The Food Contaminant Deoxynivalenol Exacerbates the Genotoxicity of Gut Microbiota

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ABSTRACT
An increasing number of human beings from developed countries are colonized by Escherichia coli strains producing colibactin, a genotoxin suspected to be associated with the development of colorectal cancers. Deoxynivalenol (DON) is the most prevalent mycotoxin that contaminates staple food—especially cereal products—in Europe and North America. This study investigates the effect of the food contaminant DON on the genotoxicity of the E. coli strains producing colibactin. In vitro, intestinal epithelial cells were coexposed to DON and E. coli producing colibactin. In vivo, newborn rats colonized at birth with E. coli producing colibactin were fed a DON-contaminated diet. Intestinal DNA damage was estimated by the phosphorylation of histone H2AX. DON exacerbates the genotoxicity of the E. coli strains producing colibactin in a time- and dose-dependent manner in vitro. Although DON had no effect on the composition of the gut microbiota, and especially on the number of E. coli, a significant increase in DNA damage was observed in intestinal epithelial cells of animals colonized by E. coli strains unable to produce colibactin or animals exposed only to DON. In conclusion, our data demonstrate that the genotoxicity of E. coli strains producing colibactin, increasingly present in the microbiota of asymptomatic human beings, is modulated by the presence of DON in the diet. This raises questions about the synergism between food contaminants and gut microbiota with regard to intestinal carcinogenesis.

IMPORTANCE
An increasing number of human beings from developed countries are colonized by Escherichia coli strains producing colibactin, a genotoxin suspected to be associated with the development of colorectal cancers. Deoxynivalenol (DON) is the most prevalent mycotoxin that contaminates staple food—especially cereal products—in Europe and North America. Our in vitro and in vivo results demonstrate that the intestinal DNA damage induced by colibactin-producing E. coli strains was exacerbated by the presence of DON in the diet. This raises questions about the synergism between food contaminants and gut microbiota with regard to intestinal carcinogenesis.

The gut is colonized by a rich ecological consortium of more than a thousand species of microorganisms that exert marked effects on basic host physiology, immunity, and metabolism (1–3). Escherichia coli bacteria are some of the pioneer bacteria that colonize the guts of mammals within a few days following birth (4). The genetic structure of the E. coli population is clonal and can be segregated into seven major
phylogenetic groups (A, B1, B2, C, D, E, and F) (5). The prevalence of the B2 group is increasing among E. coli strains persisting in the microbiota of humans in developed countries (6, 7). This change in the distribution of phylogenetic groups of the E. coli population could be a consequence of enriched dietary habits and increased levels of hygiene in industrialized countries.

E. coli genomes show evidence of a widespread acquisition of functions through horizontal transfer of genes (8). Up to 50% of E. coli strains from the phylogenetic group B2 have acquired the pks genomic island (9, 10). This gene cluster encodes a nonribosomal peptide synthase-polyketide synthase (NRPS-PKS) assembly line and produces a genotoxic secondary metabolite called colibactin (9). A short contact between mammalian cells and E. coli producing colibactin induces DNA damage, senescence, and chromosomal abnormalities (9, 11–13). Colonization of the gut by phylogroup B2 E. coli producing colibactin is associated with the presence of DNA double-strand breaks in intestinal epithelial cells (14). Phylogroup B2 E. coli bacteria producing colibactin have an impact on host physiology (14, 15) and contribute to the development of colorectal cancer in mouse models of colitis (16, 17). Infants are colonized at birth with B2 E. coli expressing colibactin, and these E. coli strains have a long-term capacity to persist in the bowel microbiota (14, 18). Except for iron availability, little is known about the environmental factors that modulate the genotoxicity of these bacteria in the gut (19).

Mycoxotins are the most frequently occurring natural food contaminants in human and animal diet (20). Of the mycoxotins, deoxynivalenol (DON) is mainly produced by Fusarium graminearum and Fusarium culmorum. This toxin frequently develops in cereals and grains. A survey of 12 European countries indicated that 57% of samples were positive for DON (21). Analyses of urine samples lead to the estimation that 98% of adults in the United Kingdom had been exposed to DON, while 80% of children in the Netherlands exceeded the tolerable daily intake for this mycotoxin (22, 23). DON targets the intestine (24–26) and interacts with the peptidyl transferase region of the 60S ribosomal subunit, inducing a “ribotoxic stress,” resulting in the activation of mitogen-activated protein kinases (MAPKs) and their downstream pathways (27, 28).

