environmental microbiology

Environmental Microbiology (2021) **00**(00), 00–00 doi:10.1111/1462-2920.15710

Editorial

Is an azole-resistant *Aspergillus* hotspot emerging in South-East Asia?

Norman van Rhijn and David W. Denning*
Manchester Fungal Infection Group, Division of Infection, Immunity and Respiratory Medicine, School of Biological Sciences, Faculty of Biology, Medicine and Health, The University of Manchester, Manchester Academic Health Science Centre, Manchester, UK.

Azole resistance in *Aspergillus* is much prevalent than it used to be and further emphasized by a paper in this issue from Vietnam (Duong *et al*., n.d.). The implications for human health are potentially profound. *Aspergillus* is an airborne pathogen and allergen, with millions of people affected annually (Bongomin *et al*., 2017). Aspergillosis is a spectrum of diseases caused by *Aspergillus* species, which include allergic, chronic or invasive disease (Kosmidis & Denning, 2015). The current gold standard, and only oral, treatments are azoles (itraconazole, voriconazole, posaconazole and isavuconazole). Untreated, the most severe forms of aspergillosis (invasive) have a mortality of >95% (Denning, 1996; Vandewoude *et al*., 2004). Infection caused by azole-resistant *Aspergillus* leaves physicians with sub-optimal therapies, assuming resistance is detected rapidly allowing time to change therapy (Howard *et al*., 2009; Verweij *et al*., 2015; Meis *et al*., 2016).

Itraconazole was the first clinically approved azole antifungal agent with activity against *Aspergillus* and licensed for use in 1991 (Schmitt *et al*., 1992). The first azole-resistant isolates of *Aspergillus fumigatus* were originally cultured in 1997 and 1998 in California in patients with subacute invasive aspergillosis, previously untreated with antifungal agents (Denning *et al*., 1997; Verweij *et al*., 1998). The methodology for determining resistance was improved in the 1990's and validated methods introduced by European Committee on Antimicrobial Susceptibility Testing (EUCAST) and Clinical and Laboratory Standards Institute (CLSI) (using these azole-resistant strains). A few itraconazole-resistant strains were described in the late 1990s and in 2002 multi-azole resistance was documented for the first time (Mosquera and Denning, 2002; Meneau and Sanglard, 2005). In 2003, a significant increase in resistance rates was observed in Manchester and in Nijmegen, and the first resistant strains were found in the environment (Bueid *et al*., 2010; Buil *et al*., 2019). These environmental strains were documented to have a different mode of resistance, the so-called TR_{34}/L98H dual genetic alteration (Snelders *et al*., 2008). The tandem repeat in the promoter of the azole target *cyp51a* increases copy number, as well as a point mutation changing the binding affinity of the azoles. Resistant strains from the environment are closely related to isolates from patients, likely reflecting environmental acquisition of resistance (Abdolrasouli *et al*., 2015; Rhodes *et al*., 2021). The potential for life-threatening infection was documented in resistant strains, heralding a major shift in thinking about the underlying reasons for development of resistance, mechanisms of resistance and routine susceptibility testing of *Aspergillus* spp. in clinical laboratories.

Several other mechanisms of resistance have been discovered, mostly related to target site mutations and/or upregulation (Diaz-Guerra *et al*., 2003; Buied *et al*., 2013; Gonzalez-Jimenez *et al*., 2020; Macedo *et al*., 2020). Other resistance mechanisms involve dysregulation via transcription factors or upregulation of efflux pumps (Slaven *et al*., 2002; Fraczek *et al*., 2013; Paul *et al*., 2018; Furukawa *et al*., 2020). With respect to environmental strains found to be resistant, all appear to have a combination of a tandem repeat upstream of the gene start codon leading to gene upregulation plus one or more 14 alpha demethylase (*cyp51a*) mutations. Over the last decade, resistance has been documented on every continent except Antarctica. While before 2000, the number of resistant isolates was close to zero, increased numbers of resistance are being reported. Prevalence data of azole resistance (~5%–10%) have been reported from several studies of *Aspergillus* from soil samples

Received 9 July, 2021; accepted 4 August, 2021. *For correspondence. E-mail ddenning@mancheter.ac.uk; Tel. +441612915353; Fax +441612915806.

across Europe, Africa, South America and Asia (Fraaije *et al*., 2020; Resendiz-Sharpe *et al*., 2021; Yerbanga *et al*., 2021). Azole resistance in the United States is becoming of great concern as over the last 20 years, azole usage in agriculture has increased fourfold (Wiederhold *et al*., 2016; Toda *et al*., 2021). Additionally, changes in climate may contribute to development of azole resistance and being exposed to *Aspergillus* (van Rhijn & Bromley, 2021).

