


SHORT REPORT

Oral application of vancomycin alters murine lung microbiome and pulmonary immune responses

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Abstract

Early life exposures to antibiotics negatively impact respiratory health and are associated with an increased risk of childhood asthma. It is explained that the lung is inclined to develop chronic inflammatory phenotypes due to early antibiotic alteration in the gut microbiome. We investigated whether a gut-targeted antibiotic has an impact on the lung microbiome and on pulmonary immunity. Fourteen-day old C57BL/6 mice were administered with vancomycin via oral gavage for 3 days (1 time/day). Control groups were treated with clarithromycin and phosphate-buffered saline (PBS), respectively. Five days after treatment, the cecum and lung microbiome, and pulmonary immune response were analyzed. Vancomycin treatment decreased the relative abundance of the genera *Clostridium* XIVa and *Alistipes* and the family *Lachnospiraceae* in the cecum. Furthermore, the relative abundance of the family *Parabacteroidetes* and the genus *Lactobacillus* were increased, whereas the abundance of the phylum Firmicutes was decreased. In the lung, vancomycin treatment reduced bacteria belonging to *Clostridium* XIVa and the family *Lachnospiraceae* as compared to those in the clarithromycin treated

Abbreviations: BALF, bronchoalveolar lavage fluid; CLA, clarithromycin; IFN- γ , interferon-gamma; IL, interleukin; OVA, ovalbumin; PBS, phosphate-buffered saline; Th2, T helper cell type 2; TNF- α , tumor necrosis factor alpha; VAN, vancomycin.

Stefan Pfeiffer, Gregor Jatzlauk, Joni V. Lund, Michael Schloter, and Susanne Krauss-Etschmann contributed equally to this study.

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group. Lung cells from the vancomycin-treated mice released higher levels of interleukin (IL)-4 and IL-13 compared to those from the PBS group, and increased levels of IL-6, IFN- γ , and TNF α compared to lung cells from the clarithromycin and PBS treated mice. Our pilot study suggests that alteration in the gut microbiome could affect bacterial composition and immunity of the lung hence proposes a gut–lung microbiome axis in early life.

KEYWORDS

early life antibiotics, gut microbiome, gut–lung axis, lung inflammation, lung microbiome

1 | INTRODUCTION

A repertoire of research studies associates exposure to antibiotics in early life with the development of allergy and asthma in childhood. Antibiotics contribute to fluctuations in the diversity of microbial communities in the host. It is discussed that the gut microbiome undergoes dynamic changes in the first 3 years of life until a more stable and diverse community is established.¹ This microbial colonization is suggested to influence the maturation and functional development of immune cells.^{2,3} These reports potentially underscore the notion that intermittent modulation of the gut microbiome with antibiotics in early life could be detrimental to the immune system. In line with this, an earlier study demonstrated that oral vancomycin (VAN) use during pregnancy followed by early postnatal treatment reduced the gut microbial diversity in infant mice, and further aggravated the severity of ovalbumin (OVA)-induced experimental asthma.^{4,5} Indeed, there is evidence that dysbiosis in the gut microbiome is a trigger for respiratory diseases. To add to what is already known, we investigated if the gut–lung microbiome axis⁶ prevails in early life and influences immune cell response. We treated mice with VAN, an antibiotic not absorbed in the gut, or clarithromycin (CLA), a macrolide that is resorbed from the gut for systemic distribution. In general, we proposed that a gut–lung microbiome axis in infants could be one contributing factor reinforcing risks to chronic inflammatory diseases later in life.

2 | RESULTS AND DISCUSSION

In this study, we applied the locally acting VAN via oral gavage to 14-day-old C57BL/6 male and female mice for 3 days and analyzed the gut and lung microbiome as well as pulmonary immune cells 5 days after the last antibiotic treatment (Figure 1A). As controls, we treated one group of infant mice with CLA and another group

with phosphate-buffered saline (PBS). Care was taken to avoid gavage-related refluxes and esophageal trauma during orogastric application of VAN, CLA, and PBS.

