Methods: The Af-Sm co-culture was prepared on an 8-chambers Lab-Tek slide as described previously[1]. Anti-GAG and anti-GM monoclonal antibodies produced at the *Aspergillus* Unit at Pasteur Institute of Paris, were used to focus on the 3D structure of GAG and GM in the ECM of the biofilm. These polysaccharides were analyzed by fluorescence confocal microscopy at 24 h.

Results and Conclusion: GM was found, as expected, in the hyphal Af cell wall but was very little in the ECM. GAG was also found in the cell wall but mainly formed a beautiful fibrillary network between the hyphae, showing the importance of this polysaccharide in cell-cell interaction and in the structuration of Af biofilm. GAG could be the surface receptor for Sm, which would promote strong adhesion between Sm and Af in the biofilm.

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S2.4a
Pythiosis: An emerging disease in Hong Kong

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S2.4 Veterinary mycology research, September 21, 2022, 3:00 PM - 4:30 PM

Oomycosis is an emerging disease of humans and animals caused by oomycetes in the Stramenopiles-Alveolata-Rhizaria super group, mainly *Pythium insidiosum* and occasionally *Lagenidium giganteum* or *Paralagenidium* species. In surface freshwater, oomycetes produce motile biflagellate asexual zoospores with marked chemotactic attraction to epithelial surfaces of vertebrate hosts. Infection is the result of encystation and invasion of damaged skin or gastrointestinal mucosa.

Of ~ 4200 cases of pythiosis reported globally between 1980 and 2021 only ~ 20% involved humans while 80% involved animals, mainly horses, dogs, and cattle. Most human cases occur in India and Thailand, whereas most animal infections were reported in the United States and Brazil. Pythiosis has been reported in mainland China, but the burden of the disease is low and comprises <1% of overall cases in humans.

Neither pythiosis nor lagenidiosis has been previously reported in humans or animals in Hong Kong. From January 2018 to January 2022, the Veterinary Diagnostic Laboratory of City University of Hong Kong diagnosed 10 cases of oomycosis (5 canine, 5 feline) after identification of non-parallel walled, irregularly branching, and poorly septate hyphae in the center of necrotic regions of histological sections of formalin-fixed paraffin-embedded tissues (FFPET). Species identity was confirmed by PCR and sequencing of 28S rDNA from DNA extracts of FFPET. There were 8 cases of *P. insidiosum* and 2 of *L. giganteum* infection. Serum ELISA was positive for Pythium antibodies in 5/6 cases tested and negative for *Pythium/Lagenidium/Paralagenidum* antibodies in a German shepherd dog (GSD) with disseminated disease caused by *L. giganteum*.

Affected dogs were young to middle-aged at presentation (9 months to 5 years old). Two dogs had focal cutaneous infections, two had extensive gastrointestinal involvement, and the GSD had disseminated disease with cutaneous, mediastinal, and abdominal involvement.

Affected cats were young (8 weeks to 18 months) and presented with subcutaneous/cutaneous disease. Three cats had a distinctive perianal ring of bulging subcutaneous granulation tissue, including one that also had an ulcerated, proliferative and necrotic lesion involving two adjacent hind-limb digits. One cat had facial subcutaneous swelling with mandibular lymph node enlargement and the remaining cat presented with extensive circumferential swelling of one hind limb from the distal paw to the mid-stifle.

All cats tested negative for the Feline leukemia virus and Feline immunodeficiency virus. Traumatic or surgical wounds preceding infection were identified in a kitten caught in a rodent glue-trap with skin wounds, in a cat with facial involvement that had an injured globe surgically enucleated, and in a dog with cutaneous pythiosis that had chronic dermatitis.

Treatment data were available for one canine case. The GSD with lagenidiosis was treated with combination antimicrobial therapy including voriconazole, terbinafine, minocycline, and azithromycin. The dog responded poorly. Mefenoxam was substituted for voriconazole and hyperbaric oxygen therapy was administered. After initial response, the dog succumbed 4 months from diagnosis. We have had success with the treatment of four feline cases using combination therapy including surgical debridement, immunotherapy with a Pythium vaccine, and combinations of antifungal drugs (posaconazole and terbinafine), and/or antimicrobials (doxycycline/minocycline and azithromycin).

