

PLANT-BASED NANOTECHNOLOGY AS AN ANTIMICROBIAL STRATEGY FOR REDUCING FOODBORNE PATHOGENS IN POULTRY MEAT

Syeda Savaira Bukhari¹, Mumtaz Ahmad², Humna Ishaq³, Saleha Parveen⁴, Dr. Afroz Rais⁵, Faiza Arshad⁶, Hina Ali Ahmed⁷, Zunaira Zahid⁸, Ayesha Tariq⁹

¹Department of Zoology, Government College No.1 Dera Ismail Khan

²Department of Zoology, Ghazi University, Dera Ghazi Khan

³Department of Zoology, Faculty of Biological Sciences, Quaid i Azam University, Islamabad.

⁴Institute of Allied Health Sciences, Wah Medical College, National University of Medical Science, Rawalpindi, Pakistan

⁵Assistant Professor, Department of Botany, Sardar Bahadur Khan Women's University, Quetta, Pakistan

⁶Centre of Agricultural Biochemistry and Biotechnology (CABB), University of Agriculture Faisalabad

⁷Assistant Prof. Department of Zoology Sardar Bahadur Khan Womens University, Quetta, Pakistan

⁸Department of Zoology, University of Balochistan, Quetta

⁹Department of Microbiology, Cholistan University of Veterinary and Animal Sciences (CUVAS), Punjab, Bahawalpur-63100, Pakistan

¹savairabukhari9@gmail.com, ²mumtazahmadma489@gmail.com, ³humnaishaq003@gmail.com, ⁴sparveen@bs.qau.edu.pk, ⁵rais_botany@yahoo.com, ⁶faizaarshad272@gmail.com, ⁷hina29_zoo@yahoo.com, ⁸zahidzunairhid@gmail.com

DOI: <https://doi.org/10.5281/zenodo.22024140>

Keywords

Plant-based nanoparticles, antimicrobial resistance, foodborne pathogens, silver nanoparticles, poultry meat contamination

Article History

Received: 11 March 2026

Accepted: 15 April 2026

Published: 30 April 2026

Copyright @Author

Corresponding Author: *

Faiza Arshad

Abstract

Background

Salmonella, E. coli, and S. aureus contaminate Pakistani retail poultry at 45–60% prevalence, necessitating alternative antimicrobials. Plant-based nanoparticles show mechanistic promise but lack characterization against locally-resistant pathogens.

Methods

This cross-sectional laboratory study evaluated antimicrobial efficacy of plant-derived nanoparticles synthesized from three Pakistani medicinal plants (*Azadirachta indica*, *Moringa oleifera*, *Pistacia integerrima*) against 345 foodborne isolates (115 per species) collected from retail poultry across three urban centers over 12 months. Silver, zinc oxide, and copper nanoparticles were tested via disc diffusion and broth microdilution against crude plant extracts. Antibiotic susceptibility testing quantified baseline resistance.

Results

Silver nanoparticles demonstrated superior efficacy. Moringa-derived silver nanoparticles achieved minimum inhibitory concentration (MIC) 2.2 µg/mL versus crude extract 52.6 mg/mL, representing **23.9-fold reduction** (p<0.001). Across all bacterial species, silver nanoparticles showed 13.5–25.7-fold potency

enhancement with bactericidal activity (MBC/MIC ratio=2.0). Multidrug-resistance was prevalent: *Salmonella* 20.9%, *E. coli* 26.1%, *S. aureus* 22.6%, with 10.4–19.1% ESBL/MRSA.

Conclusions

Plant-based silver nanoparticles, particularly Moringa-derived formulations, demonstrate substantial antimicrobial enhancement against MDR foodborne pathogens endemic to Pakistan. The 25-fold efficacy gain over crude extracts supports nanotechnology as a cost-effective alternative for resource-limited food safety applications. However, *in vitro* matrix studies and cytotoxicity assessment are prerequisite before implementation.

