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Accelerating the understanding of *Aspergillus terreus*: Epidemiology, physiology, immunology and advances

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ABSTRACT

Aspergillus species encompass a variety of infections, ranging from invasive aspergillosis to allergic conditions, contingent upon the immune status of the host. In this spectrum, *Aspergillus terreus* stands out due to its emergence as a notable pathogen and its intrinsic resistance to amphotericin-B. The significance of *Aspergillus*-associated infections has witnessed a marked increase in the past few decades, particularly with the increasing number of immunocompromised individuals. The exploration of epidemiology, morphological transitions, immunopathology, and novel treatment approaches such as new antifungal drugs (PC945, olorofim) and combinational therapy using antifungal drugs and phytochemicals (Phytochemicals: quercetin, shikonin, artemisinin), also using immunotherapies to modulate immune response has resulted in better outcomes. Furthermore, in the context COVID-19 era and its aftermath, fungal infections have emerged as a substantial challenge for both immunocompromised and immunocompetent individuals. This is attributed to the use of immune-suppressing therapies during COVID-19 infections and the increase in transplant cases. Consequently, this review aims to provide an updated overview encompassing the epidemiology, germination events, immunopathology, and novel drug treatment strategies against *Aspergillus terreus*-associated infections.

1. Introduction

In the past few decades, a notable surge in fungal infections worldwide has been observed (Bongomin and Gago, 2017). Among the array of fungal pathogens, infections caused by *Aspergillus* species have earned significant attention, affecting not only humans but also animals and birds (Elad and Segal, 2018). *Aspergillus* infections give rise to a spectrum of health complications, ranging from acute bronchopulmonary aspergillosis to allergic sinusitis and IgE-associated asthma (Thompson and Young, 2021). Fungi belonging to *Aspergillus* genus including *Aspergillus fumigatus, A. flavus,* and *A. terreus,* represent some of the most important spore-bearing fungi that cause severe infections in individuals with compromised immune systems, and certain immunocompetent individuals (Sugui et al., 2014; Escobar et al., 2016). This susceptibility has increased due to factors such as augmented transplantation cases, cancer occurrences, and disorders leading to immune suppression (Bongomin and Gago, 2017). The global burden of *Aspergillus*-associated infections has been further exacerbated by the emergence of drug-resistant strains within *Aspergillus* species, Recent global estimates have found 3,000,000 cases of chronic pulmonary aspergillosis and ~250,000 cases of invasive aspergillosis (Bongomin and Gago, 2017; Fisher and Hawkins, 2018). Additionally, the absence of early diagnostic indicators for *Aspergilli terreus* infections poses a great risk to susceptible individuals. Moreover, in COVID-19 era fungal infections have gain attentions. Aspergillosis emerged as one of the co-infections associated with COVID-19 along with mucormycosis (Song and Liang, 2020). Therefore, it is imperative to give attention to emerging fungal pathogens which may affect immunocompromised as well as immunocompetent individuals.

Recently, *A. terreus* has been recognized as a major factor in the development of invasive aspergillosis in critically immunocompromised patients, particularly those with cancer (Hachem et al., 2014; Zoran

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et al., 2018). Furthermore, the inherent resistance of *A. terreus* to amphotericin-B (AmB) adds complexity to the treatment strategy for infected individuals, causing concerns within the medical community (Blum et al., 2013a). Successful establishment of infection in host tissue hinges on the germination of conidia, where the conidial cell wall undergoes restructuring. As germination progresses, the rodlet and melanin layers disappear, giving way to proteins, glycoproteins, and polysaccharides that facilitate effective fungal infections (Baltussen et al., 2020). Hence, the proteins released during conidial germination are pivotal for comprehending the invasion process into host tissues and predicting the prognosis of *A. terreus* and related *Aspergilli* species infections. Likewise, these proteins, at specific stages of conidial germination, might hold promise as potential targets for drugs or vaccine candidates.

As the lungs are the prime organs in humans for these conidia that continuously encounter through inhalation. Upon success in evading the primary immune response, it starts to germinate which led to the onset of invasive infections (Latgé and Chamilos, 2019). Bronchial and alveolar epithelial cells serve as the primary barrier to negate the development of invasive fungal infections. These cells counteract the conidia with the aid of pathogen-recognition receptors (PRRs) e.g., dectin-1 and toll-like receptors (TLRs). Receptors on immune cells recognize pathogen-associated molecular patterns (PAMPs) associated with fungal pathogens, thus, activating the secretion or triggering of effector molecules such as antimicrobial peptides and cytokines to eliminate the pathogens (Goyal et al., 2018; Patin et al., 2019). In immunocompromised individuals, weakened phagocytic abilities of immune cells lead to conidial germination. Therefore, lung epithelial cells play a pivotal role in safeguarding against fungal infections and their conidia (Croft et al., 2016; Richard et al., 2018). Moreover, individuals with respiratory conditions including asthma, allergies, or chronic obstructive pulmonary disease are more susceptible to fungal infections, including *A. terreus* and other *Aspergilli*-associated infections. In such cases, these fungal pathogens come into contact with respiratory or lung epithelium cells, triggering the production of immune-regulatory molecules and the activation of various signaling pathways (Han et al., 2011; Sun et al., 2012).

The integration of high-throughput technologies like microarray and RNA-seq can aid in identifying critical components and biochemical pathways involved in virulence and invasion within host tissues (Oosthuizen et al., 2011; Escobar et al., 2018; Watkins et al., 2018). Additionally, dual transcriptomics or proteomic of ex-vivo/ in-vivo studies during host-pathogen interactions can provide a better understanding of the pathophysiology of infections associated with *A. terreus* (Bozza et al., 2002; Bonnett et al., 2006; Bouzani et al., 2012; Shankar et al, 2018).

Thus, this review article starts with the aspect of covering the *Aspergillus* burden throughout the world in recent years along with *A. terreus*, followed by its germination process that will help to understand the key regulatory pathways and proteins involve in conidial germination and transition to hyphal form. Further, this is followed by how host immune response tackle these fungi, specifically cytokines and T-cells? Moreover, review will highlight the current treatment used and new developed drugs as well as new antifungal phytochemicals which will followed by the discussion on COVID-19 era and *Aspergilli*, and its challenge for immunocompromised and immunocompetent individuals. In addition, it will also aim to increase the existing knowledge, and simultaneously discuss the novel findings in the *A. terreus* and other *Aspergilli*.

2. Epidemiology of *Aspergillus terreus*

Aspergillus species have become a growing concern to both immunocompetent and immunocompromised individuals on a global scale (Fedorova et al., 2008; Fernández et al., 2013). After *A. fumigatus, A. terreus* has emerged as a clinically important fungal pathogen worldwide, particularly in cancer hospitals (Slesionaet al., 2012a; Hachem et al., 2014). The recent worldwide estimates of chronic pulmonary aspergillosis cases are 3,000,000 and invasive aspergillosis is approximately 250,000 (Bongomin and Gago, 2017). Interestingly, *A. terreus* is the cause of invasive aspergillosis in a notable percentage of cases, ranging from 1 % and 30 % (Slesiona et al., 2012a). The infections due to *A. terreus* species complex were identified in 21 countries and 38 centres having overall prevalence of 5.2 % among different fungal infections and also it was detected that *Aspergillus* section *Terrei* was most commonly isolated from patients suffering from chronic lung diseases and their proportion was 39.2 %. (Risslegger et al., 2017). Further, it has been observed that *A. terreus* intriguingly appears as a prominent cause of invasive aspergillosis in several medical settings, like Houston, Texas, and Innsbruck, Austria (Risslegger et al., 2017; Lass-Flörl et al., 2005; Lass-Flörl et al., 2021). Furthermore, four-year research at a hospital in New Delhi, India, observed that the prevalence rate of *A. terreus* isolates was 6.6 %. Medical concerns have been raised in the medical community due to the rise in *A. terreus* infections, which have high in-vitro AmB resistance (Minimum Inhibitory Concentration $>$ 2 mg/l) and a higher mortality rate of invasive aspergillosis (51 versus 30 %) when compared to non-*terreus Aspergillus* species (Kathuria et al., 2015). Moreover, *A. terreus* strains are frequently observed in clinical cases such as neutropenia, HIV infection, severe immune suppression, and in organ transplant cases or chemotherapy for malignancies. The work of Denning et al. has documented the concerning prevalence of *Aspergillus*-associated infections in people with asthma and pulmonary tuberculosis (Denning et al., 2013a). Every year, at least 372,385 people who had received therapy for chronic pulmonary tuberculosis become infected with pulmonary aspergillosis globally (Denning et al., 2013a). In addition to these circumstances, 193 million people with active asthma suffer including a significant number of ABPA cases (Denning et al., 2013b). There on cases are increasing globally and studies estimated increase in *Aspergillus* infection cases up to 3,000,000 cases of chronic pulmonary aspergillosis and ~250,000 cases of invasive aspergillosis (Bongomin and Gago, 2017; Fisher and Hawkins, 2018). In India, Agarwal observed a significant prevalence of 1.38 million patients with ABPA within the population of 27.6 million individuals who suffer from asthma (Agarwal et al., 2014). In another study on the distribution of *A. terreus* and related species, Dietl et *al.*, at the Medical University of Innsbruck observed 5.4 % *A. terreus* strains among the 3845 environmental samples, a greater percentage of these isolates during winter (6.8 %) than the summer (3.9 %). The resistance patterns of *A. terreus* isolates against the antifungal were determined. The overall resistance to AmB was 92 % in environmental isolates and 98 % in clinical isolates of *A. terreus*, while the respective resistance rates to posaconazole were 22.6 % and 9.8 %. Further, the resistance rate to voriconazole was 3.9 % in clinical isolates (Dietl et al., 2021). Additionally, global research including 21 nations was carried out to look at the prevalence and AmB resistance in *A. terreus*. The prevalence of *A. terreus* overall was 5.2 % (370/7116) in patients with positive fungal cultures (Risslegger et al. 2017). EFISG, ISHAM, and ECMM jointly studied a projected global burden of *A. terreus*. A total of 261 cases from Europe, 70 from the Middle East, 19 from India, 10 from South America, and 10 cases from North America were recorded. The biggest number of *A. terreus* isolates observed in patients was found in Spain and Austria (Risslegger et al., 2017). Furthermore, in the era of COVID-19 fungal infection also gain importance and aspergillosis is one of the co-infections associated with it. It has been identified that *Aspergilli* infection was associated with COVID-19 including *A. terreus*. (Salmanton-García et al., 2021). In light of these observations, it is essential to conduct further epidemiological studies worldwide to gain a comprehensive understanding of the occurrence of *A. terreus*-associated infections. Collaborative efforts are needed to identify the prevalence of *A. terreus* and implement strategies to mitigate its spread effectively. Therefore, to develop effective diagnostic tools it is essential to understand the mechanisms and especially proteins involve in the transition of *A. terreus* conidia to hyphal form. This will enable to add better and accurate diagnostic targets.

