Human disease-related pathways increasing with opportunistic pathogens bloom in intestinal microbiome after vancomycin exposure and restoration with fecal transplantation by simulator of the human intestinal microbial ecosystem (SHIME)

Lei Liu

Nankai University

Qing Wang

Nankai University

Hongmei Qi

Nankai University

Ranjit Das

Nankai University

Huai Lin

Nankai University

Jingliang Shi

Nankai University <https://orcid.org/0000-0003-0561-9083>

Siyi Wang

Nankai University

Jing Yang

Nankai University

Yingang Xue

Nankai University

Daqing Mao

Nankai University School of Medicine

Yi Luo (✉ luoy@nankai.edu.cn)

<https://orcid.org/0000-0002-6732-7935>

Research

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Abstract

Background: Antibiotics are growing reported to cause human intestinal microbial disorders with increasing abundances of opportunistic pathogens, and fecal microbiota transplantation (FMT) has been confirmed to restore the dysbiosis of gut flora in many kinds of intestinal disease. However, to date, few studies have focused on the bloomed opportunistic pathogens associated human disease-related pathways as well as antibiotic resistance genes (ARGs) after vancomycin exposure, and there is limited information on using FMT for restoration of intestinal microbiome affected by antibiotics.

Methods: This study investigated effects of vancomycin on the opportunistic pathogens, human disease-related pathways as well as ARGs in human gut, and the restoration of intestinal microbiome by FMT. Using the simulator of the human intestinal microbial ecosystem (SHIME), two reactors (representing the ascending and descending colon, respectively) were inoculated with human intestinal microbiota from a healthy adult volunteer who did not suffer from gastrointestinal diseases or take any antibiotic in the previous six months. Samples were collected from the reactors at six-time points (before treatment, after seven days of individual 10 mg/L, 100 mg/L, 1000 mg/L vancomycin treatment, 14 days following the termination of vancomycin and 14 days following FMT). The intestinal microbiome composition and function were analyzed using 16S rRNA gene sequencing and High-throughput Quantitative PCR (HT-qPCR).

Results: Vancomycin treatment substantially increased human disease-related pathways and decreased abundances of ARGs. Besides, the bloomed opportunistic pathogens including *Achromobacter*, *Klebsiella*, and *Pseudomonas*, caused by vancomycin exposure, were positively correlated with human disease-related pathways. The microbiota abundance and genes of human disease-related pathways and antibiotic resistance showed a remarkable return towards baseline after FMT, but not for natural recovery.

Conclusions: These findings suggest that impacts of vancomycin on human gut are profound and FMT will be a promising strategy in clinical application that can restore the dysbiosis of gut microbiota, which may be valuable for directing future work.

Background

The human intestine constitutes primarily a large and diverse microbial habitat that is colonized by many bacteria, as well as archaea, viruses, and eukaryotes [1]. The human intestinal microbiota has been demonstrated to provide numerous essential functions for human health including nutrient supply, modulating the immune function, protection from the infectious pathogens, and metabolism of xenobiotic drugs [2, 3]. Both human and veterinary antibiotics were detected in the collective gut of the Chinese population through our previous research [4]. The antibiotic therapies have been demonstrated paramount importance in the treatment of numerous diseases caused by bacterial infections since its discovery in the 1940s. Concerns about the use of antibiotics have traditionally focused on the killing of bacterial pathogens; however overuse and misuse of antibiotics may promote the spread of antibiotic-

resistant bacteria and the antibiotic resistance genes (ARGs) which may limit the treatment efficiency and resulting in chronic and relapsing infections [5, 6]. Recent studies have found that indiscriminately kill or prevent the growth of both pathogenic and commensal bacteria by antibiotics may cause the dysbiosis of intestinal microbiota, and then will promote pathology, including obesity, asthma and infectious diseases [7, 8].

Vancomycin, a glycopeptide antibiotic is mainly active against the gram-positive bacteria and is commonly used to treat the infections produced by multidrug-resistant *Staphylococcus aureus* and *Clostridium difficile*. However, researches have reported that the administration of vancomycin would increase the abundance of opportunistic pathogens such as *Pseudomonas* and *Klebsiella* in human intestinal microbiota [9, 10]. Furthermore, few studies have focused on the bloomed opportunistic pathogens associated human disease-related pathways as well as ARGs after vancomycin exposure. These opportunistic pathogens are ubiquitous in the environment and are known to be considered as commensal in the human gut; but the increasing numbers of their population could be a significant risk factor for the infection [11, 12]. Moreover, these opportunistic pathogens are prominently linked with human diseases such as pneumonia, colitis, cancer, diabetes, neurosurgery, and cardiovascular diseases [13, 14].