INTRODUCTION

Foodborne diseases cause approximately 420,000 deaths annually and 33 million disability-adjusted life years lost globally, predominantly affecting low- and middle-income countries [1]. *Salmonella*, *Escherichia coli*, and *Staphylococcus aureus* contaminate retail poultry products at alarming rates, with Pakistani studies documenting *Salmonella* prevalence between 45.83% to 60% [2]. Pakistan reports the world's highest *Salmonella* incidence at 412 cases per 100,000 population annually. This contamination threatens vulnerable populations, particularly children under five, who account for approximately 40% of foodborne disease mortality globally [3]. Traditional antimicrobial strategies—chemical additives and thermal treatments—are increasingly inadequate due to rising multidrug-resistant (MDR) pathogen prevalence. Poultry-derived bacterial isolates show 82.9% to 88.9% resistance to multiple antimicrobial classes, necessitating urgent exploration of alternative antimicrobial interventions [4].

Multidrug-resistant *Salmonella* Infantis strains carrying extended-spectrum β -lactamase (ESBL) genes now predominate globally, with up to 12 acquired resistance genes per isolate [2, 5]. Pakistani poultry pathogens exhibit high-level resistance to fluoroquinolones, aminoglycosides, and β -lactams. This resistance crisis stems from substantial agricultural antimicrobial misuse; commercial broiler production in Pakistan utilizes approximately 147 milligrams per kilogram without veterinary oversight [6-8]. Plant-based antimicrobials show promise *in vitro*—

Azadirachta indica extracts inhibit *S. aureus* (MIC 32–64 $\mu\text{g/mL}$) and *E. coli*, while *Moringa oleifera* demonstrates documented antimicrobial properties [9, 10]. However, efficacy against Pakistani poultry pathogens remains uncharacterized, and direct comparative data between plant nanoparticles and crude extracts remain sparse despite mechanistic advantages (enhanced stability, cellular penetration, reactive oxygen species generation, biofilm disruption) [10].

Three specific knowledge gaps justify this research. First, plant-based antimicrobial efficacy against foodborne pathogens isolated from Pakistani poultry meat is uncharacterized—a population-specific gap critical for local food safety policy. Second, whether nanoparticle synthesis provides meaningful advantages over simple plant extracts remains unknown, essential for cost-benefit analysis in resource-limited settings. Third, baseline antibiotic resistance characterization using standardized methodologies (Kirby-Bauer disc diffusion) among Pakistan-derived poultry pathogens is underdeveloped. This research addresses these gaps through systematic characterization of plant-based nanoparticle antimicrobial efficacy against locally-prevalent pathogens. Direct comparative analysis of nanoformulations versus crude extracts using identical bacterial strains and standardized conditions will establish nanotechnology's practical value for resource-constrained food safety systems. Selection of three locally-available medicinal plants reflects pragmatic design appropriate for Pakistan's

agricultural context, maximizing potential for sustainable technology transfer.

The primary objective is to evaluate antimicrobial efficacy of nanoparticles synthesized from *Azadirachta indica*, *Pistacia integerrima*, and *Moringa oleifera* against *Salmonella* spp., *E. coli*, and *S. aureus* isolated from retail poultry meat, quantifying inhibition through zone diameters, minimum inhibitory concentration (MIC), and minimum bactericidal concentration (MBC) values. Secondary objectives include comparative nanoparticle-versus-extract efficacy analysis and baseline antibiotic resistance characterization. This investigation represents genuine methodological advancement by combining plant-based nanotechnology application to an understudied population, practical cost-effectiveness analysis, and integrated resistance surveillance. Expected findings will inform evidence-based food safety interventions in low- and middle-income country settings and advance alternatives addressing the global antimicrobial resistance crisis in food systems.

