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Bioremediation of Penicillin-Contaminated Poultry Faecal Waste using Betalactamase-Producing Bacteria

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Abstract:

The widespread use of antibiotics in poultry farming has led to the contamination of the environment with antibiotic residues, posing significant risks to human health and contributing to the development of antibiotic resistance. In this study, we aimed to isolate betalactamase-producing bacteria from poultry faecal waste samples obtained from local poultry processing industries in Namakkal, Tamilnadu, India. The potential isolates were further characterized for betalactamase enzyme activity and their ability to degrade penicillin, a commonly used antibiotic in the poultry industry. Twenty poultry faecal waste samples were collected from regular poultry waste dumping sites. Microorganisms were isolated from these samples using the serial dilution and plating method on nutrient agar media. The isolated bacterial colonies were purified to obtain pure cultures for further analysis. The betalactamase-producing isolates were identified using the iodometric tube method, and four out of ten isolates showed positive results for betalactamase activity. These positive isolates were subjected to enzyme assay, and isolate 10 exhibited the highest enzyme activity with a concentration of 43U/ml, followed by isolate 7 with 30.5U/ml of enzyme. The potential betalactamase-producing isolate 10 was selected for its application in the degradation of penicillin in poultry faecal waste. The faecal waste samples were collected from the antibiotic-contaminated area of a poultry farm. After the addition of separated crude enzyme (5ml of 100U), the faecal sample was incubated for 15 days under specific conditions. HPLC analysis revealed a significant degradation of penicillin in the test sample treated with the betalactamase enzyme, with a degradation percentage of 48.6%. The results of this study indicate that betalactamase-producing bacteria can effectively degrade penicillin in poultry faecal waste. This bioremediation approach presents a potential solution to reduce antibiotic pollution in the environment and mitigate the risk of antibiotic resistance. Further research and application of such enzymatic degradation methods could contribute to sustainable and eco-friendly waste management practices in the poultry industry.

Keywords: ***Betalactamase, penicillin degradation, poultry faecal waste, antibiotic resistance, bioremediation, antibiotic pollution.***

Introduction:

Antibiotics play a crucial role in disease prevention and growth promotion in poultry farming. However, the indiscriminate use of antibiotics has resulted in the contamination of the environment, including soil and water, with antibiotic residues (Puvača et al., 2022). Poultry faecal waste, which contains antibiotic residues,

deteriorated feathers, and other organic matter, is often disposed of in open dumping sites, leading to the potential contamination of surrounding soil and groundwater (Smith et al., 2018). This antibiotic pollution poses serious health risks to humans, as well as contributing to the development of antibiotic-resistant bacteria (Wellington et al., 2013). In recent years, there has been growing interest in developing sustainable and eco-friendly approaches to tackle the issue of antibiotic contamination in the environment. Bioremediation, which involves the use of microorganisms or their enzymes to degrade pollutants, has emerged as a promising method for mitigating antibiotic pollution (Ekeoma et al., 2022). One such approach involves the use of betalactamase-producing bacteria, which produce enzymes capable of degrading betalactam antibiotics, including penicillin (Martinez, 2008). In this study, we aimed to isolate betalactamase-producing bacteria from poultry faecal waste and evaluate their potential for degrading penicillin. The use of indigenous bacterial isolates could offer a cost-effective and site-specific solution for reducing antibiotic pollution in poultry waste disposal sites. This research contributes to our understanding of antibiotic degradation and provides valuable insights into the development of environmentally friendly waste management practices in the poultry industry.

Materials and Methods:

Collection of Poultry Faecal Waste Samples:

A total of 20 poultry faecal waste samples were collected from local poultry processing industries in Namakkal, Tamilnadu, India. The samples were obtained from regular poultry waste dumping sites at a depth of 3-4 cm and placed in sterile plastic bags. The samples were transported to the laboratory for further processing.

