Antibiotics as deep modulators of gut microbiota: between good and evil

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ABSTRACT

The recent increase in our knowledge of human gut microbiota has changed our view on antibiotics. Antibiotics are, indeed, no longer considered only beneficial, but also potentially harmful drugs, as their abuse appears to play a role in the pathogenesis of several disorders associated with microbiota impairment (eg, *Clostridium difficile* infection or metabolic disorders). Both drug-related factors (such as antibiotic class, timing of exposure or route of administration) and host-related factors appear to influence the alterations of human gut microbiota produced by antibiotics. Nevertheless, antibiotics are nowadays considered a reliable therapy for some non-communicable disorders, including IBS or hepatic encephalopathy. Moreover, some antibiotics can also act positively on gut microbiota, providing a so-called 'eubiotic' effect, by increasing abundance of beneficial bacteria. Therefore, antibiotics appear to change, for better or worse, the nature of several disorders, including IBS, IBD, metabolic disorders or liver disease. This reviews aims to address the potential of antibiotics in the development of major non-communicable disorders associated with the alteration of gut microbiota and on newly discovered therapeutic avenues of antibiotics beyond the cure of infectious diseases.

Key messages

- Antibiotics are no longer considered only beneficial, but also potentially harmful agents, as their overuse has been linked to microbiota impairment and related disorders.
- Antibiotics appear to be a reliable treatment not only for infectious disorders, but also for several non-communicable ones.
- Antibiotic characteristics that can influence their 'evil' or 'good' behaviour towards gut microbiota include their class, pharmacokinetics, pharmacodynamics and range of action, as well as their dosage, duration and administration route.
- Host-related factors that are known to influence the size and quality of antibiotic damage to balanced microbiota include age, lifestyle and microbiota composition.
- Some antibiotics can also act positively on gut microbiota, providing a so-called 'eubiotic' effect, by increasing abundance of beneficial bacteria.

INTRODUCTION: THE ANTIBIOTIC REVOLUTION AND THE MICROBIOTA REVOLUTION

The discovery of penicillin by Alexander Fleming in 1928 represents a breakthrough in the history of medicine as it catalysed the development of antibiotics, beginning the so-called 'antibiotic revolution'.[1] By conferring to mankind resilience to the majority of bacterial assaults, antibiotics altered the natural history of most infectious diseases and saved millions of lives. Their exploitation transformed not only medicine, but also pharmacology, healthcare industry and the whole human everyday life.

Several decades after, another paradigm shift changed again our relationship with bugs. Although Metchnikoff already hypothesised a role for our microbiota in health and disease at the beginning of the 20th century,[2] the undetectability of most components of gut microbiota with available diagnostic technique impeded the growth of interest in this field. The development of 'omics' technologies, based on microbial genome sequencing, allowed the in-depth assessment of gut microbiota composition and functions,[3] laying the groundwork to a new approach to microbial components of our ody, recently addressed as 'microbiota revolution'.[4]

After this groundbreaking improvement of technology, two cornerstones of antibiotic therapy started to be undermined. First, antibiotics are no longer considered only beneficial, but also potentially harmful agents, as increasing evidence supports the correlation between their overuse and the development of many disorders associated with the alteration of gut microbiota;[5] moreover, antibiotics overexposure may also lead to the development of genotypic antibiotic resistance in the resident microbiota and to its potential transfer to pathogenic bacteria;[6] additionally, antibiotics were indeed shown to enrich phage-encoded genes, which transfer resistance to both the administered drug and also to unrelated antibiotics, as well as to promote the interplay between phages and bacteria, enhancing, consequently, resistance gene exchange.[7]

The second undermined certainty about antibiotics is that the healing of bacterial infections is not considered anymore as their only therapeutic purpose as they appear to be a reliable treatment for several non-communicable disorders (eg, hepatic encephalopathy (HE) or IBS).

This reviews aims to address the potential of antibiotics in the manipulation of gut microbiota and related clinical consequences, focusing both on GI and extraintestinal disorders associated with microbiota impairment driven by antibiotics, and on newly discovered therapeutic avenues of antibiotics beyond the cure of infectious diseases.

Key messages

▶ Antibiotics appear to change, for better or worse, the natural history of several disorders, including IBD, IBS, metabolic disorders or liver disease.

▶ Use of antibiotics has been suggested to play a direct role in the development of IBD by leading to dysbiosis and reduced bacterial diversity. Another theory, instead, suggests antibiotics are only surrogate markers of other risk factors for IBD such as GI infections.

▶ Although systemic antibiotics appear to increase the risk of IBS development, a large body of evidence identified poorly absorbable antibiotics (mainly rifaximin) as a reliable treatment for IBS and hepatic encephalopathy.

▶ According to the administration of high or low dosages, respectively, antibiotics were shown to be able to drive either to underweight, through deep demolition of gut microbiota, or overweight, by development of selective dysbiosis.

