap/Cotton Tip Applicators) were moistened in sterile saline, and the surfaces of benches, sinks, or floors were swabbed for 30 seconds. Three laboratories were swabbed, including the molecular laboratory, COVID laboratory, and bacteriology laboratory.

Work done in the bacteriology laboratory includes cell culture diagnostic tests of patient bacterial pathogens. It also includes the accessioning section where all patient specimens are received and enter the laboratory. A separate section of this laboratory is devoted to serological testing for antibodies and antigens from human pathogens. In the molecular laboratory, diagnostic testing involves DNA, RNA, proteins, and other small molecules from patient samples. Even though the laboratory developed a separate COVID testing laboratory, approximately 50% of the COVID testing occurred in this laboratory space. The COVID laboratory contains two sections, front and back. The front of the COVID laboratory was sampled over the entire swabbing period, and includes a row of benches near the entrance to the laboratory. The back of the COVID laboratory includes a row of benches and a sink, where SARS-CoV-2 was handled beginning on 10/13/2020. Sampling of the back of the COVID laboratory began 8/21/2020.

The benches of each laboratory were swabbed during the day when laboratory employees were likely to be present conducting work. The swabbed sink areas include the front, sides, and bottom of the sink basin, which are likely to have contact with runoff water from employees' hands during hand washing. Bacteriology includes two sinks located approximately 10 feet from the benches. Molecular has one sink located three

to five feet away from the benches. The COVID laboratory has two sinks five to fifteen feet away from the benches. The swabbed floor areas include the spaces proximal to the working benches. Samples were collected over a period of approximately an hour, and then stored at 80°C until further processing.

16S rRNA Gene Amplicon Processing

Swab tubes were thawed at room temperature, opened in a class 2 biological safety cabinet, rehydrated with 1X PBS, and incubated at room temperature for 10 minutes. DNA extraction was performed using the Purelink Viral RNA/DNA Mini Kit (Invitrogen; Waltham, MA). Many samples had relatively low DNA concentrations (n = 92) and had to be further concentrated using Zymo gDNA Clean and Concentrator-10 kit (Zymo research; Irvine, CA). Several unused swabs were included in the extraction and concentration as a negative control. A segment of the 16S rRNA region of the genomes was amplified using V3-4 region primers and Kapa Hifi Hotstart Readymix (Kapa Biosystems; Wilmington, MA), using the cycle settings: 95 °C for 3 minutes, 35 cycles of 95 °C for 30s, 55 °C for 30 s, 72 °C for 30 s, and an elongation step of 72 °C for 5 minutes. The samples were further purified with Ampure XP beads (Beckman-Coulter; Fullerton, CA), the concentrations were measured using the dsDNA High Sensitivity Kit with a Qubit Fluorometer (Thermo Fisher Scientific; Waltham, MA), and the fragments were sized using a High Sensitivity DNA Kit on a Bioanalyzer (Agilent Technologies; Palo Alto, CA). Samples were diluted to equal molarity and were pooled and run in two separate runs on an Illumina MiSeq (Illumina; San Diego, CA).

Analysis and Statistics of 16S rRNA Gene Sequences

Sequenced reads were processed with Quantitative Insights Into Microbial Ecology 2 (QIIME2; version 2021.4).[21] The Deblur plugin was used in QIIME2 to filter and denoise the data.[22] Taxonomy was analyzed using the SILVA classifier,[23] and data was visualized in R-studio (version 1.4.1717). Diversity was assessed using Operational Taxonomic Unit (OTU), Faith's Phylogenetic Diversity,[24] Shannon Index,[25] and Bray Curtis dissimilarity within QIIME2. The beta diversity metric of robust Aitchison PCA was performed using the DEICODE plugin.[26] Alpha diversity significance was determined using pairwise Kruskal-Wallis testing,[27] and beta diversity significance was determined using ANOSIM tests with 999 permutations. The significance of the Aitchison PCA was quantified using a PERMANOVA test with 999 permutations.

SARS-CoV-2 Testing

Samples were tested for the presence of SARS-CoV-2 viral RNA using the ABI7500FastDx instrument and TaqPath COVID-19 RT-PCR assay (Thermo Fisher Scientific; Waltham, MA). Some specimens tested indeterminate (N=26) and had to be repeated. Upon repeat, only 4 specimens remained indeterminate and were excluded from the study.

Results

SARS-CoV-2 Testing

SARS-CoV-2 RT-PCR testing revealed that the most common location where the virus was located was on the floor of the molecular laboratory (42.1% of tests positive), followed by the bacteriology floor (13.2% of test positive), COVID laboratory floor (2.6%), and accessioning bench (2.6%), which also returned positive SARS-CoV-2 tests (Fig. 1A). When considering the temporal distribution of test results, there were more positive tests in the first half of the time period than in the second half (Fig. 1B).