As expected, compared to PBS, both VAN and CLA administration significantly changed bacterial communities in murine ceca with regard to β -diversity (Figure 1B) and their richness (Supporting Information: Figure S1A), although VAN showed a stronger decrease in bacterial richness than CLA. Further, we assessed the impact of the antibiotics on bacterial genera (Supporting Information: Figure S2A) and made a compilation of the top-ranked bacteria (Supporting Information: Table 1) in response to the treatments. Compared to both control groups, VAN significantly reduced the relative abundance of bacteria belonging to the genera *Clostridium* XIVa and *Alistipes* as well as the family *Lachnospiraceae*, whereas the relative abundance of the family *Parabacteroidetes* and the genus *Lactobacillus* were significantly increased in the cecum (Figure 1C–G). To confirm our observations, we analyzed the impact of antibiotic treatments on the phylum level. Compared to PBS and CLA, VAN significantly reduced the relative abundance of the phyla Firmicutes (Figure 1H), under which the genus *Clostridium* XIVa and the family *Lachnospiraceae* are classified. In contrast, the phyla Bacteroidetes and Proteobacteria, and the class Bacilli, followed opposite trends (Figure 1I–K). Moreover, Figure 1L shows a 16S dendrogram describing the phylogenetic distance between samples in terms of relatedness and abundance.

VAN is not absorbed in the gut, and for this reason, we assessed whether the antibiotic could alter the lung microbiome. We speculated that changes in the lung microbiome may trigger cellular responses which could influence respiratory health in accordance with the findings by Russell et al. Surprisingly, while there was no change in bacterial richness in the lung (Supporting Information: Figure S3A), the beta diversity analyses (Figure 2A) followed the same trend as observed in the cecum (Figure 1B). Furthermore, based on the top-ranked bacteria in the murine lung (Supporting

