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Original Research Article

Viability of *Bacillus coagulans* spores with ofloxacin & ornidazole: Implications for gut health

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ABSTRACT

Background: The balance of gut microbiota significantly impacts host health. Disruption of the natural gut flora, often caused by infections or the use of broad-spectrum antimicrobial drugs, can lead to dysbiosis, causing gastrointestinal disorders, such as diarrhea. Probiotics show promising outcomes in restoring gut health, but concerns remain about their interaction with antimicrobials and the viability of spores in the intended gut location. To address these uncertainties, the current study was devised to evaluate how well spores-forming bacteria endure and develop in an environment where antimicrobial agents are present.
Materials and Methods: The study investigated the survival and growth of *Bacillus coagulans* spores under the influence of broad-spectrum antimicrobial agents, Ofloxacin and Ornidazole. To cultivate the spores in the presence of these antimicrobials, a mixture of MRS broth and PNY agar media was used. The number of colonies that developed were measured to assess the extent of spore survival and germination.
Results: In a simulated environment resembling human intestinal pH, *Bacillus coagulans* spores exhibited viability. Starting with an initial count of 1.38 billion CFU, the spores multiplied to 8.75 billion CFU at 24 hours and further reached to 86.25 billion CFU at 72 hours in the presence of Ofloxacin and Ornidazole. On the other hand, the viable count reached to the level of 88 billion CFU in the absence of antimicrobial agents.
Conclusion: This study offers evidence that *Bacillus coagulans* spores are able to remain viable and germinate when co-administered with Ofloxacin and Ornidazole.

1. Introduction

The intestinal environment hosts a complex and diverse community of microorganisms known as the gut microbiota. These microorganisms are intricately connected within a metabolic network and serve various crucial functions for human well-being. Among its numerous roles, the gut microbiota aids in digestion, regulates the immune system, defends against foreign organisms, and inhibits the growth of pathogens.[1,2]

Under normal circumstances, the gut microbiota establishes a balanced and harmonious relationship with the host, displaying stability and resilience.[3] However, the onset of infections can disrupt this equilibrium, resulting in the loss of beneficial bacteria. This disruption compromises both gut function and immune responses. Presently, acute infections are typically treated with antimicrobial drugs, which effectively eliminate the responsible pathogens. Unfortunately, these drugs can inadvertently harm the beneficial components of the gut microbiota.[4,5] Such unintended consequences can lead to dysbiosis, triggering acute diarrhea. Moreover, the presence of concurrent infections exacerbates the severity of diarrhea.[6] The restoration of dysbiosis can be achieved through the use of probiotics.[7]

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A combination of Ofloxacin and Ornidazole is commonly prescribed for managing mixed infectious diarrhea.[8] The medication has an effective broad spectrum against various types of bacteria, including both gram-negative and gram-positive varieties, as well as certain anaerobes.[9–11] This broad-spectrum antimicrobial treatment adversely affects the diversity of the gut microbiota.[12] Maintaining a balanced gut microbiome is crucial, leading to the co-administration of probiotics. By co-administering probiotics with these antimicrobials, a beneficial treatment strategy has emerged. Probiotics have effectively demonstrated their capability in addressing diarrhea, thus presenting a valuable inclusion to the treatment protocol.[13]

The Cochrane Database of Systematic Reviews highlights that probiotics lower the risk of diarrhea lasting ≥48 hours by 36%, with a relative risk of 0.64 (95% CI, 0.52 to 0.79), and reduce the average duration of diarrhea by around 21.3 hours (95% CI, 15.7 to 26.9 hours).[13] About 27% of healthcare practitioners in the Asia-Pacific region are favoring the co-prescription of probiotics with antimicrobials.[14]

For probiotics to effectively deliver their intended benefits, it's crucial for them to reach at intended site of action and maintain their viability. *Bacillus* species have gained significant attention as probiotics, but their effective use faces challenges arising from sensitivity to heat and gastric acid.[15] These inherent limitations hinder their successful transit through the digestive system, potentially compromising their beneficial effects. An exception is *Bacillus coagulans* (also known as lactic acid bacillus), particularly, which demonstrates a unique advantage due to its spore-forming ability. This characteristic enhances its resilience in the digestive system, as it withstands gastric acid and high temperatures, consequently improving its potential to deliver health benefits.[16] The remarkable resilience of *Bacillus coagulans* spores lies in their dormancy, which can persist for extended periods, germinating quickly in favorable conditions in the duodenum and flourishing in the upper small intestine.[17]

For optimal health advantages through probiotic supplementation and effective intestinal colonization, a daily intake of at least 5 billion CFUs of probiotics is recommended.[18] A recent study showed that even lower quantities of Bacillus coagulans spores can still generate viable counts that surpass the suggested threshold for establishing intestinal colonization.[19]

Although probiotics have demonstrated potential health benefits, the co-prescription of probiotics with antimicrobials is still relatively infrequent. This is primarily attributed to the lack of substantial evidence concerning the viability and germination of probiotic spores in the presence of antimicrobials.[20] To address this gap, an *in-vitro* study was conducted to evaluate the survival and germination of *Bacillus coagulans* spores when exposed with Ofloxacin and Ornidazole.

