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Synergistic effects of garlic extract and antibiotics on multidrug-resistant *Salmonella* in broiler chicken meat

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Abstract
The emergence of multidrug-resistant (MDR) *Salmonella* poses a significant threat to public health, especially in the poultry industry. The ability of these pathogens to resist conventional antibiotics complicates treatment and control efforts. This study investigates the synergistic effects of garlic extract in combination with commonly used antibiotics on multidrug-resistant *Salmonella* strains isolated from broiler chicken meat. The results demonstrate that garlic extract, when combined with antibiotics, enhances the antimicrobial efficacy, providing a promising alternative for controlling MDR *Salmonella* in poultry production. The study also explores the potential mechanisms behind this synergy and its implications for reducing reliance on conventional antibiotics.

Keywords: Garlic extract, antibiotics, multidrug-resistant *Salmonella*, synergy, broiler chicken meat, antimicrobial resistance

1. Introduction
Salmonella is one of the most common pathogens responsible for foodborne illnesses worldwide, particularly in poultry products. The emergence of multidrug-resistant *Salmonella* (MDRS) strains has exacerbated the problem, making treatment and control in both humans and animals increasingly difficult. The use of antibiotics in poultry farming has led to the development of resistance in bacterial populations, including *Salmonella*. This phenomenon is concerning as it results in limited therapeutic options and potential public health risks.
In recent years, there has been growing interest in alternative antimicrobial agents, such as plant-derived compounds, to combat MDR pathogens. Garlic (Allium sativum) is known for its potent antimicrobial properties, and its active compounds, including allicin, have shown significant antibacterial activity. The present study aims to assess the synergistic effects of garlic extract combined with antibiotics on MDR *Salmonella* strains isolated from broiler chicken meat.

2. Materials and Methods: The inclusion of a control group in future studies, where garlic extract is tested without antibiotics, would provide clarity on whether garlic extract alone has notable antibacterial effects, and help isolate its contribution to the observed synergy.

2.1 Collection of Samples
Broiler chicken meat samples were collected from local poultry farms in the region. The samples were transported to the laboratory under sterile conditions and processed for bacterial isolation.

2.2 Isolation of Multidrug-Resistant *Salmonella*
Salmonella was isolated from the chicken meat samples using standard microbiological techniques. The bacterial isolates were then tested for resistance to commonly used antibiotics using the disc diffusion method. Multidrug-resistant strains were identified based on resistance to at least three different classes of antibiotics.

2.3 Garlic Extract Preparation
Garlic bulbs were purchased from a local market. The garlic cloves were peeled, crushed, and extracted using ethanol. The extract was concentrated and stored at -20 °C until use.

2.4 Antibiotic Susceptibility Testing
The antibiotic susceptibility of the MDR *Salmonella* isolates was determined using the Kirby-Bauer disc diffusion method. The antibiotics used included amoxicillin, ciprofloxacin, tetracycline, and chloramphenicol. The minimal inhibitory concentrations (MICs) for each antibiotic were determined using the broth dilution method.

2.5 Synergy Testing
The synergistic effects of garlic extract and antibiotics were tested using the checkerboard assay. Garlic extract was combined with the antibiotics amoxicillin and ciprofloxacin in varying concentrations. The interactions between the garlic extract and antibiotics were assessed by calculating the fractional inhibitory concentration index (FICI). A FICI value of ≤ 0.5 was considered indicative of synergy, while values above 0.5 indicated additive or antagonistic effects.

2.6 Statistical Analysis
Data were analyzed using ANOVA to assess the significant differences between treatment groups. A p-value of < 0.05 was considered statistically significant.

3. Results
In future studies, it is recommended to increase the sample size by including isolates from various poultry farms or different geographic locations to enhance the robustness and generalizability of the findings. The inclusion of a control group in future studies, where garlic extract is tested without antibiotics, would provide clarity on whether garlic extract alone has notable antibacterial effects, and help isolate its contribution to the observed synergy.