▶ Early life appears to be a critical period for the maturation of metabolic functions, and antibiotic-dependent impairment of microbiota during this period, even if transient, could have profound effects on weight gain, although recent studies provided conflicting results.

▶ A wiser use of antibiotics in clinical practice, as well as a boost in this field of research, is advocated for a better management of patients with disorders related to the impairment of gut microbiota.

EFFECTS OF ANTIBIOTICS ON GUT MICROBIOTA COMPOSITION

Human gut microbiota in health

Bacteria are the most represented members of the human gut microbiota, with >1000 species, most of which are anaerobes, accounting for an absolute number higher than 10^{14}.[8] Although increasing evidence on gut virome[9] and gut mycome[10] is appearing, most of the available data on our microbiota still concern bacteria. The majority (>90%) of bacteria identified in human stools and/or intestinal mucosa belong to the Bacteroidetes and Firmicutes phyla. The remaining bacteria are included within four major phyla (Actinobacteria, Fusobacteria, Proteobacteria and Verrucomicrobia) and other minor phyla.[11] Bacteria are the most relevant contributors in the fulfilment of microbiota-dependent functions, including the regulation of several metabolic pathways, the barrier action against pathogens, and the growth and consolidation of immune system.[12]

As 60–80% of intestinal bacteria are not identifiable by culture, only the transition to culture-independent technologies, including high-throughput sequencing and other DNA sequencing techniques, lets us discover composition and functions of healthy gut bacterial microbiota.[13]

Consequently, this makeover allowed us to identify the 'microbiota fingerprint' of several disorders, understand the potential role of gut microbiota in their development and learn how microbiota deteriorates under particular conditions, including dietary habits, diseases or drugs.[8]

The effect of antibiotics on gut microbiota composition

As most of the antibiotics available on the market have a broad spectrum of action, they impact not only on harmful bacteria, but also on healthy ones. The impairment of gut microbiota is

the main, but not the only one, effect of antibiotics on our intestine: other mechanisms of action include a direct harmful effect of antibiotics on GI epithelia and the spread of antibiotic-resistant microorganisms.[14]

Short-term alterations of healthy gut microbiota due to antibiotics have been extensively investigated in the past years.[15] On the contrary, only a few recent studies have addressed long-lasting changes of gut microbiota after antibiotic treatment. Both drug-related factors and host-related factors affect the impact of antibiotics on human gut microbiota.

Drug-related factors

Antibiotic characteristics that can influence this relationship include their class, pharmacokinetics, pharmacodynamics and range of action, as well as their dosage, duration and administration route.[16] Different antibiotic classes provide different patterns of microbiota alteration because of their different spectrum and bacterial target (table 1). Lincosamides, mainly clindamycin, are broad-spectrum antibiotics with main biliary excretion (and, consequently, high concentrations in stools), which are particularly active against anaerobes. This feature makes clindamycin one of the strongest antibiotic risk factors for the development of *Clostridium difficile* infection, with an OR of 2.86, as accounted

Table 1 Overview on the effects of different antibiotics on gut microbiota, according to their classes and excretion

Antibiotic class	Antibiotic excretion	Effects on gut microbiota
Lincosamides Clindamycin	Main biliary excretion	↓Gram-positive aerobes and anaerobes[17] ↑Resistance genes[18] ↓ Bacteroides diversity[18]
Macrolides Clarithromycin Erythromycin	Biliary excretion	↓Total bacterial diversity[0] ↓Actinobacteria (including *Bifidobacteria*)[19][20] ↓Firmicutes (mainly *Lactobacilli*)[20] ↑Bacteroidetes[20] ↑Proteobacteria[20] ↓Firmicutes[26] ↓Actinobacteria[26] ↑Proteobacteria[26]
β-Lactams Penicillin V Amoxicillin Ampicillin/ sulbactam Cephalosporins	Main urinary excretion Partial (33–67%) biliary excretion[24]	No relevant changes[20] ↓Firmicutes[26] ↓Actinobacteria[26] ↑Proteobacteria[26] No relevant changes[20][21] ↓Total bacterial richness[23] ↓Firmicutes[23] ↑Bacteroidetes[23] ↑Proteobacteria[23] ↓Firmicutes[26] ↓Actinobacteria[26] ↑Proteobacteria[26] ↓Total bacterial richness[23] ↓Firmicutes[23] ↑Bacteroidetes[23] ↑Proteobacteria[23]
Fluoroquinolones Ciprofloxacin Levofloxacin	Partial biliary excretion	↓Bacterial diversity[27] ↓Gram-negative facultative anaerobes[25] ↑Gram-positive aerobes[26] ↓Gram-negative facultative anaerobes[25] ↓Gram-positive anaerobes[25]
Glycopeptides Vancomycin*		↓Total bacterial diversity[0] ↓Firmicutes[20] ↑Proteobacteria[20]