In this issue of the journal, Tra-My Duong from the Oxford University Clinical Research Unit, Ho Chi Minh City document remarkably high rates of azole resistance in strains of *Aspergillus fumigatus* from South-East Vietnam (Duong *et al*., n.d.). They cultured 62 strains from the Mekong delta and 92% of *A. fumigatus sensu stricto* were itraconazole resistant, compared to 32% in *A. fumigatus* species complex, excluding *sensu stricto*. Most strains were also resistant to posaconazole, voriconazole and/or isavuconazole and most (64%) had a combination of tandem repeat and target site mutations, as in other part of the world, although surprisingly, 32% did not manifest any target site mutations. While no azoles were detected in soil from urban areas, resistant *A. fumigatus* could be isolated, reflecting spread of azole-resistant spores via the air.

This remarkably high resistance rate in *A. fumigatus* is mirrored in China – 60% in strawberry fields in Nanjing and Hangzhou (Chen *et al*., 2020) and 80% in greenhouses growing coriander, summer squash, peas, lettuce and fennel in Kunming (Zhou *et al*., 2021). In *A. flavus* strains from the Mekong Delta in Vietnam, 85% were azole resistant and especially linked to aquaculture, an association not previously identified (Duong *et al*., 2020). Prior to 2016 (in Zeijiang), only 3 of 76 (4.1%) were found to be resistant (Ren *et al*., 2017). Antifungal susceptibility testing in *Aspergillus* is not routinely done in this part of the world, so there are few data on the frequency of resistance in clinical isolates. Between August 2012 and July 2015 in Nanjing, 4 of 126 (3.2%) *A. fumigatus* strains were azole resistant (Zhang *et al*., 2017). But overall, the extent of the clinical problem with azole resistance is not clear. In several other countries, environmental strains with azole resistance are just as capable as causing allergic and invasive aspergillosis as susceptible strains, so there is no expectation of a 'loss of fitness' with resistant strains.

There is a much circumstantial evidence linking the agricultural use of several triazole fungicides (prochloraz, difenoconazole, propiconazole, hexaconazole and tebuconazole) with the emergence of azole resistance in *A. fumigatus*. In China, the increasing frequency of azole resistance was closely linked with increasing azole fungicide residues in soil (20% resistant isolates when more than 1000 ng/g fungicide was detected) (Cao *et al*., 2021). Azole fungicide pressure drives azole resistance in *A. fumigatus*. What is not clear is whether removal of that pressure reduces the azole-resistant population sufficiently to be effective. There is a major need to curtail as much as possible non-critical use of azole fungicides in agriculture to minimize azole resistance. This article emphasizes that the time to act is now.

References

Abdolrasouli, A., Rhodes, J., Beale, M.A., Hagen, F., Rogers, T. R., Chowdhary, A., *et al*. (2015) Genomic context of azole resistance mutations in *Aspergillus fumigatus* determined using whole-genome sequencing. *MBio* **6**: e00536–e00515.

Bongomin, F., Gago, S., Oladele, R., and Denning, D. (2017) Global and multi-national prevalence of fungal diseases — estimate precision. *J Fungi* **3**: 57.

Bueid, A., Howard, S.J., Moore, C.B., Richardson, M.D., Harrison, E., Bowyer, P., and Denning, D.W. (2010) Azole antifungal resistance in *Aspergillus fumigatus*: 2008 and 2009. *J Antimicrob Chemother* **65**: 2116–2118.

Buied, A., Moore, C.B., Denning, D.W., and Bowyer, P. (2013) High-level expression of cyp51B in azole-resistant clinical *Aspergillus fumigatus* isolates. *J Antimicrob Chemother* **68**: 512–514.

Buil, J.B., Snelders, E., Denardi, L.B., Melchers, W.J.G., and Verweij, P.E. (2019) Trends in azole resistance in *Aspergillus fumigatus*, The Netherlands, 1994–2016. *Emerg Infect Dis* **25**: 176–178.

Cao, D., Wang, F., Yu, S., Dong, S., Wu, R., Cui, N., *et al*. (2021) Prevalence of azole-resistant *Aspergillus fumigatus* is highly associated with azole fungicide residues in the fields. *Environ Sci Technol* **55**: 3041–3049.