Previous studies showed that many researchers have investigated influences of vancomycin on the human intestinal microbiota in vivo [9, 10, 15]. However, in vitro experiments are very important for clarifying direct effects of antibiotics on human intestinal microbiota without the disturbances from neurohumoral regulation, the individual differences, dietary habits, and physiological status. The simulator of the human intestinal microbial ecosystem (SHIME) model is known to be a useful tool for in vitro studies as (i) interactions between the microbiota; and (ii) the effects of prebiotics and other compounds on the microbial communities and metabolic activities [16]. However, to the best of our knowledge, there are very few researches that added the antibiotics into this in vitro model [17–20]. Researches mainly focused on the benefit of the mucosal environment, high-fiber diets, probiotic, and propionate-producing consortium in human intestinal microbiota. The effects of antibiotics, including AMX and other antibiotics mixture, vancomycin, and clindamycin, were limited to microbiota composition and metabolite.

Fecal microbiota transplantation (FMT) is the introduction of feces from a healthy donor into the intestinal tract of someone with disrupted microbiota, which has been confirmed superior to oral vancomycin for the treatment of *Clostridium difficile* infection [21, 22]. And combination of FMT with vancomycin could be a better choice to treat this kind of infection [23, 24]. However, there is limited information on using fecal microbiota transplantation (FMT) for restoration of intestinal microbiome affected by antibiotics. Therefore, it is exciting for us to investigate whether FMT would restore the human intestinal dysbiosis caused by vancomycin exposure.

Since the reasonable dosage of vancomycin for adult human study is about 500 to 1500 mg day⁻¹ and only half volume of adult gut can be simulated in the used SHIME model, here the direct effects of

600 mg day⁻¹ (1000 mg L⁻¹) of vancomycin on the composition and human disease-related pathways as well as ARGs of the human gut microbiota were studied [15, 24]. And 10 mg L⁻¹ and 100 mg L⁻¹ were chosen as lower doses of vancomycin to verify whether these doses of vancomycin would affect human gut microbiota, because the vancomycin agar plate concentration for vancomycin resistant bacteria isolation is between this range according to the CLSI M07-A8 2011 guidelines. In this study, the composition of human intestinal microbiota composition and human disease-related pathways were analyzed by 16S rRNA gene high-throughput sequencing, and the ARGs were quantified by high-throughput quantitative PCR (HT-qPCR). This study achieved a systematical investigation and precisely understanding of the direct effects of vancomycin modulation on the intestinal microbiota and the restoration of FMT using a SHIME model system, which may be valuable for directing future work.

Methods

Vancomycin treatment and sample collection

The SHIME model in this study was formed by four double-jacketed reactors designated as the stomach, small intestine, ascending colon, and descending colon, respectively (Fig. S1a). The last two reactors inoculated with a mixture of fecal microbiota collected from a healthy adult volunteer, who did not suffer from gastrointestinal diseases or take any antibiotics in the previous six months, and the differences between individuals may be alleviated by same culture condition [25]. The details of the SHIME system and the startup process are summarized in the Supplementary material.

After two weeks' stabilization and another week's nutritional medium adding, the SHIME sequentially exposed to a gradient of vancomycin (from low to high concentrations) and experienced two weeks' natural recovery and two weeks' FMT. All the samples were collected and analyzed at six-time points: prior to the treatment (C_A, C_D), and after 7 days of each administration of 10 mg L⁻¹ (V10_A, V10_D), 100 mg L⁻¹ (V100_A, V100_D), and 1000 mg L⁻¹ vancomycin (V1000_A, V1000_D), after 14 days of vancomycin discontinuation (NR_A, NR_D), and after 14 days of FMT (FR_A, FR_D), and each sample is a mixture of three samples collected from 7 a.m. to 11 p.m. (Fig. S1b). The samples were stored at -80 °C for further analyses.