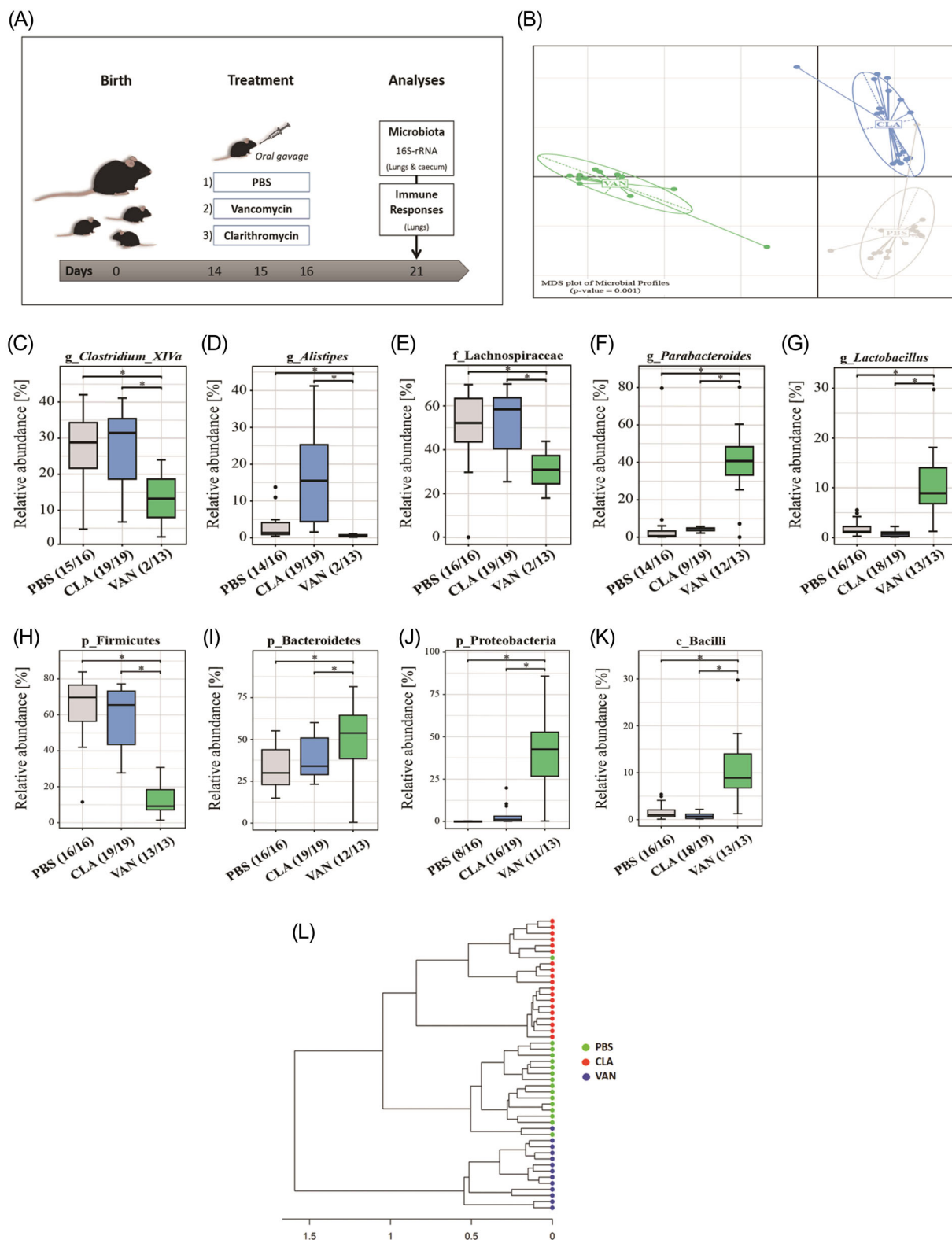


FIGURE 1 (A) Overview of experimental model. Analysis of gut microbiota (cecal samples): (B) Unconstrained nMDS plot of generalized UniFrac distances; (C–K) impact of antibiotics on particular bacterial taxa. (L) 16S dendrogram (Ward's clustering method) based on generalized UniFrac distances. PBS ($n = 16$), CLA ($n = 19$), and VAN ($n = 13$). g (genus), f (family), p (phylum), c (class). * $p < .05$ indicates significant differences. CLA, clarithromycin; nMDS, nonmetric multidimensional scaling; PBS, phosphate-buffered saline; VAN, vancomycin.

