Effects of oral antibiotics and isotretinoin on the murine gut microbiota

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ABSTRACT

Inflammatory bowel disease (IBD) may develop due to an immunogenic response to commensal gut microbiota triggered by environmental factors in the genetically susceptible host. Isotretinoin, applied in the treatment of severe acne, has been variably associated with IBD, but prior treatment with antibiotics, also associated with IBD development, confounds confirmation of this association. This study investigated the effects of doxycycline, metronidazole (frequently used in the treatment of acne and IBD, respectively) and isotretinoin on murine gut (faecal) microbiota after 2 weeks of treatment and after a 4-week recovery period. Faecal microbiota composition was assessed by 16S rRNA gene sequencing on the GS-FLX 454 platform with primers directed against the variable regions V1–V2. Doxycycline had a modest effect on bacterial richness and evenness, but had pronounced persistent and significant effects on the abundance of certain operational taxonomic units compared with the control group. In contrast, metronidazole induced a pronounced reduction in diversity after treatment, but these effects did not persist after the recovery period. This study demonstrates differential effects of antibiotics on the gut microbiota with doxycycline, unlike metronidazole, mediating long-term changes in the murine gut microbiota. Isotretinoin had no significant effect on the faecal microbiota.

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1. Introduction

The microbiota of the gastrointestinal tract has a profound influence on host physiology and nutrition, including protection of the epithelial cell barrier [1] and regulation of host fat storage [2]. Associations between alterations in gut microbiota composition and a wide variety of pathological conditions including inflammatory bowel disease (IBD), obesity and associated insulin resistance, asthma, allergy, cardiovascular disease and neurological disorders [3] have been shown over the last decade. However, in most cases, it is not clear whether alterations of the gut microbiota are causal or secondary to the disease. An increasing body of evidence rather suggests the former, including IBD-like microbial alterations in healthy siblings [4], as well as an increasing degree of hallmarks of dysbiosis in correlation with the number of genetic alterations [5]. A breakdown of host–microbial mutualism triggered by environmental factors or genetic predisposition leading to dysbiosis and an inappropriate and progressive immune response to the commensal gut microbiota [2] is assumed to be causal for the pathogenesis of IBD [6,7].

The specific pathogenesis of IBD remains unclear, to date, but appears to be multi-factorial. Genome-wide association studies have identified 201 IBD susceptibility loci [8], affecting genes involved in epithelial barrier function, mucosal immune response, autophagy and immune regulation; the majority of these genes participate in the sensing of microbial products or affect defence signalling in response to gut microbes [1]. However, host genotype only explains up to 20–25% of IBD heritability overall, and 30–40% and up to 10% of cases of Crohn’s disease (CD) and ulcerative colitis (UC), respectively [9]. Environmental factors potentially contributing to IBD include diet, appendectomy, smoking, breastfeeding, personal hygiene and medication(s) [6].

Evidence is increasing that antibiotics can influence established IBD, as well as IBD flares, and can increase the risk of developing IBD in both children and adults [10–12]. However, remarkably few studies have investigated the effect of individual antibiotics, the underlying
mechanisms or whether there are any long-term ‘persistent’ effects of antibiotics on the gut microbiota [13–15]. Furthermore, a number of reports have claimed a potential association between isotretinoin (a non-antimicrobial treatment for severe acne) and development of IBD [16,17], although a causal role has not been established [18,19]. Isotretinoin is typically used in patients unresponsive to antibiotics [11]; as such, any causal relationship with the development of IBD is difficult to confirm due to confounding antibiotic treatment.

This study investigated the effects of doxycycline (used to treat acne but associated with the development of IBD), metronidazole (one of the preferred antibiotic agents for IBD patients) and isotretinoin on murine gut (faecal) microbiota after 2 weeks of treatment (immediate effects) and after a 4-week recovery period (long-term effects). The investigations aimed to identify possible environmental stressors that might have an immediate or persistent impact on gut microbiota composition, which might affect gut homeostasis and contribute to subsequent development of IBD.

