A pilot study, randomized control trial on effect of oral calcium carbonate to fecal functional levofloxacin concentration in healthy volunteer taking oral levofloxacin

Mr. Ophat Janphet

A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science in Medicine
Department of Medicine
FACULTY OF MEDICINE
Chulalongkorn University
Academic Year 2021

การศึกษานำร่องแบบสุ่มถึงผลของยาแคลเซียมคาร์บอเนตต่อระดับยาลีโวฟลอกซาซินในอุจจาระที่ออกฤทธิ์ยับยั้งแบคทีเรียในอาสาสมัครที่มีสุขภาพดี

นายโอภาส จันทร์เพ็ชร

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
สาขาวิชาอายุรศาสตร์ ภาควิชาอายุรศาสตร์
คณะแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
ปีการศึกษา 2564

Thesis Title	A pilot study, randomized control trial on effect of oral calcium carbonate to fecal functional levofloxacin concentration in healthy volunteer taking oral levofloxacin
By	Mr. Ophat Janphet
Field of Study	Medicine
Thesis Advisor	Doctor VORAPHOJ NILARATANAKUL, M.D., Ph.D.

Accepted by the FACULTY OF MEDICINE, Chulalongkorn University in Partial Fulfillment of the Requirement for the Master of Science

..... Dean of the FACULTY OF MEDICINE
(Associate Professor Doctor CHANCHAI SITTIPUNT, M.D.)

THESIS COMMITTEE

..... Chairman
(Associate Professor Doctor LALITA WATTANACHANYA, M.D.)

..... Thesis Advisor
(Doctor VORAPHOJ NILARATANAKUL, M.D., Ph.D.)

..... Examiner
(Assistant Professor Doctor MARISA PONGPRUTTHIPAN, M.D.)

..... External Examiner
(DOCTOR Nirada Siriyakorn)

โอภาส จันทร์เพ็ชร : การศึกษานำร่องแบบสุ่มถึงผลของยาแคลเซียมคาร์บอเนตต่อระดับยาลีโวฟลอกซาซินในอุจจาระที่ออกฤทธิ์ยับยั้งแบคทีเรียในอาสาสมัครที่มีสุขภาพดี. (A pilot study, randomized control trial on effect of oral calcium carbonate to fecal functional levofloxacin concentration in healthy volunteer taking oral levofloxacin) อ.ที่ปรึกษาหลัก : ดร. นพ.วรพจน์ นิลรัตนกุล

ที่มา: ยาลีโวฟลอกซาซินนอกจากถูกใช้ในการรักษาโรคติดเชื้อแล้วยังส่งผลต่อจุลชีพในลำไส้ โดยหากนำยามาผสมกับสารประกอบแคลเซียมในหลอดทดลองพบว่ายายับยั้งเชื้อแบคทีเรียได้ลดลง แคลเซียมจึงอาจช่วยปกป้องจุลชีพในลำไส้ได้

วัตถุประสงค์: เพื่อศึกษาผลของยาแคลเซียมคาร์บอเนตว่าสามารถลดระดับยาลีโวฟลอกซาซินในอุจจาระได้หรือไม่ และผลต่อความหลากหลายทางชีวภาพของเชื้อจุลินทรีย์ในลำไส้ในกลุ่มอาสาสมัครสุขภาพดีที่รับประทานยาลีโวฟลอกซาซิน

วิธีการวิจัย: การทดลองแบบนำร่องแบบสุ่ม ในอาสาสมัครสุขภาพดี 20 ราย ซึ่งรับประทานยาเม็ดลีโวฟลอกซาซินขนาด 500 มก. ต่อวันนาน 5 วัน โดยแบ่งอาสาสมัครเป็น 2 กลุ่มคือกลุ่มทดลองรับประทานยาแคลเซียมคาร์บอเนตขนาด 1000 มก. 2 ครั้งต่อวันนาน 6 วัน และกลุ่มควบคุมไม่ได้แคลเซียมคาร์บอเนต ผลการศึกษาหลักคือระดับยาลีโวฟลอกซาซินในอุจจาระโดยวิธีการวัดระดับยาที่ต่ำที่สุดที่สามารถยับยั้งแบคทีเรียได้หรือ MIC และวิธีโครมาโทรกราฟชนิดของเหลวประสิทธิภาพสูงหรือ HPLC วัดระดับในวันที่ 2 และ 5 หลังเริ่มยาลีโวฟลอกซาซิน ผลการศึกษารองได้แก่ 1) ดัชนีแชนนอนของความหลากหลายทางชีวภาพของแบคทีเรียในอุจจาระจากการวิเคราะห์ 16-เอส ไรโบโซมอลดีเอ็นเอ 2) ระดับยาลีโวฟลอกซาซินสูงสุดในพลาสมา 3) ผลข้างเคียงไม่พึงประสงค์จากยาในระยะเวลา 4 สัปดาห์หลังเริ่มยา

ผลการศึกษา: อาสาสมัครเข้าร่วม 20 ราย พบว่าระดับยาลีโวฟลอกซาซินในอุจจาระของกลุ่มทดลองมากกว่ากลุ่มควบคุมคือ 100.50 (SD=64.88) และ 53.21 (SD =39.57) ไมโครกรัมต่อมิลลิลิตรตามลำดับ โดยการวัด MIC ของวันที่ 5 อย่างมีนัยสำคัญทางสถิติ (p = 0.024) ไม่พบความแตกต่างอย่างมีนัยสำคัญทางสถิติของระดับยาลีโวฟลอกซาซินในอุจจาระโดย HPLC ระดับยาลีโวฟลอกซาซินสูงสุดในพลาสมาและดัชนีแชนนอน แต่ในกลุ่มทดลองมีดัชนีแชนนอนหลังได้ยาแคลเซียมคาร์บอเนตลดลงอย่างมีนัยสำคัญทางสถิติ (p = 0.0019) พบเพียงผลข้างเคียงไม่รุนแรงจากยา (3 รายในกลุ่มทดลอง และ 5 รายในกลุ่มควบคุม) คือ อาการคลื่นไส้และท้องเสีย

สรุปผล: การรับประทานยาเม็ดแคลเซียมคาร์บอเนตสัมพันธ์กับระดับยาลีโวฟลอกซาซินในอุจจาระที่สูงขึ้นโดยการทดสอบ MIC แต่ไม่มีผลต่อระดับยาลีโวฟลอกซาซินสูงสุดในพลาสมา แทนที่ยาแคลเซียมคาร์บอเนตจะสามารถปกป้องความหลากหลายทางชีวภาพในลำไส้จากยาลีโวฟลอกซาซิน แต่กลับทำให้ความหลากหลายทางชีวภาพลดลง จึงอาจจะต้องระวังการจ่ายยาลีโวฟลอกซาซินร่วมกับยาเม็ดแคลเซียมคาร์บอเนตไม่ว่าจะเป็นชนิดกินหรือฉีด แม้ว่าจะบริหารยาไม่ให้มีผลต่อการดูดซึมแล้วก็ตาม ซึ่งต้องมีการศึกษาเพิ่มเติมต่อไปในอนาคต

สาขาวิชา	อายุรศาสตร์	ลายมือชื่อนิสิต .....
ปีการศึกษา	2564	ลายมือชื่อ อ.ที่ปรึกษาหลัก .....

6370116530 : MAJOR MEDICINE

KEYWORD: Levofloxacin, calcium carbonate, fecal levofloxacin concentration, gut microbiota diversity, peak plasma levofloxacin concentration

Ophat Janphet : A pilot study, randomized control trial on effect of oral calcium carbonate to fecal functional levofloxacin concentration in healthy volunteer taking oral levofloxacin. Advisor: Dr. VORAPHOJ NILARATANAKUL, M.D., Ph.D.

Objective: We conducted a pilot randomized control trial (RCT) to study whether oral calcium carbonate ($CaCO_3$) can lower fecal levofloxacin (LVX) concentration and preserve gut microbiota diversity in healthy volunteers.

Methods: The healthy volunteers received a 5-day course of once-daily 500 mg LVX oral tablet and were randomly assigned to treatment (6-day course of 1,000 mg $CaCO_3$ oral tablet twice daily) and control group (no $CaCO_3$). The primary outcome was fecal LVX concentration by MIC and high-performance liquid chromatography (HPLC) on day 2 and 5. The secondary outcomes were fecal microbiota diversity Shannon index (H) by 16s rDNA analysis, plasma LVX C_{max} by HPLC, and drug adverse events (AEs) in 4 weeks period.

Results: Total 20 volunteers were enrolled and randomly assigned to treatment and control group. Mean fecal LVX concentration was higher in treatment than control group, 100.50 (SD=64.88) vs 53.21

(SD =39.57) μg/ml by MIC at day 5 (95% CI 4.912, 89.73; p = 0.0242). No difference in mean fecal LVX concentrations by HPLC, plasma LVX C_{max}. Treatment group had significantly declined in H index (p = 0.0019). Only mild AEs included nausea and diarrhea.

Conclusion: $CaCO_3$ is significantly related to higher fecal LVX level by MIC but does not significantly affect the LVX C_{max}. However, rather than protecting gut microbiota from LVX, $CaCO_3$ may lower gut microbiota diversity in the presence of LVX. Therefore, co-prescription of LVX and $CaCO_3$ might be cautioned even without the concern about the absorption and further research is needed in the future.

Field of Study:	Medicine	Student's Signature .....
Academic Year:	2021	Advisor's Signature .....

ACKNOWLEDGEMENTS

First and foremost, I would like to thank my advisor Dr. Voraphoj Nilaratanakul for advising me in doing this project. He provided me an invaluable advice and helped me in difficult periods. His motivation and help contributed tremendously to success completion this project.

Besides, I would like to thank all co-advisor who helped me by giving me advice and providing the equipment which I needed.

Also I would like to thank my family and friends for their support. Without that support I couldn't have succeeded in completing this project.

At last but not in least, I would like to thank everyone who helped and motivated me to work on this project.

Ophat Janphet

TABLE OF CONTENTS

LIST OF TABLES

Page

LIST OF FIGURES

Page

BACKGROUND AND RATIONALE

Nowadays, antimicrobial therapy is widely used in many indications, besides killing pathogens. It can cause numerous complications, such as drug allergy, antibiotic associated diarrhea caused by *Clostridioides difficile* infection, and increasing risk of metabolic syndrome. Fluoroquinolones, especially levofloxacin, are widely used in various forms, including oral, intravenous, and eye drop forms.

Levofloxacin has a broad-spectrum coverage against gram-positive and gram-negative bacteria. It may cause dysbiosis of human gut microbiota from the fraction excreted into the upper intestine via bile for both oral and parenteral forms, reaching the cecum and colon where it can exert devastating effects on the gut microbiota. There have been many studies that demonstrated the strategies, such as oral β-lactamase and activated charcoal, to preserve the intestinal microbiota from deleterious consequence of dysbiosis during antibiotic treatment.

Based on current knowledge, metal ions can interact with fluoroquinolones and affect their solubility, pharmacokinetic and bioavailability. Bactericidal activity of fluoroquinolone-metal complex against bacteria was decreased. We hypothesized that ion-compound such as oral calcium carbonate may protect gut microbiota against excess levofloxacin in intestinal lumen. Concerning the effect of calcium carbonate on levofloxacin absorption, volunteers would take levofloxacin and calcium carbonate at least 2 hours apart. We then could measure the level of fecal levofloxacin, gut microbiota diversity and plasma levofloxacin concentration.

RESEARCH QUESTION

PRIMARY RESEARCH QUESTION

Is fecal levofloxacin functional level in healthy volunteers different between calcium carbonate and control group?

SECONDARY RESEARCH QUESTION

1. Is fecal microbiota diversity in healthy volunteers receiving levofloxacin different between calcium carbonate group and control group?
2. Does the maximal plasma levofloxacin concentration decline when taking calcium carbonate two hours apart?

OBJECTIVE

PRIMARY OBJECTIVE

To study the effect of calcium carbonate to fecal levofloxacin functional concentration by MIC method and total concentration by HPLC method at day 2 and day 5 in healthy volunteers taking oral levofloxacin tablets for 5 days. The MIC method utilizes known levofloxacin MIC value of *E. coli* ATCC 25922 strain as a comparison. The last fecal dilution that can inhibit *E. coli* ATCC 25922 will be assumed to have the same levofloxacin level as its MIC. The fecal levofloxacin functional concentration can be calculated by multiplying the MIC with the dilution factor.

SECONDARY OBJECTIVE

1. To compare the difference of fecal microbiome diversity in heathy volunteers taking levofloxacin for 5 days between calcium carbonate group and control group in day 2, 5, 14 and 30 by 16s ribosomal DNA sequencing method.
2. To study the difference of peak plasma levofloxacin concentration in heathy volunteers taking levofloxacin for 5 days between calcium carbonate group and control group in day 1 and day 5.