3. Germination events and morphological transitions in *Aspergillus terreus*

Aspergillus terreus is a widespread fungus such as in tropical and subtropical locations throughout the world with different distributions of it in soil. Conidia, which can thrive at a wide range of temperatures (11 to 48°C), are used by *A. terreus* to disperse throughout its habitat. Further, *A. terreus* is also found in coastal sand and salt marshes because its conidia can withstand high sodium chloride (NaCl) concentrations (Wang et al., 2011).

3.1. Morphological features and conidial Types

Aspergillus terreus has septate hyphae which produce two types of asexual spores, the one which is generated at the tip of a vesicle known as phialidic conidia (PC) or spore. One notable characteristic exhibited by *A. terreus* species to generate an additional form of asexual spores, known as accessory or aleurioconidia (AC) (Lass-Flörl et al., 2021). The globose-hyalinated conidia arise from the hyphae constitute second type of asexual spore, AC. Due to small size PC, they are often inhaled by individuals (Lass-Flörl, 2012; Blum et al., 2013a; Louis et al., 2014), and also remain suspended in the atmosphere. The spread of *A. terreus* to other organs are facilitated by the inhalation of the conidia, which survive in immune cells such macrophages (Slesiona et al., 2012b).

Infections frequently commence by the inhalation of PC. When comparing PC and AC, it can be observed that AC exhibit a greater size and possess a lower amount of membrane ergosterol. The increased resistance of *A. terreus* to the ergosterol-binding medication AmB remains obscure. The formation of AC and PC occurs under distinct conditions, suggesting their distinct functions. PC is known to originate from conidiophores, which are specialized structures that develop when the fungus comes into direct contact with the atmosphere. The primary purpose of PCs is to facilitate the effective and widespread dissemination of information through the air. Therefore, it is imperative for these spores to possess the capability to withstand extended periods of exposure to circumstances marked by UV light, desiccation, and oxidative stress. The structural components of PC that hold significance in this particular situation are the melanin layers and hydrophobins. The absence of these traits in AC is observed as they arise from hyphae throughout the process of growth in a liquid environment (Deak et al., 2009). Additionally, these conidia are synthesized in response to infection as noted in infected mice tissue, *Galleria mellonella* larvae, as well as in potato plants (Slesiona et al., 2012b; Louis et al., 2014; Lackner et al., 2019).

3.2. Germination process and infection

The development of ACs of *A. terreus* during the course of infection would serve as a mechanism for promoting the spread of the organism and subsequently leading to elevated mortality rates. In order to substantiate this claim, Lackner et al., conducted an analysis on 15 strains of *A. terreus*. Their findings did not provide any indication of increased virulence of AC in comparison to PC, as observed in a *G. mellonella* infection (Lackner et al., 2019). The study conducted by Deak et al., shown that AC elicits a significantly more robust inflammatory reaction in both infected macrophages and a mouse model of pulmonary aspergillosis when compared to PC. Further, they identified discrete regions of β-glucan on the surface of AC and postulated that the presentation of this prominent fungal PAMP could perhaps account for the robust immune reaction (Deak et al., 2011). Due to inadequate early diagnostic markers or techniques, diagnosing *A. terreus infections* in their early stages is challenging. Various antigens among *Aspergillus* species cell wall components are employed for the diagnosis of *Aspergillus* species including *A. terreus* (Mokaddas et al., 2010). The lack of an adequate antibody response to *Aspergilli* in immunocompromised hosts prevents the use of antibody-based detection techniques for the identification of *Aspergillus*-associated infections (Khan et al., 2000). Therefore, the characterization and interaction studies of AC conidia are important to identify the new diagnostic marker or clinical outcomes for *A. terreus* associated infections. Also, the proteins involved in germination or expressed during germination may aid in the development of novel diagnostic markers and serve to draw attention to the prevalent invasive factors in *Aspergillus* species linked to invasion of host tissues. Additionally, another on study on *A. terreus*, a total of 372 proteins were found using proteomic analysis in germinating conidia; however, only 299 of these proteins had biological roles, the remaining 102 were uncharacterized. Contrarily, only 53 proteins in conidia were found, and of these, 17 lacked any characterization (Thakur and Shankar, 2017). Possible cause of limited set of expressed protein in conidia while in dormancy. In compared to germinating conidia that are biologically quite active owing to the start of germination and morphogenesis, dormant conidia are less metabolically active and contain a lesser number of proteins that perform biological functions (Lamarre et al., 2008). To measure the stage specific proteins encoded by their mRNA during the early growth stage of spores, Thakur et al., compare the expression of four genes: tif35 (eukaryotic translation initiation factor 3 G), pcy (pyruvate carboxylase), gedJ (dihydrogeodin oxidase), and ATEG_03556 (terrelysin). They observed a substantial increase in the expression of terrelysin gene transcripts (885.28-fold), pcy transcripts (181.01-fold), tif35 transcripts (148-fold), and gedJ transcripts (26.53-fold) in germinating conidia compared to conidia (Thakur and Shankar, 2017). Further, study by Shishidia et al., compare the gene expression in mycelia extracts of *A. terreus* with that of the germination stage conidia. The genes sod (5-fold), cat (3-fold), Hsp90 (3-fold), tif35 (5.7-fold), and mpkc (6-fold) were observed to have increased expression in the mycelia morphotypes of *A. terreus* compared to the germination stage conidia. However, there was no significant change in the expression of the Hsp70 gene, and the gene encoding for terrelysin showed reduced expression in the mycelia (Shishodia and Shankar, 2020a). Myosin-1, mpkC, rRNA-processing protein, and hog1 proteins were discovered in *A. terreus* germinating conidia (Thakur and Shankar, 2017). These proteins were identified as pathogenic factors in *A. fumigatus* knockout experiments (Bhabhra et al., 2004). During germination of *A. terreus* conidia, the presence of proteins with roles in carbohydrate metabolism, such as glucan endo-β-glucosidase B, rhamno-galacturonate lyase B, β-glucan synthase component FKS1, and hog1, was also found (Thakur and Shankar, 2017). Thus, the identification of swollen conidia of *A. terreus* activates their phagocytosis, which results in their prolonged persistence within immune cells such as macrophages (Slesiona et al., 2012b). In addition, another study observed the higher expression of the genes encoding eukaryotic translation initiation factor (tif35) and pyruvate carboxylase (pcy) in germinating conidia than in *A. terreus* conidia (Thakur and Shankar, 2017). The proteins transcribed by these genes are involved in the start of protein biosynthesis (tif35) and the metabolism of carbohydrates (pcy). Hence, this data shows that the germination of *A. terreus* conidia needs glycolysis and protein synthesis. Table 1 shows the protein/ enzymes and pathways that involve in the morphological transition of *A. terreus* conidia to hyphae. Furthermore, the interacting network of proteins involved in protein synthesis (rps0, tif34, and tif1) and their transport (sec13) as well as those involved in rRNA processing (rr3, dbp10, and dbp3) aid conidia in exiting dormancy and initiating germination (Cagas et al., 2011; Suh et al., 2012). Comparative assessment of the proteomes of *A. terreus, A. flavus*, and *A. fumigatus* indicates that the germination of their conidia requires the beginning of glycolysis, protein production, and respiration (Tiwari et al., 2016).

