

ADDENDUM



The influence of proton pump inhibitors and other commonly used medication on the gut microbiota

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ABSTRACT

Proton pump inhibitors (PPIs), used to treat gastro-esophageal reflux and prevent gastric ulcers, are among the most widely used drugs in the world. The use of PPIs is associated with an increased risk of enteric infections. Since the gut microbiota can, depending on composition, increase or decrease the risk of enteric infections, we investigated the effect of PPI-use on the gut microbiota. We discovered profound differences in the gut microbiota of PPI users: 20% of their bacterial taxa were statistically significantly altered compared with those of non-users. Moreover, we found that it is not only PPIs, but also antibiotics, antidepressants, statins and other commonly used medication were associated with distinct gut microbiota signatures. As a consequence, commonly used medications could affect how the gut microbiota resist enteric infections, promote or ameliorate gut inflammation, or change the host's metabolism. More studies are clearly needed to understand the role of commonly used medication in altering the gut microbiota as well as the subsequent health consequences.

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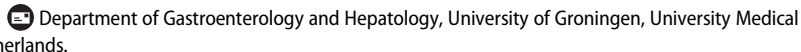
Proton pump inhibitors affect the gut microbiota

Proton pump inhibitors (PPIs), used to treat gastro-esophageal reflux and to prevent gastric ulcers, are among the most commonly used drugs in the world.^{1,2} In the Netherlands, one PPI alone (omeprazole) was the fourth most prescribed drug in 2015. In observational studies, the use of PPIs has been associated with an increased risk of enteric infections caused by *Clostridium difficile*, *Salmonella* spp., *Shigella* spp and *Campylobacter* spp.^{3–5} Since the gut microbiota can, depending on composition, increase or decrease the risk of enteric infections, we investigated the effect of PPI use on the gut microbiota.⁶ Using the 16S rRNA sequences of stool samples from 1815 individuals spanning 3 independent cohorts, we observed profound changes in the gut microbiota of PPI users. In PPI users the relative abundance of 20% of the bacterial taxa, whereof 18 bacterial families, was statistically significantly different (either increased or decreased) compared with abundances in samples from non-users.⁶

Concurrently with our research, other research groups were also investigating the influence of PPIs on the gut microbiota. A small intervention study was published a few months before ours and a similar observational study was published in the same issue of *Gut*.^{7,8} In **Table 1**, the bacterial alterations associated with PPI use from all 3 studies are presented at the family level. There are many similarities between the results of all 3 studies and, when associations at different taxonomical levels are taken into account (e.g. the decrease of order Clostridiales and increases of class Gammaproteobacteria and order Actinomycetales), a consistent profile of alterations in gut microbiota associated with PPI use emerges.

Other commonly used drugs and the gut microbiota

To ensure that our observed alterations in gut microbiota associated with PPI use were not based the confounding effects of concomitant use of other drugs,

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Table 1. PPI use associated with gut microbiota alterations in 3 studies.

Bacterial family	Imhann et al. <i>Gut</i> 2016; Figure 2 Cross-sectional study 1815 individuals	Jackson et al. <i>Gut</i> 2016; Figure 3 Cross-sectional study 1827 individuals	Freedberg et al. <i>Gastroenterology</i> 2015; Figure 1 Intervention, time series 12 healthy volunteers
Actinomycetaceae	Increased	NR	NR
Aerococcaceae	Increased	NR	NR
Anaeroplasmataceae	Decreased	NR	NR
Bifidobacteriaceae	Decreased	NR	NR
Burkholderiaceae	NR	Increased	NR
Cardiobacteriaceae	NR	Increased	NR
Carnobacteriaceae	Increased	Increased	NR
Corynebacteriaceae	NR	Increased	NR
Dehalobacteriaceae	Decreased	NR	NR
Enterobacteriaceae	Increased	NR	NR
Enterococcaceae	Increased	NR	Increased
Erysipelotrichaceae	NR	Decreased	NR
Gemellaceae	Increased	NR	NR
Lachnospiraceae	NR	Decreased	NR
Lactobacillaceae	Increased	Increased	NR
Leptotrichiaceae	Increased	NR	NR
Leuconostocaceae	Increased	NR	NR
Micrococcaceae	Increased	Increased	Increased
Pasteurellaceae	Increased	Increased	NR
Planococcaceae	Increased	NR	NR
Ruminococcaceae	Decreased	Decreased	NR
Staphylococcaceae	Increased	Increased	Increased
Streptococcaceae	Increased	Increased	Increased