16S rRNA gene sequencing and analysis

Microbial DNA was extracted from the samples using the E.Z.N.A. stool DNA Kit (Omega, USA) according to the manufacturer's protocols. The V3-V4 region of the bacterial 16S rRNA gene was amplified by polymerase chain reaction (PCR). The raw reads were deposited into the NCBI Sequence Read Archive (SRA) database under the accession number SRR9329997-9330008.

Raw Illumina fastq files were de-multiplexed, quality-filtered, and analyzed using Quantitative Insights Into Microbial Ecology (QIIME) [26]. The 16S rRNA gene sequences were classified taxonomically using the Ribosomal Database Project (RDP) classifier 2.0.1 [27]. To investigate the effects of vancomycin on alpha diversity, the taxon richness (Chao1 index), evenness (Simpson index), and diversity (Shannon

index) were calculated for all samples. In addition, beta diversity of the microbiota communities at baseline and after antibiotics were portrayed by nonmetric multidimensional scaling (NMDS) and principal coordinate analysis (PCoA) of weighted and unweighted UniFrac distances [28]. Linear discriminant analysis effect size (LEfSe) analysis was performed to determine bacterial taxa that significantly differed between the control and antibiotic group using the Galaxy application tool [29]. Functional predictions of microbial community were performed to visualize the distribution of human disease related pathways in the two parts of colon with different treatment using Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt) [30]. The accuracy of PICRUSt for the detection of these more challenging functional groups was good (min. accuracy = 0.82), suggesting that their inference of gene abundance across various types of functions was reliable, and PICRUSt predictions had high agreement with metagenome sample abundances across all body sites (Spearman $r = 0.82$, $P < 0.001$). The details of taxonomical classification, LEfSe analysis, and functional predictions were described in the Supplementary material.

High-throughput quantitative PCR (HT-qPCR) and analysis

HT-qPCR reactions were performed to visualize the variation of ARGs during the treatment using Wafergen SmartChip Real-time PCR system, which was conducted by Anhui MicroAnaly Gene Technologies Co., Ltd (Anhui, China). A total of 120 primer sets were used (Excel S1), including 114 primer sets to target almost all major classes of antibiotic resistance genes (ARGs) found in Chinese human gut microbiota [31], five mobile genetic elements (MGEs) and one 16S rRNA gene. The results were analyzed with SmartChip qPCR Software excluding the wells with multiple melting peaks or amplification efficiency beyond the range (90–110%) and then screened with conditions that a threshold cycle (CT) must be < 31 and positive samples should have three replicates simultaneously. The details of HT-qPCR analysis were described in the Supplementary material.

Antibiotic susceptibility test and serum killing resistance assays of pathogens

The Pathogenic bacterial strains were isolated from the V1000_A sample using Luria-Bertani (LB) agar plates supplemented with 100 mg L^{-1} of vancomycin. Genomic DNA of these strains was extracted using a Promega Wizard Genomic DNA Purification Kit (Promega, Madison, USA) according to the manufacturer's protocols. The isolated strains were identified by 16S ribosomal DNA sequencing conducted by BGI Tech Solutions (Beijing Liuhe) Co., Ltd (Beijing, China). Nine antibiotic resistance genes from the isolated strains were confirmed using Qualitative PCR with the primers listed in Table S1. MICs of these pathogens were determined according to the CLSI M07-A8 2011 guidelines. The serum-killing assay was performed based on the method described by David and associates [32]. Details of the antibiotic susceptibility test, and the serum killing resistance assays procedures are described in the Supplementary material.

Data analysis

Correlation between the microbiota and human disease-related pathways or ARGs were analyzed using the Spearman test in R with the vegan package. Correlations between pairs of variables were considered to be significant at $r > 0.6$, and P values were < 0.05 . The Gephi (V 0.9.1) software was used to visualize the bipartite network graphs using the Force Atlas algorithm.