2. Methods

2.1. Animals and treatment

In total, 164 female BALB/c mice were purchased from Charles River Laboratories (Sulzfeld, Germany) and housed in individually ventilated cages per treatment in the animal facility of the University Hospital Zurich, with access to rodent chow and water ad libitum (Fig. 1A). Isotretinoin (30 mg/mL, F Hoffmann-La Roche Ltd, Basel, Switzerland), vehicle (rapeseed oil, Brassica rapa, Sigma Aldrich, St. Louis, MO, USA), metronidazole (107 mg/kg, Sigma Aldrich), doxycycline (43 mg/kg, Sigma Aldrich) and water were administered orally for 2 weeks. Fig. 1 shows details of the study design, animals per group and sample collection.

2.2. Sample preparation and 16S rRNA gene sequencing

Total genomic DNA from faecal samples was extracted using the PowerLyzer PowerSoil DNA Isolation Kit (Mo Bio Laboratories, Inc., Courtaboeuf, France) according to the manufacturer’s instructions. Hypervariable regions 1–2 (V1–V2) of the 16S rRNA gene were amplified from isolated genomic DNA using bacterial specific primer Pyro_27F (Adaptor B) and the barcoded reverse primer MIDx_338R (Adaptor A) (Supplementary Table S1). The primer pair had specific eight-base-long identifiers (barcode), a linker sequence and sequencing adaptors as described earlier [20] (Supplementary Table S1).

Amplification reactions were performed in a total volume of 50 μL containing 5x HF buffer (New England Biolabs, Ipswich, MA, USA), 10 mM deoxynucleotide triphosphate (illustra solution dNTP GE

Fig. 1. Experimental design. (A) BALB/c female mice were treated with isotretinoin, rapeseed oil (isotretinoin vehicle), metronidazole, doxycycline or water (antibiotics vehicle) daily by oral gavage for 2 weeks. Faecal samples were collected before treatment, after 2 weeks of treatment (immediate effects) and after a recovery phase of 4 weeks after the cessation of treatment (long-term effects). (B) For isotretinoin and rapeseed oil, 16 animals per group were sampled before and immediately after treatment, and eight animals were sampled after the recovery phase. For metronidazole, doxycycline and water, five to 12 animals were sampled per time point and group. (C) No differences within treatment groups were registered with respect to body weight over all time points.
Healthcare, Pittsburgh, PA, USA), 2000 U/mL Phusion High-Fidelity DNA Polymerase (New England Biolabs), 10 μM Forward Primer Pyro_27F, 10 μM Reverse Primer MIDx_338R (Metabion, Planegg, Germany) and 50 ng DNA dissolved in DNA-free water.

Polymerase chain reaction (PCR) amplification was performed on a thermocycler (SenoQuest, Göttingen, Germany) with the following cycling conditions employed: 98°C for 3 min, 25 cycles at 98°C each for 10 s, 55°C for 30 s, 72°C for 30 s and a final extension at 72°C for 10 min. Amplicons were run on a 2% agarose gel to allow isolation of the bands at 400 base pairs with extraction of amplicons performed with MinElute Gel Extraction Kit (Qiagen AG, Hombrechtikon, Switzerland) and eluted in 55 μl DNase-free water. PCR products were pyrosequenced on the GS-FLX 454 platform at the Functional Genomics Centre Zurich (Zurich, Switzerland) and at Microsynth (Balgach, Switzerland).

2.3. Bioinformatics

Raw 454 sequencing reads were denoised and demultiplexed using the Amplicon Noise software [21] as implemented in mothur [22] and based on mothur's standard operating procedure for 454 data [23]. To filter for chimeric sequences, the UCHIME algorithm [24] was run in both de-novo and reference-based mode against a custom, global database of non-chimeric 16S sequences, as described previously [25]. Sequences flagged as ‘chimeric’ by both algorithms were removed from the dataset. Sequences were aligned against a secondary structure-aware model of the bacterial 16S rRNA gene (provided in the package ssu-align) using Infernal [26,27]. Alignment were pruned to positions 100–357 in the reference model, and sequences that aligned poorly within this range (>10% unaligned bases or ≥10% unaligned gaps) were excluded from further analyses. After this filtering, the dataset contained 2,098,361 aligned sequences of length 257 nt.