3.3. Role of secondary metabolites and host response

Secondary metabolite such as mycotoxins (terretonin and geodin), and citreoviridin, terrein, and lovastatin, have been observed in germinating conidia. Using quantitative Real-Time PCR, the increased

Table 1
Summarization of expressed enzymes/proteins involved in germinating conidia, hyphae/ mycelium and mycotoxin biosynthesis in *A. terreus*.

Proteins associated with germinating conidia in *Aspergillus terreus* (Thakur 2017; Thakur 2019; Tiwari 2017; Cagas, 2011; Singh 2012; Suh 2012")	
1	**Translation**
	Eukaryotic translation initiation factor 3 subunit G
	40S ribosomal protein S1
	40S ribosomal protein S0
	Eukaryotic translation initiation factor 3 subunit B
	Eukaryotic translation initiation factor 3 subunit C
	Eukaryotic translation initiation factor 3 subunit E
2	**Carbohydrate Metabolism**
	Endo-beta-1,4-glucanase D
	beta-galactosidase B
	D-xylulose kinase A
	alpha-glucuronidase A
	endopolygalacturonase I
	Ubiquitin carboxyl-terminal hydrolase CreB
	Methylthioribulose-1-phosphate dehydratase
	rhamnogalacturonate lyase B
	Mannitol-1-phosphate 5-dehydrogenase
3	**Cell Cycle**
	Histone H2A
	Histone H2B
	Nucleolar protein 58
	Nuclear distribution protein NudF
	Dicer-like protein
	endonuclease lcl3
	Structure-specific endonuclease subunit Slx1
	ATP-dependent RNA helicase Ded1
	ATP-dependent RNA helicase Fal1
	Methionine aminopeptidase 2-2
	Extracellular metalloproteinase Mep
	rRNA biogenesis protein Rrp36
4	**Energy and others**
	ATP synthase subunit beta
	ATP synthase subunit d
	Required for respiratory growth protein 9
	NADH-cytochrome b5 reductase 2
	Nascent polypeptide-associated complex subunit alpha
	Polyadenylate-binding protein, cytoplasmic and nuclear
	Pyruvate carboxylase
	Pyruvate decarboxylase
	Catalase-peroxidase
	Arginine biosynthesis bi functional protein ArgJ
Proteins associated with hyphae and mycelium in *A. terreus* (Shishodia 2019; Louis et al., 2014)	
1	**Protein and ribosomal biosynthesis related proteins**
	Ribosomal protein S26e
	Cell division cycle protein 48
	Zinc finger protein gcs1
	Translational activator GCN1
	Telomere and ribosome-associated protein Stm1
	RNA binding effector protein Scp160
2	**Cell wall and cytoskeleton organization**
	Cell wall serine-threonine-rich Galactomannoprotein (Mp1)
	UDP-N-acetylglucosamine pyrophosphorylase
	cAMP-dependent protein kinase regulatory subunit
	Cell wall biogenesis protein phosphatase Ssd1
	Arp2/3 complex subunit Arc16
	Actin cortical patch component
3	**Carbohydrate/TCA metabolism**
	Phosphoglycerate kinase
	Peptidyl-prolyl cis-trans isomerase
	Nucleoside diphosphate kinase
	Enolase/allergen Asp f 22
	Fructose-bisphosphate aldolase
	Dihydrolipoamide acetyltransferase component of pyruvate dehydrogenase complex
	Citrate synthase
	Alcohol dehydrogenase I
	Glyceraldehyde-3-phosphate dehydrogenase
	Extracellular cell wall glucanase Crf1/allergen Asp f 9
4	**Morphogenesis and Signalling pathways**
	cAMP-dependent protein kinase regulatory subunit
	Coronin-like protein crn1
	G-protein complex gamma subunit Ste18/GpgA
	Heat shock90
	Heat shock70

(*continued on next page*)

Table 1 (*continued*)

Proteins associated with hyphae and mycelium in *A. terreus* (Shishodia 2019; Louis et al., 2014)	
	Ran-specific GTPase-activating protein
	G-protein complex gamma subunit Ste18/GpgA
	Nucleoside diphosphate kinase
5.	**Pathogenesis and biofilm forming proteins**
	Septin spn3
	Profilin/allergen
	Extracellular dipeptidyl-peptidase Dpp4
	Allergen Asp f 15
	Beta-hexosaminidase
6.	**Cell redox homeostasis**
	Protein disulfide-isomerase tigA
	Cofilin
	Catalase (cat1)
	Thioredoxin-domain-containing protein
Proteins/Enzymes involved in mycotoxin biosynthesis in *A. terreus* (Thakur. 2017)	
1.	Terretonin biosynthesis
	Trt1, terpene cyclase
	Trt3, FAD- binding monooxygenase
	Trt5, Methyltransferase
	Trt6, Cytochrome P450 monooxygenase
	Trt7, Dioxygenase
	Trt9, Dehydrogenase
	Trt14, isomerase
2.	Geodin Biosynthesis
	GedA, O-methyltransferase
	GedB, Atrochrysone carboxyl ACP thioesterase
	GedC, Atrochrysone carboxylic acid synthase
	GedE, Glutathione S-transferase-like protein
	GedF, Monooxygenase
	GedH; Anthrone oxygenase
	GedI, Decarboxylase
	GedJ, Dihydrogeodin oxidase
	GedK, Questin oxidase
	GedL, Sulochrin halogenase.
	Atrochrysone carboxylic acid synthase

transcripts of the gene encoding for *gedJ* was detected in conidia that were germinating. This gene encodes the enzyme dihydrogeodin oxidase, which is involved in the geodin mycotoxin production pathway (Thakur and Shankar, 2017). The research concluded that geodin synthesis may be involved in conidial germination. Hence, mycotoxin functions during germination must be evaluated for *A. terreus*. Mycotoxin may serve as diagnostic indicators for fungal infections, according to studies. Ozdemir et al., described the use of fungal toxin for the detection of fungal infection, but did not validate their findings using clinical samples. Therefore, more research is necessary to screen for mycotoxins and examine their kinetics in patient serum samples (Ozdemir et al., 2016). In addition, Koo et al., recommended the utilization of volatile secondary metabolites for diagnosing fungal pathogens. Therefore, a number of secondary metabolites of *A. terreus* might be investigated for their potential as non-invasive diagnostic biomarkers (Koo et al., 2014). Various *Aspergilli*, including *A. fumigatus* and *A. flavus*, have been found to synthesise enzymes or proteins involved in the generation of mycotoxins (aflatoxin and gliotoxins) upon germination of conidia (Spikes et al., 2008; Tiwari et al., 2016). These mycotoxins contribute to the infection of susceptible individuals. Thus, such data showed that they may participate in the invasion of host tissue. Slesiona et al., reported the degradation of liver cells in *A. terreus* infected leucopenic mice. They reported their findings 48 hours after infection although the germinating conidia were found five days after infection (Slesiona et al., 2012b). Therefore, it seems that *A. terreus* may generate secondary metabolites prior to the germination of conidia, which may be involved in the development of fatty liver degeneration disease. Moreover, during germination of *A. terreus* conidia (Thakur and Shankar, 2017), the pathogenic protein terrelysin has been detected, which functions as a hemolysin. This protein might have a role in the pathogenesis of *A. terreus*. It has also been proposed that this protein may diffuse into the extracellular environment or media from sprouting conidia or hyphae (Nayak et al., 2011). As a hemolysin, it lyses the RBCs, thereby releasing iron into the blood. Fungi utilise this free iron for their development and survival in human hosts. Therefore, it is evident that terrelysin is a crucial virulence factor produced by *A. terreus* during early development or host tissue invasion. The high level of terrelysin protein expression during early development makes it a useful diagnostic agent (Nayak et al., 2013). Further, studies are required to evaluate the role of terrelysin as a diagnostic molecule or a marker to monitor for infection associated with *A. terreus*.

As *A. terreus* conidia expand isotopically, they germinate after 16 hours and elongate to become hyphae after 24 hours. Further, the hyphae branch forms a mycelial network after 48 hours (Suh et al., 2012). There is a wide variation in the composition of proteins, glycoproteins, and polysaccharides in different morphotypes, which causes different immunological responses during the transition of conidia to hyphae and mycelia. Analysis of *A. fumigatus* transcriptome profiles revealed that Th1 immune responses were activated 3 days after infection, whereas Th2 and Th17 immune responses were triggered 5 days after infection (Shankar et al., 2018). Furthermore, *A. terreus* conidia assimilation during contact with lung epithelial cells (Cancerous cell lines) in humans was reported by a rearrangement in cytoskeleton proteins/enzymes (Thakur and Shankar, 2019). Epithelial cells are crucial for activating downstream processes that clear these inhaled conidia. An *in-vitro* study by Thakur et al., demonstrated the interaction of germinating conidia (16h germ-time) of *A. terreus* with lung epithelial cells (A549) and observed the expression of 1253 proteins by A549 cells and only 427 proteins were reported in uninfected cells. The study identified the proteins that were involved in internalization of conidia, cadherin-24 and other various proteins that activates downstream signalling pathways and immune functions. The KEEG analysis suggested the activation of Jak/Stat, NOD, TLR, TNF and NF-kβ signalling pathways during interaction of A549 cells with germinating *A. terreus* conidia (Thakur

and Shankar, 2019). The data provided in-depth studies are required to understand the initial interaction of conidia, swollen conidia and germinating conidia with *A. terreus* that may enable to understand the specific immune response during infection. Shishodia et al., characterise the mycelial proteins of *A. terreus* at 48h to explore the biochemical and cellular mechanisms involved in development of hyphae network, and potential pathogen factors including biofilm formation machineries (Shishodia et al., 2019). Despite the importance of the morphological change in *A. terreus*, the factors that lead to the development of hyphae and mycelium remain limited. Different developmental stages of *Aspergillus terreus* and its associated molecular components have been summarized in Fig. 1.