Figure 1: *SARS-CoV-2 test results of clinical laboratory surfaces and locations.* Map of the clinical laboratory and swabbed surfaces is shown that depicts **A** the bacteriology and molecular microbiology laboratories and **B** COVID-testing laboratory. Acronym represents the surface (accessioning bench, A-BN; bench, BN; sink, SK; floor, FL). Summary of the results of SARS-CoV-2 PCR testing of the clinical laboratory **C** grouped by location **D** grouped by time. Figure coauthored with Dr. Govind Sah.

Sequencing Output

After sequences were obtained, quality filtering was performed and 329 out of 380 samples passed the filter and were included in the analysis. Samples were grouped and analyzed by a lab-wise analysis (130 for bacteriology, 100 for molecular, and 99 for COVID), or by surface-wise analysis (33 for accessioning bench, 99 for bench, 94 for floor, and 103 for sink) (Fig. S1). A total of 4,449,418 sequence reads were obtained, with a median of 10,799 sequences per sample, and an interquartile range (IQR) of 5910 sequences. A sampling depth of 6469 was chosen to account for differences in the depth of sequencing. The depth was chosen to include as many sequences as possible, while preserving the number of features present.

Alpha Diversity

Alpha diversity was quantified using observed feature count and by calculating Faith's phylogenetic diversity metric. Alpha diversity is a metric that measures diversity by examining species richness and evenness in a particular group. The different laboratory surfaces (bench, floor, sink) had significantly different (Kruskal-Wallis; $p < 0.05$) alpha diversity in both metrics from one another (Fig. 2A, S2), which shows that the microbial richness of each surface was different from other surfaces in each laboratory. The floors of each laboratory demonstrated the highest diversity (Kruskal-Wallis; $p < 0.05$), while the sinks demonstrated the lowest diversity (Kruskal-Wallis; $p < 0.05$) (Fig. S2).

Shannon's diversity index was calculated to analyze the diversity longitudinally, and this displayed that the diversity of the bacterial communities remained stable over time (Fig. S3), except for the bacteriology sink, which had a significant decrease in diversity (Pearson; $R = -0.77$, $p < 0.001$), and the molecular floor and COVID sink, which both had an increase in diversity (Pearson; $R = 0.37$, $p = 0.037$; $R = 0.46$, $p = 0.005$) This shows that for the most part, bacterial communities were stable when considered longitudinally.

