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Research article

Crosstalk between imbalanced gut microbiota caused by antibiotic exposure and rotavirus replication in the intestine

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ABSTRACT

Objective: Rotavirus (RV), one of non-enveloped double-strained RNA viruses, can cause infantile diarrheal illness. It is widely accepted that RV is transmitted mainly via feces-oral route. However, infected asymptomatic adults are becoming the source of infection. It is necessary to explore the underlying mechanism of RV replication in adult's intestine.

Methods: After recruiting healthy volunteers and RV asymptomatic carriers, we firstly investigated the association of animal-derived food intake with antibiotic level in urine samples. Secondly, we compared the difference in the structure of gut microbiota, and identified the taxa that most likely explained the difference. Finally, we investigated the impact of lipopolysaccharide (LPS), produced by gram-negative bacteria, on RV replication in vivo and in vitro.

Results: We found that 10% of participants were RV asymptomatic carriers in our study. High intake of animal-derived food was positively correlated to antibiotic level in urine samples. The disrupted gut microbiota in RV carriers was characterized by high abundance of antibiotic resistant gram-negative bacteria and high level of LPS. The disrupted gut microbiota caused by penicillin treatment was benefit to RV replication in vivo. LPS enhanced RV thermal stability in vitro.

Conclusions: Our findings suggest that the imbalanced gut microbiota caused by antibiotic exposure plays an important role in RV replication, and brings risk to health complications.

1. Introduction

RV is a non-enveloped double-strained RNA virus, which has been classified into nine groups (from A to I) on the basis of RNA sequence and antigenic difference of viral protein 6 (VP6) [1]. However, only group A causes over 90% of infection in humans. RV infection is a leading cause of infantile diarrheal illness around the world, with the potential for severe dehydration, malnutrition and even death. Despite the vaccinations for RV have been introduced, RV infection still leads to over 200,000 deaths each year, mostly in low-income countries [2].

RV is spread predominantly via the feces-oral route, close person-person contact, fomites, and contaminated water or food [3]. Only children under 5 years of age show severe, dehydrating gastroenteritis after infection, while it causes asymptomatic or mild

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symptoms in adults [4]. It has been reported that RV can cause reinfection throughout life, although the disease severity is reduced after repeat infections [2,5]. However, how RV lives in the intestine of adults is less clear.

Human gastrointestinal tract resides many types of microbes which play a crucial role in host health [6]. Microbial diversity is important for the proper role of gut microbiota. Imbalance in its composition has been linked to many human diseases, including diabetes, metabolic disorders, and infections [7]. Although many studies focus on bacteria-host interaction, the impact of imbalanced gut microbiota on enteric viruses still needs to be investigated.

Exposure to antibiotics is the most common situation because antibiotics have been widely used not only to treat infectious diseases in humans and animals, but also used as growth promoter in aquaculture and agriculture [8]. As a result, antibiotics can accumulate in human bodies through food chain, leading to the disruption of gut microbiota and induction of resistant genes in several bacterial taxa [9]. Recent findings reported that imbalanced gut microbiota enhances the infection of enteric viruses, such as poliovirus, reovirus, and mouse mammary tumor virus (MMTV) [10]. Kuss et al. found that gut microbiota can promote the replication and pathogenesis of reovirus and poliovirus. In the infection process, LPS plays a key role by enhancing virion stability and promoting environmental fitness [11].

The aim of this study was to compare the difference in the level of urinary antibiotic residues between RV carriers and healthy participants, and to characterize the structure of gut microbiota in RV carrier. Finally, we explored the possible mechanism of gut microbiota-mediated promotion of RV replication.

2. Materials and methods

2.1. Ethics statement

This study was approved by the Ethics Committee of Anhui Medical University. All participants signed a written informed consent. All of them received questionnaires and sample collections.

2.2. Samples collection and processing

Fecal samples were collected from 30 undergraduates infected with RV (named patient group) from Dec. 2020 to Nov. 2021. The basic characteristics of patient group are described in Table S1. The viral antigen in stool samples was detected by ELISA kit (YS01379B, Yaji Company, Shanghai, China) and confirmed by specific PCR reactions as previously described by Kulis-Horn et al. [12]. At the same time, 30 fecal samples were also collected from 30 healthy undergraduates as a control (named healthy group). All fecal samples were stored at −20 °C until analysis.

2.3. Antibiotic analysis of urine samples

The urine samples were obtained in morning, carried to the lab, and frozen at −20 °C. Commonly used antibiotics, including human antibiotics (HAs), veterinary antibiotics (VAs), and preferred as veterinary antibiotics (PVAs), were selected to detect in this study. The treatment and measurement of antibiotics in urine samples were presented in our paper [13].

2.4. Dietary investigation

We used a semi-quantitative food-frequency questionnaire (FFQ), reported by Zhao et al. [14], to detect the association between the types of consumed foods and antibiotic residues in urine. We calculated the daily food consumption of all participants, according to the instruction of FFQ. After that, the proportions of animal-derived foods and vegetable-derived foods were determined in the two groups.