Till lately there has been limited focus on the correlation between mycotoxins and the pathogenicity of the fungi responsible for their production. Nevertheless, numerous mycotoxins possess the capacity to modify the host's defence mechanism. Through their immunosuppressive action, these mycotoxins aid the fungus in invading the tissue of the host and acting as virulence factors. *Aspergillus* spp. produce various mycotoxins. For instance, *A. flavus* produces aflatoxin, which inhibits the activity of macrophages (Müllbacher et al., 1985). On the other hand, *Aspergillus ochraceus* produces ochratoxin, which is cytotoxic to lymphocytes. Ochratoxin not only suppresses the functions of lymphocytes, but also those of monocytes and granulocytes (Amitani et al., 1995; Müller et al., 2004). While *A. fumigatus* does not create aflatoxin, it makes other immunosuppressive mycotoxins, such as gliotoxin (326 Da), fumagillin (459 Da), helvolic acid (fumigacin) (569 Da), fumitremorgin A, and Asp-hemolysin (16 kDa). It is recognised that this substance has several immunosuppressive effects, including the inhibition of superoxide release, migration, microbicidal activity (Müller et al., 2003; Lioi et al., 2004. It also exhibits genotoxic properties and induces apoptosis in macrophages. *A. terreus* also produce toxin such as terretonin and geodin but there are no significant studies were carried out to understand the effect of these mycotoxins on host immune response or cells. Furthermore, despite their potent activity, none of these mycotoxins, including gliotoxin, has been definitively established as directly contributing to the development of *Aspergilli.* However recently they consider as virulence factors. Also, during the germination of *A. terreus* conidia, the presence of enzymes responsible for the production of mycotoxin (terretonin and geodin) as well as other secondary metabolites (terrein, lovastatin, and citreoviridin. Using qRT-PCR, it has been detected the upregulation in the expression of the gedJ gene, which encodes the dihydrogeodin oxidase enzyme involved in the geodin toxin biosynthetic pathway, during the germination of conidia in comparison to non-germinating conidia (Thakur and Shankar 2017). These observations indicate that the process of geodin production may occur during the germination stage of *A. terreus*. However, further work is required to determine the specific significance of geodin during this period. Furthermore, Ozdemir et al., proposed the utilisation of mycotoxin as a diagnostic indicator for fungal infections. However, the study's weakness was the absence of testing on clinical samples (Ozdemir et al., 2016). In addition, Lewis et al., identified the presence of gliotoxin in the blood or other bodily fluids of patients infected with *Aspergillus* species. They proposed the use of mycotoxin screening in patient samples as an early diagnostic indicator for invasive infections. Therefore, more studies are required to evaluate the role of mycotoxin of *A. terreus* in immune response and in early diagnostic.

Germination is a vital event to initiate the Aspergillosis. Each morphotype has distinct gene expression patterns and variations in RNA profiles, proteins, metabolism, and cell wall composition have been documented. The germination of fungal conidia results in the modification of cell wall-associated proteins. The cell wall of *Aspergillus* species is primarily comprised of β-glucan and chitin. Proteins implicated in the breakdown of cell wall constituents have been detected during the germination of *A. terreus*. Enzymes such as glucan endo-1,3-beta-glucosidase, endopolygalacturonase, pectate lyase, alpha/beta-glucosidase, and glucan endo-1,6-beta-glucosidase have been discovered. Further, it is revealed that morphological changes associated with conidia germination and discharge of unique chemical signals, such as terrein, terretonin, Asp–melanin, overexpression of cata-lase, and HSP70 (Shishodia and Shankar, 2020a). Further, Prior research on *A. fumigatus* utilising 2-DE and MALDI-TOF techniques revealed increased expression of proteins involved in metabolism, protein biosynthesis, transport, and translation initiation factors in germinating conidia compared to hyphae (Vödisch et al., 2009; Kubitschek-Barreira et al., 2013). The presence of proteins involved in primary metabolism, such as enolase/Aspf22 and glyceraldehyde 3-phosphate dehydrogenase, on the surface of hyphae suggests that these proteins function as adhesion agents against the host (Denikus et al., 2005; Moloney et al., 2016). The utilisation of the 2D-DIGE method revealed that germinating conidia exhibited increased expression of proteins involved in secondary biosynthetic pathways and protein production. Conversely, in hyphae, the most abundant proteins expressed were related to metabolic processes (Kubitschek-Barreira et al., 2013). Cagas et al., reported a significant upregulation of RodA, a hydrophobin protein, Abr2, a protein involved in melanin production, heat shock proteins hsp30/hsp42, superoxide dismutase SodC, and a putative carboxylase in conidia. However, the expression of these proteins decreased in other morphological forms. The process of remodelling the cell wall during isotropic

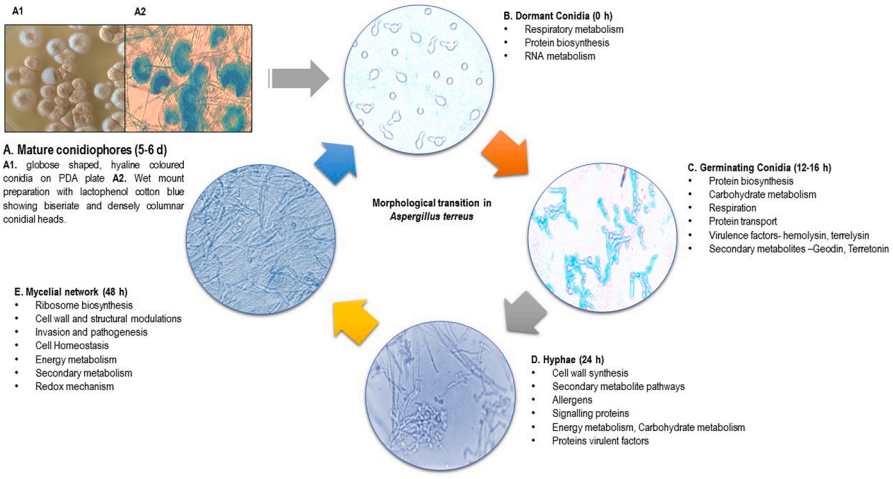

Fig. 1. Different developmental stages of *Aspergillus terreus* and underlying events. Different proteins expressed during different stages of *Aspergillus terreus* development have been shown (Thakur and Shankar 2017; Thakur and Shankar 2019; Tiwari et al., 2016; Shishodia and Shankar 2020).

development is aided by proteins that are responsible for the metabolism of polysaccharides. For example, in *A. nidulans*, VadA is engaged in this process. In *A. fumigatus*, ChiA1, Gel4, and Gel1 from the Gel family are involved in elongating β-1,3 glucans (Cagas et al., 2011).

Therefore, the early expression of proteins or cell wall components such as melanin, glucan and chitin as well as galactomannan components can be utilized for early diagnosis of *Aspergillus* species infections. Thus, the morphological transitions and germination events in *A. terreus* are critical to its life cycle, pathogenesis, and interaction with the host immune system. Understanding these processes at a molecular level can provide insights into novel diagnostic markers and therapeutic targets for *A. terreus*-associated infections. The interplay between conidia, germinating conidia, and host cells shapes the immune response and the establishment of infection, emphasizing the importance of studying these interactions for effective disease management.

4. Immune response to *Aspergillus terreus* and other *Aspergillus* species

To control *Aspergillus* species associated infections, it is imperative to understand the host immune response to determine the damage caused by infection and disease conditions in any individual. Different studies in mice demonstrated that innate and adaptive immune response is equally important against *Aspergilli* infections (Svirshchevskaya et al., 2009).

