INFECTION-REDUCTION RATES IN AMPICILLIN-RESISTANT *Escherichia coli*-INFECTED CHICKS TREATED WITH A FORMULATION OF AMPICILLIN-MEDICINAL SYNTHETIC ALUMINUM MAGNESIUM SILICATE (NANO-STABILIZING AGENT).

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ABSTRACT

Antimicrobial resistance (AMR) is a global concern that is threatening livestock production. In poultry, indiscriminate use of antibiotics has led to resistant strains of Escherichia coli, impairing treatment outcomes. Aluminum-magnesium silicate (AMS) is an approved nano-stabilizing agent. Stabilizing antimicrobials prolongs time they remain at high bioavailability. Nanoparticles also improve delivery of medicines to effect-targets. Prolonging medicines` time of high bioavailability and enhancing their delivery improve efficacy. Again, antioxidants improve hosts` immune functions. Enhanced efficacy allows lower dosages to achieve therapeutic effects, thereby minimizing immune suppression from medicines. Improving efficacy of medicines and immune response of patients could overcome AMR. Chicks were screened and assigned to five groups. Four were orally infected with 0.1 mL of E. coli (15×10□ CFU/mL), and three of these received ampicillin-MSAMS treatment for five days. One group received additional vitamin C support. Bile samples were cultured for colony-forming units (CFU), and reproductive tissues were examined histologically. Mean colony-forming units (CFU) of an ampicillin resistant E. coli infection in bile of chicks was 37.52 ± 3.13 per ml. At its 100 % dosage, Ampicillin reduced ($P \leq 0.05$) the CFU to 9.80 ± 2.83 (74 %-reduction) while at 75 %-dosage, stabilizing the medicine with Medicinal synthetic AMS reduced ($P \leq 0.01$) the CFU to 6.07 ± 0.30 (83 %-reduction,). Supporting the 75 % ampicillin-MSAMS dosage with Vitamin C reduced the CFU to 4.20 ± 0.15 (89 %-reduction). The results suggest that stabilizing Ampicillin`s 75 %-dosage with MSAMS and supporting the treatment with antioxidants restore its efficacy against AMR. We recommend incorporating nano-stabilizers and immune support in antimicrobial therapies to reduce dosage, enhance outcomes, and mitigate resistance development in poultry.

Keywords: Resistant *Escherichia coli*; Nano-stabilizing agents; Aluminum-magnesium silicate; Ampicillin; Vitamin C.

1.0 INTRODUCTION

Antimicrobial resistance R) is an escalating global health concern, threatening both human and animal populations. They cause sicknesses and mortalities in humans and animals while destroying economies (impairing livestock productivity and rendering drug-patents useless) (WHO, 2024). Recent evidence indicates that overuse and misuse of antibiotics in agriculture and livestock production continue to accelerate spread of antimicrobial-resistant pathogens through environmental contamination and genetic dissemination (Cella *et al*., 2023; Kelbrick *et al*., 2023; Han *et al*., 2023). Poultry, as one of the most widely farmed species, globally, has emerged as a significant reservoir of antimicrobial-resistant bacteria, including *Escherichia coli* (Ortega-Paredes *et al*., 2020; Ahmed *et al*., 2025; Islam *et al*., 2023). Emergence of resistant *E. coli* strains in poultry presents threats of therapeutic failure and impaired productivity (Suswati *et al*., 2025).

E. coli in poultry, specifically avian pathogenic *E. coli* (APEC), is the causative agent of colibacillosis, a disease that manifests in chickens through a variety of clinical signs, such as acute fatal septicaemia, fibrinous lesions, salpingitis and sporadic mortality (Raji *et al*., 2007; Messai *et al*., 2013; Nolan *et al*., 2013). The disease affects not only poultry health and productivity but also has severe economic implications for the poultry industry due to reduced reproductive performance, decreased egg production, and increased mortality.

Frequent administration of antibiotics such as ampicillin in poultry farming has led to emergence of ampicillin-resistant strains of *E. coli*. This resistance complicates treatment protocols, leading to poorer outcomes and reduced economic viability in poultry operations. As a result, there is an urgent need to explore alternastive treatment options that can mitigate effects of resistant *E. coli* strains.

