

Pneumonia surveillance with culture-independent metagenomics in Ugandan adults living with HIV

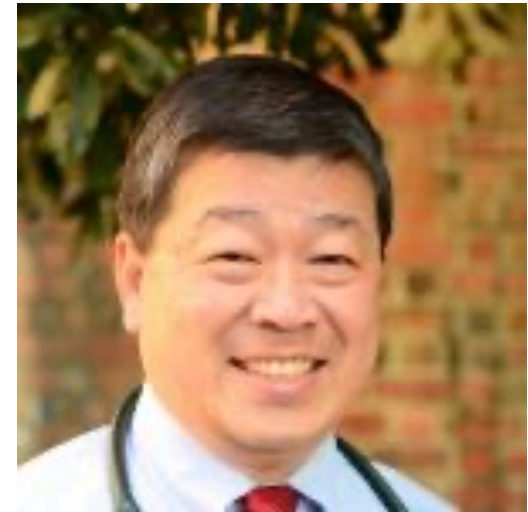
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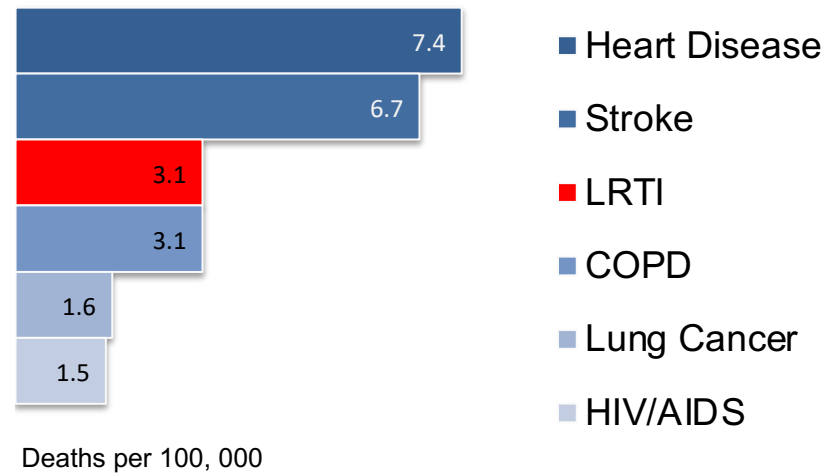
William Worodria, MD



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Lower respiratory tract infections (LRTI) are a leading cause of death worldwide

Leading Global Causes of Death Pre-COVID-19



Lower respiratory tract infections (LRTI) are the leading cause of death in HIV-infected individuals

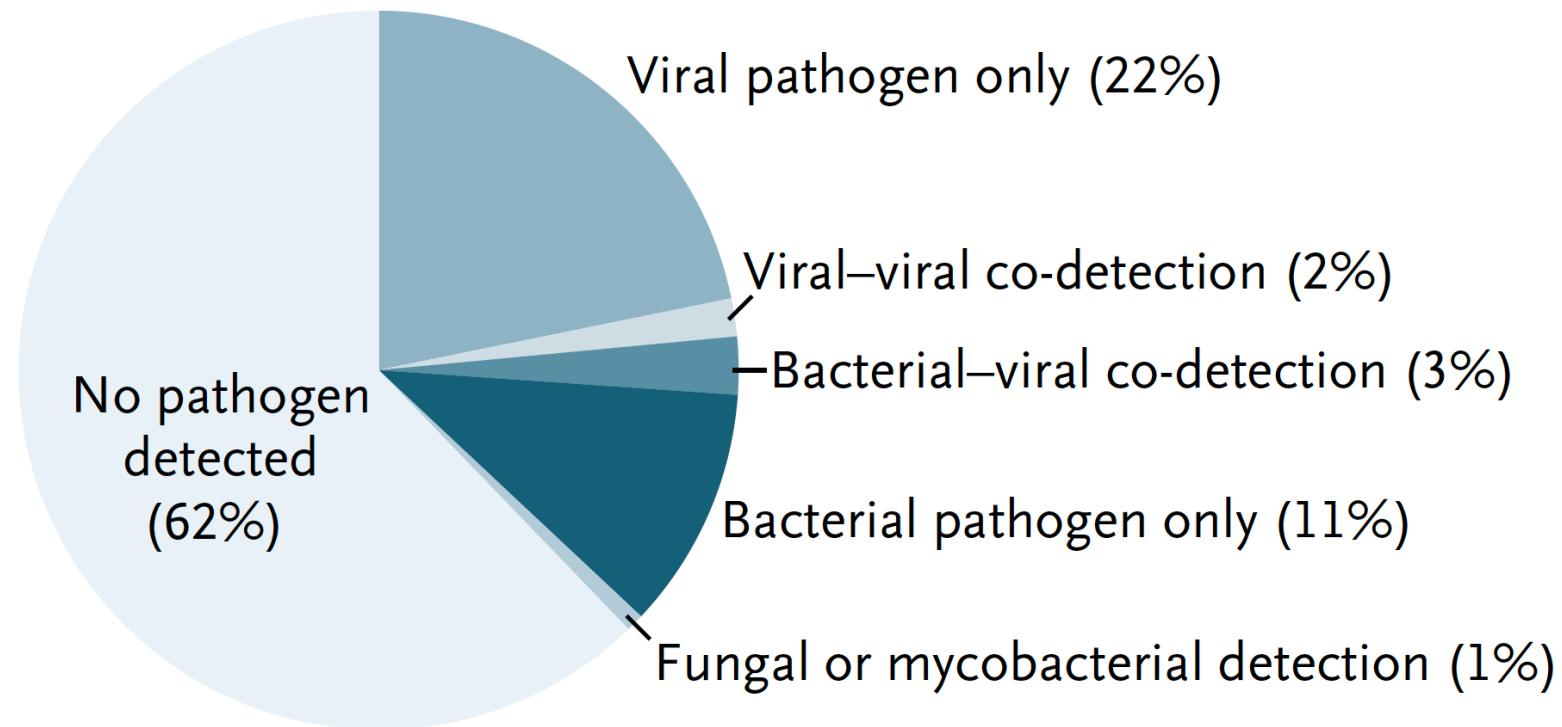
- Sub-Saharan Africa carries a disproportionate burden of the global HIV pandemic, >20,000 deaths annually in Uganda alone
- People living with HIV have a higher risk of developing LRTI, and also experience more severe disease than immunocompetent individuals
- LRTIs are the leading cause of death amongst persons with HIV in Uganda and other LMICs, with mortality rates of over 20%, even with antiretroviral and antibiotic treatment

Rationale

- Understanding causes of LRTI via epidemiological surveillance is essential for empiric treatment guidelines, especially in LMICs
- Few studies have carried out pneumonia epidemiological surveillance in Uganda or other African nations
- LRTI are challenging to diagnose

Even in the US, no pathogen is identified in most LRTI cases

U.S. CDC EPIC surveillance study, 2320 patients hospitalized for pneumonia across the US