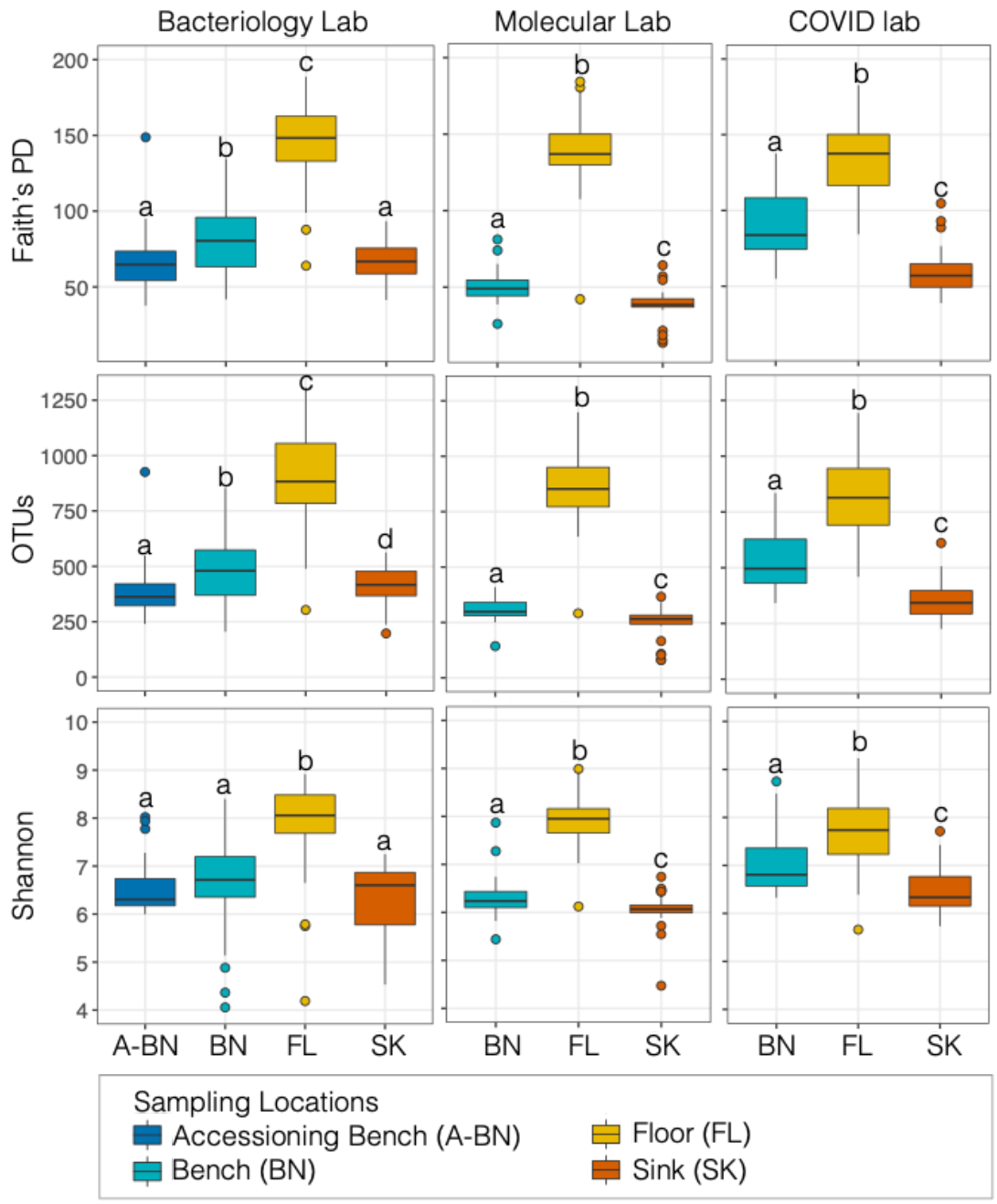

Figure 2: *Alpha diversity of clinical laboratory surfaces.* Alpha diversity boxplots of clinical laboratory surfaces depicting **A** Faith's PD **B** OTU **C** Shannon index for each surface within each laboratory group. Alpha diversity metric is shown on the y-axis, while surface group is shown on the x-axis. Letters "a", "b", "c", "d" represent significance groupings according to Kruskal-Wallis testing ($p < 0.05$). The boxplots show the Interquartile Range (IQR) between the third and first quartiles, the center line is the median, and the top whisker represents the third quartile plus 1.5 times the IQR, while the bottom whisker represents the first quartile minus 1.5 times the IQR. Outliers are depicted with circles. Figure coauthored with Dr. Govind Sah.

Beta Diversity

Beta diversity was quantified using the Bray-curtis dissimilarity index. Beta diversity is a measure of diversity that examines the relationship between different groups. This analysis demonstrated that there were minimal differences in the microbiota identified among the different laboratories (Fig. 3A). The analysis, however, did demonstrate that there were significant differences (ANOSIM; p=0.001) between the sampled surfaces. For example, there were significant differences in the microbiota identified on the floor, sink, and the benches.

