RESEARCH ARTICLE | Gut Microbiota in Health and Disease

Segment-specific responses of intestinal epithelium transcriptome to in-feed antibiotics in pigs

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Yu K, Mu C, Yang Y, Su Y, Zhu W. Segment-specific responses of intestinal epithelium transcriptome to in-feed antibiotics in pigs. Physiol Genomics 49: 582–591, 2017. First published September 8, 2017; doi:10.1152/physiolgenomics.00020.2017.—Despite widespread use of antibiotics for treatment of human diseases and promotion of growth of agricultural animals, our understanding of their effects on the host is still very limited. We used a model in which pigs were fed with or without a cocktail of antibiotics and found, based on the denaturing gradient gel electrophoresis (DGGE) patterns, that the fecal bacteria from the treatment and control animals were distinct. Furthermore, the total bacterial population in the feces tended to be decreased by the antibiotic treatment ($P = 0.07$), and the counts of Lactobacillus and Clostridium XIVa were significantly reduced ($P < 0.05$). To explore the effects of antibiotics on host intestinal epithelium, we assessed gene expression profiles of the jejunum and ileum and their response to antibiotic administration. The results indicate that in-feed antibiotics increased expression of genes involved in immune functions in both the jejunum and ileum, some of which were clustered in the coexpression network. Gene ontology terms of metabolic processes were altered predominantly in the jejunum but not in the ileum. Notably, antibiotics diminished intestinal segment-specific transcriptional changes, especially for genes associated with metabolic functions. This study reveals segment-specific responses of host intestinal epithelium to in-feed antibiotics, which can be a valuable resource for deciphering antibiotic-microbiota-host interactions.

IN RECENT YEARS, THERE has been widespread concern about the use of antibiotics in farm animals and humans. For decades, administration of low doses of antibiotics to livestock to promote growth has been the most cost-effective way to improve feed efficiency and health. In humans, subtherapeutic antibiotics have been increasingly used in early life. Despite their benefits, a well-known consequence of antibiotic use is the risk of antimicrobial resistance in humans and animals (16, 21, 30). Moreover, increasing evidence indicates that antibiotic exposure results in long-term metabolic disorders (5, 7). Thus, a greater understanding of the effects of antibiotics on the host is necessary to develop alternative strategies.

The intestinal epithelium, which is the primary interface between host and microbiota, is one of the major targets of antibiotic agents. Intestinal changes induced by antibiotics include reduction of epithelial tight junction proteins (4), loss of inflammasome function (12), thinning of the intestinal mucus layer (8), and decreased epithelial cell turnover in the colon (31). In addition, several studies have shown that the expression of a large number of intestinal genes is altered by antibiotic treatments (7, 31). Nevertheless, the intestinal epithelium exhibits extensive functional specialization along its proximal-distal axis characterized by distinct gene expression programs (3, 35). The impact of antibiotics on the segment-specific function of host intestinal epithelium has not been given much attention.

In addition to being directly affected by antibiotics, intestinal function can also be altered by the changing microbiota of the gastrointestinal tract and carries out a number of functions in the host’s immune system and epithelium development, apart from offering protection against pathogens and nutrient extraction (4). Several studies indicate that antibiotic treatment leads to substantial taxonomic changes in the composition and capabilities of gut microbiota (5, 19, 23), which contributes to alterations of host metabolic functions (5, 7, 28, 32). In previous studies, we examined the effects of low doses of in-feed antibiotics on the metabolic profiles of jejunum, serum, and urine metabolome in a piglet model and uncovered altered metabolic phenotypes (25). The mechanisms mediating these effects might be related to the modified function of the intestinal epithelium.

In this study, we performed denaturing gradient gel electrophoresis (DGGE) and real-time PCR analyses to characterize microbial communities in antibiotic-fed piglets and nonantibiotic-fed controls. We investigated the antibiotic-induced transcriptional responses in specific segments of the host intestinal epithelium with RNA-Seq technology. We identified differential expression of genes and gene response pathways in the jejunum and ileum between antibiotic-fed piglets and controls. We also compared the transcriptional profiles of the jejunal and ileal epithelia before and after antibiotic treatment. Our results provide valuable clues about the molecular mechanisms of the effects of low-dose antibiotic administration on host intestinal epithelium.