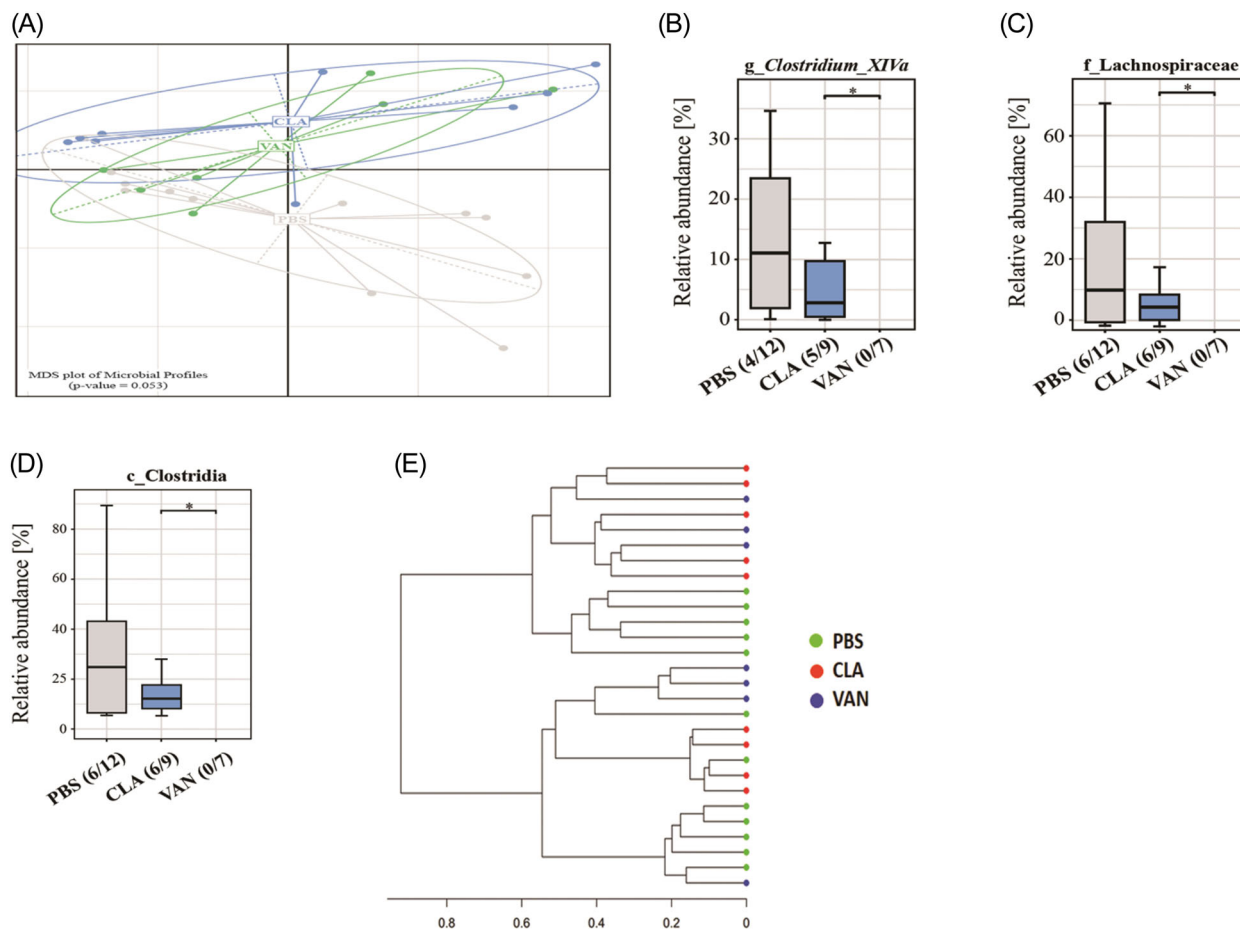


FIGURE 2 Analysis of lung microbiota. (A) Unconstrained nMDS plot of generalized UniFrac distances; (B–D) impact of antibiotics on some specific lung bacterial taxa. (E) 16S dendrogram (Ward's clustering method) based on generalized UniFrac distances. PBS ($n = 12$), CLA ($n = 9$), and VAN ($n = 7$). Numbers in brackets below the graphs show the number of samples within which a certain taxon was detected (e.g., PBS [4/12] means the taxon was detected in 4 samples out of 12). * $p < .05$ indicates significant differences. CLA, clarithromycin; nMDS, nonmetric multidimensional scaling; PBS, phosphate-buffered saline; VAN, vancomycin.

Information: Figure S2B and Table S2) VAN treatment induced a significant decrease in bacteria belonging to *Clostridium XIVa* and the family *Lachnospiraceae* as compared to CLA-treated mice (Figures 2B,C). Additionally, the relative abundance of Clostridia was significantly reduced in VAN-treated mice compared to those in the CLA group only (Figure 2D). The phylogenetic distance between samples in terms of relatedness and abundance is indicated with a 16S dendrogram in Figure 2E.

We next assessed whether the observed changes in bacterial diversity correspond with immune modulation in the lung. To do this, total cell count in bronchoalveolar lavage fluid (BALF) and T cell populations in single-cell suspensions from the lungs were analyzed. Using the flow cytometry gating strategy in Supporting Information: Figure S4, we noticed a remarkable increase in CD4+ T cells (Figure 3A) and a slight increase in CD8+ T cells (Figure 3B) in the VAN group

compared to the CLA and PBS treated mice. Further, we cultured cells isolated from the lungs and stimulated them via CD3/CD28 to investigate the levels of some selected cytokines released by the cells (Figure 3 and Supporting Information: Figure S5). The present data showed an increase in Th2-associated cytokines interleukin (IL)-4 and IL-13 in cells isolated from the VAN-treated mice compared to those from the PBS-administered mice (Figure 3C,D). Significant amounts of pro-inflammatory IL-6, IFN- γ , and TNF α , were also released by the cells from the VAN group compared to those isolated from the CLA and PBS groups (Figure 3E–G). Overall, our findings implicate an alteration of the pulmonary immune development/responses in the lungs of mice treated with VAN in early life. This is characterized by a mixed pro-inflammatory phenotype shown by increased T cell counts and not only Th1 but surprisingly common Th2 cytokines (IL-4, IL-13). These changes in immune