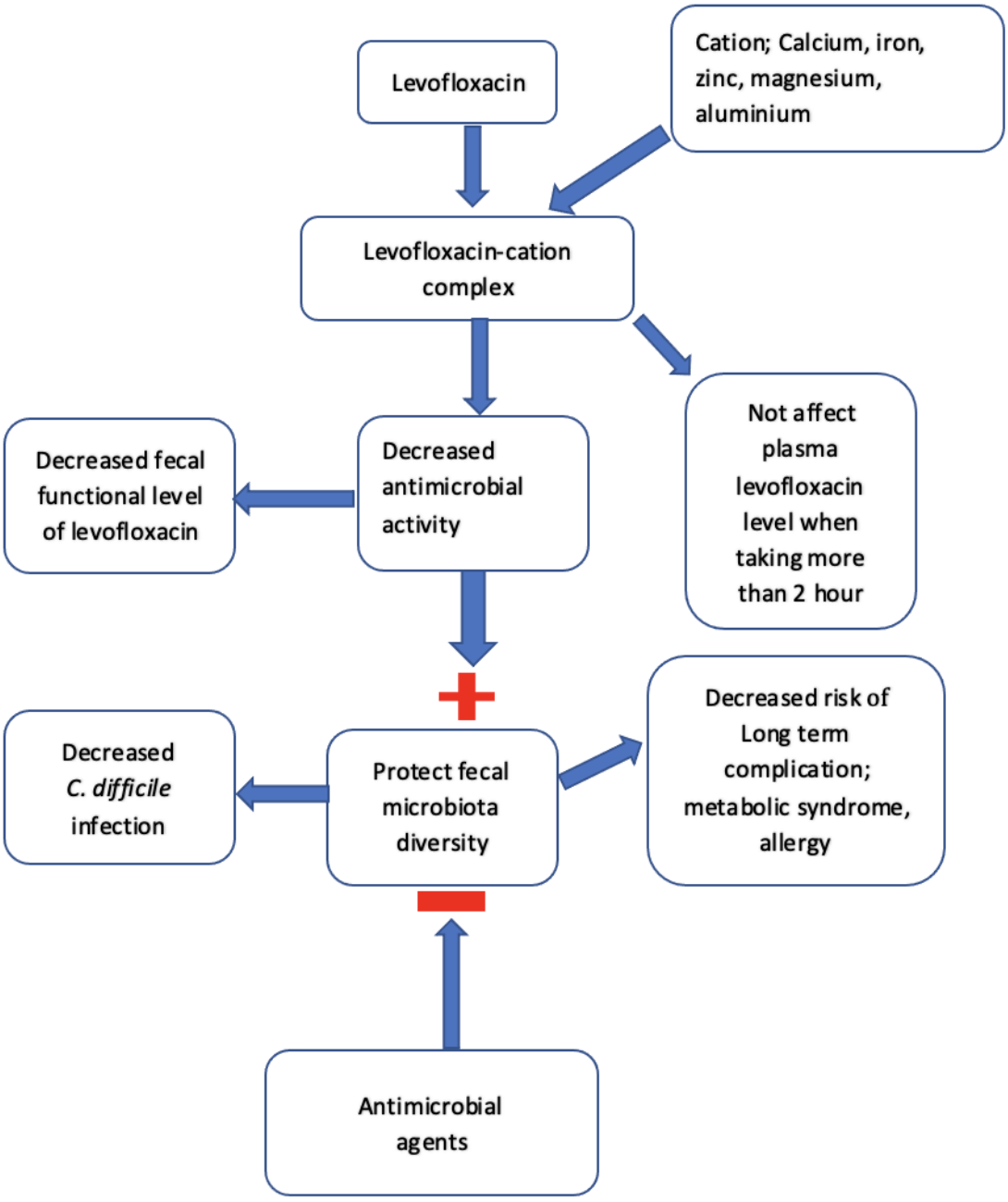
HYPOTHESIS

- Calcium carbonate can decrease fecal levofloxacin functional level (MIC method) at day 2 and day 5 in healthy volunteers taking oral levofloxacin tablet for 5 days.

- Calcium carbonate can preserve fecal microbiome diversity in healthy volunteers taking oral levofloxacin tablet for 5 days.

- There is no statistical difference of peak plasma levofloxacin concentration at 2 hours in patient treated with oral calcium carbonate and levofloxacin, taking 2 hours apart.

Figure 1. CONCEPTUAL FRAMEWORK



ASSUMPTION

Calcium ion can form complex with levofloxacin and decrease its activity. We confirmed this effect *in vitro* that mixing levofloxacin with calcium gluconate tremendously increase the levofloxacin MIC of *E. faecalis* and *E. coli*. We assumed this effect could be generalized to other bacteria.

The confounding factors should be minimized. We excluded patient with abnormal bowel habit (such as constipation and diarrhea) and informed the volunteers to have the same quantity and type of diet.

RERSEARCH VENUE:

1. Laboratory room No. 2, 5th floor, zone C, Bhumisiri Mangkhalanusorn Building

Division of Infectious Disease, Department of Medicine, Faculty of Medicine, Chulalongkorn University, Tel. 02-256-4000; 80506.

Work: sample preparation and extraction.

2. Laboratory room No. 1619, 16th floor, Aor Por Ror Building, Department of Medical Microbiology, Faculty of Medicine, Chulalongkorn University, Tel. 02-256-4132

Work: measurement of fecal levofloxacin concentration by MIC method using *E. coli* ATCC 25922 strain.

3. Pharmacology laboratory room No. 927, 9th floor, Padtayapatana Building, Department of Pharmacology, Faculty of Medicine, Chulalongkorn University, Tel. 02-251-1965

Work: measurement of fecal and plasma levofloxacin concentration by HPLC method

Duration 16 months (1 October 2020 to 30 January 2022, total 16 months)

OPERATIONAL DEFINITION

1) Fecal functional levofloxacin concentration (MIC method)

   Concentration of levofloxacin in feces that can inhibit growth of *E. coli* ATCC 25922 (levofloxacin MIC of 0.08 mcg/ml). The fecal solution is serially 2-fold diluted and then incubated with the *E. coli* ATCC 25922. The last dilutional fold that can still inhibit *E. coli* ATCC 25922 will be multiplied with 0.08 mcg/ml (MIC) to estimate the fecal functional levofloxacin concentration. More dilutional fold means more concentration in the original stool.

2) Fecal microbiota diversity

The range of different kinds of unicellular organisms using a quantitative measure that reflect how many different types of fecal bacterial microbiota using 16s rDNA sequencing analysis. The datasets are summarized as Shannon index in this study.

3) Peak plasma levofloxacin concentration (C_{max})

The concentration of levofloxacin that measured 2 hours after taking levofloxacin tablet.

RESEARCH DESIGN

Pilot open-label randomized control trial

BRIEF RESEARCH METHODOLOGY

POPULATION and SAMPLE

Healthy volunteers without current medical illness

Approach to participant

Advertisement board for healthy volunteer recruitment

Inclusion criteria

1. Healthy volunteers, male or female, more than or equal to 18 but less than 45 years old
2. No underlying disease
3. No history of receiving any medication within 1 month and no history of antibiotic use within 3 months
4. No history of drug allergy
5. Ability to swallow tablet medicine
6. No constipation
7. Body mass index 18–25 kg/m^2
8. eGFR (MDRD) > 60 ml/min/1.73m^2
9. Normal liver function test, evaluated by investigators

Exclusion criteria

1 Pregnancy or lactation

2 Use of mineral or vitamin or herbal medicine supplementation within 1 month, especially metal compound such as iron, zinc, magnesium, calcium, aluminium, etc. or any vitamins.

OBSERVATION AND MEASUREMENT

Primary outcome

Fecal levofloxacin functional level on day 2 and 5 after in healthy volunteers taking oral levofloxacin for 5 days that can inhibit *E. coli* ATCC strain 25922 and using diluted stool to calculate concentration from dilutional folds

Secondary outcome

1. Fecal gut microbiota diversity in healthy volunteers taking levofloxacin for 5 days by 16s rDNA sequencing on day 2, 5, 14 and 30

2. Peak plasma levofloxacin concentration (C_{max}) using high performance liquid chromatography (HPLC) on day 1 and day 5

Research process

There were 2 periods in this research.

1. First period: run-in period before taking levofloxacin

2. Second period: during taking levofloxacin

First period: before taking levofloxacin

1. The researcher did medical history taking, physical examination, including height, body weight, body mass index, vital sign, and blood check for liver function test and renal function test (eGFR). The volunteers were advised avoiding milk or dairy products at least 1 week before attending the study.
2. Box of four randomization was applied to assign volunteers into two arms (10 subjects each), $CaCO_3$ group and No $CaCO_3$ group. $CaCO_3$ group had 1000 mg calcium carbonate tablet twice daily before meal (1st dose 12:00-1:00 p.m. and 2nd dose 6:00-7:00 p.m.) for 6 days. Their stools were collected in the morning on day 0, 2, 5, 14, and 30 to evaluate fecal microbiota diversity.
3. During the run-in period of calcium carbonate, the volunteers were advised taking 1000 mg calcium carbonate tablet before meal twice daily for 6 days to evaluate the adverse effects of calcium carbonate including constipation or gastrointestinal irritation. If the volunteers could not tolerate the adverse effects or had constipation, they would be excluded from the study.

Second period: during taking levofloxacin

1. We randomly reassigned the first period $CaCO_3$ group and control group into 2 new groups, having (experimental group) or not having (control group) calcium carbonate during second period (10 volunteers per group) demonstrated in **Figure 3**.
2. All volunteers took 500 mg oral tablet levofloxacin once daily, 30 minutes before meal at 8:00 a.m. to avoid confounders from food/drug interactions for 5 days.

   The drug prescription is shown below:

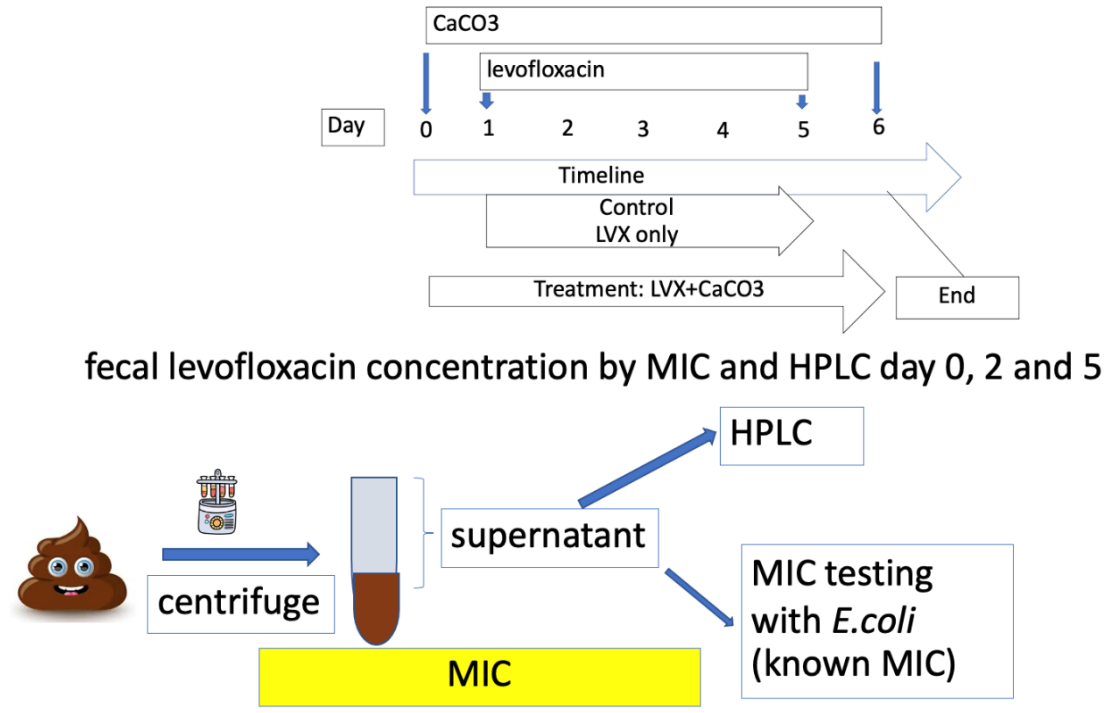
   Experimental group

- Taking a 1000 mg oral tablet calcium carbonate twice daily before meal (1st dose on 12:00 p.m. and 2nd dose 6:00 p.m.) for 6 days, starting 1 day before levofloxacin
- Taking a 500 mg oral tablet levofloxacin once daily, 30 minutes before meal at 8:00 a.m. for 5 days

   Control group

- Not receiving an oral tablet calcium carbonate
- Taking a 500 mg oral tablet levofloxacin once daily, 30 minutes before meal at 8:00 a.m. for 5 days

Figure 2. The time sequence of taking levofloxacin and calcium carbonate

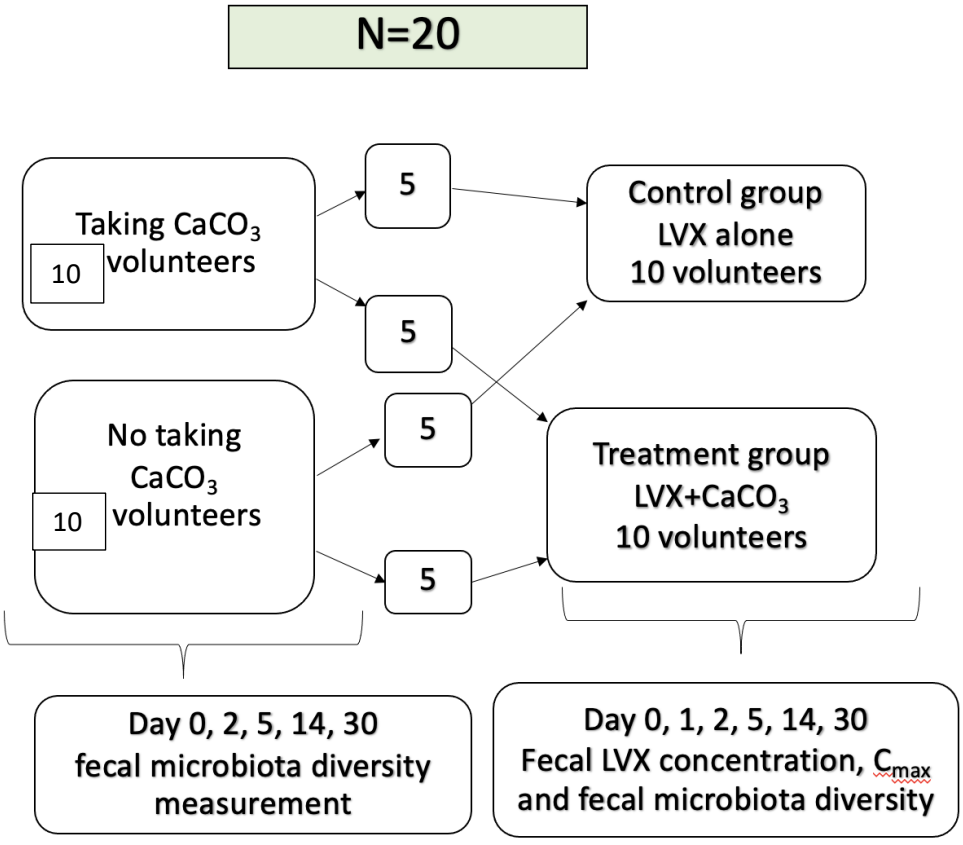


Calcium carbonate was started on day 0 in the experimental group. Levofloxacin was started on day 1 in both groups.