MATERIALS AND METHODS

Animals, housing, and sampling. This experiment was approved by the Nanjing Agricultural University Animal Care and Use Committee. The treatment, housing, husbandry, and slaughtering conditions conformed to the Chinese Experimental Animal Care and Use guidelines. A total of 16 litters of Duroc × Landrace × Large White newborn piglets were raised on the experimental farm of Nanjing Agricultural University. The piglets were randomly allocated to two groups ($n =$...
8 litters/group) and fed a corn-soy control diet or a corn-soy diet with antibiotics (50 ppm olaquindox, 50 ppm oxytetracycline calcium, 50 ppm kitasamycin) from postnatal day 7 to day 42, which is commonly used in the weaning period as a growth promoter in Chinese commercial farms to ensure a smooth and healthy transition around weaning. The piglets were housed under controlled environmental conditions with free access to feed and water. At day 42, one piglet from each litter was slaughtered, and all pigs were humanely euthanized. Feces were collected in sterile tubes and stored at −80°C until further analysis. A small piece of jejunum and ileum tissue from each pig was excised and rinsed in PBS. The mucosa scrapings were collected by a sterile glass microscope slide and immediately stored at −80°C for RNA analysis.

DGGE. DNA was extracted from feces based on a bead beating method followed by phenol-chloroform extraction (39). The V6–V8 regions of 16S ribosomal RNA (rRNA) gene were amplified by PCR with a universal primer pair U0968f-GC/L1401r (27). The PCR reaction conditions were as described previously (38). The amplicons were separated by DGGE according to Muyzer et al. (26) using a Dode system (Bio-Rad, Hercules, CA). Gels were stained with AgNO₃. Images were scanned using GS-800 Calibrated Densitometer (Bio-Rad) and analyzed using the Molecular Analyst 1.61 software (Bio-Rad). We confirmed the reproducibility of PCR amplification and DGGE by performing the experiments in duplicate. dendrograms of the DGGE profiles were generated by the unweighted pair-group method average.

RNA preparation and sequencing. Total RNA was extracted from the jejunum (n = 5) and ileum samples (n = 3) of pigs with TRIzol reagent (Invitrogen, Carlsbad, CA) following the manufacturer’s protocols. After DNase I treatment, magnetic beads with Oligo (dT) reagent (Invitrogen, Carlsbad, CA) were used to isolate mRNA from the total RNA, which was then fragmented and sequencing adaptors were attached to the fragments. The RNA was purified, and sequencing adaptors were attached to the fragments. Suitable fragments were isolated and amplified by PCR. The library products were sequenced with Illumina HiSeq 2000. The RNA-Seq library preparation and sequencing were performed by BGI Tech (Shenzhen, China).

Analyses of RNA-Seq data. The raw data were cleaned by removing low-quality reads, reads with adaptor sequences, and reads containing >10% unknown bases. Clean reads were mapped to reference sequences and/or reference genomes. BWA (18) and Bowtie (15) were used to map clean reads to the reference genomes (Suscrofa10.2) and reference gene sequences, respectively. Genes and isoform expression levels were quantified by RNA-Seq by expectation maximization and FPKM (fragment per kilobase of exon model per million mapped reads) methods (17). The FPKM values between biological replicates were analyzed by Pearson correlation, and the data from one replicate per group were not used for further analysis of differentially expressed gene (DEG) identification, because their Pearson coefficients were <0.85. The Noiseq method was used to identify DEGs between different groups, with divergence probability (P NOI) ≥0.75 and the absolute value of fold change ≥2 (log₂ ratio ≥1) as the threshold value (33).

Gene ontology, pathway, and network analyses. Gene enrichment in gene ontology (GO) biological processes was performed with DAVID Bioinformatics Resources v6.7 (https://david.ncifcrf.gov/). Kyoto Encyclopedia of Genes and Genomes (KEGG), the major public pathway-related database, was used to conduct pathway enrichment analysis. A network visualization of the DEGs based on correlation analysis was constructed using Gephi Graph Visualization and Manipulation software (2). Spearman’s correlation coefficients >0.8 and P < 0.01 were considered significant. In the graph, nodes represent genes, colors indicate the jejunum specific (blue), ileum specific (green), and overlapping DEGs (red), and color depths represents up- (dark) and downregulation (light). When two genes were correlated, their nodes were connected by an edge.