Isolation of Bacterial Isolates:

Microorganisms were isolated from poultry faecal waste samples using the serial dilution and plating method on nutrient agar media. Ten grams of poultry faecal waste samples were mixed thoroughly in 90 mL of sterilized water. The sample was serially diluted up to a 10-5 dilution, and the diluted sample (0.1 mL) was inoculated onto nutrient agar medium, spread evenly using a spreader, and incubated at 37°C for 24 to 48 hours. The resulting bacterial colonies were purified using the streak plate method, and pure cultures were obtained by further subculturing on nutrient agar.

Determination of Betalactamase-Producing Isolates:

The potential isolates were subjected to the iodometric tube method to determine their betalactamase production (Devapriya et al., 2013). Penicillin solution was dispensed in 0.5 ml volume in small test tubes. Test bacteria were removed with a loop from an overnight culture on solid medium and suspended in the penicillin solution to give a density of at least 104 CFU/ml. After one hour at room temperature, two drops of starch indicator were added to the suspension, followed by one drop of iodine reagent. A positive reaction was indicated by the disappearance of blue color immediately, while persistence of blue color for longer than 10 minutes constituted a negative test.

Extraction of Betalactamase Enzyme:

The potential betalactamase-producing isolates were inoculated into nutrient broth with the presence of ampicillin (20 µg/ml). After the incubation period, cells were harvested by centrifugation (4000× g, 15 minutes at 4°C) and washed twice in phosphate buffer (0.01 M, pH 7.0). The cells were disrupted using an ultrasonic disintegrator for 3 minutes at 4°C. Cell debris was removed by centrifugation at 12,000 rpm for 40 minutes at 4°C. Beta-lactamase activity was determined by a microiodometric assay (Devapriya et al., 2013). The absorbance was read spectrophotometrically at 620 nm, and the international unit (IU) was defined as the amount of enzyme needed to hydrolyze 1 µmol of penicillin G per minute at 25°C and pH 7.0.

Protein Estimation:

Lowry's method was used to estimate the protein content in the samples (Lowry et al., 1951). Bovine serum albumin (BSA) was used as a reference for protein assay. The necessary reagents, including Reagent A (2% sodium carbonate in 0.1 N sodium hydroxide), Reagent B (0.5% copper sulphate in 1% potassium sodium tartarate), Alkaline copper solution (Reagent C), and Diluted Folin's reagent (Reagent D) were prepared. A standard curve was plotted using different concentrations of BSA along the X-axis and the corresponding absorbance at 660 nm along the Y-axis. The concentration of protein in the test sample was calculated from the standard curve.

Application of Betalactamase on Poultry Faecal Waste for Degradation of Penicillin:
The faecal waste samples collected from the antibiotic-contaminated area of a poultry farm were air-dried and passed through a 2 mm mesh sieve before the experiment. Penicillin contamination in the faecal waste samples was determined using high-performance liquid chromatography (HPLC) (L-2000, Hitachi) with standard penicillin purchased from Himedia, India.
For the degradation of penicillin, the potential betalactamase-producing isolate 10 was selected. Separated crude enzyme (5 ml of 100 U) was added to a cup containing the faecal waste and thoroughly mixed by hand with a spatula. The faecal sample was then incubated for 15 days under conditions reported by Wang et al. (2019). Sterile water was supplied to each pot every two days to maintain 30% of faecal moisture during the incubation period.
After the incubation time, the faecal waste was subjected to HPLC analysis to determine the degradation level of penicillin. The chromatographic separation was performed using a Shimadzu C18 reverse-phase column with a flow rate of 1.00 mL/min at 25°C. The mobile phase consisted of an 85:15 (v/v) mixture of acetonitrile and pure water containing 0.02% formic acid with a pH of approximately 3.2.

Results:

Isolation of Betalactamase-Producing Isolates:
Among the ten bacterial isolates obtained from poultry faecal waste samples, four showed positive results for betalactamase production using the iodometric tube method (Table 1).

Identification of Potential Isolates by Enzyme Assay:
The four betalactamase-producing isolates were further subjected to enzyme assay, and their enzyme activity was measured. Isolate 10 exhibited the highest concentration of betalactamase enzyme, with 43 U/ml, followed by isolate 7 with 30.5 U/ml (Table 2).