3. Fecal levofloxacin functional level, the primary outcome, was measured (micrograms per kilogram) in both groups on day 0, 2, 5 during 6:00–10:00 a.m. The amount of stool should be at least 1 gm or 1 ml. The fecal specimens were sent immediately to the infectious disease laboratory within 1 hour. They were diluted 1:10 in water and then centrifuged at 3000g for 10 minutes. The supernatants were double-filtered with a 0.4-micron syringe filter and 100kDa centrifugal filter to eliminate bacterium, phage, antibody, and other macromolecule contaminations. The filtrates were sent to the microbiology lab for measuring fecal levofloxacin functional level by MIC method using *E. coli* ATCC 25922. The dilutional

folds were calculated back for fecal levofloxacin functional level. Total levofloxacin level was also measured by high performance liquid chromatography (HPLC) in the pharmacology lab.

4. Secondary outcome
    - Blood test for plasma levofloxacin C_{max} on day 1 and day 5, 2 hours after first dose levofloxacin (at 10:00 a.m.)
    - Stool Collection for fecal microbiota diversity testing by 16S ribosomal DNA sequencing on day 0, 2, 5, 14 and 30 (amount at least 1 gm or 1 ml)



Remark: Amount of stool sample must be at least 1 gram.

ETHICAL CONSIDERATION

1) Respect for person

Volunteers had freedom for decision making to participate in the study. Informed consents were signed by all volunteers.

2) Beneficence/Non-maleficence

The investigator informed the volunteers there was no direct benefit to them, but new medical knowledge might be gained from this study. The adverse effects from levofloxacin were fully explained to the volunteers, including drug allergy, tendinopathy, and nausea/vomiting. The adverse effects were closely monitored by the investigator and medical service would be provided immediately if the incidence had occurred.

The volunteers were advised stopping the culprit drug if adverse event had occurred.

The investigators would protect the privacy and security of the volunteers' personal information.

3) Justice

There were both inclusion and exclusion criteria, and we did randomized assignment for equal risk exposure to intervention.

Informed consent process

1. The investigator informed the volunteers about the objectives, rationale, principle, risk/benefit of the study, drug information of both levofloxacin and calcium carbonate, and the protocol for blood check and specimen collection.
2. The leaflet of research information was provided to all volunteers. The volunteers independently decided whether to participate in the study or not.

LIMITATION

1) The sample size may not be enough to distinguish the outcomes between experimental and control groups.

2) The volunteers may have constipation during treatment, resulting fecal specimen collection error.

3) Unexpected illness may occur to volunteers, including antibiotic prescription during the study.

EXPECTED BENEFIT AND APPLICATION

1. Discovering the effect of oral tablet calcium carbonate on fecal levofloxacin functional level. If the results support evidence that calcium carbonate can decrease fecal levofloxacin functional level, we may utilize oral tablet calcium carbonate to protect gut microbiota from nonabsorbable/excreted levofloxacin.

2. Confirming the negligible effect of calcium carbonate on levofloxacin absorption when taking at least 2 hours apart.

3. Demonstrating the effect of calcium carbonate on fecal microbiome diversity in healthy volunteers taking levofloxacin.

OBSTACLES AND STRATEGIES TO SOLVE THE PROBLEMS

Obstacles

1) Some volunteers might experience constipation from oral calcium carbonate. This might interrupt the schedule of stool collection.

2) There might be some errors from specimen collection, including environmental contamination, delayed specimen transportation, affecting fecal microbiome study and fecal levofloxacin functional level.

3) Some volunteers might experience illness during the study period, such as diarrhea for which antibiotic might be prescribed, altering fecal microbiota population.

STRATEGIES TO SOLVE THE PROBLEMS

1) There was a manual for self-collection of specimens with easy wording. The volunteers would be advised for correct specimen collection. Standard specimen containers were distributed to all volunteers.

2) Delayed specimen transport would be solved by making an appointment to laboratory and transportation staff.

3) If the volunteers experienced medical illness during study period or had any antibiotic prescription, the volunteers would have been withdrawn from study. They had to wait for at least 3 months after stopping the antibiotic to be enrolled again.

4) If the volunteer had some problems with their specimen collection, they could directly consult the investigator 24 hours every day.

REVIEW OF RELATED LITERATURES

Antimicrobial agents constitute one of the most medically important and effective class of drugs. However, during systemic treatments, the nonabsorbable part of orally administered drugs, as well as a fraction excreted via bile into upper intestine for both oral and parenteral forms, can reach the large intestine and cause devastating effects on gut microbiota with both short- and long-term consequences. Short-term effects include diarrhea, *Clostridioides difficile* infection (CDI)[1] and selection of antibiotic-resistant microorganisms. Long-term effect links to allergy[2], insulin resistance,[3] and obesity.[4-7] This long-term effect has been shown to correlate with a gut microbiota of lower bacterial richness than in healthy individuals. Solutions to protect intestinal microbiota from deleterious consequences of dysbiosis during antibiotic treatment would be benefits to prevent patients from short- and long-term consequences. Many strategies, including oral β-lactamase, prevented the impact of parenteral β-lactam on microbiota.[8, 9] Delivery of nonspecific adsorbent to the colon can also partially decrease fecal concentration of ciprofloxacin without significantly affecting its plasma pharmacokinetics in rats.[10, 11]

De Gunzburg et al, performed a randomized controlled trial using activated charcoal (DAV 132) in 28 humans divided into two groups, with or without DAV 132 coadministration. Both groups received moxifloxacin for 5 days in 2 parallel groups. Primary outcome was the decrease of free moxifloxacin fecal concentrations by 99%, while plasmatic levels were unaffected. Shotgun quantitative metagenomics showed the richness and composition of the intestinal microbiota were largely preserved in subjects co-treated with DAV 132 in addition to moxifloxacin. No adverse effect was observed. Richness of gut microbiota was failed to return to initial value even at day 37 in the control group.[12]

Levofloxacin is a third-generation fluoroquinolone available in many formulations, including parenteral, intravenous and eye drop forms. Chemical structure is L-isomer of ofloxacin. Levofloxacin oral tablet and solution formulations are bioequivalent. Food and Drug Administration (FDA) approved levofloxacin for treatment many infectious conditions including bacterial conjunctivitis, bacterial sinusitis, community-acquired pneumonia, skin and soft tissue infection, urinary tract infection, and bone/joint infection.[13]

Levofloxacin has bactericidal property by inhibiting DNA-gyrase enzyme, resulting in inhibition of replication and transcription. Levofloxacin has concentration-dependent bactericidal activity, peak/MIC, and AUC/MIC have been identified as possible pharmacodynamic predictors of clinical and microbiological outcome as well as development of bacteria resistance. Levofloxacin also has bactericidal activity against gram negative bacteria, even though plasma level is under MIC, the phenomenon called prolonged post-antibiotic effect.[14, 15]

Levofloxacin has broad spectrum activity against gram-positive and gram-negative bacteria, including Enterobacteriaceae and non-glucose fermenter such as *Pseudomonas aeruginosa*. Common gut microbiota, *Escherichia coli* CLSI clinical breakpoint for susceptibility is 0.5 mcg/ml or below and resistance if equal to or more than 2 mcg/ml. Main oral absorption is via small bowel absorption to portal system then contributed to systemic circulation.(16) Peak plasma level is 1 to 2 hours. Maximal plasma concentration (C_{max}) is 5.7± 1.4 mg/L in levofloxacin 500 mg tablet. Protein binding is 24-38%. It has good lipophilic property and its tissue to plasma concentration is 2-5 folds including connective and lung tissues. Levofloxacin is eliminated mainly via urinary excretion up to 87% and feces less than 4% (little via hepatic metabolism). Its half-life is 6-8 hours.[17, 18]

Edlund et al. performed the randomized control trial involving 20 volunteers, they were randomly assigned to receive oral levofloxacin and ofloxacin, the result

demonstrated that both levofloxacin and ofloxacin decreased intestinal microbiota, mainly toward against gram negative bacteria. The pharmacokinetic study showed that peak plasma levofloxacin concentration was steady in day 4 and 7, mean fecal levofloxacin concentration was 20.9-87.4 mcg/ml. They found that fecal levofloxacin concentration was much higher than MIC of fecal microbiota like *Enterococcus* spp. and *E. coli*.[18]

Ziegler et al. performed the retrospective cohort study in 60 patients admitted for chemotherapy in tertiary hospital. All of them receive levofloxacin or broad-spectrum-beta-lactam (BSBL) for bacterial prophylaxis. The study showed that the Shannon index diversity was higher in population exposed to levofloxacin than BSBL group. The impact of antibiotics on the gut microbiome varies by class, and levofloxacin may disrupt the gut microbiome less than BSBLs in this patient population.[19]

Levofloxacin has drug interaction with metal compound including magnesium, calcium, and iron. It forms levofloxacin-metal complex with these metal compounds, resulting gastrointestinal absorption is interfered and lose of bactericidal activity. Thus, calcium carbonate may have some property to trap nonabsorbable and excreted parts of intraluminal levofloxacin and protect gut microbiomes. [14, 15]

Manjunath P. Pai et al. performed an open-label, cross-over study matching cystic fibrosis patient and healthy volunteer receiving 750 mg of oral levofloxacin alone daily for 5 days and 2 hours apart calcium carbonate supplementation 500 mg oral thrice daily with meal in random sequence. This study showed no significant interaction in healthy volunteer, while cystic fibrosis group showed significant interaction (C_{max} decreased by 19% and time to C_{max} increased to 37%).[20]

Antimicrobial agents affect gut microbiota diversity or richness (by shotgun metagenome sequencing). 16s ribosomal RNA sequencing analysis is used to analyze

the microbiota diversity by mean of alpha index calculation, including Shannon diversity index or Simpson diversity index.[21-23]

Shannon diversity index (H' or Shannon-Wiener diversity index or Shannon entropy) is the way to measure the diversity of species in a community. The higher value of H', the higher the diversity in particular diversity. The lower value of H', the lower diversity.

The formulation of H' is calculated as

$$H' = - \sum p_i \ln (p_i)$$

Where:

the ∑: A Greek symbol that means sum.

ln: natural log

p_i: the proportion of the entire community made up of species *i*

The diversity index is presented in box plot, to compare the difference in diversity between two population by Bray-Curtis dissimilarity.

We did preliminary in *vitro* study to demonstrate effect of levofloxacin and calcium gluconate interaction. Adding calcium gluconate to levofloxacin solution increased the levofloxacin MIC (minimum inhibitory concentration) of *E. coli* ATCC 25922 with (Table1). The result supports the hypothesis that calcium ion may inhibit nonabsorbable/excreted part of levofloxacin in gut and protect intestinal microbiota.

Table 1. MIC change after mixing of 10% calcium gluconate and levofloxacin

Tested agents	*E. coli* ATCC (MIC, mcg/ml)	*E. Faecalis* ATCC (MIC, mcg/ml)
Levofloxacin	0.08	0.32
10%calcium gluconate	> 9.28	>9.28
Levofloxacin+10%calcium gluconate 20 mcg: 4.64 mg/ml	Levofloxacin > 41.8	Levofloxacin>41.8

We conducted the randomized control trial to study the effect of oral calcium carbonate to fecal levofloxacin concentration that could inhibit the bacteria (fecal functional level) in healthy volunteers taking 5-day course of levofloxacin. Our study was based on

de Gunzberg et al.'s RCT that studied the effect of activated charcoal on free fecal moxifloxacin concentration in healthy volunteers taking 5-day course of moxifloxacin.

