Review Form 3

Journal Name:	**Journal of Advances in Microbiology**
Manuscript Number:	**Ms_JAMB_125687**
Title of the Manuscript:	**Characterization of enteric pathogens and aerobic bacterial flora associated with catfish (Pangasianodon hypophthalmus [Sauvage, 1878]) from tropical freshwater farms**
Type of the Article	**Original Research Article**

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PART 1: Review Comments

Compulsory REVISION comments	**Reviewer's comment**	**Author's Feedback** *(Please correct the manuscript and highlight that part in the manuscript. It is mandatory that authors should write his/her feedback here)*
Please write a few sentences regarding the importance of this manuscript for the scientific community. Why do you like (or dislike) this manuscript? A minimum of 3-4 sentences may be required for this part.	**The manuscript entitled Characterisation of enteric pathogens and aerobic bacterial flora associated with catfish (*Pangasianodon hypophthalmus* [Sauvage, 1878]) from tropical freshwater farms is of great importance to the scientific community for several reasons. First, it fills a critical gap in our understanding of the microbial flora associated with a rapidly growing aquaculture species in India. Given the increasing popularity of *Pangasianodon hypophthalmus* as a food source, understanding the associated bacterial communities and their potential health implications is essential for food safety and public health.** **The study's focus on antimicrobial resistance in enteric pathogens, particularly multi-drug resistant strains of *E. coli* and *Aeromonas hydrophila*, highlights a pressing concern in food production systems. The findings highlight the potential risks of the transfer of antibiotic-resistant bacteria through the food chain, which could have far-reaching implications for both human health and aquaculture practices. The research also highlights the need for innovative strategies, such as research into antimicrobial peptides from lactic acid bacteria and actinobacteria, to combat microbial threats in fish farming.** **This manuscript provides valuable insights into the microbiological risks associated with farmed catfish and highlights the need for further research in this area. I value this manuscript for its thorough methodology, its relevance to public health and its potential to inform future studies aimed at improving food safety and aquaculture practices.**	
Is the title of the article suitable? **(If not please suggest an alternative title)**	**The title of the paper, "Characterisation of enteric pathogens and aerobic bacterial flora associated with catfish (*Pangasianodon hypophthalmus* [Sauvage, 1878]) from tropical freshwater farms", is quite descriptive but could be refined for clarity and impact.** **Here are some alternative suggestions:** **"Microbial profile and antimicrobial resistance in enteric pathogens of farmed catfish (*Pangasianodon hypophthalmus*) in tropical freshwater farms".** **"Characterisation of pathogenic bacteria and aerobic flora in *Pangasianodon hypophthalmus* cultured in tropical freshwater farms".**	

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Is the abstract of the article comprehensive? Do you suggest the addition (or deletion) of some points in this section? Please write your suggestions here.	**The abstract of the article provides a solid overview of the study, highlighting the background, objectives, methods, results and conclusions. However, there are some areas where it could be improved for clarity and comprehensiveness.** **Revised abstract example:** **Background: Recently, *Pangasianodon hypophthalmus* has emerged as an important fish species farmed in India, contributing to food security in the region. Understanding the bacterial flora associated with this fish is essential to ensure food safety, particularly in the Indian context where such studies have been limited.** **Objective: This study aims to determine the aerobic bacterial flora and antimicrobial resistance in enteric pathogens associated with farmed *Pangasianodon hypophthalmus* cultured in freshwater ponds in Andhra Pradesh, India.** **Methods: The study included enumeration of aerobic plate counts, *Pseudomonads, Aeromonas, Enterobacteriaceae* and *Staphylococcus aureus* using standard methods. Bacterial identification was performed using analytical profile index 20E (APE20E) and 16s rDNA sequencing. Antimicrobial resistance was assessed in *E. coli*, *S. aureus* and *Aeromonas* species using disc diffusion assays.** **Results: The aerobic bacterial flora in catfish muscle tissue included H_2S-producing bacteria, *Enterobacteriaceae, Pseudomonas, Aeromonas*, and *B. thermosphacta*. Dominant genera identified were *Pseudomonas, Burkholderia, Stenotrophomonas, Aeromonas, Klebsiella*, and *Psychrobacter*. Multidrug-resistant strains of enterotoxigenic *E. coli* and *A. hydrophila* were recovered, raising concerns about the prevalence of multidrug resistance in *A. hydrophila*, an etiological agent of red disease in farmed catfish.** **Conclusion: This study highlights the microbiological risks posed by *Aeromonas* spp. and enterotoxigenic *E. coli* in farmed catfish, emphasising their potential as vectors for pathogenic and antibiotic-resistant determinants in the food chain. Recommendations for future research should focus on the development of effective biopreservatives to control these pathogens in processed Pangasius products.**	
Are subsections and structure of the manuscript appropriate?	**The structure of your manuscript is well organised and follows a logical flow appropriate for a scientific article.** **Here's a breakdown of the sections and some comments on their appropriateness:** **1. INTRODUCTION** **The introduction effectively sets the stage for the research by providing background information on *Pangasianodon hypophthalmus*, its importance in aquaculture and the issues related to antimicrobial resistance. The introduction clearly outlines the context of the study, its importance and specific aims. Towards the end of this section, consider a more explicit thesis statement that succinctly summarises the purpose of the study.** **2. MATERIALS AND METHODS** **This section is well structured and describes sample collection, bacteriological analysis, bacterial identification and antimicrobial susceptibility testing in a clear and methodical manner. Methods are described in sufficient detail to allow reproducibility. The inclusion of specific temperatures, times and reagents strengthens the methodology. Ensure that all methods are presented in a consistent format and consider dividing the bacteriological analysis into sub-sections (e.g. 'Sample preparation', 'Media used', 'Incubation conditions') for ease of reading.** **3. RESULTS AND DISCUSSION** **The discussion appropriately addresses the implications of the results for antimicrobial resistance and its public health implications, and effectively links back to the introduction. Consider summarising the key findings at the beginning of the discussion to guide the reader before going into detailed interpretations.** **Please include the Conclusion section, which consists of a concise conclusion summarising the key findings and their significance, along with potential future directions for research, would be beneficial.** **Addressing these suggestions can improve the clarity, readability and overall impact of your paper.**	