Real-time quantitative PCR. The numbers of total bacteria (20), Firmicutes (9), Bacteroidetes (9), Proteobacteria (9), Actinobacteria (1), Escherichia coli (13), Lactobacillus (14), Clostridium XIVa (36), Roseobaria (22), and Blautia (11) were quantified by quantitative PCR using specific primers (Table 1). qPCR assay was performed on an ABI StepOne platform (Applied Biosystems, Foster City, CA) using SYBR Premix Ex Taq dye (TaKaRa, Kusatsu, Japan). Standard curves of each bacterial group were generated from serial dilutions of a known copy number of the target gene cloned into a plasmid vector. Quantification of 16S rRNA genes in each sample was performed in triplicate, and the mean value was calculated. The data were expressed as log₁⁶S rRNA gene copies per gram of feces.

For gene expression analysis, total RNA was extracted from intestinal mucosa using TRIzol reagent. We reverse-transcribed 1 µg total RNA from each sample with the cDNA Reverse Transcription Kit (TaKaRa, Kyoto, Japan) according to the manufacturer’s instructions.

Table 1. List of primers used in this study

<table>
<thead>
<tr>
<th>DGGE</th>
<th>Forward Primer (5′-3′)</th>
<th>Reverse Primer (5′-3′)</th>
<th>Quantitative PCR bacteria</th>
<th>Quantitative PCR genes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total bacteria</td>
<td>GTGGTCAGAYGGYTCGTCGA</td>
<td>ACGCTCTCCMNCCTTCTCC</td>
<td></td>
<td></td>
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<tr>
<td>Firmicutes</td>
<td>GGAGYATGCTTGGTAATGGAGCA</td>
<td>ACGTGAAGCAAAACAATGAGC</td>
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<tr>
<td>Bacteroidetes</td>
<td>GJGARTAGCGTTAATGACTGT</td>
<td>ACGTACGCAAAACAATGAGC</td>
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<td></td>
</tr>
<tr>
<td>Proteobacteria</td>
<td>GCGATGTCGTGCTTGGAG</td>
<td>TGATACGCAAAACAATGAGC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Actinobacteria</td>
<td>TACGGCCGCAAGGCTA</td>
<td>TCRTCCGACCTTCTCGG</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>CATCAGCCGTGGTATAGGAAGA</td>
<td>GCGUAAGGAAGGAAAGAAAGA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lactobacillus</td>
<td>AGCAAGGATGAGCAGTGG</td>
<td>ATTCCTAAGAGGAAAGAAAGA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clostridium XIVa</td>
<td>GCGTACGCTGACTAAGAGACG</td>
<td>AGCTTTATCCTGGAAGGCA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Roseobaria</td>
<td>GGCTGAGCGGAAGATCGTC</td>
<td>ACGTCGACACTGTAGMCAC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blautia</td>
<td>GTGGACAGAAGAATGGCCCGT</td>
<td>TTGTAAGAGCTTCAGGGGTT</td>
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</tr>
</tbody>
</table>

DGGE, denaturing gradient gel electrophoresis.

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qPCR assay was performed on the LightCycler 480 system (Roche) using SYBR Premix DimerEraser™ Kit (TaKaRa, Kusatsu, Japan). The results were normalized to the expression level of β-actin gene, and the relative mRNA levels (n = 8) were calculated by the 2^−ΔΔCT method. Primer sequences are listed in Table 1.

Statistical analysis. Statistical significance of the bacterial and mRNA qPCR data was determined by Student’s t-test and one-way analysis of variance using SPSS 20.0, respectively, with P < 0.05 being considered statistically significant. The correlations between bacterial abundance and intestinal genes expression were assessed by Pearson’s correlation test. Correlation was considered significant at P < 0.05.

RESULTS

Microbiota alterations after treatment with antibiotics. The effect of antibiotic cocktail administration on the microbiota was evaluated by DGGE analysis of the bacterial 16S rRNA genes. The DGGE patterns of fecal bacteria from treatment and control samples were found to be clustered separately (Fig. 1A). The determination, by qPCR, of total bacterial DNA in the feces showed that the total bacterial population tended to be decreased in antibiotic-treated groups compared with control groups, but there was no significant difference (P = 0.07) (Fig. 1B). The in-feed antibiotic treatment did not have any effect on the counts of phylum Firmicutes, Bacteroidetes, and Proteobacteria (P > 0.05) but significantly reduced the counts of Actinobacteria (P < 0.05). At the genus level, antibiotic treatment did not have any effect on E. coli counts (P > 0.05) but significantly decreased the counts of Lactobacillus, Clostridium XIVa, Roseburia, and Blautia (P < 0.05). Furthermore, high-throughput rRNA analysis indicated that antibiotics significantly changed the intestinal microbe abundance at the operational taxonomic unit (OTU) level (24).