MATERIALS AND METHODS

STUDY DESIGN

From October 18th, 2021 through December 8th, 2021, we conducted a pilot open-label randomized control trial in 20 healthy volunteers enrolled at King Chulalongkorn Memorial Hospital. The volunteers were randomly assigned to 2 groups, either not receive or receive calcium carbonate (1,000 mg oral b.i.d. ac from day 0 to day 5). Both treatment and control groups were assigned to receive levofloxacin (500 mg oral once daily at day 1 to day 5). Before undergoing trial procedures, all participants signed their written informed consents. The trial was approved by the Institutional Review Board (IRB No. 299/64) and Institutional Biosafety Committee (MDCU-IBC011/2021), Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand. This study has been registered with Thai Clinical Trial Registry, TCTR20210419003.

PARTICIPANTS

The investigator enrolled healthy volunteers, both male and female, no underlying disease, age between 18-45 years, no history of receiving any medication in the previous 3 months, eGFR (MDRD) > 60 ml/min/1.73m^2, and normal liver function test. Volunteers were ineligible if they were pregnant, received supplement medications, vitamins or trace elements including zinc, iron, magnesium, calcium, aluminium, and gastric acid lowering agents.

RANDOMIZATION

The investigators randomly assigned the participants (1:1) using computer-generated randomization to treatment group (having calcium carbonate) and control group (no calcium carbonate).

PROCEDURE

At first baseline visit, all participants were assessed by physical examination, laboratory tests (CBC, LFT and GFR). All participant entered a 4-week run-in period and randomly assigned into two groups, including receiving calcium carbonate (1,000 mg po b.i.d. for 6 days) and not receiving calcium carbonate (ten per group). The purpose of the run-in period was to study adverse events (constipation or abdominal discomfort) and tolerability of calcium carbonate tablet ingestion. After run-in period, all participants were again randomly allocated into two groups, including intervention (receiving calcium carbonate 1,000 mg po b.i.d. pc at 12:00 PM and 6:00 PM for 6 days) and control group (not receiving calcium carbonate). One day after oral calcium carbonate was initiated, all participants took oral levofloxacin tablet 500 mg once daily at 8:00 AM for 5 days. At least 1 gram of stools per subjects were collected on day 0, 2, 5, 14 and 28 for fecal microbiota diversity (using 16S ribosomal DNA V3-4 analysis). The fecal levofloxacin concentration was measured by HPLC and MIC methods on day 0, 2, and 5. EDTA blood samples for peak plasma levofloxacin concentration (C_{max}) were collected at 10:00 AM (2 hours after ingestion of levofloxacin) on day 1 and 5. Stool samples were processed by mixing with sterile water at 1:10 dilution, double-filtrated with 0.2-micron syringe filter and 100kDa centrifugal filter, for levofloxacin concentration measurement. Blood samples were centrifuged for plasma at 4°C and tested for levofloxacin concentration by HPLC method. Fresh stool samples were kept in -80 °C for 16s-rDNA microbiota diversity analysis by an outsourcing laboratory service. All data was recorded into case record form (**Appendix, Table A).**

OUTCOMES

The primary outcome was fecal levofloxacin concentration by MIC and HPLC methods on day 2 and day 5 of 5-day cause.

The secondary outcomes were (1) gut microbiota diversity by 16s rDNA V3-4 analysis on day 0, 2, 5, 14 and 28; (2) peak plasma levofloxacin concentration on day 1 and 5; (3) drug adverse events in 4-week period after levofloxacin intake.

STATISTICAL ANALYSIS

This study is a pilot study. We approximated the sample size of 20 volunteers based on the study of de Gunzburg et al.(12)

Baseline characteristic, demographic data, adverse events, and laboratory abnormality were summarized descriptively.

For repeated measures of fecal and plasma levofloxacin concentration in unit mcg/ml, mixed-effects model was used to analyze the difference between treatment and control group. The secondary outcomes including mean of peak plasma levofloxacin concentration in unit mcg/ml and stool microbiota diversity index were also analyzed by mixed-effects model to determine the difference between treatment and control group. Wilcoxon matched-pairs signed rank test was used to analyze the significance of diversity change in different time points. We used SPSS version 28.0.1.0 and Prism 9 GraphPad Version 9.3.1 for all analysis. We determined the two-sided significance level of 0.05.

ROLE OF FUNDING AND RESOURCE

This study was funded by Fundamental Fund (CUFRB65_hea(47)_054_30_35), Chulalongkorn University and the Royal College Physicians of Thailand (No. 6/2564). The funders were not involved in data collection, analysis, writing report, or data interpretation.

RESULTS

Between October 18th and December 8th, 2021, 20 volunteers were screened and randomly assigned to either treatment (calcium carbonate) or control group (without calcium carbonate). Demographic data and baseline clinical characteristics were balanced between two groups (**Table 2**). All participants were Thai, 7 males and 13 females. The mean age, BMI, and eGFR were 34.15 ± 1.21 years, 22.58 ± 0.97 kg/m^2, 102.99 ± 2.96 ml/min/1.73m^2, respectively.

Table 2. Baseline characteristics

Characteristic	Treatment group (N=10)	Control group (N=10)	Total (N=20)
Age (years)	34.60 ± 5.68	33.70 ± 5.37	34.15 ± 1.21
Sex			
Male	3 (30%)	4 (40%)	7 (35%)
Female	7 (70%)	6 (60%)	13 (65%)
Characteristic	Treatment group (N=10)	Control group (N=10)	Total (N=20)
BMI (kg/m^2)	22.86 ± 3.30	22.30 ± 5.39	22.58 ± 0.97
Thai eGFR (ml/min/1.73m^2)	105.27 ± 12.93	100.71 ± 13.92	102.99 ± 2.96

Mean fecal levofloxacin levels were shown in **Table 3.1 and 3.2**. By MIC method or functional levofloxacin concentration, all pre-treatment levofloxacin levels were below 1:8 by geometric mean titer. The geometric mean titer was higher in treatment group than in control group with statistical significance on day 5, 1:376 (100.50 µg/ml) vs 1:149 (53.21 µg/ml), respectively (95% CI 4.912, 89.73; p = 0.0242). The fecal levofloxacin level difference between groups by MIC method showed no statistical significance on day 2. All pre-treatment levofloxacin levels were below HPLC

detection limit (0.5 µg/ml). By HPLC method, mean fecal levofloxacin concentration on day 2 in the treatment group was 55.90 mcg/ml, not significantly different from 34.23 µg/ml in the control group (p=0.273, 95% CI -18.91, 62.24). Mean fecal levofloxacin concentration day 5 was 108.23 mcg/ml, also not significantly different from 60.75 µg/ml in the control group (p=0.128; 95% CI -14.97, 109.93).

Table 3.1 Fecal levofloxacin concentration on day 0, 2, and 5 by MIC method

Mean fecal functional levofloxacin concentration by MIC method	Treatment group		Control group		Adjusted *p*-value (95% CI)
	mcg/ml	Dilutional fold titer (geometric mean titer)	mcg/ml	Dilutional fold (geometric mean titer)	
Day 0	< 1.56	<1:8	< 1.56	<1:8	> 0.99
Day 2	41.50	1:128	30.26	1:97	0.87 (-29.00, 51.46)
Day 5	100.50	1:376	53.21	1:149	0.0242 (4.91, 89.73)

Table 3.2 Fecal levofloxacin concentration on day 0, 2, and 5 by HPLC method

Mean fecal levofloxacin concentration by HPLC	Treatment group (mcg/ml)	Control group (mcg/ml)	Adjusted *p*-value (95% CI)
Day 0	< 0.5	< 0.5	> 0.99
Day 2	55.90	34.23	0.645 (-28.52, 71.91)
Day 5	108.23	60.76	0.069 (-2.73, 97.69)

Figure 3 The plot of fecal levofloxacin concentration in day 0, 2 and 5 by MIC method

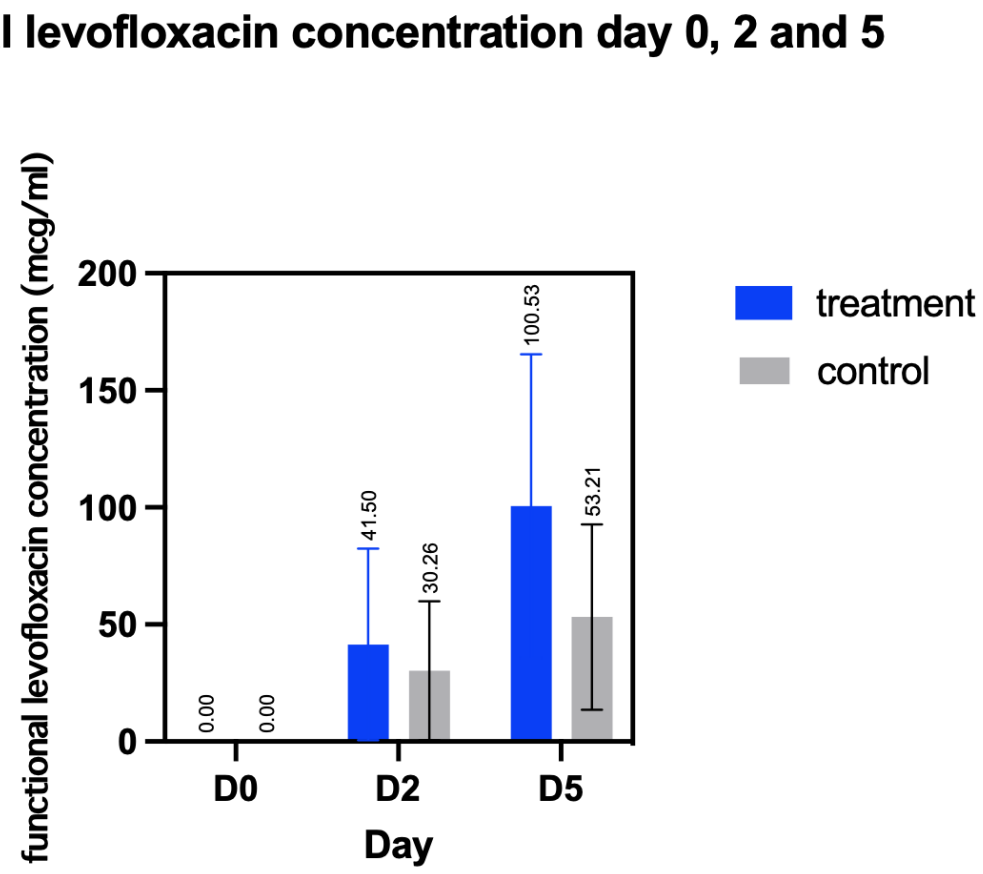


Figure 4. The plot of geometric mean of dilutional fold titer of levofloxacin concentration by MIC method in day 2 and 5

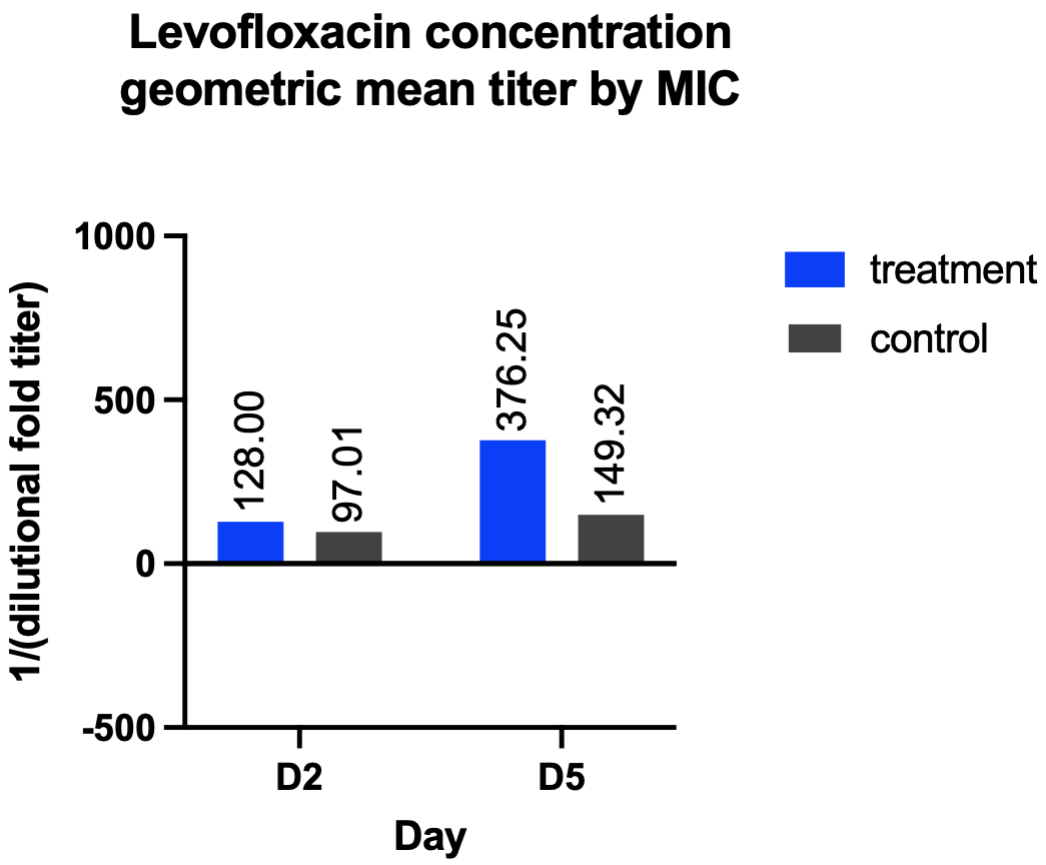


Figure 5. The plot of fecal levofloxacin concentration in day 2 and 5 by HPLC method