4.1. Innate immune response: recognition and defence

Once the *Aspergilli* conidia are inhaled, they are entrapped in the lungs' alveoli and if not cleared, infection is initiated due to their germination and subsequently spread to other organs like the brain and kidney (Wasylnka and Moore, 2003). Recognition of inhaled conidia and germinating conidia initiated through a diverse group of PRRs. These PRRs sense various moieties present on the cell wall of *Aspergilli* like carbohydrate moieties, glycoproteins, and proteins called PAMPs (Inoue and Shinohara, 2014). Critically host immune response depends upon the type of PRRs associated with *Aspergilli* recognition. Once PAMPs is recognized by PRRs, intracellular signaling started that lead to activation of immune cells and secretion of immune regulatory molecules. Among different PRRs, TLRs, C-type lectin and pentraxin-like receptors are essential to initiate an immune response (Netea et al., 2004). They are expressed on the membranes of various immune cells, where they can detect certain moieties on the surface of *Aspergilli* that are present during the germination of conidia and the development of hyphae (Hatinguais and Willment, 2023). When TLRs engage with various adaptor proteins, such as My88, signaling pathways are activated. This adaptor protein stimulates the generation of cytokines and reactive oxygen species by activating the interferon regulatory factor (IRF), nuclear factor-k (NF-k), and mitogen-associated protein kinase (MAPK). While Dectin-1 signals through the immuno-receptor tyrosine-based motif (ITAM), activating the NF-k signaling pathway, C-type lectins do not (Kawasaki and Kawai, 2014). Thus, in turn, stimulates cytokines production. The innate immune cells (alveolar macrophages, neutrophils, and antigen-presenting cells like dendritic cells or monocytes) are the first line of defense against respiratory tract pathogens because they have receptors that enable them to recognize respiratory pathogens, including conidia of fungi (Morton et al., 2012). Alveolar macrophages in human lungs are crucial phagocytic cells that provide defense against *Aspergillus* species conidia that enter lung alveoli (Slesiona et al., 2012b). After being identified as *Aspergillus* conidia by PRRs, they begin the phagocytosis of inhaled conidia followed by acidifying by phagolysosome and inducing the production of antimicrobial enzymes. Because of their high melanin concentration, conidia can occasionally evade phagocytosis by preventing the phagolysosome from becoming acidified (Thywißen et al., 2011). Additionally, macrophages secrete other cytokines (INF-γ, TNF-α) that attract additional immune cells to the infection site (Segal, 2007).

Conidia of *A. terreus* have a unique capacity for long-term persistence in alveolar macrophages; as a result, they travel through macrophages to secondary organs and carry out a wait-and-watch role (Slesiona et al., 2012b). Individuals carrying *A. terreus* conidia in secondary locations begin to germinate and develop infections when their immune systems are impaired. Another first-line phagocytic cell, neutrophils, clears fungal spores and hyphae if fungal conidia and hyphae escape the macrophage. Immune cells called neutrophils assist in clearing contaminated areas of fungus spores and hyphae. In the lungs, cytokines, primarily IL-8, attract neutrophils to the infection site and cause an inflammatory response that destroys the fungus' hyphae (Mircescu et al., 2009). As a result, invasive aspergillosis is a significant threat to those with neutropenia. Neutrophils are essential for the elimination of a fungus infection, according to in-vitro research and animal models. Moreover, to eliminate the conidia from the infected location, neutrophils adhere to the hyphae, and their degranulation causes inflammation, therefore clearing the pathogen from infected sites (Bonnett et al., 2006; Sugui et al., 2008). Through pathogen recognition receptors including toll-like receptors and dectin-1, neutrophil cells may identify the fungus. These cells are also capable of removing fungal hyphae via neutrophil extracellular traps (NET), which are created as a result of neutrophil autolysis and release DNA into the environment to impede the spread of infection (Bruns et al., 2010). In additional to these defense mechanisms, neutrophil granules contain many antimicrobial compounds, including defensins, lysozyme, lactoferrin, and proteases (Rogan et al., 2006). Most of the time, conidia are immune to neutrophil assault, but recent research indicates that these immune cells first came into contact with the conidia through lactoferrin-mediated iron sequestration. Since iron is required for immune cell function, immune cells that sequester iron eliminate fungal conidia because free iron is not available for conidia germination. Conversely, fungi that sequester iron can bypass the first line of defense (Sugui et al., 2008). Studies on the transcriptome of *A. fumigatus* conidia interacting with neutrophils showed the multifaceted response of oxidative stress-related genes. These investigations revealed an up-regulation of the catalase and superoxide dismutase genes (Sugui et al., 2008). Further, studies are required to fully understand how *A. terreus* conidia interact with neutrophils. When *Aspergilli* conidia engage with macrophages and neutrophils, an inflammatory immunological response is triggered, which draws antigen-presenting cells from surrounding tissues or blood to the infection site by secreting cytokines and chemokines (Park and Mehrad, 2009).

Dendritic cells and monocytes are the main antigen-presenting cells. Based on their markers, such as $CD14^{+}CD16^{+}$ and $CD14^{+}CD16^{-}$, monocytes are divided into a subpopulation. A cytokine called TNF-α is secreted by 90 % of these cells, which are $CD14^{+}$, $CD16^{-}$ and phagocytose *Aspergilli* conidia (Serbina et al., 2009). Additionally, it has been proposed that PRRs (Dectin-1, Pentraxin-3, and TLRs) are capable of inducing an adaptive immunological response through T cells (Morton et al., 2012). Pentraxin-3 and chemokine receptors (CCL3, CCL20, CXCL2, and CCL4) were shown to be up-regulated in transcriptomic analyses of monocytes interacting with *A. fumigatus* (Mezger et al., 2008). In addition, dendritic cells, a type of antigen-presenting cell, monitor the human system in its immature state until it interacts with pathogens and become matures. These cells display the microbial antigens on their surfaces and trigger the T-cell adaptive immunological response (Morton et al., 2012). Using a transcriptomic approach, the interaction study between mature dendritic cells and conidia of *A. fumigatus* revealed the up-regulation of genes encoding dectin-1, pentraxin-3, CCL20, IL-1B, and IL-8 (Mezger et al., 2008). Direct contact between dendritic cells and *A. fumigatus* conidia revealed that 48 % of conidia were phagocytosed after two hours of contact.

4.2. Adaptive immune response: shaping the immune landscape

The most crucial cells that link innate and adaptive immunity are dendritic cells. They trigger the adaptive branch of the immune response by displaying the antigens of the pathogen on their surface. They alter the T-cell immune response during *Aspergillus* species infections to be either protective (Th1 or Th17) or distracting (Th2 or Th9) (Romani, 2008). IL-12 and INF-γ cytokines are upregulated during the adaptive immune response by Th1 helper cells (Chotirmall et al., 2013). When invasive aspergillosis occurs, the cytokine INF-γ level rises initially. This limits the action of other cytokines, such as IL-4 and IL-17, which prevents the activation of Th2 and Th17 T-cells (Espinosa and Rivera, 2012). Contrarily, if IL-4 predominates during the early stages of *Aspergilli* infection, it begins the activation of Th2 cells by blocking IL-12 and therefore stimulates the inflammatory response by activating eosinophils and basophils (Cenci et al., 2000). In response to invasive pulmonary aspergillosis, the roles of several chemokines, chemokine receptors, and other immune cells have also been identified (Latgé, 1999). Additionally, both allergic broncho-pulmonary aspergillosis and invasive aspergillosis are accompanied by a variety of T-helper immunological responses (Anand and Tiwary, 2010; Anand et al., 2013; Anand et al., 2015. However, little is known about immune responses that are mediated by cells, particularly T-helper cell immunity against *A. terreus*. According to studies, individuals inhale several conidia every day, and they may be isolated from the sputum of immunocompetent hosts (Latgé, 1999). In the lungs, *Aspergillus* conidia come to encounter bronchial or alveolar lung epithelial cells. In addition to serving as a physical barrier against infections of the respiratory tract, airway cells are now a crucial component of cells that protect against fungi (Knight and Holgate, 2003). Type-I cells make up around 95 % of the surface of the lung's alveoli, whereas type-II cells only make up 5 % of the lung epithelial cells. Since type-II cells secrete surfactant proteins and also, they are the progenitors of type-I cells, they are crucial for the health of the alveolar surface (Ruaro et al., 2021). In addition, type II lung epithelial cells are currently thought to be the first cells to contact with fungi and trigger an immune response. The interaction of alveolar type-II cells (A549-carcinoma cell type) with *A. fumigatus* conidia has been the subject of several investigations, and these studies have shown that A549 cells absorbed conidia and activated downstream immune response against inhaled conidia (Han et al., 2011). Moreover, these cells offer a model system for researching the interactions between hosts and infections, particularly those that affect the respiratory tract. Prior reports said that the activation of the protein's phospholipase D and cadherins caused the cytoskeleton of these cells to rearrange, which in turn caused the internalization of conidia to begin (Han et al., 2011; Xu et al., 2012). The innate immune system is activated once conidia have become internalized. At later phases of *Aspergillus*-related illnesses, the interaction of inhaled *Aspergilli* conidia with lung epithelial cells has major implications and is multidimensional and very complicated. The first line of defense against fungal infections is comprised of lung epithelial cells. Additionally, these cells produce chemokines, which influence both innate and adaptive immunity. Epithelial cells (A549) exposed to *A. fumigatus* conidia in previous research employing the RNA-seq approach upregulated genes involved in preventing oxidative damage to cells and the repair mechanism (microsomal glutathione S-transferase 1) (Chen et al., 2015). These cells furthermore express the PRR dectin-1 and secrete pentraxin-3, which act as opsonins and activate the conventional complement pathway by binding to the C1q component. Conidia are recognized by the dectin-1 receptor, which also triggers the production of the pro-inflammatory cytokine IL-8 and draws immune cells to the site of pathogen detection (Croft et al., 2016). In addition, it has been hypothesized that lung injury is caused by the loss of dectin-1 from lung epithelial cells. A recent study revealed that the accessory conidia (AC) of *A. terreus* is readily engulfed by macrophages and induce a strong TNF-α response. Galactomannan was observed as a major component of AC conidia but not the β-glucan (Hen et al., 2022). Therefore, the role of galactomannan in the interaction with different immune cells or immune systems remains an open issue and required more studies.