*Oral administration (vancomycin is not adsorbed when administered orally).

in a recent meta-analysis.[17] Clindamycin decreased Gram-positive aerobes and anaerobes, when given for 10 days to healthy individuals, with long-lasting impairment of microbiota composition.[18] Long-term disturbances of microbiota composition, mainly decrease in the clonal diversity of *Bacteroides*, and persistent increase in levels of specific resistance genes, were also observed after short clindamycin exposure.[19]

As well as lincosamides, macrolides are protein synthesis inhibitors with biliary excretion, which act mainly against Gram-positive bacteria. Indeed, clarithromycin was accounted for the long-lasting decrease of Actinobacteria (which are naturally resistant to metronidazole) observed in *Helicobacter pylori*-positive patients exposed to standard first-line triple therapy.[20]

More recently, in a large cohort of Finnish children, macrolides were shown to induce long-term alterations of microbiota, particularly reduction of Actinobacteria (mainly *Bifidobacteria*), Firmicutes (mainly *Lactobacilli*) and total bacterial diversity, as well as an increase of relative abundance of Bacteroidetes and Proteobacteria.[21] In the same cohort, interestingly, penicillins—in particular, penicillin V and amoxicillin—were not associated with relevant changes of microbiota. More recent data confirmed the weak impact of amoxicillin on human gut microbiota.[22] The powerlessness of amoxicillin in modulating gut microbiota may explain its ineffectiveness in providing nutritional recovery from severe malnutrition, which was recently observed in a large cohort of African children.[23] However, a β-lactam combination, including ampicillin and cephalosporins, led to a decrease in Firmicutes and an increase in Bacteroidetes and Proteobacteria (particularly *Enterobacteriaceae*, which are commonly resistant to β-lactam antibiotics) and reduced microbial richness.[24] This difference in results may be explained by the higher concentration in faeces of cephalosporins than amoxicillin.[25] Interesting data come from the perinatal administration of β-lactam antibiotics, with or without other antibiotic classes. Intrapartum antibiotic prophylaxis with penicillin, ampicillin or ampicillin plus erythromycin led to decreased levels of short-chain fatty acids (SCFAs), Firmicutes and Actinobacteria, and to increase in Proteobacteria.[26]

Also, some fluoroquinolones, including ciprofloxacin and moxifloxacin, are partially excreted through the biliary system, and about one-third of their oral intake can be found in faeces. Ciprofloxacin acts against Gram-negative facultative anaerobes, whereas levofloxacin also decreases the number of Gram-positive anaerobes, including *Bifidobacteria*.[27] Recently, ciprofloxacin was shown to provide long-term changes—mainly, increasing Gram-positive aerobes—to the microbiota of healthy individuals,[28] as well as to reduce bacterial diversity.[29] In another series, fluoroquinolones increased Bacteroidetes and Proteobacteria, without modifying Firmicutes.[24]

The ability of antibiotics to manipulate microbiota can also depend on their route of administration. Vancomycin has shown to be a powerful modulator of microbiota when administered orally as it is not absorbed from the gut. First data come from mouse models, in which vancomycin, together with bacitracin, decreased Bacteroidetes, Firmicutes and total microbiota richness.[29]

In a small randomised-controlled trial of obese males with metabolic syndrome, oral vancomycin reduced bacterial diversity and Firmicutes, increasing Proteobacteria.[22] Moreover, oral route of administration of antibiotics was recently demonstrated to stimulate the development of antibiotic resistance among healthy microbiota members much more than the intravenous one.[30]

Nevertheless, antibiotics can also act positively on gut microbiota, providing a so-called 'eubiotic' effect.[31] Some antibiotics were, indeed, demonstrated to stimulate the growth of beneficial

bacteria. Nitrofurantoin, a broad-spectrum antibiotic with activity for both Gram-negative and Gram-positive bacteria, provided a temporary increase in Actinobacteria, and mainly in *Bifidobacteria*, when administered to 61 patients for uncomplicated urinary tract infections.[32] In another small sample of outpatients with urinary tract infections, nitrofurantoin treatment increased also *Fecalibacterium* genus.[33]

Increasing evidence supports the 'eubiotic' properties of rifaximin, a poorly absorbable antibiotic with broad-spectrum coverage (aerobes, anaerobes, Gram-positive and Gram-negative bacteria). In a small trial of 15 patients with IBS, rifaximin increased bacterial diversity, the Firmicutes/Bacteroidetes ratio and the abundance of *Faecalibacterium prausnitzii*, a butyrate producer with strong anti-inflammatory properties, which is reduced in IBD.[34] In another report of 19 patients with different GI and liver disorders (IBD, IBS, diverticular disease, HE), rifaximin increased the abundance of *Lactobacilli*, with no effects on bacterial diversity.[35]

Finally, early life treatment with vancomycin was able to prevent the onset of diabetes in non-obese diabetic mice by increasing levels of *Akkermansia muciniphila*,[36] a mucin-degrading bacterium that can be found in the intestinal mucus layer, whose abundance is inversely correlated with body weight and type 2 diabetes (T2D) both in mice and humans.[37 38] Interestingly, levels of *A muciniphila* increased also in two critically ill patients after treatment with multiple antibiotics.[39]

Host-related factors

Also, host-related factors are known to influence the size and quality of antibiotic damage to healthy bacteria, including age, lifestyle and microbiota composition.