S2.4d
Combination antifungal effects of eugenol with voriconazole against *Candida tropicalis* and *Candida krusei* strains isolated from the genital tract of mares

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S2.4 Veterinary mycology research, September 21, 2022, 3:00 PM - 4:30 PM

Objectives: The objective of the current study was to investigate the possibility that eugenol synergizes the antifungal effects of voriconazole on genital isolates of *Candida krusei* and *Candida tropicalis* from mares.

Methods: The antifungal activity of eugenol and voriconazole was evaluated using the broth microdilution assay (CLSI-M27-A3). The synergism of eugenol and voriconazole against genital *Candida* isolates was evaluated by the microdilution checkerboard method.

Results: Minimum inhibitory concentration (MIC) values for eugenol and voriconazole ranged from 400 to 800 μg/ml and 1 to 8 μg/ml, respectively, for *C. tropicalis* isolates, and from 200 to 400 μg/ml for eugenol and 2 to 16 μg/ml for voriconazole against *C. krusei* isolates. Eugenol decreased the arithmetic mean of MIC for voriconazole against *C. tropicalis* and *C. krusei* isolates from 2.66 to 0.46 μg/ml and 7.77 to 0.41 μg/ml respectively. The fractional inhibitory concentration index (FICI) values for the eugenol voriconazole combination ranged from 0.25 to 0.88 and 0.19 to 0.63 for *C. tropicalis* and *C. krusei* isolates respectively. A synergistic effect of eugenol in combination with voriconazole was observed for 83.3% of *C. tropicalis* and 77.7% of *C. krusei* isolates. The antagonistic activity was not seen in any of the isolates tested.

Conclusions: Eugenol showed fungistatic and fungicidal effects against genital *Candida* isolates and, in combination, synergized the antifungal effects of voriconazole. The eugenol-voriconazole combination can lay the foundation for a therapeutic approach against isolates in which azole resistance has increased over time.

S2.5a
***Magnusiomyces* spp.**

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S2.5 Rare yeasts, September 21, 2022, 3:00 PM - 4:30 PM

Magnusiomyces clavatus and *Magnusiomyces capitatus* are emerging yeasts with intrinsic resistance to many commonly used antifungal agents. Identification is difficult, and the determination of susceptibility patterns with commercial and reference methods is equally challenging. For this reason, few data on invasive infections by *Magnusiomyces* spp. are available. We, therefore, determined the epidemiology and susceptibility of *Magnusiomyces* isolates from bloodstream infections (BSI) isolated in Germany and Austria from 2001 to 2020. A total of 34 *Magnusiomyces* BSI were analyzed; isolates were identified by internal transcribed spacer (ITS) sequencing and matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS). Antifungal susceptibility was determined by EUCAST broth microdilution and gradient tests. Of the 34 isolates, *M. clavatus* was more common ($n = 24$) than *M. capitatus* ($n = 10$). BSI by *Magnusiomyces* spp. were more common in men (62%) and mostly occurred in patients with hemato-oncological malignancies (79%). The highest *in vitro* antifungal activity against *M. clavatus*/*M. capitatus* was observed for voriconazole (MIC50, 0.03/0.125 mg/l), followed by posaconazole (MIC50, 0.125/0.25 mg/l). *M. clavatus* isolates showed overall lower MICs than *M. capitatus*. With the exception of amphotericin B, a low essential agreement between gradient test and microdilution was recorded for all antifungals (0%-70%). Both species showed distinct morphologic traits on ChromAgar Orientation medium and Columbia blood agar, which can be used for differentiation if no MALDI-TOF MS or molecular identification is available. In conclusion, most BSI were caused by *M. clavatus*. The lowest MICs were recorded for voriconazole. Gradient tests demonstrated unacceptably low agreement and should preferably not be used for susceptibility testing of *Magnusiomyces* spp.

S2.5c
***Exophiala* spp.**

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S2.5 Rare yeasts, September 21, 2022, 3:00 PM - 4:30 PM

The black yeast *Exophiala dermatitidis* is an opportunistic pathogen, causing phaeohyphomycosis in immunosuppressed patients, chromoblastomycosis, and fatal infections of the central nervous system in otherwise healthy Asian patients. In addition, it is also regularly isolated from respiratory samples from cystic fibrosis patients, with rates varying between 1% and 19%. Melanin, as part of the cell wall of black yeasts, is one major factor known to contribute to the pathogenicity of *E. dermatitidis* and increased resistance against host defense and anti-infective therapeutics. Further virulence factors, e.g., the capability to adhere to surfaces and to form biofilm were reported.