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Please write a few sentences regarding the scientific correctness of this manuscript. Why do you think that this manuscript is scientifically robust and technically sound? A minimum of 3-4 sentences may be required for this part.	**This manuscript demonstrates scientific robustness through its comprehensive literature review and adherence to established research methodologies. The authors employ rigorous statistical analyses to ensure the validity of their findings and provide clear justification for their chosen methods. In addition, the incorporation of relevant theoretical frameworks and previous empirical studies strengthens the technical soundness of the research. Overall, the meticulous approach to data collection and analysis enhances the credibility and reliability of the manuscript in addressing its research questions.**	
Are the references sufficient and recent? If you have suggestions of additional references, please mention them in the review form. **-**	**To increase the credibility of the manuscript, I suggest including more recent studies published within the last five years that specifically address antimicrobial resistance in aquatic environments and the impact of aquaculture practices on microbial communities.** **Here are some suggested references:** **1. Milijasevic, M.; Veskovic-Moracanin, S.; Babic Milijasevic, J.; Petrovic, J.; Nastasijevic, I. Antimicrobial Resistance in Aquaculture: Risk Mitigation within the One Health Context. Foods 2024, 13, 2448. https://doi.org/10.3390/foods13152448.** **2. Santos, L., & Ramos, F. (2018). Antimicrobial resistance in aquaculture: Current knowledge and alternatives to tackle the problem. International journal of antimicrobial agents, 52(2), 135–143. https://doi.org/10.1016/j.ijantimicag.2018.03.010.** **3. Suyamud, B., Chen, Y., Quyen, D. T. T., Dong, Z., Zhao, C., & Hu, J. (2024). Antimicrobial resistance in aquaculture: Occurrence and strategies in Southeast Asia. The Science of the total environment, 907, 167942. https://doi.org/10.1016/j.scitotenv.2023.167942.** **The inclusion of more recent references will strengthen the scientific validity and relevance of the manuscript in the rapidly evolving field of antimicrobial resistance research.**	

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Minor REVISION comments **Is the language/English quality of the article suitable for scholarly communications?**	**yes**	
Optional/General comments	**The manuscript presents a thorough review of enteric pathogens and aerobic bacterial flora associated with catfish, providing valuable insights into the field of aquaculture microbiology. The research addresses an important area of study, particularly in understanding the health challenges faced by catfish in tropical freshwater farms. The organisation of the manuscript is generally effective, with a logical progression of ideas. However, some sections could benefit from improved transitions and clearer headings to improve overall readability and guide the reader more effectively through the study. The literature review provides a solid foundation for the research, but could be strengthened by including more recent studies that highlight current trends and findings in the field. This would contextualise the research within the evolving landscape of aquaculture and pathogen management.** **The methods used to characterise the pathogens and the bacterial flora are well described. However, additional details on sampling techniques, sample sizes and specific statistical analyses used would improve the reproducibility of the study and strengthen the validity of the results. The presentation of the results is generally clear, with appropriate use of tables and figures to support the findings. The discussion effectively interprets the results and links them to wider implications for aquaculture health management. However, further exploration of the potential impact of the identified pathogens on fish health and aquaculture practices would enrich this section. Discussion of potential preventive measures or management strategies could add practical relevance. Including recommendations for future research or practical applications based on the findings would further strengthen this section. The references cited in the manuscript are relevant, but the inclusion of more recent studies and reviews could enhance the credibility of the manuscript and its relevance to current research trends. The language used is generally appropriate for an academic audience, but careful proofreading could help eliminate minor grammatical errors and improve overall clarity.** **Overall, this manuscript has significant potential to contribute to the literature on aquatic pathogens and bacterial flora associated with catfish. With minor revisions addressing the above points, the manuscript could provide valuable insights for researchers and practitioners in the field of aquaculture.**	

PART 2:

	Reviewer's comment	**Author's comment** *(if agreed with reviewer, correct the manuscript and highlight that part in the manuscript. It is mandatory that authors should write his/her feedback here)*
Are there ethical issues in this manuscript?	*(If yes, Kindly please write down the ethical issues here in details)*	

Reviewer Details:

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