The consequence of exposure to this food contaminant on the genotoxic potential of the gut microbiota has never been addressed. In the present study, we investigated the impact of DON on the genotoxicity of E. coli producing colibactin. Using both in vitro and in vivo experiments, we demonstrated that DON exacerbates the intestinal DNA damage induced by genotoxic strains of E. coli.

RESULTS

Genotoxicity of DON and colibactin-producing E. coli on intestinal epithelial cells. The genotoxicity of DON and colibactin-producing E. coli was first examined in cultured rat intestinal epithelial cells (IEC-6) using an in-cell Western (ICW) technique that assessed the phosphorylated form of histone H2AX (γH2AX) as a marker of DNA double-strand breaks. DON alone did not exert detectable genotoxicity on IEC-6 cells, except at high doses (12.5 to 50 μM for at least 8 h) where low levels of γH2AX were observed (2.27 ± 0.22 relative fluorescence units [RFU] in control cells versus 4.64 ± 1.37 and 5.21 ± 1.30 RFU in cells treated with 25 μM and 50 μM DON, respectively [P < 0.05 and P < 0.001, respectively]) (Fig. 1A; see Fig. S1A and S2A in the supplemental material). In contrast to DON-treated cells, IEC-6 cells were highly susceptible to the genotoxicity of colibactin-producing E. coli (colibactin-producing wild-type E. coli [E. coli WT]). As shown in Fig. 1B, the infection of IEC-6 cells with E. coli WT induced a dose-dependent increase in γH2AX (2.27 ± 0.22 RFU in control cells versus 9.1 ± 3.92 RFU [P < 0.05] in E. coli WT with a multiplicity of infection [MOI] of 25 and 29.03 ± 8.19 RFU [P < 0.001] in E. coli WT with an MOI of 100 compared to noninfected cells).

DON exacerbates the genotoxicity of colibactin-producing E. coli. To determine whether the genotoxic effect of colibactin can be modulated by exposure to the food contaminant DON, IEC-6 cells were infected with a low dose of E. coli WT (MOI of 25) and coexposed to DON (25 μM). Exposure of cells to DON exacerbated the genotoxicity of E. coli producing colibactin on IEC-6 cells compared to cells exposed solely to DON.
FIG 1  Quantification of DNA double-strand breaks in intestinal epithelial cells in vitro. (A) IEC-6 cells were treated with increasing doses of DON (0 to 50 μM) for 8 h, and γH2AX was quantified by an in-cell Western (ICW) method. Untreated cells (white bar) and cells exposed to DON (dotted white bars) are indicated. (B) IEC-6 cells were infected with wild-type E. coli producing colibactin (E. coli WT) for 4 h with increasing multiplicities of infection (MOIs [m.o.i. in the figure] of 0 to 100 bacteria per cell) before ICW analysis 4 h after infection (black bars). Mean values plus standard errors of the means (SEM) (error bars) from two independent experiments are shown. Values that are significantly different by one-way ANOVA with Bonferroni’s multiple-comparison correction are indicated by asterisks as follows: *, P < 0.05; **, P < 0.01; ***, P < 0.001.

