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COVID-19 Associated Pulmonary Aspergillosis isolates exhibit high genomic heterogeneity but are more similar to each each other in their response to infection-relevant stresses

Background

- **Purpose:** Coronavirus disease 2019 (COVID-19) continues to be a global health issue and superinfections involving fungi have widely been reported in patients with severe cases of this novel disease. COVID-19 Associated Pulmonary Aspergillosis (CAPA) is one of such superinfections and while many CAPA fungal isolates have been reported, there is a dearth of publicly available whole genome sequences and phenotypic characterizations for those isolates. This includes whether, or to what extent, patient isolates exhibit “strain heterogeneity” for these traits. We set out to increase the number of whole genome sequences of CAPA isolates available to the community and characterize 11 new isolates using both phylogenomic and phenotypic analyses.

- **Methods:** 11 newly isolated CAPA clinical isolates from Europe were sequenced. Standard pipelines were used to assemble these genomes and predict gene annotations.

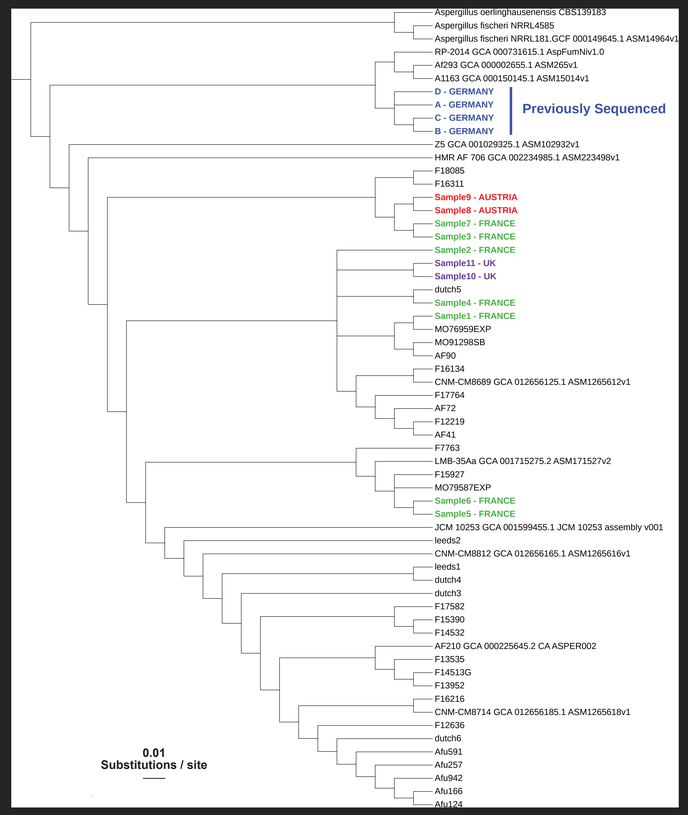
  To build a phylogenomic tree that included the 11 new CAPA isolates, four previously reported European CAPA clinical isolates, 43 non-CAPA clinical and environmental *Aspergillus fumigatus* isolates that span the known diversity of the species, and three outgroup *Aspergillus* strains from two different species, we used a previously constructed data set of 4,525 single-copy orthologous genes. Of the 4,525 orthologs, we found 4,515 to be present in single copy in all the newly sequenced CAPA genomes. These 4,515 single-copy orthologs from each of the 61 strains were then aligned, trimmed, concatenated, and used to construct a phylogenetic tree with maximum likelihood methods.

  In addition, we characterized the efficiency of macrophages to kill the CAPA strains and two reference strains 6 hours post-infection.

- **Results:** The resulting phylogenetic tree showed that the 11 new CAPA strains are more genetically diverse than the previously sequenced CAPA strains and appear to span *A. fumigatus* genomic diversity based on their placements on the phylogeny. There also appears to be low correlation between geographic location and genetic relatedness in these CAPA strains.