2.5. Resistant gene identification by PCR

To detect resistant genes existed in *Escherichia coli* (*E. coli)* and *Staphylococcus aureus* (*S. aureus*), respectively, we performed PCR assay using different primers listed in Table S2. Total DNA, which was extracted from fecal samples, was used as the template for PCR amplification. The 50 μL of PCR mixture contained 25 μL of Takara Taq™ HS Perfect mix (R300S, Takara Company, Beijing, China), 0.6 μM of each primer, 1 μL of template DNA, and 23 μL of water. PCR steps was as follow: 30 s at 94 °C for DNA denaturation, followed by 30 cycles of 94 °C for 10 s, 55 °C for 10 s, and 72 °C for 20 s. Amplicons were visualized by electrophoresis in agarose gel stained by 4S Green Plus (A616694, Sangon Company, Shanghai, China).

2.6. DNA extraction and sequencing of 16S rRNA gene amplicons

DNA was extracted from fecal samples using QIAamp Fast DNA Stool Mini Kit (code no. 51604, QIAGEN Company, Hilden, Germany). Next, qualified DNA was amplified using 341F and 806R primers. Finally, qualified PCR products were sent to Shanghai Personalbio Technology Co., Ltd. for sequencing of 16S rRNA gene amplicons and data analysis.

2.7. LPS determination in fecal samples

LPS level was determined using Bioendo Limulus Amebocyte Lysate kit (EC32545, Xiamen Bioendo Company, Xiamen, China) according to the manufacture's instruction. Briefly, 0.1 g of fecal sample was dissolved in LPS-free water. The mixture was vortexed for 1 min and centrifuged at 10,000 rpm for 5 min to obtain supernatant. The calibration curve was constructed using LPS isolated from *E. coli* O111:B4. LPS level in samples was determined based on the calibration curve.

2.8. Antibiotic treatment and RV infection

C57BL/6 mice (3 months old) were purchased from animal center of Anhui Medical University. After 1 week of adaptive feeding, 24 mice were randomly divided into 4 groups (6 mice in one cage): (1) untreated control, (2) antibiotic-treated group received antibiotics cocktail (including four antibiotics: ampicillin (1 g/L), vancomycin (0.5 g/L), metronidazole (1 g/L) and neomycin (1 g/L)) ad libitum in drinking water, (3) RV-infected group received antibiotics cocktail 1 week before receive 50 μL of 10^6 PFU/mL Mouse RV strain EC by oral gavage. (4) LPS-treated group received antibiotics cocktail for 1 week, then administered LPS at 1 g/L in drinking water ad libitum for 3 days before receiving 50 μL of 10^6 PFU/mL Mouse RV strain EC by oral gavage.

2.9. Quantification of RV in faecal sample

Nucleic acids were extracted from fecal samples by Mag-MK Virus RNA Extraction Kit (B518767-0050, Sangon Company, Shanghai, China) according to the manufacturer's instructions. RV quantity was determined by the Rotavirus Real-time PCR Kit (AP4351-50T, Fusheng Company, Shanghai, China) according to the manufacturer's instructions.

2.10. Particle Stability Thermal Release assay (PaSTRy)

PaSTRy was conducted to investigate RV thermal stability, as described elsewhere [15]. Briefly, Reaction mixture contained 1 μg of RV, 3 × SYPRO red, 5 μM SYTO9 and adding buffer (10 mM HEPES, 200 mM NaCl) to a final volume of 50 μL. Samples were heated from 25 °C to 95 °C on a 0.5 °C stepwise gradient in a Roche LightCycler 96 real-time instrument with fluorescence reading.

Table 1
Detected antibiotics in urine samples.

Antibiotic type	Antibiotics	Healthy group (ng/mL)	Patient group (ng/mL)	*p*-Value
HAs	Levofloxacin	6.79	6.28	NS
	Clarithromycin	0.96	0.91	NS
	Cefaclor	196.44	202.78	NS
VAs	Enrofloxacin	2.99	2.62	NS
	Sulfachloropyridazine	41.34	71.86	*
	Sulfamonomethoxine sodium hydrates	4.65	7.50	*
	Sulphaquinoxaline	1.13	2.99	*
	Sulfamethazine	1.82	1.97	NS
	Sulfaclozine sodium monohydrate	13.49	61.64	*
	N4-acetylsulfamonomethoxine	4.49	4.05	NS
	Cefquinome sulfate	102.94	49.77	*
	Ceftiofur	0.00	1.99	*
	Cyadox	306.38	309.81	NS
PVAs	Lomefloxacin hydrochloride	4.03	8.16	*
	Ofloxacin	0.00	14.77	*
	Pefloxacin mesilate dihydrate	9.35	67.89	*
	Ciprofloxacin	14.78	15.82	NS
	Norfloxacin	2.80	5.13	*
	Trimethoprim	5.00	4.47	NS
	Sulfametoxydiazine	7.65	25.73	*
	Sulfamethoxazole	8.37	5.02	*
	Sulfadiazine	123.23	65.74	*
	Erythromycin	58.43	83.63	*
	Lincomycin hydrochloride	12.54	28.29	*
	Spectinomycin hydrochloride	69.00	12.08	*
	Doxycycline hydrochloride	81.39	119.91	*
	Oxytetracycline hydrochloride	2.00	3.80	*
	Tetracycline hydrochloride	3.34	2.39	*
	Chlortetracycline hydrochloride	155.25	232.62	*
	Cefotaxime sodium	32.21	24.19	*
	Amoxicillin trihydrate	32.12	249.61	*
	Penicillin-G sodium salt	143.06	441.53	*
	Penicillin V	0.00	5.23	*

