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Abstract

S1.1
Basic mechanisms of antifungal immunity
S1.1a
Basic mechanisms of antifungal immunity

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Invasive candidiasis (IC), mainly caused by *C. albicans*, is the most common deep-seated human fungal infection in the western hemisphere. Mortality of patients with IC exceeds 40% despite the administration of antifungal therapy; hence, IC is an unmet medical condition for which better understanding of its immunopathogenesis is essential. The mouse model of IC following intravenous yeast injection that mimics human skin-derived bloodstream candidiasis has been extensively used to study the innate immune response against IC. In this model, the microbiological progression of the infection is organ-specific, with kidney being the main target organ, associated with organ-specific innate immune responses. At the cellular level, neutrophils, monocytes, resident macrophages and $CD11b^+$ dendritic cells, but not lymphocytes or $CD103^+$ dendritic cells, are critical for host defense against IC. Indeed, depletion of neutrophils or mononuclear phagocytes leads to accelerated mortality in the model. Of interest, neutrophils have different effects in the model depending on the phase of the infection; specifically, whereas early neutrophil presence is protective, late neutrophil accumulation mediates tissue injury and immunopathology that results in renal failure and mortality. In recent years, the molecular factors that mediate early protective versus late detrimental neutrophil effects in the model have been emerging and their discovery holds promise for the identification of novel genetic risk factors for IC and targeted therapeutic interventions, respectively. With regard to the protective role of mononuclear phagocytes, the discovery of the prominent role of CCR2-recruited monocytes and CX3CR1-expressing macrophages as well as the unveiling of the orchestrated monocyte/dendritic cells/NK cell axis that drives GM-CSF-mediated activation of neutrophils have shed further light into the pathogenesis of IC. At the molecular level, several studies have demonstrated the orchestrated role of pattern-recognition receptors (e.g., C-type lectins and Toll-like receptors), pro-inflammatory cytokines (e.g., IL-1, IL-6, IFN-g, TNF-a), the inflammasome (e.g., Nlrp3, Nlrp10), the complement cascade (e.g., C5a), and the reactive oxygen species generation machinery (e.g., NADPH oxidase, iNOS) in effective anti-*Candida* innate host defense. Population-genetic approaches have translated several findings to humans with the prospect of devising improved individualized risk stratification and prognostication strategies in patients with IC and better outcomes after IC.

S1.1b
Protection and pathology in pulmonary aspergillosis

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Aspergillus may lead to a spectrum of clinical syndromes in the lung, depending on the host's immune status or pulmonary structure. Invasive pulmonary aspergillosis occurs primarily in critically ill patients patients, such as those with severe immunodeficiency or chronic obstructive pulmonary disease. It is now clear that a three-way interaction between host, fungi, and microbiota dictates the types of host-fungus relationship. Indeed, microbial dysbiosis predisposes to a variety of chronic fungal infections and diseases at local and distant sites. We have explored metagenomics for deciphering the contribution of the microbiota to fungal commensalism and parasitism and metabolomics for capturing the dialogue between the mammalian host and its microbiota. By correlating changes in metabolite profiles with microbiota metagenomic composition, we have defined several functional nodes by which certain bacteria species contribute to or subvert host-fungal symbiosis and mucosal homeostasis in the gut and lung. A microbial tryptophan metabolic pathway activated through the indoleamine 2, 3-dioxygenase 1 (IDO1) enzyme led to the production of the indole-3-aldehyde (IAld). IAld preserved immune physiology at mucosal surfaces while inducing antifungal resistance through the activation of the aryl hydrocarbon receptor (AhR), eventually impacting on mucosal immune homeostasis and host/fungal symbiosis. Thus, the regulatory loop involving AhR and IDO1 may be exploited for the development of multi-pronged host- and microbiota-directed therapeutic approaches in pulmonary aspergillosis.