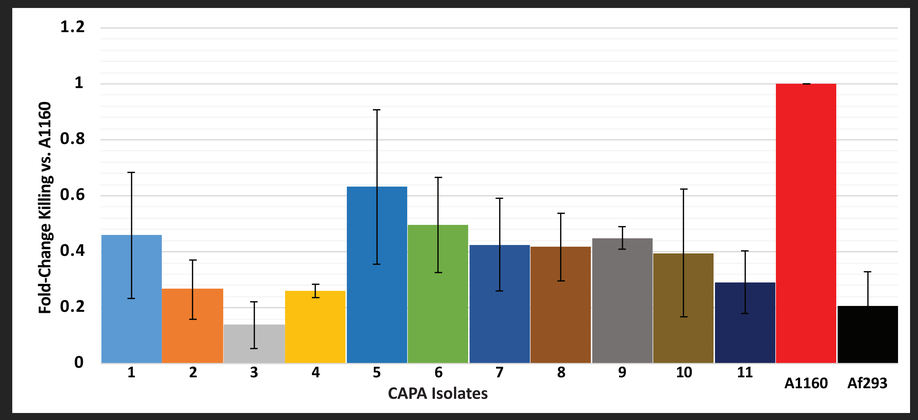
  Comparative analyses of CAPA strains and the reference strains Af293 and A1160 demonstrated that CAPA strains were less susceptible to macrophage killing compared to A1160 (a sister strain to A1163) but were just as susceptible as Af293.

- **Conclusion:** Our results suggest that CAPA strains exhibit genomic heterogeneity amongst each other yet are similar to other *A. fumigatus* strains both in their genetic diversity and phenotypic response to disease-relevant stresses; a novel finding compared to our previous studies that utilized a smaller number of CAPA strains. This information will likely be of great interest to molecular biologists, as well as clinicians and epidemiologists as they continue to address the fungal disease implications of the COVID-19 pandemic.



New CAPA isolates are not closely related to previously analyzed isolates

CAPA isolates are less efficiently killed by macrophages compared to A1160, but at levels comparable to Af293



DNA from 11 new CAPA isolates was obtained

Isolate Name	City/Country of Origin	Patient Condition	Patient Age
Sample 1	Rennes, France	Chronic myeloid leukemia, ARDS	79
Sample 2	Rennes, France	Chronic myelo-monocytic leukemia, ARDS	78
Sample 3	Rennes, France	ARDS	75
Sample 4	Rennes, France	ARDS	58
Sample 5	Rennes, France	ARDS	78
Sample 6	Rennes, France	ARDS, Obesity, Hypertension	71
Sample 7	Rennes, France	ARDS, Hyptertension	73
Sample 8	Graz, Austria	ARDS	65
Sample 9	Graz, Austria	ARDS	60
Sample 10	Manchester, UK	ECMO	41
Sample 11	Manchester, UK	ECMO	41

ARDS = Acute Respiratory Distress Syndrome
ECMO = Extra-Corporeal Membrane Oxygenation

Genomes were assembled and annotated for the 11 CAPA isolates

Analysis Pipeline

Read Trimming → Assemble Genomes → Annotate Genomes → Quality Control

Trimmomatic | SPAdes | Augustus | BioKIT and BUSCO

Isolate Name	Assembly Size (nts)	# Big Scaffolds (>500nts)	# Genes	BUSCO %
Sample 1	29,587,884	219	9,124	96.52
Sample 2	29,792,764	264	9,149	96.35
Sample 3	28,427,726	137	8,825	96.56
Sample 4	29,156,450	308	9,007	96.40
Sample 5	27,969,377	207	8,854	96.16
Sample 6	27,716,363	174	8,810	96.09
Sample 7	28,566,337	211	8,866	96.69
Sample 8	28,318,560	113	8,810	96.64
Sample 9	28,663,298	123	8,841	96.56
Sample 10	28,676,383	164	8,930	96.35
Sample 11	28,682,101	135	8,931	96.33
Af293	29,420,142	8 Chromosomes	10,130	96.87

The pipeine used to construct a previous phylogeny containing CAPA strains was modified for this dataset

Analysis Pipeline

Identify genes in new isolates → Align orthologs → Trim and concatenate alignments → Construct ML tree

Orthofisher | MAFFT | ClipKIT and PhyKIT | IQ-TREE

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