Invited Review

Microbiome, antibiotics and irritable bowel syndrome

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Accepted 9 September 2016

Abstract

Introduction: Irritable bowel syndrome (IBS) is the most prevalent functional gastrointestinal (GI) disorder. Increasing evidence implicates the GI microbiota in IBS pathogenesis and its modulation represents an emerging therapeutic strategy.

Sources of data: Original and review articles were identified through selective searches performed on PubMed and Google Scholar.

Areas of agreement: The role of gut microbiota in IBS is supported by evidence from animal and human studies. Randomized controlled trials demonstrate efficacy of the non-systemic antibiotic rifaximin in reducing IBS symptoms.

Areas of controversy: Existing studies on microbiota alterations are often inconsistent and limited by the heterogeneity of IBS. The exact mechanism of rifaximin remains to be elucidated. Identifying predictors of response to rifaximin and treatment strategies for symptom recurrence are important clinical questions.

Growing points: High-throughput molecular methods are leading to rapid advances in our understanding of GI microbiota in IBS

Areas timely for developing research: Future well designed longitudinal studies are required to identify characteristic microbial signatures and potential biomarkers to identify therapeutic targets and predict clinical response.

Key words: irritable bowel syndrome, microbiota, dysbiosis, inflammation, antibiotics
Introduction

Irritable bowel syndrome (IBS) is one of the commonest diagnosed gastrointestinal (GI) conditions. It is associated with reduced health-related quality of life and considerable societal costs. It is subclassified according to the ROME III criteria for functional GI disorders. This categorizes patients according to the predominant stool type; diarrhoea predominant (IBS-D), constipation predominant (IBS-C), mixed (IBS-M) or unsubtyped (IBS-U).

The aetiology and underlying pathophysiology remain unclear although a number of hypotheses have been proposed which include visceral hypersensitivity, abnormal brain-gut axis, GI dysmotility and chronic low grade inflammation. More recently there has been an appreciation that the bacterial composition of the GI tract, also known as the intestinal microbiota, has a role in GI health. Alterations in the microbiota could contribute to the pathogenesis of IBS. This review will consider the role of microbiota in IBS and the evidence for its modulation with antibiotic therapy.

The intestinal microbiota

The GI tract hosts a complex community of bacteria collectively known as the microbiota. At birth the neonatal intestine is believed to be sterile and soon becomes colonized by environmental bacteria, initially by aerobes that predominantly reflect the maternal vaginal or skin flora depending on mode of delivery. Over the first year of life there is dense colonization and variable composition of GI microbiota until it stabilizes to resemble an adult profile.

The adult human microbiota is estimated to consist of $10^{14}$ cells, which is 10-fold greater than the number of human cells in the entire body. Culture based techniques developed much of our early understanding of the microbiota; however, this was relatively labour intensive and limited by the inability to culture many species. More recent culture-independent methods, which include high-throughput sequencing and 16S rRNA-based micro-arrays, have revolutionized the field. Metagenomics involves large scale sequencing and analysis of microbial DNA obtained from the lumen or stool samples. To gain more functional information, the approaches of metatranscriptomics, metaproteomics and metabolomics provide an insight into active gene expression, microbial proteins and metabolic profiles.

Ten bacterial phyla have been identified in the human gut, of which the majority of bacteria belong to two, Firmicutes and Bacteroidetes. Density varies throughout the GI tract with $10^4$–$10^7$ cells per gram in the jejunum and ileum, to up to $10^{12}$ cells in the colon representing the highest known density of any natural ecosystem. It has been proposed that GI microbiota can be categorized into three clusters known as enterotypes, identified by their enrichment in Bacteroides (Enterotype 1), Prevotella (Enterotype 2) and Ruminococcus (Enterotype 3). Moreover, they appeared to be unrelated to host properties such as age, gender, body mass index or nationality. However, this concept remains controversial.

The GI microbiota constitutes an ecosystem with a symbiotic relationship with the host playing a role in metabolic, structural and protective functions. This has been highlighted by studies on germ-free animals where the absence of microbes led to increased susceptibility to infection and poor development of the mucosal immune system. In addition to acting as a simple physical barrier, the microbiota has an immunomodulatory effect via regulation of inflammatory cytokines. It also demonstrates metabolic activity that includes the production of short-chain fatty acids, extraction of energy from otherwise indigestible polysaccharides and modulation of host fat storage.