Sequences were clustered into operational taxonomic units (OTUs) at different similarity thresholds according to the average linkage algorithms as implemented in hpc-clust [28]. Both algorithms have been shown to provide consistent OTUs that approximate clustering of full-length sequences for the 16S rRNA gene subregions targeted in this study [25]. Sequence taxonomy was inferred using the ribosomal database project (RDP) Classifier [29] with default parameters. A maximum likelihood phylogenetic tree of unique sequences was obtained using FastTree2 employing default parameters [30]. Community diversity calculations and statistical analyses were performed in R [31], and in particular using the packages phyloseq [32], vegan [33] and edgeR [34]. All data used in this study are available online [National Center for Biotechnology Information (NCBI) Sequence Read Archive Project SRP065320].

2.4. Community richness and evenness

Estimates of intergroup and intermouse community richness and evenness were assessed per sample in terms of Chao’s Abundance-based Coverage Estimator (ACE), the Gini–Simpson Index and the evenness were assessed per sample in terms of Chao’s Abundance-based Coverage Estimator (ACE), the Gini–Simpson Index and the

2.5. Detection of individual differentially abundant OTUs (taxonomic analysis)

The R package edgeR [34] was used to investigate the fine-scale community composition at the level of individual OTUs, as suggested previously [35]. This approach provides a statistical framework for comparisons between treated and non-treated groups by quantifying the abundances of individual OTUs found in each dataset. Representative sequences for every OTU per treatment and time point that was significantly different from the respective control group was blasted against the NCBI 16S rRNA reference database for fine-scale taxonomic annotation, employing the following thresholds: |log2(fold change)| ≥ 1, false discovery rate ≤ 0.001 and match-ID ≥ 97%.

3. Results

3.1. Immediate and long-term effects on microbiota phylum-level composition

The microbiota composition of all 164 animals at phylum level, across all time points and treatment conditions of the study, were as generally expected for murine gut microbiota (Fig. 2A). The dominant phyla were Bacteroidetes and Firmicutes, with very low abundances of Proteobacteria and Actinobacteria; approximately 10% of sequences per sample could not be classified confidently at phylum level when using the RDP Classifier with default parameters. In faecal samples collected prior to the start of treatment, there were no notable differences between the treatment and vehicle-control groups for either of the antibiotics (metronidazole, doxycycline and water; multi-variate analysis of variance as implemented in the ‘adonis’ function of the ‘vegan’ package in R [36]). This approach provides a statistical framework for comparisons between treated and non-treated groups by quantifying the abundances of individual OTUs found in each dataset. Representative sequences for every OTU per treatment and time point that was significantly different from the respective control

3.1. Immediate and long-term effects on community composition

The trends observed above at a very coarse resolution of phylum-level taxonomic composition were consistent at the level of individual unique sequences (i.e. highest possible taxonomic resolution). Principal coordinate analysis of weighted UniFrac distances between samples calculated on a maximum likelihood phylogenetic tree at single nucleotide resolution are shown in Fig. 3. Before treatment onset, phylogenetic community structure did not differ significantly between groups, either for antibiotic (permutational multi-variate analysis of variance as implemented in the ‘adonis’ function of the ‘vegan’ package in R [36]) or for isotretinoin treatment (R2 = 0.0442, P = 0.223). As expected, both antibiotic treatments led to significant shifts in community composition immediately after treatment relative to controls (R2 ≤ 0.4499, P ≤ 0.001); in particular, metronidazole treatment induced a distinct shift relative to both doxycycline-treated mice (R2 = 0.479, P ≤ 0.001) and mice administered water alone (R2 = 0.445, P ≤ 0.001). The change in community composition of doxycycline-treated mice was less pronounced (R2 = 0.252, P = 0.015). After the recovery period, community composition in doxycycline-treated mice remained significantly different
from control mice ($R^2 = 0.115, P = 0.046$). Interestingly, the observed changes in community composition directly after metronidazole treatment did not persist after the recovery period, with metronidazole-treated animals being indistinguishable from controls ($R^2 = 0.025, P = 0.73$). In contrast, isotretinoin treatment did not lead to significant alterations in community composition either directly after treatment ($R^2 = 0.0435, P = 0.229$) or after the recovery period ($R^2 = 0.0678, P = 0.384$).