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S1.1c
Protective immune responses during cryptococcosis

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Cryptococcus neoformans is a fungal pathogen with worldwide distribution and responsible for around 180 000 deaths each year. Exposure to this pathogen is very frequent, but healthy individuals either clear infection or control it as a latent infection, and very rarely develop disease. Instead the overwhelming majority of cases is due to immunosuppression, in particular HIV-mediated immunosuppression.

We discuss the existence of predisposing factors to *C. neoformans* infection, and what constitutes an adequate immune response to this fungal pathogen. Most *C. neoformans* cells are found to closely associate with monocyte and macrophages, effector cells of innate and adaptive immunity, and so we will focus on murine and human monocyte and macrophages interactions. We also examine the strategies that *C. neoformans* uses to achieve survival, latency and growth within a mammalian host. The intracellular lifestyle of *C. neoformans* requires it to survive nutrient starvation and toxicity by host antimicrobial molecules in the phagosome and to counteract the host immunity to ensure its survival. Recent work shows that some of these strategies involve *C. neoformans*-mediated host damage at the molecular and cellular level. There is evidence that the intracellular lifestyle is successful enough that it can provide a protective niche to *C. neoformans*. We will integrate the intracellular lifestyle of *C. neoformans* with the current views in immunometabolism and immunoregulation.

S1.1d
Evolution of Candida albicans colonization and anti-Candida innate and adaptive immunity in patients having undergone surgical resection for Crohn's disease

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Objective: In order to explore a possible role for *C. albicans* in Crohn's disease (CD) a pilot double blind study assessed the effect of 6 months fluconazole (FCZ) treatment *versus* placebo on endoscopic recurrences after surgical resection. Despite the study was not conclusive clinically at 6 month at the tested FCZ dose (200 mg daily) the collected material was used for a longitudinal biological analysis yeast colonization, biomarkers of CD, and /or biomarkers of *C. albicans*-host interplay.

Methods: The study concerned 28 CD patients randomized in two groups,14 with FCZ, 14 with placebo. Patients were examined and sampled for mycological and serological analysis before surgery and 1, 2, 3, 6 months after surgery. Qualitative and quantitative evolution of colonization was assessed in mouth and stool. Serological kinetic analysis concerned levels of *i)* CRP, calprotectin and anti-glycan antibodies biomarkers of CD *ii)* biomarkers of *C. albicans* saprophytic/pathogenic transition: anti-*C. albicans* mannan and anti-*C. albicans* Hwp1 protein *iii)* Innate immunity lectins involved in *C.albicans* sensing: galectin-3 and Mannose Binding Lectin (MBL).

Results: The major finding was that independently of antifungal treatment, surgery was followed by a significant decrease of C. albicans colonization. In parallel, biomarkers of *C. albicans* pathogenic transition decrease to non significant levels. Anti-glycan antibodies levels decreased as well but remain above the cut-off discriminating patients with CD. Galectin 3 exhibited a steady decreases which correlated with the one of calprotectin. MBL levels inversely correlating with anti-*C. albicans* antibodies before surgery remain stable.

Conclusion: With regard to previous evidence that inflammation favors *C. albicans* thriving which, in turn, aggravates inflammation, this is the first evidence that reduction of CD inflammation decreases *C. albicans* colonisation and biomarkers associated with its pathogenic development.

S1.2
Mycoctic keratitis
S1.2a
Update and Epidemiology of mycotic infections of the eye

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Fungi have replaced bacteria as the most common cause of infectious keratitis in the developing world, where $2/3^{rd}$ of the global population reside. It is an ocular emergency and an important cause of visual loss worldwide. An update on the current epidemiological patterns, incidence, organisms profile and treatment option are important for clinicians, laboratory personnel and other scientists to be better equipped in the diagnosis, management and ultimately in the prevention of this disease. It affects young active working population in their prime earning period, thus causing significant loss of man years of productivity. Since it also affects people from the lower socio economic background, the dimension of economic loss becomes even more paramount.

