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Impact of mycotoxin deoxynivalenol on antibiotic resistance induction – a proof-of-concept study#

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Abstract

With nearly 60-80 per cent of global food commodities contaminated with mycotoxins, long-time exposure to mycotoxins is highly likely. In-addition to causing toxic effects in humans and animals these mycotoxins are shown to cause gut-microbiota dysbiosis and increased pathogenic bacterial infection. However, despite their antibiotic properties, until now mycotoxins were usually not-suspected to induce resistance to antibiotics (ABR). To provide a proof-of-concept, we carried out experiments using mycotoxin deoxynivalenol (DON) in both extended-spectrum-beta-lactamase Escherichia coli (ESBLEC) and American-type-culture-collection E. coli (ATCCEC) following standard protocols. In-coherence with the postulate ESBLEC showed induction of ABR against one antibiotic "Piperacillin-Tazobactum" out of 19 tested antibiotics, as compared to control group on day 11. However, in ATCCEC no new resistance was induced. Nevertheless, against 3 antibiotics there was retention of minimal-inhibitory-concentration (MIC) in ATCCEC in different pattern. Further rigorous experiments using more statistically significant models employing different doses and types of mycotoxins in different bacteria would be helpful to definitively establish the role of mycotoxins in ABR.

Keywords: *Mycotoxins, deoxyivalenol (DON), Escherichia coli (E. coli), antibiotic resistance (ABR)*

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Mycotoxins are secondary fungal metabolites, frequently encountered as food contaminants. Mycotoxins contaminate nearly 60-80% of global food commodities (Eskola *et al.*, 2020). Currently over 400 mycotoxins are identified and global survey indicate that among them deoxynivalenol (DON) is most commonly prevalent mycotoxin indicating likely significant lifetime exposure (Rodrigues and Naehrer, 2012). Previous studies have shown that mycotoxins can cause microbial dysbiosis in intestines also shown to increase pathogenic bacterial infection (Baines *et al.*, 2013). Indeed mycotoxins are also known to possess antibiotic activity since decades (Bisht *et al.*, 2011). However, until now mycotoxins were usually not-suspected to induce antibiotic resistance (ABR). Thus, to test this postulate, preliminary trials were conducted.

Following standard protocols (Hong *et al.*, 2012), ABR induction assay was performed using *Escherichia coli (E. coli)* as model organism by culturing in Luria-Bertani (LB) broth. Both American-type-culture-collection *E. coli* (ATCCEC) and extended-spectrum-beta-lactamase positive *E. coli* (ESBLEC) were used. Antibiotic sensitivity (AST) of *E. coli* was determined through MIC (minimal inhibitory concentration, µg/ml), using Vitek 2 system from Biomerieux against a total of 19 antibiotics specified for *E. coli* on day 0. From day 1, test group of bacteria were continuously cultured in presence of DON (10µg/ml) for 10 days and control group without DON using a fresh LB broth each day. The MIC value (µg/ml) for antibiotics (AST values) of bacterial culture was again determined on day 11 (transfer 10) for both the control and test groups.

In our experiments, out of total 19 antibiotics tested, ESBLEC showed induction of ABR against only one antibiotic, "Piperacillin + Tazobactum" as compared to AST results on day 0 of ESBLEC and such induction was not noticed in control group on day 11 (Table 1). This is acceptable as one antibiotic will not induce resistance against all class of antibiotics.

However, ATCCEC, contrary to ESBLEC, showed no ABR induction on day 11,

Table 1: Results of AST (Antibiotic Sensitivity) in ESBL *E. coli* strain showing induction of Antibiotic resistance through MIC (minimal inhibitory concentration, µg/ml)

		Control MIC	Test MIC
Piperaciliin + Tazobactum	Day 0	8	8
	Day 11	8	64

Table 2: Results of AST (Antibiotic Sensitivity) in ATCC *E. coli* strain showing retention of MIC (minimal inhibitory concentration, in µg/ml) in three antibiotics

		Control MIC	Test MIC
Cefixime	Day 0	0.5	0.5
	Day 11	0.25	0.5
	Day 22	0.25	0.5
Amoxicillin + Clavulanic acid	Day 0	4	4
	Day 11	4	4
	Day 22	< 2	4
Ampicillin	Day 0	8	8
	Day 11	4	8
	Day 22	< 2	4

Note: The data for remaining antibiotics, showing negative results are not shown (as there is no difference in AST levels between control and test group).

thus we extended our study in ATCCEC up to day 22. This ATCCEC showed retention of MIC against 3 antibiotics (cefixime, ampicillin and amoxicillin plus clavulanic acid) compared to day 0 levels and as compared to control group without DON exposure. There was slightly different pattern of MIC retention for these 3 antibiotics as presented in Table 2.

To the best of our knowledge, induction of ABR by mycotoxin exposure in bacteria is a novel data that is interesting and alarming simultaneously, yet our results are still proof-of-concept (PoC). Further, though preliminary, our results also provide PoC that even when there is no new induction of ABR, exposure of bacteria to mycotoxins might enhance the retention period of MIC-level against antibiotics. But this needs further rigorous experimental data and more statistically significant study design to establish definitive conclusion. Interestingly, while trying to mitigate mycotoxin-toxicity scientists identified that probiotic enteric bacteria detoxify DON. And, bacteria employ molecular mechanisms that were similar to the molecular processes involved in development of ABR mechanism against antibiotics, that included activation of transport proteins, mycotoxin-specific (DON-specific) deactivation enzymes, regulation of accessory proteins porins, phosphotransferases, etc. (Hassan *et al.*, 2019). This provides a clear plausible mechanistic link for mycotoxin-induced ABR, scientifically supporting our experimental results by substantiating the plausible phenomenon involved in the role of mycotoxins in ABR. Further as *E. coli* is one of the common food-borne pathogen, strains like shiga-toxin producing *E. coli* (STEC) causing life-threatening disease and multi-drug resistant ESBLEC being a common primary pathogens in clinical cases (Naushad *et al.*, 2022). The present results highlight the serious nature of the public health burden of mycotoxins and discovered a previously unidentified effect of mycotoxins.

Summary

Using ESBL *E. coli* and ATCC *E. coli* the study provides PoC for the possible potential of mycotoxin DON in ABR induction and retention of ABR. Further testing of DON with more replicates for statistical significance and investigating induction of ABR by different mycotoxins, in different doses and different combination both in-vitro and in-vivo models in different bacteria (gram positive and negative) would aid in establishing definitive conclusion on this phenomenon. Finally, prospective epidemiological studies (based on exposure and induction) would be more appropriate to add more detailed understanding to the ABR-induction potential of mycotoxins.

Conflict of interest

There is no conflict of interest

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