Chen, Y., Dong, F., Zhao, J., Fan, H., Qin, C., Li, R., *et al*. (2020) High azole resistance in *Aspergillus fumigatus* isolates from strawberry fields, China, 2018. *Emerg Infect Dis* **26**: 81–89.

Denning, D.W. (1996) Therapeutic outcome in invasive aspergillosis. *Clin Infect Dis* **23**: 608–615.

Denning, D.W., Venkateswarlu, K., Oakley, K.L., Anderson, M.J., Manning, N.J., Stevens, D.A., *et al*. (1997) Itraconazole resistance in *Aspergillus fumigatus*. *Antimicrob Agents Chemother* **41**: 1364–1368.

Diaz-Guerra, T.M., Mellado, E., Cuenca-Estrella, M., and Rodriguez-Tudela, J.L. (2003) A point mutation in the 14α-sterol demethylase gene cyp51A contributes to itraconazole resistance in *Aspergillus fumigatus*. *Antimicrob Agents Chemother* **47**: 1120–1124.

Duong, T.M.N., Nguyen, P.T., Le, T.V., Nguyen, H.L.P., Nguyen, B.N.T., Nguyen, B.P.T., *et al*. (2020) Drug-resistant *Aspergillus flavus* is highly prevalent in the environment of Vietnam: a new challenge for the management of aspergillosis? *J Fungi* **6**: 296.

Duong, T.M.N., Van, L.T., Linh, T.H.K., Tuyen, N.P., and Phuong, N.T., et al., Unprecedented prevalence of azole-resistant *Aspergillus fumigatus* identified in the environment of Vietnam, with marked variability by land use type.

Fraaije, B., Atkins, S., Hanley, S., Macdonald, A., and Lucas, J. (2020) The multi-fungicide resistance status of

Aspergillus fumigatus populations in arable soils and the wider European environment. *Front Microbiol* **11**: 3199.

Fraczek, M.G., Bromley, M., Buied, A., Moore, C.B., Rajendran, R., Rautemaa, R., *et al*. (2013) The cdr1B efflux transporter is associated with non-cyp51a-mediated itraconazole resistance in *Aspergillus fumigatus*. *J Antimicrob Chemother* **68**: 1486–1496.

Furukawa, T., van Rhijn, N., Fraczek, M., Gsaller, F., Davies, E., Carr, P., *et al*. (2020) The negative cofactor 2 complex is a key regulator of drug resistance in *Aspergillus fumigatus*. *Nat Commun* **11**: 1–16.

Gonzalez-Jimenez, I., Lucio, J., Amich, J., Cuesta, I., Sanchez Arroyo, R., Alcazar-Fuoli, L., and Mellado, E. (2020) A Cyp51B mutation contributes to azole resistance in *Aspergillus fumigatus*. *J Fungi* **6**: 315.

Howard, S.J., Cerar, D., Anderson, M.J., Albarrag, A., Fisher, M.C., Pasqualotto, A.C., *et al*. (2009) Frequency and evolution of azole resistance in *Aspergillus fumigatus* associated with treatment failure. *Emerg Infect Dis* **15**: 1068–1076.

Kosmidis, C., and Denning, D.W. (2015) The clinical spectrum of pulmonary aspergillosis. *Thorax* **70**: 270–277.

Macedo, D., Devoto, T.B., Pola, S., Finquelievich, J.L., Cuestas, M.L., and Garcia-Effron, G. (2020) A novel combination of CYP51A mutations confers pan-azole resistance in *Aspergillus fumigatus*. *Antimicrob Agents Chemother* **64**: e02501–e02519.

Meis, J.F., Chowdhary, A., Rhodes, J.L., Fisher, M.C., and Verweij, P.E. (2016) Clinical implications of globally emerging azole resistance in *Aspergillus fumigatus*. *Philos Trans Roy Soc* **371**: 20150460.

Meneau, I., and Sanglard, D. (2005) Azole and fungicide resistance in clinical and environmental *Aspergillus fumigatus* isolates. *Med Mycol* **43**: S307–S311.

Mosquera, J., and Denning, D. (2002) Azole cross-resistance in *Aspergillus fumigatus*. *Antimicrob Agents Chemother* **46**: 556–557.

Paul, R.A., Rudramurthy, S.M., Dhaliwal, M., Singh, P., Ghosh, A.K., Kaur, H., *et al*. (2018) Magnitude of voriconazole resistance in clinical and environmental isolates of *Aspergillus flavus* and investigation into the role of multidrug efflux pumps. *Antimicrob Agents Chemother* **62**: e01022–e01018.