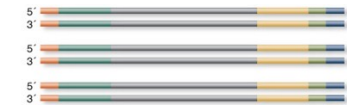
Metagenomic next generation sequencing (mNGS) is a tool for infectious disease surveillance

- Detects of microbial RNA or DNA
- Biospecimen type independent
- A single test to detect multiple pathogens, including novel, emerging microbes
- Less affected by antibiotics versus microbial culture



BAL fluid

RNA



Library
Prep



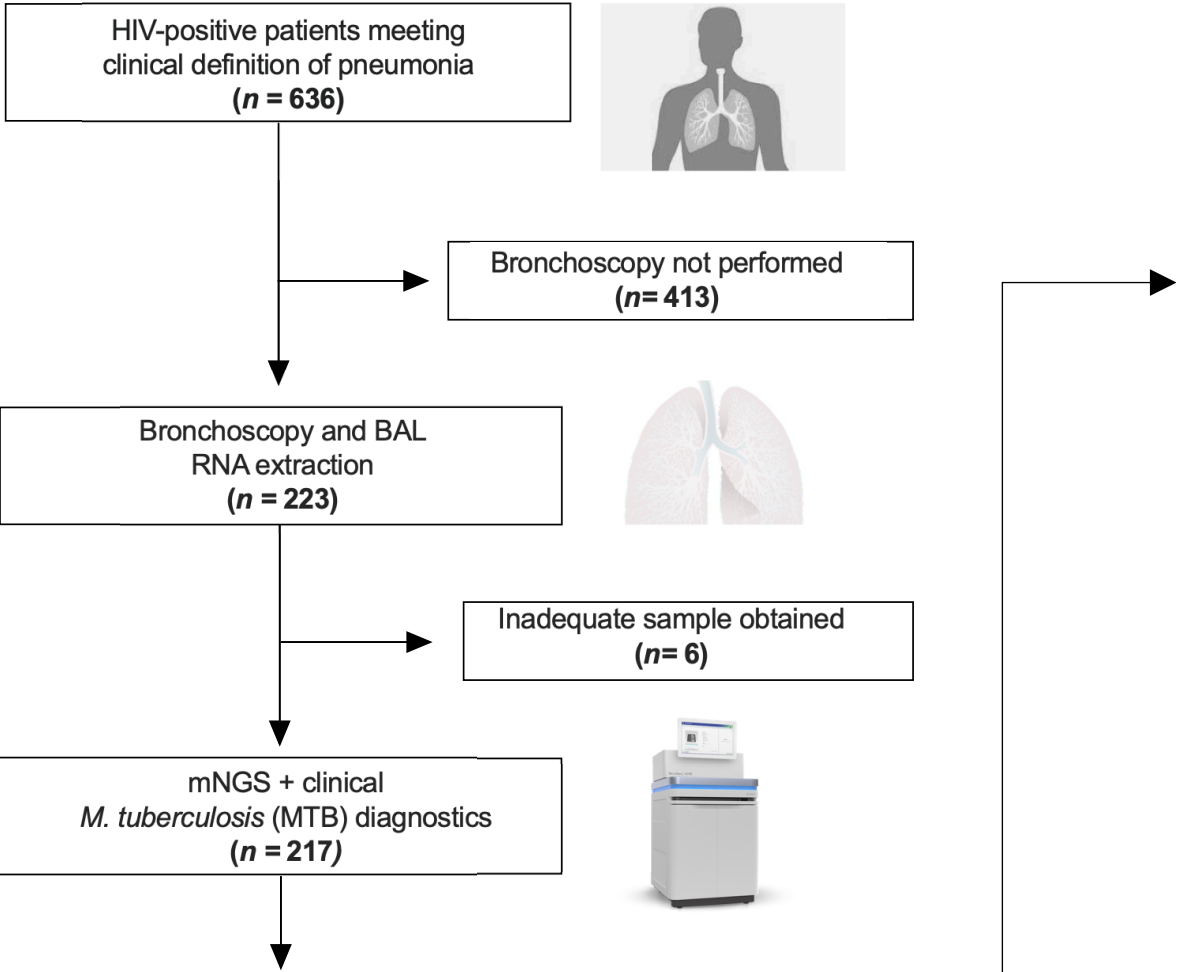
Sequencing

Study aims

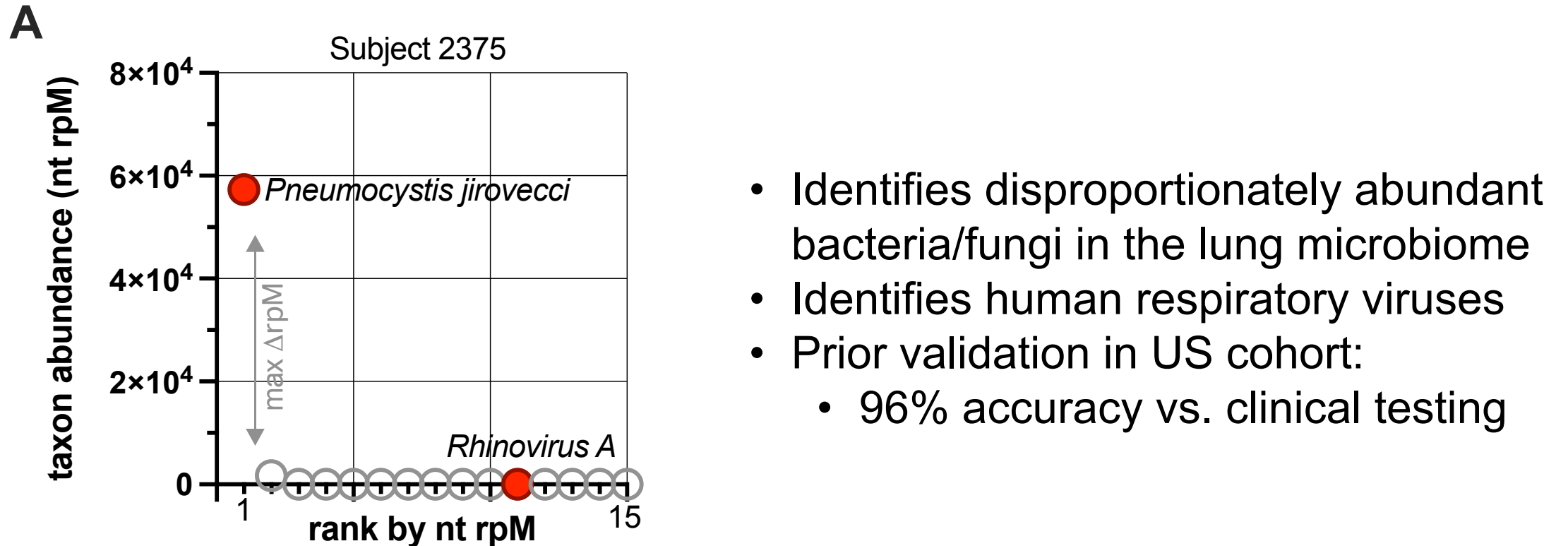
- Goal:
Assess the feasibility of mNGS for epidemiological surveillance of pneumonia in Ugandan patients with HIV.
- Location:
Mulago Hospital,
Kampala, Uganda
2009-2011



