Identifying healthcare transmission routes of nontuberculous mycobacteria with whole genome sequencing: A systematic review. Schrank SD, et al.

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Study Variable
Study design
Study duration
Facility Type
Epidemiologic case definition
Control group definition (if applicable)
Patient frequency - cases
Patient frequency - controls
Species
Species-isolate frequency
Anatomical site of infection frequency
Healthcare-associated infection (HAI) type (as reported by author)
HAI frequency
Epidemiologic exposure
Single nucleotide polymorphism (SNP) cutoff
WGS as a reactive tool (add information to help interpret 94 epidemiological findings from an investigation
of a previously defined cluster)
WGS as active surveillance tool (perform WGS on all isolates of a group prior to identifying a cluster based
on epidemiological criteria)
Transmission route identification
Effectiveness of WGS in NTM transmission investigations (as interpreted by the author)

## Supplemental Table 2. Anatomical sites of isolates

1 <sup>st</sup> Author, Year	Anatomic Site	Frequency
Bryant, 2013	Respiratory	168
Harris, 2015	Respiratory	27
Robinson, 2016	Respiratory	1
Meera, 2016	Cardiac	11
	SST	3
	Disseminated	3
	Bone	1
Everall, 2017	SST	1
Yan, 2020	Respiratory	118
Hasan, 2019	NR	
Decalonne, 2019	SST	1
Gu, 2020	SST	4
	Respiratory	3
Labuda, 2020	Blood	52
Doyle, 2020	Respiratory	134
	Gastrointestinal	11
	Blood	1
Ghodousi, 2020	Blood	19
	Gastrointestinal	2
	CNS	1
	Respiratory	1
Wetzstein, 2020	Respiratory	38
	Lymph node	1
Xu, 2021	SST	1
	Cardiac	4
	Deep fluid collection	2
	Blood	1
	Bone	1

	Respiratory	1
	Stool	1
Lipworth, 2021	Respiratory	1997
	BAL	135
	Unknown	71
	Tissue Unclear Source	14
	Blood	19
	Cutaneous Tissue	10
	Pus	9
	Peritoneal dialysis catheter	8
	Swab	8
	Breast	4
	Aspirate unclear source	2
	Fluid unclear source	4
	Intraabdominal	4
	Pleural fluid	3
	Chin implant	2
	Lung tissue	2
	Lymph biopsy	1
	Spinal Aspirate	1
	Tibia aspirate	1
Lecorche, 2021	Blood	2
	Bone	1
Gross, 2022	Respiratory	27
Fujiwara, 2022	Respiratory	25
Waglechner, 2022	Respiratory	238
Daniau, 2022	SST	31
	Bone	15
	Blood	14
	Ocular	4

	Gastrointestinal	2
	Urogenital	1
Inkster, 2022	Blood	2
Davidson, 2022	Respiratory	16
	Pleural Fluid	2
	SST	2
	Intraabdominal	1
	Blood	1
Gross, 2023	Respiratory	11
Nils, 2022	Respiratory	154
Dedrick, 2023	Respiratory	NR
	SST	NR
Nagano, 2023	Respiratory	22
Komiya, 2023	Respiratory	52
Faury, 2023	Blood	1
Kling, 2023	Respiratory	6
Groenewold, 2023	Bone	8
Klompas, 2023	Blood	2
	SST	2

Note: SST, Skin/Soft Tissue Infection; NR, Not Reported; CNS, Central Nervous System; BAL, Bronchoalveolar Lavage

**Supplemental Table 3.** Frequency of author defined hospital-associated infections (HAI) type among non-tuberculous mycobacterial infections in cluster investigations aided by whole genome sequencing

Publication First Author, Year	Surgical Site Infection (% total infections)	Central Line Associated Blood Stream Infection (% total infections)	Hospital Acquired Pneumonia (% total infections)	Total study infections
Chand, 2016	18 (83%)			15
Everall, 2017	188 (100%)			188
Decalonne,	1 (100%)			1
2019				
Gu, 2020	2 (29%)			7
Labuda, 2020	52 (100%)			52
Xu, 2021	12 (67%)		1 (6%)	18
Daniau, 2022	51 (60%)	17 (20%)		85
Inkster, 2022		1 (50%)		2
Faury, 2023		1 (100%)		1
Groenewold,	7 (88%)			8
2023				
Klompas, 2023	2 (50%)			4
Total	333 (87%)	19 (5%)	1 (0.3%)	381

Note: Publications not listed did not explicitly document hospital-associated infections.