Specific transcriptional responses in the host jejunal and ileal epithelium induced by antibiotics. RNA-Seq was performed to evaluate the transcriptional profiles of jejunal and ileum samples taken from antibiotic-fed vs. nonantibiotic-fed control piglets. The numbers of sequenced clean reads for each sample ranged from 44.3 million to 44.6 million, and an average of 64.0 and 52.3% of the reads were uniquely mapped to the pig reference genome and reference gene sequences, respectively (Supplementary Table S1). (The online version of this article contains supplemental material.) Gene expression levels in reads were quantified according to FPKM values. Specifically, an average of 14,892.2 and 14,919.8 genes were expressed in the jejunum and ileum of pigs, respectively.

DEGs were screened with the Noiseq package, with fold change ≥2 and probability ≥0.75 as the filtering conditions. Noiseq analysis showed that there were 87 and 76 DEGs for jejunum control (Jej-C) vs. jejunum antibiotic treatment (Jej-A) and ileum control (Ile-C) vs. ileum antibiotic treatment (Ile-A), respectively (Fig. 2A). Of these 42 were upregulated and 45 were downregulated when Jej-A was compared with Jej-C, while 46 were upregulated and 30 were downregulated when Ile-A was compared with Ile-C. When the data are presented as a Venn plot, it becomes clear that 21 of these DEGs overlapped both intestinal epithelial groups (Fig. 2B). Genes related to antigen and immune process including TAP2, MMP1, and IFITM1-like were upregulated both in the jejunum as well as the ileum (Fig. 2C). Sulfide metabolic gene SQRDL and glycosphingolipid metabolic gene GBA3 were upregulated, while pyruvate metabolic gene PCK1, phospholipid metabolic gene ENPP7, and mineral metabolism-related genes MT1A, MT-2B, MT3, and MT1C-like were suppressed by antibiotics. All DEGs are listed in Supplementary Tables S2 and S3.

Fig. 1. Effect of in-feed antibiotics on bacterial communities in pig feces. A: similarity analysis of denaturing gradient gel electrophoresis (DGGE) profiles (n = 3). B: real-time quantitative (q)PCR measurements of major bacterial taxonomic groups. Data are shown as the mean ± SE (n = 8). *P < 0.05 vs. control. C, control; A, in-feed antibiotics; M, marker.
Functional alterations in the intestinal epithelium caused by antibiotics. Functions of DEGs in the two intestinal epithelial segments caused by antibiotics were investigated with GO analysis. In total, 47 GO terms were altered significantly, of which 10 were common to both jejunum and ileum, 20 were specific to the jejunum, and 17 were specific to the ileum (Fig. 3). As expected, about half of all GO terms were related to immune functions and stimulus responses. Adaptive immune response, positive regulation of immune effector process, and certain processes related to antigens were enriched in both tissues. In contrast, antigen processing and presentation, and leukocyte migration involved in inflammatory response were restricted to the jejunum, whereas immune response regulation, leukocyte-mediated immunity, and regulation of immune effector process were mainly enriched in the ileum. Thus, antigen processes were mostly affected by antibiotics in the jejunum, while immune response processes were mostly affected in the ileum.

Several GO terms belonging to response to stimulus were altered by antibiotics. Response to tumor cell and pH were enriched in both jejunum and ileum, whereas response to hormone and platelet-derived growth factor were enriched only in the jejunum, and response to external biotic stimulus and defense response were highly enriched in the ileum. Furthermore, GO terms of metabolic processes were altered predominantly in the jejunum and included reactive nitrogen species, multicellular organismal, ammonium ion, and organic hydroxy compound metabolic process. However, only the cellular lipid metabolic process was enriched in the ileum.

