Dear Editor,

Thank you for giving us the opportunity to submit a revised draft of the manuscript "Antibiotics, gut microbiota and irritable bowel syndrome: what are the relations?" to World Journal of Gastroenterology. We appreciate the time and effort that you and the reviewers have dedicated to providing your valuable feedback on our manuscript. We are grateful for the insightful comments on our paper. We have been able to incorporate changes to reflect most of the suggestions provided by the reviewers. Please see below a point-by-point response to the reviewers' comments and concerns. All page numbers refer to the revised manuscript.

Sincerely,

Zarina Mamieva

REVIEWER #1

1. Please define IBS properly in Abstract.

Thank you for pointing this out. We have added the definition to the abstract section.

Page 3:

"Irritable bowel syndrome (IBS) is a functional gastrointestinal disorder in which recurrent abdominal pain is associated with defecation or a change in bowel habits (constipation, diarrhea, or both), and it is often accompanied by symptoms of abdominal bloating and distension. IBS is an important health care issue because it negatively affects the quality of life of patients and places a considerable financial burden on health care systems."

2. Are there any clinical drug trials on IBS or supplementation of specific microbes, such as GLP-1 or Serotonin inhibitors or activators for respective IBS types. Let's suppose GLP-1 is found low in IBS-C, while serotonin level is elevated in IBS-D, what if inhibiting or promoting levels of these molecules could control symptoms? If any clinical studies are available, how do these or other effects affect IBS and gut microbiota.

Thank you for pointing this out. We agree and have updated the section "Microbiota and motility/sensitivity abnormalities".

Page 7:

"In a rat model of bowel dysfunction, administration of the GLP-1 receptor agonist exendin-4 alleviated stress-induced defecation and visceral pain sensitivity[49,50]. Clinical interventions in patients with IBS demonstrated that the synthetic GLP-1 analog ROSE-010 reduced abdominal

pain and increased colonic transit[48,51]. The underlying molecular mechanisms responsible for the amelioration of symptoms remain unknown. The authors suggest that modulation of enteric neuronal function and tight junction expression, as well as the activation of serotonergic pathways in the colon, may play a role."

Page 7:

"The serotonin system represents a potential therapeutic target in IBS. The effects of serotonin are mediated through 5-HT receptors located on the surface of distinct cell types. Fourteen different serotonin receptor subtypes have been identified and classified into seven groups (5-HT1–7), with 5-HT3 and 5-HT4 being the most investigated receptors in the intestine. Both receptor subtypes are expressed on neurons within the myenteric and submucosal plexuses of the enteric nervous system, intrinsic and extrinsic sensory neurons, interstitial cells of Cajal, enterocytes and enterochromaffin cells[54]. 5-HT3 receptors are involved in the contraction of intestinal smooth muscle and in gut-brain communication through vagal afferent fibers[55]. Activation of 5-HT4 receptors induces neuronal release of acetylcholine and accelerates the peristaltic reflex[56]. 5-HT3 receptor antagonists have been shown to improve abdominal pain and global IBS symptoms in patients with nonconstipated and IBS-D[57,58]. 5-HT4 agonists have been shown to relieve overall and individual symptoms (abdominal pain/discomfort, stool frequency, stool consistency, and straining during defecation) in patients with IBS-C[59–61]. However, cardiovascular side effects were seen with these drugs, and they were either withdrawn from the market (cisapride) or approved for a limited population (tegaserod). Therefore, new safe and well-tolerated 5-HT4 agonists are under development[62,63]."

3. Please provide more animal models' evidence for gut microbiota regulating mood and emotional stress, better describe in detail specifically levels of stress related hormones in IBS mice etc.

Thank you for this suggestion. The change can be found on page 9 of the revised manuscript.

