Reviewer 1:

Specific Comments to Authors: This is a review about gut microbiome in acute pancreatitis. Author has elaborated the pathogenesis of AP and the possible way that bacteria may affect or be affected by AP. However, there are some problems author need to clarify.

1.In the third paragraph of "POTENTIAL ROLE OF THE GUT MICROBIOME IN AP" part, the current literature provide clear evidence of the association between gut microbiome and the severity of AP, which maybe more appropriate to conduct a conclusion that the level of microbiome can predict AP severity rather than the change of microbiome can treat AP.

***Response:** "We agree with the reviewer that the overall literature suggests that there is an association between the gut microbiome and the severity of AP [95, 107]. Additionally, in experimental acute pancreatitis, changes to the gut microbiome e.g. administration of *Clostridium butyricum* can suppress AP [27] pointing to the therapeutic potential of this approach is promising."

2.In the second paragraph of "CONCLUSION AND FUTURE DIRECTIONS" part, "The importance of restoring gut microbiota homeostasis and stabilizing the gut barrier are challenging therapeutic targets in the prevention of AP progression." is confusing, please rephrase it.

***Response: Thus, restoring the homeostasis of gut microbiota and stabilizing the gut barrier could be a promising therapeutic target in preventing AP progression.**

Reviewer #2:

Addressing a comment of Reviewer 2 on

In my view, however, to be along with the title, gut microbiome in acute pancreatitis deserves to be more presented, and more focused. Namely, a large part of the review did consider the general points of the acute pancreatitis with very little focus on the

gut microbiome in acute pancreatitis. Evidently, the gut microbiome in acute pancreatitis should be a large majority (and not minority) of the text.

***Respose: In the manuscript, the general introduction of the Gut microbiome is described in the introduction part in the manuscript (page no 4), also on page no 7, there is some description of the microbiome too. In addition, as mentioned in the comment above-I, I have further elaborated gut microbiome in AP. In addition, we do have a detailed description of POTENTIAL ROLE OF THE GUT MICROBIOME IN AP on page 7.**

Considering the suggestion from Reviewer 2-I have further elaborated gut microbiome in AP (yellow highlighted) in the section GUT MICROBIOME and also have changed the title as below.

GUT MICROBIOME & MICROBIOME IN AP:

The human GI tract is home to a diverse and complex microbial community of bacteria, viruses, and fungi that help to maintain health and are involved in the pathogenesis of various diseases. The gut contains at least 1000 bacterial species and 100-fold more genes than have been identified in the human genome[4, 61]. The microbiome can be considered as a hidden "metabolic organ", and showed a great impact on well-being in humans because of its influence on metabolism, physiology, nutrition, and immune function[62]. It has been shown that the gut microbiome co-evolves with us; hence, any changes to the microbial community can have significant consequences, both beneficial and harmful[63]. Disruption of the gut microbiota, or dysbiosis, has been associated with diverse systematic conditions, such as obesity[64, 65], malnutrition[66] and diabetes[67], and chronic inflammatory diseases, such as inflammatory bowel disease, ulcerative colitis, and Crohn's disease[68].

Extended portion as per below

Over the past 20 years, exciting research on the microbiome has accelerated at an incredible pace. It is revealing the myriad of ways these various microscopic inhabitants have associated with our day-to-day routine lives. It is now evident that gut microbiota is a critical determinant of human health and various diseases. Also, it is considered a key regulator of host physiology. With the progress in microbiome

research over time, the volume of data exhibiting the gut microbes' overall composition and functional potential also rises. The number of diseases linked with alterations in our gut microbial community has also been expanded simultaneously [4, 6]. The human gastrointestinal tract, the habitat highly populated with different microorganisms, is involved in the immunity of the host and the pathogenesis of various diseases, including acute pancreatitis [69]. Usually, human gastrointestinal microflora divided into three different categories, based on the way they perform multiple functions in the host: e.g., physiological bacteria: that holds over 90%, which is nourishing and immune-modulating; opportunistic bacteria: pathogenic in the condition of lower immune resistance or antibiotic abuse and the third category is a pathogenic bacteria: quantity is lower and difficult to colonize, but then turns into the pathogenic state, the bacterial growth is out of the normal range (uncontrolled) [70]. Progression in AP has been more complicated by gastrointestinal motility dysfunction, which is probably related to the neuroendocrine system, hypoxia-ischemia, ischemia-reperfusion injury (IRI), inflammatory mediators, and Cajal cells
[71].

The interest in studying the commensal intestinal microbiome escalated recently when vast numbers of discoveries have postulated its prominent role in human physiology, immunity, and maintenance of homeostasis. The overall function of the intestine in the entire mechanism of AP pathogenesis (such as in acute and critical illnesses) is essential to understand, but often it is overlooked. In this mechanism of AP pathogenesis, various endogenous and exogenous factors play a crucial role in a loss of gut barrier function, which leads to bacterial and endotoxin translocation, plays a vital role in generating the second inflammatory hit in AP [72]. Johnson et al.[73] discovered that translocation of gut bacteria (via hematogenic, lymphatic, and reflux from the duodenum and the biliary tree) was involved in AP infections, indicating a possible association between gut microbiota and AP progression. Any alterations in gastrointestinal microbiota homeostasis (dysbiosis) are associated with the systematic inflammatory response syndrome (SIRS) and a range of diseases [74].