FIG 2  Dose-dependent synergistic genotoxicity of DON and E. coli. (A) IEC-6 cells infected for 4 h with E. coli strains producing colibactin (E. coli WT) or not producing colibactin (E. coli ΔclbA and E. coli ΔclbP) (MOI of 25) and coexposed to 25 μM DON 8 h before quantification of γH2AX by in-cell Western (ICW). (B) ICW pictures of IEC-6 cells exposed or not exposed to 25 μM DON and infected (MOI of 25) with E. coli strains producing colibactin (E. coli WT) or not producing colibactin (E. coli ΔclbA or ΔclbP). DNA is artificially colored red, and γH2AX is shown in green. N.I., not infected. (C) IEC-6 cells infected for 4 h with E. coli WT or not infected with E. coli and treated with increasing doses of DON (0 to 50 μM) 8 h before ICW. Control (not treated) cells (white bars) and cells infected with E. coli WT (black bars), E. coli ΔclbP (light gray bars), and E. coli ΔclbA (dark gray bars) are shown. Dotted white, black, light gray, and dark gray bars represent cells exposed to DON and coinfected with different E. coli strains (dotted black, WT; dotted light gray, ΔclbP; dotted dark gray, ΔclbA) or not coinfected with E. coli strains (dotted white). Mean values plus SEM from three independent experiments are shown. Values that are significantly different from the values for cells infected with colibactin-producing E. coli and exposed to DON with all other groups by one-way ANOVA with Bonferroni’s multiple-comparison correction are indicated by asterisks as follows: *, P < 0.05; **, P < 0.01; ***, P < 0.001.
or infected solely by *E. coli* WT as demonstrated by the expression of γH2AX by an in-cell Western method (Fig. 2A and B) and Western blotting (data not shown). This exacerbation was dependent on the dose of DON (Fig. 2C) and was observed only when DON was present for 8 h during and after infection (Fig. S2B). The DNA double-strand breaks in intestinal cells was confirmed by the expression and quantification of p53 binding protein 1 (53BP1 [Fig. S3]) and was associated with an increased phosphorylation of the MAP kinase extracellular signal-regulated kinase 1 or 2 (ERK1/2) (data not shown).

Using two independent *E. coli* mutants (*E. coli* ΔclbA and *E. coli* ΔclbP) that did not produce colibactin, we verified that the genotoxicity observed in the infected cells depended specifically on the production of colibactin. As shown in Fig. 2A, no genotoxicity was observed in IEC-6 cells infected with the mutants and exposed to DON. To verify that DON did not modulate the physiology or gene expression in *E. coli*, the effects of DON on bacterial growth and expression of the *pks* island genes were evaluated. Treatment of *E. coli* with increasing doses of DON for 24 h had no effect on bacterial growth, nor did it modify the expression of genes of the *pks* island coding for enzymes required for the production of colibactin (data not shown).

**Contamination of the diet with DON does not impair the colonization of the gut by *E. coli* strains producing colibactin or not producing colibactin.** The next aim was to assess whether DON could exacerbate the genotoxicity exerted by colibactin-producing *E. coli* present in the gut microbiota. To this end, animals were colonized at birth with *E. coli* WT or *E. coli* ΔclbA and *E. coli* ΔclbP mutants. After weaning, the rats were fed for 4 weeks with a diet contaminated with DON or not contaminated with DON (Fig. S1B). We first examined the effect of DON on the colonization of the gut by *E. coli* and more generally on the microbiota. Ingestion of a DON-contaminated diet from weaning to adulthood did not modify the levels of enterobacteria in the feces.
compared to those in animals fed a normal diet or the fecal E. coli counts (Fig. 3A and B). A 16S microbiota analysis indicated that ingestion of the DON-contaminated diet did not significantly alter the composition or diversity of the gut microbiota of rats colonized at birth with E. coli strains producing colibactin or not producing colibactin or not colonized by E. coli and coexposed to a DON-contaminated diet (10 mg · kg⁻¹) or not coexposed to a DON-contaminated diet. Mean values plus SEM are shown (n = 8 to 10). Values for animals fed a DON-contaminated diet (10 mg · kg⁻¹) that are significantly different for the values for animals fed a control diet by one-way ANOVA with Bonferroni’s multiple-comparison correction are indicated by symbols as follows: * and $, P < 0.05; ## and **, P < 0.01; *** and ⚫ ⚫ ⚫, P < 0.0001. Values for control animals (*), animals colonized with E. coli WT (#), and animals colonized with the E. coli ΔclbP mutant (¤) or with the E. coli ΔclbA mutant (●) are indicated.

FIG 4 Exposure to DON reduces body weight gain and alters the jejunal tissue in adult animals independently of the colonizing E. coli strain. (A) Body weight gain was evaluated in the progeny from days 0 to 28. (B and C) Histological score (B) and villus length (C) in the jejunum of rats colonized since birth by E. coli strains producing colibactin or not producing colibactin or not colonized by E. coli and coexposed to a DON-contaminated diet (10 mg · kg⁻¹) or not coexposed to a DON-contaminated diet. Mean values plus SEM are shown (n = 8 to 10). Values for animals fed a DON-contaminated diet (10 mg · kg⁻¹) that are significantly different for the values for animals fed a control diet by one-way ANOVA with Bonferroni’s multiple-comparison correction are indicated by symbols as follows: * and $, P < 0.05; ## and **, P < 0.01; *** and ⚫ ⚫ ⚫, P < 0.0001. Values for control animals (*), animals colonized with E. coli WT (#), and animals colonized with the E. coli ΔclbP mutant (¤) or with the E. coli ΔclbA mutant (●) are indicated.

