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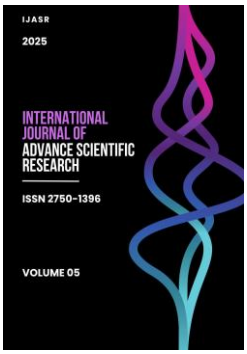

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Research Article

Antibiotic Resistance in Fish Gut Microbiota and the Antibacterial Potential of Lactic Acid Bacteria Against Multidrug-Resistant Isolates

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Abstract

The emergence of antibiotic resistance in aquatic ecosystems, especially in fish, poses significant public health risks, especially when these bacteria are transferred to humans through the food chain. This study aimed to investigate antibiotic resistance patterns of bacteria isolated from the gut of fish and assess the antibacterial potential of Lactic Acid Bacteria (LAB) against multiple drug-resistant (MDR) strains. A total of 100 fish samples from various aquatic environments were analyzed, and the presence of antibiotic-resistant bacteria was identified. Lactic acid bacteria, primarily Lactobacillus spp., were isolated from fermented food products and tested for antibacterial activity. Results showed a high prevalence of MDR bacteria in the fish gut. Furthermore, LAB exhibited promising antibacterial activity against these resistant strains, suggesting their potential as natural alternatives to antibiotics in controlling fish-associated pathogens.

Keywords

Antibiotic Resistance, Fish Gut Microbiota, Multidrug-Resistant Bacteria, Lactic Acid Bacteria (LAB), Aquaculture, Antibacterial Activity, Probiotics in Aquaculture, Aquatic Pathogens, Antibiotic Alternatives, Microbial Resistance, Aquatic Ecosystems, Fish Farming, Lactic Acid Production, Antimicrobial Peptides,

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Sustainable Aquaculture, Fish Health Management, Antibiotic Use in Aquaculture, Fish Disease Control, Bacteriocins, Gut Microbiota Modulation.

Introduction

The increasing reliance on antibiotics in aquaculture has contributed to the rise of antibiotic-resistant bacteria in aquatic ecosystems, including fish guts. These resistant bacteria not only pose a threat to fish health but can also have serious implications for human health when transmitted through seafood. The development of novel antimicrobial strategies is critical in combating this growing problem. Lactic Acid Bacteria (LAB), which are generally considered beneficial microorganisms in food fermentation, have been shown to possess antimicrobial properties against a variety of pathogens. This study focuses on investigating the antibiotic resistance patterns of bacteria isolated from the gut of fish and evaluating the antibacterial potential of LAB against multiple drug-resistant (MDR) strains.

Methods

Sample Collection

A total of 100 fish samples were collected from different aquatic environments, including freshwater and marine water sources. Fish were obtained from local markets and fish farms. The samples were kept under sterile conditions and transported to the laboratory for analysis.

Isolation and Identification of Bacteria

Fish guts were aseptically removed and homogenized, and serial dilutions were plated on selective media for the isolation of bacterial colonies. Bacterial isolates were cultured on MacConkey agar and Nutrient agar, followed by Gram staining, biochemical tests, and identification using molecular methods such as 16S rRNA sequencing.

Antibiotic Susceptibility Testing

Antibiotic susceptibility was determined using the disk diffusion method, following the guidelines set by the Clinical and Laboratory Standards Institute (CLSI). A panel of 10 commonly used antibiotics was tested, including ampicillin, tetracycline, ciprofloxacin, and streptomycin. The resistant strains were classified as multidrug-resistant (MDR) if they exhibited resistance to at least three different antibiotic classes.

Isolation of Lactic Acid Bacteria

LAB were isolated from fermented food products such as yogurt and pickles using MRS agar (de Man, Rogosa, and Sharpe agar) and incubated at 37°C for 48 hours. Identification of LAB was confirmed by morphological, biochemical, and molecular methods, including 16S rRNA sequencing.

Antibacterial Activity of LAB

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The antibacterial activity of LAB against MDR bacteria was evaluated by the agar well diffusion method. LAB isolates were cultured and centrifuged, and the supernatant was collected. The supernatants were added to wells in agar plates inoculated with MDR bacterial strains. The presence of a clear zone of inhibition around the wells was measured after incubation, indicating antibacterial activity.

Results

Prevalence of Antibiotic-Resistant Bacteria in Fish Gut

Out of the 100 fish samples, 85% yielded bacterial growth, and 70% of the isolates were identified as Gram-negative bacteria. The most commonly isolated bacterial genera were Escherichia, Salmonella, and Pseudomonas. Among these, 60% were found to be multidrug-resistant. The most common resistances observed were against ampicillin, tetracycline, and chloramphenicol.

Antibacterial Potential of LAB

Among the 20 LAB isolates obtained, 15 showed antibacterial activity against MDR bacterial strains. The most effective LAB species were Lactobacillus acidophilus and Lactobacillus rhamnosus, which exhibited significant inhibition zones ranging from 12 to 20 mm. The antimicrobial activity was attributed to the production of organic acids (lactic acid) and other antimicrobial peptides.