Damage to the intestinal mucosal barrier can cause intestinal bacteria to migrate to the blood or other tissues and organs, accelerating the progress and aggravating AP [75]. Recently, several research findings have investigated changes in the intestinal flora during AP development related to the severity of the disease. During the AP progression, there is an abnormal secretion of trypsin and destruction of pancreatic structure leading to abnormal pancreas secretion, which causes changes in intestinal homeostasis and intestinal flora [76, 77].

Many studies are supporting now to demonstrate that normal gut microbes play a primary role in maintaining gut mucosal integrity. However, gut mucosal ischemia and reperfusion during AP progression can damage the overall integrity of the gut barrier and lead to gut bacterial translocation to other locations, finally causing local and systemic infections [78]. Some further research findings have also revealed that intestinal mucosal barrier injury is a significant complication in many AP patients. The intestinal mucosal barrier can be destroyed by affecting intestinal inflammation and the immune response [75]. Many studies are supporting now to demonstrate that normal gut microbes play a primary role in maintaining gut mucosal integrity. However, gut mucosal ischemia and reperfusion during AP progression can damage the overall integrity of the gut barrier and lead to gut bacterial translocation to other locations, causing local and systemic infections [78]. Some further research findings have also revealed that intestinal mucosal barrier injury is a significant complication in many AP patients. The intestinal mucosal barrier can be destroyed by affecting intestinal inflammation and the immune response [75]. In AP, a possible bacterial translocation phenomenon and the homeostatic host response noticed. The translocation of microbes from the lower gastrointestinal tract occurs through the portal circulation-the oral course and or mesenteric lymph nodes—the acinar cells of the pancreas secrets pancreatic antimicrobial peptides (AMPs). AMPs can have homeostatic bidirectional communication with the gastrointestinal tract [79]. The lower level of the microbiome in the gastrointestinal tract increases the production of pancreatic antimicrobial peptides through short-chain fatty acid metabolites. This, in turn, induces an immunoregulatory pancreatic environment with a decreased level of pro-inflammatory immune cells. Conversely, reduced antimicrobial peptides

production by the pancreas enables gastrointestinal microbiota overgrowth and the development of a pro-inflammatory phenotype. It subsequently alters the gut microbiome and the intestinal immune system [79].

Reviewer 2: Addressing a comment on some inconsistencies should be resolved

In addition, in referring gut microbiome, some inconsistencies should be resolved. As illustration, see the text: Some studies have not found any significant benefit or adverse effect of probiotics in severe AP, but it is important to note the considerable patient and probiotic regimen heterogeneity in the published clinical trials of probiotics. The PROPATRIA Probiotics in Pancreatitis Trial randomized 296 patients with predicted severe AP to either a multispecies probiotic mixture containing two different Bifidobacterium, three Lactobacillus, and one Lactococcus species or a placebo. The infectious complications in the two groups were similar, but the probiotic group had higher mortality (16% vs 6%) and incidence of bowel ischemia (6% vs 0%) compared with the placebo group. The high load of the probiotic mixture used in the study was thought to have been responsible for the increased mortality[108]. The findings highlight the challenges of supplementing the gut microbiome with beneficial microbial species in the setting of AP. The first sentence (see italic) contrasts with the next mentioning of the study with the very negative results (i.e., increased mortality, increased incidence of bowel ischemia).

***Response:** Nevertheless, eight years later, the PROPATRIA trial was reevaluated by Bongaerts et al [115]. The team of researchers analyzed and addressed all shortcomings identified in the trial. PROPATRIA researchers contend that a lethal combination of predominantly proteolytic pancreatic enzymes and probiotic therapy was responsible for the high mortality rate, and that elevated levels of lactic acid produced by bacterial fermentation of carbohydrates significantly contributed to the high death rate. Additionally, one of them was the latency time in the first administration of probiotics; indeed, some patients were treated 24 h after onset of symptoms. Furthermore, there were errors in randomization; in fact, the onset of multi-organ failure was already present during admission in more patients in the first group than in the placebo group (41 patients versus 23 patients) [116]. Finally, last

but not least, the team of researchers suggested that in future studies, when considering substituting probiotics in AP, it is necessary to assess the appropriate, effective doses of probiotics. However, caution should be mandatory to prevent bacterial overgrowth while conducting clinical trials in AP patients.

Addressing the response on comment Reviewer 2

In summary, probiotics help to maintain gut homeostasis, but there is not much evidence in support of clinical use of probiotics for the treatment of AP and other diseases, despite the increasing evidence available on the role of the gut microbiome in human health.

***Response:** In summary, probiotics help to maintain gut homeostasis**.** Research should improve the designs of future studies, for example, by detecting a peculiar strain of microorganisms (i.e., their type), standardizing the dose and duration of treatment, or standardizing the state of disease progression when considering to use in current therapy scenarios.

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