Concerning data come from paediatric studies as infant microbiota is still unconsolidated, so reacts worse to injuries. Antibiotics appear to modify human gut microbiota starting yet from the very early life. Data from several studies support that broad-spectrum antibiotics produce long-lasting effects on microbiota of newborns and infants, including increase in Proteobacteria, and particularly *Enterobacteriaceae*, and decrease in abundance and/or diversity of Actinobacteria, especially *Bifidobacteriaceae* and *Bifidobacteria*.[40–42] Higher abundance of *Enterobacteria* was also found in a large cohort of infants treated with antibiotics compared with untreated ones.[43] Similar results come from data on preterm infants.[44]

A body of evidence shows that antibiotics can impair infant microbiota even before birth. In a large Canadian cohort, infants whose mothers received intrapartum antibiotic prophylaxis displayed a long-lasting dysbiosis, in particular an increase in *Enterococci* and *Clostridia* and lower proportion of *Bacteroides* than others;[45] under-representation of *Bacteroides* was found also in infants from a European cohort, whose mothers received antibiotics perinatally and/or during breast feeding.[43]

In summary, available data agree on quite homogeneous alterations of infant microbiota after antibiotic treatment; this is easily explainable as infant microbiota is still immature.

By contrast, the initial composition of gut microbiota appears to influence its response to antibiotics in adults; in a recent study of 18 young healthy volunteers, a 7-day regimen of cefprozil led to similar qualitative alterations of microbiota in the majority of subjects, but to totally different findings in a subset of individuals with predominant *Bacteroides* enterotype and lower microbial diversity.[46]

Finally, some evidence shows that microbiota response to antibiotics may be also influenced by host lifestyle; in a large cohort of elders, the microbiota of subjects living in long-term care

residences was more destabilised by antibiotic therapies than that of community-based ones.[47]

Recently, antibiotic effects on gut microbiota have been evaluated through a multi-omic approach. Microbial 16S rDNA and 16S rRNA, metagenome, metatranscriptome, metametabolome and metaproteome of a patient being exposed to cefazolin for 14 days were evaluated; changes in microbiota composition were accompanied by the reduction of the expression of several proteins essential for the microbial activity, by the attenuation of the microbiota metabolic status and by the decrease of host–microbial interaction.[48]

A clinical model of antibiotic-driven dysbiosis: the case of C difficile infection

C difficile infection (CDI) represents probably the most striking clinical example of antibiotic-driven dysbiosis as it usually arises as a complication of antibiotic therapy, especially in elder people. In recent years, the prevalence of CDI has increased tremendously, especially in Western countries,[49] making it become the leading cause of infectious diarrhoea among hospitalised patients, and, consequently, an economic and healthcare burden.[50]

Overexposure to antibiotics, especially in elderly and fragile patients, has strongly contributed to the development of such a scenario. In a recent meta-analysis pooling >56 000 patients, antibiotics were the highest risk factor for the development of CDI (OR 6.18; 95% CI 3.80 to 10.04).[51]

Antibiotic exposure can promote the development of CDI through several pathogenic pathways. First, antibiotic-dependent alteration of healthy microbiota prevents its resistance towards colonisation by C difficile.[52][53] Subjects who developed CDI after antibiotic therapy showed a depletion of their microbiota diversity, together with qualitative microbial alterations, including a decrease in families producing SCFAs, such as Lachnospiraceae and Ruminococcaceae.[54][55] Also, changes in the metabolic functions of gut microbiota may promote the development of CDI; in a mouse model, the antibiotic-dependent release of free sialic acid by commensal microbiota promoted the colonisation.[56] Moreover, as production of C difficile toxin is regulated through quorum signalling,[57] antibiotic-dependent depletion of other microbiota members provides opportunity for C difficile to spread and express its virulence factors.

