Manuscript Title

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Abstract

As ubiquitous, naturally multidrug-resistant, nontuberculous mycobacteria (NTM) are a growing concern in cystic fibrosis (CF) due to their increasing prevalence in people with CF (PWCF). The pathophysiological process, colonization, and progression of NTM disease still need to be better understood. At the same time, in recent years, the lung microbiome has been shown to be a cornerstone of CF disease progression. This case-control study aims to investigate the lung microbiome associated with NTM infection in PWCF and to explore some potential dysbiosis that may set the stage for NTM colonization, development, and treatment response. We compared the microbiome composition of sputum samples from 36 NTM-positive CF patients and 72 CF NTM-negative controls (1 case versus 2 controls). Slight differences of α-diversity were observed between cases and controls, but no clear stratification was noticed in analyzing β-diversity between NTM-positive and NTM-negative patients. Analyses have highlighted some bacterial taxa, mainly anaerobes, more abundant in NTM-positive patients. Co-occurrence network analysis has helped determine specific interactions of these taxa in specific bacterial communities within the pulmonary microbiome. This article highlights the complexity of polymicrobial interactions within the CF pulmonary microbiome and unveils the potential link between NTM and anaerobes, which should be investigated further

Introduction

Nontuberculous mycobacteria (NTM) are ubiquitous environmental bacteria in part of the phylum Actinobacteria, usually found in soil, air, water, or animals [1]. However, in recent years, NTM has become a growing concern in patients with cystic fibrosis (PWCF), responsible for NTM pulmonary disease. Its diagnosis is based on clinical, radiological, and microbiological criteria [2]. NTM prevalence has been reported with a wide variation (4 - 40 %), with a significant increase in PWCF in the last few years [3–5]. NTM occurrence has also been shown to vary with age, ranging from 19% for patients aged 12 to 17 years old to 29% for patients older than 60 in a cohort of PWCF from the United States monitored between 2010 and 2014 [6]. The prevalence of NTM also varies according to geographic region and environmental conditions [5]. The causes involved in NTM increase over the last years are widely discussed and probably associated with a myriad of factors: (1) the strengthening of NTM virulence, (2) an alteration of host-pathogen interactions, (3) an enhancement of the monitoring of NTM disease, (4) an improvement of the diagnostic procedures and (5) the aging of the CF population [7,8]. The most commonly isolated NTM species in the United States and Europe belong to the Mycobacterium avium complex (M. avium, Mycobacterium fortuitum, and Mycobacterium chimaera) and the Mycobacterium abscessus complex (M. abscessus subspecies abscessus, M. abscessus subspecies boletii, and M. abscessus subspecies massiliense) [9].

This manuscript is a template (aka “rootstock”) for Manubot, a tool for writing scholarly manuscripts. Use this template as a starting point for your manuscript.

The rest of this document is a full list of formatting elements/features supported by Manubot. Compare the input (`.md` files in the `/content` directory) to the output you see below.

Basic formatting

Bold text

Semi-bold text

Italic text

Combined *italics and* ***bold***

~~Strikethrough~~

1. Ordered list item
2. Ordered list item
    a. Sub-item
    b. Sub-item
        i. Sub-sub-item
3. Ordered list item
    a. Sub-item

- List item
- List item

- List item

subscript: H_2O is a liquid

superscript: 2^{10} is 1024.

unicode superscripts^{0123456789}

unicode subscripts$_{0123456789}$

A long paragraph of text. Lorem ipsum dolor sit amet, consectetur adipiscing elit, sed do eiusmod tempor incididunt ut labore et dolore magna aliqua. Ut enim ad minim veniam, quis nostrud exercitation ullamco laboris nisi ut aliquip ex ea commodo consequat. Duis aute irure dolor in reprehenderit in voluptate velit esse cillum dolore eu fugiat nulla pariatur. Excepteur sint occaecat cupidatat non proident, sunt in culpa qui officia deserunt mollit anim id est laborum.

Putting each sentence on its own line has numerous benefits with regard to editing and version control.

Line break without starting a new paragraph by putting
two spaces at end of line.

Document organization

Document section headings:

Heading 1

Heading 2

Heading 3

Heading 4

Heading 5

Heading 6

A heading centered on its own printed page

Horizontal rule:

`Heading 1`'s are recommended to be reserved for the title of the manuscript.