Note. Bacterial families associated with PPI use in 3 gut microbiota studies.⁶⁻⁸; Consistent changes in 2 or all studies are marked in bold; NR = Not reported.

which could also potentially influence the gut microbiota, we grouped the most commonly used drugs and analyzed possible associations with the gut microbiota. This analysis identified several changes in the gut microbiota associated with other commonly used drugs. (See the most commonly prescribed medication in the Netherlands in Table 2 and the gut microbiota associations in Table 3). However, all gut microbiota alterations associated with PPI use remained statistically significant even after statistical correction for the use of other commonly used drugs.

Our group published an elaborate metagenomic sequencing analysis of 1135 participants of the same general population cohort used in our first study. In this

Table 2. Most commonly used medication in the Netherlands (17 million inhabitants) in 2015.

Rank	Medication name	Medication group or use	Users (millions)
1	diclofenac	NSAIDS	1.29
2	amoxicillin	Antibiotics	1.22
3	simvastatin	Statins	1.17
4	omeprazole	PPIs	1.16
5	metoprolol	β -blockers	1.11
6	macrogol	Stimulating bowel movements/anti-constipation	1.06
7	inert dermal creams	Skin creams for eczema	1.02
8	salbutamol	Dilate airways	0.90
9	colecalfiferol	Prevent osteoporosis	0.83
10	acetylsalicylic acid	Platelet aggregation inhibitor	0.81

Table 3. Commonly used medication associated with gut microbiota alterations. Table from Imhann et al. *Gut* 2016.

Medication category	Taxon	Direction
Antibiotics	<i>g__Holdemanina</i>	Increased
Antidepressants (SSRI, SNRI, mirtazapine and TCA)	<i>f__Bacteroidaceae</i>	Increased
Antidepressants (SSRI, SNRI, mirtazapine and TCA)	<i>g__Bacteroides</i>	Increased
Antidiabetic medication (both oral and insulin)	<i>o__Bacillales</i>	Increased
Changes bowel movement/stool frequency*	<i>p__Firmicutes</i>	Decreased
Changes bowel movement/stool frequency*	<i>o__Clostridiales</i>	Decreased
Changes bowel movement/stool frequency*	<i>c__Clostridia</i>	Decreased
Changes bowel movement/stool frequency*	<i>g__Coprococcus</i>	Decreased
Changes bowel movement/stool frequency	<i>o__Bacteroidales</i>	Increased
Changes bowel movement/stool frequency	<i>p__Bacteroidetes</i>	Increased
Changes bowel movement/stool frequency	<i>f__Bacteroidaceae</i>	Increased
Changes bowel movement/stool frequency	<i>g__Bacteroides</i>	Increased
Cholesterol lowering medication (statins)*	<i>o__Bacillales</i>	Increased
Cholesterol lowering medication (statins)	<i>g__Dorea s__longicatena</i>	Decreased
Cholesterol lowering medication (statins)	<i>g__Ruminococcus</i>	Increased
Triglyceride lowering medication (Fibrates)*	<i>g__Ruminococcus s__gnavus</i>	Increased

Note. All associations are statistically significant at FDR < 0.05 k__, kingdom; p__, phylum; c__, class; o__, order; f__, family; g__, genus; s__, species.

*Statistically significant FDR<0.05 after correction for PPI use.