In health, the GI microbiota composition appears to remain relatively stable over time within individuals. However, changes attributable to environmental factors and disease are increasingly being recognized. The concept of dysbiosis has evolved, where perturbations in the intestinal microbiota have been linked to a number of conditions including IBS.
Role of microbiota in pathogenesis of IBS

Post-infectious IBS

The observation that IBS develops in a subset of patients following an acute episode of infectious gastroenteritis supports the hypothesis that the microbiota is implicated in the pathogenesis of IBS. A meta-analysis demonstrated a strong association between intestinal infection and development of IBS, with an odds ratio (OR) of 5.9 (95% CI: 3.6–9.5) taken from data pooled from nine prospective studies. The risk remained significantly elevated for up to 3 years following the acute gastroenteritis. Risk factors associated with an increased risk included young age, psychological disturbance and prolonged fever during the acute gastroenteritis.

Studies have evaluated the inflammatory response in post-infectious IBS (PI-IBS) but very few have considered changes in microbiota. The faecal microbiota of PI-IBS patients differed from healthy controls and resembled that of patients with IBS-D in one study using phylogenetic microarray and selected quantitative polymerase chain reaction assays. A more recent study found that the faecal microbial composition of PI-IBS patients differed significantly from healthy subjects and general IBS patients. In addition there was reduced diversity of microbes in the PI-IBS group in both mucosal and faecal samples when compared to healthy controls.

Activation of host mucosal immunity

The role of the gut microbiota in establishing a healthy functioning enteric immune system leads to the appealing hypothesis that dysbiosis may lead to a low grade inflammatory state in IBS. There is evidence that both the innate and adaptive immune systems are involved.

Activation of the mucosal innate immune system is suggested by the observation that activated mast cells appear to be increased in mucosal biopsies from IBS patients. In one study, mast cells were found in close proximity to enteric nerves and correlated with abdominal pain perception. It is possible that mast cell mediators induce visceral hypersensitivity as demonstrated in mesenteric sensory nerves in rats.

Toll-like receptors (TLRs) are key components of the innate immune system, where pathogen-associated molecular patterns are recognized. TLR expression appears to increased or decreased depending on TLR subclass in IBS patients. There were specific increases in TLR-4 and TLR-5 which are involved in the recognition of lipopolysaccharide and flagellin from gram negative bacteria. Furthermore, an increase in anti-flagellin antibodies has been observed in PI-IBS patients. Some have suggested that this is indicative of an exaggerated immune host-microbial response due to underlying increased epithelial permeability. Supporting this concept is the finding of increased pro-inflammatory cytokines, including interleukin (IL)-6, IL-8 and tumour necrosis factor-alpha in IBS patients.

Small intestinal bacterial overgrowth

A number of studies have suggested that small intestinal bacterial overgrowth (SIBO) is associated with symptoms in IBS patients. SIBO has traditionally been defined on the basis of a bacterial density ≥10^5 colony forming units (cfu) per mL measured by jejunal culture. However, there has been much debate over diagnostic criteria. Some have suggested a lower concentration of ≥10^3 cfu per mL was more representative.

Moreover, many studies have based their findings on culture-independent methods for diagnosing SIBO such as breath tests. During these tests, hydrogen gas concentration is measured following lactulose or glucose ingestion. Hydrogen is produced by gut microbes as a metabolic by-product. An increase in hydrogen concentration of >20 parts per million within 90 min is considered to be indicative of SIBO. However, a combined scintigraphic scanning and lactulose hydrogen breath test study demonstrated that the abnormal rise in hydrogen may simply reflect rapid oro-caecal transit time rather than SIBO.

Meta-analyses have suggested that SIBO is observed more frequently in patients with IBS.
However, the role of SIBO in IBS remains uncertain and controversial given the degree of trial heterogeneity and lack of validation of diagnostic methods.

Altered composition of the GI Microbiota in IBS

Several studies have described differences in the microbiota of patients with IBS when compared to healthy controls. The majority of these analysed faecal samples which are readily accessible although others utilized biopsy samples which better reflect mucosa associated microbiota. Overall studies lack reproducibility and general consensus. This perhaps is due to a combination of variable methodology, non-standardized recruitment and small cross-sectional studies. These do not account for possible temporal instability in microbiota and lack correlation with symptoms. Moreover, the influence of external factors such as diet is difficult to control.