S.No	Isolates	Enzyme	Protein	Specific enzyme activity
1.	Isolate 3	21± 0.81	0.97± 0.1	21.6±1.52
2.	Isolate 4	19± 1.52	0.82± 0.13	23.1±1.62
3.	Isolate 7	22± 1.63	0.72±0.1	30.5± 2
4.	Isolate 10	29 ±1.24	0.53±0.15	43.0±1.2

Table 2: Isolation of Betalactamase-Producing Potential Isolates

Application of Betalactamase on Poultry Faecal Waste for Degradation of Penicillin:
After the incubation period, HPLC analysis was performed to assess the degradation of penicillin in the faecal waste samples treated with the betalactamase enzyme. The chromatograms revealed a significant reduction in the penicillin peak in the test sample (Figure 5), indicating a degradation percentage of 48.6% compared to the control sample (Figure 4).

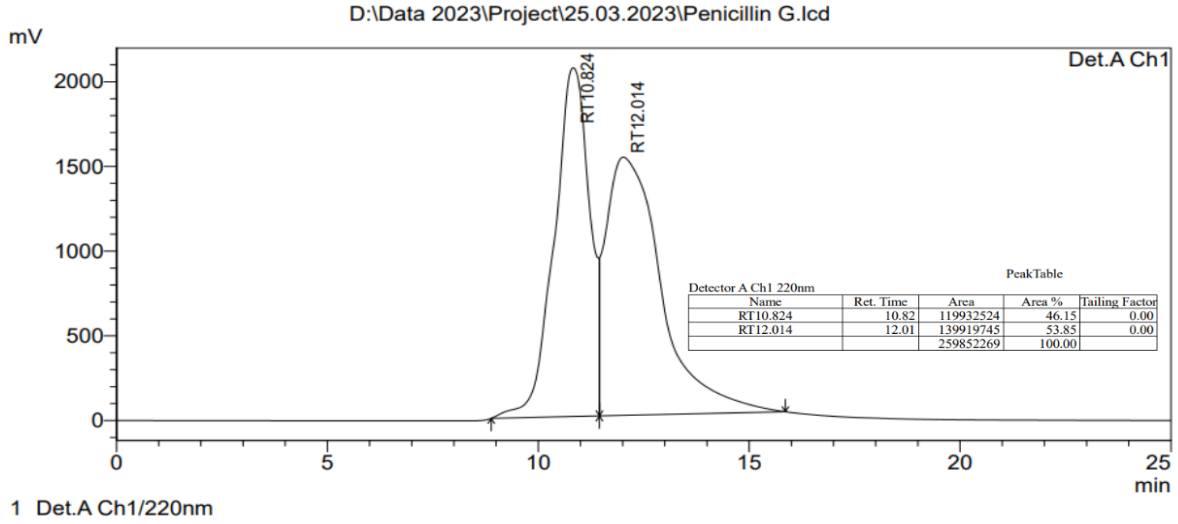

Figure No.3: The standard HPLC chromatogram of Penicillin-G antibiotic assayed in the study for the comparison.