In response to this challenge, recent research has focused on the use of medicinal formulations such as aluminum-magnesium silicate compounds. These compounds enhance efficacy of antibiotics like ampicillin by improving drug delivery and binding bacterial toxins. Investigating the infection-reduction rates in ampicillin-resistant *E. coli*-infected chicks treated with a formulation of ampicillin-medicinal synthetic aluminum magnesium silicate (nano-stabilizing agent) could offer valuable insights into improving both health outcomes and economic sustainability in poultry farming. This study aims to evaluate therapeutic potentials of this formulation in managing antimicrobial-resistant infections in poultry.

Ampicillin trihydrate (AT), a semi-synthetic β-lactam antibiotic, has long been used in poultry farming as a broad-spectrum treatment for bacterial infections, particularly those caused by Gram-negative organisms such as *E. coli*. Its mechanism of action involves inhibiting bacterial cell wall synthesis, leading to cell lysis and death. However, the frequent and often indiscriminate use of AT in livestock has contributed to the development of resistance in bacterial populations, particularly in *E. coli* strains commonly found in poultry (Monaghan *et al*., 2021;Veloo *et al*., 2025; Nyolimati *et al*., 2025).

In poultry, antibiotics like AT are widely employed not only for therapeutic purposes but also as growth promoters and prophylactics, further exacerbating the resistance problem. These practices, particularly in intensive poultry production systems, have created an environment where antimicrobial resistance can rapidly emerge and spread within the flock, posing significant challenges to animal health management and food safety.

Since the introduction of ampicillin trihydrate, the resistance rates among bacterial pathogens have escalated sharply. Studies have shown that approximately 70 % of *E. coli* isolates from broiler chickens in the European Union are now resistant to ampicillin (Burow *et al*., 2020; European Food Safety Authority, 2025). This resistance is largely attributed to the overuse of antibiotics in poultry, often without adequate veterinary oversight, which allows resistant bacteria to thrive and proliferate.

Globally, the problem of antimicrobial resistance (AMR) is not confined to specific regions. For instance, a meta-analysis in Ethiopia reported that *E. coli* showed the highest resistance to ampicillin (83.81 %) and amoxicillin (75.79 %) among tested antibiotics (Tuem *et al*., 2018). Similarly, a World Health Organization (WHO) report analyzing data from 87 countries in 2020 revealed a global median AMR level of 42 % for *E. coli*, underscoring the widespread prevalence of resistance (WHO, 2022). This alarming trend is further supported by recent reviews that revealed diverse mechanisms by which *E. coli* pathotypes acquire and propagate antibiotic resistance (Nasrollahian, Graham, & Halaji, 2024). In regions such as sub-Saharan Africa, where veterinary oversight and regulation of antibiotic use are often limited, resistance rates may be even higher. A study conducted in Nigeria revealed widespread resistance of *E. coli* to ampicillin trihydrate, followed by trimethoprim-sulphamethoxazole, streptomycin, cephalexin, and gentamicin (Enabulele *et al*., 2010).

The emergence of ampicillin-resistant *E. coli* presents a multifaceted challenge, not only in terms of animal health and productivity but also in the broader context of public health. The potential transmission of resistant bacteria from poultry to humans through the food chain or environmental contamination underscores the urgency of finding effective treatment alternatives.

Medicinal synthetic aluminum-magnesium silicate® (MSAMS®) is an innovative formulation derived from two pharmacologically approved compounds, aluminum silicate (AS) and magnesium silicate (MS). Both of these are medicines approved by the United States Pharmacopeia (2020) and the European Pharmacopoeia (2020) and also as stabilizing agents for drug formulations. The unique composition of MSAMS, with molecules structured as aluminum-magnesium silicate { Al□ Mg□ (SiO□)□}, consist of *nanoparticles*, measuring approximately 0.96 nm in thickness. These *nanoparticles* significantly enhance drug delivery by facilitating more precise transport of active ingredients to target cells (Ezeibe *et al*., 2021; Song *et al*., 2024).

Dextrose monohydrate, a simple sugar, was incorporated in the MSAMS to assist in conveying the charged particles across mucosal barriers, ensuring that they reach the bloodstream by aided transport mechanisms (Murray, 2000). This combination improves bioavailability and ensures more efficient systemic distribution of the drug. In addition, MSAMS serves as a stabilizing agent for the active pharmaceutical ingredients (APIs), helping to protect them from rapid degradation thereby, extending their therapeutic effectiveness (Walwante *et al*., 2025). This stabilization prolongs duration of action of antibiotics, enhancing their efficacy against resistant bacterial strains.