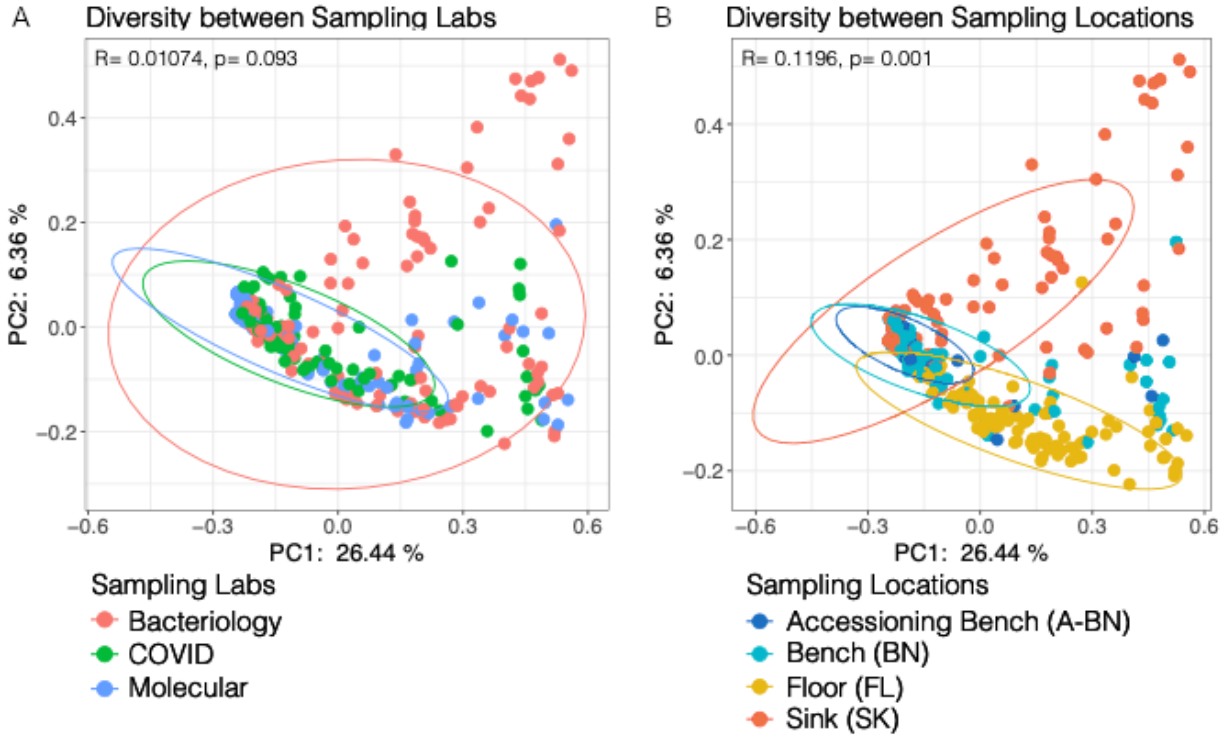

Figure 3: *Beta diversity of clinical laboratory surfaces.* Principal coordinates of microbes on different surfaces in different **A** laboratories, color represents laboratory location (bacteriology, orange; COVID, green; molecular, blue) and **B** surfaces, color represents surface (accessioning bench, dark blue; bench, teal; floor, yellow; sink, orange). Significance determined by ANOSIM with 999 permutations and is displayed in the top left corner of each panel. Figure coauthored with Dr. Govind Sah.

A robust Aitchison PCA plot was used to investigate which microbes might be responsible for the differences identified in beta diversity. In the bacteriology laboratory, differences in beta diversity of floor samples were driven by the γ-proteobacteria, Lactobacillales, and *Dickeya*, while the sink diversity was driven by *Comamonas*, *Staphylococcus*, and γ-proteobacteria (Fig. 4A). For the molecular laboratory, floor diversity was driven by *Streptococcus*, *Nocardia*, and Actinobacteria, and Lactobacillales.

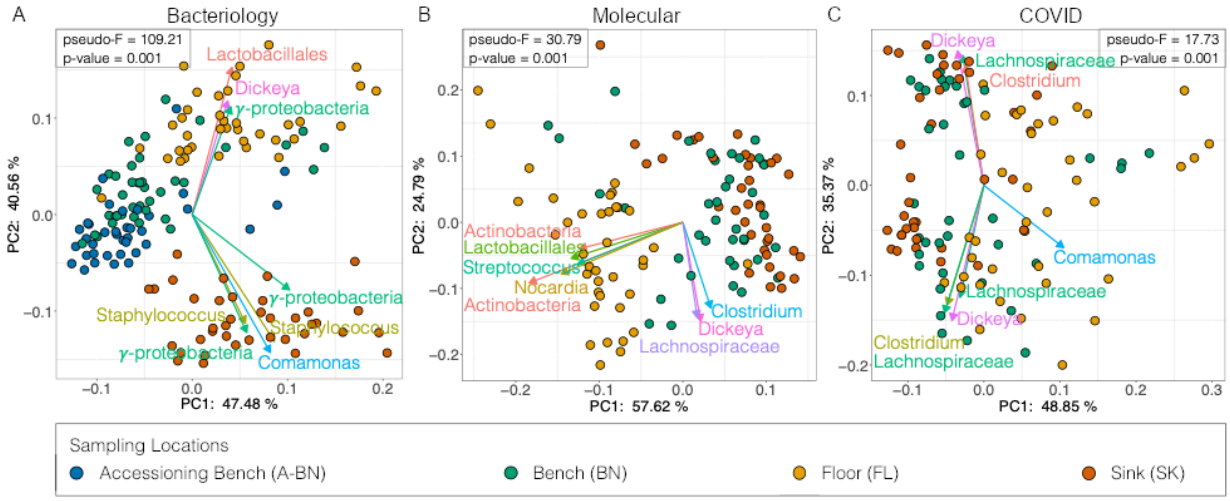

Figure 4: *Taxonomic groups driving laboratory beta diversity.* Aitchison compositional biplot for **A** bacteriology **B** molecular and **C** COVID laboratories. Color represents surface (accessioning bench, blue; bench, green; floor, yellow; sink, orange). Arrows represent clusters of diversity driving taxa. Significance was determined by PERMANOVA with 999 permutations and is displayed in the bottom right hand corner of each panel. Figure coauthored with Dr. Govind Sah.

Discussion

Even though the COVID-19 pandemic is winding down due to the introduction of effective vaccines, the number of tests performed has held steady, and even increased

in the United States. In early 2021, the height of testing was during January, when around 2,100,000 tests were being performed per day, as opposed to late 2021 in November, which saw daily test counts between 500,000 and 3,100,000 tests.[28] Although at UCSD CALM, the number of tests has decreased from around 4,500 per day at its peak, to around 1,000 tests per day as of November 2021,[8] testing will likely continue far into the future, therefore our findings of SARS-CoV-2 on the surfaces of the clinical microbiology testing laboratory will remain relevant. Although it is unknown whether the identified SARS-CoV-2 on the floors of the laboratory (Fig. 1A) represents infectious or inert virus, its presence suggests that labs processing large numbers of SARS-CoV-2 specimens may require improved floor sanitation practices. The virus may originate from outside the laboratory and be tracked in via foot to the laboratory floor or may be from aerosols generated from the testing of SARS-CoV-2. A third possibility that this virus appeared from infected employees seems highly unlikely, as during this study, UCSD had weekly testing practices of all hospital employees, and there were no positive results among the clinical microbiology laboratory staff during this period. UCSD performs all sample handling in biosafety hoods, and these findings stress the importance of strict biosafety techniques when working with potential aerosols.