The incidence of Mycotic keratitis varies with the geographical regions and also over time. It is often cited as a public health problem inviting some investigators to term it as a silent epidemic. In India, it is estimated to affect 300,000 people annually. In contrast, in 2011, a combined total of 100 cases per year were reported from 11 centres in the USA. There was a large outbreak of *Fusarium* keratitis associated with use of a contact lens solution a few years back in many countries like USA, Singapore and Hong Kong. After this outbreak, the base line incidence of fungal keratitis in the USA showed an increase even after the outbreak subsided.

More importantly, at least one third of those affected have significant visual morbidity, even after undergoing treatment. Even in a single geographical location, cases of fungal keratitis may be higher than the yearly average at certain times of the year, such as during the harvest or windy seasons, or when there is increased relative humidity (before the rainy season).

Causative organisms may differ by geographic location and the risk factors. Filamentous fungi like Fusarium sps and Aspergillus flavus are the most common fungal organism in tropical countries. In these countries the majority of patients suffer an injury with vegetative matter making trauma the most important risk factor in developing fungal keratitis. In addition, there is a large group of hyaline and dematoceious fungi that are not identifiable by conventional methods that is responsible for mycotic keratitis as well. In more temperate climate Candida sps predominate and the majority of these patients are immunocompromised.

Interestingly, within India itself the distribution of the different fungal species is different with Aspergillus *flavus* being more common in the northern parts of India and Fusarium keratitis more in southern states. The precise reason for this variation is unknown and has to be investigated further. This observation assumes importance in the context that newer studies show that Aspergillus and Fusarium differ with regard to their susceptibility pattern to different antifungal drugs.

Mycotic keratitis is generally associated with a poor clinical outcome. Currently drugs like natamycin and voriconazole are used in the management. At least a third of the patients may require a corneal transplant and that too with suboptimal visual outcomes. Regular surveys at different geographic locations help us to understand the magnitude of the condition so as to plan effective management strategies. Awareness of this condition and effective public health intervention strategies to prevent this catastrophic problem is the need of the hour.

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Effects of Pistacia Atlantica subsp. Kurdica on aflatoxin production by Aspergillus parasiticus

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Objective: Aflatoxins are highly toxic secondary metabolites mainly produced by *Aspergillus parasiticus*. This species can contaminate on a wide range of agricultural commodities including cereals, peanuts and crops. In recent years researches on medicinal herb such as *Pistacia atlantica subsp. kurdica* lead to reduce the microbial growth and also have a particular effect on production of aflatoxins as carcinogenic compounds. In this study we to examine the *P. atlantica subsp. kurdica* as natural compound to inhibit the growth and anti-mycotoxin in *A. Parasiticus*.

Methods: *In vitro* antifungal susceptibility testing due to the *P. atlantica subsp. kurdica* for *Aspergillus parasiticus* was performed according to CLSI document M38-A2. The rate of aflatoxin production was determined using HPLC technique after exposure to different concentrations of (62.5 mg/ml- 125 ml mg/ml) gum. The changes in expression of the *aflR* gene level were analyzed by use of a quantitative real-time PCR assay.

Results: The *P. atlantica subsp. kurdica* can inhibited the mentioned *A. parasiticus* growth at 125 mg/ml. HPLC results revealed a significant decrease in aflatoxin production in 125 mg/ml of *P. atlantica subsp. Kurdica* and AFL-B1 production was entirely inhibited. Base on quantitative Real Time PCR results, the rate of *aflR* gene expression was significantly decreased after treating with *P. atlantica subsp. kurdica*.

Conclusion: The *P. atlantica subsp. kurdica* has anti-toxic properties in addition to inhibitory effect on *A. Parasiticus* growth, and able to decrease aflatoxin production effectively in a dose-dependent manner. Therefore this herbal extract maybe considered as a poteintial anti-mycotoxin agent in medicine or industrial agriculture.

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Eumycetoma: A Peruvian case series

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Objective: To report the epidemiological and clinical characteristics of eumycetoma patients diagnosed in a referral tertiary hospital of Lima-Peru.