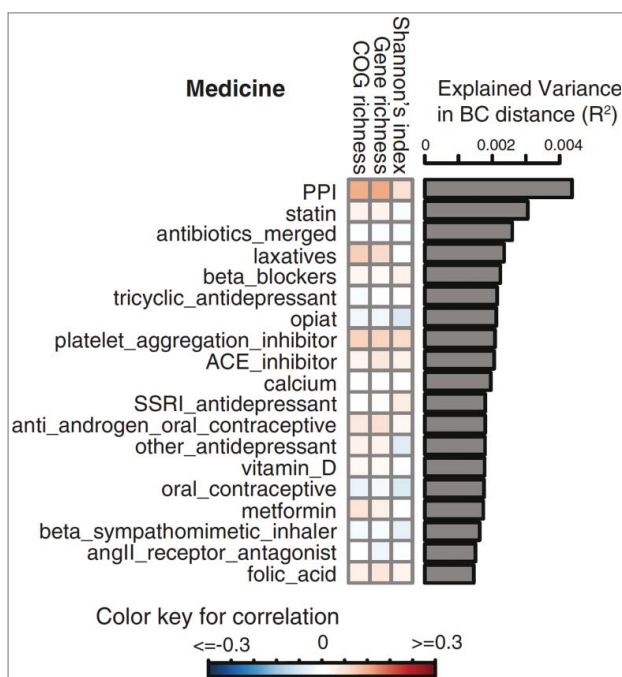


Figure 1. Inter-individual gut microbiota variation (Bray-Curtis distance) explained by commonly used medication at FDR < 0.1. Figure from Zhernakova et al. *Science* 2016. © *Science*. Reproduced by permission of *Science*. Permission to reuse must be obtained from the rightsholder.

metagenomics study, we showed that several other commonly used drugs are also associated with gut microbiota alterations (See Fig. 1).⁹ Consistent with our earlier results, the variance in the gut microbiota that could be explained by PPI use was the largest of all the commonly used drugs. However, many other drugs also had a statistically significant effect on the gut microbiota composition.

Consequences of gut microbiota changes caused by medication

What is becoming increasingly clear is that antibiotics, PPIs and metformin affect the gut microbiota and that other commonly used medication, like statins and SSRIs and are associated with distinct gut microbiota signatures.⁶⁻¹¹ As a consequence, these types of medication could have an effect on the risk of developing enteric infections or gut inflammation, and they may also have an effect on host metabolism.¹²⁻¹⁵

Susceptibility to enteric infections

A well-known example of reduced enteric infection resistance through alteration of the gut microbiota caused by medication is the increased risk of *Clostridium difficile*

infections after (repeated) treatment with antibiotics.¹⁶ Antibiotics kill off a large proportion of the gut microbiota, creating an empty niche that allows *Clostridium difficile* to colonize and overgrow.¹⁶ Recent observational studies indicated that it is not only antibiotic use, but also PPI use, that is associated with increased risk of *Clostridium difficile* infection.^{5,17} Subsequent gut microbiota studies attributed the increased risk to unfavorable gut microbiota alterations caused by PPI use.⁶⁻⁸ Human, animal and *in vitro* studies show an overlap between the specific gut microbiota alterations associated with PPI use (as found in Imhann et al. *Gut* 2016) and the bacterial changes that lead to increased susceptibility to *Clostridium difficile* (as depicted in Fig. 2).^{14,15,18-24} The US Food and Drug Administration (USFDA) already aims to limit the use of antibiotics to reduce the number of *Clostridium difficile* infections in the USA.²⁵ Since PPIs are often prescribed, and these prescriptions renewed, without evidence-based indication, a reduction of unnecessary PPI use could also contribute to this aim.¹⁷

PPI are also associated with an increased risk of other enteric infections caused by *Salmonella*, *Shigella* and *Campylobacter* species.⁴ In the Netherlands, recent trends toward increased incidence of campylobacter infections closely follow trends in the number of PPI prescriptions: both increased rapidly from 2004 to 2011 then showed small decrease in 2012.³ Since PPIs diminish the gastric acid barrier, pathogenic microbial species that would not otherwise survive the gastric acid could more easily be introduced into the gut microbiota.^{6,8} The overrepresentation of oral microbiota in the gut microbiota of PPI users supports this hypothesis.^{6,8} The effects of antibiotics and PPIs on the gut microbiota are currently the clearest examples of how medication can change susceptibility to enteric infections and have an effect on human health. The influence that other gut-microbiota-changing medication have on the susceptibility to enteric infections is still unclear.