Page 9:

"In recent studies, germ-free mice have been widely used as a tool for assessing the role of intestinal microbes in brain function and behavior. Studies on germ-free and specific pathogen-free mice indicate that intestinal microbes can cause imbalances of the HPA axis, resulting in an anxiety-like behavioral phenotype[78]. Fecal microbiota transplantation studies have indicated the rodent-to-rodent and human-to-rodent transfer of anxiety-like behaviors[79,80]. Moreover, animal studies have shown that transplantation of microbiota from depressed patients to rodents is able to induce depression-like behavior. The authors linked microbiota-induced depression in

mice to alterations in the cAMP-response element binding protein (CREB) signaling pathway in the olfactory bulb[81] and alterations in carbohydrate and amino acid metabolism[82]."

4. It is believed that stress is a secondary effect triggered due to IBS (any cause), please state any difference of stress hormones in bacterial IBS or other causes.

We appreciate your insightful suggestion and agree that this information would be helpful. Unfortunately, we found no data on this issue.

5. The use of some antibiotics favour the growth of some of bacteria such as (amoxicillin, amoxicillin/clavulanate, cephalosporins, lipopolyglycopeptides, macrolides, ketolides, clindamycin, tigecycline, quinolones and fosfomycin) increase the abundance of Enterobacteriaceae, mainly Citrobacter spp., Enterobacter spp. and Klebsiella spp etc. How do authors explain it? Beneficial for gut microbiota and IBS? Could these or other antibiotics be used in IBS? Any remarks about safety of the antibiotics in IBS.

We appreciate the suggestions. Changes can be found on pages 14 and 15 of the revised manuscript. Moreover, proinflammatory properties of Enterobacteriaceae were already mentioned in the submitted manuscript(revised manuscript page 16).

Page 14:

"These bacteria contain molecules that directly enhance the inflammatory response of the host and may play a significant role in the alteration of bile acid metabolism[136]. Moreover, expansion of bacteria belonging to the Enterobacteriaceae family was associated with inflammatory bowel diseases, both in animal models and in humans[137,138]."

Page 15:

"However, nonabsorbable antibiotics can be used to treat IBS. In a double-blind, randomized, placebo-controlled study, treatment with neomycin resulted in a 35% improvement in composite scores of IBS symptoms, compared with only 11% for placebo ($p < 0.05$)[139]. Nonetheless, the use of this antibiotic is limited by the risk for Clostridium difficile infection and systemic adverse events. A recent meta-analysis of four studies and 1803 patients showed that rifaximin was more effective than placebo in the overall improvement of IBS symptoms (OR== 1.19; 95% CI: 1.08–1.32 and OR = 1.36; 95% CI: 1.18–1.58, respectively, $P < 0.05$ for both). There was no difference in adverse events between rifaximin and placebo[140]. Due to its safety, rifaximin was approved by the Food and Drug Administration (FDA) for the treatment of IBS-D."

6. Please also describe how to recover the IBS, thorough probiotics and medicines.

We appreciate the reviewer's feedback. However, our review focuses on the pathogenesis of IBS and the role of antibiotics in the development of IBS. In our manuscript we have discussed treatment options (non-absorbable antibiotics, probiotic strains, 5-HT3 receptor antagonists, 5-HT4 agonists) in the context of IBS pathogenesis.

7. Please provide your concluding remarks based on your analysis of the literature for every headings of the manuscript, so it could make this manuscript more reader friendly. Please also provide the future prospects and recommendations.

We agree with this assessment. Accordingly, throughout the manuscript, we have added our remarks. We have also revised the conclusion section to provide future prospects.

Page 16:

"There is clear and consistent evidence from a variety of studies that patients with IBS have altered composition of gut microbiota and that these alterations are related to the generation of gastrointestinal symptoms. However, studies comparing fecal microbiota in patients with IBS and healthy controls produced variable findings. To date, there is still no consensus on distinct microbiome signatures in IBS. Although some common threads reviewed here were found, prospective large-scale studies need to be carried out to shed light on this issue. Independent analysis of the gut microbiota and its metabolites will help to develop novel microbiota-based treatment strategies that target the underlying pathophysiology of IBS rather than focusing on symptom alleviation.

A number of recent studies have addressed the effects of antibiotics on gut microbiota composition, and these effects were found to be quite similar to those observed in IBS. We suggest that the Rome V criteria could provide a new definition of postantibiotic IBS. As major disruptors of the gut microbiota, antibiotics seem to contribute to all aspects of IBS pathogenesis. However, further research in this area is definitely warranted."