Note: NS: not significant; *: $p < 0.05$; HAs: human antibiotics, VAs: veterinary antibiotics, PVAs: preferred as veterinary antibiotics.

2.11. Statistical analysis

Data analyses were conducted using SPSS version 22.0. All data were expressed as the mean ± standard deviation (SD). Statistical significance was considered at the $p < 0.05$ level. Except when indicated otherwise, three biological replicates were performed in this study.

3. Results

3.1. High consumption of animal-derived food results in more antibiotic residues in urine

Although all participants did not receive any drugs, we detected a total of 33 types of antibiotics in urine samples (Table 1). Of them, 3 of HAs, 10 of VAs, and 20 of PVAs were identified. Interestingly, there was no difference in the level of HAs between the two groups. However, the concentration of 5 VAs and 13 PVAs was significantly higher, while the level of 1 VA and 5 PVAs was significantly lower in patient group than that of healthy group. Moreover, 4 VAs and 2 PVAs were only detected in patient group.

Since animal-used antibiotics have been widely utilized, a variety of antibiotic residues can deposit in the human bodies via animal-derived foods, including meat, milk et al. To investigate the association of antibiotic level in urine samples with the difference in food intake, we analyzed the difference in food consumption. As shown in Fig. 1, patient group had high proportion of animal-derived food (56.3%) and low proportion of vegetable-derived food (43.7%), while healthy group had high proportion of vegetable-derived food (65.4%) and low proportion of animal-derived food (34.6%). Based on participants' medication record and food consumption, our finding suggested that excessive consumption of animal-derived food may result in the high concentration of drug residues in urine.

3.2. More resistant genes are detected in E. coli rather than S. aureus in patient group

Being part of endogenous microbiota, both *E. coli* and *S. aureus* can easily be induced resistant genes after exposure to antibiotics. As a result, they are usually regarded as the indicators of antibiotic resistance. Since patient group exposed to VAs and PVAs more than healthy group, we investigated whether more resistant genes are induced in *E. coli* and *S. aureus*. We performed a PCR assay to amplify the resistant genes. As shown in Table 2, we found that several resistant genes existed in *E. coli* were only detected in the fecal samples of patient group, including *cml*A gene for chloramphenicol, *tet*A gene for tetracycline, *TEM*1 gene for β-lactam, two genes for quinolone (*qnr*-S and *qnr*-B), and genes for coding multidrug efflux pumps (*mdt*F and *mdt*B). *OXY* gene for β-lactam was detected in both the two groups. Interestingly, only one resistant gene from *S. aureus*, *erm*B gene, was detected in both the two groups. These results suggested that both *E. coli* and *S. aureus* were widely distributed in the intestine of young adults, and *E. coli* harbored more resistant genes than *S. aureus* in patient group.

3.3. The gut microbiota diversity in patient group is lower than that in healthy group

To compare the difference in gut microbiota composition between healthy group and patient group, we performed Illumina high throughput sequencing for all samples. Qualified reads ranging from 41,109 to 45,279 were obtained, and rarefaction curve reached to peak (data not shown). These results indicated that sequencing depth was enough to analyze gut microbiota.

We noticed that healthy group and patient group had completely different composition in gut microbiota, which was reflected by the measurements of within-sample diversity (α-diversity) and between-sample diversity (β-diversity). Shannon index was remarkably lower in patient group than that in healthy group (Fig. 2a). The decreased α-diversity in patient group meant that less bacterial species was recruited than that in healthy group. As shown in Fig. 2b, unconstrained principle coordinate analysis (PCoA) uncovered that gut microbiota in patient group and healthy group formed two distinct clusters, which were separated along the first coordinate axis.

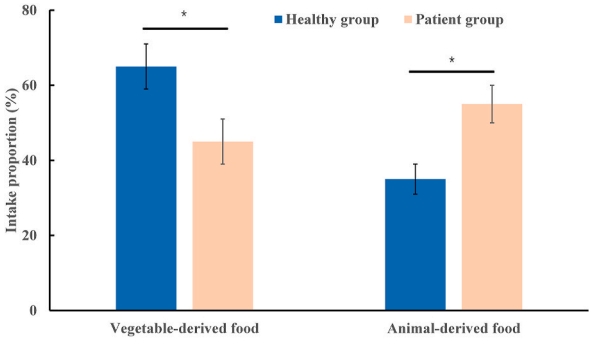

Fig. 1. The comparison of proportion of animal-derived food and vegetable-derived food consumed by the patient group and healthy group. *: $P < 0.05$.