Results

Human disease-related pathways and ARGs

The human disease-related pathways and ARGs in samples from the SHIME model were assessed at different time points, which included samples prior to antibiotic treatment (C), sequential doses (10, 100, and 1000 mg L⁻¹) of antibiotic treatments at seven days interval designated as V10, V100, and V1000, followed by 14 days after termination of vancomycin (NR) and 14 days after FMT (FR). The hierarchy cluster heatmap showed that gene numbers of human disease-related pathways, including drug resistance, cancer, cardiovascular diseases, immune system diseases, infectious diseases, metabolic diseases, and neurosurgery diseases were more abundant in antibiotic exposure groups than that in control group, which was especially apparent in ascending colon (Fig. 1). For instance, gene numbers of bladder cancer, renal cell carcinoma, primary immunodeficiency, type II diabetes mellitus, and prion diseases were nearly 1.3–1.5 times more enriched in the V1000 group than in control. Moreover, gene numbers of these human disease-related pathways still maintained at a higher level (about 1.2–1.3 times enriched than control) after natural recovery and FMT treatment could restore them to baseline level.

The relative abundance of ARGs such as aminoglycoside, beta-lactam, multidrug, and tetracycline resistance genes were lower in vancomycin exposure groups than that in control samples, which was also especially obvious in ascending colon (Fig. 2). For instance, log relative abundance reduction of *aac6ie* (aminoglycoside) was about 2.9 log units after 1000 mg L⁻¹ vancomycin treatment than in control, and *bl2b_tem1* (beta_lactam), *ermB* (MLSB), and *qacdelta1* (multidrug) were about 2.0, 2.3, and 1.5 log units, respectively. Besides, the ARGs such as *ant3ia* (aminoglycoside), *bl1_ec* (beta_lactam), *ermf* (MLSB), *mexf* (multidrug), *tetq* (tetracycline), and *sul1* (sulfonamide) were not detected after vancomycin treatment. Similarly, these ARGs were unable to return to the baseline level following the natural recovery with about 2.2 to 3.8 log units lower of relative abundance. FMT would restore most of them to some extent (about 1.7 log units lower to 0.6 log units higher).

Microbiota community composition and diversity

In this study, the effects of vancomycin on gut microbial communities' composition were also investigated. Based on the 16S rRNA gene sequence analysis, the most abundant taxonomic groups assigned at the phylum level were Proteobacteria (64.7–98.7%), Bacteroidetes (0.1–18.7%) and Firmicutes (0.5–12.4%) present in the ascending colon (total of 92.5–99.4%), followed by Synergistetes, Fusobacteria, and Verrucomicrobia (Fig. S2). However, the most three abundant bacterial phyla in the

descending colon (total of 79.8–94.8%) were Proteobacteria (53.6–83.4%), Bacteroidetes (0.1–23.0%) and Synergistetes (2.3–10.8%). Moreover, an obvious decrease of Bacteroidetes (from 17.7–18.7–0.1%) and Firmicutes (from 5.9–12.4% to 0.5–0.9%), and increase of Proteobacteria (from 68.3–68.9% to 83.4–98.7%) were seen after 1000 mg L⁻¹ vancomycin treatment. The significant changed communities' composition at the phylum level was sustained after natural recovery and returned to baseline after FMT treatment. At the genus level, the antibiotic-treated subjects were shown to be substantially overgrown by Burkholderia (from 0.2–3.2% to 2.8–17.9%) and Achromobacter (from 0.3–0.4% to 6.3–8.9%) with the dose of vancomycin, while the percentage of Pseudomonas was increased in the V10 group (from 6.9–45.5% to 17.0–53.2%) and Klebsiella increased in the V1000 group (from 15.0–39.7% to 22.3–51.4%). Similarly, following the natural recovery period, the gut microbiota was incompletely restored, and the complete recovery observed after FMT. These results were in line with human disease-related pathways and ARGs results.

Meanwhile, fecal microbiota alpha diversity was assessed. The taxon richness (Chao1 index), evenness (Simpson index), and diversity (Shannon index) are shown in Fig. S3a to c. Compared with control sample C_A, a drop in microbiota richness (Chao1), evenness (Simpson), and diversity (Shannon) was observed in sample collected from ascending colon after 1000 mg L⁻¹ of vancomycin treatment; however opposite change was observed in sample V10_A. As shown in Fig. S3, the decreasing of microbiota richness, evenness, and diversity caused by vancomycin treatment restored after two weeks of vancomycin discontinued (NR). Besides, the beta diversity of the microbiota communities and weighted UniFrac distance was affected by antibiotic treatment. As shown in Fig. 3, the samples that collected after vancomycin treatments were differed from the control group in both UniFrac NMDS and PCoA analyses. The beta diversity results also showed that gut microbial composition remained comparable after two weeks of vancomycin discontinued (NR) because these two samples were clustered together with vancomycin treatment groups. Meanwhile, after two weeks of FMT treatment, the samples clustered to control group for both UniFrac NMDS and PCoA analyses. Moreover, weighted UniFrac distance between 1000 mg L⁻¹ vancomycin treatment group and control group (V1000 vs. C) were higher than that within the control group (C vs. C), and that between the recovery groups and the control group (NR vs. C, FR vs. C) were slightly higher (Fig. S4).