Study overview

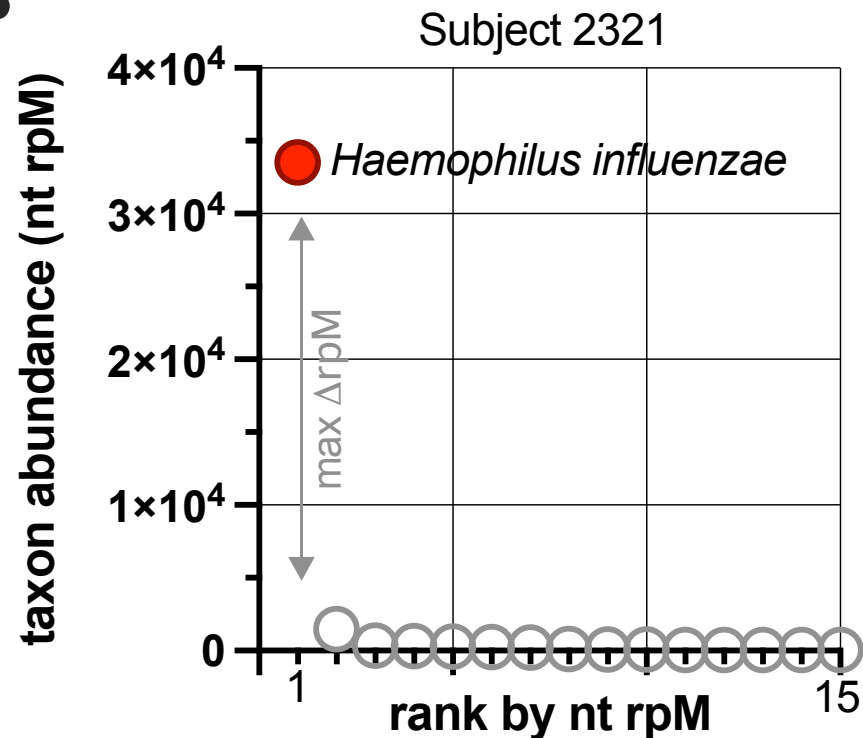


mNGS rules-based model for detecting LRTI pathogens and differentiating them from commensals



mNGS rules-based model for detecting LRTI pathogens and differentiating them from commensals

B



Demographic characteristics of participants

Characteristic	Number (%)*
Age in years (mean [stdev])	36 [10]
CD4 count (mean [stdev])	139 [151]
Female	131 (60%)
Male	86 (40%)
ART	48 (22%)
PJP prophylaxis	122 (56%)
Previous MTB diagnosis	16 (7%)
Prior antibiotic use	183 (84%)
70-day mortality	47 (24%)

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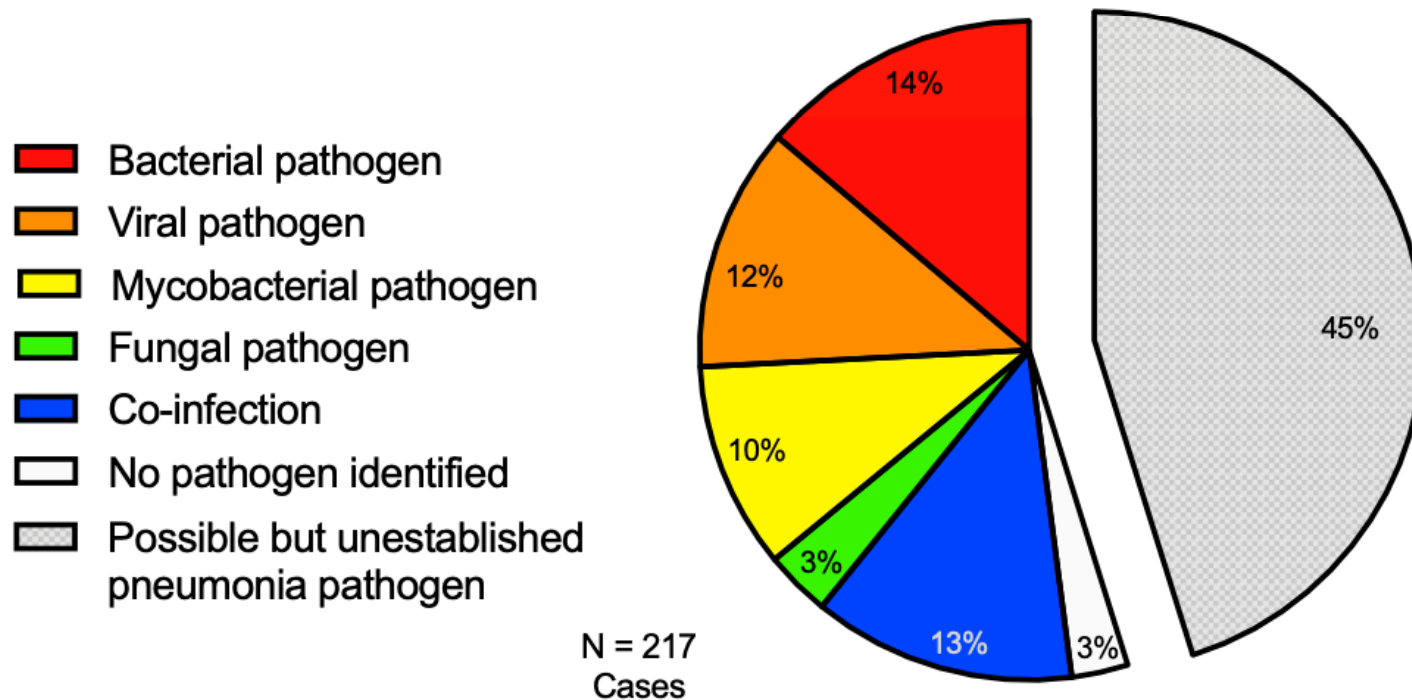
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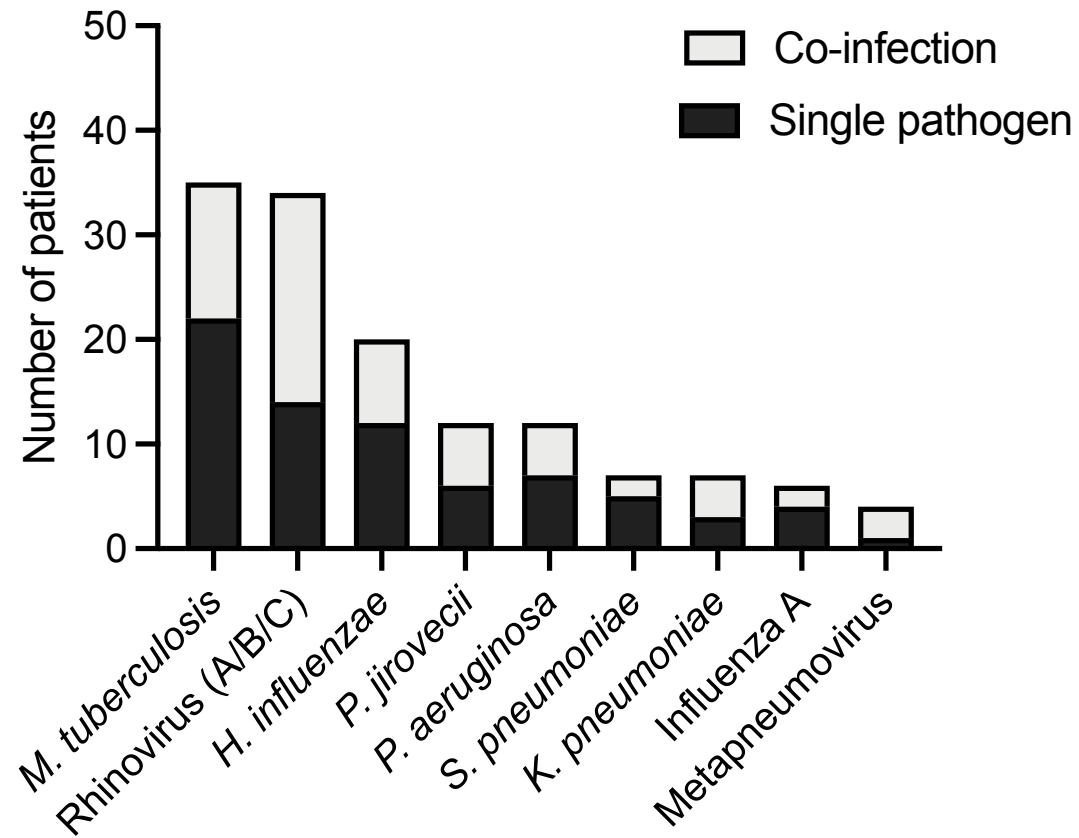
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LRTI Pathogens Detected