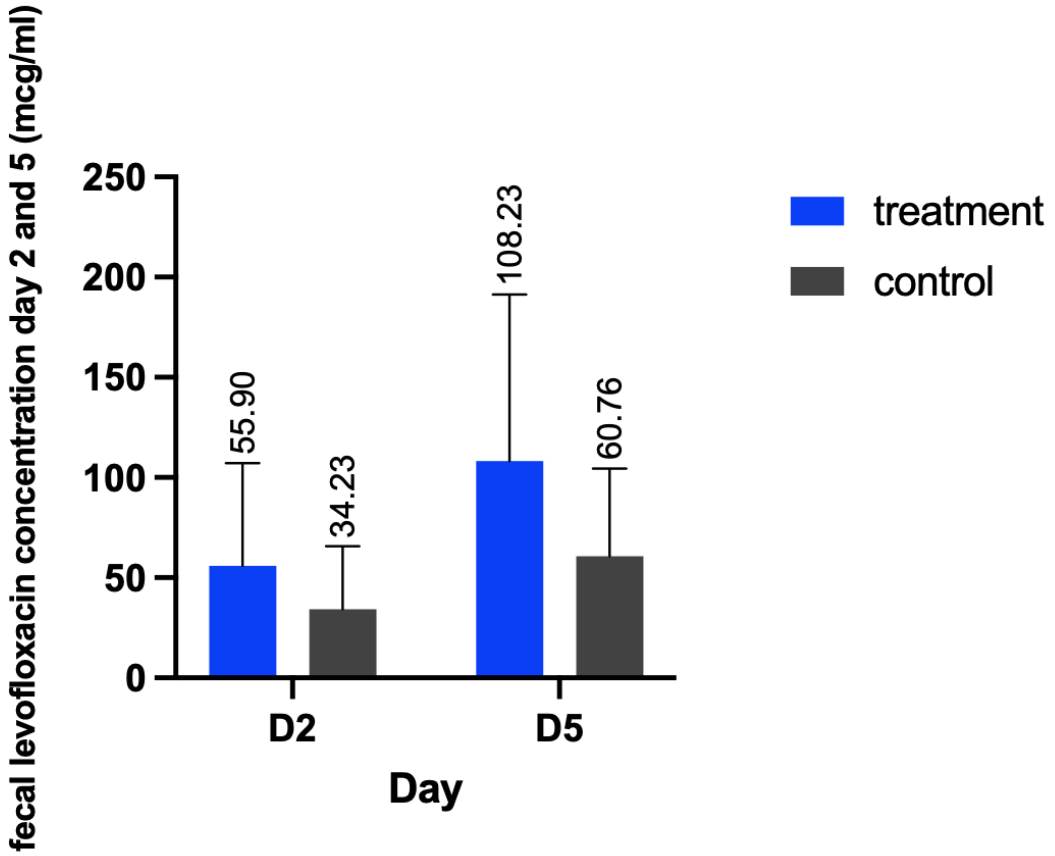


Table 4 and **Figure 6, 7 and 8** showed the Shannon diversity index. The mean diversity index of treatment group at day 0 was a bit higher than control group without statistical significance (p = 0.923). On day 2 and 5 of levofloxacin treatment, mean diversity index was higher in control group than in treatment group, also without statistical significance (p = 0.426 on day 2 and 0.237 on day 5). On day 14, mean diversity index was higher in treatment group. On day 28, mean diversity index was returned close to baseline at day 0. We used Wilcoxon matched-pairs signed rank test to analyze the difference of diversity changes on day 2 and day 5 compared to day 0. On both day 2 and 5, the treatment group diversity declined

significantly from day 0, while the control group, the diversity did not change significantly (**Table 6**). There were no significant differences of mean peak plasma levofloxacin concentration at day 1 and day 5 between treatment and control group (**Table 5**).

Table 4. Shannon diversity index

Mean gut microbiota diversity	Treatment group volunteers	Control group volunteers	*p*-value (95% CI)
Day 0	3.86	3.73	0.923 (-0.28, 0.55)
Day 2	3.58	3.84	0.426 (-0.68, 0.16)
Day 5	3.30	3.62	0.237 (-0.75, 0.11)
Day 14	4.17	4.02	0.912 (-0.29, 0.58)
Day 28	4.13	4.04	0.988 (-0.33, 0.51)

Table 5. Peak plasma levofloxacin concentration

Mean plasma levofloxacin level (mcg/ml) by HPLC	Treatment group volunteers	Control group volunteers	*p*-value (95% CI)
day 1	3.66	3.25	0.88 (-1.17, 2.60)
day 5	3.59	2.21	0.27 (-0.80, 3.56)

Figure 6. Shannon diversity during run-in period

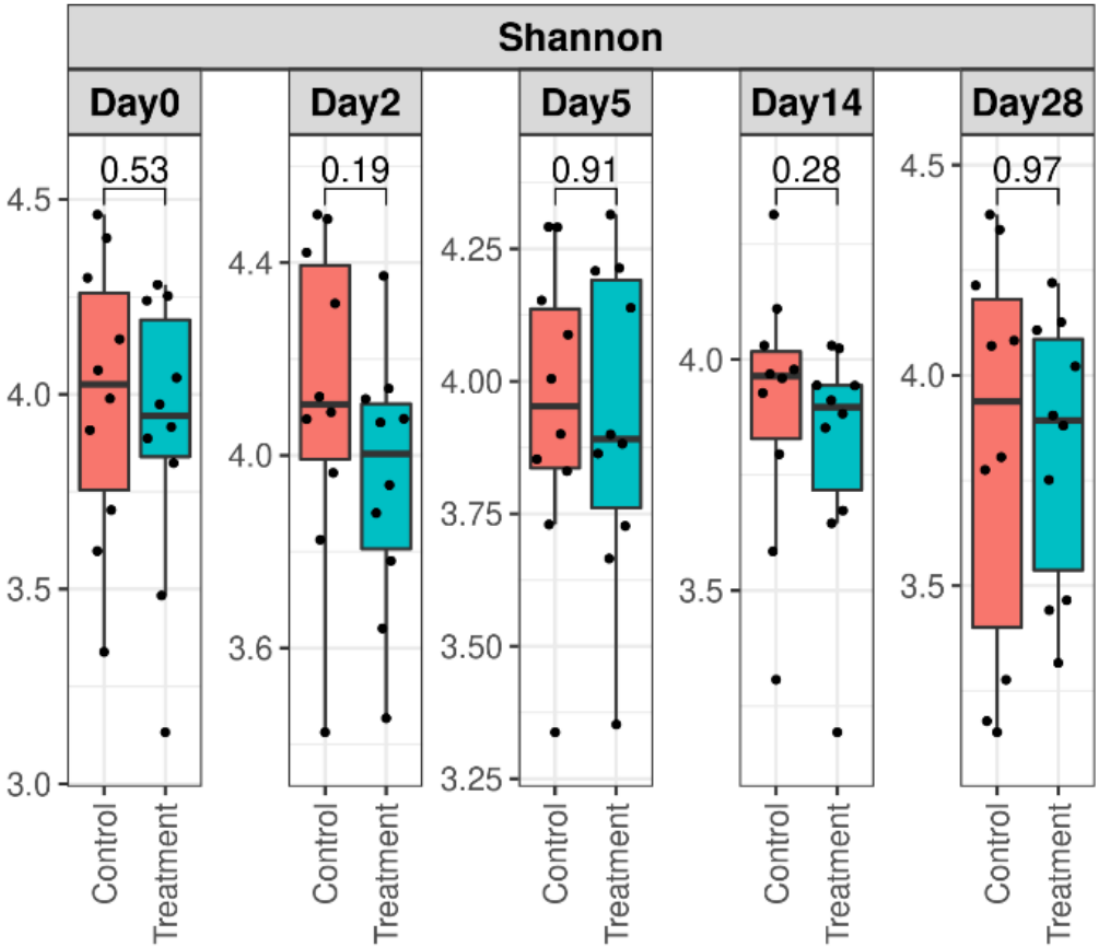


Figure 6 demonstrated the Shannon diversity index during run-in period. The diversity was lower, but not significantly, in treatment group (receiving calcium carbonate) compared to control group in day 2.

Figure 7. Shannon index diversity in treatment vs control group at each time points.

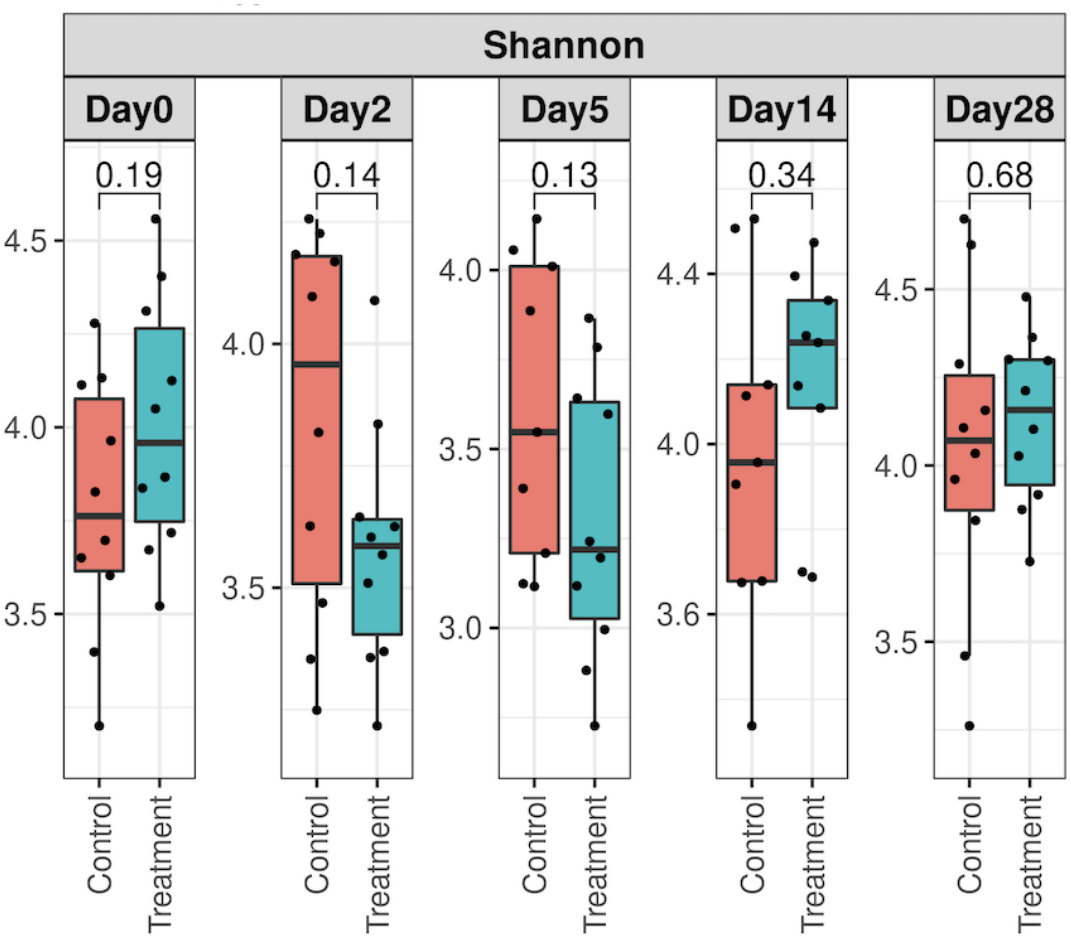
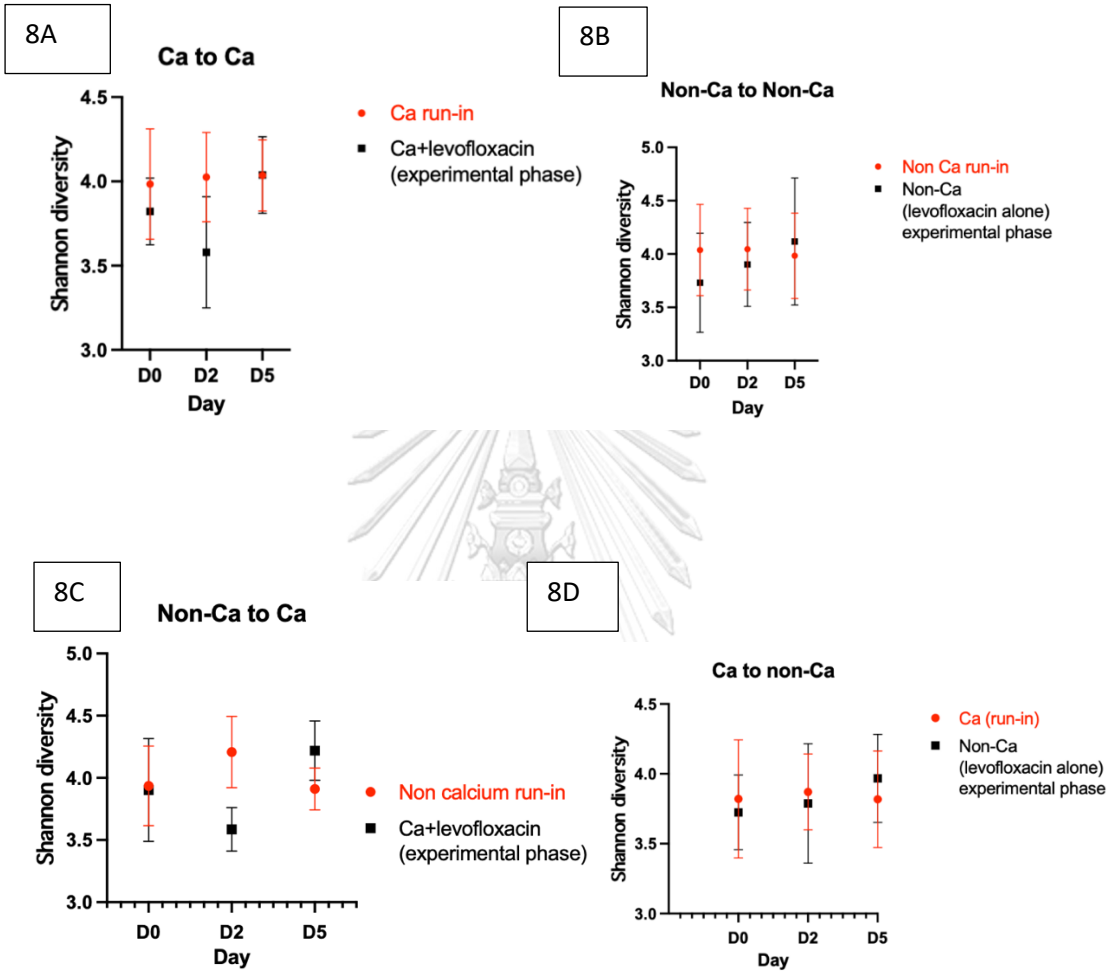