Bloomed opportunistic pathogens

The linear discriminant analysis effect size (LEfSe) comparison analysis between the control and antibiotic groups was shown in Fig. 4. LEfSe analysis indicated that vancomycin resulted in significant decreases in several taxa, including the members of Bacteroidetes (Bacteroides, Parabacteroides), Firmicutes (Clostridium, Dialister) and Campylobacter. The changes were accompanied by increases in the relative abundance of Proteobacteria phylum, including Achromobacter (LSD = 4.61), Pseudomonas (LSD = 3.18), and Klebsiella (LSD = 3.34), which were consistent with the gut microbial communities' composition results.

Moreover, five opportunistic pathogens were isolated from V1000_A sample (Table S2), which included *Klebsiella aerogenes* (NKU-Kae), *Klebsiella pneumoniae* (NKU-Kpn7), *Klebsiella oxytoca* (NKU-Kox6), *Achromobacter xylosoxidans* (NKU-Axy), and *Pseudomonas aeruginosa* (NKU-Pae). Table S3 represents the minimal inhibitory concentration (MIC) of these opportunistic pathogens. Data suggested that all the isolated opportunistic pathogens were resistant to vancomycin with MIC ≥ 256 mg L⁻¹, as well as beta-lactam antibiotics including ampicillin (MIC ≥ 64 –256 mg L⁻¹) and amoxicillin (MIC ≥ 64 –256 mg L⁻¹), sulfonamides (sulfamethoxazole MIC ≥ 128 –256 mg L⁻¹) and MLSB (erythromycin MIC ≥ 8 –64 mg L⁻¹). However, *Klebsiella oxytoca* (NKU-Kox6), *Achromobacter xylosoxidans* (NKU-Axy) and *Pseudomonas aeruginosa* (NKU-Pae) showed the more comprehensive resistant properties to several kinds of antibiotics than *Klebsiella aerogenes* (NKU-Kae) and *Klebsiella pneumoniae* (NKU-Kpn7), including kanamycin, tetracycline, and streptomycin. Table S4 showed a total of nine ARGs detecting in these opportunistic pathogens. The results showed that all the opportunistic pathogens carried *qnrS* and *bl2be_shv2*, and except NKU-Kae, all others contained *aac3iia*, *vang*, *arna*, *roSB*, and *ant2ia* ARGs. Therefore, their intrinsic ARGs such as *vang*, *aac3iia*, *sul1*, and *bl2be_shv2* might be attributed to high values of MICs to vancomycin, gentamicin, sulfamethoxazole, ampicillin, and amoxicillin. As shown in Table S5, a considerable variability in serum resistance for the opportunistic pathogens was observed, which constituted an important virulence trait that allowed these opportunistic pathogens to persist in vivo. And the survival of *Pseudomonas aeruginosa* (NKU-Pae) strain was significantly higher (12–54 times) than other strains with a virulence level of Grade 5.

Correlation between microbial taxa and human disease-related pathways or ARGs

Figure 5 showed the results of co-occurrence patterns between microbial taxa and human disease-related pathways. It can be seen that significantly increased bacteria after vancomycin exposure such as *Achromobacter* and *Klebsiella* were positively associated with almost all of those human disease-related pathways, and the decreased bacteria were negatively associated with these pathways. Specifically, the correlation coefficients of *Achromobacter* with colorectal cancer, viral myocarditis and toxoplasmosis were about 0.8 ($P < 0.01$) and the correlation coefficient of *Klebsiella oxytoca* with *Staphylococcus aureus* infection was 0.7 ($P < 0.01$).