- A potential microbial etiology identified in 97% of LRTI cases
- Established respiratory pathogens: 52% of LRTI cases
- Possible LRTI pathogens: 45% of LRTI cases



Established LRTI Pathogens Detected

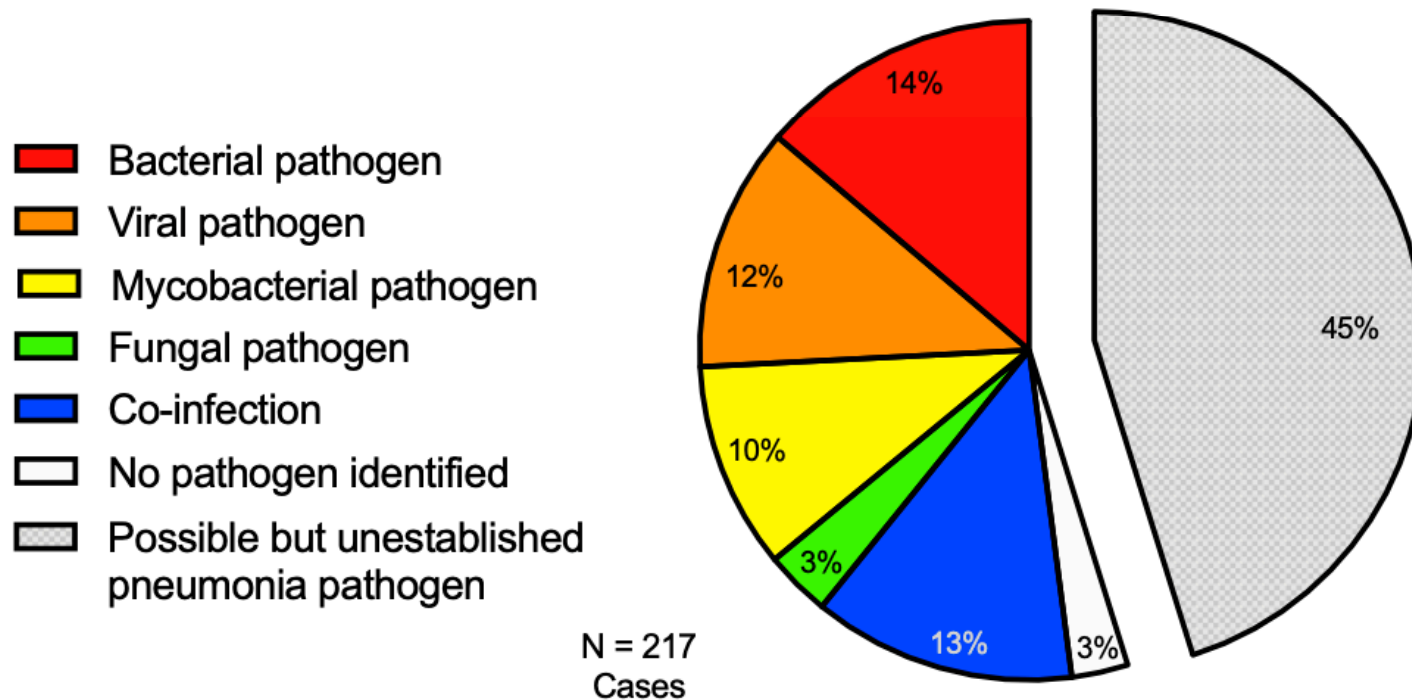


Top 10 by prevalence

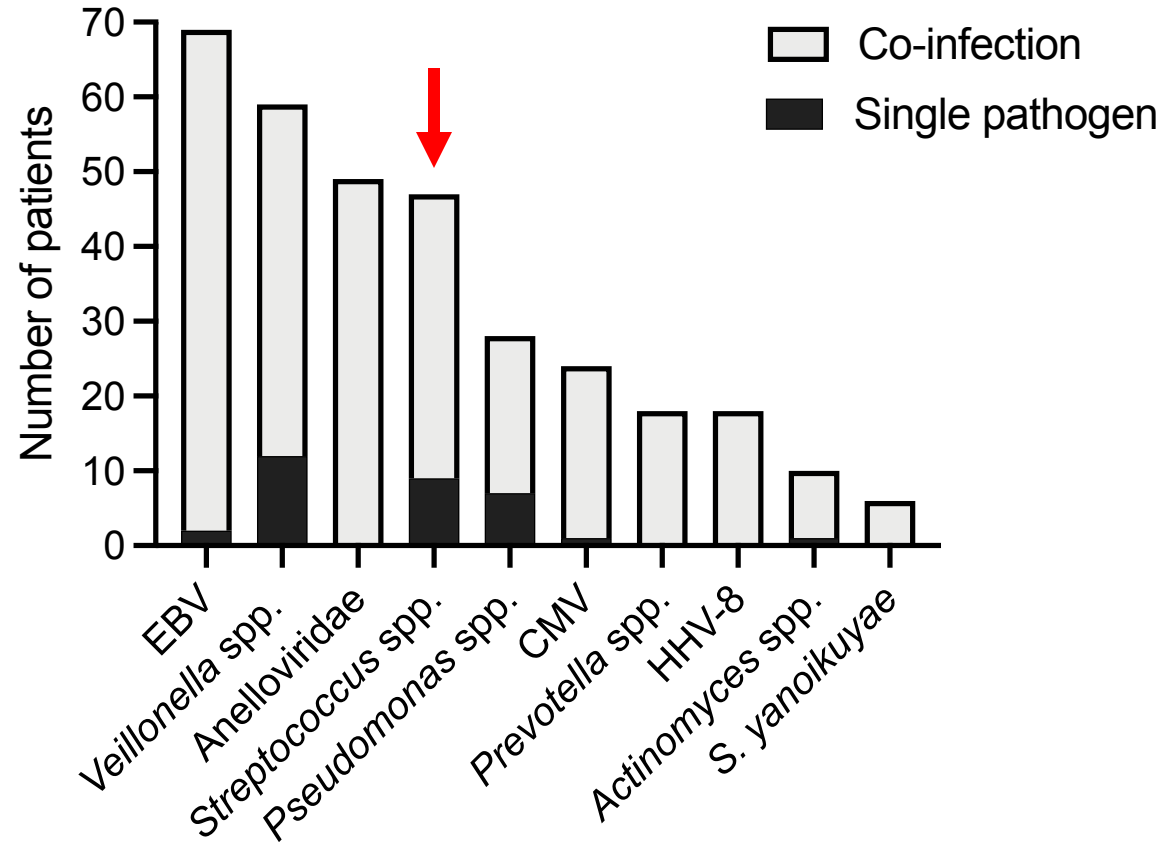
Established pathogens	Established pathogen detected (n, %)	Present as co-infection (n, %)	Total patients with pathogen (n, %)
Bacterial pathogens			
<i>Escherichia coli</i>	1 (1%)	1 (1%)	2 (1%)
<i>Haemophilus influenzae</i>	12 (6%)	8 (4%)	20 (9%)
<i>Klebsiella pneumoniae</i>	3 (1%)	4 (2%)	7 (3%)
<i>Mycoplasma pneumoniae</i>	0 (0%)	1 (1%)	1 (1%)
<i>Neisseria meningitidis</i>	0 (0%)	1 (1%)	1 (1%)
<i>Pseudomonas aeruginosa</i>	7 (3%)	5 (2%)	12 (6%)
<i>Stenotrophomonas maltophilia</i>	1 (1%)	0 (0%)	1 (6%)
<i>Streptococcus pneumoniae</i>	5 (2%)	2 (1%)	7 (3%)
Viral pathogens			
Coronavirus HKU1	1 (1%)	0 (0%)	1 (1%)
Coronavirus 229E	1 (1%)	0 (0%)	1 (1%)
Influenza A	4 (2%)	2 (1%)	6 (3%)
Influenza B	1 (1%)	0 (0%)	1 (1%)
Metapneumovirus	1 (1%)	3 (1%)	4 (2%)
Parainfluenza virus 2*	1 (1%)	0 (0%)	1 (1%)
Parainfluenza virus 3*	1 (1%)	0 (0%)	1 (1%)
Parainfluenza virus 4*	1 (1%)	0 (0%)	1 (1%)
Respiratory syncytial virus	0 (0%)	1 (1%)	1 (1%)
Rhinovirus A*	6 (3%)	10 (5%)	16 (7%)
Rhinovirus B*	5 (2%)	6 (3%)	11 (5%)
Rhinovirus C*	3 (1%)	6 (3%)	9 (4%)
Mycobacterial pathogens			
<i>Mycobacterium tuberculosis</i>	22 (10%)	13 (6%)	35 (16%)
Fungal pathogens			
<i>Pneumocystis jirovecii</i>	6 (3%)	6 (3%)	12 (6%)
<i>Histoplasma capsulatum</i>	1 (1%)	0 (0%)	1 (1%)

LRTI Pathogens Detected

- A potential microbial etiology identified in 97% of LRTI cases
- Established respiratory pathogens: 52% of LRTI cases
- **Possible LRTI pathogens: 45% of LRTI cases**