2. Materials and Methods

The viability of *Bacillus coagulans* spores was assessed under two different conditions. Group A involved the examination of *Bacillus coagulans* spores in the presence of a combination tablet of Ofloxacin (200 mg) and Ornidazole (500 mg). On the other hand, Group B focused only on *Bacillus coagulans* spores without the antimicrobial agents. In both groups, the powder containing *Bacillus coagulans* spores comprised 1 billion spores, with a weight of 166.66 mg. Further, the same procedural steps were applied to both groups to observe bacterial dynamics over the incubation period.

The amalgamation of spores and tablet was dissolved in 5-liter phosphate buffer at a pH of 6.8. The resulting phosphate buffer solution was diluted with 0.9% saline solution in a 10^{-2} dilution. The diluted solution was then heated at 75°C for 30 minutes and cooled to 45-50°C, termed 'Solution A' for further use. Subsequently, 1 mL of Solution A was added to three sterile petri dishes, followed by 20 mL of sterilized PNY agar medium, and incubated at 37°C for 72 hours to find the colony count.

Simultaneously, another 1 mL aliquot of 'Solution A' was added to three test tubes containing 9 mL sterile MRS broth medium. These tubes were incubated at 37°C for 24, 48, and 72 hours to promote germination.

Following initial incubation, sequential dilutions were performed up to 10^{-10} using 0.9% saline solution. 1 mL of each dilution was mixed with pre-cooled PNY agar medium in sterile petri dishes, after solidification, these were incubated at 37°C for 72 hours, and colonies were counted.

3. Results

The bacterial count after incubation was 1.38 billion CFUs in Group A and 1.45 billion CFUs in Group B. A consistent increase in the bacterial count was observed over time. Notably, this pattern of spore germination remained consistent between the two groups at each of the assessed time points throughout the study duration.

The findings highlight a noteworthy augmentation in the count of *Bacillus coagulans* following incubation periods of 24, 48, and 72 hours. After 24 hours, the counts of 8.75 billion CFUs in Group A and 9.10 billion CFUs in Group B were evident, indicating the germination and proliferation of spores even in the presence of antimicrobial agents.

This growth continued at the 48 hours, where *Bacillus coagulans* counts reached at 36 billion CFUs in Group A and 37.35 billion CFUs in Group B whereas, at the 72 hours, it reached at 86.25 billion CFUs and 88.0 billion CFUs in Group A and B respectively (Figure 1).

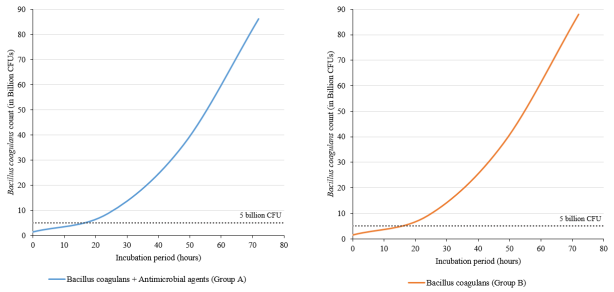

Figure 1: Trend of germination and multiplication of Bacillus coagulans viable count

4. Discussion

The use of *Bacillus coagulans* spores along with antimicrobial agents like Ofloxacin and Ornidazole remains a relatively unexplored area. This study, therefore, aimed to delve into the viability and germination capacity of *Bacillus coagulans* spores in the presence of these antimicrobial agents.

The outcomes reveal that *Bacillus coagulans* spores exhibit resilience and successful germination even when exposed to Ofloxacin and Ornidazole. Notably, after 72 hours, *Bacillus coagulans* count reached 86.25 billion CFUs in presence of antimicrobials and 88.0 billion CFUs without antimicrobials. From these findings, it becomes evident that the presence of Ofloxacin and Ornidazole did not significantly impact the survivability and growth of *Bacillus coagulans* spores. The observed patterns highlight the spores' resilience to antimicrobial agents, providing insights into their effectiveness in maintaining their intended benefits.

The study emphasizes that 1 billion spores in the formulation adequately achieve a viable count of more than 5 billion CFUs in 24 hours that can effectively colonize the gut. This observation challenges the notion that formulation should contain more than 5 billion probiotic counts for successful colonization. A corroborative study that supports the results reinforces the notion that optimal colonization can be achieved without resorting to high doses of probiotics. [19]

In essence, this study bridges the gap in understanding the interplay between *Bacillus coagulans* spores and commonly used antimicrobial agents. By showcasing the spores' ability to withstand and germinate even in the presence of Ofloxacin and Ornidazole, it expands the potential applications of probiotics in contexts where antimicrobial treatment is administered. In addition, the observations that lower levels of spores at the beginning can still result in successful colonization has important implications for probiotic supplementation strategies.

5. Conclusion

This study provides evidence indicating that the co-administration of *Bacillus coagulans* spores with Ofloxacin and Ornidazole may help in normalizing the gut flora. In fact, the administration of *Bacillus coagulans* spores resulted in viable count of 86.25 billion after 72 hours which exceeds the minimum requirement of 5 billion CFU per day for successful intestinal colonization. The study demonstrates the robust survivability and growth of *Bacillus coagulans* even in the presence of antimicrobials.

6. Source of Funding

None.

7. Conflict of Interest

None.

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