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Posted Date: 2 September 2024

doi: 10.20944/preprints202409.0031.v1

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Whole-Genome Sequencing, Phenotypic Characterization, and Antifungal Susceptibility Profiles of Three *Aspergillus hortae* Clinical Isolates from Colombia

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Abstract: The *Aspergillus* genus comprises fungi that are widely distributed in nature. The *Terrei* section is particularly important due to its functions in recycling organic matter. *Aspergillus hortae* is a species from this section that has been isolated from clinical samples, but its role as a pathogenic agent is unclear. In this study, three clinical *A. hortae* isolates from Colombia, initially identified as *A. terreus*, were characterised by micro and macro-morphological analysis at 26°C and 37°C. A bioinformatic pipeline was used for molecular typing, which included analysis of whole genome sequences for accurate species identification. Susceptibility assays were also conducted using the microdilution broth method (EUCAST). It was confirmed that this species is intrinsically resistant to Amphotericin B with a minimum inhibitory concentration (MIC) of 2-4 mg/L, and is also thermotolerant. We obtained the in vitro susceptibility profiles for three azole drugs (MIC, 0.25-1 mg/L). The three isolates showed phylogenetic divergence from the reference genome strain. A mutation (M769K) in the MelA ortholog of a hypopigmented isolate, which had the lowest MIC value for AmB (2 mg/L), was identified. This study presents the morphological characterization, molecular typing through whole-genome analysis and identification of susceptibility profiles to azoles and amphotericin B of three clinical isolates of *A. hortae* from Colombia.

Keywords: *Aspergillus hortae*; Genome; Pathogenic fungi; antifungals susceptibility

1. Introduction

Aspergillus section *Terrei* represents an important taxonomic group of the *Aspergillus* genus due to its biological diversity and functions in natural ecosystems. Species belonging to section *Terrei* are saprophytic, frequently occurring in soil and organic matter [1]. Furthermore, they are utilized in the industry as producers of secondary metabolites, including drugs and other bioactive compounds [2,3]. However, some isolates from this section are often isolated in clinical samples and linked with diseases in both human and animals [4]. One of the species described within the section *Terrei* is *Aspergillus hortae*, which is phylogenetically close to *A. terreus* [5]. *A. hortae* has been briefly mentioned in the literature as a human pathogenic species [4,6] and has been isolated from clinical samples such as ear secretions [5]. Currently, knowledge about the diversity of this fungus remains limited. As an organism with pathogenic capacity, it is necessary to acquire additional knowledge regarding its phenotypic and genotypic characteristics, as well as its geographical distribution.

 doi:10.20944/preprints202409.0031.v1

The wide genotypic diversity present within the *Aspergillus* genus makes the section/species that the patient is exposed to a key parameter when selecting an appropriate antifungal treatment. This is because *Aspergillus* spp. do not have homogeneous susceptibility profiles [7]. In the section *Terrei*, for example, an intrinsic resistance to amphotericin B has been reported [4]. Nonetheless, the molecular and evolutionary mechanisms behind this resistance remain unclear. The issue of resistance to antifungal treatment is on the rise, with azoles in particular posing a worldwide challenge [8,9]. It is urgent to characterise the pathogenic species of the genus *Aspergillus* and understand their susceptibility profiles to different antifungal drugs.

Currently, whole-genome sequence approaches have expanded the knowledge about the genetic characteristics of pathogens [10]. These approaches enable simultaneous species identification, prediction of drug resistance, and discovery of genetic variants [11,12]. Despite the enormous risk that antifungal-resistant isolates from the genus *Aspergillus* can pose to human health, whole-genome sequences of this genus are still limited, particularly in non-*fumigatus Aspergillus* species [11]. The aim of this study was to provide a morphological characterization, perform molecular typing through whole-genome analysis, and identify susceptibility profiles to azoles and amphotericin B of three clinical isolates of *A. hortae* from Colombia.

2. Materials and Methods

Fungal isolates: Three clinical isolates of *Aspergillus* section *Terrei* were obtained from the Fungi Collection at the Corporación para Investigaciones Biológicas (CIB, Medellin; Colombia). Upon receiving the isolates, they were identified at the species level by microscopic identification technique based on morphological features [1]. These isolates were initially identified and reported as *A. terreus*, and were named as MC7, MC8, and MC10. Cottony colonies with a beige to cinnamon brown colour were observed with. Prior to this work, in Colombia *A. terreus* was the only species within the section *Terrei* reported in clinical samples.