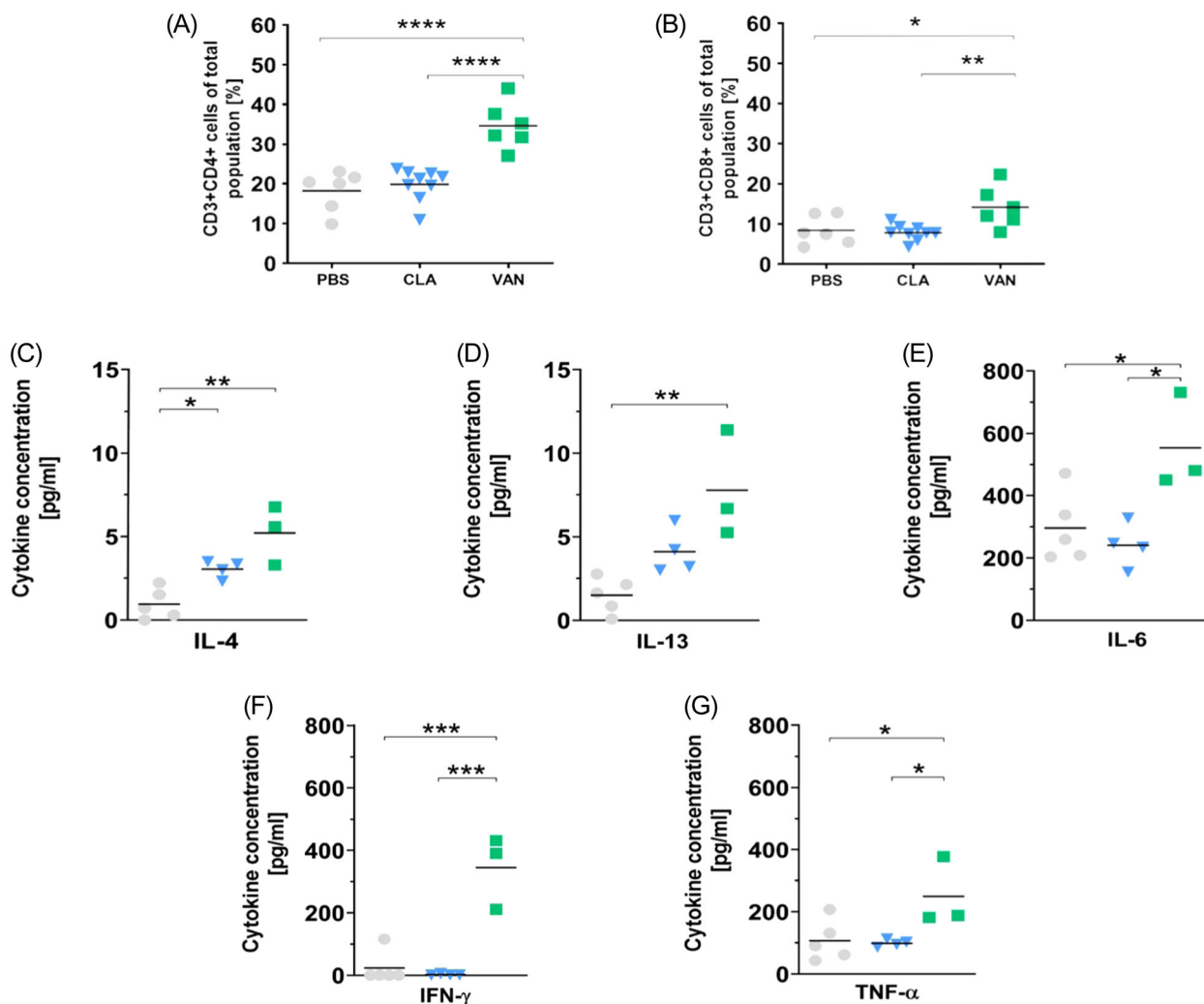


FIGURE 3 (A, B) T cell population in the lungs. n for PBS, CLA, and VAN are 6, 9, and 6, respectively. (C–G) cytokines in the supernatant of CD3/CD28 stimulated cell cultures of lung cells (The scales differ according to the variable abundance of the cytokines). $n = 5$ (PBS), $n = 4$ (CLA), $n = 3$ (VAN), and each n represents pooled samples. * $p < .05$, ** $p < .01$, *** $p < .001$, and **** $p < .0001$ represent significant differences. CLA, clarithromycin; PBS, phosphate-buffered saline; VAN, vancomycin.

response may support the idea that an antibiotic-induced shift in the gut microbiota could provoke Th2 hyperreactivity to trigger the development of atopic diseases later in life.

3 | CONCLUDING REMARKS

In brief, orally administered VAN acts locally in the gut, however, simultaneous trends in the lung microbial community probably suggest systemic adaptability to treatment. We understand that the translocation of bacteria or bacterial fragments,⁷ release of bacterial metabolites, for example, short-chain fatty acids,⁸ and the trafficking of immune cells from the gut to the lung⁹ are possible explanations for the observations in our study. Moreover, we speculate a gut–lung axis to that

effect. In addition to what was previously explained,⁶ here, the gut microbial changes may have induced moderate alterations in the lung environment or comparatively, showed similar trends as observed in the lung—pointing to a possible gut–lung–microbiome axis. This raises the question of the extent to which microbes at different body sites connect with each other and how a local antibiotic treatment could affect other microbial communities. Further studies are therefore needed for sufficient evidence and clarity on the concept before probing into the mechanistic aspect. Taken together, our pilot study suggests that changes in the gut microbiome could have consequences on the bacterial composition and immunity of the lung and this may contribute to the reported increase in asthma and allergies in individuals given antibiotics in the first years of life.