3.2. Immediate and long-term effects on community richness and evenness

Estimates of community richness and evenness assessed based on 98% average linkage OTUs using Chao’s ACE, the Gini–Simpson Index and the Shannon Entropy Index (Fig. 2B–D) supported a generally large intragroup variation in these parameters (Fig. 4, left panel), and even shifts in diversity per animal over time based on paired observations were generally unspecific within a given treatment group (Fig. 4, right panel). Directly after treatment, metronidazole induced a significant decrease in community richness and evenness (ACE, Fig. 4A, left panel; Shannon Entropy Index, Fig. 4C, left panel) in comparison with all other groups. The observed shift in Gini–Simpson Index was not statistically significant. Similarly, directly after treatment, richness as estimated by ACE showed a significant decrease in doxycycline-treated animals compared with isotretinoin-treated animals ($P = 0.029$), the water-treated controls ($P = 0.027$) and the rapeseed-oil-treated ($P = 0.003$) controls (Fig. 4A). Isotretinoin-treated mice showed significantly increased ($P = 0.032$) richness as estimated by the Gini–Simpson Index relative to rapeseed-oil-treated controls directly after treatment, which appeared to be due to a decrease in diversity in rapeseed-oil-treated controls. No significant differences in richness or evenness were evident between any of the treatment groups after the recovery period.

Changes in gut microbiota richness and evenness over time based on paired t-tests for individual animals are shown in Fig. 4A–C (right panel). Treatment with metronidazole induced a significant decrease
intra-animal evenness directly after treatment (Shannon Entropy Index, $P = 0.025$), which recovered to pretreatment richness levels (ACE, $P = 0.034$) and even more diverse composition (Shannon Entropy Index, $P \leq 0.001$) after the recovery period. Microbiota richness and evenness showed no significant change directly after treatment with doxycycline, but was significantly increased after the recovery period compared with directly after treatment (ACE, $P = 0.044$; Gini–Simpson Index, $P = 0.008$; Shannon Entropy Index, $P = 0.006$) and before treatment (Gini–Simpson Index, $P = 0.033$). Isotretinoin treatment did not induce any significant changes per animal over time. Interestingly, small but significant changes in gut microbiota evenness and richness were also seen in the vehicle-control groups over time. Relative to pretreatment baseline, diversity showed a significant drop (Gini–Simpson Index, $P = 0.008$; Shannon Entropy Index, $P = 0.013$) in rapeseed oil controls directly after treatment, but recovered after the recovery period. In animals administered water, there was a modest but significant increase ($P = 0.043$) in community richness (estimated by ACE) after the recovery period relative to pretreatment levels, but otherwise there were no significant effects per animal over time.

3.3. Identification of individual OTUs associated with treatment conditions

To identify individual taxa–treatment associations, this study quantified significant OTU abundance shifts per treatment relative to the respective vehicle-control group using the edgeR statistical framework [34]. The results of this analysis at different levels of taxonomic resolution per treatment group and time points are shown in Fig. 5. Within the phylum Bacteroidetes, OTUs of the genera Alistipes spp., Marinilabilia spp. and three other Bacteroidales were highly abundant directly after the treatment period, and this increase persisted after the recovery period. At the same time, a pronounced decrease in OTUs of Clostridiales spp. and Lachnospiraceae spp. was observed.