Phenotypic characterization: After obtaining a pure culture, the isolates were inoculated at three points using a micropipette and an inoculum size of 1 µl per spot into Petri dishes containing Malt Extract Agar (MEA), Potato Dextrose Agar (PDA), Sabouraud Agar (SB) incubated at 25°C, and other dishes with Czapek Yeast Agar (CYA) , were incubated at 26°C and 37°C. After 7 days of incubation, the macroscopic characters of the colonies on each medium and the microscopic characteristics on MEA were evaluated. Macroscopic characteristics including colony diameters and texture, obverse and reverse colony colours, degree of sporulation, production of soluble pigments, and exudates were determined. After 10 days of incubation, microscopic characters such as the shape of conidial heads, the presence or absence of metulae between vesicle and phialides, colour of stipes, and the dimension, shape, and texture of stipes, vesicles, metulae, phialides, and conidia were evaluated. Each isolate was morphologically characterised using standardised and recommended methods for laboratories working with *Aspergillus* spp., as described by Samson et al. (2014) [1]. Photographic records of the colonies were obtained using a digital camera, and images of the microscopic structures were captured using the Leica ICC50 W microscope. The colony diameters were analysed in SPSS 24 software through a Klustal-Wallis test.

Antifungal Susceptibility Test: The antifungal susceptibility test was carried out via a 2X microdilution broth method following EUCAST instructions [13]. The antifungals evaluated in this study were Amphotericin B (AmB), Itraconazole (ITC), Voriconazole (VRC), as well as Posaconazole (POS). To test antifungal susceptibility, a suspension of each isolate was prepared in RPMI 1640 broth medium. The four antifungals mentioned above were diluted in dimethyl sulfoxide (DMSO). The strains were tested against 10 concentrations (0.03-8 mg/L) in 96-well plates by 2x dilution and incubated at 35°C for 48 h.

DNA Extraction: Isolates were cultured in Brain Heart Infusion (BHI) at 30°C, at 120 rpm. The biomass was collected during the exponential growth phase after 96 hours of incubation. Cell lysis was performed by mechanical disruption using liquid nitrogen. Genomic DNA was obtained using phenol/chloroform extraction, and RNA was eliminated by treatment with 10 µg of RNase A (Thermo Fisher Scientific, USA) for 120 min at 37°C [14]. DNA quality was evaluated using a NanoDrop 2000

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spectrophotometer (Thermo Fisher Scientific, USA) to determine concentration and purity, using the default setting (1 OD = 50 mg/mL dsDNA). In addition, the integrity of the DNA recovered from isolates was evaluated through 1% agarose gel electrophoresis.

Whole Genome Sequencing: Library preparation was carried out using Illumina Nextera DNA Library Preparation Kit (Illumina Inc. San Diego, CA, USA), with 500 ng of DNA per sample. Next Generation Sequencing was performed using the Illumina Xten (Inc. BGI Hong Kong), generating 150-bp paired-end sequencing. The samples were run on one sequencing line on the Illumina platform, generating around 7 million paired-end reads per isolate and producing an average genome coverage of 30X. Raw data is available on the Sequence Read Archive (SRA) website from National Center for Biotechnology Information (NCBI) (Bioproject PRJNA975750), Biosamples codes SAMN35344990, SAMN35344991, SAMN35344992.

Pre-assembly Analysis: FastQC v0.11.8 program was used to analyse the FASTQ quality code of the short paired-end raw reads [15], Trimmomatic v0.39 was employed to filter out low-quality (Q<30) sequences and adapters [16].

Genome Assembly: The SPAdes 3.10 software [17] with the BayesHammer module for error correction [18] was used to process data. *De novo* assembly of the short reads (2x150) was performed, and iterative k-mer lengths (21, 33, 55, 77 bp) were used to take advantage of the paired-end reads. The draft genome assembly's quality was evaluated using QUAST 5.2.0 [19], comparing the metrics with representative genomes. Three parameters were utilized to verify the quality of each genome: the average coverage of paired-end reads, histograms of the distribution of the percentage of Guanine-Cytosine (% GC), and sequence alignments using the genes of the *A. hortae* IBT 26384 as a reference. The genomes available were downloaded from the JGI MycoCosm database (https://genome.jgi.doe.gov/programs/fungi/index.jsf).

Gene homology Analysis: The *Aspergillus terreus* model was used for *ab initio* gene prediction using Augustus v3.0.1 [20]. The predicted protein sets were then compared using OrthoFinder v2.0.9 pipeline [21] to analyse sequence homology with the representative genomes of *Aspergillus* section *Terrei* reported in the databases (Table S1).

Species Identification by barcoding: To identify the species of *Aspergillus* isolates, ITS, *CaM*, and *BenA* sequences were identified in the assembly using a local blastn search. The query reference sequences from GenBank®, *A. terreus BenA* (EF669520.1), *A. hortae CaM* (KP987054.1), and *A. hortae* ITS (OL711861.1), were used. A web blastn search was then performed on these sequences in the Nucleotide Collection (nr/nt) of NCBI and EMBL-bank databases with default settings (Figure S1).