Exposure to DON reduces body weight gain and induces intestinal modifications in adult rats, independently of neonatal colonization by E. coli strains producing colibactin or not producing colibactin. As expected, ingestion of the DON-contaminated diet (10 mg · kg⁻¹) significantly decreased body weight compared to animals fed a control diet (Fig. 4A). In addition, animal weight decreased independently of neonatal colonization with the E. coli strain producing colibactin or not producing colibactin. Similarly, as previously observed, in the jejunum of animals fed the DON-contaminated diet, increased histological alterations were observed, demonstrating moderate intestinal lesions and breakdown (Fig. 4B). A decreased villus height in the jejunum was also observed in these animals (Fig. 4C). These histological and morphological modifications occurred independently of colonization of animals by E. coli strains producing colibactin or not producing colibactin (Fig. 4B and C). These results suggest that colibactin-producing strains did not impact the classical effects of DON in relation to weight gain and morphology of the gut.

Exposure to DON exacerbates the intestinal DNA damage induced by colibactin-producing E. coli in a dose- and time-dependent manner. The in vivo effect of a diet contaminated with DON and colibactin-producing E. coli on intestinal DNA damage was evaluated next. To this end, jejunal sections from animals colonized
at birth with *E. coli* producing colibactin or not producing colibactin that were fed a control diet or a DON-contaminated diet for 4 weeks were stained for the phosphor-
ylated form of H2AX. Dietary exposure of animals to 10 mg · kg$^{-1}$ DON alone did not
induce detectable DNA damage in intestinal epithelial cells (Fig. 5A, dotted white bar,
and B). Similarly, adult rats colonized with *E. coli* strains producing colibactin and fed
the control diet did not exhibit significant intestinal DNA damage (Fig. 5A, black bar).
However, in animals colonized with *E. coli* WT and fed a diet contaminated with DON
(10 mg · kg$^{-1}$), a significant increase in γH2AX-positive cells was observed
compared to rats exposed to DON or rats colonized with *E. coli* WT (Fig. 5A, dotted black
bar, and B). As observed in vitro, no genotoxicity was observed in animals fed a
DON-contaminated diet and colonized with non-colibactin-producing mutants (*E. coli*
ΔclbA and *E. coli* ΔclbP) (Fig. 5A and B). The increase in DNA double-strand breaks
observed in intestinal epithelial cells (IECs) of animals colonized since birth by
colibactin-producing *E. coli* and exposed to a DON-contaminated diet was associated
with a significant increase in activation of phosphorylated MAP kinase ERK p42/p44 (data
not shown).

We next investigated the effect of the dose or duration of DON exposure on the
genotoxic effect of colibactin. We first assessed the effect of the dose of DON on DNA
damage in control animals or animals colonized with *E. coli* WT and fed diets contam-
inated with 2 or 10 mg · kg$^{-1}$ DON (Fig. 6A). In rats exposed for 4 weeks to the lowest
dose of DON contamination, exacerbation of DNA damage induced by the genotoxic
*E. coli* strain was already observed (Fig. 6A). This effect was significantly less than the
one observed at 10 mg · kg$^{-1}$ DON. We then determined the minimal duration of DON
exposure required to exacerbate the genotoxic effect of colibactin. A significant
increase in DNA damage in intestinal epithelial cells of rats colonized with *E. coli* WT was
observed from 2-week exposure to 10 mg · kg$^{-1}$ DON, and DNA damage was increased

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**FIG 5** DON exacerbates DNA damage in jejunal epithelial cells of animals colonized by colibactin-
producing *E. coli*. Immunofluorescence analysis of the jejunal epithelium of adults (PND 58) colonized
since birth by *E. coli* strains (*E. coli* WT, *E. coli* ΔclbA, or *E. coli* ΔclbP strain) or treated with PBS (control
group) and coexposed to a DON-contaminated diet (10 mg · kg$^{-1}$) for 4 weeks or not coexposed to a
DON-contaminated diet for 4 weeks. (A) Quantification of the percentage of γH2AX-positive cells in
jejunal crypts. Mean values plus or minus SEM are shown (n = 8 to 10). Values that are significantly different (P <
0.0001) by one-way ANOVA with Bonferroni’s multiple-comparison correction are indicated by four
asterisks. (B) Representative jejunal frozen sections at PND 58. DNA was stained in blue. γH2AX foci are
shown in green. Bars = 10 μM.
after a 4-week exposure to the food contaminant (Fig. 6B). Taken together, these results indicate that ingestion of a DON-contaminated diet exacerbates the intestinal DNA damage induced by colibactin-producing \textit{E. coli} in a dose- and time-dependent manner.