Table 6. Wilcoxon matched-pairs signed rank test of Shannon index at day 2 and 5 compared to day 0

Day 0 vs day 2	P value	Sum of pos. ranks	Sum of neg. ranks	Sum of signed ranks (W)	q value
Control	0.263672	16	-39	-23	0.133154
Treatment	0.001953	55	0	55	0.001973

Day 0 vs day 5	P value	Sum of pos. ranks	Sum of neg. ranks	Sum of signed ranks (W)	q value
Control	0.734375	26	-19	7	0.370859
Calcium	0.001953	55	0	55	0.001973

Figure 8. Subgroup analysis of 4 divided group of volunteers comparing the Shannon diversity index in run-in calcium carbonate phase to experimental phase



In **Figure 8,** Graph B and D showed no difference in Shannon diversity index between run-in phase and experimental phase. In Graph A and C, the Shannon diversity index was lower after taking calcium carbonate and levofloxacin on day 2, only reaching statistically significance in graph C (95% CI 0.17, 1.08, p = 0.0054).

Details of microbiota composition in different taxa were demonstrated in (**Figure B**, **Appendix**). Different color represented different taxa compositions at genus level, which were also presented in Krona plot (**Figure C, Appendix**).

The bacteria of phylum Firmicutes were highly prevalent in all samples (avg. 0.79±0.01), followed by Actinobacteriota, Bacteriodota, and Proteobacteria, respectively. A total of 11 different phyla were identified. Overall, 234 genera were detected among the fecal samples. The relative abundance of bacteria in members of *Faecalibacterium*, *Streptococcus*, *Sellimonas*, *Phascolarctobacterium*, *Megasphaera*, *Lactobacillus*, *Lachnoclostridium*, and *Catenibacterium* were higher in the control group when compared to the treatment group at day 0. In contrast, the *Parabacteroides*, *Clostridium* and *Monoglobus* were significantly increased in treatment group at day 0. The abundance of *Blautia*, *Romboutsia*, *Lachnospiraceae ND3007 group*, *Escherichia-Shigella*, and *Dorea* were increased in treatment group at 2 days after treatment compared to the control group. The *Blautia* was also highly increased in gut microbiota of treatment group at day 5. The relative abundance of *Blautia, Butyricicoccus*, *Dialister, Dorea*, *Enterobacter*, *Faecalibacterium*, *Megamonas*, *Parabacteroides, Roseburia*, and *Lachnoclostridium* were increased in fecal samples of treatment group compared to the control group at day 14. Moreover, the compositions of *Agathobacter*, *Romboutsia*, *Negativibacillus*, *Dorea*, and *Dialister* were also increased in the treatment group at day 28 after experiment when compared to the control group at day 28. **Table 7** demonstrated adverse event in volunteers, after an exposure to levofloxacin with or without calcium carbonate within 4 weeks. Participants in both groups had similar numbers of adverse events (3 participants in treatment group and 5 participants in control group) (p-value = 0.388). No serious adverse event was reported. Most adverse events were mild and the common were nausea, dizziness, and diarrhea.

Table 7. Reported adverse events

Adverse effect	Treatment group volunteers (%) (N=10)	Control group volunteers (%) (N=10)	*p*-value
Any adverse event	3 (30%)	5 (50%)	0.388
nausea	0	2	0.168
dizziness	1	1	-
myalgia	0	1	0.343
diarrhea	1	1	-
belching	1	0	0.343
dyspepsia	0	1	0.343

DISCUSSION

Fecal levofloxacin concentration was higher in treatment group on day 2 and day 5 for both MIC and HPLC methods, but only the difference on day 5 by MIC method reached statistical significance.

From fecal microbiota diversity results, the treatment group had significantly declined level of Shannon index diversity compared to the control group. This suggested that calcium carbonate pills might have direct effect on fecal microbiota diversity or enhance dysbiosis when co-administered with levofloxacin. Low-diversity microbiota, with increases in proportion of facultative anaerobes, has been observes with acute diarrhea disease, inflammatory bowel disease, C. difficile infection (CDI), liver disease and in cancer patients.(24)

The results of fecal levofloxacin concentration and fecal microbiota diversity were opposite to our original hypothesis that calcium carbonate might lower fecal levofloxacin and protect gut microbiota diversity. We re-hypothesize that calcium carbonate oral tablet might form complex with levofloxacin and induce more levofloxacin retention in the intestinal lumen by inhibition of enterohepatic recirculation and worsen the dysbiosis.

From the previous data of intervention that could protect and decrease the intraluminal antibiotic concentration, de Gunzberg et al. had showed that DAV-132 (nonspecific adsorbent-activated charcoal) was highly effective to protect gut microbiota of moxifloxacin-treated healthy volunteers by decreasing free moxifloxacin fecal concentration by 90%, but our study had the opposite result.

There was no significance in difference between treatment and control groups regarding peak plasma concentration. The result was similar to the previous study from Manjunath P. Pai et al.(20)

There has been limited evidence of the effect of calcium carbonate on the gut microbiota. Most data are from the effect of phosphate binder treatment in end

stage renal disease patient with hyperphosphatemia.(24) Trautvetter et al. found an increase in fecal excretion of short chain fatty acid and increased relative abundance of species from *Clostridium* cluster XVIII in those supplemented with calcium carbonate.(25)

Figure 8C showed the effect of calcium carbonate to gut microbiota diversity in a run-in period. Though not statistically significant, microbiota diversity was decreased after taking calcium carbonate on day 2. However, the decreased diversity in calcium carbonate group was more remarkable in the experimental phase, when the participants also had oral levofloxacin.

There is the evidence from end stage kidney disease patients receiving ferric citrate vs calcium carbonate as the phosphate binders. The overall results supported the data from our treatment group that calcium carbonate treated patients had a significantly reduced microbial species diversity (Shannon index and Simpson index). However, the influence of iron and calcium-containing phosphate binder on the gut microbiota is still largely unknown.[25] Though calcium can protect some specific bacteria from levofloxacin *in vitro*, the levofloxacin-calcium complex may still active against some gut flora. The very high variety of fecal components might also affect the diversity outcome. Now, there is no concrete evidence that calcium carbonate can lower gut microbiota diversity in healthy volunteers. We hypothesize that calcium-levofloxacin complex may inhibit levofloxacin absorption, leading to accumulation of intraluminal levofloxacin which can then destroy the gut microbiota.

The change of taxonomic profile was showed, after treatment with calcium carbonate plus levofloxacin compared to levofloxacin alone, the abundance of the bacteria named *Blautia* spp. was increased and the causal relationship of *Blautia* spp. and diseases is not yet clear. Whether *Blautia* spp. play a direct regulatory role in diseases requires further intervention studies and more detailed evidence.[26] From

our study there was no increased abundance of pathogenic bacteria like *Clostridium* spp..

There was no serious adverse event in all participants. Only mild adverse events were observed. We conclude that coadministration of levofloxacin and calcium carbonate is reasonably safe. Constipation, the common adverse effect of calcium carbonate was not reported in the treatment group.

There were some advantages from our study. First, it was the first randomized control trial studying the effect of calcium carbonate on the effect of levofloxacin fecal concentration and gut microbiota diversity. Second, we minimized the confounding factors to gut microbiota diversity by using healthy volunteers, not receiving any medications in the past 3 months and during the study.

There were two limitations in our study. First, there might be food-drug interactions interfering the results of fecal levofloxacin concentration, plasma levofloxacin and gut microbiota diversity. Second, there is an internal variation in each volunteer, so the cross-over study to compare the diversity after expose to calcium carbonate in each volunteer may be preferred.

This study emphasizes the complexity of antibiotic-induced dysbiosis. Many drugs, foods, or compounds might either prevent or worsen dysbiosis caused by antibiotics. Further exploration in this field will be very beneficial, especially on the struggling against the rapid rising of many multidrug-resistant organisms.

CONCLUSION

In healthy volunteer taking 5-day course of oral levofloxacin 500 mg once daily, coadministration with calcium carbonate 1,000 mg oral twice daily is safe and does not significantly alter peak plasma levofloxacin concentration. However, this combination might increase fecal levofloxacin level and lower fecal microbiota diversity. Therefore, co-prescription of levofloxacin and calcium should be cautioned even without the concern about the absorption, like when levofloxacin is administered intravenously or when both drugs in oral forms are taken at different times.

APPENDIX

TABLE A. CASE RECORD FORM

Code number

Age……..Sex……..

V/S BT…. BP….. HR……. RR……..

BW…….. Height……. BMI……

day	0	1	2	5	14	30	remark
Fecal functional level of levofloxacin							
Shannon diversity							
Plasma C_{max} levofloxacin							
Adverse event Rash tendinopathy N/V diarrhea And other illness							