Phylogenetic Analysis: The resulting sequences were aligned with reference sequences from *Aspergillus* section *Terrei* (Table S1) using the ClustalW v-2.1 program. For phylogenetic reconstruction, the Maximum Likelihood (ML) method [22] was employed with IQtree v1.4.4 software, and the best nucleotide substitution model was estimated. Phylogenetic analysis was conducted using individual genes and a concatenated matrix with *BenA* and *CaM* markers. The Bootstrap method with 1000 replications was used to evaluate the internal branches.

Whole-genome single-nucleotide polymorphism (SNP) calling and phylogenetic analysis of *A. terreus* clade

We download and available Illumina reads of species from this clade (SRA NCBI database) (Table S2). Each of the 10 Illumina data sets was independently aligned to the *A. terreus* reference genome using BWA version 0.5.9 [23] with default settings. SNPs and indels were called with Pilon version 1.4 using the haploid ploidy default setting. Variant call format (VCF) files were filtered using VCFtools version 0.1.1 (minimum depth 4). Alignments were constructed from SNP matrices extracted from the VCF files.

3. Results

3.1. Morphological Analysis

The colonies grown on CYA and MEA culture media at 26°C exhibit compact, columnar-shaped heads. The conidiophores are short, hyaline, and have smooth walls. *A. hortae* vesicles take the form

 doi:10.20944/preprints202409.0031.v1

of half-heads with extensive cylindrical metulae, from which the phialides emerge. The conidia are round, hyaline, and have smooth walls. When grown on CYA and MEA culture media at 37°C, the colonies display a dense cottony appearance with a beige to cinnamon brown colour. As the colony matures, a dark center with a lighter periphery becomes evident. The isolated MCA7 strain shows lighter colony pigmentation and a lower minimum inhibitory concentration (MIC). These morphological characteristics are consistent with what has been described in the literature for *Aspergillus* section *Terrei* (Figure 1).

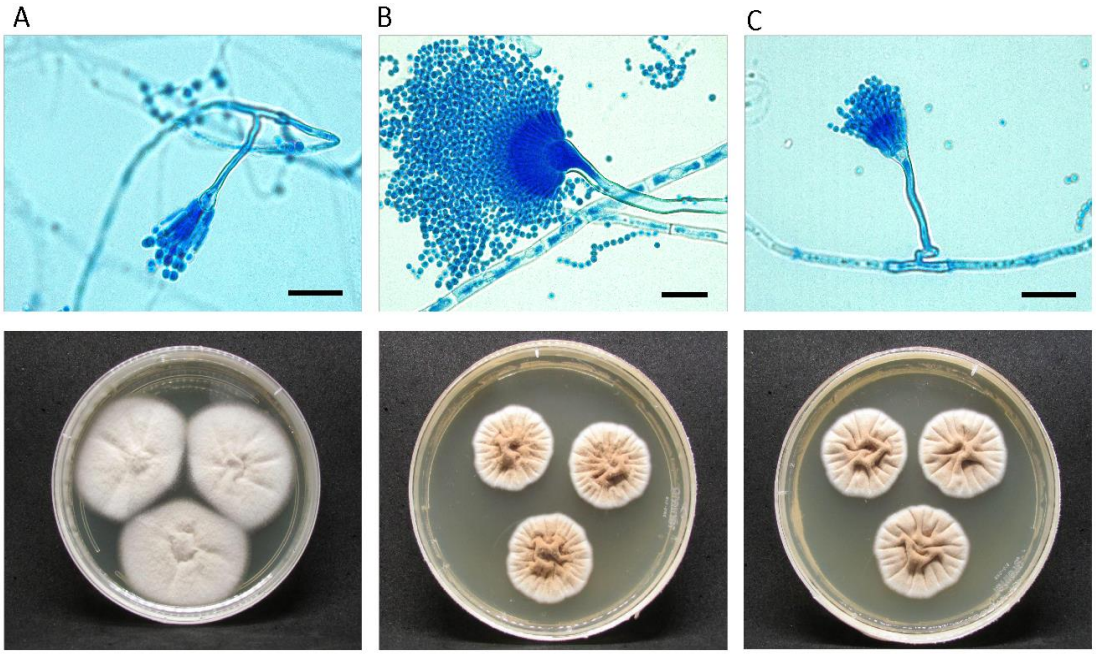

Figure 1. Morphological characteristic of clinical isolates of *A. hortae*. MCA-7 (**A**), MCA-8 (**B**) and MCA-10 (**C**). Microscopic view Lactophenol cotton blue mount in 100X (top) and Macroscopic view of colonies in MEA (down). Scale bars = 10 μm.