Treatment with doxycycline led to higher numbers of significantly over- or under-represented taxa in samples taken directly after the treatment period as well as in samples taken after the recovery period. In samples taken directly after the treatment period, 18 OTUs showed a significant increase in abundance and 33 OTUs showed a significant decrease in abundance. The greatest number of OTUs with
decreased abundance directly after doxycycline treatment was found within the phylum Firmicutes. Additionally, OTUs of the genera Ruminococcus spp. and Hespellia spp. were less abundant directly after doxycycline exposure; a change that persisted after the recovery period. Moreover, after the recovery period in doxycycline-treated animals, 12 OTUs showed significantly increased abundance and 16 OTUs showed significantly reduced abundance. Notably, the abundance of individual Butyrivibrio spp. and Proteobacteria spp. OTUs was found to be decreased after the recovery period, but not directly after doxycycline treatment.

Fig. 4. Effects on community richness and evenness ('alpha diversity'). Left panel: Abundance-based Coverage Estimator (A), Gini–Simpson Index (B) and Shannon Entropy Index (C) indices are shown per time point and treatment; differences between groups were assessed using one-way analysis of variance (data not shown), followed by unpaired, two-sided t-tests. Right panel: within-group comparisons of per-animal diversity, relative to pretreatment levels; each point indicates the absolute shift of diversity in a given animal with respect to time point 0. Significance of shifts was assessed using paired, two-sided t-tests, also for comparisons of per-mouse levels immediately upon treatment to after recovery (indicated as dashed brackets).
Fig. 5. Identification of individual operational taxonomic units (OTUs) associated with treatment conditions using differential abundance analysis. For each time point and treatment group, differentially abundant OTUs with respect to control groups (i.e. animals treated with water or rapeseed oil, respectively) were detected using edgeR at a false discovery rate of $\alpha < 0.001$. Significantly differential OTUs are shown, coloured by phylum, sorted by log2 (fold change) in abundance relative to control levels (x-axis). Species-level taxonomic annotations were obtained, where possible, by assigning OTU representative sequences to their closest BLAST hit against the National Center for Biotechnology Information’s 16S rRNA database at a tolerance of 97% identity. Individual OTUs with significant abundance shifts for several groups or time points are indicated using running numbers per phylum ($B_{01}, B_{02}$, etc.).
In metronidazole-treated animals, 20 OTUs (e.g. of Enterococcus gallinarum and Parabacteroides goldsteinii) were observed to be highly abundant directly after treatment, while 11 were less abundant (e.g. of Hespilia spp. and Ruminococcus spp.). Notably, two OTUs representing Proteobacteria spp. were highly abundant in samples taken directly after treatment, corresponding to findings in this study for taxonomic composition by RDP Classifier. Comparable with changes seen in community composition, richness and evenness, only seven OTUs showed differential abundances after the recovery period.

Isotretinoin treatment had a markedly less-pronounced effect on taxonomic composition than the other agents evaluated. Directly after the treatment period, only eight classifiable OTUs showed a decrease in abundance (e.g. Bacteroides acidifaciens, Ruminococcus spp., Anaerotruncus spp.) relative to control animals, with only a single OTU showing an increase in abundance (Lachnospiraceae spp.). After the recovery period, no OTUs in isotretinoin-treated animals showed a significant change in abundance.