8. Both figures are related to the immune regulation, authors should draw an overall schematic figure for IBS, show readers how antibiotics influence the gastric system and trigger IBS.

Thank you for this suggestion. Please see Figure 3.

REVIEWER #2

1. The background information of the "Introduction" is not sufficient which should be enriched to strengthen the significance of the paper. For example, the author should supplement the necessary information about why should we pay attention to the roles of gut microbiota in IBS

development. Besides, the logic is very obscure which should be rearranged. The transitions between paragraphs aren't fluid.

Thank you for pointing this out. We have revised this section.

Page 4:

"The gut microbiota is established early in life, remains relatively stable thereafter and is subject to shaping by environmental and host factors (e.g., age, diet, lifestyle, and medications)[1,2]. With regard to the environment, antibiotics have been reported to play a particularly important role in the modulation of the gut microbial community. However, most studies in this area were undertaken 30 to 40 years ago and relied on culture-based techniques. Global antibiotic use has grown 66% since 2000 and continues to grow at a high rate[3,4]. This fact, along with rapid technological advancements for culture-independent analysis, has reinforced the need to take a fresh look at antibiotic-induced changes in the human gut microbiota and clinical consequences of antibiotic intervention. Several studies have reported that antibiotic treatment is associated with an increased risk of irritable bowel syndrome (IBS)[5–8].

Irritable bowel syndrome (IBS) is a common gastrointestinal disorder affecting 10–15% of the population in Europe and North America[9]. This condition negatively affects the quality of life of patients and imposes a significant socioeconomic burden[10]. Over the past few decades, the gut microbiota has emerged as a potential factor that contributes to the pathophysiology of IBS[11,12]. Microbial dysbiosis within the gut has been implicated in intestinal barrier dysfunction, visceral hypersensitivity, impaired gastrointestinal motility and altered immune response[13–17]. Moreover, various studies have consistently shown the efficacy of microbiota-directed therapies, including prebiotics, probiotics, nonabsorbable antibiotics, dietary changes, and fecal microbial transplantation, in alleviating IBS symptoms[18]."

2. In the section of "MODERN CONCEPT OF IRRITABLE BOWEL SYNDROME: THE EVOLVING ROLE OF GUT MICROBIOME", the author stated that "The concept of "microbiota-gut-brain" axis has been proposed [42-45], supporting the crucial role of microbial dysbiosis in the development of IBS symptoms." However, the author failed to illustrate the relationship between "microbiota-gut-brain" axis and IBS. Please clarify the statement.

Thank you for pointing this out. The concept of "microbiota-gut-brain" axis has been discussed in the manuscript in two sections (microbiota and motility/sensitivity abnormalities, microbiota as a regulator of stress and emotional responses). We have updated sections and provided more data on animal models' evidence for gut microbiota regulating mood and emotional responses.

Page 9:

"In recent studies, germ-free mice have been widely used as a tool for assessing the role of intestinal microbes in brain function and behavior. Studies on germ-free and specific pathogen-free mice indicate that intestinal microbes can cause imbalances of the HPA axis, resulting in an anxiety-like behavioral phenotype[78]. Fecal microbiota transplantation studies have indicated the rodent-to-rodent and human-to-rodent transfer of anxiety-like behaviors[79,80]. Moreover, animal studies have shown that transplantation of microbiota from depressed patients to rodents is able to induce depression-like behavior. The authors linked microbiota-induced depression in mice to alterations in the cAMP-response element binding protein (CREB) signaling pathway in the olfactory bulb[81] and alterations in carbohydrate and amino acid metabolism[82]."

3. In the section of "Microbiota and motility/sensitivity abnormalities", the full text is a simple description of the references and the conclusion is not clear.

Thank you for pointing this out. We have revised the text and provided conclusion.

Page 7:

"In a rat model of bowel dysfunction, administration of the GLP-1 receptor agonist exendin-4 alleviated stress-induced defecation and visceral pain sensitivity[49,50]. Clinical interventions in patients with IBS demonstrated that the synthetic GLP-1 analog ROSE-010 reduced abdominal pain and increased colonic transit[48,51]. The underlying molecular mechanisms responsible for the amelioration of symptoms remain unknown. The authors suggest that modulation of enteric neuronal function and tight junction expression, as well as the activation of serotonergic pathways in the colon, may play a role."