The significant differences in alpha diversity (Fig. 2) between each surface within the laboratory sections suggests that there are differential abundances of microbes and their distribution is distinct among the surfaces. Indeed, we expected to find a distinct microbiota diversity in the sink because of the abundance of water-associated organisms that live in this habitat. Those microbes differ in diversity from those found on

the floor, which was highest in alpha diversity. We believe that the floor has so many microbes because many of them are brought inside through contact with the outside environment. Our largest concern was the diversity of microbes on the bench surfaces in the laboratory. These laboratory surfaces are constantly cleaned to keep contaminating microbes away from potentially being planted on culture medium. The relatively low diversity of these surfaces compared to the floors, suggests that the frequent bench sanitation practices in the laboratory likely are affecting the contents of the bench microbiomes significantly.

Many of the microbes that were shown to drive diversity are either environmental or human-associated microbes. The diversity seen on the floor of the bacteriology laboratory is driven primarily by the class γ-proteobacteria, order Lactobacillales, and genus *Dickeya* (Fig. 4A) *Dickeya* is a common plant pathogen,[29] so it is not surprising to find it as a driver of floor diversity, where it may have been tracked into the lab on foot. Also of note in the bacteriology laboratory are the drivers of microbiome diversity in the sink. Notably, *Comamonas* and multiple species of *Staphylococcus* (Fig. 4A). *Comamonas* is a common environmental bacterium occasionally found in mud or water,[31] therefore its presence in the sink is not unexpected. *Staphylococcus* is a human-associated skin bacteria and a normal member of the microbiome, and is sometimes associated with human disease, so their presence on the surfaces suggests that they occur there as a result of human contact. Its presence on the sink surfaces may be due to hand washing.

Many of the microbes found on the surfaces of the bacteriology laboratory appeared to be human-associated, and consistent with this representing an area where there is constant cultivation of bacterial pathogens from patient specimens. Some of these species, such as the multiple species of *Staphylococcus*, are often normal members of the human microbiome, but can be pathogenic under the right circumstances. Environments have been shown to pick up human-associated bacteria through normal interaction, as is evidenced from cell phones taking on the microbiota associated with the hand and door knobs/light switches appearing the same,[16] so it is likely that the presence of these microbes is due to human transfer. Therefore, further investigation is needed to determine whether changes in sanitization protocols may lower the risk that potentially pathogenic microbes such as *Staphylococcus* may be transferred to unwitting individuals utilizing these surfaces.

Within the molecular laboratory, the taxa accounting for diversity include *Streptococcus*, *Nocardia*, and *Actinobacteria*. Streptococcus is a human-associated bacteria and a normal member of the human microbiome on both the skin and in the oral cavity.[32] *Nocardia* are usually associated with the soil, and are occasionally pathogenic in immunocompromised hosts.[33] *Actinobacteria* also are common environmental bacteria that are closely related to *Nocardia*.[34] The presence of these two microbes driving diversity of the molecular laboratory suggests that environment-associated microbes predominate in the molecular laboratory, and probably arrive in the laboratory because they have been tracked in on foot.

Conclusions

This is the first study to investigate the microbial communities and their succession in the clinical microbiology laboratory, and the first to assess for the presence of SARS-CoV-2 outside of biosafety cabinets in the laboratory. SARS-CoV-2 was found primarily on the floors of the laboratory, largely in the molecular microbiology laboratory (where much of the SARS-CoV-2 testing was taking place), however it is unknown if the virus we identified was infectious on the laboratory surfaces at the time they were found. Microbiome analysis of workbenches, sinks, and floors revealed unique communities of primarily human-associated and environmental bacteria. The characterization of the clinical microbiology laboratory provides a greater understanding of what microbes come to inhabit the surfaces of the laboratory. The discovery of SARS-CoV-2 on the floors should be taken into consideration when determining sanitization protocols and for assessing the potential risk for exposures for any laboratory processing a large number of SARS-CoV-2 tests.

References

1. Rhoads DD, Sintchenko V, Rauch CA, Pantanowitz L. Clinical Microbiology Informatics. *Clinical Microbiology Reviews.* 2014;27(4):1025-1047. doi:10.1128/cmr.00049-14

2. Bayot ML. Clinical laboratory. StatPearls. https://www.ncbi.nlm.nih.gov/books/NBK535358/. Published July 30, 2021.

3. Huang X, Wei F, Hu L, Wen L, Chen K. Epidemiology and clinical characteristics of COVID-19. *Archives of Iranian Medicine*. 2020;23(4):268-271. doi:10.34172/aim.2020.09

4. Katella K. Our Pandemic Year - A COVID-19 Timeline. Yale Medicine. https://www.yalemedicine.org/news/covid-timeline. Published March 9, 2021.