Table 2
The list of detected antibiotic resistant genes.

	OXY	MdtB	MdtF	TEM-1	cml-A	qnr-B	qnr-S	tet-A
Health group	P	N	N	N	N	N	N	N
Patient group	P	P	P	P	P	P	P	P
Targeting the antibiotics	β-Lactam	Efflus pump	Efflus pump	β-Lactam	Chloramphenicol	Quinolone	Quinolone	Tetracycline

Note: P: gene was detected in fecal sample; N: gene was not detected in fecal sample.

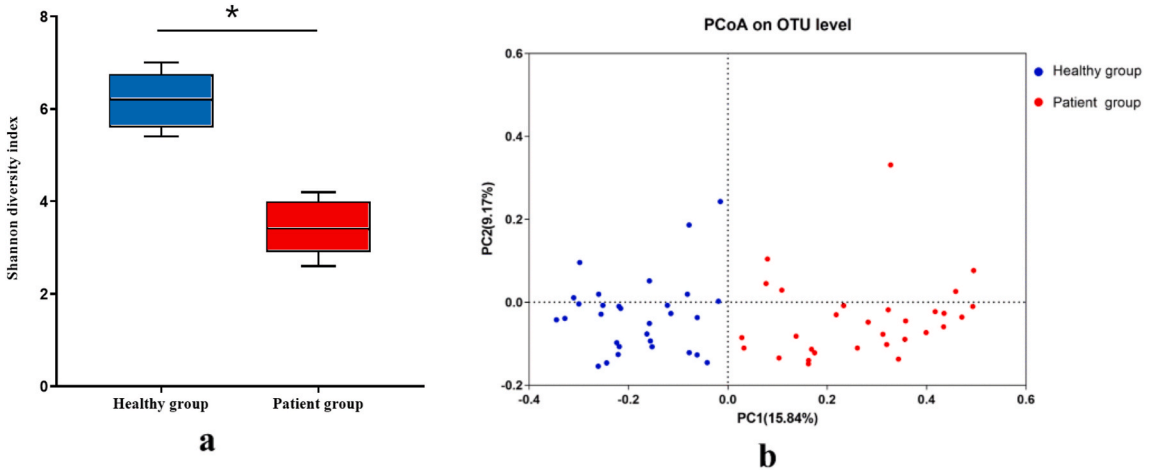

Fig. 2. The difference in the diversity of gut microbiota between patient group and healthy group according to the data of 16S rRNA. (a) The comparison of Shannon diversity index between patient group and healthy group; (b) Principal coordinate analysis (PCoA) with unweighted UniFrac distance showing that the gut microbiota of patient group separate from that of healthy group in the first axis.

3.4. The structure of gut microbiota and abundant species are different between the two groups

There was detectable and significant difference in the composition of gut microbiota between the two groups. At the phylum level (Fig. 3a), patient group was characterized by high percentage of Bacteroidetes (68.8%) and Proteobacteria (16.4%), whereas healthy group had high percentage of Firmicutes (49.6%) and low Bacteroidetes (47.2%). At the genus level (Fig. 3b), patient group was characterized by high percentage of *Bacteroides* (52.4%), *Sutterella* (14.2%), and *Parabacteroides* (4.5%), whereas healthy group had high percentage of *Proabacteroides* (20.8%), *Dialister* (13.0%), and *Alistipes* (4.2%).

After that, we conducted LEfSe analysis to discover the taxa that most likely explained the difference between the two groups. As shown in Fig. 4, the predominant bacteria showed changes at the different taxonomic levels which were illustrated by the result of