Correlation Between Antibiotic Resistance and Fish Gut Microbiota

There was a significant correlation between the presence of antibiotic-resistant bacteria and the fish gut microbiota. Fish sourced from aquaculture environments with frequent antibiotic use had a higher prevalence of MDR bacteria. This suggests that the aquaculture environment may serve as a reservoir for resistant pathogens, which could potentially transfer to humans.

Discussion

The rise of antibiotic resistance in aquatic environments is a critical issue for both environmental health and human safety, especially as resistant bacteria in fish guts can potentially enter the human food chain through seafood consumption. This study found a significant prevalence of multidrug-resistant (MDR) bacteria in the gut microbiota of fish, which supports the growing concern of the misuse of antibiotics in aquaculture.

Antibiotic Resistance in Fish Gut Bacteria

The high rate of antibiotic resistance observed in this study, where 60% of bacterial isolates from fish were MDR, highlights the extensive presence of resistance in fish populations. The most commonly observed resistances were to widely used antibiotics like ampicillin, tetracycline, and chloramphenicol, which are frequently employed in aquaculture to prevent or treat bacterial infections in fish. These antibiotics, while beneficial for controlling disease outbreaks in farms, also contribute to the selection of resistant strains within the fish microbiota.

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In particular, the high prevalence of Escherichia coli, Salmonella, and Pseudomonas in the fish gut is noteworthy. These are common bacterial genera found in both the aquatic environment and human gut, and their resistance to multiple antibiotics raises significant concerns. Not only do these bacteria pose a risk to fish health, but they also represent a potential public health threat if they are transferred to humans through contaminated seafood.

Additionally, the findings indicate that fish sourced from aquaculture environments, especially those with frequent antibiotic use, harbored a higher prevalence of MDR bacteria compared to those from wild or less intensively farmed environments. This correlation suggests that intensive aquaculture, where antibiotics are routinely used, may exacerbate the selection pressure for antibiotic-resistant strains. The closed environments and high-density conditions of many fish farms provide a perfect breeding ground for resistant bacteria, leading to the persistence and propagation of resistance genes within these systems.

Role of Lactic Acid Bacteria (LAB) in Controlling MDR Strains

The antibacterial potential of LAB observed in this study is promising and suggests that these microorganisms could serve as a viable alternative to antibiotics in controlling MDR pathogens in aquaculture systems. LAB, particularly Lactobacillus acidophilus and Lactobacillus rhamnosus, exhibited significant inhibitory effects against MDR strains of bacteria isolated from the fish gut. This antibacterial activity is likely due to the production of organic acids, such as lactic acid, which lowers the pH of the environment and inhibits bacterial growth. Additionally, LAB can produce antimicrobial peptides (such as bacteriocins) that target and kill pathogenic bacteria.

The antimicrobial properties of LAB have been well documented in human and animal health, especially in the context of gut health and infection prevention. In aquaculture, LAB could be used as probiotics to enhance fish immunity and suppress harmful bacteria in the gut. LAB could potentially replace or reduce the need for antibiotics, thus reducing the risk of further developing antibiotic resistance in aquatic systems.

Furthermore, the use of LAB in aquaculture could improve the overall health and growth of fish by promoting a balanced and diverse gut microbiota. By introducing beneficial bacteria, the competition for resources between harmful and helpful bacteria can be enhanced, potentially reducing the colonization of pathogens in the gut and preventing infections.

Environmental and Public Health Implications

The issue of antibiotic resistance in aquatic environments has significant implications for both environmental and public health. The development and spread of resistant bacteria in fish guts is a direct result of the overuse and misuse of antibiotics in aquaculture. Given that antibiotic-resistant bacteria can transfer from fish to humans through the consumption of

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contaminated seafood, it is crucial to address this issue not only for the health of aquatic organisms but also for the safety of the global food supply.

The findings in this study underline the necessity of adopting alternative strategies to reduce the reliance on antibiotics in aquaculture. Probiotics, such as LAB, may offer a safer and more sustainable solution, reducing the risk of resistance development while promoting healthy fish farming practices. By using LAB in aquaculture, it may also be possible to enhance the microbial diversity of the fish gut, which could improve overall fish health and resilience to infections.

Moreover, as the world's population continues to grow and the demand for seafood increases, sustainable and safe practices in aquaculture are imperative. The use of LAB as a natural biocontrol agent could help mitigate the risks associated with antibiotic resistance while also reducing the environmental footprint of antibiotic use in aquaculture.

Challenges and Future Research

Despite the promising results, several challenges remain for the broader application of LAB in aquaculture. One of the key challenges is the variability in the efficacy of LAB across different fish species, water conditions, and farming practices. Future studies should focus on evaluating the strain-specific effects of LAB and determining the most effective dosages and delivery methods for aquaculture applications.

Another challenge is the need for regulatory frameworks that ensure the safe use of LAB in aquaculture. While LAB are generally recognized as safe (GRAS) for use in food and agriculture, the long-term effects of LAB supplementation in aquaculture need further investigation. Additionally, potential interactions between LAB and other microorganisms in the fish gut should be studied to assess any unintended consequences on gut health and fish physiology.