After incubating the colonies at both 26°C and 37°C for five days in darkness , it was observed higher biomass production in all cultures at 37°C. The cultures grown at 26°C had a lower colony diameter (56.67 ± 5.60 mm), with significantly better growth ($p<0.05$) in all evaluated media at 37°C (86.92 ± 9.07 mm). Additionally, the highest colony diameter was observed in CYA at 37°C (98.7 ± 10.69 mm) while the lowest was observed in MEA at 26°C (51.67 ± 3.51 mm) (Figure 2).

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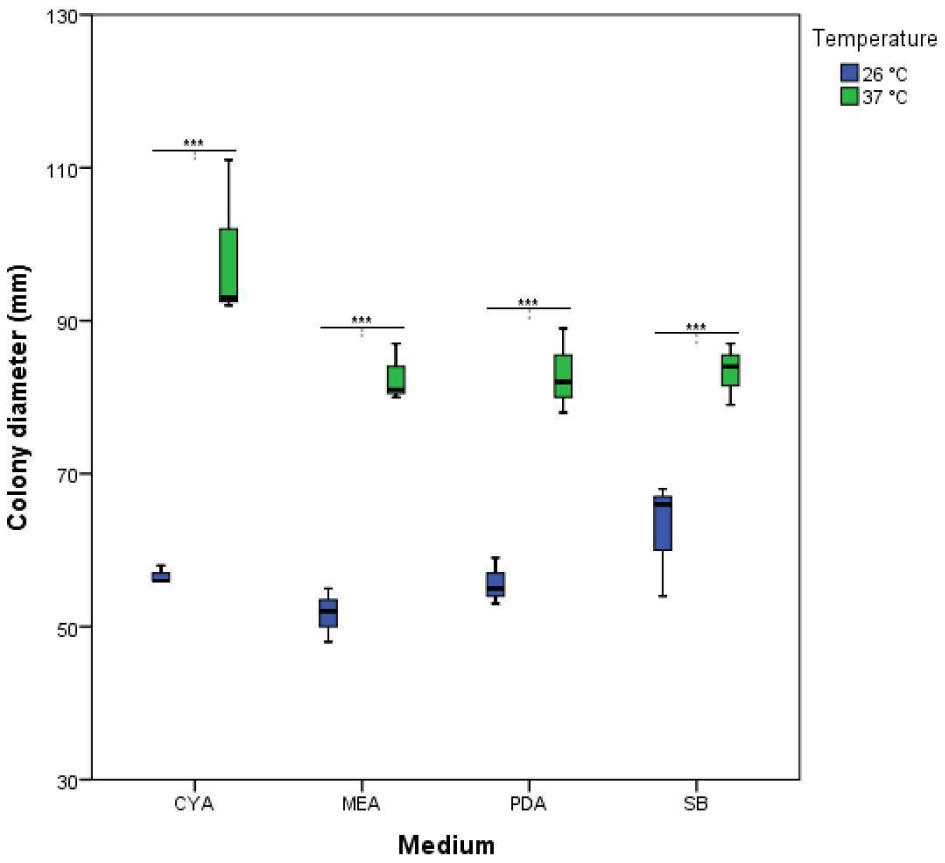

Figure 2. Radial growth box plot of *A. hortae* clinical isolates in different culture media and temperatures.(t-test) *** statistical significance p<0.001.

3.2. Antifungal Susceptibility Testing

The three *A. hortae* isolates exhibited high MIC values upon AmB exposure (2-4 mg/L), with MCA7 displaying the lowest MIC value (2 mg/L), whereas the other isolates showed twice the MIC value (4 mg/L). In contrast, we observed the lowest MIC values with POS (0.25 mg/L), which were consistent across all three isolates. Similarly, the MIC values with ITC were also consistent at 1 mg/L. However, the MIC values with VRC were varied, with two isolates (MCA7 and MCA8) demonstrating a result of 0.5 mg/L and MCA10 having twice this value (1 mg/L) (Table 1).

Table 1. Antifungal susceptibility test of *Aspergillus hortae* isolates.

		VRC		ITC		POS		AmB	
Species	**Isolate**	**MIC**	**INT**	**MIC**	**INT**	**MIC**	**INT**	**MIC**	**INT**
A. hortae	**MCA-7**	0.5	S	1	S	0.25	S	2	R
A. hortae	**MCA-8**	0.5	S	1	S	0.25	S	4	R
A. hortae	**MCA-10**	1	S	1	S	0.25	S	4	R

VRC: Voriconazole. ITC: Itraconazole, POS: Posaconazole, AmB: Amphotericin B. S: Sensible. R: Resistant. MIC: Minimum Inhibitory Concentration (mg/L). INT: interpretation. VRC: Voriconazole. ITC: Itraconazole. POS: Posaconazole. AmB: Amphotericin B.