The network analysis of co-occurrence patterns between microbial taxa and ARG subtypes was shown in Fig. 6. Interestingly, a very similar pattern of results was observed that a significantly decreased the bacteria was positively associated with the most ARGs. For example, the correlation coefficients of *Bacteroides fragilis* with *bl2e_cepa aac6ie* and *tet32* were about 1.0 ($P < 0.001$), and the correlation coefficient of *Parabacteroides* with *ermB* was about 0.8 ($P < 0.01$). Only two significantly increased bacterial genera were positively associated with several ARGs. For example, the strong correlations were found in *Klebsiella oxytoca* with *yidY/mdtL* and *tetC* (about 0.9, $P < 0.01$), and in *Pseudomonas* with *ermF* and *tetQ* (about 0.8, $P < 0.05$).

Discussion

Vancomycin exposure significantly increased the opportunistic pathogens

The SHIME model was stably operated in this study because the predominant phyla of Bacteroidetes, Proteobacteria, Synergistetes, and Firmicutes in the gut microbiome was previously demonstrated by Yu's group [33]. These predominant phyla had also been observed in fecal samples from human and animals [34, 35]. Moreover, the inconsistent bacterial phyla and genera were identified in different colon regions, providing further evidence that colon regions play an essential role in shaping the structure of gut microbiota [18].

Numerous in vivo studies have reported the similar phenomena as shown in our research that significantly decreased of Bacteroidetes and Firmicutes phyla and predominantly detected human intestinal microbiota from Proteobacteria phylum such as *Pseudomonas* and *Klebsiella* following vancomycin administration [9, 10, 15]. Some studies have also reported a drop in richness and alpha diversity and significant effect on beta diversity pursued by vancomycin exposure [9, 36]. *Achromobacter*, *Pseudomonas*, and *Klebsiella* genera were all confirmed to increase after vancomycin exposure, although these bacteria are all considered commensal in the human gut, fecal carriage of these opportunistic pathogens was confirmed to be a severe risk factor for the infection [11, 12]. *Achromobacter* and *Klebsiella* intrinsically carry ARGs such as *vang*, *aac3iia*, *sul1*, and *bl2be_shv2*, which make them inherently resistant to vancomycin, aminoglycosides, sulfonamides, and beta-lactam antibiotics, consequently pose a significant therapeutic challenge [37]. Additionally, the multidrug resistant *Pseudomonas aeruginosa* with high virulence obtained from this research has also been well recorded in many previous research papers, indicating its immense threat to human health [38]. Some commensal microbiota in the human gut that decreased after vancomycin exposure such as *Bacteroides fragilis* and *Parabacteroides* are novel probiotics [39, 40], and the decrease of these probiotics may cause dysbiosis of gut microbiota, which would lead human health problem [41, 42]. Therefore, the results of our research demonstrated a serious impact and a negative side-effect of vancomycin that enriched pathogens related to health problems, which should be considered as a fundamental aspect of the cost-benefit equation for an antibiotic prescription.

Opportunistic pathogens associated human disease-related pathways increased by vancomycin exposure

The significant increase in opportunistic pathogens associated human disease-related pathways and decrease in ARGs of human intestinal microbiota following vancomycin treatment exhibited in this study have not been reported in details yet. However, ampicillin had been found to enhance glucose tolerance, and promote the selection of antimicrobial resistance genes in intestinal microbiota using animal models [43]. Importantly, this study displayed a positive correlation between opportunistic pathogens (*Klebsiella* and *Achromobacter*) and human disease-related pathways, which suggested the bloom of these opportunistic pathogens caused by vancomycin treatment that might contribute to human diseases. For instance, some studies have indicated that *Klebsiella* was enriched in cancer and diabetes patients [44],

and be related to various infectious diseases [45] and neurosurgery disease [14]. Furthermore, *Achromobacter* was also reported to be related to human diseases such as pneumonia [46], colitis [47], cancer [48], and cardiovascular diseases [13]. All of the findings, as mentioned above, were also linked with positive correlations between the abundances of these opportunistic pathogens and human disease-related pathways in our study. Some previous studies have also reported multiple ARGs resided in these types of pathogenic bacteria [49, 50], that maybe one of the critical reason for ARGs were more abundantly present in control samples.

