PP1.207

Heterogeneity and within-host adaption observed in clinical isolates of Aspergillus fumigatus

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Objective: We explored the phenotypical and genetic variability among isolates of the ubiquitous and saprophytic fungus *Aspergillus fumigatus* which has the remarkable ability to adapt and grow in many different niches. Due to this ability it can also cause invasive and non-invasive infections in humans and animals, for example invasive pulmonary aspergillosis (IPA) in humans and sinonasal aspergillosis (SNA) in dogs. Our main objective is to understand how this fungus adapts to different niches and to find the factors and genetic traits that influences host adaptation and development in the context of fungal infections. To address this question we have compared a set of clinical and environmental isolates at a genetic and phenotypic level. Isolates were derived from sputum or bronchoalveolar lavage from human patients at the intensive care unit who were suspected to developed IPA. In addition we cultured isolates from fungal plaques isolated from the sinus of dogs suffering with SNA using endoscopy or trephination.

Methods: We have compared a set of isolates of *A. fumigatus* from A) humans (29 isolates from a preselected set of 9 patients),B) dogs with SNA (27 isolates form 9 patients) C) environmental isolates (27 isolates) with reference strains. Azole resistance was determined by microdilution assay antifungal susceptibility testing and tandem repeats in the promotor region of the *cyp51A* gene. Sequencing of calmodulin (*CaM*), beta-tubulin(*benA*) and mating type genes (*MAT1-1 and 1–2*) and microsatellite (STRAf) analysis were performed to detect genetic differences between isolates. Plating on different media was performed to observe differences in macro and micromorphology

Results: Genotyping of the different isolates showed that each human patient carried multiple fungal genotypes. In contrast, each dog suffering from SNA appeared to be infected by only one single genotype. Remarkably, different isolates from each dogs, and having the same genotype, showed a large phenotypic variability. In particular "white isolates" with apparent reduced sporulation were frequently isolated (13 out of 27 isolates) from dogs but not in human patients or in environmental isolates. In terms of azole resistance only human isolates and one of the indoor and outdoor environmental isolates were found to be resistant. Principal component analysis using colony diameter as a proxy for growth speed suggests that canine isolates might represent a subgroup of *A. fumigatus* that are responsible for SNA.

Conclusion: Our observations shows that*fumigatus* from dogs with SNA are phenotypically very diverse in contrast to their environmental and human counterparts.

Phenotypic variability seems to be generated during the chronic infection process in the sinus of the dogs. The basis of this heterogeneity might be due to genomic differences and/or epigenetic variations.

We expect that appearance of the phenotypic "white isolates" in dogs is a result of within-host adaption and is triggered by environmental factors in the sinus which we address in ongoing research.

PP1.208

Simple, low-cost micro-culture method for rapid diagnosis of mucormycosis in murine model

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Objective: Mucormycosis is a life threatening invasive fungal infection caused by mucoralean fungi and delay in diagnosis and treatment usually results to high mortality rates. This study aimed to describe a simple, low cost micro-culture method for rapid diagnosis of mucormycosis in murine model.

Methods: Infection by reference strain (*Rhizopus oryzae*), isolated from cerebral mucormycosis was induced in three groups which consist of five immunocompetent mice with three different inoculums (1×10^4, 1×10^5 and 1×10^6 cfu/ml) in a volume of 0.2 ml into the lateral tail vein. Animals were euthanized daily, at day 3 to 7 post infection. Homogenized tissues (brain and kidney) and blood samples were cultured on SDA and diphasic blood-culture bottle and incubated at 35°C. Subsequently, histopathology and molecular assay have performed for confirmation. Micro-culture sampling was adjusted by non-heparinized glass capillary tube with 50–70 μl of RPMI 1640 medium (Sigma). Homogenized tissue and blood samples (75 capillary tubes for 15 mice) were inoculated into capillary tubes and sealed with paraffin wax and incubated at 35° C. After 24 hr direct examination were performed using invert microscope.

Results: 21 out of 75 (28%) blood samples and 14 of 15 (93.3%) brain and kidney of each samples showed positive micro-culture results. From 25 micro-culture blood samples for each group, 5, 9 and 7 were positive with the inoculum sizes of 1×10^4, 1×10^5 and 1×10^6 cfu/ mouse, respectively. Neither positive blood culture, nor PCR were observed. However, PCR and tissue culture were positive for 25 of 30 (83.3%) and 27 of 30 (90%), respectively.