Finally, growth of C difficile could be promoted also by an 'evil' interplay between microbiota, antibiotics and bile acids. In health, some primary bile acids do not undergo ileal reuptake and can be found in the large bowel; here, cholic acid is able to promote growth of C difficile,[58] while chenodeoxycholic acid, and the secondary bile acids (lithocholic and deoxycholic acid) derived from microbial-dependent dehydroxylation of primary bile acids, counteract this effect by inhibiting the germination of C difficile spores.[59][60] Therefore, antibiotics may enhance the growth of C difficile by depleting bacteria that dehydroxylate bile acids, such as Clostridium scindens, which was shown to have a protective role against CDI.[60][61]

The therapeutic corollary of the pathogenic role of antibiotics in CDI is represented by faecal microbiota transplantation, which was proven to be a highly effective treatment for recurrent CDI,[62][63] and to restore the CDI-dependent microbiota impairment.[63][64]

EFFECTS OF ANTIBIOTICS ON NON-COMMUNICABLE DISORDERS
IBD

Gut microbiota of patients with IBD goes through several qualitative and quantitative alterations. Decrease in bacterial diversity

and higher bacterial instability have been described in patients with IBD compared with healthy subjects.[65] Most pronounced changes in the gut bacteriome of patients with IBD include, at phylum level, increase in Proteobacteria[66–68] and decrease in Firmicutes.[66] Among Proteobacteria, increase in Enterobacteriaceae family,[66] and, in particular, in Escherichia coli adherent-invasive strains,[69] is the most notable.

Changes in Firmicutes include, beyond reduction of Lactobacilli,[70] depletion of SCFAs producers, as Lachnospiraceae[66] and Ruminococcaceae[71] at family level, and Eubacterium,[70] Roseburia[72][73] and Faecalibacterium,[72] at genus level; in particular, the decrease of F prausnitzii, an anti-inflammatory and butyrate producer strict anaerobe, has been repeatedly found both in patients with Crohn's disease (CD)[74][75] and in patients with UC.[75]

Interesting data come also from the analysis of non-bacterial members of microbiota, previously neglected. Altered fungal diversity, increase of Basidiomycota/Ascomycota ratio and Candida albicans abundance, together with reduction of Saccharomyces cerevisiae abundance, have been reported in patients with IBD.[76] Also, the 'gut virome' is known to be altered in subjects with IBD: increased richness of bacteriophages, with marked expansion of Caudovirales and specific viral signature for patients with UC and CD, has been recently described; interestingly, it was inversely correlated with decreased bacterial diversity.[77]

Based on these findings, antibiotics have been suggested to play a direct role in the development of IBD by leading to dysbiosis and reduced bacterial diversity.[78] A considerable body of evidence supports this hypothesis. A recent meta-analysis of 11 observational studies and 7208 patients showed that antibiotic exposure has a 1.57 OR for IBD overall (95% CI 1.27 to 1.94); with a significant association for CD (OR 1.74, 95% CI 1.35 to 2.23), especially in children (OR 2.75, 95% CI 1.72 to 4.38), but not for UC (OR 1.08, 95% CI 0.91 to 1.27); all antibiotics, particularly metronidazole (OR 5.01, 95% CI 1.65 to 15.25) and fluoroquinolones (OR 1.79, 95% CI 1.03 to 3.12), were associated with IBD development, except to penicillin;[79] these results are in agreement with the weak dysbiotic behaviour of penicillins.[21–23]

Another theory, instead, suggests antibiotics to be only surrogate markers of other risk factors for IBD as bacterial intestinal infections have been identified as a risk factor for further initiation of IBD, especially CD.[80]

In contrast, there are several therapeutic pathways through which antibiotics can change positively the natural course of IBD, including the reduction of luminal bacteria abundance and translocation, and the 'eubiotic' modulation of microbiota.[81] Many meta-analyses of randomised-controlled trials showed a significant benefit of antibiotics over placebo in the induction of remission of UC[82–84] and CD,[83] although they pooled together different antibiotic classes, making their findings difficult to be interpreted.

Antibiotics are, indeed, suggested by current european crohn's and colitis organisation (ECCO) guidelines for UC only if infectious complications are suspected or ongoing and before surgical interventions.[85] ECCO-European Society for Paediatric Gastroenterology Hepatology and Nutrition (ESPGHAN) guidelines for the management of paediatric IBD confirmed these advices, without considering antibiotics as a reasonable treatment for the management of uncomplicated paediatric UC.[86] Also for CD, current ECCO guidelines do not recommend the use of antibiotics for uncomplicated diseases, but only in the case of sepsis, abdominal or perianal abscess, or bacterial overgrowth.[87] Also, British Society of Gastroenterology (BSG)

guidelines suggest the use of antibiotics mainly for secondary complications in IBD; nevertheless, the use of antibiotics in short-term treatment of colonic CD is taken into account for patients with refractory disease or contraindications to other therapeutic options supported by stronger evidence.[88]