The overuse and misuse of antimicrobial agents in livestock can lead to immune suppression, weakening animals' defense mechanisms and research has shown that antioxidants such as vitamins A, C, and E can bolster the immune response by neutralizing oxidative stress and protecting cells from free radical-induced damage (Eske, 2019; Engwa *et al*., 2022; Pisoschi *et al*., 2022; Chandimali *et al*., 2025). They play a critical role in maintaining cellular integrity and improving overall health of poultry, which is especially important when dealing with infections caused by resistant pathogens.

Integrating antioxidants into the treatment of ampicillin-resistant *E. coli*-infected chicks can mitigate the oxidative stress induced by infection and antibiotic treatment, offering immune support to the treatments.

2.0 MATERIALS AND METHODS

A total of **80 two-week-old pullets** that tested negative for *Escherichia coli* by cloacal swab screening were used for the study. The birds were orally infected (per os) with **0.1 mL of an Ampicillin-resistant *E. coli* inoculum**, containing approximately **1.5×10^7 CFU/mL**, following the method of **Murray (*2007*)**.

The chicks were **randomly allocated** into four treatment groups of **20 birds each**, using a **completely randomized design (CRD)**. Each group was further subdivided into **2 replicates of 10 birds**. The treatment groups were as follows:

1. **Infected – Treated with AT-MSAMS at 75% dosage** (7.5 mg/kg body weight of ampicillin trihydrate stabilized with MSAMS®),
2. **Infected – Treated with AT-MSAMS + antioxidants at 75% dosage** (7.5 mg/kg body weight of AT stabilized with MSAMS®, supported with Vitamin C),
3. **Infected – Treated with AT at 100% dosage** (10 mg/kg body weight),
4. **Infected – Untreated control.**

All treatments were administered **via drinking water for five (5) consecutive days**. Birds were monitored for clinical signs, and two birds per group were **sacrificed at four time points**: before treatment, day-3 of treatment, day-1 post-treatment (PT), and day-4 PT.

Bile samples were collected from the sacrificed birds to determine infection load. Serial dilutions were prepared by mixing 0.1 mL of bile with 0.9 mL of normal saline (1:10), followed by a second dilution to 1:100. From the 1:100 dilution, **0.05 mL** was plated on MacConkey agar and incubated at **37°C for 24 hours**. Colony-forming units (CFUs) were counted and expressed using the formula:

CFU/mL = (X/5) × 10,000,
where **X** is the number of colonies observed.

Infection-reduction rates were calculated using the formula:

Infection Reduction (%) = [(Mean CFU before treatment – CFU on assessment day) ÷ Mean CFU before treatment] × 100.
Positive values indicated reduction, while negative values indicated increased bacterial load.

Data Analysis:
All numerical data were analyzed using **IBM SPSS Statistics**, **version 22.0** (IBM Corp., Armonk, NY, USA; released **2013**). One-way **analysis of variance (ANOVA)** was used to compare group means. Where significant differences were detected, **Duncan's Multiple Range Test (DMRT)** was applied to separate the means. Statistical significance was accepted at **$P \leq 0.05$** and **$P \leq 0.01$**, as appropriate.

3.0 RESULTS AND DISCUSSION

3.1 Bacterial loads of Ampicillin-resistant *Escherichia coli* infected chicks treated with Ampicillin-Medicinal synthetic Aluminum-magnesium silicate® formulation (MSAMS®) and Vitamin C.

Table 1: Bacterial loads of Ampicillin-resistant *Escherichia coli* infected chicks treated with Ampicillin-Medicinal synthetic Aluminum-magnesium silicate® formulation (MSAMS®) and Vitamin C.

Treatment groups	CFU (Mean ± Standard error)
AMP-MSAMS (75% AMP- dosage)	6.07 ± 0.30^{bc}
AMP-MSAMS (75% AMP-dosage) + AOX	4.20 ± 0.15^{c}
AMP (100% dosage)	9.80 ± 2.83^{b}
Untreated (Control)	37.52 ± 3.13^{a}

Note*: Values are presented as mean ± S.E (Standard error of mean). Different superscript letters show significant ($P \leq 0.05$) differences.*

Mean colony-forming units (CFU) per ml of bile in the untreated group (Control) of ampicillin trihydrate (AT)-resistant *E. coli*-infected chicks was 37.52 ± 3.13. For the group treated with 100 % ampicillin-dosage (at a rate of 0.01 g/litre of drinking water), the mean bacterial count was reduced to 9.80 ± 2.83 CFU/ml, representing a 74 % infection reduction.