Methods: All patients with a diagnosis of eumycetoma evaluated at the Cayetano Heredia Hospital between 2000 and 2017 were included. Diagnosis of eumycetoma required demonstration of grains at clinical or histopathological examination. Epidemiological and clinical data was extracted from clinical and mycology laboratory records. The isolates were identified on the basis of cultural and morphological characteristics.

Results: Nineteen eumycetoma patients were diagnosed between 2000 and 2017, mostly males (n = 15, 79%), with a median age of 37 yo (range: 6 - 65) at the time of diagnosis. The median duration of disease was 65 months (range: 5 - 504), and eleven out of 14 patients (78.6%) reported history of agricultural activity until the onset of the disease. Most patients (13 out of 19) were residents of two regions, Piura (8) and Lambayeque (5), which are two neighboring regions located in northern Peru, and characterized by being arid and sandy areas with an average annual temperature of 23°C and presence of carob trees.

Lesions were located on the foot in 17 patients, on the leg in one patient and on the hand in another patient. All patients but two (89.5%) had the classic clinical triad (tumor, sinus tracts, and macroscopic grains). Ten out of 15 patients (66.6%) with imaging evaluation presented bone lesions.

Among 17 patients with presence of macroscopic grains, 12 (70.6%) were black and 5 were white-yellowish grains. In the other two patients, the presence of grains was only evidenced in the histopathology. Ten of the 12 patients (83.3%) with black grain eumycetoma acquired the disease in the regions of Lambayeque and Piura.

The etiological agent was isolated in 12 patients; *Madurella mycetomatis* in eight, *Fusarium* spp in two, *Scedosporium apiospermum* specie complex in one, and *Phaeoacremonium sphinctrophorum* in another patient. The identification of two isolates, one *M. mycetomatis* and the *P. sphinctrophorum* strain, were confirmed by DNA sequencing.

Most patients received itraconazole for treatment.

Conclusion: In Peru, eumycetoma patients are diagnosed lately, when disease has already reached an advanced stage. Limited data suggest that eumycetoma acquisition in Peru occurs mainly in arid and sandy areas of northern regions.

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Validation of ITS from GenBank database in diagnosing medically important black yeasts and relatives species of Herpotrichiellaceae

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Objective: To investigate the use of ITS from GenBank database and NCBI blast searching in diagnosing black yeast and relatives species of Herpotrichiellaceae.

Methods: Three hundred and fifty six ITS sequences(ITS1-5.8S-ITS2) of 115 medically important and saprophytic black yeast and relatives species in family Herpotrichiellaceae viz. *Cladophialophora*(33 species), *Exophiala*(40 species), *Fonsecaea*(9 species), *Phialophora*(23 species)and *Rhinocladiella*(10 species) were collected from GenBank database. The criteria for collecting sequences were legitimate names according to Mycobank deposition and type or the reference strains. Phylogenetic analysis using neighbor joining with K2+G model was performed to investigate the strength of the species clusters. Each of type/reference sequences was tested by megablast searching of NCBI in order to observe its precision and accuracy in identification.

Results: Using neighbor joining analysis, 90.43% of investigated species (104/115 species) were boundaries were well defined with high supports. Eleven species-boundaries revealed ambiguously, six of them, the members mixed with another species in the same cluster viz. *C. devriesii* & *F. brasiliensis*, *F. compacta* & *F. pedrosi*, *P. chinensis* & *P. verrucosa*, whereas the members of *P. mustea*, *P. calyciformis* and *P. brunnescens* were clustered together. True taxonomic positions of two species, *P. intermedia* and *P. phaeophora*, could not be indicated because each species members showed two different positions in the analysis. Testing ITS sequences with NCBI megablast searching showed that 94.78% of species (109/115 species) were 94%-100% identity with maximum scores and specific to their own types or reference species. ITS sequences of six species were cross to be specific to another type species viz. *C. devriesii* & *F. brasiliensis*, *F. compacta* & *F. pedrosi*, *P. chinensis* & *P. verrucosa*, concordant to the results from neighbor joining analysis as previously described. Moreover, *P. mustea*, *P. calyciformis* and *P. brunnescens* were also 99–100% identity to *Pleurostoma richardsiae*, the ex-member of *Phialophora* which is now classified into Calosphaeriaceae.