Ren, J., Jin, X., Zhang, Q., Zheng, Y., Lin, D., and Yu, Y. (2017) Fungicides induced triazole-resistance in *Aspergillus fumigatus* associated with mutations of TR46/Y121F/T289A and its appearance in agricultural fields. *J Hazard Mater* **326**: 54–60.

Resendiz-Sharpe, A., Dewaele, K., Merckx, R., Bustamante, B., Vega-Gomez, M.C., Rolon, M., *et al*. (2021) Triazole-resistance in environmental *Aspergillus fumigatus* in latin American and African countries. *J Fungi* **7**: 292.

Rhodes, J., Abdolrasouli, A., Dunne, K., Sewell, T.R., Zhang, Y., Ballard, E., *et al*. (2021) Tracing patterns of evolution and acquisition of drug resistant *Aspergillus fumigatus* infection from the environment using population genomics. *bioRxiv*.

Schmitt, H., Edwards, F., Andrade, J., Niki, Y., and Armstrong, D. (1992) Comparison of azoles against *Aspergilli* in vitro and in an experimental model of pulmonary aspergillosis. *Chemotherapy* **38**: 118–126.

Slaven, J.W., Anderson, M.J., Sanglard, D., Dixon, G.K., Bille, J., Roberts, I.S., and Denning, D.W. (2002) Increased expression of a novel *Aspergillus fumigatus* ABC transporter gene, atrF, in the presence of itraconazole in an itraconazole resistant clinical isolate. *Fungal Genet Biol* **36**: 199–206.

Snelders, E., van der Lee, H.A.L., Kuijpers, J., Rijs, A.J.M. M., Varga, J., Samson, R.A., *et al*. (2008) Emergence of azole resistance in *Aspergillus fumigatus* and spread of a single resistance mechanism. *PLoS Med* **5**: e219.

Toda, M., Beer, K.D., Kuivila, K.M., Chiller, T.M., and Jackson, B.R. (2021) Trends in agricultural Triazole fungicide use in the United States, 1992–2016 and possible implications for antifungal-resistant fungi in human disease. *Environ Health Perspect* **129**: 055001.

van Rhijn, N., and Bromley, M. (2021) The consequences of our changing environment on life threatening and debilitating fungal diseases in humans. *J Fungi* **7**: 367.

Vandewoude, K., Blot, S.I., Benoit, D., Colardyn, F., and Vogelaers, D. (2004) Invasive aspergillosis in critically ill patients: attributable mortality and excesses in length of ICU stay and ventilator dependence. *J Hospital Infect* **56**: 269–276.

Verweij, P.E., Ananda-Rajah, M., Andes, D., Arendrup, M.C., Brüggemann, R.J., Chowdhary, A., *et al*. (2015) International expert opinion on the management of infection caused by azole-resistant *Aspergillus fumigatus*. *Drug Resist Updat* **21**: 30–40.

Verweij, P.E., Voss, A., and Meis, J.F. (1998) Resistance of *Aspergillus fumigatus* to itraconazole. *Scand J Infect Dis* **30**: 642–642, 643.

Wiederhold, N.P., Gil, V.G., Gutierrez, F., Lindner, J.R., Albataineh, M.T., McCarthy, D.I., *et al*. (2016) First detection of TR34 L98H and TR46 Y121F T289A Cyp51 mutations in *Aspergillus fumigatus* isolates in the United States. *J Clin Microbiol* **54**: 168–171.

Yerbanga, I.W., Resendiz-Sharpe, A., Bamba, S., Lagrou, K., Nakanabo Diallo, S., Rodriguez-Villalobos, H., *et al*. (2021) First investigative study of azole-resistant *Aspergillus fumigatus* in the environment in Burkina Faso. *Int J Environ Res Public Health* **18**: 2250.

Zhang, M., Feng, C.L., Chen, F., He, Q., Su, X., and Shi, Y. (2017) Triazole resistance in *Aspergillus fumigatus* clinical isolates obtained in Nanjing, China. *Chin Med J (Engl)* **130**: 665–668.

Zhou, D., Korfanty, G.A., Mo, M., Wang, R., Li, X., Li, H., *et al*. (2021) Extensive genetic diversity and widespread azole resistance in greenhouse populations of *Aspergillus fumigatus* in Yunnan, China. *mSphere* **6**(1): 1–19.