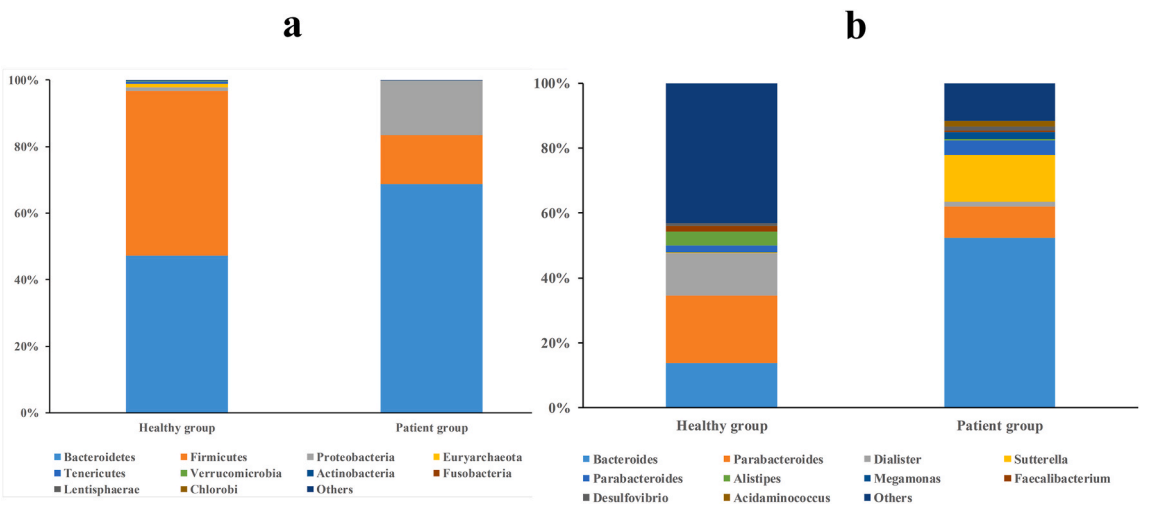

Fig. 3. The relative abundance of bacterial taxa at phylum (a) and genus (b) level in patient group and healthy group.

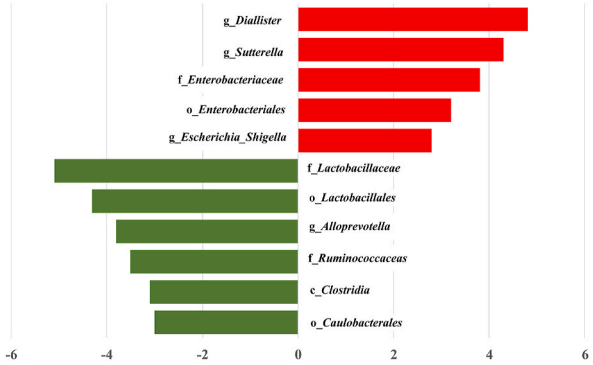

Fig. 4. The most differentially enriched taxa between patient group and healthy group which was identified via LDA score based on linear discriminant analysis effect size (LEfSe) analysis. Red bars indicate taxa were enriched in patient group, and green bars indicate taxa were enriched in healthy group.

LEfSe analysis. We observed an enrichment of *Dialister*, *Sutterella*, *Enterobacteriaceae*, *Enterobacteriales*, and *Escherichia_Shigella* in patient group, most of them were belonged to Bacteroidetes, while *Lactobacillaceae*, *Lactobacillales*, *Ruminococcaceae*, *Clostridia*, and *Alloprevotella* were enriched in healthy group.

3.5. LPS level is higher in the fecal samples of patient group than that in healthy group

Our result of bacterial abundance analysis at the phylum level indicated that Bacteroidetes and Proteobacteria, many species from them are LPS producing bacteria [16], were enriched in patient group. Now we were interested in whether there is any difference in LPS level. As shown in Fig. 5, LPS concentration in patient group was remarkably higher by 33.3% than that in healthy group.

3.6. Exposure to penicillin promotes RV replication

We noticed that RV only exists in the intestine of participants with high abundance of antibiotic residues in urine samples. To evaluate the causality between disrupted gut microbiota caused by antibiotic exposure and RV replication in the intestine, we administered orally RV to adult mice treated by penicillin for 1 week, while these mice were still receiving penicillin during RV infection. Although infecting adult mice with RV does not show severe diarrhea, it still can be used as an infection model. After oral inoculating adult mice with RV, we quantitated RV in mice feces by qPCR. As shown in Fig. 6, RV quantity started to increase in penicillin-treated mice after 2 days of inoculation, while it was delayed to 4 days in untreated mice. RV quantity reached to peak on the sixth day and kept stable after inoculation in penicillin-treated mice, while it reached to low peak on the eighth day and started to decline in untreated mice. These results suggested that penicillin exposure can promote RV replication.

3.7. LPS produced by imbalanced gut microbiota promotes RV replication in vivo

Since we found that patient grouphad high ratio of Bacteroidetes/Firmicutes and high level of LPS. We hypothesized that these changes are caused by antibiotic exposure. To confirm this hypothesis, mice were treated with penicillin and their gut microbiota was analyzed. Consistent with the result in human samples, we observed the disrupted gut microbiota caused by penicillin exposure, which

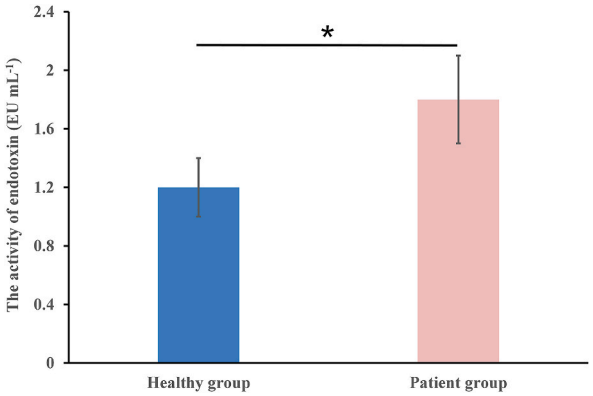

Fig. 5. The comparison of LPS level in faecal samples from patient group and healthy group. *: $P < 0.05$.