Further analysis revealed that a number of individual OTUs showed persistent trends (e.g. B_01 or F_04), particularly after treatment with doxycycline but less marked for metronidazole, suggesting an antibiotic-specific effect. Specifically, the abundance of Bacteroidetes OTUs (B_01, B_02, B_03, B_05, B_06) were elevated directly after doxycycline treatment as well as after the recovery period relative to control animals, while OTUs representing Firmicutes (F_03, F_04, F_07, F_11) were less abundant for both time points. Following treatment with metronidazole, OTUs annotated for Bacteroidetes were specific; however, after the recovery period, change in B_01 and B_02 (increase) and in B_07 (decrease) was the same as in doxycycline-treated animals. This finding could either indicate a common effect of both antibiotics or a potential time effect on these OTUs in the common reference (i.e. water-treated mice). Moreover, the abundance of Firmicutes (F_02, F_04, F_05, F_06, F_08, F_09, F_10) was decreased directly after the treatment period (comparable with doxycycline) but not after the recovery period. Interestingly, the abundance of F_12 (Lachnospiraceae spp.) was strongly decreased for both time points and for both antibiotics. Isotretinoin treatment showed no changes in OTUs common with those for the antibiotic groups.

4. Discussion

This study showed differential effects of antibiotics and isotretinoin on the gut microbiota that support a putative association between treatment with doxycycline, but not metronidazole or isotretinoin, and development of IBD. Overall, metronidazole induced a significant decrease in diversity directly following treatment, which returned to pretreatment status during the recovery period. In contrast, doxycycline induced only modest effects on diversity directly following treatment, but a persistent impact on composition after the recovery period, whereas isotretinoin had no significant impact on community composition, richness or evenness either directly after treatment or after recovery.

Distinct changes in gut microbiota composition have been reported in IBD patients [36], and alterations in gut microbiota composition are associated with increased disease risk and severity in animal models of colitis [37]. A history of bacterial gastrointestinal infections and antibiotic treatment is reported to severely affect the intestinal microbiota, and to be associated with the development of gastrointestinal disorders such as IBD in children and adults [10,12].

Recent studies have shown metronidazole, a nitroimidazole-based antibiotic frequently prescribed for gastrointestinal-related disorders, including IBD, to be strongly associated with new-onset IBD (odds ratio 5.01) [12,38], and doxycycline to be associated with CD (odds ratio 2.25) [11]. A putative association with IBD has also been reported for isotretinoin, a treatment for severe acne [11]. Confirmation of the putative association between isotretinoin and IBD has proved difficult due to confounding antibiotic treatment, and limited evidence on a potential immunogenic response to commensal gut microbiota triggered by isotretinoin, or any long-term persistent effects on the gut microbiota. This led the authors to investigate mouse faecal microbial composition before and directly after a treatment course with metronidazole, doxycycline or isotretinoin, as well as following a recovery period.

In animal models, metronidazole treatment has provided contradictory findings, such as compromised goblet cell function, decreased mucus layer thickness and increased microbial stimulation of the epithelium, as well as increased susceptibility to Citrobacter rodentium infection in mice [39], and an increase in mucus layer thickness in rats [38]. In the present study, metronidazole treatment was associated with pronounced changes in community composition and significant reduction in bacterial richness and evenness directly after treatment, which recovered during the recovery phase. This finding is seemingly at odds with the reported strong association of metronidazole with new-onset IBD [12]. However, the increases in Proteobacteria and the facultative anaerobic species (e.g. Enterococcus spp.), marked reduction in Clostridiales and relatively modest impact on Bacteroides was in accordance with the inherent specificity of metronidazole. Jakobsson et al. [15] reported an increase in Enterococcus spp. and Proteobacteria (Klebsiella spp.) and a reduction in Lachnospiraceae spp. in human samples, while Sjölund et al. [40] showed persistence of resistant enterococci for up to 3 years. A recent study investigating the effect of metronidazole on microbiota composition in a model of elderly colonic fermentation identified a very pronounced shift in gut microbiota composition. Clostridium cluster IV, Faecalibacterium prausnitzii and Roseburia spp. were the bacterial groups particularly affected by metronidazole treatment, with incomplete recovery after 10 days without treatment [41]. Despite the pronounced alteration of gut microbiota composition directly after metronidazole treatment in this study, only seven OTUs remained significantly altered after recovery. Five of these (B_01, B_02, F_07, F_12, F_13) were similarly impacted after the recovery period in doxycycline-treated animals. It might be hypothesized, therefore, that some strains are particularly sensitive to treatment with antibiotics (F_07, F_12), or are opportunistic to a disturbed microbiota (B_01, B_02, F_13).