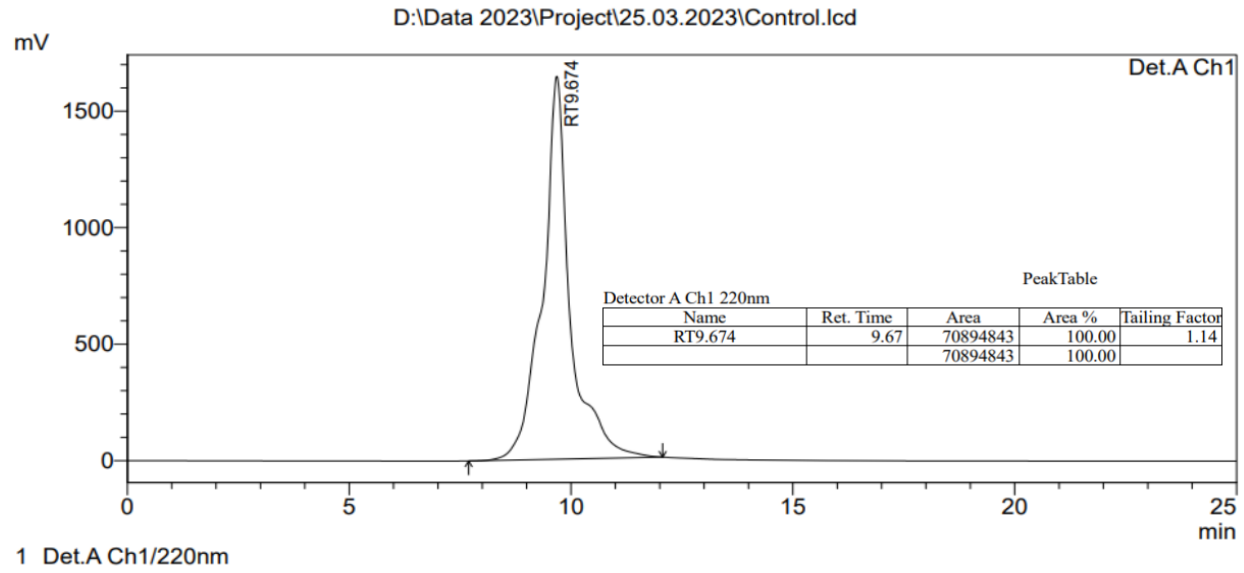

Figure No.4:The HPLC Chromatogram of Control Sample (before enzymatic treated) assayed in this study.

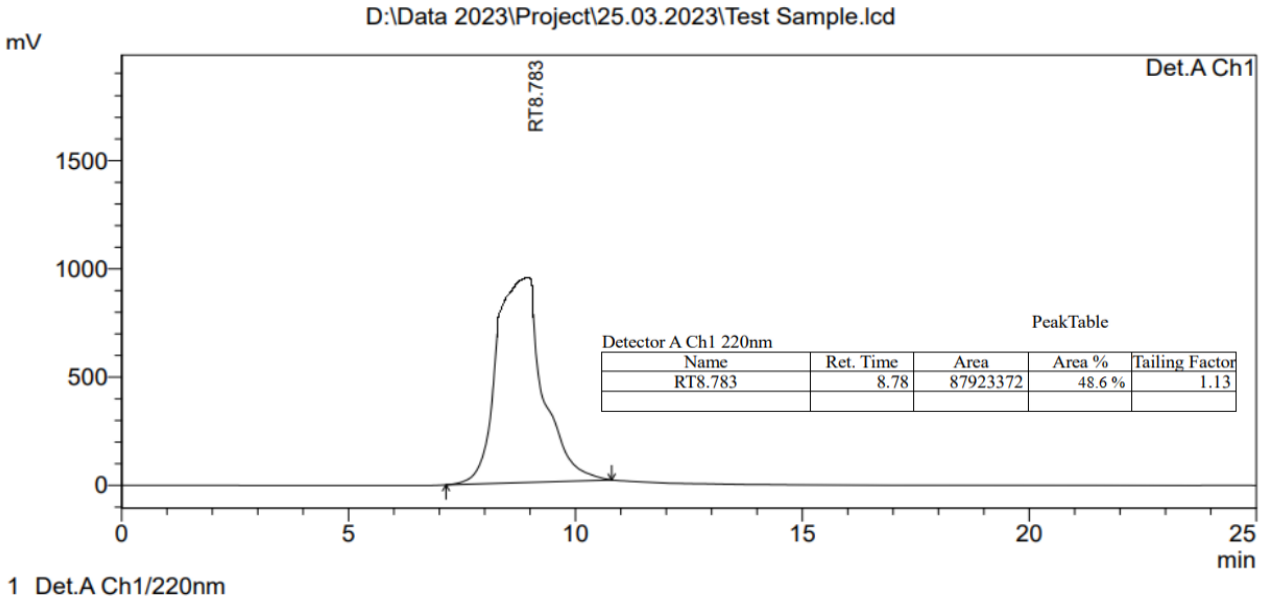

Figure No.5: The HPLC Chromatogram of Test Sample (enzymatic treated) assayed in ths study.