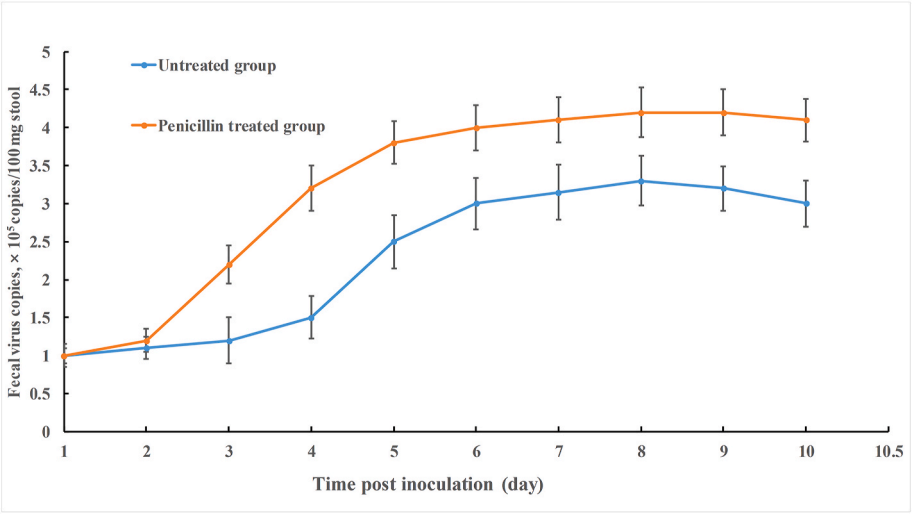

Fig. 6. RV replication in untreated mice and penicillin-treated mice at various time points tested. Feces were collected, and virus was quantified by qPCR. *: $P < 0.05$.

was characterized by high ratio of Bacteroidetes/Firmicutes. Meanwhile, we also observed the high level of LPS in penicillin-treated mice (data not shown).

Given that penicillin-treated mice still had a high bacterial load in the intestine, antibiotics cocktail was used to deplete gut microbiota for evaluating whether the absence of gut microbiota inhibits RV colonization. There was no culturable bacteria in mice feces after receiving antibiotics cocktail (data not shown). Both antibiotics cocktail-treated mice and control mice were orally inoculated with filtered RV. As shown in Fig. 7, antibiotics cocktail treatment resulted in a 2-fold reduction in RV quantity on 3 days after inoculation, confirming that absence of gut microbiota reduced RV replication in mice intestine. Interestingly, RV quantity was restored partly when LPS was added to drinking water in antibiotics cocktail-treated mice, indicating that LPS played an important role in RV replication.

3.8. LPS enhances virion thermal stability

Some findings showed that LPS enhances the stability of some enteric viruses, including poliovirus and reovirus, by increasing their thermal stability [10,11]. To validate whether LPS imposes similar effect on RV, we conducted Particle Stability Thermal Release assay (PaSTRy). As shown in Fig. 8, fluorescence intensity in PBS-treated RV reached a peak at 44 °C. However, the peak of fluorescence intensity in LPS-treated RV was increased to 48 °C, suggesting that LPS treatment stabilized the structure of RV. Interestingly, we found

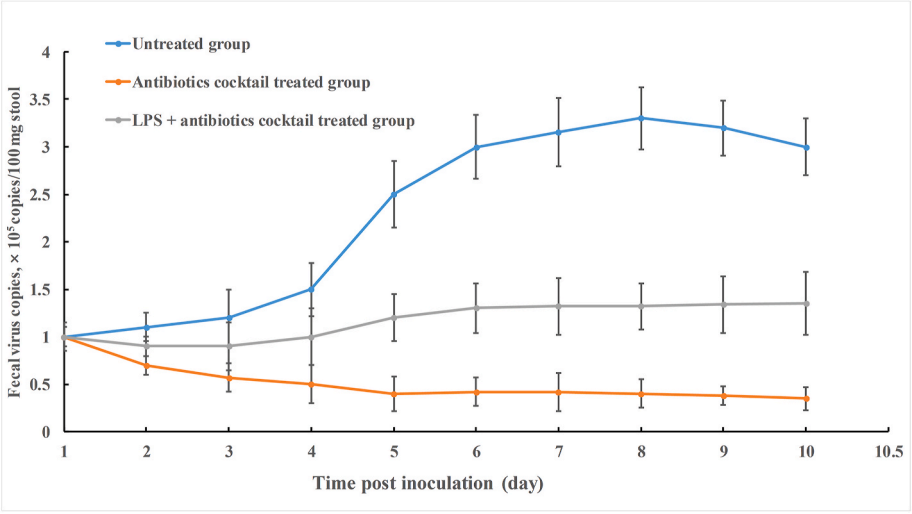

Fig. 7. RV replication in untreated mice receiving RV inoculation only and RV + antibiotics cocktail. Feces were collected, and virus was quantified by qPCR.