Conclusion: Use of micro-culture as a simple, rapid, and reliable method has the potential role to become a valuable surrogate assay for early diagnosis of mucormycosis. Further validation is required to confirm the clinical utility of this method.

PP2.009

Study the effect of honey ingestion on recognition of Aspergillus fumigatus conidia by peritoneal macrophages

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Objective: Invasive aspergillosis (IA) is one of the most severe systemic fungal diseases caused by the opportunistic fungus *Aspergillus*. It is the main reason of fungal related mortality in high risk patients. The most important predisposing factor known is quantitative and/or qualitative defect in neutrophils; however the increase in the number of IA in non-neutropenic immunocompromised patients highlights the importance of non-neutrophil defense-related factors. These mechanisms may include the recognition of the microorganism, recruitment of leucocytes other than neutrophils and effector mechanisms of recruited or resident cells. In this study we examined the effect of three Iranian honeys including Thyme, Pennyroyal and Astragalus Honeys on TLR2 and TLR4 gene expressions in mice involving with Invasive Aspergillosis.

Methods: BALB/c mice weighing between 20–30gr were divided into 10 groups (honey alone, honey and infection, negative and positive controls) each containing 10. Mice were treated with honey (1.5 g/kg BW/orally) for 10 days. At day 6, mice in infection group were infected with *Aspergillus fumigatus* conidia (5×105/ml) intravenously. Animals were sacrificed at day 11 and their peritoneal macrophages were cultured. The mRNA from macrophages was extracted and TLR2 and TLR4 gene expression was determined by semi-quantitative RT-PCR.

Results: The results showed that TLR2 expression by peritoneal macrophages had decreased in all honey treated mice in comparison to control group, however this decrease was not significant except in mice receiving Thyme honey ($P > 0.05$). Among infection groups, only infected mice treated with Pennyroyal had significantly higher TLR2 expression; while TLR2 expression had increase in all honey treated groups comparing to normal saline treated mice. TLR4 expression showed that infected groups had higher TLR4 expression comparing to non-infected groups and this increase was significant among mice treated with Pennyroyal and Astragalus honeys ($P < 0.05$). All honey treated infected groups had lower TLR4 expression than saline treated infection group ($P < 0.05$).

Conclusion: It can be concluded that honey could increase *Aspergillus* recognition by peritoneal macrophages, however in order to illuminate the exact mechanism of action of honey on innate immune responses during invasive aspergillosis, more studies are necessary in the future.

PP2.010

Occurrence of pathogenic Aspergillus species in drinking water from restaurants in Kathmandu, Nepal

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Objective: *Aspergillus* is a ubiquitously distributed opportunistic fungus that causes a wide range of infections in both immunocompetent and immunocompromised hosts. *Aspergillus fumigatus* is the global leading cause of invasive aspergillosis associated with high morbidity and mortality. *A. flavus* also causes invasive aspergillosis and is known to produce aflatoxins. *Aspergillus* species and other fungi are accounted as a significant cause of water contamination due to their ability to survive after filtration in distribution networks and during storage even when they have been treated with chlorine. The presence of *Aspergillus* in drinking water can lead to invasive infections, allergy and toxic responses, particularly in immunocompromised patients. In this study, we investigated the occurrence of pathogenic *Aspergillus* species in drinking water from restaurants in Kathmandu, Nepal.

Methods: A total of 120 drinking water samples were collected between March to June 2017 from restaurants in the centre of Kathmandu and processed using a membrane filter (MF) technique according to standard methods of American Public Health Association (2005). A volume of 100 mL water was filtered through a sterile membrane filter with 0.45 μm pore size and 47 mm diameter. The membranes were placed on Sabouraud dextrose agar plates with chloramphenicol (50 mg/L) and incubated at 37°C for up to 7 days and examined daily for any visible growth of pathogenic fungi. Pathogenic *Aspergillus* species as well as different types of other fungi were enumerated and identified to species complex level by macroscopic and microscopic morphology. Gram stain, germ-tube test and biochemical tests were also performed for identification of yeasts.