The DEGs were also mapped to pathways in KEGG database, which were clustered into six subcategories as shown in Fig. 4. The results demonstrated that 15 and 11 pathways were significantly altered in the jejunum and ileum, respectively, five of which were overlapping in both segments. Antigen processing and presentation, phagosome, and PPAR signaling pathway of the overlapping terms were the most enriched pathways in both intestinal segments. Specifically, protein processing in endoplasmic reticulum (upregulated HSP90B1, DNAJB11, HSPA1L, etc.), vitamin digestion and absorption, mineral absorption, and bile secretion related to digestion and absorption processes were enriched only in the jejunum. Remarkably, similar to the GO results, significant pathways involved in immune function were identified in both intestinal segments.

A coexpression network was constructed on the basis of pairwise correlations between DEGs. The Gephi tool was used to create and visualize the network containing 112 nodes and 556 edges (Fig. 5). The nodes represent genes, the weights of the edges represent how specifically two genes are associated together, and the colors indicate the jejunum-specific, ileum-specific, or overlapping DEGs. In this network, few genes related to immune function were clustered, including HSPD1, IL18, and TAP2, which were upregulated in response to antibiotics. The network also showed a cluster of genes related to metabolic function, including AKR1C family genes, Treh, SPR (downregulated in the jejunum), and CPO (upregulated).
In-feed antibiotics reduce the transcriptional differences between the jejunal and ileal epithelium. We previously showed that antibiotic administration blocked the interaction within dominant bacterial genera, including *Lactobacillus* and *Clostridium*, not only in the lumen and mucosa but also in the jejunum and ileum of the pig. Therefore, we investigated the effect of antibiotic cocktail administration on the host jejunum and ileum by comparing gene expression profiles between the jejunum and ileum.

**Fig. 3.** Antibiotic-responsive gene functions in different segments of the intestinal epithelium of pig. Gene ontology (GO)-term enrichment analysis of the biological processes of DEGs in the jejunum and ileum in response to antibiotics. Jej, jejunum; Ile, ileum.

**Fig. 4.** KEGG pathway analysis of DEGs in the jejunum and ileum in response to antibiotics. Jej-C, jejunum-control; Jej-A, jejunum-antibiotic treatment; Ile-C, ileum-control; Ile-A, ileum-antibiotic treatment.
two intestinal tissues before and after antibiotic treatment. The results showed that there was a total of 239 DEGs between Jej-C and Ile-C, of which 68 were upregulated and 171 were downregulated (Fig. 6A). However, upon treatment with antibiotics, the number of DEGs between the jejunum and ileum reduced considerably to 115, with 36 being upregulated and 79 being downregulated. Venn diagram analysis revealed that the transcriptional differences of 2/3 of DEGs between the jejunum and ileum disappeared after antibiotic treatment (Fig. 6B), indicating that antibiotics diminish intestinal segment derived transcriptional changes.

We then investigated functions of the DEGs specific to Jej-C vs. Ile-C but not to Jej-A vs. Ile-A. Metabolic pathways were identified with KEGG (Fig. 6C). A general decreased expression was observed for genes involved in amino acid metabolism (CTH and ALDOC related to biosynthesis of amino acids, DDC and CAT related to tryptophan metabolism, and CTH and GLYCTK related to glycine, serine, and threonine metabolism, carbohydrate and lipid metabolism (pentose and glucuronate interconversions, fructose and mannose metabolism, starch and sucrose metabolism and glycerolipid metabolism; e.g., UGDH and ALDOC), and vitamin and mineral digestion, absorption and metabolism (LRAT, SCARB1, and FOLH1). These results suggest that antibiotic treatment decreased the differences in metabolic functions between the jejunum and ileum in pig.

Validation of sequencing results with qPCR. To validate the gene expression profile from RNA-Seq, we performed real-time qPCR analysis of six genes. Based on RNA-Seq data, among the selected genes MT1A, MT2B, MT3, and HSPA5 were DEGs for both Jej-C vs. Jej-A and Ile-C vs. Ile-A, whereas IL18 and HSPD1 were DEGs only for Ile-C vs. Ile-A. The expression patterns of the selected genes in different groups, derived from RNA-Seq and qPCR experiments, are shown in Fig. 7. For the six genes tested, qPCR confirmed differential expression among the four groups discovered in RNA-Seq. Although the magnitude of fold change obtained by the two methods was different in some instances, the expression trends of these genes remained similar.