Discussion:

The present study focused on the isolation and characterization of betalactamase-producing bacteria from poultry faecal waste and their potential application in the degradation of penicillin, a widely used antibiotic. The findings of this study are significant considering the increasing concerns over antibiotic resistance and the environmental impact of antibiotic residues in waste disposal sites. The discussion will delve into the implications of the results, compare them with recent literature, and highlight the potential benefits of using betalactamase-producing isolates as a bioremediation strategy.

The isolation of betalactamase-producing bacteria from poultry faecal waste is an important step towards understanding the prevalence of antibiotic resistance in this environment. The positive identification of betalactamase-producing isolates (Isolate 3 and Isolate 4) confirms the presence of enzymes capable of hydrolyzing penicillin, which contributes to the degradation of the antibiotic. The results are consistent with previous studies that have reported the existence of betalactamase-producing bacteria in various environmental settings (Martinez, 2020) (Wellington et al., 2013). This highlights the potential role of poultry faecal waste as a reservoir for antibiotic-resistant bacteria, which can further disseminate resistance genes to other environments (Roca et al., 2021, Tiedje.,2022).

The enzyme assay results showed that Isolate 10 exhibited the highest concentration of betalactamase, indicating its potential efficacy in degrading penicillin. The significant enzymatic activity of Isolate 10 aligns with the findings of Tong et al. (2022), who reported a positive correlation between enzymatic activity and antibiotic degradation. Additionally, the isolation of betalactamase-producing bacteria from poultry faecal waste suggests that these isolates might play a crucial role in mitigating the antibiotic burden in the environment.

Comparing the results of the degradation of penicillin in faecal samples with and without the application of betalactamase-producing isolates, the data revealed a substantial reduction in antibiotic residues in the treated samples. The HPLC analysis demonstrated a degradation rate of 48.6% in the treated samples, which is promising and supports the potential applicability of betalactamase-producing isolates as a bioremediation tool. Similar studies have shown the effectiveness of enzymatic degradation in reducing antibiotic residues in the environment (Hong et al., 2018). This implies that harnessing the enzymatic potential of betalactamase-producing bacteria can be an environmentally friendly approach to tackle antibiotic pollution.

The application of betalactamase-producing isolates for antibiotic degradation has several advantages. Firstly, this approach addresses the concern of antibiotic resistance by directly targeting and degrading the antibiotics. Unlike conventional treatments that might inadvertently promote antibiotic resistance by selecting for resistant bacteria, enzymatic degradation offers a more targeted and specific approach. Secondly, the use of betalactamase-producing isolates as a bioremediation tool is relatively cost-effective and can be implemented at the site of waste disposal, reducing the need for expensive treatment processes (Wang et al., 2019, Ott et al.,2021). Furthermore, it provides an eco-friendly solution that does not involve the use of harmful chemicals or energy-intensive methods.

However, it is essential to acknowledge some limitations of this study. The degradation efficiency observed in the current research might vary under different environmental conditions, and the potential impact of the treated faecal waste on the surrounding ecosystem needs further investigation. Additionally, the study focused on penicillin degradation, but other antibiotics and their residues present in poultry waste should also be considered for a comprehensive assessment of antibiotic pollution.

In conclusion, the findings of this study provide valuable insights into the presence of betalactamase-producing bacteria in poultry faecal waste and their potential application in antibiotic degradation. The use of betalactamase-producing isolates as a bioremediation strategy presents a promising approach to mitigate antibiotic pollution in the environment. The study aligns with the growing body of research addressing antibiotic resistance and the environmental consequences of antibiotic residues. Further research and field-scale trials are necessary to explore the practical implementation and long-term efficacy of this enzymatic degradation approach.

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Conflict of Interest:
The authors declare no conflicts of interest related to this research.

Ethical Approval:
Ethical approval for this study was not required

Data Availability:
The datasets generated and analyzed during the current study are available from the corresponding author upon reasonable request.

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