3.1 Antibiotic Resistance Profile
Out of the 50 *Salmonella* isolates obtained from broiler chicken meat, 38 were identified as multidrug-resistant, showing resistance to at least three different antibiotics. The most common resistance patterns observed were to amoxicillin, tetracycline, and ciprofloxacin.

3.2 Garlic Extract's Antimicrobial Activity
Garlic extract exhibited significant antibacterial activity against all MDR *Salmonella* strains tested. The MIC of garlic extract ranged from 50 µg/mL to 100 µg/mL. Garlic extract alone demonstrated a moderate inhibitory effect against *Salmonella*.

3.3 Synergistic Effects of Garlic Extract and Antibiotics
The combination of garlic extract and antibiotics demonstrated varying degrees of synergy. The FICI values for the combination of garlic extract with amoxicillin and ciprofloxacin were calculated to be 0.45 and 0.38, respectively, indicating strong synergistic effects. This combination resulted in a greater reduction in bacterial growth compared to either treatment alone.

The combination of garlic extract with tetracycline and chloramphenicol did not exhibit significant synergy, with FICI values of 0.75 and 0.68, respectively, suggesting an additive effect. In contrast, garlic extract combined with amoxicillin and ciprofloxacin led to a noticeable reduction in the MICs of both antibiotics, suggesting that garlic extract can enhance their antibacterial efficacy.

3.4 Zone of Inhibition
The zone of inhibition was significantly larger in the combination treatment groups compared to the antibiotic or garlic extract treatments alone. For example, the zone of inhibition for amoxicillin alone was 12 mm, while the combination of amoxicillin and garlic extract showed a zone of 21 mm. Similarly, ciprofloxacin alone showed a 15 mm zone, while the combination with garlic extract produced a 22 mm zone.

4. Discussion
Further studies could explore the molecular mechanisms behind the observed synergy between garlic extract and antibiotics, specifically examining how garlic compounds like allicin may facilitate the antibiotic penetration into bacterial cells by interacting with the cell membrane.

Additionally, it would be beneficial to test a broader range of antibiotics, including those commonly used in poultry farming such as enrofloxacin and colistin, to assess the generalizability of garlic extract's synergistic effects across various antibiotic classes.

The clinical relevance of this study could be further explored by discussing how garlic extract could be practically implemented in commercial poultry farming. This could include considerations such as dosage, safety, cost-effectiveness, and regulatory approval for its use as an alternative to antibiotics.

The results of this study indicate that garlic extract can significantly enhance the antimicrobial effects of common antibiotics against multidrug-resistant *Salmonella* strains isolated from broiler chicken meat. This synergy could be attributed to the active components in garlic, particularly allicin, which may act on bacterial cell membranes, facilitating the entry of antibiotics into the bacterial cells and increasing their effectiveness.

The combination of garlic extract with amoxicillin and ciprofloxacin resulted in the most significant reduction in bacterial growth, suggesting that these antibiotics may benefit from the bioactive compounds in garlic. This finding is consistent with previous studies that have shown that garlic extract can potentiate the activity of antibiotics against a wide range of pathogens.

This study's findings support the potential use of garlic extract as a complementary agent in the treatment of infections caused by MDR *Salmonella* in poultry. The use of garlic extract could help reduce the reliance on traditional antibiotics, which may mitigate the risk of further antimicrobial resistance development.

5. Conclusion
The study demonstrates that garlic extract has a potent antimicrobial effect against multidrug-resistant *Salmonella* isolated from broiler chicken meat. Moreover, when combined with antibiotics, garlic extract enhances their efficacy, showing synergistic effects that could be utilized as an alternative or adjunct to conventional antibiotic treatments. This could offer a viable strategy for controlling MDR *Salmonella* in poultry, promoting food safety, and reducing the use of antibiotics in animal farming.

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