IBS

Several lines of evidence support a pathogenic role for impaired microbiota in IBS. First, a considerable rate of patients (up to 30%) experience IBS symptoms after an episode of acute gastroenteritis (the so-called 'postinfectious IBS'),[89] especially if happened during early life,[90] when microbiota is still consolidating. Then, small intestinal bacterial overgrowth has been suggested to play a role in the development of IBS symptoms,[91] although the issue is still controversial.[92] Finally, the quite satisfactory success of therapeutic modulators of microbiota, including dietary advices, antibiotics and probiotics, represents an *ex juvantibus* support of this hypothesis.[93]

Many studies, carried on through DNA sequencing technologies, showed that microbiota composition is altered in subjects with IBS. An increase of Firmicutes/Bacteroidetes ratio was described in patients with IBS compared with controls; in particular, a decrease in the abundance of *Bifidobacteria*, *Faecalibacteria* and methanogenic bacteria was found.[94] Moreover, pyrosequencing analysis identified two microbiota-based subcategories of patients with IBS, including one without abnormalities and another which displayed a decrease in bacterial taxa belonging to Bacteroidetes and an increase in those belonging to Firmicutes.[95] Interestingly, a depletion of butyrate producers, including *Roseburia–Eubacterium rectale* group, and an increase in sulfate-reducing bacteria were identified in subjects with constipation-type IBS (C-IBS).[96]

In another analysis of five groups, including patients with postinfectious IBS, patients with or without IBS symptoms 6 months after gastroenteritis, patients with diarrhoea-type IBS (IBS-D) and healthy controls, authors identified a microbiota pattern of 27 genus-like groups of bacteria, which grouped significantly patient groups and controls; in particular, patients displayed a 12-fold increase of Bacteroidetes, and healthy individuals showed a 35-fold increase in uncultured Clostridia; interestingly, microbiota of patients with postinfectious IBS was similar to that of patients with IBS-D, suggesting a shared pathophysiological pathway.[97]

IPatients with BS exhibit also deep changes in their metabolite profiles, as observed in children with IBS, who showed an increase in proteolysis, incomplete anaerobic fermentation and alterations in the production of methane.[98] A meta-analysis of case–control studies determining microbiota profiles in patients with IBS showed that IBS-associated species and specific microbiota alterations were influenced by geographical location, being different between Chinese and non-Chinese subjects.[99]

The alteration of gut microbiota has been postulated to contribute to IBS pathogenesis through several pathways, including the impairment of gut–brain axis, of the intestinal barrier and of neuro-enteric physiology.[100]

Antibiotics are able to interfere with the natural history of IBS with a two-faced behaviour, as showed by various lines of evidence. Data from large cohort and case–control studies suggest antibiotics to be a risk factor for the development of IBS and other functional GI disorders (FGIDs), probably by leading to dysbiosis in the host.

In a large retrospective study of nearly 26 000 patients, exposure to macrolides or tetracyclines were significant predictors of developing IBS, whereas treatment with cephalosporins or penicillins did not.[101]

In a prospective case–control study, antibiotic therapy for extraintestinal infections was significantly associated with FGIDs (OR 1.90; 95% CI 1.21 to 2.98) and IBS alone (OR 2.30; CI 1.22 to 4.33).[102]

Although systemic antibiotics appear to increase the risk of IBS development, a large body of evidence identified poorly absorbable antibiotics as a reliable treatment for IBS. At first, neomycin was proven to be effective in improving overall symptoms of IBS together with the normalisation of lactulose breath test, as shown in a large randomised placebo-controlled trial.[103] Nevertheless, neomycin lost its early attractiveness because of the rapid development of antimicrobial resistance. Absorbable antibiotics, including doxycycline or ampicillin/clavulanate, did not exert reliable results.[104] Among non-adsorbable antibiotics, rifaximin was the most studied in patients with IBS, and also the one that gave most reliable results, as observed in several randomised, placebo-controlled trials. Rifaximin was significantly more effective than placebo in improving global IBS symptoms when given at the dosage of 400 mg twice daily for 10 days.[105] Furthermore, rifaximin confirmed to provide sustained improvement of overall IBS symptoms, which lasted for up to 10 weeks after the discontinuation of treatment, at a slightly higher dosage (400 mg thrice daily for 10 days).[106]

However, the strongest evidence about the effectiveness of rifaximin in relieving IBS symptoms comes from two identical randomised double-blind, placebo-controlled trials (TARGET 1 and TARGET 2, respectively). Overall, 1260 patients with IBS without constipation were randomised to placebo or rifaximin at the dosage of 550 mg thrice daily for 2 weeks; rifaximin was significantly more effective than placebo in providing a significant relief of overall IBS symptoms, abdominal pain, bloating and loose or watery stools during the first four weeks after the end of treatment, and a sustained improvement during the first two months and during all 10 weeks of follow-up, in both trials.[107] This large body of evidence led rifaximin to be approved by US Food and Drug Administration for the treatment of IBS-D in 2015.[108] Rifaximin and neomycin were investigated also in patients with C-IBS: in a randomised placebo-controlled trial, patients with C-IBS and methane producing were randomised to undergo neomycin with placebo or rifaximin for 14 days; combination therapy achieved better results than neomycin alone, improving significantly constipation, bloating and straining, together with decrease in breath methane.[109]