Vancomycin effects were “SHIME-compartment” different and persistent

The phenomenon observed in the SHIME model that a larger shift of microbiota found in the ascending colon was also revealed by previous reporters. They demonstrated that changes of intestinal microbiota by vancomycin or mixed antibiotics exposure were “SHIME-compartment” different [17, 18]. Meanwhile, more extensive shifts in bloomed opportunistic pathogens associated human disease-related pathways as well as ARGs found in this study were in line with other research that had clarified the primary effect observed at the level of the microbiota could also be found at genomic and metabolic levels [51, 52]. The intestinal microbiota is a key “organ” for the individual’s health and their different shifting in the ascending colon and descending colon suggested that more studies should focus on the proximal colon for the uniform data sheets of colon microbiota. However, in vivo experiments usually analyses the feces that standing for the distal intestinal microbiota. Furthermore, the vancomycin effects were still evident for at least two weeks after the vancomycin discontinued, although the resilient reduction of opportunistic pathogens and human disease-related pathways was observed. Reijnders and colleagues revealed that a markedly decreased of microbial diversity, decreased relative abundance of most Gram-positive bacteria of the Firmicutes, and increased Gram-negative Proteobacteria caused by vancomycin treatment persisted 56 days after cessation of antibiotic therapy [15]. Isaac and colleagues also reported a similar phenomenon, which revealed that 14 days treatment with vancomycin induced persistent reduction of the abundances of OTU13 and OTU23, and diminishment of richness till postnatal weeks 22 after antibiotic treatment discontinuation [9].

FMT restored microbiome composition and genes

Finally, this study revealed that the FMT would restore human intestinal microbiome composition, human disease-related pathways, and ARGs that profoundly affected by vancomycin. The restoration of microbiome composition find in our study may refer to the findings by Lee and associates that germ-free mice mono-associated with a single *Bacteroides* species are resistant to colonization by the same, but not different, species [53]. FMT may also colonize missing flora after vancomycin exposure with priority to restore the dysbiosis. For recurrent of *Clostridium difficile* infection, several researchers have suggested that FMT is a superior method to oral vancomycin for the treatment [21, 22], and combinations of them would be a better choice attributed to restore the intestinal microbiota [23, 24]. The clinical application of FMT also includes inflammatory bowel disease, diabetes mellitus, liver cirrhosis, gut-brain disease, and others [54]. Moreover, the FMT has become a completely novel way to eradicate drug-resistant bacteria

from the intestinal reservoir, which would include decolonization of extended-spectrum beta-lactamase (ESBL)-producing and carbapenemase-producing Enterobacteriaceae, vancomycin-resistant Enterococci, or methicillin-resistant *Staphylococcus aureus* [55]. However, the clinical utility of FMT to displace the multidrug-resistant bacteria or treat patients with ulcerative colitis (UC) is still controversial because of little benefit shown in other papers, and the use of probiotics maybe better with potentially lower risks of adverse events [56–59]. Thus, there would be much work to reveal whether FMT will be promising in clinical application that limit opportunistic pathogens overgrowth and restore human intestinal microbiota composition and genes that profound affected by antibiotic exposure.

Perspectives

Typical antibiotics have been detected in the collective gut of the Chinese population in our previous research which provided a reference for this study of the effects of vancomycin on human gut microbiota [4]. However, there were several limitations of this study. First, repeating these experiments at least a number of times more would be better for currently stating that vancomycin causes a significant disruption on gut microbiota and confirming the benefit of FMT on vancomycin induced dysbiosis, which were not taken attributed to the costs associated with system operation and samples detection. On the other hand, the comparison of opportunistic pathogens isolated from control sample with vancomycin treated sample will also be necessary to reveal its effect in bacteria characteristic. It is also of interest to inoculate the microbiota mixture from several healthy humans as well as patient to discover the different effects of vancomycin between individuals. Further in vivo studies are also needed to verify whether the findings in this in vitro study reflect the real recovery in vivo. Moreover, research on the restoration effects of some prebiotics, probiotics, and synbiotics during and after antibiotic therapy will also be promising, which may help discover several clinical strategies to restore side effects caused by antibiotics. As the increasing of opportunistic pathogens and human disease-related genes by antibiotics exposure, which may pose a significant therapeutic challenge and take some efficient measures to reduce or even eliminate the effects after antibiotic treatment should be more critical.

