Institution: The University of Manchester

Unit of Assessment: 5 (Biological Sciences)

Title of case study: Defining global antifungal azole resistance in *Aspergillus* – enhancing diagnostics and driving drug discovery

Period when the underpinning research was undertaken: 2000 - 2020

Details of staff conducting the underpinning research from the submitting unit:

Name(s):	Role(s) (e.g. job title):	Period(s) employed by submitting HEI:
David Denning	Professor of Infectious Diseases in Global Health Senior Lecturer	2005 - present 1990 - 2005
Peter Warn	Senior Lecturer Translational Medicine	1997 - 2008
Mike Bromley	Reader Senior Lecturer Lecturer	2019 - present 2016 - 2019 2013 - 2016
Paul Bowyer	Professor Senior Lecturer in Molecular Biology	2020 - present 2005 - 2020
Caroline Moore	Honorary Research Associate	1992 - present

Period when the claimed impact occurred: August 2013 - July 2020

Is this case study continued from a case study submitted in 2014? N

1. Summary of the impact

The University of Manchester (UoM) has led internationally in describing the methodology, mechanisms and implications of azole resistance in *Aspergillus*, a common airborne fungus causing life-threatening lung diseases called aspergillosis. Such resistance is a major clinical problem because the best (and only oral) antifungal drugs are azoles. The UoM team's research has led to the following impacts: i) developed and validated a standardised antifungal susceptibility testing method, now a global reference method, ii) enabled documentation of resistance in every continent, iii) driven new antifungal drug development (>USD300,000,000 investment) and iv) spawned one commercial product detecting resistance, sold in >25 countries.

2. Underpinning research

Millions of people are treated with azoles for aspergillosis annually. Denning and colleagues at UoM were the first to describe multi-azole resistance in *Aspergillus*, in patient samples tested in the lab, in 2002 [1]. In subsequent, iterative *in vitro*, *in vivo* and mechanistic research, the group demonstrated direct links between mechanisms of resistance and susceptibility testing results, and confirmed intra- and inter-laboratory reproducibility - all key to the development of standardised methodology. This led to the development of resistance testing methods for *Aspergillus* that were validated and reproduced in Europe and the US [2], using the UoM strains as quality controls.

The UoM team described the most common mechanism of resistance (target site mutations) [3]. UoM has also identified many other mechanisms of resistance (all since 2000 and reviewed in Bowyer, Bromley and Denning, Proc Natl Acad Sci 2020, but not cited in section 3 due to space limitations). These include transposon driven up-regulation of the CYP51A

target protein (2002), additional target mutations (2006, 2010, 2011), up-regulation of the primary azole target orthologue CYP51B (2013), the key efflux transporter CDR1B (2013), alternative mechanisms of resistance related to mitochondrial function (2016), the role of the SrbA gene and the CCAAT binding complex (2016) and the role of iron and CybE (2017). The remarkably broad range of resistance mechanisms makes them difficult to overcome, revealing the need for prevention, rapid detection and new antifungal drugs with novel chemistries. UoM has shown that one such drug (aminocandin) was effective in a mouse model of fungal infection [4].

The international use of azole fungicides on crops, seeds and bulbs has directly led to resistance arising in the environment but this is fortunately less common in the UK. UoM's research showed that most UK cases have emerged directly in patients on azole therapy [3], with multiple mechanisms of resistance found [5]. The team used molecular typing to document *in vivo* resistance in fungal balls (aspergillomas) surgically removed from human lungs. They further characterised the rising rates of azole resistance in Manchester for chronic pulmonary aspergillosis [5] and contributed to the world's first multi-country survey showing highly variable resistance rates across Europe.

Another notable challenge is that the sputum of over 50% of patients with active *Aspergillus* infection detected by PCR has false negative fungal cultures; subsequently the UoM team also showed, for the first time, that resistance could be directly detected with molecular markers in culture-negative clinical specimens, by detecting azole target mutations [6]. Their research also found detrimental outcomes for these patients [3].