`Heading 2`'s are recommended for broad sections such as *Abstract*, *Methods*, *Conclusion*, etc.

`Heading 3`'s and `Heading 4`'s are recommended for sub-sections.

Links

Bare URL link: https://manubot.org

Long link with lots of words and stuff and junk and bleep and blah and stuff and other stuff and more stuff yeah

Link with text

Link with hover text

Link by reference

Citations

Citation by DOI [2].

Citation by PubMed Central ID [3].

Citation by PubMed ID [4].

Citation by Wikidata ID [5].

Citation by ISBN [6].

Citation by URL [7].

Citation by alias [8].

Multiple citations can be put inside the same set of brackets [2,6,8]. Manubot plugins provide easier, more convenient visualization of and navigation between citations [3,4,8,9].

Citation tags (i.e. aliases) can be defined in their own paragraphs using Markdown's reference link syntax:

Referencing figures, tables, equations

Figure 1

Figure 2

Figure 3

Figure 4

Table 1

Equation 1

Equation 2

Quotes and code

> Quoted text

> Quoted block of text
>
> Two roads diverged in a wood, and I—
> I took the one less traveled by,
> And that has made all the difference.

Code `in the middle` of normal text, aka `inline code`.

Code block with Python syntax highlighting:

```
from manubot.cite.doi import expand_short_doi

def test_expand_short_doi():
    doi = expand_short_doi("10/c3bp")
    # a string too long to fit within page:
    assert doi == "10.25313/2524-2695-2018-3-vliyanie-enhansera-copia-i-
        insulyatora-gypsy-na-sintez-ernk-modifikatsii-hromatina-i-
        svyazyvanie-insulyatornyh-belkov-vtransfetsirovannyh-geneticheskih-
        konstruktsiyah"
```

Code block with no syntax highlighting:

```
Exporting HTML manuscript
Exporting DOCX manuscript
Exporting PDF manuscript
```

Figures

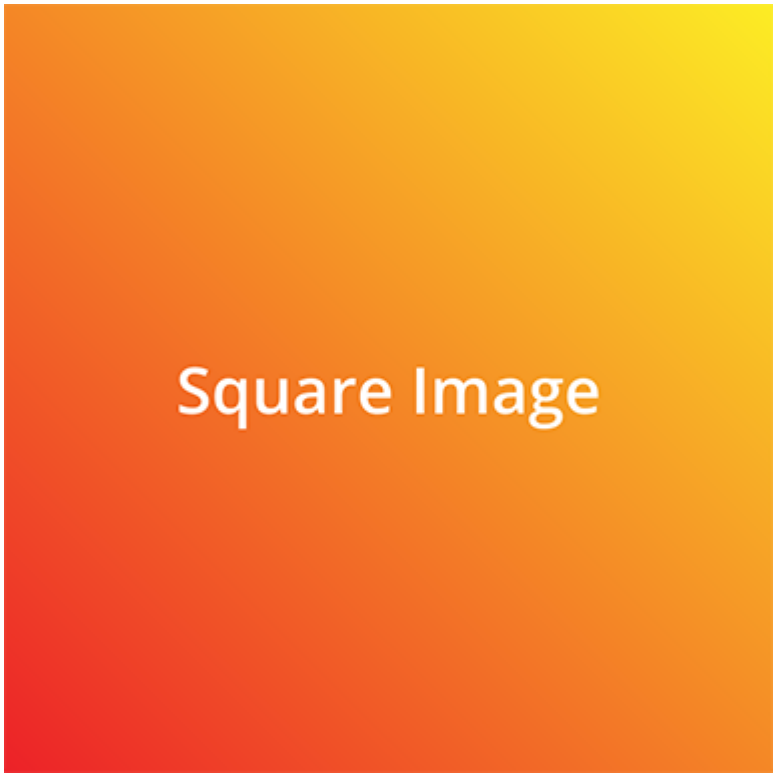

Figure 1: A square image at actual size and with a bottom caption. Loaded from the latest version of image on GitHub.