**Supplemental Table 5.** Details of authors' assessment of the importance of whole genome sequencing in investigations of clusters of infections due to non-tuberculous mycobacteria

Publication	Did sequencing aid	Author comments
First Author,	in identifying point	
Year	source of	
	outbreak?	
Bryant, 2013	Yes	Whole genome sequencing has provided the first convincing
		evidence for transmission of <i>M. abscessus</i> .
Harris, 2015	Yes	In conclusion, we could not demonstrate cross-transmission of <i>M</i> .
		abscessus within our hospital, except between 1 sibling pair that had
		intense exposure both in the hospital and, perhaps more importantly,
		the home environment. Two patients were infected with highly
		genetically similar strains but appeared to be epidemiologically
		unrelated.
Robinson, 2016	Yes	Whole-genome sequencing (WGS) revealed the phylogenetic
,		relationships, dominant circulating clones (DCCs), the likelihood of
		patient-to-patient transmission and the presence of prophages.
Chand. 2016	Yes	WGS analysis detected very low genetic diversity across probable
,		cases, which were restricted to a single lineage within the M.
		chimaera species. The absence of any genetically divergent M.
		chimaera among probable cases and high genetic similarity to HCU
		isolates is consistent with a role for HCUs in transmission.
Everall, 2017	Yes	The clear geographical and temporal structure within the phylogeny,
,		is a pattern more consistent with the introduction of the lineage to
		different locations occurring through transmission, rather than
		dissemination from a point source.
Yan, 2020	Yes	This cluster of isolates with a difference of <7 SNPs (i.e. equivalent
		to or less than same-patient isolate difference) suggests a shared
		recent ancestor and high probability of cross-transmission.
Hasan, 2019	Yes	The application of WGS has advanced our understanding of M.
		chimaera present in US LivaNova 3T HCUs and patient cases after
		the initial analysis of suspected cases in Pennsylvania and Iowa.
Decalonne,	Yes	Sampling a large amount of water and WGS have been decisive for
2019		the root cause analysis of the infection. Our findings prove, if
		necessary, the usefulness of WGS for typing purposes in the M.
		chelonae species.
Gu, 2020	Yes	We show that all M. chelonae isolates tested were highly dissimilar,
		as indicated by high pairwise SNV values, consistent with
		environmental acquisition and not a nosocomial point source.
Labuda, 2020	Yes	For phylogenetic analyses, 13 isolates were nearly identical. Isolate
		14 was also highly related. Together, these data provide evidence of
		a common source.
Doyle, 2020	Yes	We found WGS to be useful to confirm whether different patients'
		strains are unrelated, even within the dominant clones, but it has

Publication	Did sequencing aid	Author comments
Year	source of	
i cui	outbreak?	
		been far more difficult to reach definite conclusions about cross-
		transmission
Ghodousi, 2020	Yes	Finally, we shown that WGS is the preferred method to distinguish
		whether a clinical strain is related to the HCU outbreak strain.
Wetzstein,	Yes	We found no evidence for nosocomial patient-to-patient
2020		transmissions, although patients with closely related strains and
		treated at the same center were identified.
Xu, 2021	Yes	The WGS of 11 available M. chimaera patient isolates and 13
		available M. chimaera environmental isolates indicated that all were
		closely related to each other and to previous isolates from the
		international outbreak identified in 2015.
Lipworth, 2021	Yes	Over the past 6 years, Public Health England (PHE) has implemented
		whole-genome sequencing (WGS) to replace existing reference
		aboratory techniques for all mycobacteria, producing a near-
		Complete dataset of all sequenced <i>M. dbscessus</i> clinical isolates in
		England from patients with and without cystic horosis. These data
		nave been linked to routinely collected field in-care usage datasets,
		transmission of M. abscessus on a national scale across all national
		arouns
Lecorche 2021	Yes	We found very low diversity in the epidemic cluster for the M
		chimaera isolates in France, with a mean pairwise distance of 4
		SNPs, and confirmed that the 2 suspected patients were infected by
		the M. chimaera epidemic strain.
Gross, 2022	Yes	Using integrated analysis of epidemiologic investigations, healthcare
		environmental sampling, mapping home of residence watershed,
		and determination of strength of cluster relatedness with pan-
		genome analysis, this study suggests healthcare-associated
		transmission of NTM is uncommon in our center.
Fujiwara, 2022	Not reported	
Waglechner,	No	We show WGS data is necessary but not sufficient to establish
2022		patient-to-patient M. abscessus transmission
Daniau, 2022	Yes	WGS appears to be a suitable tool for the molecular investigation of
		NTM infections, but might need expert rules and standardization to
		be used further.
Inkster, 2022	Yes	WGS is a promising adjunct for the investigation of outbreaks due to
		atypical mycobacteria.
Davidson, 2022	Yes	Prompt and thorough investigation of HCFA <i>M. abscessus</i> clusters is
		essential for mitigation of potential outbreaks and prevention of
		new infections. Molecular techniques, including WGS, have become
		critical components of such investigations; however, despite its
		power, WGS of NTM isolates has limitations.