**DISCUSSION**

In the present study, we demonstrated that DON exacerbates the DNA damage induced by \textit{E. coli} producing colibactin both \textit{in vitro} on cultured intestinal epithelial cells and \textit{in vivo} in animals colonized with colibactin-producing \textit{E. coli} and fed DON-contaminated diets.

Several long-term studies showed that DON is not a carcinogenic compound (29, 30); consequently, this mycotoxin has been classified in group 3 ("not classifiable as to its carcinogenicity to humans") by the World Health Organization (WHO) International Agency for Research on Cancer (IARC). In the present study, DON, used at realistic levels (31, 32), did not induce detectable DNA damage in the intestine. \textit{In vitro}, genotoxicity was observed only upon exposure to very high nonrealistic levels of DON. On the other hand, our \textit{in vitro} and \textit{in vivo} data demonstrated that DON exacerbates the genotoxicity induced by colibactin-producing \textit{E. coli}. This raises questions about the synergism between food contaminants and gut microbiota with regard to intestinal carcinogenesis.

There is overwhelming evidence that DON induces a systemic and intestinal inflammatory response at both the systemic and intestinal levels (33–35). Through this inflammatory effect, DON may predispose the gut epithelium to DNA damage. Indeed, the genotoxic effect of colibactin requires bacterium-host cell contact (9). By decreasing protective mucins and antimicrobial peptide production (36, 37), the inflammation induced by DON could also create an environment in which colibactin-producing \textit{E. coli} bacteria more readily access the epithelium and express their genotoxic potential. This hypothesis has already been proposed to explain the genotoxicity and tumorigenicity of colibactin-producing \textit{E. coli} in azoxymethane-treated IL-10−/− mice (16). Likewise, numerous studies demonstrated that DON induces oxidative stress (38); it stimulates the production of reactive oxygen species (ROS) (39) but has no effect on the production of nitric oxide (40). The rapid generation of ROS was proposed as one of the mechanisms for DNA damage in hepatocytes and lymphocytes exposed to high doses of toxin (41, 42). Secher et al. demonstrated that colibactin-producing \textit{E. coli} strains also trigger the production of intracellular and mitochondrial ROS in infected cells (12), and...
a recent study shows, in jejunal explants exposed to DON, an increase in the production of COX-2 in the tissue (43). The induction of production of ROS by the mycotoxin and bacteria could explain the exacerbated DNA double-strand breaks in intestinal epithelial cells exposed to both stressors. Finally, DON is also known to activate MAPKs through the phosphorylation of protein kinase R (PKR) (44–46). Recent data suggest that PKR promotes genomic instability by inhibiting DNA damage response signaling and double-strand break repair (47). Increased expression of PKR has also been reported in patients with colon cancer (48). Thus, DON-induced phosphorylation of PKR may exacerbate the genotoxicity induced by colibactin and explain the observed synergism between colibactin and DON. Indeed, in the present study, an increased activation of a MAP kinase was observed in animals colonized by colibactin-producing E. coli and exposed to DON.

The prevalence of the specific phylogenetic B2 group, which encompasses E. coli strains producing colibactin, is increasing among E. coli strains persisting in the microbiota of humans from developed countries (6). DON is the most prevalent fungal toxin present in the food chain in Europe and North America (49, 50). The worldwide incidence of trichothecene contamination and especially of DON has increased in the last years because of climate change, increased use of no-till farming to prevent soil erosion, nonoptimal crop rotations, and inadequate fungicide treatments (51). Since DON is resistant to milling, processing, and heating, this mycotoxin remains present in final food products, such as bread and pasta, obtained from contaminated grain (52).

