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DOI
10.1016/j.jgar.2015.01.001

Publication date
2015

Document Version
Final published version

Published in
Journal of Global Antimicrobial Resistance

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Citation for published version (APA):

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Letter to the Editor

De novo induction of resistance against voriconazole in Aspergillus fumigatus

Sir,

The opportunistic pathogen Aspergillus fumigatus causes invasive aspergillosis in patients with reduced immune function [1]. Infections caused by this organism are difficult to treat, particularly in critically ill patients. Therapy prospects become very dismal when the pathogen is resistant to the various azole compounds that are the drugs of choice against this organism.

The same resistance mechanism, designated TR34/L98H, was uncovered in clinical isolates from patients who had not been treated with azoles previously as in A. fumigatus exposed toazole fungicides used for crop protection and material preservation [2]. This observation suggests that the origin of this resistance mechanism may lie in the use of azoles in the environment, since resistant conidia can easily reach the human population transported by air or in any other way. What is not understood at present is whether azole resistance in A. fumigatus can be induced de novo by exposure to sublethal drug concentrations, as was shown for Escherichia coli [3], or whether horizontal transfer of resistance genes is essential. The development of resistance during patient therapy suggests that de novo emergence of resistance is possible [4,5], but since genetic exchange cannot entirely be ruled out, this needs to be proven under conditions that do not allow gene transfer.

An azole-susceptible strain of A. fumigatus, designated AZN 8196, was grown on plates as described previously [2] with sublethal concentrations of voriconazole and itraconazole. Following 7 days of incubation, the strains were transferred to (i) a plate with the same drug concentration and (ii) a plate with a two times higher level. When the plate with the higher drug concentration showed growth within 1 week, the plate with the lower level was discarded and the procedure was repeated. At every transfer, the minimum inhibitory concentration (MIC) of the strain was measured by following growth during 48 h in a set of liquid cultures having drug concentrations stepwise increasing by a factor of 2.

Exposure of A. fumigatus to stepwise increasing concentrations of voriconazole starting at 0.125 μg/mL resulted in a rapid increase of the MIC once the concentration of the drug exceeded the initial MIC (Fig. 1). After 5 weeks, the MIC reached 8 μg/mL, even though the concentration in the plate was only 2 μg/mL. A similar attempt to induce itraconazole resistance was not successful. The MIC for itraconazole went up only by a factor of 2, from 0.125 μg/mL to 0.25 μg/mL. The sharp increase in MIC for voriconazole suggests that a genetic mutation may play a role. This idea was investigated by sequencing PCR products of the cyp51A and cyp51B genes that contained known hotspots forazole resistance in A. fumigatus [5]. The wild-type and voriconazole-resistant sequence were unchanged compared with the published sequences of the cyp51A and cyp51B genes as recorded in GenBank under accession nos.

AF338659 and AF338660, respectively. The only mutations found were in the cyp51B gene of the strains grown on plates that had an itraconazole concentration of 0.25 μg/mL; these were two silent mutations (S274S and P394P). Hence, if any mutations were involved in developing this de novo azole resistance, they were not in the cyp51A and cyp51B genes.

On the one hand it is possible that A. fumigatus adjusts itself by differentially regulating expression levels to induce, for example efflux pumps, as was found for de novo acquisition of antibiotic resistance in E. coli [6]. Even this type of adaptation has lasting effects and is not readily reversed when the drug is no longer present. On the other hand it is equally likely that resistance to voriconazole is caused by different mutations than the well known ones in cyp51A and cyp51B. Regardless, in both cases the main conclusion is that azole resistance in A. fumigatus is readily induced by exposure to sublethal concentrations of these drugs. Use of a single strain may limit the general applicability of this conclusion, but the proof of principle remains valid. Especially in agricultural practice, where the application is often not overly precise, sublethal concentrations might be encountered. It is therefore essential that unnecessary exposure of A. fumigatus to these essential drugs for human medicine is prevented.

Funding

Funding was provided by The Netherlands Food and Consumer Product Safety Authority (Utrecht, The Netherlands).

Competing interests

BHtK is employed by The Netherlands Food and Consumer Product Safety Authority. All other authors declare no competing interests.

http://dx.doi.org/10.1016/j.jgar.2015.01.001
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Ethical approval

Not required.

Acknowledgment

The expert technical assistance of Mr. A. Rijs is greatly appreciated.

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9 June 2014