Studies using a molecular analysis on faecal samples have failed to demonstrate a clear unifying signature for IBS patients but did reveal an increased ratio of *Firmicutes* to *Bacteroidetes*. Ruminococcaceae spp., *Dorea* spp and *Clostridium* cluster XIVa phylotypes were particularly increased. *Actinobacteria* forms the other main phyla of the GI microbiota, where a decrease in numbers was reported. This includes *Bifidobacteria* which is frequently used as a probiotic. Remarkably, the most prominently elevated bacteria were uncultured phylotypes related to *Ruminococcus torques*.

Unfortunately most studies are cross-sectional and merely report on observed differences in microbial profiles in patients with IBS when compared to controls. Functional information relating to microbiota and indeed symptom correlation is limited. A positive correlation in intestinal symptoms has been observed for *Gammaproteobacteria* species while a negative correlation was reported for *Bifidobacteria* and various *Firmicutes*. A *R. torques* like phylotype was found to be more abundant in faecal samples of patients with IBS who were more likely to self-report increased symptoms on the basis of an adapted inflammatory bowel disease symptom questionnaire. Analysis of rectal mucosa associated bacteria found no significant association between bloating and any particular genera, but did demonstrate an inverse relationship between stool frequency and numbers of *lactobacillus* and *bifidobacteria*.

Data are lacking on mechanisms through which host-microbiota may explain pathophysiology and generate symptoms. Most are limited to pre-clinical studies in animals and its worthwhile considering a notable few. An intriguing study on germ-free rats revealed that visceral hypersensitivity was induced following the transfer of faecal microbiome from IBS patients. Furthermore, visceral hypersensitivity was normalized in rat models of post-inflammatory colonic hypersensitivity by the administration of a probiotic. The mechanism by which this effect is mediated remains unclear with some speculating that bacterial metabolites may be responsible in the absence of any observed mucosal alterations. Others have suggested an increase in mediators such as proteases and histamine leads to activation of enteric neurons possibly facilitated by increased epithelial permeability.

Microbiota as a therapeutic target in IBS

A number of therapeutic approaches have been described in an attempt to modulate the microbiota in IBS. This review will focus on the use of non-systemic antibiotics. Other therapies include dietary modification, probiotics, prebiotics and faecal microbiota transplantation which are beyond the scope of this article and reviewed elsewhere.

Antibiotics

Although some small studies have reported that antibiotic use is associated with functional bowel disorders and IBS development, more recent studies have demonstrated a beneficial effect particularly with non-absorbable antibiotics such as neomycin and rifaximin. One small randomized trial did describe significant symptom improvement in patients with IBS following treatment with metronidazole. The key studies are outlined in Table 1.

A randomized, double-blind placebo-controlled trial of 111 non-selected IBS patients according to
Rome I criteria compared neomycin with placebo. Neomycin resulted in a 35% improvement in composite scores of IBS symptoms when compared with 11.4% for placebo \((P < 0.05)\). When the 39 patients with IBS-C were specifically considered in a subgroup analysis, a more striking improvement was observed in terms of global improvement of IBS with neomycin \((32.6\% \text{ vs. } 5.0\%, P < 0.001)\). 

Rifaximin is a non-systemic derivative of rifampicin. It is highly soluble in the presence of bile acids and contains an extra pyrido-imidazole ring which minimizes systemic absorption. A number of mechanisms of action have been proposed for rifaximin including those beyond a direct effect on microbiota composition. These include anti-inflammatory actions or modulation of gut microbiota functions such as adherence, virulence and metabolism.

