

Response to Reviewers

Dear Editors and Reviewers,

First of all, the authors greatly appreciate all the suggestive and helpful comments from the reviewers. The comments will help the authors to make better modifications, deepen the interpretation of behaviors and improve the quality of the paper. The authors have taken into account of the comments and made the modifications. All modified or added parts are filled with blue in the revised manuscript. The main corrections in the paper and the responds to the reviewer's and editor's comments are as following:

Reviewer 1

1. Comments of Reviewer

The authors stated in the conclusion of the abstract that intestinal bacteria were the main source of biliary bacteria. However, the authors mentioned in the introduction that the bacteria may also invade through the blood and lymphatic system. What make the authors sure to address such confirmation in the abstract?

Reply from Authors

Thank you for the reviewer's comment. In the introduction, we introduce the source of biliary bacteria, such as intestinal tract, the blood and lymphatic system. And in the discussion, we added the description, some articles introduce the intestinal flora distribution, such as *E. coli*, *K. pneumoniae*, *Enterococcus*. which is consistent with our results. However we didn't perform bacterial profile in the intestine, and We didn't compare various sources of bacterial species, we agree with that the conclusion "intestinal bacteria were the main source of biliary bacteria" was not so accurate and we changed it.

2. Comments of Reviewer

In Materials and methods section, it is usually convenient to start with the "patient's characteristics" subsection (Clinical characteristics).

Reply from Authors

Thank you for the reviewer's suggestion. We have changed the Materials and methods section and started with the "patient's characteristics" subsection.

3. Comments of Reviewer

In many occasions the names of bacterial species should be mentioned in italic.

Reply from Authors

Thank you for the reviewer's comment. We have checked the whole article, and changed the mistake.

4&6&7. Comments of Reviewer

In result section, the title "Distribution of the bacteria identified in 738 bile samples with positive bile culture based on the different diseases caused by them" should better read: "Distribution of bile bacteria and diseases cause"

In the discussion section, the sentence "Microbiological profiles may be different account for different diseases." Should be reformulated to read for example: Microbiological profiles may be related to different diseases.

In the discussion section, the sentence "...but the evidence was little definite and less of

fundamental researches. This sentence is meaningless.

Reply from Authors

Thank you for the reviewer's suggestions. They have been changed the sentences in the article.

5 Comments of Reviewer

In the discussion section, the authors stated "Therefore, at the species level, the pathogenic bacteria in the biliary tract were basically identical to intestinal bacteria in the biliary tract..." This sentence is confusing. The biliary tract seems duplicated. Do the authors perform bacterial profile in the intestine?

Reply from Authors

Thank you for the reviewer's comment. we didn't perform bacterial profile in the intestine, we just quoted the results of some research. We have checked the sentence and changed it.

The sentence has changed to: It has been reported that the biliary pathogenic bacteria may be associated with intestinal flora distribution, such as E. coli, K. pneumoniae, Enterococcus^[10-12]. In our study, we analyzed 1339 bile samples of ten years and established that biliary separation was mainly gram-negative bacteria, accounting for 74.94%, the rest includes 22.88% of gram-positive bacteria and 2.18% of fungus. E. coli (37.78%) and K. pneumoniae (10.29%) were the most common gram-negative bacteria, and Enterococcus (13.20%) and Staphylococcus (7.38%) were the main gram-positive bacteria. Therefore, at the species level, which is consistent with the results.

"In conclusion, the Gram-negative bacteria were the most commonly isolated biliary bacteria."

Reviewer 2

1 Comments of Reviewer

How the authors explain the high positive culture rate among benign disease compared to patients with malignant disease ?

Reply from Authors

Thank you for the reviewer's comment. In the former article, we wrote some reasons, maybe it is simple, so I add the description. "This may be owing to some reasons. According to some investigators, Oddi's sphincter function of patients is normal before the onset of the malignant disease. Normal function can adjust the flow of bile and pancreatic juices to maintain the normal bile duct pressure. In addition, it can prevent the duodenal contents reflux. Once Oddi's sphincter dysfunction, it will lead to obstruction of biliary tract and growth of bacteria. Further, patients with benign diseases had higher rates of bile duct stones than those with malignant diseases in our study, which would also lead to obstruction of biliary tract and the growth of biliary pathogenic bacteria.

2 Comments of Reviewer

In the "Distribution of the bacteria identified in 738 bile samples with positive bile culture based on the different diseases caused by them" section, the authors stated that "in contrast" - i disagree with that, in fact both for benign and malignant disease - almost the same micro-organisms were positively cultured - please modify the paragraph !!