The group treated with 75 % of the recommended ampicillin-dosage (0.006 g/litre), in combination with the MSAMS, recorded a mean bacterial count of 6.07 ± 0.30 CFU/ml, (83 % infection reduction).

The group treated with 75 % ampicillin-MSAMS formulation plus Vitamin C (10 mg per 25 kg feed : National Research Council, Washington DC, 1994) achieved a mean bacterial count of 4.20 ± 0.15 CFU/ml (89 % infection reduction).

E. coli CFUs per ml of bile of the treated chicks are as presented on Table 1, while their infection-reduction rates are as in Figure 1.

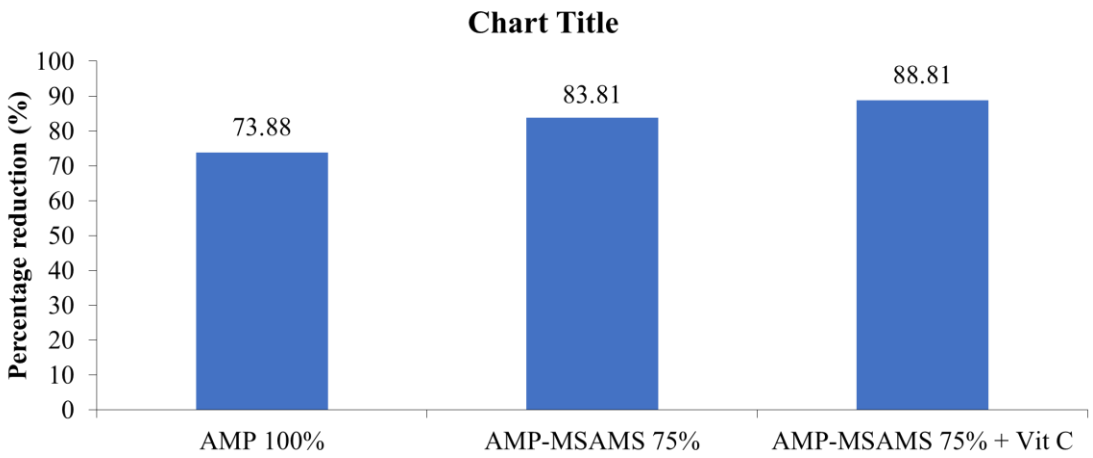

Fig 1: Reduction rates (%) in colony forming units of Ampicillin-resistant *Escherichia coli* in chicks treated with Ampicillin-Medicinal synthetic Aluminum-magnesium silicate formulation and Antioxidants.

Discussion

Results of this study suggest that formulating ampicillin trihydrate (AT) with Medicinal Synthetic Aluminum-Magnesium Silicate® (MSAMS®) restores its efficacy against resistant *E. coli* infections. In the untreated control group, the mean bacterial count in bile was 37.52 ± 3.13 CFU/mL, reflecting the full burden of infection. In contrast, all treated groups had significant reductions in bacterial load, confirming antibacterial activity of ampicillin.

Treatment with 100 % of the recommended ampicillin dosage reduced bacterial count to 9.80 ± 2.83 CFU/mL, representing only 74 % reduction while ≥ 80 % reduction is needed for clinical recovery (DeJong *et al*., 2012), thereby confirming the *E. coli* isolate as resistant to ampicillin.

When Ampicillin was stabilized with MSAMS® and administered at 75 % of its dosage, the bacterial count further dropped to 6.07 ± 0.30 CFU/ml (≥ 83 %-reduction). This improvement suggests that MSAMS potentiated the antimicrobial activity of ampicillin, enabling the reduced dosage to achieve curative efficacy against the resistant infection.

MSAMS®, a pharmaceutical stabilizing agent, may have extended duration at which ampicillin remained at high bioavailability. Being of *nanoparticles*, MSAMS may also have enhanced drug delivery across physiological barriers, improving access to infection sites. The synergy of prolonged bioavailability and enhanced drug delivery likely contributed to the observed therapeutic success.

The results agree with results of earlier studies that showed that MSAMS® improves efficacies of antimicrobials (Ezeibe, 2023).