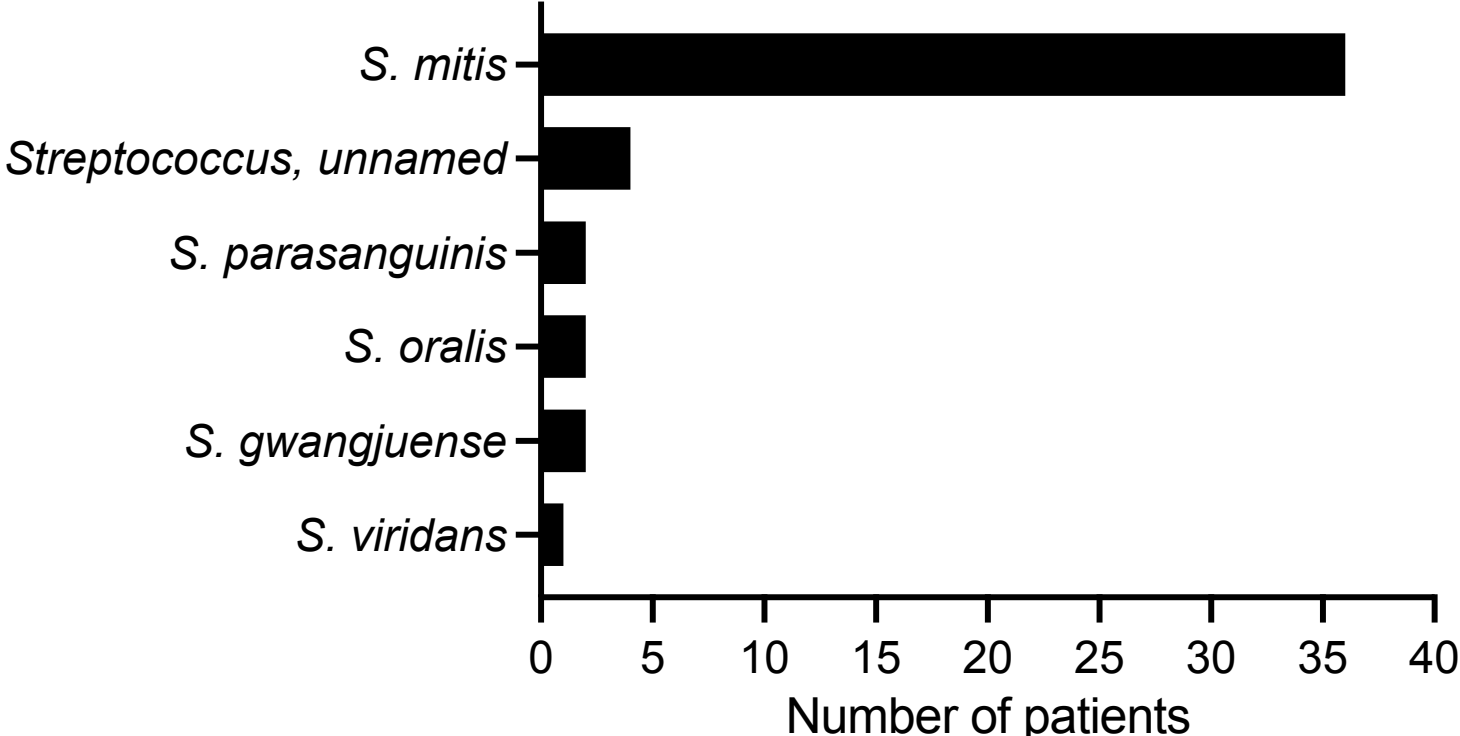


Possible LRTI Pathogens Detected



Top 10 by prevalence

***Streptococcus mitis*:** **a previously unrecognized potential LRTI pathogen**



Possible pathogens: other viruses

Rubella virus

- Detected in 1 patient
- Preventable with MMR vaccine, but outbreaks still occur
- Major concern in USA is congenital syndrome
- Sometimes causes encephalitis, but not usually LRTI

HSV-1

- Detected in 5 patients
- Prevalent, causes 'cold sores' in immunocompetent hosts
- May reactivate in immunosuppressed patients, cause pneumonitis
- HSV-1 has been associated with longer ICU stays and mortality

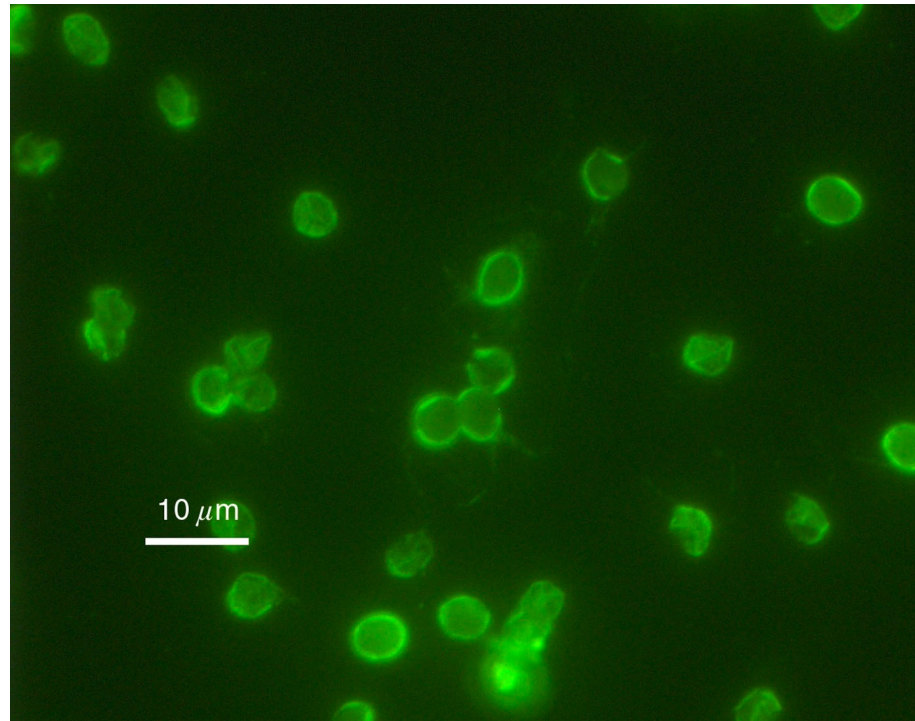
Possible LRTI pathogens: *T. whipplei*

> Taxon ^	Score ^	Z S... ^	rPM ^	r ^	con... ^	con... ^	%id ^	L ^	log... ^	NT NR