4.3. Distinctive characteristics of Aspergillus terreus: accessory conidia and galactomannan

Aspergillus terreus possesses the distinctive capability to synthesize AC both in- vitro and in-vivo. Deak et al., conducted an analysis of the characteristics of these two conidia by the utilization of transmission electron microscopy, assessment of metabolic activity, and β -1,3 glucan staining. The researchers provided clear evidence regarding the distinct features of these two types of conidia and proposed that they may exhibit differential behaviour throughout the process of infection. Further, they have demonstrated that these different conidia have shown difference in binding capacity with dectin-1. Moreover, their findings demonstrate that AC cells exhibit multi-nucleation and experience hyperpolarization during the process of germination (Deak et al., 2009). Consequently, it has been revealed that the immune response triggered by *A. terreus* AC is more robust to ex-vivo alveolar macrophages culture and in-vivo experiments including intra-tracheal challenges in mice (Deak et al., 2011). The potential impact of these immunological variations on the survival outcomes of mice exposed to different strains of *A. terreus* has yet to be identified. Additionally, it is important to note that there are inherent variations not just between various species of *Aspergillus*, but also within individual species. Research that establishes a correlation between alterations in the fungal cell wall and variations in immune responses offers valuable insights into the intricate host reactions to this particular emerging fungal pathogen.

Additionally, a study by Henß et al., demonstrated that the galactomannan has been found as a prominent surface antigen on AC, exhibiting a notable absence on the surface of PC. The distribution of galactomannan is uniform across the entire surface of AC, and it can even be detected on newly formed AC that is present on the surface of hyphae. Further they have identified that during *A. terreus* infection of murine J774 macrophages, AC are easily phagocytosed and elicit a robust TNF-α immune response. In the phagosome, it was observed that galactomannan, but not β-glucan, is released from the conidial surface and subsequently transported to the cytoplasm of the host cell. AC exhibits persistence within phagolysosomes with a significant proportion of them initiating germination within 24-hour timeframe. Furthermore, galactomannan has been observed on AC as a significant antigen that distinguishing them from PC of *A. terreus* (Henß et al., 2022). The precise function of galactomannan, which is peculiar to *A. terreus*, in its interactions with the immune system remains a topic that requires further investigations.

Over the past ten years, several enigmatic *Aspergillus* species have been identified as the primary cause of invasive aspergillosis. Specifically, *Aspergillus quadrilineata, Aspergillus lentulus, Aspergillus alliaceus, Aspergillus tubingensis, Aspergillus calidoustus, Aspergillus viridinutans, Aspergillus udagawae, and Aspergillus felis* have been identified as causing invasive aspergillosis, mostly in pulmonary infections (Varga et al., 2008; Verweij et al., 2008; Vinh et al., 2009; Howard et al., 2014; Gautier et al., 2016). Further, it has been documented that these species cause severe disseminated infections. Less common occurrences, *Aspergillus quadrilineata* and *Aspergillus granulosus* (a species belonging to the Usti section that is infrequently associated with human disorders), have been documented as causing cerebral aspergillosis (Houbraken et al., 2007). Still there are limited studies on immune response and about the pathophysiology of these emerging *Aspergilli* and more studies are required to elucidate their mode of pathogenesis and immune invasion along with immune response.

5. Drug resistance and biofilm formation in *Aspergillus terreus*

Excessive use of antifungals increases the risk of drug resistance. This is mostly due to extended or partial dosages, although it may also differ by *Aspergillus* species, antifungal compounds, and geographic settings (Gonçalves et al., 2016; Rivero and Alastruey, 2016). In addition, certain *Aspergillus* species are naturally resistant to particular antifungals. Though information about drug-resistant genes and genome mutations exists, there is insufficient data to accurately determine molecular mechanisms involved in drug resistance. For this reason, it is quite difficult to battle invasive fungal secondary infections using limited therapeutic options with resistant isolates. Concerning *A. fumigatus* which causes a majority of aspergillosis cases, more exploration has been conducted into the molecular aspects of drug resistance compared to other species. The key types of drug resistance mechanisms in *Aspergilli* are target modification (mutations that impair the binding efficiency of drugs), lack of drug potency, enhanced drug effluxing, biofilm formation and antifungal agent sequestration or overexpression (Figs. 2 and 3). Azole resistance in *A. fumigatus* is primarily caused by a mutation/overexpression of the cyp51A gene, which encodes 14-sterol-demethylase, a critical enzyme in ergosterol production; as well as mutations or modifications in the cyp51 genes or promoter regions (TR34/L98H, TR56/Y121F/T289A) (Wiederhold et al., 2016). Mutational analyses of *A. fumigatus* biofilms also revealed that the glycophosphatidylinositol-anchored cell wall protein 'cspA' is required for biofilm development, integrity, and drug response (Fan et al., 2015). Also, In *A. fumigatus*, the S678P substitution in Fks1p is associated with echinocandin resistance (Rocha et al., 2007), while high levels of gliotoxin are thought to explain drug resistance in biofilms (Bruns et al., 2010).

5.1. Intrinsic resistance of Aspergillus terreus to amphotericin-B

The intrinsic resistance of *A. terreus* to AmB is still a major clinical issue. The interaction of *A. terreus* with AmB revealed that some *A. terreus* isolates are resistant to AMB while others are tolerant. AMB results obtained with *A. terreus* isolates MICs of 1 mg/L (susceptible range) may exhibit AMB-tolerant phenotypes, which may explain the poor outcome of AMB therapy in phenotypically susceptible isolates. However, MICs alone are not an adequate metric for tolerance detection, and relying solely on MICs may result in suboptimal or inappropriate therapy (Vahedi-Shahandashti et al., 2022). The precise mechanism is unclear, how *A. terreus* become intrinsically resistant to AmB. The possible mechanism of resistance includes the upregulation of ERG5, ERG6, and ERG25 (ergosterol biosynthesis genes) (Graybill et al., 2004; Deak et al., 2009) or better protection from oxidative damage (Blum et al., 2013b). Additionally, AmB drug resistance isolates were found to have higher superoxide dismutase and catalase activity than sensitive strains (Jukic et al. 2017), as well as greater response to Hsp70 and Hsp90 inhibitors when combined with azole medicines and AmB (Blatzer et al., 2015; Jacob et al., 2015), Fig. 3A. Sonia and Shankar have analysed the relevant specific proteins for critical roles in AmB resistance include Cat1, Prx1/LsfA, enolase, thioredoxin peroxide (Aspf 3), Sod2, cytochrome C, RodA, PhiA, Hsp70, and Hsp90 (Shishodia and Shankar, 2020a). It remains to be determined how heat shock proteins may contribute to AmB drug resistance in *A. terreus*.

5.2. Biofilm formation

Microorganisms are known to grow on a variety of surfaces such as sediment, skin, bark, rock, and mucosal tissues (Kolter and Greenberg, 2006; Desai et al., 2014). A large percentage of bacterial and fungal pathogens take the form of biofilms in human bodies, interfering with drug response and host immunity. It's thought that the protective matrix of the biofilm shields the fungal cells from the effects of antifungal agents. However, research into biofilm formation by *A. terreus* is relatively limited compared to other species. There are evidences suggesting biofilm formation by *A. fumigatus* containing parallel-packed hyphae (Seidler et al., 2008), along with the development of an extracellular matrix (ECM) (Al Abdallah et al., 2012). To eliminate the biofilm structures, a higher MIC of antifungal drug is required, which is one of the reasons for drug resistance in *A. fumigatus* against existing drugs (Mowat et al., 2008; Seidler et al., 2008). It has been proposed that the extracellular matrix in biofilm confers drug resistance by absorbing antifungal molecules, preventing their diffusion to fungal cells. This has been supported by the formation of ECM in *C. albicans*, which sequesters antifungal drugs and reduces drug susceptibility (Nett et al., 2010). In biofilm structure, activation of multidrug resistance protein that pumps out antifungal drugs has been reported. As per the present knowledge cell wall protein cspA is associated with biofilm development and drug resistance (Fan et al., 2015). Biofilms consisting of various components such as α-1,3-glucans, galactomannan, melanins, and some proteins along with encasing hyphal cells have been observed on dialysis catheters (Loussert et al., 2010; Ramage et al., 2011; Rajendran et al., 2013), Fig. 3B. In addition to this evidence, Transcription factors have been studied for their role in biofilm formation; the involvement of agglutinin-like sequence (ALS) proteins, proteins Hyr1-like CFEM proteins suggests their participation in this process (Nobile and Mitchell, 2005; Desai et al., 2014). Additionally, the role of the efflux-pump in azole resistance has been well demonstrated, which may be one of the reasons for treatment failure in cases of aspergillosis (Rajendran et al., 2011). Furthermore, metabolic alterations thought to be connected with virulence are seen during biofilm formation (Muszkieta et al., 2013). Unfortunately, current treatments are largely inadequate for resolving these dangerous biofilm-related infections; therefore, more attention is needed for understanding the exact influence of biofilms on the drug resistance mechanism. Another study on *A. terreus* conducted by Rayón-López et al on-biofilm formation showed the presence of nucleic acid, carbohydrates, proteins, and different lipid components. It has been observed that the lipid-like biofilm may be associated with increased AmB resistance in some *A. terreus* isolates (Rayon-Lopez et al., 2023). Similarly, Sonia et al demonstrated biofilm formation in

