Project Details	
Project Code	MRC23IIARBa Brown
Title	How are antifungal usage and environment change on our farms driving cross-resistance in clinical pathogens?
Research Theme	Infection, Immunity, Antimicrobial Resistance and Repair
Summary	Fungal pathogens cause deadly human infections, destroy our crops, and contaminate our food with toxins. Fungal pathogens of people are also present on our farms. Worryingly, we rely on a few antifungals to cure human infection and to secure our safe food supply. Here, we aim to understand how our changing environments and use of antifungals on farms may drive human pathogens to evolve cross-resistance to clinical treatments that result in poor patient outcomes.
Description	THE FUNGAL THREAT: Fungal pathogens cause deadly human infections, destroy our crops, and poison our food with harmful toxins. Despite our best efforts, we still lose ~10% of our crops to fungal diseases, ~25% of all food is contaminated with fungal toxins, and human infections have extremely high mortality rates. Worryingly, our population is becoming more vulnerable to infection, as the numbers of susceptible patients with immune disorders, major trauma, or viral co-infections rises. ENVIRONMENTALLY ACQUIRED RESISTANCE: We rely on a few antifungal drugs to secure our safe food supply and to cure human infection. This has created a perfect storm for the evolution of antifungal resistance (AFR), as many of the major fungal pathogens of people are also present on our arable farms. It is believed that the exposure of pathogens to agricultural antifungals has driven the evolution of cross-resistance to similar antifungals in hospitals, termed environmentally acquired resistance. Examples include Aspergilli and Fusaria which causes toxic cereal rots and life-threatening pulmonary, skin and eye infections, where antifungal resistance has been reported to contribute to poor treatment outcomes. But what is driving the increased threat of environmentally acquired resistance, is it changes to our environment or altered agricultural practices? Also, where are these cross-over pathogens acquiring AFR on our farms? DIRECTED EVOLUTION: We will evolve Aspergillus and Fusarium species under conditions replicate our changing agricultural environments in the presence of differing levels of agricultural antifungals. These environmental stresses will replicate the impacts of climate change and agricultural intensification, i.e. temperature, humidity, salt, and pH stress. Evolved and non-evolved strains will harbour constitutive GFP or RFP markers to facilitate comparative assays. Minimum inhibitory concentration (MIC) will be used to evaluate adaptations to different stresses along the evolutionary timeframe, and how this confers cross-resistance to clinical antifungals. Competition assays and fitness cost experiments will be used to model how these adaptations may influence the structure of the fungal population. This will enable us to determine which scenarios are driving the rise in environmentally acquired resistance on our farms. For example, does the use of irrigation in agriculture, which increases soil salinity, drive soil dwelling fungi such as Aspergilli and Fusaria to evolve stress tolerance mechanisms that promote AFR to both agricultural and clinical antifungals? Or, will future climatic environmental stress increase the rate at which AFR evolves? We will use genomics and epigenetic

	(bisulfite) sequencing to identify genetic changes acquired through exposure to stress and antifungals, in multiple fungal lineages with phenotypic adaptations. Finally, CRISPR-Cas9 genome editing will be used to confirm these genetic adaptations confer phenotypic adaptations that enhance environmental stress tolerance and AFR evolution. CROSS-OVER PATHOGEN COMMUNITIES: We will sample distinct arable environments (crops, soils, residues) throughout the cycles of a farming year to create a collection of Aspergillus and Fusarium species. This will be used to monitor how pathogen abundance and AFR profiles change in response to altered practices and the environment. Comparative genomics will be used to identify the genetic basis of adaptation in natural pathogens, which will be correlated with our lab-evolved strains. IMPORTANCE: This research will help us determine what is driving environmentally acquired resistance on our farms and where cross-over pathogens are becoming antifungal resistant. This knowledge will support then development of improved farming practices to mitigate the risk of environmentally acquired resistance, protecting the shelf-life of our limited antifungal drugs, to the benefit of our food security and human health.
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