Gut inflammation

Gut microbiota can promote or ameliorate gut inflammation. Favorable microbiota can ameliorate gut inflammation through the induction of regulatory T-cells (T_{regs}), interleukin 10 (IL-10) and the production of the short-chain fatty acid butyrate.^{26,27} In contrast, unfavorable microbiota can produce toxins that promote inflammation of the gut epithelium.²⁸ A

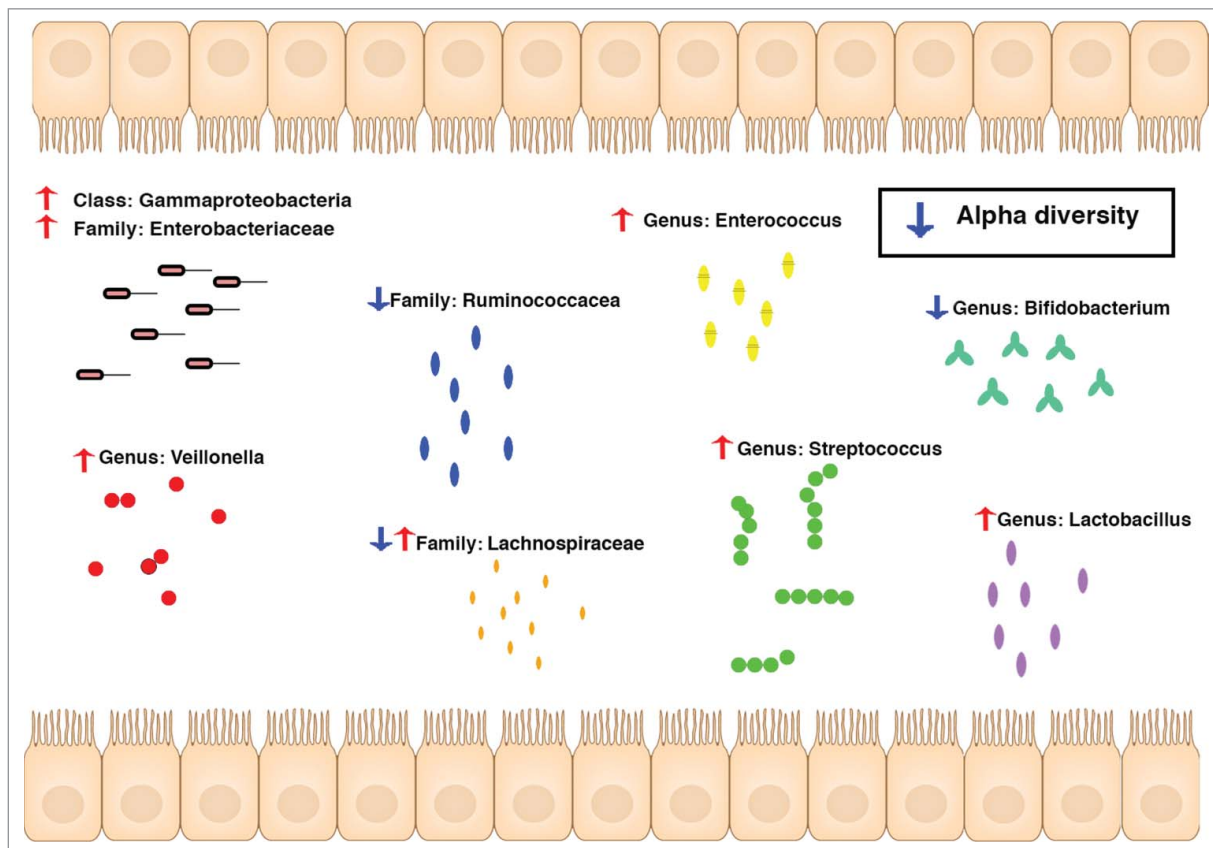


Figure 2. Gut microbiota changes associated with PPI use that increase the risk of *Clostridium difficile* infection.^{14,15,18-24}

profoundly disturbed balance between favorable and unfavorable gut microbiota, resulting in gut inflammation, is seen in Inflammatory Bowel Disease (IBD).²⁹ IBD is a common disorder of which the incidence continues to rise, and is attributed to the transition to a sedentary, high fat, high calorie 'Western lifestyle'.³⁰ Aside from changes in the habitual diet, prior *Salmonella* or *Campylobacter* gastroenteritis and prior use of antibiotics are also presumed IBD risk factors.^{30,31} Whether increased use of other commonly used medication that influence the gut microbiota could contribute to the increasing incidence of IBD is unknown. Links have been reported between the PPI use and increases in the gut inflammation marker, fecal calprotectin, but the mechanism by which PPI cause this increased fecal calprotectin, i.e. if they are due to gut microbiota changes, is not clear.³² Aside from the influence of PPIs, NSAIDs are known to sometimes cause enteropathy, ulceration and elevation of fecal calprotectin. How other commonly used medications affect inflammation in the gut still has to be elucidated.