Conclusions

Exposure to vancomycin significantly altered the taxonomic composition and diversity, and it subsequently increased opportunistic pathogens and human disease-related pathways. The changes were “SHIME-compartment” different with a more significant effect on the ascending colon. Moreover, bacteria that increased after vancomycin exposure were positively associated with the human disease-related pathways, while bacterial decreased were positively related to quantified ARGs. The significant shifted human gut microbiota was not recovered after two weeks of vancomycin discontinuance; however, restored thoroughly after FMT. These results may be valuable for directing future work, which may open up new perspectives for assessing the direct effects of antibiotics on the intestinal microbiota and suggested that FMT may be promising in clinical application.

Declarations

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Authors' contributions

LL carried out the laboratory work and wrote the manuscript. QW revised this manuscript. DR edited the language and improved the clarity of this manuscript. HQ, HL, JS, SW and JY carried out the laboratory work. YX provided funding support. DM and YL revised this manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

Please contact the author for data requests.

Ethics approval and consent to participate

The study was approved by the Biomedical Ethics Committees of Nankai University. The participant has given written informed consent to understand the study purpose, procedures, risks, benefits, and rights.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Author details

liulei19880114@qq.com (L. Liu)

wangqing@hebeu.edu.cn (Q. Wang)

898975347@qq.com (H. Qi)

anmicro_85@yahoo.co.in (R. Das)

447446985@qq.com 18567774811 (H. Lin)

sjl_net@163.com (J. Shi)

15330856579@163.com (S. Wang)

yangjing1@sust.edu.cn (J. Yang)

zxyg@126.com (Y. Xue)

maodq@nankai.edu.cn (D. Mao)

luoy@nankai.edu.cn (Y. Luo)

Abbreviations

ARGs: Antibiotic resistance genes; ESBL: Extended-spectrum beta-lactamase; FMT: Fecal microbiota transplantation; HT-qPCR: High-throughput quantitative PCR; LB: Luria-Bertani; LEfSe: Linear discriminant analysis effect size; MGEs: Mobile genetic elements; MIC: Minimal inhibitory concentration; NMDS: Nonmetric multidimensional scaling; PCoA: Principal coordinate analysis; SHIME: Simulator of the human intestinal microbial ecosystem; PCR: Polymerase chain reaction; PICRUSt: Phylogenetic Investigation of Communities by Reconstruction of Unobserved States; QIIME: Quantitative Insights Into Microbial Ecology; RDP: Ribosomal Database Project; SRA: Sequence Read Archive; UC: Ulcerative colitis

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Figures

Figure 2

Heatmap of human disease-related pathways in the two parts of colon with different treatment. Heatmap colors reflect gene numbers of human disease-related pathways from low (blue) to high (red).

Figure 4

Heatmap of antibiotic resistance genes (ARGs) in the two parts of colon with different treatment. Heatmap colors reflect relative abundance of ARGs from low (blue) to high (red).

Figure 6

Effect of vancomycin treatment on gut microbiota beta diversity of the control (green), vancomycin (red), and recovery (blue) groups. Unweighted (a) and weighted (b) PCoA, and unweighted (c) and weighted (d) NMDS of UniFrac distances of samples in six groups (C, V10, V100, V1000, NR and FR).

Figure 8

LDA score (a) and cladogram of LefSe (b) comparison analysis between the control and antibiotic groups. The red, green shading depicts bacterial taxa that were significantly higher in either the control or antibiotic groups, as indicated. Selection of discriminative taxa between the control and antibiotic groups were based on an LDA score cutoff of 3.0, and differences in the relative abundances of taxa were statistically determined based on a Mann-Whitney test at a significance level of 0.05.

Figure 10

Network analysis revealing the co-occurrence patterns between microbial taxa and human disease-related pathways. The nodes in Network were colored according to types of human disease-related pathways and microbial genera that increased (red) or decreased (green) after vancomycin exposure. The edges were colored according to positive (red) or negative (blue) correlation. A connection represents strong and significant (P value < 0.05 , $r > 0.6$) correlation. The size of each node is proportional to the number of connections, that is, degree.

Figure 11

Network analysis revealing the co-occurrence patterns between microbial taxa and ARG subtypes. The nodes in Network were colored according to ARG types and microbial genera that increased (red) or decreased (green) after vancomycin exposure. The edges were colored according to positive (red) or negative (blue) correlation. A connection represents strong and significant (P value < 0.05 , $r > 0.6$) correlation. The size of each node is proportional to the number of connections, that is, degree.

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