∨ Tropheryma (1 bacterial species)	249,815	99.0 9.4	25.5 0.3	2,568 33	5 0	2,519 0	99.6 94.8	1,475. 48.	302.3 253.4	
Tropheryma whipplei	249,815	99.0 9.4	25.5 0.3	2,568 33	5 0	2,519 0	99.6 94.8	1,475. 48.	302.3 253.4	

- We normally think of *T. whipplei* causing a difficult-to-diagnose syndrome with diarrhea, steatorrhea, cardiac, arthralgias, neuralgias
- Recently shown that it can be found in the lungs of patients with HIV

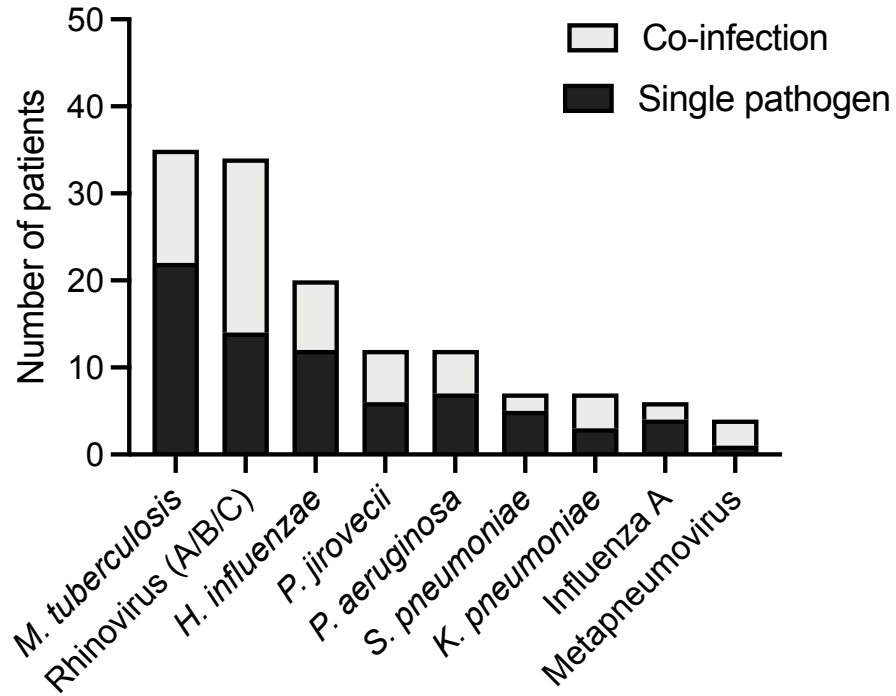
Possible pathogens: Cryptosporidium

- Two patients with *Cryptosporidium parvum*, a protozoal parasite.
- Normally presents as human diarrheal illness
- Rarely (case reports) has been identified as a causative pathogen of pulmonary disease in HIV-infected patients

