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JOURNAL CLUB

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## ARTICLE

"Genomic Analysis of a Hospital-Associated Outbreak of *Mycobacterium abscessus*: Implications on Transmission." Davidson et al., *J Clin Microbiol*. 2022 Jan 19;60(1):e0154721. doi: 10.1128/JCM.01547-21. PubMed PMID: 34705540; PubMed Central PMCID: PMC8769749. https://pubmed.ncbi.nlm.nih.gov/34705540/

## CLINICAL QUESTION

It is currently unclear how to interpret genomic comparisons of *Mycobacterium abscessus* isolates between patients to infer transmission. This study provides a baseline for genomic comparisons by analyzing *M. abscessus* isolates from a well-characterized hospital associated outbreak, including environmental isolates from the suspected point source and clinical isolates collected during the outbreak period.

## SUMMARY

***Background:*** Acquisition of *M. abscessus* is generally thought to occur through independent exposure to soil, air, or water [1-3]. Recent studies using whole genome sequencing (WGS) to examine clinical *M. abscessus* isolate populations have challenged traditional hypotheses on transmission. For example, genomic studies of *M. abscessus* isolates from people with cystic fibrosis (CF) identified clones across Europe, Australia, and the US that differed by fewer than 20 single-nucleotide polymorphisms (SNPs) in their core genomes [4-6]. Some investigators have concluded that acquisition of *M. abscessus* likely occurs through person-to-person transmission [4,7]. However, other studies with WGS and epidemiologic analyses of *M. abscessus* determined that SNP thresholds do not accurately predict transmission and that healthcare associated transmission of *M. abscessus* between patients is rare (in 3-10% of subjects studied) [8-12]. Each of the preceding studies did not include genomic analyses of environmental *M. abscessus* isolates for comparison.

Authors of this study previously investigated a large, biphasic outbreak that occurred from 2013 to 2015 at Duke University Hospital in North Carolina (NC) [13]. This outbreak affected over 100 patients, including lung transplant recipients and cardiac surgery patients, and was epidemiologically linked to a new-hospital-addition water system colonized with *M. abscessus*. Interventions designed to prevent hospital tap water contact mitigated the outbreak, and incidence rates of hospital-associated *M. abscessus* acquisition returned to baseline [13,14].

During the outbreak investigation, genetic screening with non-WGS fingerprinting methods revealed that over 75% of patient isolates and all environmental isolates represented a specific clone of *M. abscessus* subsp. *abscessus*. In the current study, a subset of isolates collected during this outbreak were analyzed by WGS with the hypothesis that core and accessory genome analyses would improve interpretation of genetic distances between *M. abscessus* isolates and understanding of *M. abscessus* acquisition.

***Methods and Results:***

1. *Description of Study Isolates (Table 1):* A total of 26 isolates with specific non-WGS genetic fingerprints (inclusion criteria) were analyzed including environmental (n=4) and clinical isolates (n=7) from phase 1 or phase 2 of the outbreak, outbreak hospital clinical isolates collected before and after the outbreak period (n=4), regional clinical isolates from a neighboring hospital (n=2), and clinical isolates obtained from outside laboratories (n=9).
2. *Hypothesis #1 (Figure 1): Isolates with similar non-WGS genetic fingerprints will cluster together by phylogenomics.* Study isolates (n=26) with genetic inclusion criteria were compared to isolates without genetic inclusion criteria (n=22). Study isolates clustered together and were distinct from isolates without the criteria supporting the hypothesis.
3. *Hypothesis #2 (Figure 2): Outbreak hospital patients within the outbreak period acquired M. abscessus from the same source as environmental isolates.* No significant differences in genome-wide SNP distances (Figure 2A) or % accessory genes (Figure 2B) were observed between environmental and 'within outbreak' isolate groups supporting the hypothesis.
4. *Hypothesis #3 (Figure 2): Outbreak strains predated or remained in the geographic region after the outbreak period.* Significant differences in SNP distances and % accessory genes were observed between environmental and pre/post isolate groups. These data partially support the hypothesis, and also reveal genetic drift in a clonal population over time.
5. *Hypothesis #4 (Figure 2): Control isolates from a neighboring hospital and remote outside laboratories have genome-wide SNP distances similar to those of outbreak isolates as predicted by non-WGS genetic fingerprinting methods.* A wide range of genetic distances were observed among control isolates compared to environmental outbreak isolates suggesting that non-WGS fingerprinting methods do not fully reflect genomic distances.
6. *Hypothesis #5 (Figures 2 and 3): Accessory genome comparisons provide additional information about isolate relatedness beyond SNP thresholds.* Integrated analyses of % accessory genes (gene content variation) and SNP distances (Figure 2C) provided a refined method for identifying 90% of clinical isolates associated with the outbreak.

***Conclusions***:

- Non-WGS genetic fingerprinting methods are a good screening tool to identify genetically related isolates during an outbreak investigation.
- Genomic comparisons provided a higher resolution evaluation of isolate relatedness in terms of recent acquisition compared to non-WGS methods.
- Genome-wide SNP distances alone did not clearly differentiate the mechanism of acquisition of outbreak versus non-outbreak isolates.
- However, integrated analyses of genome-wide SNPs distances and shared accessory genes identified 90% of outbreak isolates.
- Successful investigation of *M. abscessus* clusters requires **molecular** and **epidemiologic** components, ideally complemented by **environmental sampling.**

***Remaining questions:***

- Genetically similar *M. abscessus* isolates have been observed among patients from geographically disparate regions, between CF and non-CF patients, and in situations with limited evidence of transmission. The mechanism(s) of spread remain unclear.
- Analysis of longitudinally-sampled *M. abscessus* isolates from patients over time show clonality and genetic stability. However, it is unknown if this stability also occurs among *M. abscessus* isolates from environmental sources.
- This study confirms the source of *M. abscessus* as water exposure, but there are few recent examples of *M. abscessus* isolated from the environment. Future environmental studies are needed to fully understand exposure risks to vulnerable patient groups.

## GROUP OPINION

WGS has revolutionized the way researchers study pathogen populations and investigate potential outbreaks. Each bacterial species has its own population structure, lifestyle, and environmental niche that should be considered when interpreting genomic comparisons. As with any new method, there is a lag in fully understanding the results and implications for clinical care. The WGS-enabled discovery of *M. abscessus* dominant clones in disparate regions of the world is an extraordinary finding. However, hypotheses that challenge paradigms should be accompanied by rigorous testing of previously held dogma. In this study, genome-wide SNP distances alone were unable to differentiate hospital plumbing *M. abscessus* acquisition from interhuman transmission, neighboring hospital acquisition, or remote community acquisition. Use of genomic comparisons combined with systematic epidemiologic analyses provide the best opportunity for timely investigation of outbreak clusters and successful interventions.

Summary Statement: Genome sequencing has allowed scientists to identify dominant strains of *M. abscessus* in many regions of the world and clusters of similar strains within healthcare centers. However, epidemiological investigations have shown that healthcare settings are an unlikely source for acquisition of *M. abscessus*. Ecological studies are needed to get a better understanding of where *M. abscessus* occurs in the environment to help minimize exposures for at-risk patients.

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