FIGURE B. TAXONOMIC PROFILE IN TREATMENT GROUP AND CONTROL GROUP EACH TIME POINT

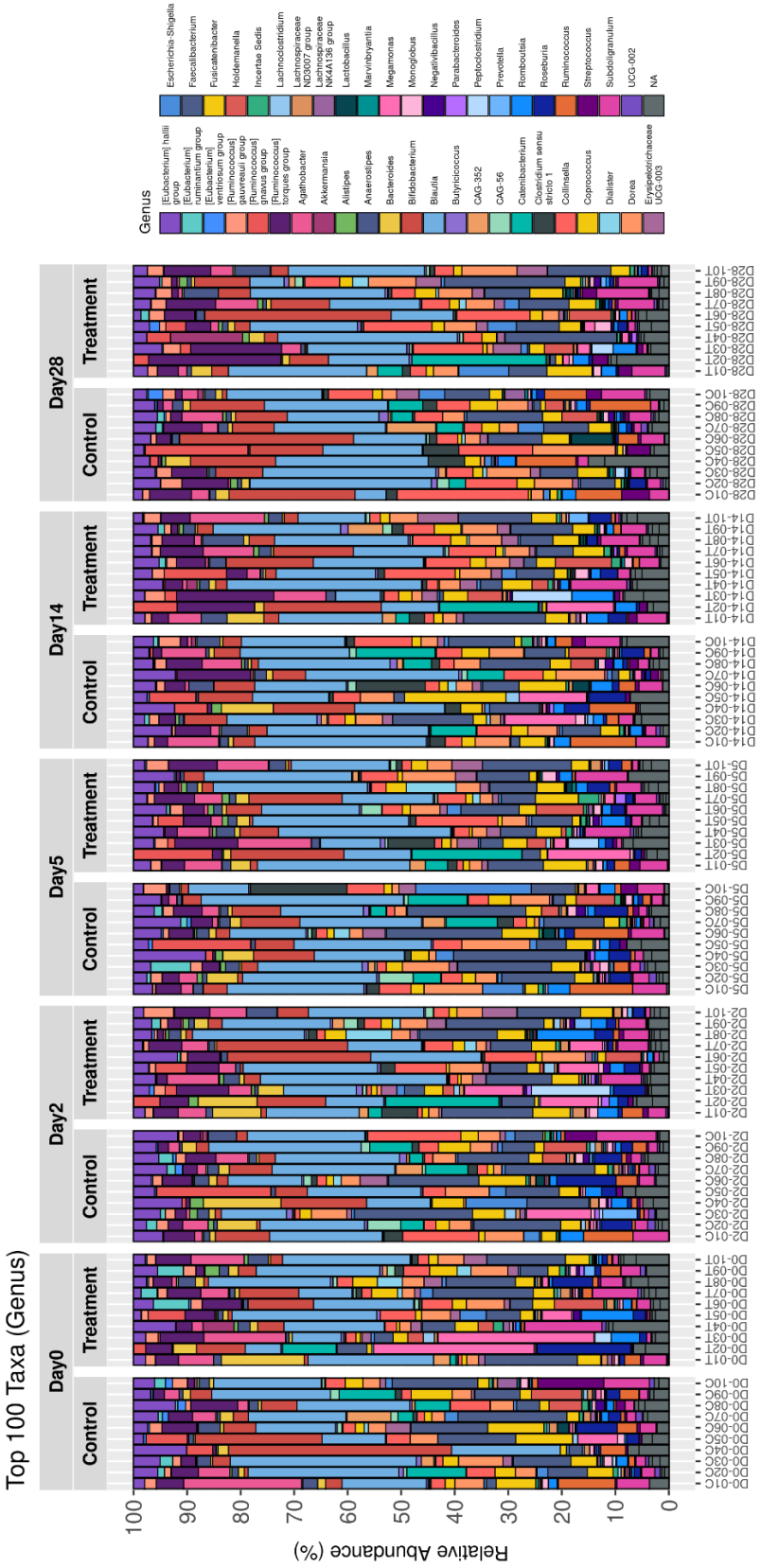
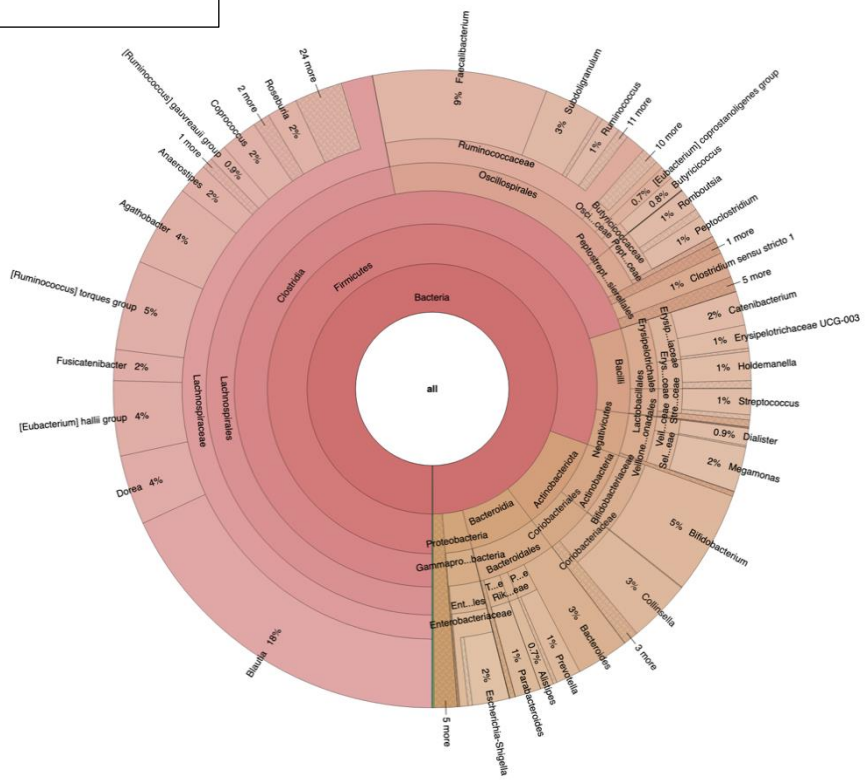
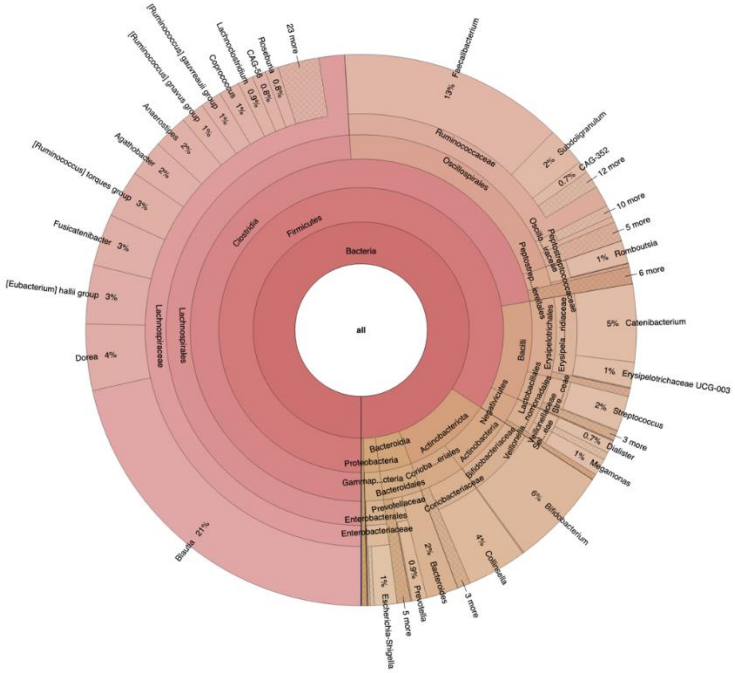