Page 7:

"The serotonin system represents a potential therapeutic target in IBS. The effects of serotonin are mediated through 5-HT receptors located on the surface of distinct cell types. Fourteen different serotonin receptor subtypes have been identified and classified into seven groups (5-HT1–7), with 5-HT3 and 5-HT4 being the most investigated receptors in the intestine. Both receptor subtypes are expressed on neurons within the myenteric and submucosal plexuses of the enteric nervous system, intrinsic and extrinsic sensory neurons, interstitial cells of Cajal, enterocytes and enterochromaffin cells[54]. 5-HT3 receptors are involved in the contraction of intestinal smooth muscle and in gut-brain communication through vagal afferent fibers[55]. Activation of 5-HT4 receptors induces neuronal release of acetylcholine and accelerates the peristaltic reflex[56]. 5-HT3 receptor antagonists have been shown to improve abdominal pain and global IBS symptoms in patients with nonconstipated and IBS-D[57,58]. 5-HT4 agonists

have been shown to relieve overall and individual symptoms (abdominal pain/discomfort, stool frequency, stool consistency, and straining during defecation) in patients with IBS-C[59–61]. However, cardiovascular side effects were seen with these drugs, and they were either withdrawn from the market (cisapride) or approved for a limited population (tegaserod). Therefore, new safe and well-tolerated 5-HT4 agonists are under development[62,63]."

4. In the section of "Microbiota as a regulator of stress and emotional responses", the author illustrates the role of gut microbiota in stress and emotional responses, and briefly describes the possible symptoms of stress in the IBS patient. However, the key point that How microbiota influence the stress responses and IBS pathology wasn't clarified.

Thank you for pointing this out. We have updated this section.

Page 9:

"In recent studies, germ-free mice have been widely used as a tool for assessing the role of intestinal microbes in brain function and behavior. Studies on germ-free and specific pathogen-free mice indicate that intestinal microbes can cause imbalances of the HPA axis, resulting in an anxiety-like behavioral phenotype[78]. Fecal microbiota transplantation studies have indicated the rodent-to-rodent and human-to-rodent transfer of anxiety-like behaviors[79,80]. Moreover, animal studies have shown that transplantation of microbiota from depressed patients to rodents is able to induce depression-like behavior. The authors linked microbiota-induced depression in mice to alterations in the cAMP-response element binding protein (CREB) signaling pathway in the olfactory bulb[81] and alterations in carbohydrate and amino acid metabolism[82]."

5. In the section of "Microbiota and host immunity", the author expounds the relationship between IBS and immune system, and the role of gut bacteria in immune response, but the logic is not strong. The author should focus on the role of gut bacteria in immune response in the context of IBS.

In the section "Microbiota and host immunity" we discuss how gut bacteria modulate the immune response, focusing on changes that were observed in IBS. We appreciate your suggestion and modified this section.

Page 11:

"The importance of the interaction between the gut microbiota and host immune system in IBS is highlighted by a number of studies in patients with postinfectious IBS, indicating activation of the gastrointestinal immune system after acute gastroenteritis[94,95]. Moreover, animal studies have shown that stress-induced changes in the gut microbiota are associated with altered immune response and increased susceptibility to enteric pathogens[96,97]."

6. In the section of "Microbiota and intestinal barrier integrity", since the behavior of gut microbiota will be different under the different physiology status, the author should focus on how gut bacteria regulate intestinal epithelial barrier integrity in the context of IBS.

Thank you for pointing this out. In this section paragraph 2 describes changes observed in IBS (increased density of epithelial gaps, decreased expression of tight junction proteins). Paragraph 3 illustrates how certain gut bacteria modulate expression of tight junction proteins and affect the mucus layer thickness/composition. Increased intestinal permeability is one of the major mechanisms involved in the pathogenesis of IBS. Therefore, data provided in this section are intrinsically linked to the pathogenesis of IBS.