Publication	Did sequencing aid	Author comments
First Author,	in identifying point	
Year	source of	
	outbreak?	
Gross, 2023	Yes	However, this investigation does support healthcare-associated M.
		chimaera acquisition on the basis of a genetic match with a hospital
		water fountain isolate.
Dedrick, 2023	Not reported	
Nagano, 2023	Yes	In conclusion, we used the WGS analysis to genetically demonstrate
		a pseudo-outbreak of <i>M. lentiflavum</i> in our hospital.
Komiya, 2023	Yes	Whole-genome sequencing revealed a potential nosocomial
		outbreak among patients who were ventilator dependent and
		without CF.
Faury, 2023	Yes	Whole-genome sequencing showed that the same strain was
		isolated from the shared shower water of the unit.
Kling, 2023	Yes	Our findings suggest that isolation of <i>M. xenopi</i> from pulmonary
		specimens may be increasing, further defines the genomic
		population structure of this potentially emerging infection and
		establishes WGS as a useful tool for outbreak investigation strain
		typing.
Groenewold,	Yes	We conducted environmental sampling of the HCW's home and
2023		identified an outdoor hot tub at the HCW's home as the possible
		source of <i>M. wolinskyi</i> . These results were confirmed by PFGE and
		WGS analyses comparing clinical and environmental mycobacterial
		isolates.
Klompas, 2023	Yes	Whole-genome sequencing confirmed the presence of a genetically
		identical element in ice and water machine and patient specimens.

Publication First	WGS (Yes/No)	Accessory Genome	Signature SNP
Author, Year		(Yes/No)	(Yes/No)
Bryant, 2013	Yes	No	No
Harris, 2015	Yes	No	No
Robinson, 2016	Yes	No	No
Chand, 2016	Yes	No	No
Everall, 2017	Yes	No	No
Yan, 2020	Yes	No	No
Hasan, 2019	Yes	No	No
Decalonne, 2019	Yes	No	No
Gu, 2020	Yes	Yes	No
Labuda, 2020	Yes	No	No
Doyle, 2020	Yes	Yes	No
Ghodousi, 2020	Yes	No	Yes
Wetzstein, 2020	Yes	No	No
Xu, 2021	Yes	No	No
Lipworth, 2021	Yes	No	No
Lecorche, 2021	Yes	No	No
Gross, 2022	Yes	Yes	No
Fujiwara, 2022	Yes	No	No
Waglechner, 2022	Yes	No	No
Daniau, 2022	Yes	No	No
Inkster, 2022	Yes	No	No
Davidson, 2022	Yes	Yes	No
Gross, 2023	Yes	Yes	No
Dedrick, 2023	Yes	No	No
Nagano, 2023	Yes	Yes	No
Komiya, 2023	Yes	Yes	Yes
Faury, 2023	Yes	No	No
Kling, 2023	Yes	No	No
Groenewold, 2023	Yes	No	No
Klompas, 2023	Yes	No	No

Supplemental Table 6. Genomic methods used as part of NTM transmission investigation