S2.5d
Exploring multidrug resistance, fitness compensation, and collateral sensitivity in *Candida auris* : Fight fire with fire?

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S2.5 Rare yeasts, September 21, 2022, 3:00 PM - 4:30 PM

Candida auris (*C. auris*) is a recently emerged human fungal pathogen of growing concern due to its ability to acquire extensive multidrug resistance (MDR) to all four antifungal drug classes. The unprecedented extent of MDR in *C. auris*, suggests accelerated resistance evolution, novel mechanisms of resistance, and/or potential fitness compensation. Despite being the first fungus to be officially considered an urgent antimicrobial resistance threat by the CDC (US), insights into the resistance mechanisms and evolutionary dynamics of *C. auris* are still scarce.

By using high-throughput *in vitro* experimental evolution with various antifungal drugs, we have obtained a library of resistant strains from four different clades. Through both genome and targeted sequencing, we have discovered novel mutations, especially for polyene resistance, which indicate new mechanisms of resistance and fitness compensation. For the validation of mutations, we have optimized a recyclable CRISPR/Cas9 tool for *C. auris* based on the *C. albicans* HIS-FLP system.

By mapping drug susceptibility responses of evolved strains across a library of several antifungals and repurposed drugs, we have discovered trends of cross-resistance and collateral sensitivity. Both phenomena have been extensively studied in tumors and bacteria but remain unexplored in fungi. In the light of these observations, we explore novel treatment schemes that prevent antifungal drug resistance development in *C. auris* and other pathogenic fungi.

S3.1a
Update in mycetoma control

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S3.1 Neglected implantation mycoses, September 21, 2022, 4:45 PM - 6:15 PM

Mycetoma is a badly neglected tropical disease, reported globally but endemic in many tropical and subtropical, and Sudan has the highest disease burden. Mycetoma epidemiological features remain uncharacterized. Hence, there is no preventive or control program for mycetoma worldwide. To reduce the disease burden and its socio-economic impacts on patients, families, communities, and the health system in the endemic regions, the Mycetoma Research Center, University of Khartoum, Sudan, WHO Collaborating Center on Mycetoma has adopted a unique holistic approach to management and mycetoma patients, and it gained a great experience on that.

In this presentation, we will share the MRC's experience in dealing with the patients at the endemic villages level, supporting the affected communities and collaborating with the local health authorities to improve the life quality of the affected patients.

S3.1d
High histoplasmosis incidence in kidney transplant recipients in Santa Fecity, Argentina

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S3.1 Neglected implantation mycoses, September 21, 2022, 4:45 PM - 6:15 PM

Objectives: Histoplasmosis is endemic in the central/northeast region of Argentina. No data on the incidence of Histoplasmosis are available in most countries. It is estimated that the incidence of this mycosis is low in solid organ transplant recipients. In endemic areas of the USA (Ohio), the incidence of histoplasmosis in kidney transplant recipients is 0.25%. The objectives of this work are to describe the epidemiology, clinical forms, and evolution of kidney transplant recipients' diagnoses with histoplasmosis in Santa Fe city, Argentina.

Methods: A retrospective study was carried out between July 2017 and July 2020 at the Nephrology, Urology, and Cardiovascular Diseases Clinic, Santa Fe (Argentina). Demographic, clinical, and laboratory data were obtained and analyzed. Histoplasmosis diagnosis was performed by means of histopathology (intracellular yeasts), recovery of Histoplasma spp. by culture, and/or positive nested PCR specific for Histoplasma Hc100 gene. No antigen detection method was available in Argentina at the time of the study.

Results: During the 36 months of the study, 225 kidney transplantations were performed. Out of these patients, 10 were diagnosed with histoplasmosis (4.44%). All the patients were Santa Fe province inhabitants. Patients' median age was 47 years old and 90% were male. A total of 9 patients (90%) presented the disseminated form of the disease and 1 the pulmonary form; 8 were recipients of their first transplant and 2 were second transplant recipients. All received thymoglobuline induction as immunosuppressive therapy. In all, 4 were diagnosed with histoplasmosis in their first-year post-transplantation (mostly 6-12 months) and the rest after 1-year post-transplantation. At the time of the histoplasmosis diagnosis, five patients presented glomerular filtration between 30 and 60 ml/min, two <15 ml/min, two between 30 and 15 ml/min, and only one with glomerular filtration >90 ml/min. A total of 7 retained graft function at the end of treatment, 3 lost the graft (1 due to death). Histoplasmosis