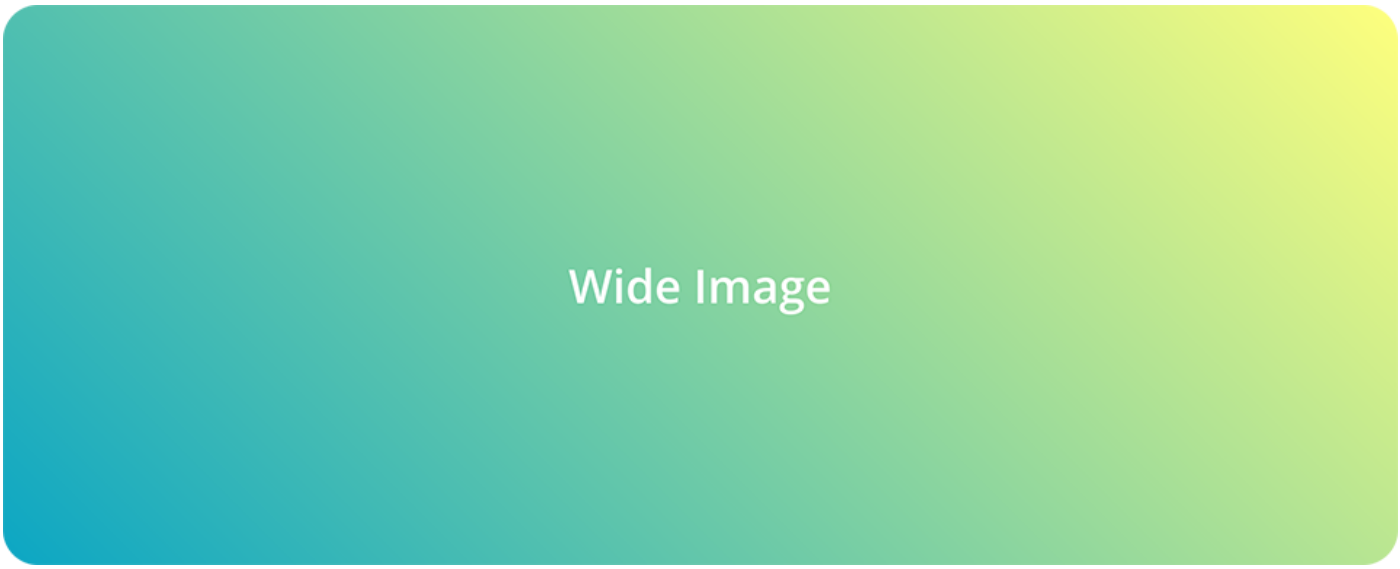

Figure 2: An image too wide to fit within page at full size. Loaded from a specific (hashed) version of the image on GitHub.

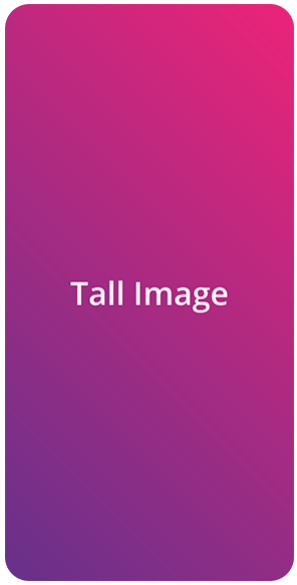

Figure 3: A tall image with a specified height. Loaded from a specific (hashed) version of the image on GitHub.

Figure 4: A vector `.svg` image loaded from GitHub. The parameter `sanitize=true` is necessary to properly load SVGs hosted via GitHub URLs. White background specified to serve as a backdrop for transparent sections of the image.

Tables

Table 1: A table with a top caption and specified relative column widths.

Bowling Scores	**Jane**	**John**	**Alice**	**Bob**
Game 1	150	187	210	105
Game 2	98	202	197	102
Game 3	123	180	238	134

Table 2: A table too wide to fit within page.

	Digits 1-33	**Digits 34-66**	**Digits 67-99**	**Ref.**
pi	3.14159265358979323 846264338327950	28841971693993751 0582097494459230	78164062862089986 2803482534211706	`piday.org`
e	2.71828182845904523 536028747135266	24977572470936999 5957496696762772	40766303535475945 7138217852516642	`nasa.gov`

Table 3: A table with merged cells using the `attributes` plugin.