Reply from Authors

Thank you for the reviewer's comment. We have checked and modified it. "We found that the most common strains in patients (n = 554) with benign diseases were E. coli (231; 41.7%), P.

aeruginosa (55; 9.93%), *K. pneumoniae* (55; 9.93%), and *E. faecium* (39; 7.04%). The predominant strains identified in patients (n = 184) with malignant diseases were *E. coli* (81; 44.02%), *K. pneumoniae* (30; 16.30%), *P. aeruginosa* (19; 10.33%), and *E. faecium* (12; 6.52%). Both for benign and malignant disease, the prevalence is almost the same. ”

3 Comments of Reviewer

in the discussion: the statment "and found a slight difference. *E. coli*, *Enterococcus*, *P. aeruginosa*, and *K. pneumoniae* were the most common bacteria present in patients with benign diseases. Conversely, *E. coli*, *Enterococcus*, *K. pneumoniae*, and *Staphylococcus* were the predominant bacteria in patients with malignant diseases. This maybe meaningful for doctors to choose accurate therapy account for microbiological profiles in different diseases. " is not accurate - the prevalence is almost the same - please modify the conclusion.

Reply from Authors

Thank you for the reviewer’s comment. We have checked it and modify the conclusion. “We suspected microbiological profiles may be related to different diseases. We also analyzed the difference in microbiological profiles of patients with benign and malignant diseases. In the study, we found almost the same micro-organisms were positively cultured, *E. coli*, *Enterococcus*, *P. aeruginosa*, and *K. pneumoniae* were the most common bacteria present in patients with malignant and benign diseases.”

4 Comments of Reviewer

It seems that the most important point in this study is the susceptibility profiles of the micro-organisms as most of them were resistant to cephalosporins, quinolones and ampicillin - this findings is important and should be further discussed - please elaborate more

Reply from Authors

Thank you for the reviewer’s comment. We have elaborated more content. “*In the past, the combination of ampicillin and an aminoglycoside was considered to be the first choice for treatment of biliary tract infection. But due to the increasing resistance of penicillin and the kidney toxicity of aminoglycoside, the empiric therapy was changed. And now current guidelines recommend treatment with third-generation cephalosporins or a penicillin/beta-lactamase inhibitor-based agent for empiric therapy for biliary bacteria by intravenous infusion*^[17]. The bacteria resistance has changed. In our study, gram-negative strains had low susceptibility to ceftriaxone, quinolones and ampicillin, which is inconsistent with the guidelines. The high resistance may be related to commonly inappropriate use of these antibiotics, the selection of third-generation cephalosporins and no classification of quinolones. Maybe ceftriaxone and ampicillin were not recommend. On the other hand, they were reasonably susceptible to piperacillin/tazobactam and carbapenems. However, the resistance rates of *E. faecium* were exceedingly high. In our series, gentamicin and piperacillin/tazobactam led to insignificant susceptibility rates, and only narrow-spectrum antibiotics such as vancomycin were effective. This all should be considered during future empiric antibiotic treatments. ”

5 Comments of Reviewer

sub-analysis should be performed to assess what are the risk factors for this high resistance rate, since not all patients with biliary infections are going to be treated with carbapenems, piperacillin and gentamicin due to cost issues and emerging resistance, it is crucial to identify predictors for patients with high resistance for the traditional used antibiotics (cephalosporins and others) so those patients will be administered the more potent antibiotics.

Reply from Authors

Thank you for the reviewer's comment. We agree with you very much. So we used current data to analysis the risk factors for high resistance rate of *E. coli* which is resistant to Ceftriaxone. Unfortunately, we did not find relevant risk factors. The specific form would be displayed in the supplementary material. However we would discuss the result in the second paragraph of discussion.

6 Comments of Reviewer

the authors reported in that "Minimum inhibitory concentrations (MICs) for antibiotic were determined using the broth dilution method, E test (bioMérieux), or disk diffusion methods according to Clinical Laboratory Standards Institute guidelines" - where the authors reported the MICs in the results or tables ?

Reply from Authors

Thank you for the reviewer's comment. The minimum inhibitory concentrations usually were used to assess the sensitivity to drugs. And the related results were presented in "Antibiotic susceptibility and resistance profile of bacteria"(Table1).

7 Comments of Reviewer

the manuscript need english language editing

Reply from Authors

Thank you for the reviewer's suggestion. Someone has edited the article and I will uploade the certificate of editing.

Yours sincerely,

End of Reply by Xue-Xiang Gu, Meng-Pei Zhang, Yan-Feng Zhao, and Guang-Ming Huang