5. WHO Coronavirus (COVID-19) Dashboard. World Health Organization. https://covid19.who.int/.

6. Irons NJ, Raftery AE. Estimating SARS-COV-2 infections from deaths, confirmed cases, tests, and random surveys. *Proceedings of the National Academy of Sciences*. 2021;118(31). doi:10.1073/pnas.2103272118

7. Hortaçsu A, Liu J, Schwieg T. Estimating the fraction of unreported infections in epidemics with a known epicenter: An application to COVID-19. *Journal of Econometrics*. 2021;220(1):106-129. doi:10.1016/j.jeconom.2020.07.047

8. Stites EC, Wilen CB. The interpretation of SARS-COV-2 diagnostic tests. *Med*. 2020;1(1):78-89. doi:10.1016/j.medj.2020.08.001

9. Pride DT. What to do with expanded molecular testing capacity in a post-pandemic world. https://www.mlo-online.com/molecular/mdx/article/21235174/what-to-do-with-expanded-molecular-testing-capacity-in-a-postpandemic-world. Published August 25, 2021.

10. Laboratory biosafety guidance related to coronavirus disease (COVID-19). World Health Organization. https://www.who.int/publications-detail-redirect/laboratory-biosafety-guidance-related-to-coronavirus-disease-(covid-19). Published February 12, 2020.

11. van Doremalen N, Bushmaker T, Morris DH, Holbrook MG, Gamble A, Williamson BN, Tamin A, Harcourt JL, Thornburg JL, Gerber SI. Aerosol and surface stability of SARS-COV-2 as compared with SARS-COV-1. *New England Journal of Medicine*. 2020;382(16):1564-1567. doi:10.1056/nejmc2004973

12. Goldman E. Exaggerated risk of transmission of COVID-19 by Fomites. *The Lancet Infectious Diseases*. 2020;20(8):892-893. doi:10.1016/s1473-3099(20)30561-2

13. A Timeline of COVID-19 Developments in 2020. AJMC. https://www.ajmc.com/view/a-timeline-of-covid19-developments-in-2020. Published January 1, 2021.

14. Bergquist S, Otten T, Sarich N. COVID-19 pandemic in the United States. *Health Policy and Technology*. 2020;9(4):623-638. doi:10.1016/j.hlpt.2020.08.007

15. CDC Museum COVID-19 Timeline. Centers for Disease Control and Prevention. https://www.cdc.gov/museum/timeline/covid19.html. Published August 4, 2021.

16. Marotz C, Belda-Ferre P, Ali F, Das P, Huang S, Cantrell K, Jiang L, Martino C, Diner RE, Rahman G, McDonald D, Armstrong G, Kodera S, Donato S, Ecklu-Mensah G, Gottel N, Garcia MCS, Chiang LY, Salido RA, Shaffer JP, Bryant MK, Sanders K, Humphrey G, Ackermann G, Haiminen N, Beck KL, Kim H, Carrieri AP, Parida L, Vázquez-Baeza Y, Torriani FJ, Knight R, Gilbert J, Sweeney DA, Allard SM. SARS-CoV-2 detection status associates with bacterial community composition in patients and the hospital environment. *Microbiome*. 2021;9(1). doi:10.1186/s40168-021-01083-0

17. Multistate outbreak of salmonella typhimurium infections linked to exposure to clinical and teaching Microbiology Laboratories. Centers for Disease Control and Prevention. https://www.cdc.gov/salmonella/typhimurium-07-17/index.html. Published July 19, 2017.

18. Wurtz N, Papa A, Hukic M, Di Caro A, Leparc-Goffart I, Leroy E, Landini MP, Sekeyova Z, Dumler JS, Bădescu, D, Busquets N, Calistri A, Parolin C, Palù G, Christova I, Maurin M, La Scola B, Raoult D. Survey of laboratory-acquired infections around the world in Biosafety Level 3 and 4 laboratories. *European Journal of Clinical Microbiology & Infectious Diseases*. 2016;35(8):1247-1258. doi:10.1007/s10096-016-2657-1

19. Singh K. Laboratory‐acquired infections. *Clinical Infectious Diseases*. 2009;49(1):142-147. doi:10.1086/599104

20. Yarbrough ML, Kwon JH, Wallace MA, Hink T, Shupe A, Fraser VJ, Dubberke ER, Burnham CA. Frequency of instrument, environment, and laboratory

technologist contamination during routine diagnostic testing of infectious specimens. *Journal of Clinical Microbiology*. 2018;56(6). doi:10.1128/jcm.00225-18