### Table 1: Summary of published randomized, double-blind, placebo-controlled studies for antibiotics in IBS

<table>
<thead>
<tr>
<th>Study</th>
<th>Participants</th>
<th>Design</th>
<th>Key outcomes</th>
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<tbody>
<tr>
<td>Pimentel et al.</td>
<td>IBS according to Rome I criteria ((n = 111))</td>
<td>Double-blind RCT Neomycin 500 mg b.d ((n = 55)) vs. placebo ((n = 56)) for 10 days</td>
<td>Greater composite score improvement in neomycin group ((35.0% \text{ vs. } 11.4%, P &lt; 0.05))</td>
</tr>
<tr>
<td>Pimentel et al.</td>
<td>IBS-C according to Rome I criteria ((n = 39))</td>
<td>Subgroup analysis of IBS-C patients in Double-blind RCT Neomycin 500 mg b.d ((n = 19)) vs. placebo ((n = 20)) for 10 days</td>
<td>Larger percentage of patients reported global improvement in IBS symptoms ((36.7% \text{ vs. } 5.0%, P &lt; 0.001)).</td>
</tr>
<tr>
<td>Pimentel et al.</td>
<td>IBS according to Rome I criteria ((n = 87))</td>
<td>Double-blind RCT Rifaximin 400 mg t.d.s ((n = 43)) vs. placebo ((n = 44)) for 10 days</td>
<td>Global IBS symptoms improvement more marked in rifaximin group ((36.4% \text{ vs. } 21.0%, P = 0.02))</td>
</tr>
<tr>
<td>Sharara et al.</td>
<td>Patients with chronic (&gt;12 weeks) bloating and/or excessive flatulence</td>
<td>Double-blind RCT Rifaximin 400 mg b.d. ((n = 63)) vs. placebo ((n = 61)) for 10 days</td>
<td>Global symptom improvement among subgroup with IBS defined by Rome II criteria ((n = 70)) ((40.5% \text{ vs. } 18.2% \text{ for placebo, } P = 0.04))</td>
</tr>
<tr>
<td>Pimentel et al.</td>
<td>Non-constipated IBS according to Rome II criteria ((n = 1260))</td>
<td>Two identically designed double-blind RCTs Rifaximin 550 mg t.d.s ((n = 625)) vs. placebo ((n = 635)) for 14 days</td>
<td>Significantly higher proportion of patients achieving adequate relief of global IBS symptoms for at least 2 of the first 4 weeks after treatment in rifaximin group ((40.7% \text{ vs. } 31.7% \text{ for placebo, pooled; } P &lt; 0.001)). Larger percentage of patients in rifaximin group achieved relief of bloating Effects durable for the 10-week drug-free follow-up period.</td>
</tr>
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RCT, Randomized controlled trial.
patients who previously responded to open label rifaximin but developed symptom recurrence during an 18-week observation period were again randomized to receive either rifaximin 550 mg b.d or placebo for 2 weeks, then followed by a 4-week treatment-free period. The composite end point consisted of the percentage of patients who experienced a decrease from baseline of at least 30% in mean abdominal pain score and a decrease of at least 50% in the number of days per week with a stool consistency of Type 6 or 7. Although fully published results from this trial are awaited, an abstract has reported promising results with rifaximin demonstrating significant efficacy again over placebo.

It should be noted that patients starting rifaximin re-treatment had lower symptom severity scores compared with their baseline before initial treatment.

Another group evaluated rifaximin at a dose of 400 mg twice daily for relieving chronic bloating and excessive flatulence in a randomized, placebo-controlled study. Among unselected IBS patients according to ROME II criteria, a significantly greater improvement in symptoms was observed in the rifaximin group after 10 days of treatment (40.5% vs. 18.2% for placebo, \( P = 0.04 \)).

A systematic review and meta-analysis published in 2012 evaluated the efficacy of Rifaximin in IBS. Five randomized, placebo-controlled trials (1803 participants) met the eligibility criteria. Heterogeneity was assessed using \( \chi^2 \) test and inconsistency index statistic (I^2) with significance defined as \( I^2 \geq 25\% \). Rifaximin was found to more efficacious than placebo for global IBS symptom improvement (OR = 1.57, 95% CI = 1.22–2.01, number needed to treat (NNT) = 10.2) with mild heterogeneity. In terms of secondary outcome, data were available from four studies demonstrating that rifaximin was significantly more likely to improve bloating over placebo (OR = 1.55, 95% CI = 1.23–1.96, NNT = 10.1) with no significant heterogeneity. The authors reported that there was no evidence of publication bias and concluded that the modest therapeutic gain was similar to that yielded by other therapies for IBS available at that time.

Adverse event rates appear to be similar between rifaximin and placebo. A meta-analysis suggested that one patient would discontinue rifaximin because of an adverse event for every 846 patients who would benefit (number needed to harm = 8971, NNT = 10.6). Despite initial concerns, evidence from existing studies does not suggest emergence of pathogenic or resistant organisms with rifaximin.

**Conclusions**

Growing evidence indicates that the gut microbiota is altered in patients with IBS. The precise mechanisms by which this may relate to aetiology and pathophysiology remain to be elucidated. A number of therapeutic modalities have attempted to modulate the microbiota and demonstrated efficacy, notably non-systemic antibiotics such as rifaximin. Studies are limited by the heterogeneity that IBS patients represent along with the complexity of microbiota being influenced by a number of environmental factors. Major advances are possible with larger, well designed longitudinal studies using high-throughput molecular methods to characterize perturbations that correlate with fluctuating symptoms. This may identify characteristic microbial signatures and potential biomarkers that both identify therapeutic targets and predict clinical response.

**References**