3. References to the research

1. Mosquero J, **Denning DW**. Azole cross resistance in *Aspergillus fumigatus*. Antimicrob Ag Chemother 2002;46:556-557. https://doi.org/10.1128/AAC.46.2.556-557.2002 [*Google Scholar (GS) citations = >142*]
2. Subcommittee on Antifungal Susceptibility Testing of the ESCMID European Committee for Antimicrobial Susceptibility Testing: Rodriquez-Tudela JL, Donnelly JP, Arendrup MC, Arikan S, Barchiesi F, Bille J, Chryssanthou E, Cuenca-Estrella M, Dannaoui E, **Denning D**, Fegeler W, Gaustad P, Lass-Flörl C, **Moore C**, Richardson M, Schmalreck A, Velegraki A, Verweij P. EUCAST Technical Note on the method for the determination of broth dilution minimum inhibitory concentrations of antifungal agents for conidia-forming moulds. Clin Microbiol Infect 2008;14:982-4. https://doi.org/10.1111/j.1469-0691.2008.02086.x. [*GS citations = >210*]
3. Howard SJ, Cesar D, Anderson MJ, Albarrag AM, Fisher M, Pasqualotto AC, Laverdiere M, Arendrup MC, Perlin DS, **Denning DW**. Frequency and evolution of azole resistance in *Aspergillus fumigatus* associated with treatment failure. Emerg Infect Dis 2009;15:1068-76. https://doi.org/10.3201/eid1507.090043 [*GS citations = >681*]
4. **Warn PA**, Sharp A, Morrissey G, Lowther J, **Denning DW**. Activity of aminocandin (IP960; HMR3270) compared to amphotericin B, itraconazole and caspofungin and micafungin in neutropenic murine models of disseminated infection caused by itraconazole susceptible and resistant strains of *Aspergillus fumigatus.* Int J Antimicrob Ag 2010;35:146-51. https://doi.org/10.1016/j.ijantimicag.2009.09.029 [*GS citations = >38*]
5. Bueid A, Howard SJ, **Moore CB**, Richardson MD, Harrison E, **Bowyer P, Denning DW**. Azole antifungal resistance in *Aspergillus fumigatus*: 2008 and 2009. J Antimicrob Chemother 2010;65:2116-8. https://doi.org/10.1093/jac/dkq279 [*GS citations = >282*]
6. **Denning DW**, Park S, Lass-Florl C, Fraczek MG, Kirwan M, Gore R, Smith J, Bueid A, **Bowyer P**, Perlin DS. High frequency triazole resistance found in non-culturable *Aspergillus fumigatus* from lungs of patients with chronic fungal disease. Clin Infect Dis 2011;52:1123-9. https://doi.org/10.1093/cid/cir179 [*GS citations = >291*]

4. Details of the impact

Context

Antifungal resistance is of great importance to an estimated >300,000 new cases of invasive (life-threatening) aspergillosis p.a., approximately 3,000,000 chronic pulmonary aspergillosis and 6,000,000-15,000,000 allergic aspergillosis, worldwide. Infection with a resistant strain of *Aspergillus* doubles mortality rate to approximately 90% and the aspergillosis becomes untreatable with oral therapy as triazoles are the only oral class of antifungal with anti-*Aspergillus* activity.

Pathway to impact

Susceptibility testing of fungi is difficult and affected by many factors. UoM's confirmation of the validity of resistance in animal models [4] and good intra- and inter-laboratory reproducibility has allowed standardised methodology internationally. Denning demonstrated resistance with clinical observations [3,5], which other groups confirmed. Denning led the founding of the European Committee on Antimicrobial Susceptibility Testing (EUCAST) Antifungal Susceptibility Testing (AFST) group (2000-2002) (ESF grant 2000-2002) [2]. EUCAST provides regulatory authorities, clinical laboratories and companies with reproducible methods and breakpoints (the concentration of an antifungal which defines whether a microorganism is susceptible or resistant to it) for antimicrobials.

Reach and significance of the impact

i) Development of a global reference method for susceptibility testing for Aspergillus and regulatory approval of new antifungal agents

UoM work which identified multi-azole resistance [1] and developed antifungal susceptibility testing methods [2] led directly to the development of quality control strains and an international standardised method for susceptibility testing for *Aspergillus*. [Ai and Aii]. Both reference methods and associated breakpoints are used extensively by industry in preclinical assessment and in submissions to regulatory authorities to demonstrate the relative activity of a proposed new antifungal compound. A network of 14 European laboratories, including UoM, collaborate to assess breakpoints and new methods.

Chair of the EUCAST Antifungal Susceptibility Testing Subcommittee said: "*The early initiatives in Europe to standardise antifungal susceptibility testing in Aspergillus were led by Manchester in the early 2000's. Publication by the Manchester team of multi-azole resistant strains, using a reproducible method was the foundation of the EUCAST method* [which] *is an intrinsic and necessary part of any submission for regulatory approval with the European Medicines Authority for any new antifungal drug* [and] *… includes the use of an azole resistant QC strain of Aspergillus fumigatus from Manchester F6919*" [1]. "*... the importance of this early development work cannot be over-emphasised*." [B]. Chair of the Antifungal Susceptibility Testing Subcommittee (ASTS) of the US CLSI said: "… *the 2002 publication of Mosquera et al.* [1] *… was pivotal in driving awareness of, and a response to, the problem of azole resistance….These strains and the associated testing methods from the Manchester group underpinned development of standardized reference susceptibility testing methods for Aspergillus*." [C].

ii) Worldwide detection of azole resistance

The international reference method derived from UoM research has made it possible to document the emergence of azole resistance in patients and the environment globally. For example, azole resistance was found in 1.1-30% of isolates of *Aspergillus* from patients in Europe, including 13.2% in London [D].

iii) New antifungal drug development and economic impact from Manchester spin-out

Azole resistance is a key regulatory concern, and is a major driver for the development of new antifungal agents. The clinical development pipeline now has nine new candidates. Chair of the CLSI ASTS said: "*Documentation of the emergence of azole resistance in Aspergillus was made possible by the CLSI M38 reference method. This was, in turn, instrumental in highlighting the clinical need and thereby making it possible to fund several new companies*

and antifungal developments. And as a further result, several promising new antifungal agents are now in the clinic." [C].