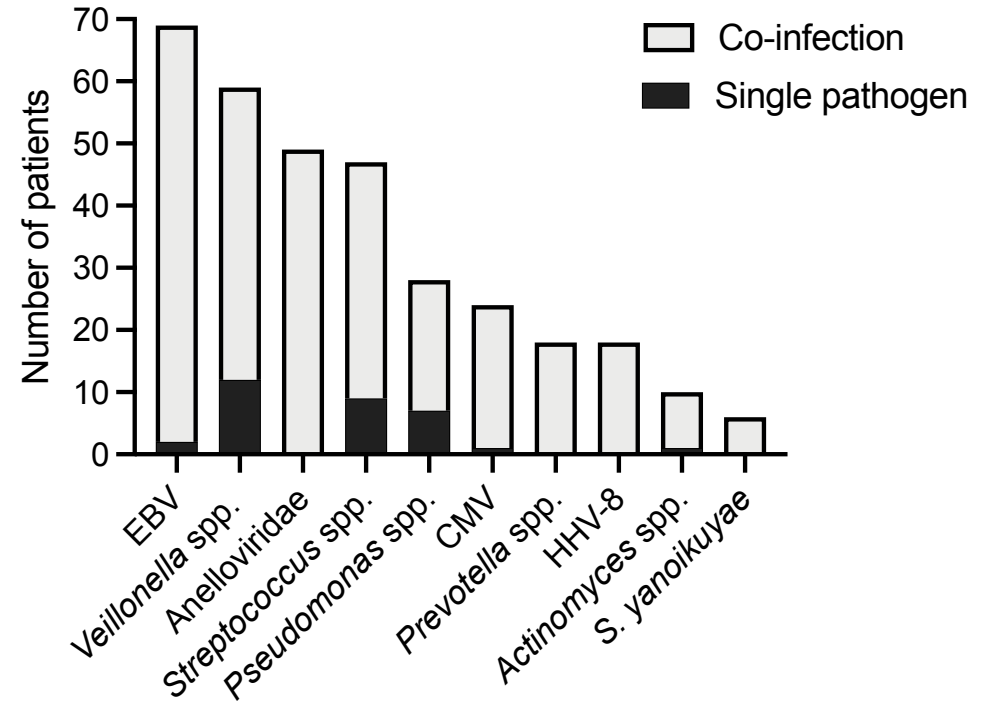


Co-infections are common

Established Pathogens



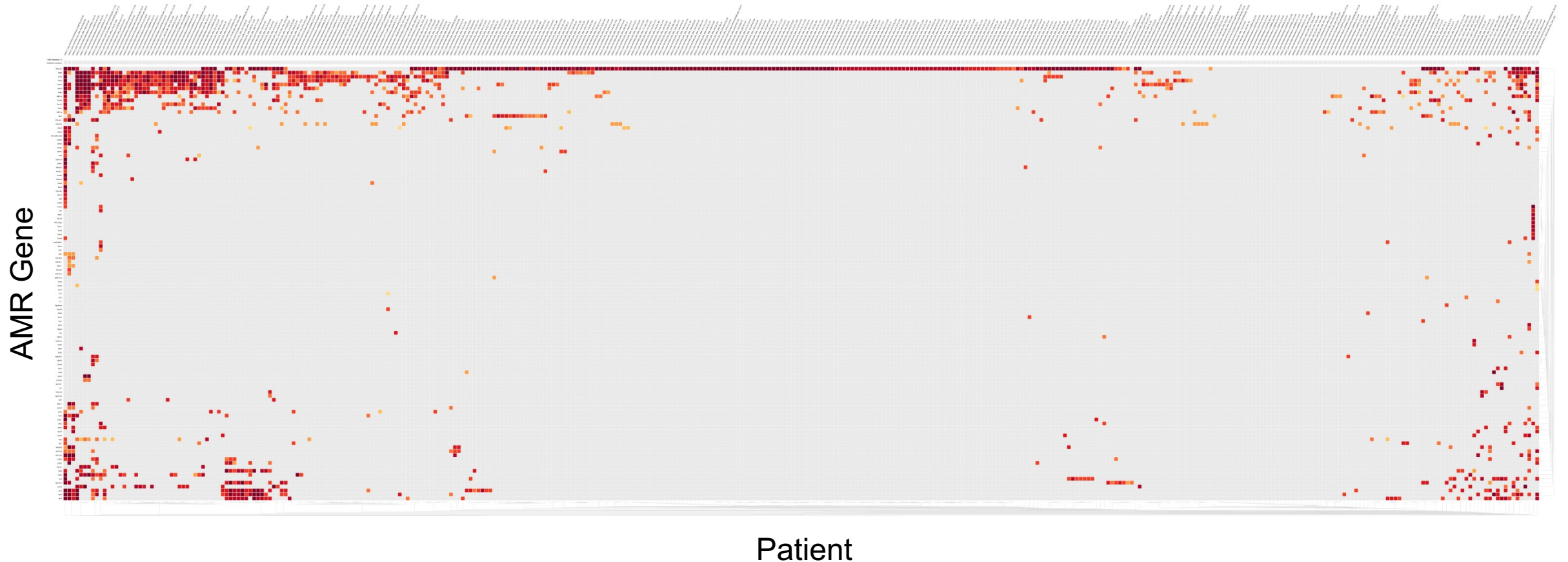
Possible Pathogens



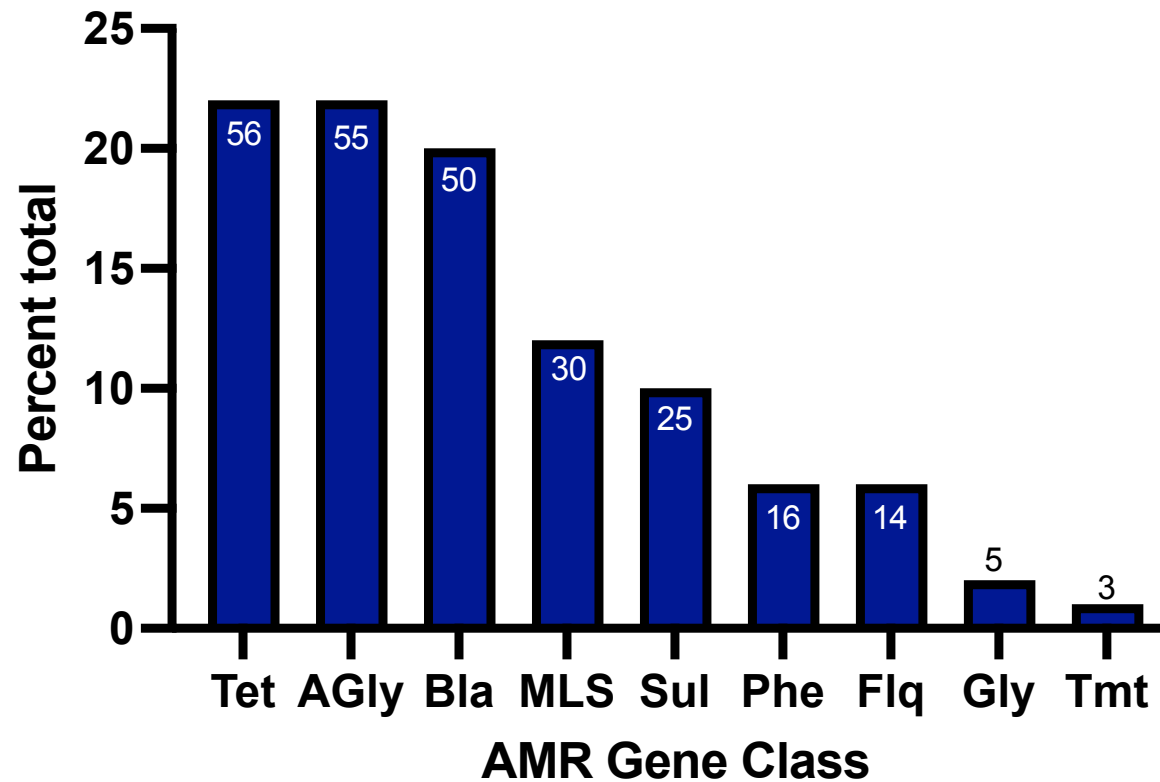
MTB co-infections are common

	N patients (percentage)
Patients with <i>M. tuberculosis</i> detected	35
<i>M. tuberculosis</i> only pathogen detected	22 (62%)
Co-infection with established pathogen	13 (38%)
Rhinovirus (A/B/C)	6 (17%)
<i>Pseudomonas aeruginosa</i>	4 (11%)
<i>Haemophilus influenzae</i>	3 (9%)
<i>Klebsiella pneumoniae</i>	1 (3%)
<i>Neisseria meningitidis</i>	1 (3%)
Metapneumovirus	2 (6%)
<i>Pneumocystis jirovecii</i>	2 (6%)
3+ established pathogens	4 (14%)

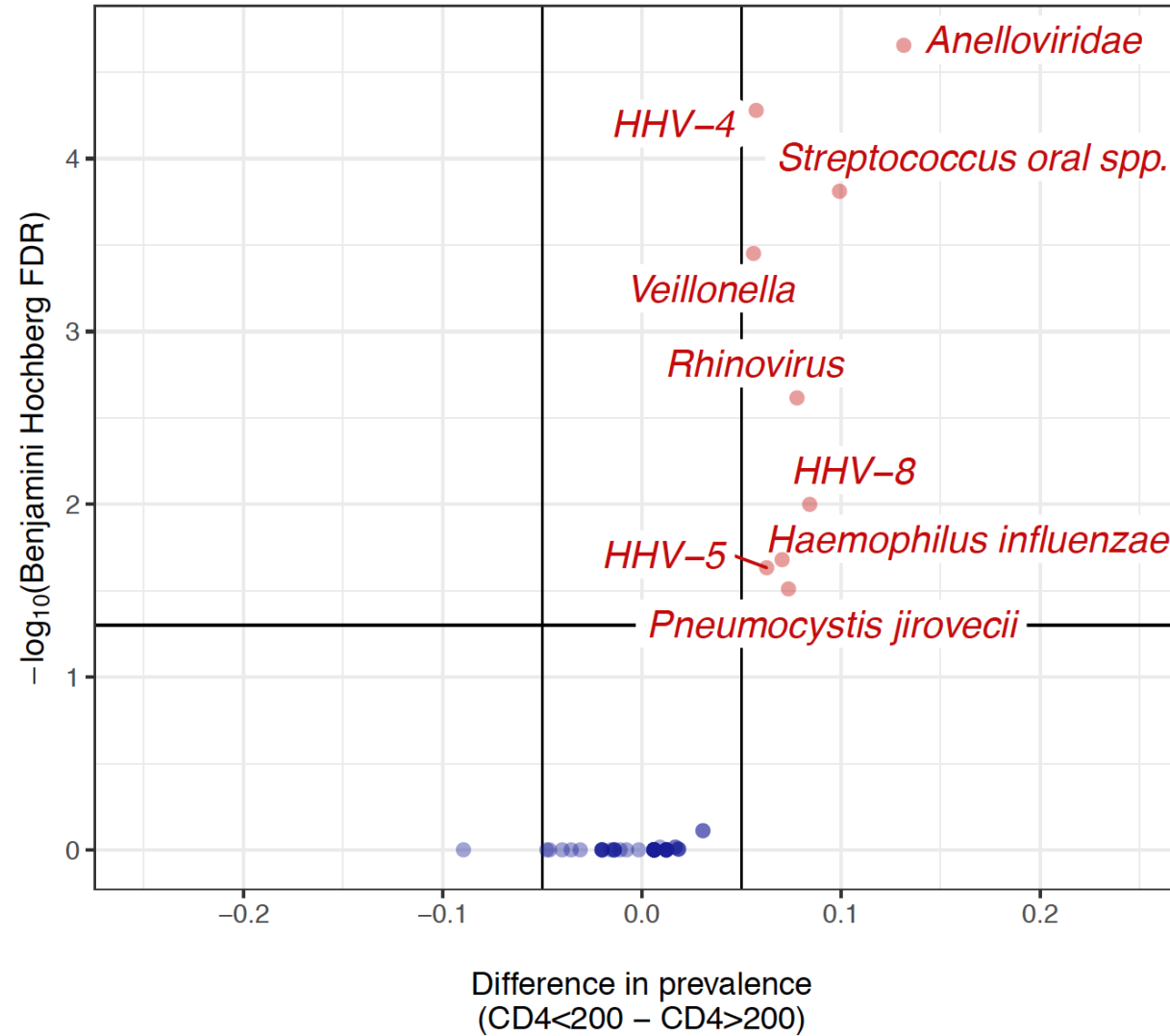
Antimicrobial resistance genes in the lower respiratory microbiome



Antimicrobial resistance genes in the lower respiratory microbiome



Opportunistic pathogens associated with low CD4 T-cell count



Study conclusions:

- A potential microbial etiology identified in 97% of LRTI cases
- MTB was the most common in 16% of LRTI cases
- Bacterial or viral co-infections found in 37% of MTB cases
- *Streptococcus mitis*, not previously reported as a cause of LRTI in patients with HIV, was the most commonly identified bacterial organism (17%).
- Haemophilus influenzae was the next most commonly identified established bacterial pathogen (9% of patients).

Key points

- **mNGS can identify LRTI etiology in cases where:**
 - traditional methods are negative
 - infrastructure needed to perform comprehensive infectious disease testing is not available
 - outbreaks of novel or emerging pathogens occur
- **mNGS can generate enhanced population-level data that can inform public health policies:**
 - pandemic preparedness
 - vaccine efficacy
 - burden of antimicrobial resistance
 - clinical empiric treatment guidelines

Acknowledgements

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Thank you!

Questions?