21. Bolyen E, Rideout JR, Dillon MR, Bokulich NA, Abnet CC, Al-Ghalith GA, Alexander H, Alm EJ, Arumugam M, Asnicar F, Bai Y, Bisanz JE, Bittinger K, Brejnrod A, Brislawn CJ, Brown CT, Callahan BJ, Caraballo-Rodríguez AM, Chase J, Cope EK, Da Silva R, Diener C, Dorrestein PC, Douglas GM, Durall DM, Duvallet C, Edwardson CF, Ernst M, Estaki M, Fouquier J, Gauglitz JM, Gibbons SM, Gibson DL, Gonzalez A, Gorlick K, Guo J, Hillmann B, Holmes S, Holste H, Huttenhower C, Huttley GA, Janssen S, Jarmusch AK, Jiang L, Kaehler BD, Kang KB, Keefe CR, Keim P, Kelley ST, Knights D, Koester I, Kosciolek T, Kreps J, Langille MGI, Lee J, Ley R, Liu YX, Loftfield E, Lozupone C, Maher M, Marotz C, Martin BD, McDonald D, McIver LJ, Melnik AV, Metcalf JL, Morgan SC, Morton JT, Naimey AT, Navas-Molina JA, Nothias LF, Orchanian SB, Pearson T, Peoples SL, Petras D, Preuss ML, Pruesse E, Rasmussen LB, Rivers A, Robeson MS, Rosenthal P, Segata N, Shaffer M, Shiffer A, Sinha R, Song SJ, Spear JR, Swafford AD, Thompson LR, Torres PJ, Trinh P, Tripathi A, Turnbaugh PJ, Ul-Hasan S, van der Hooft JJJ, Vargas F, Vázquez-Baeza Y, Vogtmann E, von Hippel M, Walters W, Wan Y, Wang M, Warren J, Weber KC, Williamson CHD, Willis AD, Xu ZZ, Zaneveld JR, Zhang Y, Zhu Q, Knight R, and Caporaso JG. 2019. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nature Biotechnology 37: 852–857. doi:10.1038/s41587-019-0209-9

22. Amir A, McDonald D, Navas-Molina JA, Kopylova E, Morton JT, Xu ZZ, Kightley EP, Thompson LR, Hyde ER, Gonzalez A, Knight R. Deblur rapidly resolves single-nucleotide community sequence patterns. *mSystems*. 2017;2(2). doi:10.1128/msystems.00191-16

23. Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, Peplies J, Glöckner FO. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Res. 2013;41(Database issue):D590-D596. doi:10.1093/nar/gks1219

24. Faith DP. Conservation evaluation and phylogenetic diversity. *Biological Conservation*. 1992;61(1):1-10. doi:10.1016/0006-3207(92)91201-3

25. Shannon CE. A mathematical theory of communication. *ACM SIGMOBILE Mobile Computing and Communications Review*. 2001;5(1):3-55. doi:10.1145/584091.584093

26. Martino C, Morton JT, Marotz CA, Thompson LR, Tripathi A, Knight R, Zengler K. A Novel Sparse Compositional Technique Reveals Microbial Perturbations.

mSystems. 2019;4(1):e00016-19. Published 2019 Feb 12. doi:10.1128/mSystems.00016-19

27. Kruskal WH, Wallis WA. Use of ranks in one-Criterion Variance Analysis. *Journal of the American Statistical Association*. 1952;47(260):583-621. doi:10.1080/01621459.1952.10483441

28. Daily State-by-State Testing Trends. Johns Hopkins Coronavirus Resource Center. https://coronavirus.jhu.edu/testing/individual-states.

29. Hugouvieux-Cotte-Pattat N. Metabolism and virulence strategies in Dickeya–host interactions. *Progress in Molecular Biology and Translational Science*. 2016:93-129. doi:10.1016/bs.pmbts.2016.05.006

30. Patterson MJ. Chapter 13 Streptococcus. In: *Medical Microbiology. 4th Edition*. University of Texas Medical Branch at Galveston; 1996.

31. Willems A, De Vos P. Comamonas. *The Prokaryotes.* 2006:723-736. doi:10.1007/0-387-30745-1_31

32. Glaeser SP, Kämpfer P. The Family Sphingomonadaceae. *The Prokaryotes*. 2014:641-707. doi:10.1007/978-3-642-30197-1_302

33. Wilson JW. Nocardiosis: Updates and clinical overview. *Mayo Clinic Proceedings*. 2012;87(4):403-407. doi:10.1016/j.mayocp.2011.11.016

34. Barka EA, Vatsa P, Sanchez L, Gaveau-Vaillant N, Jacquard C, Klenk HP, Clément C, Ouhdouch Y, van Wezel GP. Taxonomy, Physiology, and Natural Products of Actinobacteria. *Microbiology and Molecular Biology Reviews*. 2016;80(1):1-43. doi:10.1128/mmbr.00019-15

Supplementary

	number of samples before filtering				
	acessioning	bench	floor	sink	total
bacteriology	38	38	38	38	152
molecular		38	38	38	114
COVID		38	38	38	114
total	38	114	114	114	380
	number of samples after filtering				
	acessioning	bench	floor	sink	total
bacteriology	33	34	29	34	130
molecular		34	32	34	100
COVID		31	33	35	99
total	33	99	94	103	329

Figure S1: *Sample count before and after filtering of different laboratories and surfaces.* Figure coauthored with Dr. Govind Sah.