Venture funding for antifungals in development is a direct result of UoM's pioneering work on antifungal resistance, notably Ibrexafungerp (Scynexis), Fosmanogepix (Amplyx) and Olorofim (F2G), three examples each attracting >GBP100,000,000 in funding [E], [F] and [G]. "*At Scynexis … we recognize the ground-breaking and world-class scientific progress made in Manchester on aspergillosis...*" (CEO, Scynexis [E]). "*This unmet need of "resistance" was a major driver for the investment in Amplyx …*" (>USD90,000,000). (CMO, Amplyx [F]). "*F2G has really focussed primarily on Aspergillus infections as a major area of unmet need. A key driver in this area was the early descriptions of azole resistance in Aspergillus from Manchester .... F2G's many investors have been sufficiently impressed by both the clinical need and F2G's progress that they have invested GBP83.3 million* [GBP83,000,000] *to date".* (CEO, F2G Ltd) [G].

Warn's translational research correlating *in vitro/in vivo* antifungal activity [4] led him to set up spin-out Euprotec in 2009. Euprotec employed 15 people with an annual turnover of GBP1,500,000 until acquired by Evotec in 2014 [H]. Evotec expanded and now approximately 250 people work in anti-infectives, pharmacology and toxicology. In 2018 Warn led the expansion to consolidate Sanofi infectious diseases research in Lyon, Toulouse, Germany and Verona to form the largest industrial group in infectious diseases discovery worldwide. Dr Werner Lanthaler, Chief Executive Officer of Evotec, said: "*With the signing of this transaction, Evotec will have created the highest qualified translational footprint in infectious disease research globally with approx. 180 scientists*." As part of the transaction, Sanofi paid Evotec EUR60,000,000 upfront to progress the anti-infectives portfolio [H].

iv) Improved resistance detection and diagnosis

UoM's finding that azole resistance can be detected by PCR in clinical specimens with negative cultures [6] has led to the commercial development of a new diagnostic kit, AsperGenius®, by PathoNostics, the first on the market able to differentiate *A. fumigatus* wildtype from the most prevalent azole resistance markers, now sold in >25 countries [I]. The CSO for PathoNostics stated "*The research into antifungal resistance at ... Manchester had a major impact and has given us scientific input for the development of improved diagnostics of resistant fungi*" [I].

5. Sources to corroborate the impact

A. International reference methods for susceptibility testing for *Aspergillus* based on UoM research:
   (i) European Committee on Antimicrobial Susceptibility Testing (EUCAST) 'Method for the determination of broth dilution minimum inhibitory concentrations of antifungal agents for conidia forming moulds' (April 2020) - cites references [3] and [5].
   (ii) Clinical and Laboratory Standards Institute, USA (CLSI) 'M38 Reference Method for Broth Dilution Antifungal Susceptibility Testing of Filamentous Fungi' (November 2017).

B. Letter from Chair of the EUCAST Antifungal Susceptibility Testing Subcommittee (2 October 2019), confirming that the UoM research was the foundation of the EUCAST reference method.

C. Letter from Chair of the CLSI Antifungal Susceptibility Testing Subcommittee (30 June 2020), confirming that the UoM research was the foundation of the CLSI reference method.

D. Review of the frequency of resistance: Verweij et al., Azole Resistance in Aspergillus fumigatus: Can We Retain the Clinical Use of Mold-Active Antifungal Azoles? Clinical Infectious Diseases 2016;62(3):362–8. https://doi.org/10.1093/cid/civ885 – cites Denning et al. 2011 [6].

E. Letter from President and CEO, Scynexis (3 July 2020), confirming investment in antifungal development as a direct result of UoM's documentation of resistance.

F. Letter from Chief Medical Officer, Amplyx (30 June 2020), confirming investment in antifungal development as a direct result of UoM's documentation of resistance.

G. Letter from Chief Executive Officer, F2G Ltd. (27 July 2020), confirming investment in antifungal development as a direct result of UoM's documentation of resistance.

H. Evotec webpages evidencing the acquisition of Euprotec in 2014, and the Sanofi agreement in 2018: https://www.evotec.com/en/invest/news--announcements/p/evotec-to-establish-an-anti-infectives-platform-with-the-acquisition-of-euprotec-ltd-5211; https://www.evotec.com/en/invest/news--announcements/press-releases/p/evotec-and-sanofi-sign-definitive-agreement-to-combat-infectious-diseases-5695

I. Letter from Chief Scientific Officer, PathoNostics B.V. (28 October 2019), confirming that the UoM research provided the original scientific basis for development of their AsperGenius® diagnostic kit.