However, it is also possible that there is a potential time effect on these OTUs in the common reference (i.e. water-treated animals). An increase or decrease of these OTUs in the water-treated group would skew the abundances in both treatment groups (doxycycline and metronidazole) as they are compared with each other.

In the present study, doxycycline was only associated with moderate changes in microbiota richness and evenness immediately after treatment but, based on taxonomic composition analysis, a strong shift mainly in Clostridiales (up to 500-fold downregulation), with only a few strains of the Firmicutes phylum showing a marked increase in abundance (e.g. Clostridium fusiformis and Lactobacillus murinus). Directly after treatment with doxycycline, Firmicutes strains were reduced whereas Bacteroidetes showed a distinct pattern of OTUs that were either increased or decreased in abundance, possibly mirroring the sensitivity of individual strains towards doxycycline. As the gut microbiota is a complex ecosystem with networks of co-dependence between different strains, any niche resulting from a reduction in Firmicutes, for example, will very likely be re-occupied by other strains. The findings here, in fact, confirm the overall reduction in Firmicutes reported recently in patients suffering from Q-fever endocarditis administered long-term doxycycline and hydrochloroquine [42]. Notably, this study did not find the reduction in Bacteroidetes reported by Angelakis et al., representing the only other study investigating the effect of doxycycline on gut microbiota [42]. Tetracyclines have been used as animal growth-promoting agents in productive livestock for several decades, and the impact on gut microbiota composition has been reviewed recently [43,44]. Most studies investigated the impact
of low-dose chlorotetracycline in pigs. Zhang et al. showed an increase in the phylum Firmicutes and the genus Prevotella [45], while Holman et al. only observed minor alterations with subtherapeutic doses of chlorotetracycline [46]. A study investigating the impact of combined administration of chlorotetracycline and sulfamethazine on the bovine gut microbiota did not identify any differences in bacterial community fingerprints or bacterial load in comparison with the control group. [47]

In the present study, complete recovery of the microbiota was not observed, even 4 weeks following cessation of doxycycline treatment. The treatment groups showed little differentiation in community richness or evenness but changes in OTU abundances. Overall community composition remained different from controls, most notably the Bacteroides OTUs that were elevated directly after treatment remained elevated after recovery (B.01, B.02, B.03, B.05, B.06), whereas the Firmicutes OTUs that were reduced directly after treatment (F.03, F.04, F.07, F.11, F.12) remained so by 4 weeks after cessation of treatment.

Such persistent changes at OTU level might be speculated to promote the development of colitis in a susceptible host. To date, reduction in diversity, temporal instability and over- (e.g. Desulfovibrio) or under-representation (e.g. F. prausnitzii) of individual strains have been shown in UC and new-onset CD. In addition, decreased abundance of the genera Faecalibacterium, Roseburia and Clostridiales, and increased abundance of Enterobacteriaceae, are a consistent finding in CD patients [36,48]. Thus, these findings of a persistent reduction of Clostridiales OTUs are congruent with these reported alterations in microbiota in CD patients. Nevertheless, further investigations are needed to establish a causal relationship.

In summary, these findings demonstrate differential effects of antibiotics on gut microbiota community composition and diversity, with doxycycline mediating long-term changes in the murine gut microbiota. In contrast, the microbiota profile of isotretinoin-treated animals was not significantly affected, providing no evidence that isotretinoin impacts the risk for IBD development through effects on the gut microbiota.

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Ethical approval: All animal experiments were approved by the Cantonal Veterinary Office of Zurich under Licence Numbers ZH-54-2011 and ZH-214-2016. All animal experiments were performed in accordance with Swiss national law for animal welfare, and in accordance with the minimal standards for laboratory animals defined by the Institute for Laboratory Animal Science of the University of Zurich.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version at doi:10.1016/j.jantimicrob.2017.03.017.

References