AUTHOR CONTRIBUTIONS

Conceptualization: Susanne Krauss-Etschmann, Sabine Bartel, and Michael Schloter. *Methodology:* Stefan Pfeiffer and Gregor Jatzlauk. *Formal analysis and investigation:* Stefan Pfeiffer, Gregor Jatzlauk, Joni V. Lund, and Draginja Kovacevic; *Resources:* Susanne Krauss-Etschmann. *Data curation:* Gregor Jatzlauk, Joni V. Lund, Draginja Kovacevic, Machteld N. Hylkema, Michael Schloter, and Susanne Krauss-Etschmann. *Writing—original draft preparation:* Susanne Krauss-Etschmann, Stefan Pfeiffer, Gregor Jatzlauk, Joni V. Lund, and Eistine Boateng. *Writing—review and editing:* Susanne Krauss-Etschmann, Stefan Pfeiffer, Gregor Jatzlauk, Joni V. Lund, Eistine Boateng, Draginja Kovacevic, Machteld N. Hylkema, Sabine Bartel, and Michael Schloter. *Visualization:* Draginja Kovacevic. *Supervision:* Susanne Krauss-Etschmann and Michael Schloter. *Funding acquisition:* Susanne Krauss-Etschmann and Michael Schloter. All authors have read and agreed to the published version of the manuscript.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest except for Sabine Bartel who reports grants and personal fees from Bencard Allergie GmbH which had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

DATA AVAILABILITY STATEMENT

Sequences are currently in the process of submission to the NCBI Sequence Read Archive.

ETHICS STATEMENT

The animal study protocol was approved by the Institutional Review Board of the Government of the District of Schleswig—Holstein (V 244—14538/2016 (10-1/16)).

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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