3.3. Genome Assembly and Analysis

To compare genome structures and gene contents between Aspergilli, we sequenced, assembled, and predicted the proteins of the three *A. hortae* isolates included in this study. In the assemblies, we found characteristics like those reported for the *A. hortae* reference genome, including a genome size

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ranging from 29.86 - 31.90 Mb and a GC content of 52.05 - 52.19% (Table S1). The *ab initio* predictions showed a range of 13,120 - 13,300 predicted protein-coding genes, with a high proportion (>99%) of homologs found in the genus *Aspergillus*.

Using the local blastn tool, the sequences of the markers (ITS, *CaM*, *BenA*) were found within the assemblies, which are commonly used for phylogenetic analysis and identification of the *Aspergillus* genus [1]. Only one copy of these genes was found in each of the assemblies. Subsequently, a web search was conducted to compare these sequences with the databases, leading to the successful identification of the species as *A. hortae*/*A. terreus*. The best hit with *BenA* was with *A. hortae* species (100% identity and 99.81% coverage), while with ITS and *CaM*, we obtained similar hits for *A. terreus* species as well. The 99.81% coverage is for the gene. This result showed that the isolates had a high probability of being classified as *A. hortae*, and it was confirmed after phylogenetic analysis.

The alignment of three *de novo* assemblies from Colombian isolates to the reference genome (Asphor1_AssemblyScaffolds) showed conserved synteny when comparing the Locally Collinear Blocks (LCB) generated in mauve. However, in the alignment, LBC in the reverse panel (R) is observed in the three new assemblies, and these are relatively conserved in them, mainly in MCA8 and MCA10 isolates (Figure 2). After homology analysis using Orthofinder, we identified the orthologs associated with melanin production in *A. terreus*, MelA (XP_001212741), and TyrP (XP_001212742.1). These two proteins are conserved in the *Terrei* and *Flavipedes* sections, and TyrP orthologs were found in almost all evaluated species, while MelA orthologs were found only in 13 species (Figure 2A). MelA possesses a conserved Thioesterase domain of type I polyketide synthase (EntF), however, during protein alignment, a K769M point mutation was observed in the hypopigmented isolate MCA7. The Methionine at position 769 is conserved in all species, while MCA7 is the only isolate with a change in this amino acid (Figure 2B) (Figure 3 #hypopigmented isolated).

When the whole genome is aligned to *A. hortae*, *TyrP* and *MelA* are located contiguously within a LCB in the alignment of the four *A. hortae* genome assemblies. However, the two highly pigmented isolates (MCA8 and MCA10) showed the LBC with melanin-associated genes in an inverted orientation (Figure 2C). Finally, we zoomed into the *MelA* locus, where the nucleotide substitution t2307a (XM_001212741.1) was found in the MCA7 isolate.