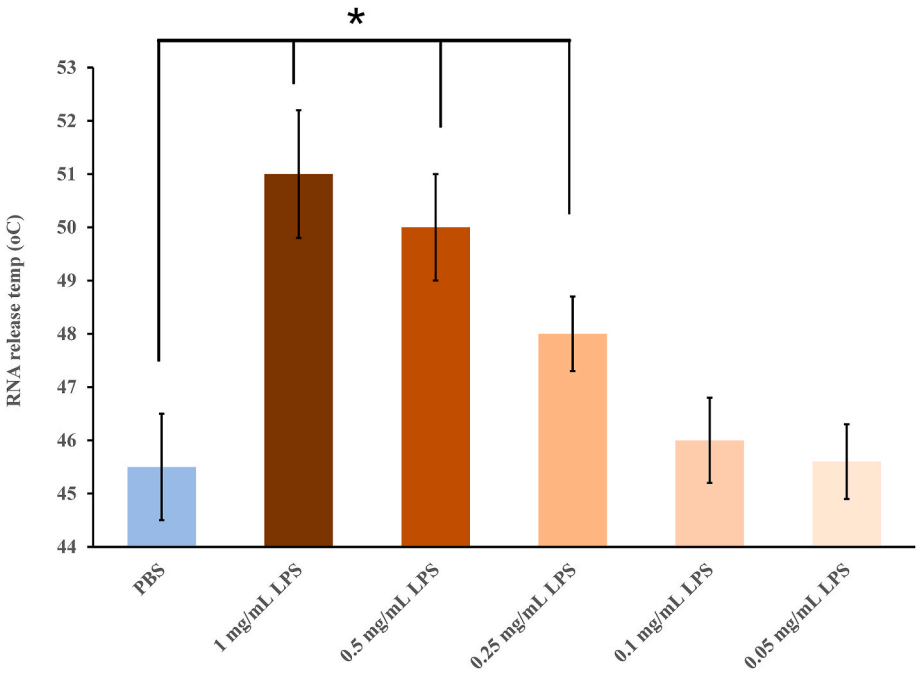

Fig. 8. Quantification of PaSTRy experiments indicating LPS promotes RV thermos stability in a LPS concentration dependent way. *: $P < 0.05$.

that thermal stabilization imposed by LPS was dose dependent, as the peak of fluorescence intensity was similar to control when RV was incubated in 0.1 mg/mL of LPS instead of 1 mg/mL. Overall, these results indicated that exposure to LPS enhanced RV thermal stability.

4. Discussion

Based on the current knowledge, RV is transmitted mainly by feces-oral route [2]. However, we found that 10% of young adults are asymptomatic RV-infected in our study. Perhaps adults with asymptomatic or mild symptoms are widely distributed in the population. Human-to-human dissemination is becoming a more important route than food chain for RV infection. According to our results, we found that RV carriers had high intake of animal-derived food which resulted in high load of VAs and PVAs in urine samples. As a result, it leaded to the disrupted gut microbiota characterized by abundant gram-negative bacteria and high level of LPS. We demonstrated that imbalanced gut microbiota caused by penicillin exposure enhances RV replication in vivo.

4.1. The relationship of dietary composition and antibiotic residues in urine samples

It has been extensively reported that antibiotic residues can be found in human urine [17]. The harmful effects of antibiotic residue on human health have been deeply discussed [18]. Since exposure to residual animal-used antibiotics from foods is continuous and hardly avoidable, it is necessary to explore its additional harmful effects, such as its effects on the interaction of gut microbiota and enteric viruses. Firstly, we detected high concentration of animal-used antibiotics in urine samples from RV carriers without record of drug administration. Since animal-used antibiotics are widely used, next we investigated the difference in food consumption between healthy group and patient group. We found that RV carriers had high intake of animal-derived foods. Several studies have described the linkage of intake animal-derived foods and antibiotic burden [19,20]. Our results provide evidence that excessive intake of animal-derived food may involve in the interaction of gut microbiota and enteric viruse.

4.2. The dysbiosis of gut microbiota in RV carriers

A balanced gut microbiota contributes to nutrition, metabolism, and pathogen resistance of a host. However, many factors, such as dietary and drugs, can cause the dysbiosis of gut microbiota [9,21]. Xiong et al. reported that RV infected infants had low diversity of gut microbiota, whereas healthy control had high diversity of gut microbiota [22]. Moreover, a reduction in gut microbiota diversity has also been found in RV infected children [23]. In our study, we also observed the shift in the structure of gut microbiota and decreased diversity in patient group. The decreased α-diversity implies the imbalance of gut microbiota, some predominant taxa are impaired while some rare taxa are promoted [24]. This finding is further illustrated by LEfSe analysis, indicating that some rare species became abundant in patient group. These findings suggest that gut microbiota with low diversity is a biomarker of RV carriers regardless of their ages.