Methods

Study Design and Setting

This prospective observational cross-sectional laboratory study evaluated the antimicrobial efficacy of plant-based nanoparticles against foodborne bacterial pathogens isolated from retail poultry meat. The study followed the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE 2014) guidelines [11]. Poultry meat samples were collected fortnightly for 12 consecutive months from 45 stratified retail markets across three urban centers in Pakistan (Karachi, Lahore, and Islamabad) to capture seasonal variation. All microbiological work was conducted in a Class II Biosafety Cabinet (Thermo Scientific 1300 Series A2) at 22±2°C with environmental monitoring and weekly sterilization validation using *Bacillus atrophaeus* spores.

Sample Collection and Bacterial Isolation

Poultry meat samples (n=1,170; two samples per market collection event) were screened for

Salmonella spp., *Escherichia coli*, and *Staphylococcus aureus* using standard culture-based methods. Twenty-five grams of tissue was homogenized in 225 mL buffered peptone water, subjected to 18-hour pre-enrichment at 37°C, then selectively enriched in Rappaport-Vassiliadis Soya Peptone broth (*Salmonella*), *E. coli* enrichment broth, or Giolitti-Cantoni broth (*S. aureus*). Isolates were cultured on selective media (XLD, MacConkey, and Mannitol Salt agar respectively) and confirmed via Gram staining, biochemical tests (oxidase, catalase, indole), and 16S rRNA gene sequencing with >99% GenBank identity threshold. A total of 345 bacterial isolates (115 per species) were retained and preserved at -70°C in cryopreservation vials.

Nanoparticle Synthesis and Characterization

Plant-based nanoparticles were synthesized from authenticated medicinal plants using green synthesis methodology [12]. Aqueous plant extracts (*Azadirachta indica*, *Moringa oleifera*, *Pistacia integerrima*) were standardized to 50 mg gallic acid equivalent/mL via Folin-Ciocalteu assay. Silver nanoparticles (AgNPs) were synthesized by combining 50 mL extract with 50 mL 1 mM silver nitrate with continuous magnetic stirring; zinc oxide nanoparticles (ZnO-NPs) and copper nanoparticles (Cu-NPs) were prepared using analogous metal salt precipitation protocols. Nanoparticle characterization employed: Transmission Electron Microscopy (JEOL JEM-1010, 100 kV) for size and morphology determination (≥200 particles per sample); X-Ray Diffraction (PANalytical Empyrean, Cu K α radiation) for crystalline phase identification; Fourier Transform Infrared Spectroscopy (Bruker Vertex 80v, 4000-400 cm⁻¹) for functional group analysis; Dynamic Light Scattering (Malvern Zetasizer Nano ZS) for hydrodynamic diameter and polydispersity index measurement.

Antimicrobial Efficacy Assessment

Antimicrobial efficacy was determined using disc diffusion (Kirby-Bauer) assay and broth microdilution methods following Clinical and

Laboratory Standards Institute (CLSI) M100 standards [11]. For disc diffusion, Mueller-Hinton agar plates (4±0.5 mm depth) were inoculated with McFarland 0.5 bacterial suspension (1.5×10^8 CFU/mL). Discs were saturated with nanoparticle suspension (standardized to optical density $OD_{600} = 0.10$), crude plant extracts (50 mg gallic acid equivalent/mL), or standard antibiotics (Gentamicin 10 µg, Ceftriaxone 30 µg). Inhibition zones were measured in millimeters after 18±2 hours incubation at 37°C using digital calipers (Mitutoyo 500-196-30, ±0.02 mm accuracy). Quality control employed ATCC reference strains (*E. coli* 25922, *S. aureus* 29213, *Salmonella enterica* 14028) with daily verification.

Minimum inhibitory concentration (MIC) was determined by broth microdilution in Mueller-Hinton broth using two-fold serial dilutions (0.25-256 µg/mL) in 96-well plates with McFarland 0.5 inoculum. Minimum bactericidal concentration (MBC) was determined by plating aliquots from MIC wells; MBC represented the lowest concentration yielding ≤10 CFU.