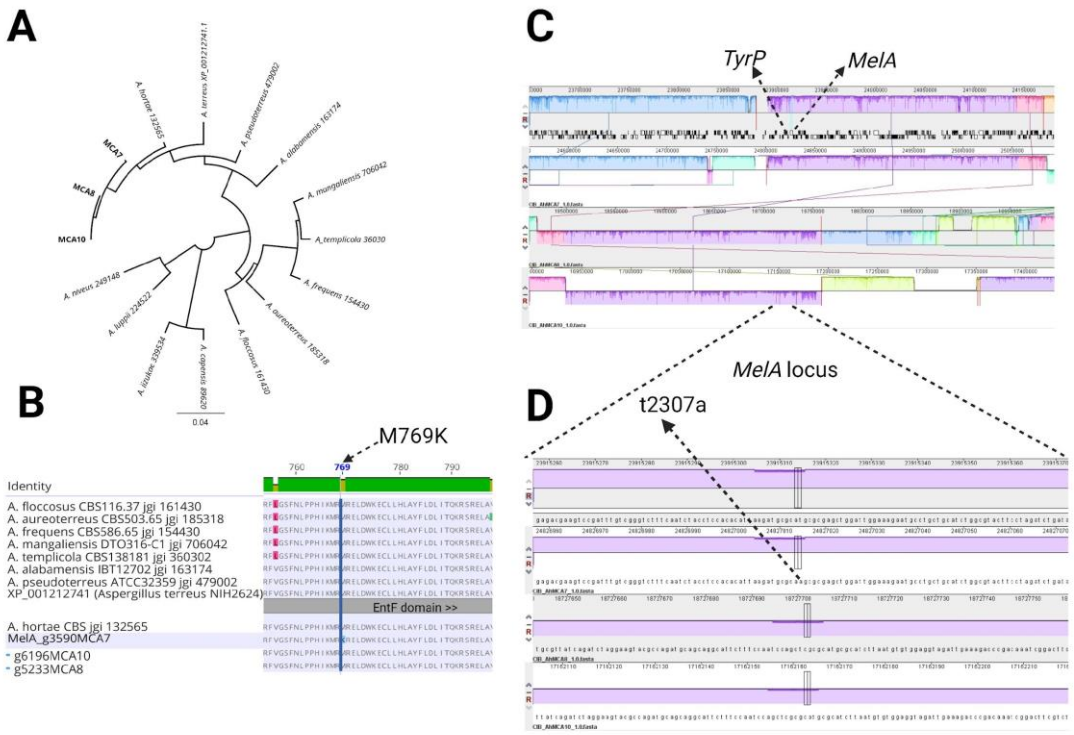

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Figure 3. Genomic variation analysis in *A. hortae*. (A) Neighbor joining phylogenetic tree of MelA in *Terrei* and *Flavipedes* sections. **(B)** Alignment of 12 MelA (XP_001212741) predicted orthologs from the *Aspergillus* section *Terrei*, highlighting a K679M substitution in the hypopigmented isolate MCA7. **(C)** Alignment of whole genome sequences of *A. hortae*. Three clinical isolates were aligned to the genome reference Asphor1_AssemblyScaffolds, and Mauve analysis reveals possible inverted regions (red R) in two of the clinical isolates. These regions contain the melanin associated genes (*MelA* and *TyrP* orthologs from *A. terreus*). **(D)** *MelA* locus exhibits a SNP, with an A to T substitution, in the MCA7 isolate.

3.4. Phylogenetic Analysis

Our best phylogenetic reconstruction was achieved using concatenated sequences of *BenA* and *CaM* in a partitioned matrix of 59 OTUs (Table S4) and 1344 characters. Modelfinder was used to determine the best nucleotide substitution model for each partition (Table S2). The three isolates were grouped with reference isolates from *A. hortae*, creating a monophyletic clade (Bs=88%) with IBT 6271, IBT 6271, HEGP06, PSL01, and SAT02. The reference genome strain IBT 26384 is observed as an outgroup in another monophyletic clade (Bs=94%) with strains IBT 16744 and CMV004A9. The *A. hortae* species is closely related to the clade formed by *A. terreus* and *A. citrinoterreus* species (Figure 4). The phylogenetic reconstruction using sequences from ITS marker, does not provide clear genotypic differentiation between *A. hortae* and other species from *Aspergillus* section *Terrei* (Supplementary Figure S2). In the phylogenetic tree of the clade *A. terreus*, the three Colombian isolates of *A. hortae* cluster with the reference strain IBT 26384 and are positioned as a sister species to *A. terreus*, with *A. pseudoterreus* serving as the outgroup.