FIGURE c. KRONA PLOT DEMONSTRATE TAXONOMIC PROFILE CLASSIFICATION IN EACH TIME POINT

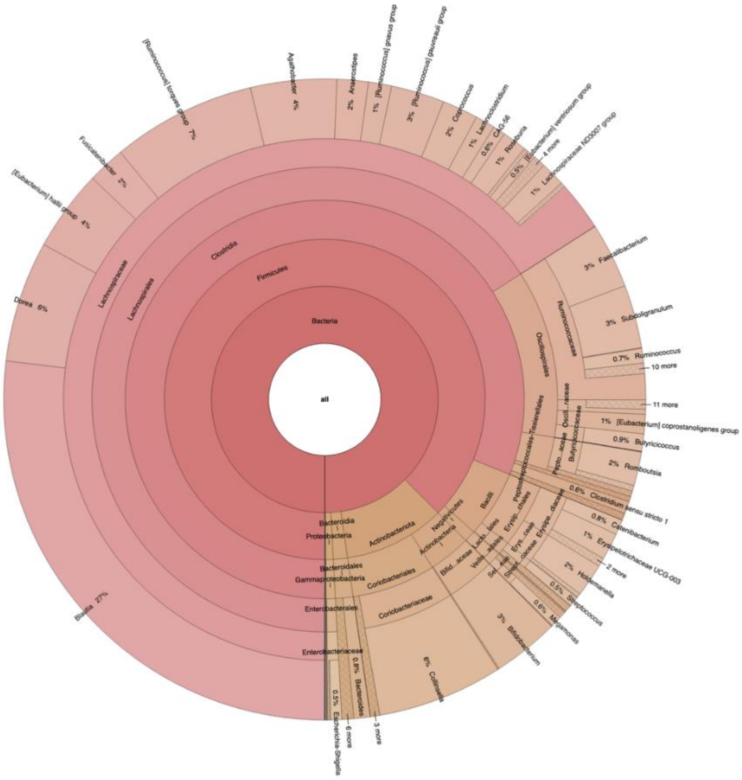
Treatment day 0



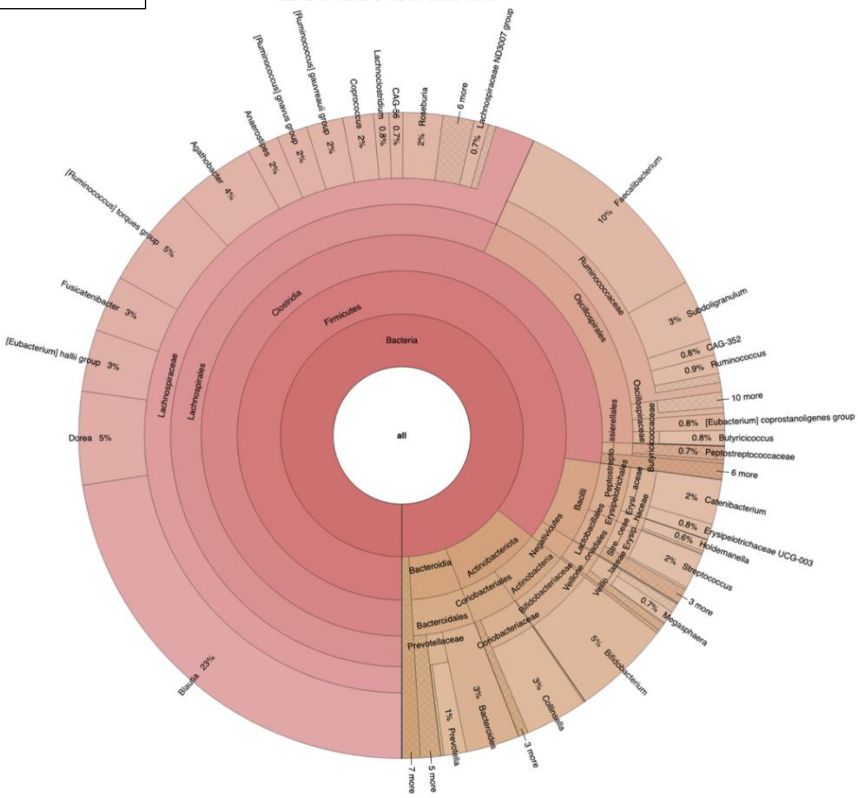
Control day 0



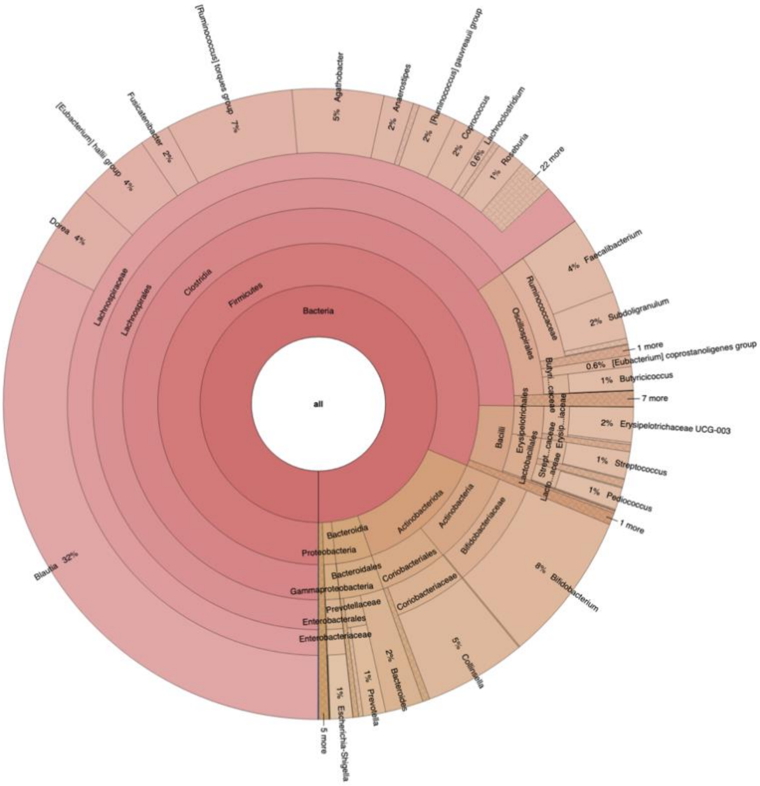
Treatment day 2



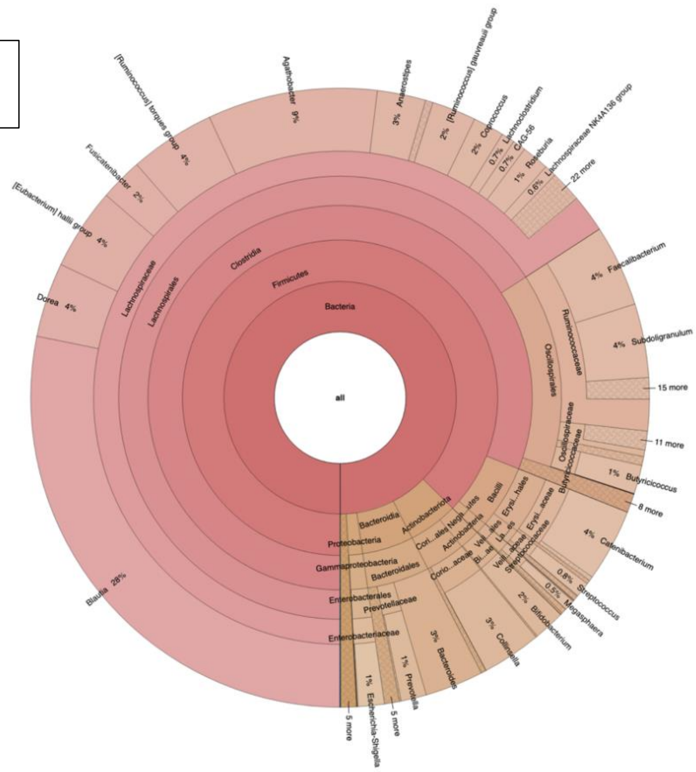
Control day 2



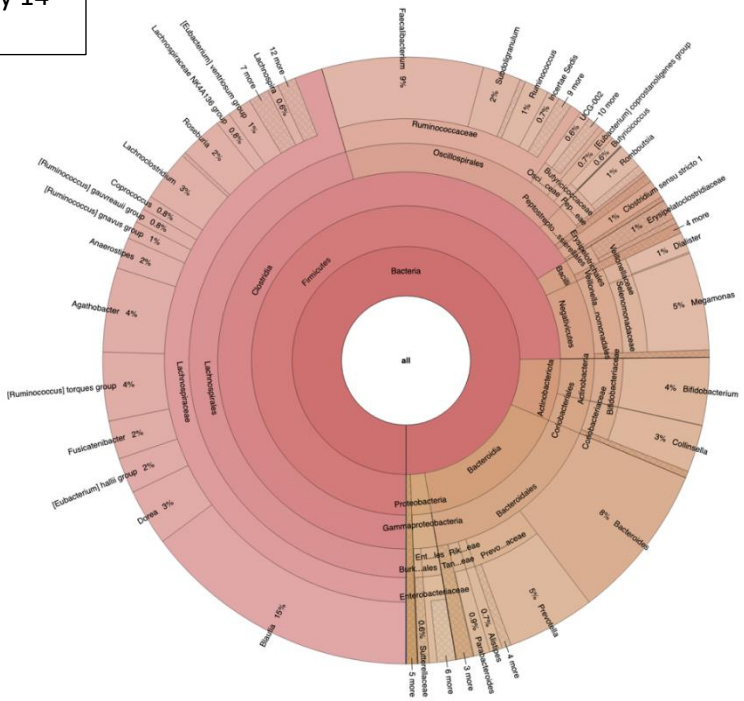
Treatment day 5



Control day 5



Treatment day 14



Control day 14

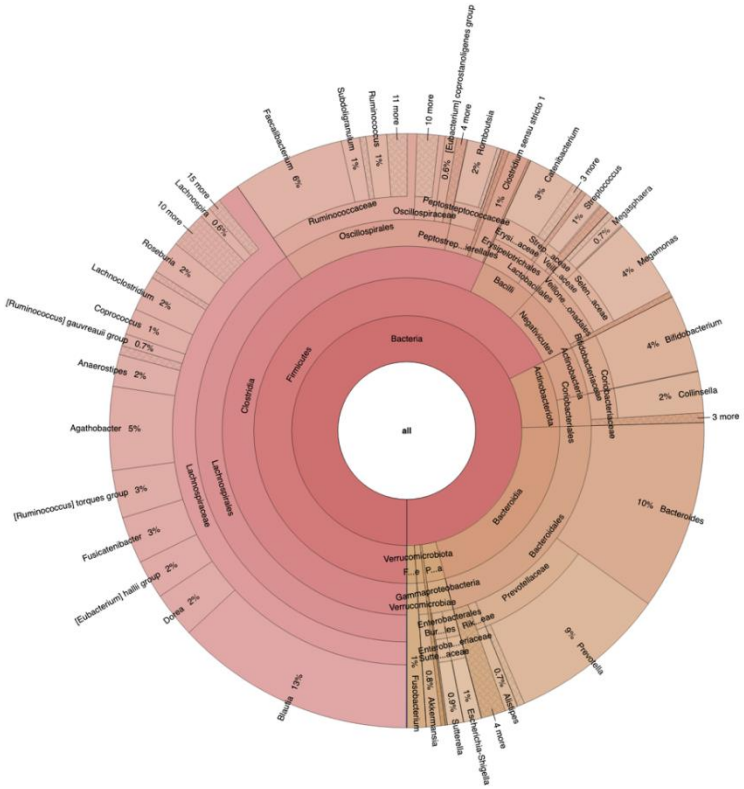
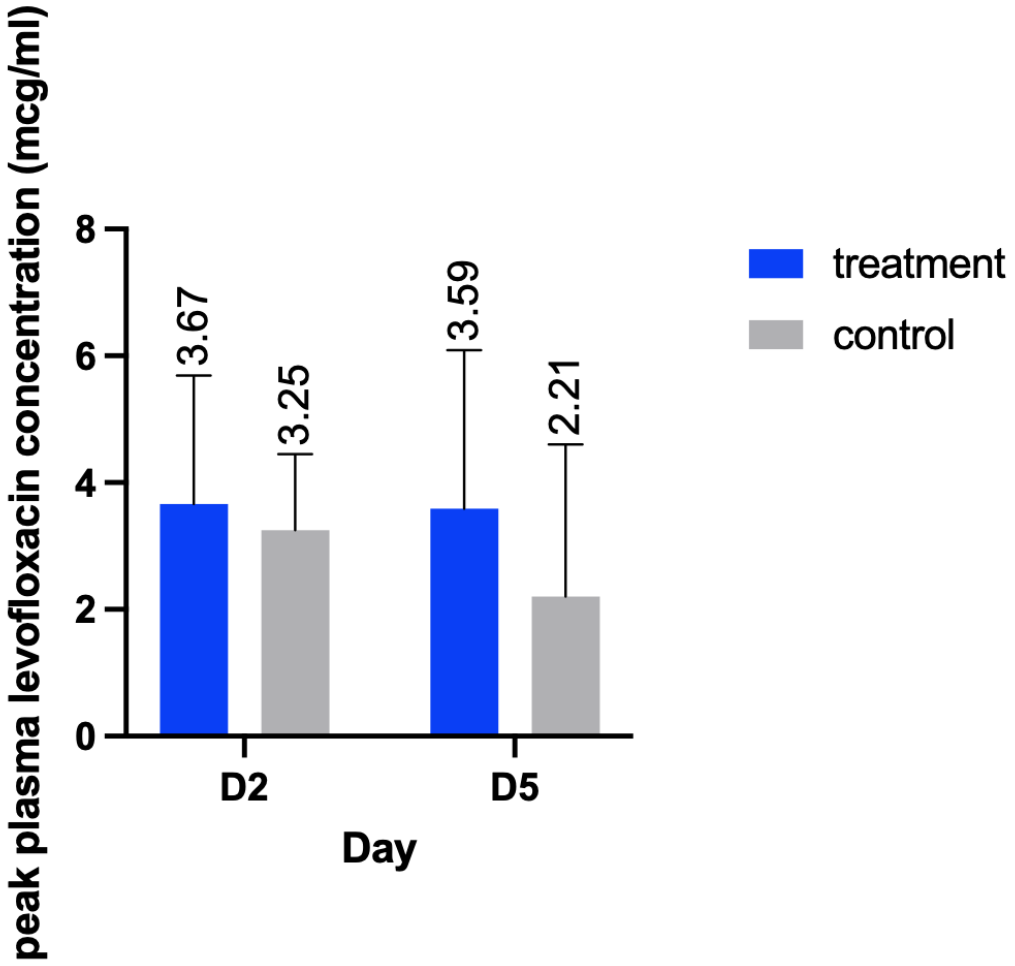


FIGURE D. MEAN PEAK PLASMA LEVOFLOXACIN CONCENTRATION ON DAY 1 AND DAY 5



REFERENCES

1. Leffler DA, Lamont JT. Clostridium difficile infection. N Engl J Med. 2015;372(16):1539-48.

2. Belkaid Y, Hand TW. Role of the microbiota in immunity and inflammation. Cell. 2014;157(1):121-41.

3. Pedersen HK, Gudmundsdottir V, Nielsen HB, Hyotylainen T, Nielsen T, Jensen BA, et al. Human gut microbes impact host serum metabolome and insulin sensitivity. Nature. 2016;535(7612):376-81.

4. Cox LM, Blaser MJ. Antibiotics in early life and obesity. Nat Rev Endocrinol. 2015;11(3):182-90.

5. Cox LM, Yamanishi S, Sohn J, Alekseyenko AV, Leung JM, Cho I, et al. Altering the intestinal microbiota during a critical developmental window has lasting metabolic consequences. Cell. 2014;158(4):705-21.

6. Jernberg C, Lofmark S, Edlund C, Jansson JK. Long-term impacts of antibiotic exposure on the human intestinal microbiota. Microbiology (Reading). 2010;156(Pt 11):3216-23.

7. Dethlefsen L, Relman DA. Incomplete recovery and individualized responses of the human distal gut microbiota to repeated antibiotic perturbation. Proc Natl Acad Sci U S A. 2011;108 Suppl 1:4554-61.

8. Leonard F, Andremont A, Leclerq B, Labia R, Tancrede C. Use of beta-lactamase-producing anaerobes to prevent ceftriaxone from degrading intestinal resistance to colonization. J Infect Dis. 1989;160(2):274-80.

9. Stiefel U, Nerandzic MM, Koski P, Donskey CJ. Orally administered beta-lactamase enzymes represent a novel strategy to prevent colonization by Clostridium difficile. J Antimicrob Chemother. 2008;62(5):1105-8.

10. Le Chatelier E, Nielsen T, Qin J, Prifti E, Hildebrand F, Falony G, et al. Richness of human gut microbiome correlates with metabolic markers. Nature. 2013;500(7464):541-6.

11. Khoder M, Tsapis N, Domergue-Dupont V, Gueutin C, Fattal E. Removal of residual colonic ciprofloxacin in the rat by activated charcoal entrapped within zinc-pectinate beads. Eur J Pharm Sci. 2010;41(2):281-8.

12. de Gunzburg J, Ghozlane A, Ducher A, Le Chatelier E, Duval X, Ruppe E, et al. Protection of the Human Gut Microbiome From Antibiotics. J Infect Dis. 2018;217(4):628-36.

13. Davis R, Bryson HM. Levofloxacin. A review of its antibacterial activity, pharmacokinetics and therapeutic efficacy. Drugs. 1994;47(4):677-700.

14. David C. Hooper JS. Quinolones. Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases, Ninth Edition. 2020:426-48.

15. Hooper DC. Mechanisms of fluoroquinolone resistance. Drug Resist Updat. 1999;2(1):38-55.

16. Fujieda Y, Yamaoka K, Ito T, Nakagawa T. Local absorption kinetics of levofloxacin from intestinal tract into portal vein in conscious rat using portal-venous concentration difference. Pharm Res. 1996;13(8):1201-4.

17. Inc JP. Levaquin (levofloxacin) tablet package insert. 2020.

18. Edlund C, Sjostedt S, Nord CE. Comparative effects of levofloxacin and ofloxacin on the normal oral and intestinal microflora. Scand J Infect Dis. 1997;29(4):383-6.

19. Ziegler M, Han JH, Landsburg D, Pegues D, Reesey E, Gilmar C, et al. Impact of Levofloxacin for the Prophylaxis of Bloodstream Infection on the Gut Microbiome in Patients With Hematologic Malignancy. Open Forum Infect Dis. 2019;6(7):ofz252.

20. Pai MP, Allen SE, Amsden GW. Altered steady state pharmacokinetics of levofloxacin in adult cystic fibrosis patients receiving calcium carbonate. J Cyst Fibros. 2006;5(3):153-7.

21. Reese AT, Dunn RR. Drivers of Microbiome Biodiversity: A Review of General Rules, Feces, and Ignorance. mBio. 2018;9(4).

22. Spellerberg F. A tribute to Claude Shannon (1916–2001) and a plea for more rigorous use of species richness, species diversity and the 'Shannon–Wiener' Index. Global Ecology and Biogeography. 2003;3:177-9.

23. Kriss M, Hazleton KZ, Nusbacher NM, Martin CG, Lozupone CA. Low diversity gut microbiota dysbiosis: drivers, functional implications and recovery. Curr Opin Microbiol. 2018;44:34-40.

24. Merino-Ribas A, Araujo R, Bancu I, Graterol F, Vergara A, Noguera-Julian M, et al. Gut microbiome in hemodialysis patients treated with calcium acetate or treated with sucroferric oxyhydroxide: a pilot study. Int Urol Nephrol. 2021.

25. Wu PH, Liu PY, Chiu YW, Hung WC, Lin YT, Lin TY, et al. Comparative Gut Microbiome Differences between Ferric Citrate and Calcium Carbonate Phosphate Binders in Patients with End-Stage Kidney Disease. Microorganisms. 2020;8(12).

26. Ozato N, Saito S, Yamaguchi T, Katashima M, Tokuda I, Sawada K, et al. Blautia genus associated with visceral fat accumulation in adults 20–76 years of age. npj Biofilms and Microbiomes. 2019;5(1):28.

REFERENCES

VITA

NAME	Ophat Janphet
DATE OF BIRTH	5 June 1988
PLACE OF BIRTH	Surin Hospital, Surin
INSTITUTIONS ATTENDED	Doctor of Medicine, Khon Kaen University Dip., Thai Board of Internal Medicine, Chulalongkorn University
HOME ADDRESS	71 Troktalon, Krungsrinai road, Tambon Nai Muang, Amphoe Muang, Surin 32000
PUBLICATION	-
AWARD RECEIVED	-