The DON concentrations tested in this study are in accordance with the levels plausibly encountered in the gut after consumption of contaminated food (32). A large percentage of the human population can be exposed to both factors. In conclusion, our results demonstrate that DON exacerbates the genotoxicity of colibactin. Food contaminants and microbial factors act together to impact host physiology and especially intestinal epithelial cells. This finding raises questions about the interaction between food contaminants and gut microbiota in intestinal carcinogenesis and underlines that the impact of food contaminants, especially mycotoxins, must be evaluated together with the host microbiota.

MATERIALS AND METHODS

Bacterial strains and toxins. E. coli bacterial strains (E. coli strain M1/5 [14]), bacterial growth conditions, and the use of toxins used in this study are listed in Text S1 and Table S1 in the supplemental material. Purified DON was purchased from Sigma-Aldrich (Saint-Quentin Fallavier, France).

Cell culture. Nontransformed rat intestinal epithelial IEC-6 cells (ATCC CRL-1592) were cultured as described before (14). Experimental procedures were described in Text S1 and Fig. S1A. At the end of the treatments, in-cell Western (ICW) procedure and immunofluorescence analysis were performed to analyze DNA damage via phosphorylation of the histone H2AX.

The ICW procedure was performed as previously described (53). The cells were fixed, permeabilized, blocked, and then incubated overnight (ON) with rabbit monoclonal anti-H2AX 1/200 (20E3; Cell Signaling, Saint-Quentin en Yvelines, France). An infrared fluorescent secondary antibody (IRDye 800CW; Rockland) (1/500) was applied simultaneously with RedDot2 (1/500) (Biotium, Interchim, Montluçon, France) for DNA labeling. The DNA and γH2AX were visualized using an Odyssey infrared imaging scanner (LI-COR Science Tec, Les Ulis, France). All experiments were carried out in triplicate.

Experimental animal model. Pregnant Wistar female rats (obtained from Janvier Labs, Le Genest Saint-Isle, France) were treated with streptomycin (5 g/liter) and inoculated twice with 109 bacteria by intragastric gavage ([E. coli strain M1/5]). Animals were given noncontaminated control food or a DON-contaminated diet (10 mg or 2 mg of DON kg of body weight–1) for 1 to 4 weeks (Fig. S1B and Text S1).

Colonic bacterial load and 16S microbiota analysis. The colonic bacterial load in feces was analyzed before exposure to a DON-contaminated diet at postnatal day 28 (PND 28) and upon completion of the experiment (PND 58). For 16S microbiota analysis, feces were taken at the end of the experiment. Total DNA was isolated from the individual fecal contents using the QiAamp DNA stool minikit (Qiagen, Courtaboeuf, France) (Text S1).

Histological analysis. The jejunum fixed in 10% buffered formalin were dehydrated and embedded in paraffin in accordance with standard histological procedures. Sections (5 μm) were stained with hematoxylin–eosin for histopathological evaluation and intestinal morphometry. A lesion score was designed to compare histological changes (44). Images were acquired with a Leica DMRB microscope. Analyses were performed using a Motic Image Plus 2.0 image analysis system.

Immunofluorescence analysis. Jejunal samples were immediately placed in optimum-cutting-temperature (OCT; Sakura) compound and snap-frozen at −80°C (14). Sections (5 μm) were fixed, permeabilized, blocked with phosphate-buffered saline (PBS) containing 0.1% Tween 20 and 5% normal
DON Exacerbates DNA Damage Induced by Colibactin

goat serum (NGS) and stained with rabbit anti-phospho-H2AX antibody (Cell Signaling) followed by Alexa Fluor 546-labeled goat anti-rabbit antibodies (Invitrogen). Slides were mounted in Vectashield containing 4',6-diamidino-2-phenylindole (DAPI) (Vector Laboratories). Images were acquired with an Apotome (Zeiss Inc.). γH2AX-positive cells were counted and expressed as a percentage of total epithelial cells. One investigator, blind to the treatment, analyzed all slides.

Statistical analysis. All statistical analyses were performed using GraphPad Prism 4.0. The differences between the experimental groups were evaluated using one-way analysis of variance (ANOVA) followed by Bonferroni posttest (which allows for the comparison of all group pairs). All the data were expressed as means ± standard errors of the means (SEM). A P value below 0.05 was considered significant.

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at https://doi.org/10.1128/mBio.00007-17.

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