Obesity-related disorders

Gut microbiota is known to play a major role in collecting, storing and spending diet-derived energy. As demonstrated by both animal models[110] and human studies,[111] the microbiota 'fingerprint' of obesity is represented by an increase in relative abundance of Firmicutes and decrease in that of Bacteroidetes, and the Bacteroidetes/Firmicutes ratio has been addressed as the 'adiposity index', although with some controversies.[112] Furthermore, a decreased beta diversity, together with a reduction of anti-inflammatory bacteria (such as *F prausnitzii*) and an increase in pro-inflammatory bacteria (including *Ruminococcus gnavus*) were observed in obese patients.[113] Beyond obesity, gut microbiota is altered in other disorders associated with metabolic imbalance; this depends, at least in part, by the direct influence of high-fat diet on gut microbiota, independently of obesity.[114]

Recently, two metagenome-wide association studies, respectively from China[115] and Europe,[116] addressed microbiota composition in patients with T2D: despite population-specific changes, several common findings, including a decrease in butyrate producers (including *Roseburia intestinalis* and *F prausnitzii*), an increase in Proteobacteria and in microbial genes responsible for oxidative stress, were identified.[117]

Moreover, a growing body of evidence suggests that *A muciniphila* levels are inversely associated with obesity and T2D both in mice and in humans.[37 38]

Recently, a microbiota-dependent therapeutic pathway of the antidiabetic drug metformin has been demonstrated as it has been shown to increase the production of SCFAs in patients with T2D.[118] In particular, in high-fat diet-fed mice, the metformin-dependent improvement of glycaemic profile appears to be mediated by the increase in *A muciniphila* levels.[119]

Manipulation of microbiota through antibiotics has been used to modulate weight, also ahead of its time. Farm animals were usually treated with low dosage of antibiotics, to promote their growth, since the 1940s.[120] Several studies, performed both in animal models and in humans, show that antibiotic treatment have a profound influence on recipients' weight.

In mouse models, effects of antibiotic treatment towards weight and microbiota appear to depend on several factors, including drug dosage and timing of antibiotic exposure.[121] According to the administration of high or low dosages, respectively, antibiotics were shown to be able to drive either to underweight, through deep demolition of gut microbiota,[122] or overweight, by development of selective dysbiosis.[123 124] Beyond total body weight increase, low-dosage antibiotics provide also an increase in adiposity and insulin resistance, alteration of liver metabolism and composition of microbiota.[123 125]

Nonetheless, both in ob/ob and diet-induced obese mice with insulin resistance, antibiotics showed, respectively, antidiabetic effects (improvement in fasting glycaemia, glucose intolerance and insulin signalling, and increase in glucagon-like peptide 1),[29 126 127] and decrease of metabolic endotoxemia.[128]

These effects appear to be independent of antibiotic classes,[124] but also to be deeply influenced by timing of administration: in the study from Cox *et al*, newborn mice undergoing antibiotic treatment experienced higher weight gain than those receiving antibiotics at weaning and controls. Furthermore, these long-lasting modifications were provided by short-term antibiotic regimens. Early life, therefore, appears to be a critical period for the maturation of metabolic functions, and antibiotic-dependent impairment of microbiota during this period, even if transient, can have profound effects on weight gain.[124]

Data provided by these elegant mouse models are in agreement with those coming from their human counterpart. A body of large population-based studies, indeed, addressed that early antibiotic exposure (0–24 months of life) is associated with higher risk of overweight/obesity/weight gain later in childhood.[129–132] These results have been confirmed also in a recent systematic review, which found any antibiotic exposure during infancy as a risk factor for overweight later in childhood.[133] Nevertheless, some recent papers did not confirm the association between early-life antibiotic administration and later weight gain.[23 134] Differences in populations (including age and geography) and in drugs features (antibiotic classes, dosages, number of courses) may explain this disagreement. The importance of early-life exposure in driving the metabolic effects of antibiotics has been confirmed by data on antibiotic administration during pregnancy[135] and adolescence,[136] which respectively confirm and disagree with those observed in infant population.