	Colors	
Size	**Text Color**	**Background Color**
big	blue	orange
small	black	white

Equations

A LaTeX equation:

$$\int_0^\infty e^{-x^2} dx = \frac{\sqrt{\pi}}{2} \tag{1}$$

An equation too long to fit within page:

$$x = a + b + c + d + e + f + g + h + i + j + k + l + m + n + o + p + q + r + s + t + u + v + w + x + y + z + 1 + 2 + 3 + 4 + 5 + 6 + 7 + 8 + 9 \tag{2}$$

Special

⚠ **WARNING** *The following features are only supported and intended for* `.html` *and* `.pdf` *exports. Journals are not likely to support them, and they may not display correctly when converted to other formats such as* `.docx`*.*

LINK STYLED AS A BUTTON

Adding arbitrary HTML attributes to an element using Pandoc's attribute syntax:

Manubot Manubot Manubot Manubot Manubot. Manubot Manubot Manubot Manubot. Manubot Manubot Manubot. Manubot Manubot. Manubot.

Adding arbitrary HTML attributes to an element with the Manubot `attributes` plugin (more flexible than Pandoc's method in terms of which elements you can add attributes to):

Manubot Manubot Manubot Manubot Manubot. Manubot Manubot Manubot Manubot. Manubot Manubot Manubot. Manubot Manubot. Manubot.

Available background colors for text, images, code, banners, etc:

`white` `lightgrey` `grey` `darkgrey` `black` `lightred` `lightyellow` `lightgreen` `lightblue` `lightpurple` `red` `orange` `yellow` `green` `blue` `purple`

Using the Font Awesome icon set:

✔ ? ★ 🔔 ⊗ •••

Light Grey Banner
useful for *general information* - manubot.org

Blue Banner
useful for *important information* - manubot.org

Light Red Banner
useful for *warnings* - manubot.org

References

1. **Non-tuberculous mycobacteria and the rise of Mycobacterium abscessus**
Matt D Johansen, Jean-Louis Herrmann, Laurent Kremer
Nature Reviews Microbiology (2020-02-21) https://doi.org/gh87nq
DOI: 10.1038/s41579-020-0331-1 · PMID: 32086501

2. **Sci-Hub provides access to nearly all scholarly literature**
Daniel S Himmelstein, Ariel Rodriguez Romero, Jacob G Levernier, Thomas Anthony Munro, Stephen Reid McLaughlin, Bastian Greshake Tzovaras, Casey S Greene
eLife (2018-03-01) https://doi.org/ckcj
DOI: 10.7554/elife.32822 · PMID: 29424689 · PMCID: PMC5832410

3. **Reproducibility of computational workflows is automated using continuous analysis**
Brett K Beaulieu-Jones, Casey S Greene
Nature biotechnology (2017-04) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6103790/
DOI: 10.1038/nbt.3780 · PMID: 28288103 · PMCID: PMC6103790

4. **Bitcoin for the biological literature.**
Douglas Heaven
Nature (2019-02) https://www.ncbi.nlm.nih.gov/pubmed/30718888
DOI: 10.1038/d41586-019-00447-9 · PMID: 30718888

5. **Plan S: Accelerating the transition to full and immediate Open Access to scientific publications**
cOAlition S
(2018-09-04) https://www.wikidata.org/wiki/Q56458321

6. **Open access**
Peter Suber
MIT Press (2012)
ISBN: 9780262517638

7. **Open collaborative writing with Manubot**
Daniel S Himmelstein, Vincent Rubinetti, David R Slochower, Dongbo Hu, Venkat S Malladi, Casey S Greene, Anthony Gitter
Manubot (2020-05-25) https://greenelab.github.io/meta-review/

8. **Opportunities and obstacles for deep learning in biology and medicine**
Travers Ching, Daniel S Himmelstein, Brett K Beaulieu-Jones, Alexandr A Kalinin, Brian T Do, Gregory P Way, Enrico Ferrero, Paul-Michael Agapow, Michael Zietz, Michael M Hoffman, … Casey S Greene
Journal of The Royal Society Interface (2018-04) https://doi.org/gddkhn
DOI: 10.1098/rsif.2017.0387 · PMID: 29618526 · PMCID: PMC5938574

9. **Open collaborative writing with Manubot**
Daniel S Himmelstein, Vincent Rubinetti, David R Slochower, Dongbo Hu, Venkat S Malladi, Casey S Greene, Anthony Gitter
PLOS Computational Biology (2019-06-24) https://doi.org/c7np
DOI: 10.1371/journal.pcbi.1007128 · PMID: 31233491 · PMCID: PMC6611653