Results: All treated drinking water samples were positive for the growth of pathogenic fungi. *Aspergillus* species were recovered from 63% of water samples from restaurants but yeasts (83.7%) were more predominant than filamentous fungi (16.3%). Total count of *Aspergillus* species ranged from 1 to 38 colony forming units (cfu)/100 mL, with an average of 5 cfu/100 mL. The most abundant genera of filamentous fungi identified were *Aspergillus* (10.2%) but *Fusarium* (1.0%), *Penicillium* (0.8%), *Rhizopus*(0.4%), *Mucor* (0.4%), *Curvularia* (0.3%) and *Trichoderma* (0.2%) were also isolated. The genera *Rhodotorula* (35.1%) and *Candida* (25.5%) were detected in a high frequency. Among *Aspergillus* isolates, *A. fumigatus* (5.3%), *A. flavus* (15.8%) and *A. niger* (78.9%) were recovered from drinking water samples.

Conclusion: Pathogenic *Aspergillus* species were the most frequently isolated filamentous fungi in treated drinking water sources in Kathmandu. The occurrence of opportunistic fungal pathogens in drinking water is a potential threat to human health and indicated increased risk of *Aspergillus* infections. Awareness of drinking water quality and water safety and the availability of improved drinking water treatment systems should be emphasized to maintain microbial drinking water safety.

PP2.011

Transcriptomic analysis of non -invasive infections by Aspergillus fumigatus: the case of sino-nasal aspergillosis (SNA) in dogs

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Objective: The form of human fungal sinusitis that most closely approximates the disease occurring in the dog is chronic erosive non-invasive fungal sinusitis. This disease is characterized by final destruction of bone in the absence of tissue invasion by the fungus and requires both removal of fungal plaques, necrotic tissue and medical therapy with antifungals. Remarkably, these fungal plaques are white indicated that asexual development does not proceed in the patients. Immune response in SNA infections has been studied via biopsy and cytokine profiling as well as transcriptomic analysis of the host tissue. However, a transcriptomic study of this fungal pathogen growing in patients causing a non-invasive infection has never being performed. We obtained fungal plaques directly from canine patients suffering from SNA and characterize the transcriptome of the causative fungus *A. fumigatus* in order understand gene expression in the context of the host and particularly in the field of in-host adaptation

Methods: Four different fungal plaques were isolated from dogs suffering from SNA. After surgical removal using endoscope or trephination part of fungal plaques were immediately frozen in liquid nitrogen and stored at -80°C for RNA isolation and sequencing. RNA isolation was performed using RNeasy Mini Kit® from Qiagen and sequencing was performed by ServiceXS (Leiden, The Netherlands). RNA-seq analysis involved quality check with fastQC. Cleaning and trimming of reads with Fastx-toolkit.Kallisto was used for transcript quantification(TPM) with *A.fumigatus* Af293 (AspGD) as reference.

For functional characterization of the transcriptome, highly variable expressed genes between samples were removed, and 3 subjective levels of expression were established: low (1 to 39.8 TPM), median (39.8 to 1584.8 TPM) and high (1584.8 to 79432.8 TPM), for each group an enrichment analysis was performed. Additionally a more targeted categorization of the transcriptome was done using published lists of genes involved in stress, reproduction and virulence.

Results: According to the used criteria 17% of the expressed genes presented a stable expression across all 4 fungal plaques. Careful examination of this group of genes showed genes previously reported to be involved in SskA-Hog/SakA signalling pathway with some of them (bck1 and hdaA) also involved in the regulation of secondary metabolism. Interestingly, central regulators of asexual reproduction like BrlA,WetA and AbaA showed variable or null expression. A similar pattern was observed for catalases and superoxide dismutases. Finally comparison with published biofilm expression data showed that 18% of the stable expressed genes were also differentially expressed in *A. fumigatus* biofilm, of which approximately half of them were described to be differentially expressed in an "mature" biofilm (48 h).

Conclusion: 1-To our knowledge this is the first transcriptomic study of *A. fumigatus* in the context of a natural non-invasive infection in dogs suffering from SNA.

2-Variability in gene expression in SNA fungal plaques could be caused by several factors like time of infection, host response, and genomic differences.

3-SNA fungal plaques resemble a non-sporulation mature biofilm, explained partially by low expression of central regulators of sporulation and the expression of some genes related to previously reported biofilm formation.