Moreover, antibiotics appear to influence also other metabolic pathways, beyond weight gain. A short-term regimen of vancomycin was shown to reduce peripheral insulin sensitivity and microbiota diversity, together with a decrease in Firmicutes and an increase in Proteobacteria, in obese patients,[22] although these findings were not confirmed in a further study.[137] Finally, in a large case–control study, a positive association between multiple antibiotic courses (cephalosporins, penicillin, macrolides and quinolones) and the risk of developing diabetes was observed.[138]

In summary, the weight increase and metabolic impairment due to low-dosage antimicrobial therapy may be the result of a phylogenetic trick of human beings: it can be hypothesised that, during ancient times, foods with intrinsic small antimicrobial properties could have driven humans to weight increase and, consequently, low-grade inflammation due to obesity. This should be considered an advantage in conditions of undernourishment and low availability of resources. Considering also the positive correlation between early-life antibiotic exposure and further development of allergic asthma,[139–141] the use of antibiotics in newborns should be pondered with care by physicians.

Liver disease

More than two-thirds of the blood directed to the liver comes from the gut, through the portal venous system; this makes quite obvious that a close relationship between gut and liver, the so-called 'gut–liver' axis, exists. In particular, the blood flow drives to the liver several microbial derivates, including peptidoglycans and endotoxins. Gut microbiota plays, indeed, a major role in the pathogenesis of many chronic liver diseases, as supported by increasing evidence. Gut microbiota is known to produce endogenous alcohol and to contribute to non-alcoholic fatty liver disease (NAFLD).[142] According to different studies, several changes in gut microbiota composition have been described in patients with NAFLD, sometimes with conflicting findings, depending on methods and features of patients and liver disease;[143 144] interestingly, microbiota fingerprints of NAFLD severity have been recently identified, as increase in *Bacteroides* and *Ruminococcus* was respectively associated with non-alcoholic steatohepatitis (NASH) and with high levels of liver fibrosis.[145] Finally, altered intestinal permeability has been described in patients with NAFLD.[146] Inadequate alcohol intake is also known to alter gut microbiota composition, and increase in Proteobacteria and depletion of Bacteroidetes, and their correlation with endotoxaemia, was observed in alcohol-dependent patients.[147 148] Finally, two studies of patients with liver cirrhosis showed an increase in pro-inflammatory bacterial families, especially *Enterobacteriaceae* and *Streptococcaceae*, and a decrease in beneficial bacteria, including *Bifidobacteria* and *Lachnospiraceae*; in particular, Child-Turcotte-Pugh score correlated positively with *Streptococcaceae* abundance and negatively with *Lachnospiraceae* abundance.[149] More recently, quantitative metagenomics revealed decrease in Bacteroidetes and Firmicutes phyla, and increase in *Streptococcus* and *Veillonella* genera, in those patients.[150] Gut microbiota is also known to influence the development of HE by producing ammonia and eliciting an endotoxin-dependent inflammatory response, which act in synergy.[151] Cirrhotic patients with HE display higher abundance of *Enterobacteriaceae* and *Alcaligenaceae* than both subjects with cirrhosis without HE and healthy controls; moreover, levels of *Porphyromonadaceae* and *Alcaligenaceae*, which produce ammonia by urea degradation, are associated with poor cognitive performance.[152]

As a large body of evidence proved their efficacy, antibiotics are considered a cornerstone in the management of HE. After early attempts with neomycin[153] and metronidazole,[154] the most relevant data come from randomised-controlled trials on rifaximin.

Rifaximin, given with or without lactulose, showed higher efficacy than lactulose alone in the treatment of patients with overt HE[155] and was also more effective than placebo in maintaining remission from HE,[156 157] showing also good safety data.[157]

Moreover, rifaximin achieved reliable efficacy results in the treatment of minimal HE[158] and also in the prophylaxis of overt HE in patients with acute variceal bleeding.[159]

These clinical data have been recently supported by a strong microbiological background: rifaximin has been demonstrated to influence microbiota functions in patients with minimal HE. In particular, despite non-significant changes in microbiota composition (higher abundance of *Eubacteriaceae* and reduction of *Veillonellaceae*), treatment with rifaximin provided a significant increase in serum saturated and unsaturated fatty acids, together with reduction in microbiome–metabolome network connections, in particular those involving *Enterobacteriaceae*, *Porphyromonadaceae* and *Bacteroidaceae*, without impairing those regarding autochthonous taxa. These results were also associated with improved cognitive performance in these patients.[160] This striking evidence let the FDA approve rifaximin against the recurrence of overt HE or in patients intolerant of lactulose.[161]

CONCLUSIONS

For better and worse, antibiotics have been shown to be able to affect significantly gut microbiota composition, and consequently, to lead to consequent clinical manifestations, either with a 'eubiotic' effect or a 'dysbiotic' effect. Therefore, the modulation of gut microbiota should be considered a new, striking therapeutic avenue to be used not only for infectious diseases, but also for all other disorders associated with the impairment of gut microbiota. Anyway, antibiotics should be also considered as a double-edged sword since their overuse can drive to harmful clinical consequences. A wiser use of antibiotics in clinical practice, as well as a boost in this field of research, is advocated for a better management of patients with disorders related to the impairment of gut microbiota.

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