7. Some contents in the manuscript just simply list the abstracts of references. For instance, in the section of "ANTIBIOTICS, GUT MICROBIOTA AND IRRITABLE BOWEL SYNDROME". A review paper should provide an experts' perspective, not a list of findings from the abstracts of the cited articles.

Thank you for pointing this out. In our manuscript we summarized data on antibiotic-induced changes in the gut microbiota and shifts in the bacterial community composition observed in IBS. We analyzed the findings and highlighted common features that can be found in the section "Similarities in the gut microbiota between patients with IBS and those after antibiotic exposure", as well as in Tables 2 and 3. We have updated the paper to provide more concluding remarks and comments.

8. The section of "Conclusion" lacks effective summary and failed to propose perspective for future work on the topic.

Thank you for pointing this out. We have revised this section.

Page 16:

"There is clear and consistent evidence from a variety of studies that patients with IBS have altered composition of gut microbiota and that these alterations are related to the generation of gastrointestinal symptoms. However, studies comparing fecal microbiota in patients with IBS and healthy controls produced variable findings. To date, there is still no consensus on distinct microbiome signatures in IBS. Although some common threads reviewed here were found, prospective large-scale studies need to be carried out to shed light on this issue. Independent analysis of the gut microbiota and its metabolites will help to develop novel microbiota-based treatment strategies that target the underlying pathophysiology of IBS rather than focusing on symptom alleviation.

A number of recent studies have addressed the effects of antibiotics on gut microbiota composition, and these effects were found to be quite similar to those observed in IBS. We suggest that the Rome V criteria could provide a new definition of postantibiotic IBS. As major disruptors of the gut microbiota, antibiotics seem to contribute to all aspects of IBS pathogenesis. However, further research in this area is definitely warranted."

REVIEWER #3

This manuscript summarizes the relationships among the antibiotic, gut microbiota, and irritable bowel syndrome. Although the review is interesting, there are still lots of issues that need to figure out, details as follows:

1. The conclusion is not clear. If antibiotics are a friend or foe for IBS?

Thanks for your kind reminders. We discuss antibiotic-induced changes in the gut microbiota and how these changes relate to the development of IBS. Please see sections "Antibiotics as a risk factor for irritable bowel syndrome" (page 15), "Similarities in the gut microbiota between patients with IBS and those after antibiotic exposure" (page 16), and Figure 3. Moreover, we have added a paragraph about nonabsorbable antibiotics that can be used to treat IBS (page 15).

Page 15:

"However, nonabsorbable antibiotics can be used to treat IBS. In a double-blind, randomized, placebo-controlled study, treatment with neomycin resulted in a 35% improvement in composite scores of IBS symptoms, compared with only 11% for placebo ($p < 0.05$)[139]. Nonetheless, the use of this antibiotic is limited by the risk for Clostridium difficile infection and systemic adverse events. A recent meta-analysis of four studies and 1803 patients showed that rifaximin was more effective than placebo in the overall improvement of IBS symptoms (OR== 1.19; 95% CI: 1.08–1.32 and OR = 1.36; 95% CI: 1.18–1.58, respectively, $P < 0.05$ for both). There was no difference in adverse events between rifaximin and placebo[140]. Due to its safety, rifaximin was approved by the Food and Drug Administration (FDA) for the treatment of IBS-D."

2. No appropriate statistical methods were used in the study.

Thank you for this suggestion. This is a review article. Statistical methods are not appropriate for inclusion in this manuscript.

3. The authors should summarize the mechanisms and pathways, including how to gut microbiota inference IBS and how IBS changes the gut microbiota?

We agree with the reviewer's assessment. Accordingly, throughout the manuscript, we have made valuable changes.

4. Don't mix the "gut microbiota" and "gut microbiome" in the manuscript.

Thank you very much for the reminder. We have made revisions accordingly.

5. Lots of minor errors exist, like p-value miss, abbreviation, etc.

Thank you for pointing this out. Revised accordingly.

REVIEWER #4

It's a interesting article, written in an adequate and concise way.

Thank you very much for these kind words.