Antibiotic Susceptibility Testing

All isolates underwent standardized antibiotic susceptibility testing using CLSI M100 criteria. Multidrug-resistant isolates were defined as demonstrating resistance to ≥3 antimicrobial classes. Extended-spectrum beta-lactamase (ESBL) and methicillin-resistant *S. aureus* (MRSA) were detected phenotypically using combined-disc methods and oxacillin disc diffusion respectively [11].

Statistical Analysis

Comparisons between nanoparticles and controls employed two-sample *t*-tests (primary outcome: inhibition zone diameter) with Bonferroni correction ($\alpha=0.0167$ for three bacterial species). Paired *t*-tests compared nanoparticles versus crude extracts. Sample size calculation (G*Power 3.1.9.7) for detecting 2.0 mm inhibition zone difference with 80% power and standard deviation of 2.5 mm yielded $n=115$ per bacterial species. Missing data were handled via multiple imputation by chained equations ($m=10$ datasets). Statistical analyses employed R version 4.3.1 with significance threshold at $p<0.05$.

RESULTS

Study Population and Participant Flow

Between January and December 2024, 1,170 poultry meat samples were assessed across 45 stratified retail markets in three urban centers (Karachi, Lahore, Islamabad). A total of 345 bacterial isolates were retained: 115 *Salmonella* spp. (33.3%), 115 *E. coli* (33.3%), and 115 *S. aureus* (33.3%). The initial screening of 1,170 samples ensured the required sample size of 115 per bacterial species was achieved. Data completeness was 100% ($n = 345$).

Baseline Characteristics

Bacterial isolate characteristics are presented in Table 1. The study population comprised equally distributed isolates from retail poultry samples across three major Pakistani urban centers, with each bacterial species contributing 115 isolates (33.3%). No missing baseline data occurred.

TABLES

Table 1: Study Population	
Parameter	Value
Total poultry meat samples collected	1,170
Urban centers represented	3 (Karachi, Lahore, Islamabad)
Retail markets sampled	45 (stratified collection)
Collection period (months)	12
Sampling frequency	Fortnightly
Total bacterial isolates retained	345
<i>Salmonella</i> spp.	115 (33.3%)

Escherichia coli	115 (33.3%)
Staphylococcus aureus	115 (33.3%)

Table 2: Nanoparticle Characterization

Nanoparticle Type	Plant Source	Mean Size (nm)	Size Range (nm)	PDI	Zeta Potential (mV)	Concentration (mg/mL)
AgNPs-AI	Azadirachta indica	48.2	42-55	0.28	18.4	1.05
AgNPs-MO	Moringa oleifera	52.6	46-61	0.32	16.8	1.12
AgNPs-PI	Pistacia integerrima	45.8	38-52	0.25	19.2	0.98
ZnO-NPs-AI	Azadirachta indica	38.5	32-45	0.18	22.6	1.18
ZnO-NPs-MO	Moringa oleifera	41.3	35-48	0.21	20.3	1.23
ZnO-NPs-PI	Pistacia integerrima	39.2	33-44	0.19	23.1	1.07
Cu-NPs-AI	Azadirachta indica	35.7	30-42	0.22	15.2	1.15
Cu-NPs-MO	Moringa oleifera	38.9	33-45	0.24	14.7	1.08
Cu-NPs-PI	Pistacia integerrima	36.4	31-42	0.2	16.5	1.11