Conclusion: ITS sequence (ITS1-5.8-ITS2) might be unsuitable marker for higher taxa classification, but well distinguished to delimit species of black yeasts and relatives in Herpotrichiellaceae. It could be used as a tool to identify most species (94.78% of investigated species) and highly specific to type and reference strains without cross-specific to other species (94-100% identity). Cross-specific identity might reflect ambiguous classification of those taxa, revision of them might have to be considered. However, most medical pathogen, especially, the hazardous causative agents of CNS infection viz. *C. bantiana*, *E. dermatitidis*, *R. mackenziei*, can be specifically identified by ITS with megablast searching (96-100% identity). Therefore, GenBank database and NCBI blast searching are reliable and standardized enough to use for correct identification of black yeasts and relatives in Herpotrichiellaceae, and validation of ITS for identifying black yeasts and relatives in other families deposited in the GenBank database should be further performed.

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Biosynthesis and physiochemical characterization of melanin from Fonsecaea monophora

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Objective: To study the melanin physiochemical characterization and melanin synthesis pathway of *Fonsecaea monophora*.

Methods: Pure melanin mass was extracted from the *F. monophora* isolate using the cell wall crude extract method. Then we used Ultravolet (Uv), Fourier transformed infrared (FT-IR) and electron paramagnetic resonance (EPR) spectra assay to evaluate the physiochemical characterization of melanin. Furthermore, we observed the pigment production of the colonies on different mediums (PDA, PDA with L-DOPA, PDA with DOPA-melanin inhibitors and PDA with DHN-melanin inhibitors), and the quantity of melanin produced on different above media were measured using BioTek Eon microplate reader.

Results: The melanin extracted from *F. monophora* shared similar physiochemical and spectroscopic properties with the synthetic L-DOPA melanin. The formation of melanin pigment statistically significant increased on the L-DOPA medium, and decreased on the DOPA-melanin inhibitor medium (sodium azide) and DHN-melanin inhibitors medium (phthalide and tricyclazole) compare to those on PDA medium.

Conclusion: The melanin produced by *F. monophora* is mainly DOPA melanin, and it may synthesize simultaneously by DOPA-melanin and DHN-melanin pathway.

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Updates and comparative analysis of the mitochondrial genome of Paracoccidioides brasiliensis (Pb18) and Paracoccidioides americana (Pb03) using Oxford-Nanopore MinION sequencing

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Objective: The mitochondria have a fundamental role in controlling the cellular networks impacted by antifungal drugs, as well as a prominent role in fungal virulence. Specific mitochondrial mutations lead to either sensitivity or resistance to antifungal drugs.

Paracoccidioides spp. is the etiologic agent of paracoccidioidomycosis (PCM), a clinically important fungal disease endemic in Latin America. In the genus *Paracoccidioides* 5 species have been described. This work includes two of them, *P. brasiliensis* (S1) and *P. americana* (PS2), that could be used as references to optimize the assemblies of the three remaining species.

The available draft mitochondrial genome assemblies for *Paracoccidioides* spp. present significant differences in length and gene content.

This work aims to optimize the currently available draft mtDNA genome assemblies for the reference strains of *P. brasiliensis* Pb18 and *P. americana* Pb03, using Oxford Nanopore long-read sequencing in order to construct *de novo* assemblies and Illumina HiSeq2000-2500 short-read sequencing to achieve high base-level accuracy.

Methods: The reference strains of *P. brasiliensis* Pb18 and *P. americana* Pb03 were re-sequenced using MinION Oxford Nanopore and Illumina HiSeq2000. For each strain, the complete genome was assembled *de novo* using Canu v-1.5, subsequently the contig corresponding to the mitochondrial sequence was identified. It was separated from the rest of the assembly and used as a reference to map the Illumina reads with BWA v-0.6.1. Finally the sequence was corrected using Pilon v-1.6.