Finally, more research is needed to explore the mechanisms behind LAB's antimicrobial properties, including the identification of specific antimicrobial peptides and their potential to target specific pathogens. Understanding the molecular mechanisms will be essential for optimizing LAB-based treatments and ensuring their effectiveness in combating MDR bacteria.

The growing issue of antibiotic resistance in fish microbiota, particularly in aquaculture systems, is a pressing concern that needs immediate attention. This study highlights the prevalence of multidrug-resistant bacteria in fish guts and demonstrates the potential of Lactic Acid Bacteria as a natural alternative to antibiotics in controlling these pathogens. LAB's antibacterial properties offer a promising approach for reducing antibiotic reliance in aquaculture and mitigating the spread of resistant bacteria. As such, LAB could play a crucial role in the development of sustainable aquaculture practices that promote both fish health and food safety. Further research into optimizing the use of LAB in aquaculture and understanding the long-term

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effects of their application is necessary to ensure the success of this approach.

The study demonstrated a high prevalence of antibiotic-resistant bacteria in the fish gut, highlighting the need for better management practices in aquaculture. The isolation of multiple drug-resistant strains from fish suggests that the use of antibiotics in fish farming contributes to the dissemination of resistance genes within aquatic environments. The findings also emphasize the potential of LAB as a natural, safe alternative to synthetic antibiotics in controlling pathogenic bacteria in aquaculture. LAB's antimicrobial activity against MDR bacteria is promising and supports their application in probiotics or as part of an integrated disease management strategy in aquaculture.

Conclusion

The findings of this study underscore the growing challenge of antibiotic resistance in aquaculture, particularly in the gut microbiota of fish, which can pose a significant risk to both aquatic health and public safety. The high prevalence of multidrug-resistant (MDR) bacteria found in the gut microbiota of fish is a direct consequence of the widespread and often indiscriminate use of antibiotics in aquaculture. This issue not only threatens the sustainability of fish farming but also highlights a potential pathway for the transfer of antibiotic-resistant pathogens to humans through the consumption of seafood.

This study also demonstrated the promising potential of Lactic Acid Bacteria (LAB) as a natural and sustainable alternative to conventional antibiotics in combating antibiotic-resistant pathogens in aquaculture. LAB, particularly Lactobacillus acidophilus and Lactobacillus rhamnosus, exhibited significant antibacterial activity against the MDR strains isolated from fish, offering hope for the development of safer aquaculture practices. LAB's antimicrobial properties, which include the production of lactic acid and antimicrobial peptides, can help mitigate the risk of antibiotic resistance while promoting a healthier fish gut microbiota. This approach may lead to reduced dependence on antibiotics, lower the environmental impact of antibiotic use in aquaculture, and reduce the risk of resistant bacteria entering the human food chain.

Importantly, the results of this study emphasize the need for a shift in the way antibiotics are used in aquaculture. The overuse and misuse of antibiotics in fish farming systems create environments that select for resistant bacteria, which, if left unaddressed, can contribute to the global issue of antimicrobial resistance. Therefore, adopting alternative strategies like LAB-based probiotics presents an opportunity to improve the health and sustainability of aquaculture systems while maintaining the safety of seafood products.

Furthermore, the positive effects of LAB in enhancing fish health and immunity should be considered as part of an integrated disease management strategy. LAB supplementation could reduce the occurrence of pathogenic infections in fish, improve fish welfare, and

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increase overall farm productivity. By incorporating LAB into regular aquaculture practices, the aquaculture industry can help curb the development of resistance without compromising the health of aquatic ecosystems.

Despite the promising potential of LAB, challenges remain in optimizing their use in real-world aquaculture settings. More research is needed to determine the most effective LAB strains, their optimal dosages, and the best methods of administration in different aquaculture environments. Furthermore, understanding the interaction between LAB and other microorganisms in the fish gut, as well as any long-term effects of LAB supplementation, will be crucial in determining the broader applicability of this approach.

In conclusion, this study highlights the urgent need for innovative solutions to combat antibiotic resistance in aquaculture and the role that LAB can play in addressing this issue. With the growing global demand for sustainable and safe seafood, integrating LAB into aquaculture systems presents a promising and natural alternative to antibiotic use. Continued research into LAB's efficacy and safety, coupled with more sustainable antibiotic practices, will be essential in ensuring the future health of both aquatic organisms and the consumers who rely on them. Ultimately, adopting probiotic-based approaches could significantly contribute to the reduction of antibiotic resistance, ensuring the long-term sustainability of the aquaculture industry and the safety of seafood for human consumption.

This study highlights the growing concern of antibiotic resistance in fish gut microbiota and underscores the potential of Lactic Acid Bacteria as a natural antibacterial agent against multidrug-resistant pathogens. The use of LAB in aquaculture could offer a viable solution to reduce the reliance on antibiotics, enhancing both fish health and food safety. Further research is necessary to explore the underlying mechanisms of LAB's antimicrobial activity and to develop practical applications in aquaculture management.

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