Further enhancement was observed when Vitamin C was added to the 75 % MSAMS-stabilized ampicillin formulation. The mean bacterial count dropped to 4.20 ± 0.15 CFU/mL, indicating an 89 % reduction. This finding agrees with previous reports that Vitamin C, through its antioxidant properties, enhanced immune responses and improved efficacies of MSAMS-stabilized streptomycin against resistant *Salmonella typhimurium* (Ezeibe, 2023).

The triple actions: MSAMS stabilization; Reduced ampicillin dosage and Vitamin C support restored efficacy to ampicillin against the resistant infection. It not only restored antibiotic effectiveness but did so at a lower dosage, minimizing side effects of medicines to avoid immune suppression. The strengthened immune response further contributed to the clearance of infection, suggesting a dual mechanism involving both improved pharmacodynamics and host immunity.

Results of trials of antimicrobials-MSAMS (Nano-stabilizing agent) formulations and antioxidants on both sensitive and resistant infections of different bacteria, protozoa, and helminths suggest that formulating antimicrobials with Nano-stabilizing agents and supporting their treatments with antioxidants may be an effective treatment strategy for prevention and treatment of AMR. (Ezeibe *et al.,* 2011; Ezeibe *et al.,* 2023).

Combining certain medicinal formulations with regular antibiotics can enhance antibiotic efficacy in animals. These combinations may allow for reduced antibiotic dosages and shorter treatment durations to minimize antimicrobial residues in human foods of animal origin (Ezeibe *et al*., 2023). This is of significant public health importance, as drug residues in food animals are recognized pathways for transmission of resistant bacteria to humans.

This strategy aligns with global efforts to mitigate antibiotic overuse in livestock in order to curb development of antimicrobial resistance through responsible antibiotic use and the integration of alternative measures to safeguard animal health and productivity (Wickramasuriya *et al*., 2024)

4.0 CONCLUSION AND RECOMMENDATIOS

4.1 Conclusion

Stabilizing antimicrobials with Medicinal Synthetic Aluminum Magnesium Silicate (MSAMS®) and supporting them with antioxidants such as Vitamin C significantly restores the efficacy of conventional drugs against resistant infections, even at reduced dosages. This strategy enhances drug bioavailability, improves delivery to target sites, and strengthens host immune response, resulting in improved therapeutic outcomes. The findings of this study demonstrate that a 75% dosage of ampicillin, when stabilized with MSAMS® and supported with Vitamin C, achieved greater bacterial clearance than the full dosage of the antibiotic alone.

This approach offers a cost-effective and sustainable solution to the global challenge of antimicrobial resistance (AMR), with potential for wide application in both human and veterinary medicine. It aligns with One Health principles by reducing drug residues in animal products and the environment, and limiting the emergence of multidrug-resistant pathogens.

4.2 Recommendations:

1. **Incorporation of MSAMS® in Antimicrobial Formulations:** Pharmaceutical industries and veterinary drug manufacturers should consider formulating existing antibiotics with MSAMS® to enhance efficacy, especially for resistant infections.
2. **Co-administration with Antioxidants:** Clinicians and veterinary professionals should support antimicrobial therapy with immune-boosting antioxidants such as Vitamin C to improve recovery and reduce immune suppression.
3. **Reduction in Antibiotic Dosage:** Stabilizing agents like MSAMS® can allow for the use of reduced dosages of antibiotics without compromising efficacy. This will reduce drug costs and minimize side effects or toxicities.
4. **Policy and Regulation:** Regulatory bodies should evaluate and approve nano-stabilizer-supported therapies for use in livestock and poultry industries as part of integrated AMR mitigation strategies.
5. **Further Research:** More in vivo and clinical studies are recommended to explore the application of MSAMS® in combination with various antibiotics across different animal species and infection types.
6. **Awareness and Training:** Veterinary practitioners, animal health workers, and farmers should be trained on the benefits and usage of nano-stabilized drug therapies and antioxidant support in combating AMR.

Declarations

Ethics approval and consent to participate

All animal procedures were conducted in accordance with the ethical standards of the Michael Okpara University of Agriculture, Umudike, Nigeria. The study protocol was reviewed and approved by the the Ethical Committee, College of Veterinary Medicine, MOUAU. Appropriate measures were taken to minimize animal discomfort and ensure humane treatment throughout the study.

Availability of data and materials

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

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

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Consent to Publish declaration:

All images included in this manuscript are original or have been used with the necessary permissions. The authors affirm that appropriate consent was obtained for publication where required.

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