Supplemental Table	7. Genomic anal	ysis performed b	y study authors in	addition to SNP analy	/sis
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Publication First Author, Year	Additional genomic analysis
Bryant, 2013	Mutation Rate
Harris, 2015	Multilocus sequence typing
	Antibiotic resistance
Robinson, 2016	None
Chand, 2016	Phylogenetic analysis
Everall, 2017	Phylogenetic analysis
Yan, 2020	None
Hasan, 2019	Phylogenetic analysis
Decalonne, 2019	None
Gu, 2020	Resistance gene identification
Labuda, 2020	None
Doyle, 2020	Multilocus sequence typing
	Phylogenetic analysis
Ghodousi, 2020	Phylogenetic analysis
Wetzstein, 2020	None
Xu, 2021	None
Lipworth, 2021	Phylogenetic analysis
Lecorche, 2021	Phylogenetic analysis
Gross, 2022	Phylogenetic analysis
Fujiwara, 2022	Phylogenetic analysis
Waglechner, 2022	No
Daniau, 2022	Yes
Inkster, 2022	None
Davidson, 2022	Phylogenetic analysis
	Pangenome analysis
Gross, 2023	Phylogenetic analysis
	Pangenome analysis
Dedrick, 2023	Dominant circulating clones
	Prophage identification
	Phylogenetic analysis
Nagano, 2023	Multilocus sequence typing
Komiya, 2023	Phylogenetic analysis
Faury, 2023	None
Kling, 2023	Phylogenetic analysis
Groenewold, 2023	Pulsed-field gel electrophoresis
Klompas, 2023	None

Supplemental Table 4. Anatomical sites of non-tuberculous mycobacterial isolates from cluster investigations aided by whole genome sequencing												
Anatomic Site												
Publication First Author, Year												
Bryant, 2013												
Harris, 2015												
Robinson, 2016												
Everall, 2017												
Decalonne, 2019												
Yan, 2020												
Gu, 2020												
Labuda, 2020												
Doyle, 2020												
Ghodousi, 2020												
Wetzstein, 2020												
Lecorche, 2021												
Waglechner, 2022												
Gross, 2022												
Inkster, 2022												
Davidson, 2022												
Gross, 2023												
Wetzstein, 2022												
Nagano, 2023												
Komiya, 2023												
Faury, 2023												
Kling, 2023												
Groenewold, 2023												
Klompas, 2023												

Total sequenced isolates % of all sequenced isolates by anatomic site % of sequenced isolates implicated in transmission by anatomic site % of all implicated sequenced isolates by anatomic site Note: A publication not listed indicates the data was not available to report

	Pulmonary		Skin/Soft Tissue			Blood			Bone			Gastrointestinal/Intraabdominal			Lymph node			Tissue			CNS		
Fotal Sequenced Study Case Isolates	Sequenced Isolates Transmitted	s - Sequenced Isolates - Not Transmitted	Total Sequenced Study Case Isolates	Sequenced Isolat Transmitted	es - Sequenced Isolates - Not Transmitted	Total Sequenced Study Case Isolates	Sequenced Isolat Transmitted	es - Sequenced Isolates - Not Transmitted	<ul> <li>Total Sequenced</li> <li>Study Case Isolates</li> </ul>	Sequenced Isola Transmitted	ates - Sequenced Isolates - Not Transmitted	<ul> <li>Total Sequenced</li> <li>Study Case Isolate</li> </ul>	Sequenced Isola es Transmitted	ates - Sequenced Isolates Not Transmitted	<ul> <li>Total Sequenced</li> <li>Study Case Isolates</li> </ul>	Sequenced Isola Transmitted	tes - Sequenced Isolates Not Transmitted	<ul> <li>Total Sequenced</li> <li>Study Case Isolates</li> </ul>	Sequenced Isola <sup>.</sup> Transmitted	tes - Sequenced Isolates Not Transmitted	<ul> <li>Total Sequenced</li> <li>Study Case Isolates</li> </ul>	Sequenced Isolat Transmitted	tes - Sequenced Isolate Not Transmitted
31	17	14																					
27	0	0																					
1	Ţ	Ū	188 1	188 1	0 0																		
17	7	10																					
3	0	3	4	0	4			•															
134 1	0 1	134 0				14 1 19	14 0 19	0 1 0				11	0	11				2	2	0	1	1	0
38	0	38						2		4	0				1	0	1						
169	7	162				2	2	0	1	1	0												
80	5	75																					
4	4	0	1 2	1 2	0 0	1 1	1 1	0 0															
23 154	8 0	15 154																					
22 52	18 52	4 0																					
6	0	6				1	1	0															
			2	2	0	2	2	0	8	8	0												
							_																
762	120	642	198	194	4	41	40	1	9	9	0	11	0	11	1	0	1	2	2	0	1	1	0
74.3	15 7		19.3	98.0		4.0	97 6		0.9	100.0		1.1	0.0		0.1	0.0		0.2	100.0		0.1	100.0	
	32.8			53.0			10.9			2.5			0.0			0.0			0.5			0.3	