After comparing the structure of gut microbiota between healthy group and patient group, we observed alterations in patient group. The proportion of Bacteroides and Parabacteroides was increased, while the proportion of Firmicutes and Euryarchaeota was decreased when compared to healthy group. Interestingly, several studies based on RV-caused diarrhea in infants or neonatal mice observed a decrease in the abundance of Bacteroides [22,25], which is contradict with our finding. Since gut microbiota in 5-year-old children has not reached adult complexity yet [26], perhaps it exerts its impact on RV replication in a different way with adults. Our findings are based on adult RV carriers and adult mice model, and their gut microbiota receive low-dose antibiotic exposure. We postulate that these differences lead to the contradiction. Based on the result of LEfSe analysis, we found that some probiotic genera, including Parabacteroides, Lactobacillus spp., and Alloprevotrlla were totally undetectable in patient group. Previous studies have demonstrated that probiotics can pose protective effects on human health, such as eliminating infections, restoring gut microbiota and producing lactate and short chain fatty acids [27,28]. The decrease of these probiotics in patient group maybe a result of antibiotic exposure because it has been proved that probiotics are more vulnerable than other genera [29]. Meanwhile, we noted a remarkable increase of *Escherichua_shigella* in patient group. Some studies have found a tight linkage between high abundance of *Escherichua_shigella* in intestine and many diseases. A study reported an expansion of *Escherichua_shigella* in nonalcoholic fatty liver disease patients, the increase of this genus is associated with disease severity [30]. Another study observed that high abundance of this genus impairs response to warfarin anticoagulation therapy in heart valve replacement patients [31]. Our results suggest that the disrupted gut microbiota, characterized by high abundance of gram-negative bacteria and low abundance of probiotics, play a critical role in RV replication in the intestine.

Since high abundance of VAs and PVAs was detected in the urine samples of patient group, we are interested in whether resistant genes are induced in gut microbes. *E. coli* and *S. aureus* were chosen as target species because both of them are commensal microbes and representative antibiotic-resistant bacteria in gram-negative bacteria and gram-positive bacteria, respectively [32,33]. In our study, they showed different response to VAs and PVAs exposure. *E. coli* exhibited more resistant genes while *S. aureus* did not in patient group. It is reasonable to deduce that emerging many resistant genes leads to the dominance of gram-negative bacteria in the intestine of patient group.

The adult mouse model can be used to investigate the role of gut microbiota during RV infection. After eliminating gut microbiota via antibiotics cocktail, we observed a significant decrease in RV quantity. This result indicated that presence of gut microbiota is essential for RV replication. It is in accordance with the finding that microbiota elimination delay infection and reduce infectivity of RV [34]. However, the mechanism of gut microbiota promoting RV replication remains unclear.

4.3. The effect of LPS on RV stability

The mechanism of LPS promoting the stability of enteric viruses, including poliovirus and reovirus, has been described in some studies [11,35]. Since all of them are RNA viruses, we deduced that RV stability is promoted by LPS in the same way. In our study, we observed an increment of LPS level in fecal samples of patient group and penicillin-treated mice. RV thermal stability promoted by LPS was validated in vitro. Our findings are consistent with previous reports. These data demonstrated that exposure to antibiotics resulted in LPS increase, which leaded to the enhancement of RV replication.

5. Conclusion

In this study, we illustrated the remarkable difference in gut microbiota structure caused by VAs and PVAs exposure. Gut microbiota plays a critical role in RV infection. We verified that LPS promotes RV replication by enhancing RV thermal stability. Our research provides useful data regarding the mechanism of enteric virus replicating in the intestine of adult human.

However, there are still several limitations. First, the major limitation is the small sample size. Larger cohort is needed to address this limitation. Second, we did not determine the concentration of antibiotics in food and drinking water. The main contributive foods to antibiotic level in urine are not surveyed. Third, only *E. coli* and *S. aureus* were chosen for resistant gene detection. Metagenomics is likely to provide insight into the microbiome members involving in drug resistance. Fourth, only the impact of LPS on RV thermal stability was tested. More impacts of LPS on RV are needed to be investigated.

Declarations

Author contribution statement

Yuhui Li: Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials; Wrote the paper.

Yifan Wu, Jie Wu, Lingling Yu: Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

Xin Li, Ke Xie, Mingyi Zhang: Contributed reagents, materials, analysis tools or data.

Lingling Ren: Analyzed and interpreted the data; Wrote the paper.

Yanli Ji, Yehao Liu: Performed the experiments; Conceived and designed the experiments; Wrote the paper.

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Data availability statement

Data included in article/supp. material/referenced in article.

Declaration of interest's statement

The authors declare no competing interests.

Additional information

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