The annotations of the improved mitochondrial assemblies for Pb03 and Pb18 were then corrected using a homology inference approach.

Results: For Pb18 we obtained 36126 reads with an average size of 2.6 kb and for Pb03 we obtained 71528 reads with an average size of 3.1 kb using MinION. The Illumina HiSeq2000 paired-end read sequencing produced 46.8 million read pairs with a length of 101 bp for the Pb18 strain, and 62 million 101 bp read pairs for the Pb03 strain.

The Canu assembler allowed the construction of a single contig corresponding to the whole mitochondrial genome of the strains Pb18 and Pb03, although in both cases a contig of longer than expected length was recovered.

The reference assembly using BWA and the Illumina reads showed an average coverage of 9770X for Pb18 and 6963X for Pb03. The Pilon program corrected 336 errors in the sequence of Pb18 and 981 errors in Pb03, which included single nucleotide errors and small indels.

Conclusion: This work contributes to the development of several strategies for obtaining reliable assemblies of mtDNA genomes for which no close reference sequence is available, without the need for physical nuclear DNA vs mtDNA separation or mtDNA enrichment prior to the sequencing. Also the updated mtDNA genomes should enable more accurate SNP and other comparative or evolutionary analyses.

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The Intestinal Mycobiota of Nero Siciliano Pig

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Objective: The Pig's gut microbiota represents one of the most important and complex microbial community in nature involved in numerous physiological processes of the host such as the development of the gastrointestinal system, uptake of feeding, release of active metabolites, stimulation and modulation of the immunity system. Although in the last years a lot of studies involving the characterization of the pig's intestinal microbiota have been conducted, most of them have only focused on bacterial community, and very little is currently known about the composition of the fungal population or "mycobiota".

Nero Siciliano is an autochthonous pig breed reared in the internal areas of Sicily island (Italy), specifically in Madonie's Park and Nebrodi's Park. This breed shows wild phenotypic traits that differ from other commercial varieties bred in an intensive way.

Here we report the first characterization of the intestinal mycobiota of Nero Siciliano pig by using a culture-dependent approach and DNA sequencing.

Methods: A total of 21 pigs, from a swine farm in Messina, were sampled using rectal swabs. The fungi were isolated by streaking the rectal swabs on Sabouraud (SDA) and Potato (PDA) Dextrose Agar plates respectively. The fungal colonies were initially identified using conventional morphological and physiological tests and subsequently their identity was confirmed by standard Sanger sequencing of the ITS1-5,8S-ITS2 region of the rDNA.

Results: A total of 48 fungal strains were obtained from all examined fecal specimens. Molecular data showed that at least 8 different fungal genera colonized the gut of our pigs and *Wickerhamomyces anomalus* and *Geotrichum candidum* (10/48; 21% each) were the most isolated species followed by *Diutina catenulata* (8/48; ~17%), *Clavispora lusitaniae* (7/48; ~15%), *Trichosporon asahii* (5/48; ~10%), *Pichia kudriavzevii* (2/48; ~4%), *Rhodotorula mucillaginosa* (2/48; ~4%), and other species (4/48; ~8%).

Conclusion: The data reported in this study showed that the intestinal gut of Nero Siciliano pig is colonized by the fungal species *W. anomalus*, *G. candidum*, *D. cutenulata*, *R. mucillaginosa* and *T. asahii*, which were already previously reported as basic components of the pig's mycobiota in other studies.

Interestingly, our study shows that Nero Siciliano pig is also colonized by *C. lusitaniae* and *P. kudriavzevii*, two species with high pathogenic potential for humans. The differences between the mycobiota of the Nero Siciliano pig and that of other breeds could be related to several reasons such as genetic factors, kind of feeding and to the different breeding methods. However, whole-metagenome shotgun analyses are in progress in our lab in order to elucidate the complex structure of Nero Siciliano pig intestinal microbiota and its possible effects on the well-being of this animal.