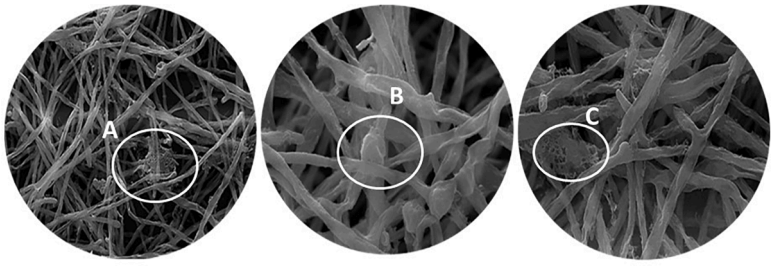

Fig. 2. Represents the different features of biofilm formation in *Aspergillus terreus*. Scanning Electronic Microscopy (SEM) images of biofilm formation in *Aspergillus terreus* at 48h (inoculums concentration 1×10^6 conidia/mL,) (**A**) mycelium networks embedded in ECM (Extracellular Matrix), 1000X (**B**) Hyphae covered with ECM showing superficial appearance, 5000 × (**C**). *Aspergillus terreus* biofilm showing porous ECM, 5000 ×.

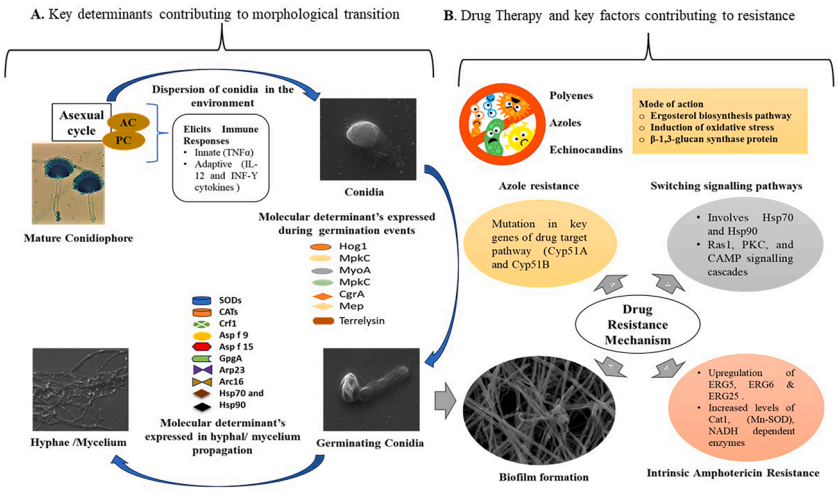

Fig. 3. Depicting the key determinants contributing to morphological changes as well as factors contributing drug resistance in *Aspergillus terreus.* (Shishodia et al. 2019; Louis et al., 2014; Thakur and Shankar 2017; Thakur and Shankar 2019; Shishodia and Shankar 2020; Lass-Flörl et al., 2021) **3A.** Various Key factors and virulence factors during Germination and hyphae/mycelium formation in *Aspergillus terreus*, along with the innate/adaptive responses in host cell are summarized. **3B.** Key modulators in drug resistance mechanism in *Aspergillus terreus* are summarized.
*AC; Accessory conidia, PC; phialidic conidia CgrA; Nucleolar rRNA-processing protein, Mep; metalloproteinase, Hog1; Putative MAPK role in oxidative stress, Mpkc; Mitogen-activated protein kinase, MyoA; Type I myosin, SOD; superoxide, CAT; Catalase, Asp f 9; Extracellular cell wall glucanase, Asp f 15; Allergen, Arp2/3 and Arc16; Arp2/3 complex-mediated actin nucleation. Hsp70; Heat shock protein 70, Hsp90; Heat shock protein 90, ras1; Ras proteins, cAMP; cAMP-dependent protein kinase regulatory subunit, PKC; Protein kinases, NADH dependent proteins; nicotinamide adenine dinucleotide hydrogen dependent proteins, ERG5, ERG6 & ERG25; ergosterol biosynthesis genes, cyp51A and cyp51B;14-alpha sterol demethylase-related genes

Amb-resistant isolate of *A. terreus*, suggesting secretory proteins play important role in induction or establishment of biofilms in *A. terreus* (Shishodia et al., 2020b). Further studies are required to elucidate the role of lipids in biofilms in filamentous fungi.

6. Current treatment regime for *Aspergillus terreus*

Aspergillus species vary in their sensitivity to antifungal medications, making it challenging to treat the invasive aspergillosis caused by different species. Along with the usual medication AmB, voriconazole was suggested for the treatment of invasive aspergillosis in the previous decade (Rocha et al., 2007; Lewis, 2011; Pianalto and Alspaugh, 2016). Lipid-based AmB and micafungin therapy is the other potential treatment (Botero et al., 2015). Despite the use of antifungal medication to treat invasive aspergillosis, the death rate is significant, ranging from 27 % to 80 % (Karthaus, 2011). Clinical breakpoints (CBPs) have been established for antifungal drugs such as azoles, echinocandins, and AmB through a variety of approaches (Sanglard, 2016).

6.1. Polyenes therapy

The clinical threshold for antifungal medication resistance and sensitivity to *Aspergillus* species has been established by the European Committee on Antimicrobial Susceptibility Testing in recent years (Alastruey et al., 2015). However, *A. terreus* possesses intrinsic resistance to this drug, and the exact mechanism behind is unknown (Blum et al., 2013a). According to recent investigations, *A. terreus* resistance to AmB may be influenced by heat-shock protein (Hsp90) and oxidative stress (Blum et al., 2013b; Blatzer et al., 2015). AmB must be present in quantities greater than 2 mg/L to inhibit *A. terreus*. AmB is therefore ineffective in treating invasive aspergillosis caused by *A. terreus* (Blum et al., 2013a). Additionally, AmB has harmful side effects including glomerular filtration rate reduction, hypokalemia, hepatotoxicity, and cell toxicity (Ramu et al., 2021). Clinical trials have indicated that AmB treatment has a significant failure rate (80 %–90 %) against invasive aspergillosis induced by *A. terreus* (Walsh et al., 2003). Furthermore, AmB's lipid formulation notably does not improve the efficacy of this medication therapy (Hamill, 2013). As a result, the AmB is not a viable treatment option for *A. terreus* infections.

6.2. Azole therapy

For invasive aspergillosis caused by *A. terreus*, voriconazole is the primary line of treatment (Lass-Flörl et al., 2005). In contrast to other medications, which have a failure rate of 65.3 %, voriconazole has a treatment failure rate of 52.9 %. Additionally, *A. terreus* developed resistance to voriconazole. The mutation M217I in Cyp51 that *A. terreus* acquired causes a high MIC of this medication against *A. terreus* (Rivero-Menendez and Alastruey-Izquierdo, 2016). Posaconazole and echinocandins are the alternative options for treating IA brought on by *A. terreus*, and these medications are said to be more effective in preventing IA brought on by *A. terreus* (Graybill et al., 2004; Langner et al., 2008). Antifungal medications have a high rate of failure in treating *A. terreus* induced invasive aspergillosis. Patients having *A. terreus* infections are usually immunocompromised and receive co-medication (chemotherapy in cancer patients). Therefore, co-medication results in drug-drug interactions and antifungal treatment failure. Further, a study by Fakhim et al observed the trends of AmB resistance in *A. fumigatus, A. flavus, A. terreus* between 2016 and 2020. They identified more resistant AmB *A. terreus* and *A. niger* in Asian studies as compared to American and European studies (Fakhim et al., 2022). Therefore, future studies should also a focus on analysing the trends of AmB resistance on a geographical basis.