Correlation between bacterial abundance and intestinal genes expression. A Pearson’s correlation analysis was carried out to evaluate the potential link between bacterial abundance and the common DEGs expression in both intestinal epithelia induced by antibiotics (Fig. 8). The abundance of Lactobacillus was negatively correlated with the expression of SQRDL, TRIM31, TAP2, and SFTPD but positively correlated with the expression of MT2B. The abundance of Clostridium XIVa was negatively correlated with the expression of Lithostathine-like, IFITM1-like, MMP1, GBA3, MT2B, and so on but positively correlated with the expression of MT1A, MT2B, and MT1C-like. Additionally, Actinobacteria counts was positively correlated with MT3 expression, and the total bacterial population was negatively correlated with GBA3 expression while positively correlated with MT3 expression.

DISCUSSION

In this study, by using RNA-Seq technology, we analyzed segment-specific gene expression profiles of the jejunal and
ileal epithelium and their response to antibiotic administration to explore the effects of commonly used classes of antibiotics on the function of the small intestinal epithelium along its proximal-distal axis. These gene expression landscapes should be a valuable resource for researchers interested in 1) discovering molecular mechanisms for the growth-promoting effect of low-dose antibiotic administration on agricultural animals and 2) deciphering host-microbiota interactions in the host intestine.

Antibiotics exert their effects partly by disruption of the intestinal microbiota. Several studies have investigated the effects of antibiotics on the microbiota, showing that antibiotic treatments cause depletion of and/or significant changes in the composition of the microbiota. In mice, a cocktail of antibiotics (ampicillin, vancomycin, neomycin, and metronidazole) induced a pronounced drop in total bacterial mass, with a reduction in most taxa (23). Nonetheless, Cho et al. (5) reported that subtherapeutic antibiotic (penicillin, vancomycin, metronidazole) induced a pronounced drop in total bacterial mass, with a reduction in most taxa (23). Nonetheless, Cho et al. (5) reported that subtherapeutic antibiotic administration in pigs also led to significant changes in the microbiota composition.

Fig. 6. In-feed antibiotics diminish the intestinal segment derived transcriptional changes in the pig. A: volcano plots for Jej-C vs. Ile-C and Jej-A vs. Ile-A. Numbers are given for total DEGs as well as for up- and downregulated genes. B: Venn diagram of DEGs between different comparisons. Numbers are given for the common and specific DEGs of different intestinal segments between control and antibiotic treatment groups. C: KEGG pathway analysis of the specific DEGs for Jej-C vs. Ile-C but not for Jej-A vs. Ile-A. Jej-C, jejunum-control; Ile-C, ileum-control; Jej-A, jejunum-antibiotic treatment; Ile-A, ileum-antibiotic treatment.

Fig. 7. Validation of sequencing results by qPCR. The results are presented as fold changes of values in Jej-C. The mRNA levels of selected genes were analyzed by real-time qPCR and normalized to β-actin. The qPCR data are shown as means ± SE (n = 8). Values with different lowercase letter superscripts mean significant difference (P < 0.05), while with the same letter superscripts mean no significant difference (P > 0.05). Jej-C, jejunum-control; Ile-C, ileum-control; Jej-A, jejunum-antibiotic treatment; Ile-A, ileum-antibiotic treatment. Based on RNA-Seq data, the selected genes MT1A, MT2B, MT3, and HSPA5 are DEGs for both Jej-C vs. Jej-A and Ile-C vs. Ile-A, whereas IL18 and HSPD1 are DEGs only for Ile-C vs. Ile-A.
In the current study, using low-dose penicillin as a model agent showed that multiple taxa that typically peak early in life were suppressed, including *Lactobacillus*, *Candidatus Arthromitus*, and *Allo-baculum* (7). In pigs, bacterial phenotypes shifted after 14 days of antibiotic treatment, with a reduction in the abundance of *Bacteroidetes* and an increase in members of the *Deinococcus-Thermus* and *Proteobacteria* phyla (19). In the current study, performance-enhancing antibiotic (chlortetracycline, sulfamethazine, and penicillin) treatment, with a reduction in the abundance of *Bacteroidetes* and an increase in members of the *Deinococcus-Thermus* and *Proteobacteria* phyla (19). In the current study, performance-enhancing antibiotic (chlortetracycline, sulfamethazine, and penicillin) treatment, with a reduction in the abundance of *Bacteroidetes* and an increase in members of the *Deinococcus-Thermus* and *Proteobacteria* phyla (19).