BMI and lipid metabolism

Gut microbiota alterations have large effects on body mass index (BMI), lipid- and cholesterol-levels.^{12,33,34} The effects of some medications on the development of obesity have received considerable attention, for example, the effect of antibiotic use during childhood on obesity.³⁵ Use of other drugs is also related to weight gain and obesity, including several classes of antidepressants: SSRIs, SNRIs and TCAs.³⁶ These antidepressants are also associated with gut microbiota alterations, which could potentially be a mechanisms by which these drugs cause weight gain.^{6,9} PPI use is associated with an increased risk of cardiovascular disorders.³⁷ Although other mechanisms have been proposed,³⁸ the effects of PPIs on the microbiota could affect lipid metabolism and an thereby an individual's subsequent risk of cardiovascular events.^{9,12} The effects of commonly used medication and the effects on the host metabolism therefore warrant further investigation.

Intervention studies and animal studies are required to clarify the influence of commonly used medication on the gut microbiota

Intervention studies

One way to study the effects of medication on the gut microbiota is to perform an intervention study. Intervention studies in humans in which the pre-medication gut microbiota can be compared with the post-medication gut microbiota are relatively easy to implement. All commonly prescribed drugs have already been approved by the USFDA and the European Medication Agency (EMA). There are, by definition, a large number of patients who begin using these types of medication for the first time every day. Moreover, stool sampling is not an invasive procedure. One small scale intervention study investigating the role of PPI on the gut microbiota has already been performed.⁷

Animal studies

In addition to intervention studies in humans, mouse experiments are required in which gut microbiota changes can be studied in isolation and the effects in tissues and blood markers observed.³⁹ The effects on enteric-infection-resistance to *Clostridium difficile* through gut microbiota alterations have already been tested using a mouse model in which mice were exposed to pathogens after antibiotic treatment.¹⁵ This model could be used to investigate how other commonly used drugs affect enteric infection resistance. The effects on gut epithelial inflammation and inflammatory markers can also be studied using IBD mouse models.⁴⁰ Finally, mouse experiments can be used to investigate the metabolic effects of gut microbiota alterations.⁴¹

Commonly used medication should be part of the core phenotype set for human gut microbiota research

PPI use is overrepresented in many disorders and conditions, including obesity, non-alcoholic steatohepatitis (NASH), Irritable Bowel Syndrome (IBS), rheumatoid arthritis and IBD. NSAID use is overrepresented in rheumatoid arthritis. Antibiotics are much more often prescribed in Crohn's disease. Many of the current studies relating the gut microbiota and disease do not take into account the potential confounding effects of medication use. The poor reproducibility of

gut microbiota studies thus far could partially be explained by lack of collection or inadequate collection of confounding phenotypes.

To begin to resolve these issues, a new core set of phenotypes needs to be used as covariates in microbiota studies. We think this new core phenotype set should at least consist of:

- Age
- Sex
- BMI
- Commonly used medication including antibiotics, PPIs, laxatives, antidepressants, statins, metformin^{6,9}
- Bristol stool form chart and stool frequency⁴²

Conclusions

The use of PPIs, antibiotics, NSAIDs, SSRIs, metformin and other commonly used medication are associated with specific gut microbiota compositions. As a consequence, the use of these types of medication could affect how the gut microbiota can resist enteric infections, promote or ameliorate gut inflammation, or change the host metabolism. While the effects of antibiotics have been relatively well-studied, and the effects of PPI use are starting to become clear, the effects of many other commonly used medications on the gut microbiota remain unknown. More investigations of the role of commonly used medication on the gut microbiota and the subsequent health consequences is certainly needed.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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Author contributions

FI and AGLM wrote the manuscript. AVV designed the graphics. AVV, MJB, DPYK, JF, CW, AZ and RKW critically reviewed the manuscript.

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