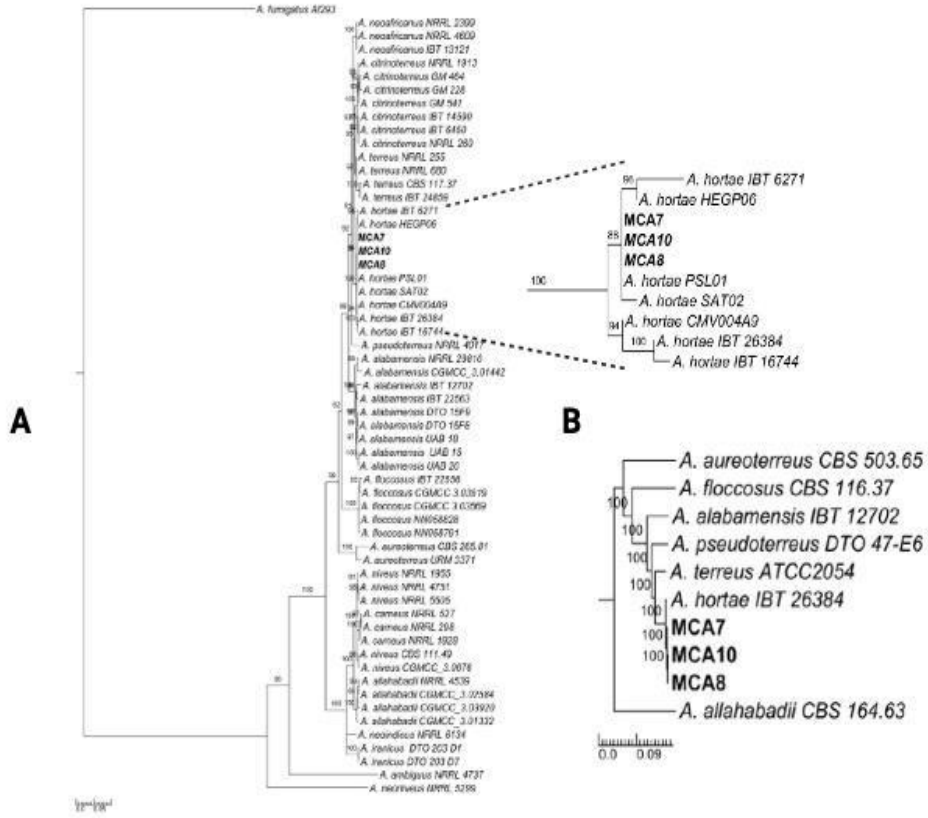

Figure 4. Phylogenetic Analysis. Phylogenetic tree of *Aspergillus* section *Terrei* using concatenated sequences from the *BenA-CaM* markers, *A. fumigatus* Af293 *CaM* was utilized as an outgroup. (**A**). Phylogenomic tree of *A. terreus* clade based on 124,027 SNPs position (**B**). The trees were inferred using the Maximum Likelihood method. The numbers close to branches are % of supported bootstrap after 1,000 replications. Colombian clinical isolates of *A. hortae* are shown in bold.

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4. Discussion

In this study, we described the first report of susceptibility profiles of *A. hortae* isolated in Colombia. Upon analysing the MIC of the isolates MCA-7, MCA-8 and MCA-10, and comparing them with the cut-off established in EUCAST, we confirmed that these isolates exhibit intrinsic resistance to AmB. As previously reported. Isolates from different species of *Aspergillus* section *Terrei*, including *A. hortae*, display a high tolerance to this antifungal *in vitro*. Consequently, patients do not respond when tAmB is employed to treat Aspergillosis caused by members of this section [4,24]. However, the AmB susceptibility profiles of the isolates were not uniform; MCA7 exhibited higher susceptibility to AmB than the other two isolates. Imbert et al, in 2018 also reported two *A. hortae* French isolates with low AmB MIC values (0.25 mg/L) [6], indicating genetic variability within this species.

The AmB resistant phenotype has been linked to changes in the cell membrane of the fungus alterations in the expression of membrane transporters, or modifications in the structure of the drug target [25]. Nevertheless, the exact resistance mechanisms in these species are still unclear. Further investigation of the genomes of a high number of species from this section, as well as the phylogenetically related sections *Fumigati* and *Flavi*, whose susceptibility profiles were heterogeneous, could aid in the elucidation of this matter [26,27].

The fact that we found isolates with heterogeneous AmB resistance and pigmentation levels indicates genetic diversity in this fungal species. Interestingly, the isolate with the lowest pigmentation had the lowest MIC value with AmB, and harboured a mutation in a melanin pathway gene. In *Cryptococcus* spp., high melanin pigmentation isolates are associated with decreasing AmB susceptibility [28]. Similarly, in *W. dermatitidis*, strains with *PSK1* gene knocked out displayed increased susceptibility to both AmB and VRC [29]. This suggests that AmB susceptibility in *A. hortae* could also be associated with melanin production. Geib et al, 2016 demonstrated in *A. terreus* that the pigment produced by MelA and TyrP protects conidia from biotic and abiotic stress factors [30]. However, the role of the melanin in the AmB resistance was not analysed. A potential area for further research is an investigation into the susceptibility of MelA and TyrP knockout strains from section *Terrei* to AmB susceptibility. This study could help to understand the importance of this pigment in the antifungal resistance.

The three isolates were susceptible to the three evaluated azole drugs (ITC, POS, VRC). ITC and POS showed the lowest MIC values, in agreement with previous studies on this fungal species [8,24]. Meanwhile, VRC displayed a heterogeneous profile. This patron could be considered as a caution sign. Despite the fact that all isolates evaluated are classified as susceptible to this drug, its VRC resistance has been observed in other species that exhibit the same profile [8]. The isolated (MCA10) with VRC higher MIC value also had differences in the LCB profiles with respect to the other isolates (MCA7 and MCA8), when their draft assemblies were aligned to the reference genome. Intra-chromosomal rearrangements are a molecular mechanism recently associated with antifungal resistance evolution in *C. auris* [31].