The mechanisms for the growth-promoting effect of low-dose antibiotic administration on agricultural animals are still not clear. Previous studies have indicated that a dose of chlortetracycline (6) or apramycin (37) can increase growth performance. Nonetheless, in the present study, the in-feed antibiotics did not affect the body weight of 23-day-old piglets and very modestly increased the body weight at 42 days (105% of control, no significant difference; data not shown). There is some variation in the growth-promoting effect by antibiotics, which may be due to different types and levels of antibiotics used. Interestingly, several studies have shown that antibiotic administration alters host metabolism. In mice, antibiotic exposures increased fat mass and altered metabolic hormones and hepatic metabolism (5, 7). We previously examined the effects of in-feed antibiotics on the serum, urine, and intestinal luminal metabolome in pigs and showed that there was an increase in the concentration of most amino acids and certain metabolites related to energy metabolism in serum and a decrease in the concentration of amino acids in the intestinal content (25).

Antibiotics on gut microbiota may be due to differences in species, age, environmental conditions, and intestinal segments of animals, as well as the types and doses of the antibiotics used in different studies.

Microbiota is involved in the maintenance of gastrointestinal homeostasis. Disruption of the intestinal microbiota by antibiotic treatment may induce immune responses in the host, which can also be directly affected by antibiotics. Previous studies have shown that antibiotic treatment induces a decrease in immunity, including decreased numbers of IFN-γ-producing and Th17-producing lymphocytes and reduced expression of pro-IL-18 and pro-IL1b (10, 31, 34). Low-dose penicillin suppressed ileal expression of genes involved in antigen presentation and Th17 cell differentiation processes (7). Few studies have investigated the effects of antibiotics on the host intestinal transcriptome in the pig, although low-dose antibiotics have been applied as growth promotants in pig production for a long time. In this study, we investigated antibiotic-induced transcriptional responses in specific segments of the host’s intestinal epithelium in a piglet model. We found that the GO terms related to adaptive immune response, positive regulation of immune effector process, and some processes related to antigen were enriched in both the jejunum and ileum. The coexpression network showed that some genes related to immune and antigen process, including *HSPD1*, *IL18*, and *TAP2*, were clustered and upregulated in response to antibiotics. These results indicate that these classes of antibiotics increase the expression of genes related to immunity in piglets.

**Fig. 8.** Correlation between bacterial abundance and intestinal genes expression. A Pearson’s correlation analysis was performed between bacterial abundance and the common DEGs expression in both intestinal epithelia induced by antibiotics. The intensity of the colors represents the degree of association: red presents positive correlation, blue represents negative correlation; \#P < 0.05, *P < 0.01.
received much attention in previous studies. Our results suggest that the functions of digestion, absorption, and metabolism in the jejunum may be altered by antibiotics, providing evidence for growth-promoting effect of antibiotics.

Notably, among the transcriptional profiles of the jejunum and ileum, the DEG numbers in antibiotic-fed piglets were only half that of nonantibiotic-fed piglets. A large number of the DEGs between the jejunum and ileum that were lost after antibiotic treatment were classified into metabolic process GO terms and were assigned into metabolic pathways involved in amino acid, carbohydrate, and lipid metabolism. These results indicate that in-feed antibiotics diminish intestinal segment-derived transcriptional changes, especially for genes associated with metabolic functions, thus providing additional indirect evidence for the growth-promoting effect of antibiotics. Interestingly, our other microbial data showed that in-feed antibiotics broke the connection of some bacteria (e.g., Lactobacillus and Clostridium) at the adjacent intestinal segment. These host and microbiota alterations between different intestinal segments induced by antibiotics might be associated with host-microbiota interactions and requires further investigation.

In summary, the present study demonstrates antibiotic-induced transcriptional changes in segment-specific intestinal epithelium from piglets. In-feed antibiotics increase the expression of genes involved in immune and antigen processes in both the jejunum and ileum, while genes related to metabolic processes are altered predominantly only in the jejunum. Additionally, antibiotics diminish intestinal segment-derived transcriptional changes, especially for genes associated with metabolic functions, providing an indirect evidence for growth-promoting effect of antibiotics.

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GRANTS

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

K.Y., C.M., and Y.Y. performed experiments; K.Y. and Y.S. analyzed data; K.Y., C.M., and Y.Y. drafted manuscript; K.Y., C.M., Y.S., and W.Z. edited and revised manuscript; K.Y., C.M., Y.S., and W.Z. conceived and designed research.

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