7. Emerging treatment approaches for *Aspergillus*-related illnesses

Aspergillus infections are accompanied by restricted antifungal medications and toxicity, which contribute to a high death and morbidity rate (Scorzoni et al., 2017). Due to the sequence similarity of genes belonging to *Aspergillus* to human, it adds challenge to identify novel treatment targets against fungi (Shankar et al., 2004; Roemer and Krysan, 2014). Antifungal drugs usually interact with the cell wall components or membrane components and also inhibit transcription. Therefore, the means of discovering new drug targets must be investigated further (Scorzoni et al., 2017). Pathogenic fungi have also

developed a resistance mechanism to existing antifungal medicines, caused by the production, over-expression, and growth of biofilm-associated proteins (da Silva Ferreira et al., 2004; Mowat et al., 2009). The calcineurin signaling pathway has become a target for anti-*Aspergillus* treatments; this protein interacts with different biological processes, induces morphogenesis and tolerance to antifungals, as well as regulating ergosterol biosynthesis, chitin and β-glucan (Juvvadi et al., 2017). Activation of calmodulin can also be blocked through triphenylethylene compounds (Cramer et al., 2008; Liu et al., 2015), while microtubule synthesis is inhibited with antimicrobials. The inhibition of microtubule synthesis and heat shock proteins (Hsp90 and Hsp70) has become novel antifungal targets (Jacob et al., 2015). To address toxicity issues, N-methyl-N-D-fructose AmB methyl ester, an AmB derivative, has been employed in the past decade as a less harmful antifungal (Antillón et al., 2016). Another antifungal drug belongs to class- echinocandins- which prevent the biosynthesis of cell wall glucose from fungi (Pianalto and Alspaugh, 2016) have also been infused. In addition, novel azole compounds (PC945 and PC1244) to treat aspergillosis and counter-resistance in *A. fumigatus* strains were synthesized (Colley et al., 2017). Moreover, CD101 echinocandin, and olorofim (F901318) were designed to treat severe fungal infections such as candidemia as well as fatal *Aspergillus* infections respectively (Buil et al., 2017). Unfortunately, current measures to regulate antifungal resistance or produce new antifungal compounds with high efficacy yet low toxicity are inadequate. Therefore, to overcome the drug resistance in *A. terreus*, the screening of effective and potent phytochemicals is required. In pursuit of more effective antifungals, plants are a major source of natural products with therapeutic potential for managing fungal diseases. To overcome drug resistance in *A. terreus*, research needs to focus on screening potent phytochemicals that could be employed either alone or in conjunction with existing treatments. Tocopherol, phenolic, thiols, flavonoids, anthocyanin, and carotenoids were found to be promising phytochemicals for their synergistic antifungal effects (Kumar et al., 2006). For instance, quercetin (QRT) has been explored for its ability to limit the development of *A. parasiticus* and *A. flavus* (Tiwari et al., 2017), while shikonin has been studied in inhibiting *C. albicans* isolates and fluconazole-resistant *C. albicans* (Miao et al., 2012), and for the *A. terreus* using proteomic approach (Shishodia et al., 2020a). Additionally, Artemisinin (ART) and a synthetic coumarin derivative (SCD-1) were investigated for their efficacy against *A. fumigatus* (Singh et al., 2012). Thus, to overcome the drug resistance in *A. terreus*, more screening of effective and potent phytochemicals is required that can be used alone or in combination with exiting drugs to improve therapeutic approaches. Also, along with these compounds immune modulators can be use as combinational therapies to overcome *Aspergillus sp.* infections. Colony stimulating factor (CSF) enhances the ability of the innate immune system to eliminate *Aspergillus* infection. This could potentially enhance the population of innate immune cells, specifically in cases such as neutropenia. Consequently, enhance the cellular response to combat fungal infections (Lauruschkat et al., 2018). Further, study investigates the genetically modified T cells to express a chimeric antigen receptor (CAR) specific to *A. fumigatus* that shown these modified T cells are capable of exhibiting antifungal activity in both laboratory and animal settings. Chimeric antigen receptor (CAR) that specifically targets the AB90-E8 domain, which is capable of identifying a highly conserved protein antigen located in the cell wall of *A. fumigatus* hyphae. T lymphocytes expressing the Af-CAR receptor specifically identified *A. fumigatus* strains and clinical isolates and demonstrated a direct fungicidal activity against *A. fumigatus* hyphae. Specifically, CD8+ Af-CAR T cells secreted perforin and granzyme B, causing harm to *A. fumigatus* hyphae. CD8+ and CD4+ Af-CAR T cells secreted cytokines that stimulated macrophages to enhance the antifungal response (Seif et al., 2022). Moreover, in order to increase the Th1 response of patients, FDA-approved forms of IFN-γ might be administered. In-vivo, IFN-γ is secreted by T and NK cells. It has the capacity to induce protective responses of the innate and adaptive immune systems against *Aspergillus* (Dewi et al., 2017). These immunomodulators or cells can be used in combination with antifungal drugs to effectively eliminate *Aspergillus* sp. infection in future studies.

7.1. COVID-19-associated pulmonary aspergillosis (CAPA)

A significant epidemic brought on by the coronavirus SARS-CoV-2 has been sweeping the globe. Patients with coronavirus disease 2019 (COVID-19) require hospitalisation in intensive care units (ICUs) in a non-negligible proportion of 5–15 % of cases (Wu and McGoogan 2020). A recent meta-analysis estimated the overall mortality of COVID-19-positive critically sick patients to be 35.5 % (0-85 %), although the attributable mortality was not disclosed (Armstrong and Kane 2021). Moreover, patients with COVID-19 appear to have a greater prevalence of respiratory co-infection with gram-negative organisms (Schauwvlieghe et al. 2018). It is no longer thought to be a rare consequence because influenza-associated pulmonary aspergillosis (IAPA) has been seen in patients with influenza and severe acute respiratory distress syndrome (ARDS) in the ICU (Paramythiotou et al. 2021). Similar to this, reports of potential COVID-19-associated pulmonary aspergillosis (CAPA) cases emerged early in the pandemic (Bartoletti et al. 2021). There are questions over whether the pulmonary aspergillosis (CAPA) caused by the 2019 coronavirus illness (COVID-19) increases mortality. Prevalence of CAPA varies greatly between hospitals and between nations, in part due to the challenges in making a trustworthy diagnosis. Respiratory viruses directly harm the airway epithelium, allowing *Aspergillus* to enter the tissue. The risk factors for ventilator-associated pneumonia and CAPA include immune dysregulation, therapy with systemic corticosteroids or anti-interleukin-6 drugs, and diffuse alveolar damage in severe SARS-CoV-2 infection (Somers et al. 2021). The precise incidence of CAPA is unknown, however, as patients with CAPA do not typically exhibit the host, clinical, or radiological characteristics that are frequently used to diagnose invasive fungal diseases (Donnelly et al. 2020). Mycological evidence is instead based on positive *Aspergillus* cultures in non-bronchoalveolar lavage (BAL) samples like bronchial secretions, tracheal aspirates, and sputum, which may indicate colonisation rather than infection. Lackner et al., used Galactomannan (GM) detection from serum and/or bronchoalveolar lavages (BAL) to routinely check respiratory specimens in COVID-19 ICU patients for *Aspergillus* species and found that 7 % of ICU patients treated between March 2020 and April 2021 had CAPA. *A. fumigatus, A. flavus, A. niger,* and *Aspergillus nidulans* made up the spectrum of *Aspergillus* species (Lackner et al. 2022). In immunocompromised hosts, such as transplant recipients, individuals with hematologic malignancies, patients using long-term or high-dose steroids, or patients receiving various immunosuppressant medications, invasive aspergillosis is frequently diagnosed (Ullmann et al. 2018). Influenza A (H1N1) pandemic has led to frequent Aspergillosis associated with severe influenza virus infection (IAPA). According to one of the studies, patients with immunocompetent (14 %) and immunocompromised conditions (31 %), respectively, were impacted (Schauwvlieghe et al. 2018). ICU patients with severe pulmonary diseases increased as a result of the current severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic (Lai and Yu 2021). As a result, there have been multiple instances of pulmonary aspergillosis (CAPA) linked to the coronavirus disease 2019 (COVID-19) (Koehler et al. 2021), which has sparked worries that this superinfection may be causing an increase in mortality. But it was discovered that the number of CAPA cases varied greatly between hospitals and nations; ICU reported rates ranging from 3 % to 33 % (Arastehfar and Carvalho 2020). Since radiological characteristics are not unique and diagnostic bronchoscopy is less frequently utilised in COVID-19 patients due to the risk of infectious aerosols, these discrepancies may in part be the result of challenges in making a valid diagnosis and the lack of a specific clinical presentation (Koehler et al. 2021). Moreover, the identification of *Aspergillus* sp. in upper respiratory specimens does not differentiate between fungal colonisation and infection due to its limited sensitivity

for circulating galactomannan (GM) in serum (Arastehfar and Carvalho 2020; Koehler et al. 2021; Lass-Flörl et al. 2021). It can be generalized that there has been modest rise in culture-positive specimens (*Aspergillus* sp.) during the first wave of the pandemic. In a way for possible optimized patient care, using culture and GM tests from blood or bronchoalveolar lavages (BAL), a routine screening of respiratory specimens from COVID-19 ICU patients for *Aspergillus* sp. should be introduced.

8. Concluding remarks

Fungal infections are arising continuously over the years and post-COVID-19 era has significantly highlighted the importance of fungal infections. *Aspergillus* infections are among the leading cause of fungal infections worldwide. Also, the emergence of resistance strains of *Aspergilli* helps to exploratory the situation further. More continuous epidemiological studies are required to monitor emerging fungal pathogens such as *A. terreus* and *C. aureus*. Transitional and biofilm studies will further help to explore new drug targets for *Aspergilli* or other fungal infections. The emerging immunotherapies or T-cell studies will pave a new way to treating fungal infections. Simultaneously, the development of novel antifungal molecules such as CD101, olorofim and PC945, offers a renewed perspective on antifungal strategies. However, it is noteworthy that new strains of *Aspergillus* with diverse mechanisms to evade both the immune response and antifungal therapies have been identified. Consequently, a multifaceted approach to studying various *Aspergillus* species presents a hopeful avenue that could revolutionize the management and outcomes of *Aspergillus* infections

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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