	Faith's PD				Observed OTU				Shannon Index			
Bacteriology		bench	floor	sink		bench	floor	sink		bench	floor	sink
	accession	3.76E-03	8.98E-10	2.54E-01	accession	1.67E-03	2.31E-09	3.44E-02	accession	6.52E-02	3.52E-06	8.31E-01
	bench		3.66E-09	1.43E-02	bench		1.39E-08	4.43E-02	bench		1.14E-05	1.14E-01
	floor			5.46E-10	floor			2.10E-09	floor			6.61E-07
	sink				sink				sink			
Molecular		floor	sink			floor	sink			floor	sink	
	bench	5.86E-11	5.61E-07		bench	3.17E-11	6.35E-05		bench	1.17E-10	1.55E-03	
	floor		2.19E-11		floor		1.67E-11		floor		2.62E-11	
	sink				sink				sink			
COVID		floor	sink			floor	sink			floor	sink	
	bench	1.80E-07	4.12E-08		bench	1.12E-07	3.29E-08		bench	1.56E-04	1.56E-04	
	floor		8.35E-12		floor		7.00E-12		floor		3.09E-09	
	sink				sink				sink			

Legend
significant
non-significant

Figure S2: *Q-values of Kruskal-Wallis pairwise testing for different alpha diversity metrics.* Significance is denoted with yellow highlight. Figure coauthored with Dr. Govind Sah.

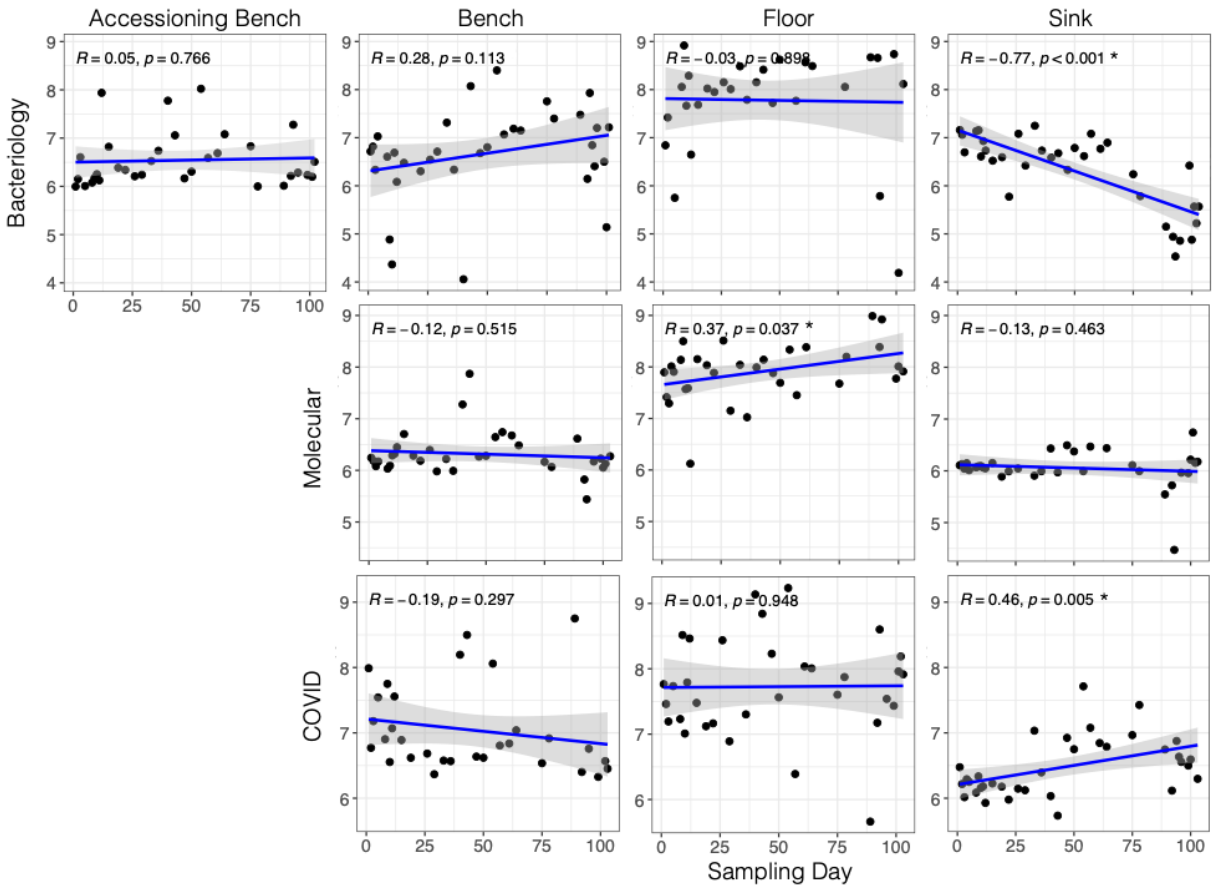

Figure S3: *Scatterplot showing the correlation between Shannon index (y-axis) and sampling day (x-axis) of different laboratories and their surfaces.* Significance determined by Pearson testing with stars denoting significant p-values. Figure coauthored with Dr. Govind Sah.