Here, we reported three new genome assemblies from clinical isolates of *A. hortae*, which exhibit significant phylogenetic divergence from the reference genome strain (IBT 26384, a clinical isolate from Brazil). It is noteworthy that this is the first time that genomic variability has been found in this *Aspergillus* species. Steenwyk et al. (2022), using WGS analysis, demonstrated an extensive misidentification of species and low novel lineages detection in the genus *Aspergillus* when only a few barcode markers were used [23]. Despite the phenotypic differences, the phylogenetic reconstruction has revealed that the three isolates are clonal. We suggest that these phenotypic differences may be due to a potential variation in chromosome structure in *A. hortae*, although the finding requires analysis using long-read sequencing technologies that have enhanced precision and resolution in the identification of structural variations [32].

At the moment, the whole genome data for this species continues to be limited for phylogenomic reconstruction to examine the genetic diversity within this species. The species tree generated after homology analysis from section *Terrei* only showed that the three Colombian isolates are related to the only available *A. hortae* reference genome, whereas the *BenA-CaM* phylogeny with 10 isolates

 doi:10.20944/preprints202409.0031.v1

showed evidence of genetic divergence from this isolate. Further research with more isolates sequenced at genome level could be conducted to elucidate the true evolutive history and genetic diversity of *A. hortae*.

On the other hand, the ITS barcode marker has been used as a panfungal molecular test in the diagnostic laboratory [33,34]; however, it showed very low-resolution power in the *A. terreus*/*A. hortae*/*A. citrinoterreus* clade. It may have overestimated the epidemiology of *A. terreus* impeded our understanding of the actual impact on health of new cryptic species, such as *A. hortae*. The epidemiology of Aspergillosis has changed in recent years, with the emergence of new species, which could be attributed more to the improvement of typing techniques that have allowed for accurate classification [23,35]. Salem-Bango et al. (2023) propose the use of WGS in specialized laboratories to accurately identify *Aspergillus* species [12]. We agree that the adoption of WGS could be an effective solution for correct species identification in *Aspergillus* Section *Terrei*, and we also suggest using at least the *BenA* as a second barcode marker for more precise identification of species within the section.

A. hortae is a thermotolerant fungal species, a critical characteristic for microbial pathogens to thrive in human and animal hosts. This characteristic demonstrates how this species has evolved to adapt to the stress of growth in the host [36,37], and is also linked to virulence factors in other fungal pathogens, particularly in *A. fumigatus [38],* which tolerates up to 60°C, the upper temperature limit for eukaryotic organisms [39]. Lacker et al. (2019) also demonstrated that *A. hortae* at 37 °C exhibited the highest growth rates, and some isolates had high virulence potential in *G. mellonella* larvae [40]. These findings indicate that *A. hortae* could be an emergent human pathogenic fungal species. Furthermore, there is significant phenotypic and genetic variability among the isolates of this species, requiring further exploration.

5. Conclusions

This study presents the whole genome sequences, phenotypic characteristics, and susceptibility profiles to antifungals of three thermotolerant clinical isolates of *A. hortae* in Colombia, representing the country's first such documentation. Such studies aim to expand our understanding of pathogens and their reactions to current drug therapies, with the ultimate goal of enabling safer and more efficient dosage strategies for patient treatment.

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org., Figure S1: Bioinformatic workflow for species identification; Figure S2: Phylogenetic tree of *Aspergillus* section *Terrei* using sequences from the ITS marker; Table S1: Reference Proteomes used in OrthoFinder; Table S2: Assembly metrics MCA-7, MCA-8 y MCA-10; Table S3: List of best-fit models per partition for phylogenetic reconstruction; Table S4: *Aspergillus* section *Terrei* SRA codes for phylogenomic reconstruction.

Author Contributions: For research articles with several authors, a short paragraph specifying their individual contributions must be provided. The following statements should be used "Conceptualization, O.M.G and S.M.C; methodology, O.M.G. and A.M. L.; formal analysis, S.M.C, M.Q.T., A.M. L and O.M.G ; investigation, S.M.C, M.Q.T. and O.M.G.; resources, O.H.R, A.L.R, J.G.M and A.M.G.; data curation, O.M.G, M.C.Z and S.T.; writing—original draft preparation, S.M.C, M.Q.T. and O.M.G; writing—review and editing, O.H.R, S.T., M.C.Z., J.G.M, C.L.B. and A.M.G .; visualization, X.X.; supervision, O.H.R, J.G.M, C.L.B, A.L.R. and A.M.G.; project administration, A.M.L. and O.H.R; funding acquisition, O.H.R. and A.M.G. All authors have read and agreed to the published version of the manuscript.".

Funding: This study was financed in part by To Ministerio de Ciencia, Tecnologia e Innovación by financial support (Grant code 221384467683) from Colombia.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: It was no necessary because in this work, personal information of patients was not used.

Data Availability Statement: The sequencing data will be made available when the manuscript is publicly available.

Conflicts of Interest: The authors declare no conflict of interest.

 doi:10.20944/preprints202409.0031.v1

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