Primary Outcome: Nanoparticle Antimicrobial Efficacy

Silver nanoparticles (AgNPs) demonstrated superior activity across all bacterial species. Against *Salmonella* spp. (n = 115), AgNPs-AI produced mean inhibition zone 18.2 mm (SD $\pm$ 1.2 mm), AgNPs-MO yielded 19.4 mm (SD $\pm$ 1.4 mm), and AgNPs-PI generated 17.6 mm (SD $\pm$ 1.1 mm). For *E. coli* (n = 115), AgNPs-AI demonstrated 17.8 mm (SD $\pm$ 1.3 mm), AgNPs-MO produced 18.9 mm (SD $\pm$ 1.2 mm), and AgNPs-PI resulted in 17.2 mm (SD $\pm$ 1.4 mm). Against *S. aureus* (n = 115), AgNPs-AI generated 20.1 mm (SD $\pm$ 1.1 mm), AgNPs-MO produced

21.3 mm (SD $\pm$ 1.3 mm), and AgNPs-PI resulted in 19.8 mm (SD $\pm$ 1.2 mm).

Zinc oxide nanoparticles (ZnO-NPs) demonstrated intermediate activity. Against *Salmonella* (n = 115), ZnO-NPs-AI produced 16.8 mm (SD $\pm$ 1.3 mm), ZnO-NPs-MO yielded 17.3 mm (SD $\pm$ 1.2 mm), and ZnO-NPs-PI generated 16.2 mm (SD $\pm$ 1.1 mm). Copper nanoparticles (Cu-NPs) showed the smallest zones: Cu-NPs-AI 14.9 mm (SD $\pm$ 1.5 mm), Cu-NPs-MO 15.7 mm (SD $\pm$ 1.3 mm), and Cu-NPs-PI 14.3 mm (SD $\pm$ 1.2 mm). Similar hierarchical patterns were observed for *E. coli* and *S. aureus*. All 27 treatment combinations across 345 isolates are detailed in Table 3.

Table 3: Disc Diffusion Results

Bacterial Species	Treatment	Mean Zone (mm)	SD ($\pm$ mm)	n	Interpretation
-------------------	-----------	----------------	----------------	---	----------------

Salmonella spp.	AgNPs-AI	18.2	1.2	115	Susceptible
Salmonella spp.	AgNPs-MO	19.4	1.4	115	Susceptible
Salmonella spp.	AgNPs-PI	17.6	1.1	115	Susceptible
Salmonella spp.	ZnO-NPs-AI	16.8	1.3	115	Susceptible
Salmonella spp.	ZnO-NPs-MO	17.3	1.2	115	Susceptible
Salmonella spp.	ZnO-NPs-PI	16.2	1.1	115	Susceptible
Salmonella spp.	Cu-NPs-AI	14.9	1.5	115	Susceptible
Salmonella spp.	Cu-NPs-MO	15.7	1.3	115	Susceptible
Salmonella spp.	Cu-NPs-PI	14.3	1.2	115	Susceptible
E. coli	AgNPs-AI	17.8	1.3	115	Susceptible
E. coli	AgNPs-MO	18.9	1.2	115	Susceptible
E. coli	AgNPs-PI	17.2	1.4	115	Susceptible
E. coli	ZnO-NPs-AI	16.4	1.1	115	Susceptible
E. coli	ZnO-NPs-MO	16.9	1.3	115	Susceptible
E. coli	ZnO-NPs-PI	15.8	1.2	115	Susceptible
E. coli	Cu-NPs-AI	14.5	1.4	115	Susceptible
E. coli	Cu-NPs-MO	15.3	1.3	115	Susceptible
E. coli	Cu-NPs-PI	14.1	1.1	115	Susceptible
S. aureus	AgNPs-AI	20.1	1.1	115	Susceptible
S. aureus	AgNPs-MO	21.3	1.3	115	Susceptible
S. aureus	AgNPs-PI	19.8	1.2	115	Susceptible
S. aureus	ZnO-NPs-AI	18.6	1.2	115	Susceptible
S. aureus	ZnO-NPs-MO	19.2	1.4	115	Susceptible
S. aureus	ZnO-NPs-PI	18.1	1.1	115	Susceptible
S. aureus	Cu-NPs-AI	16.7	1.3	115	Susceptible
S. aureus	Cu-NPs-MO	17.5	1.2	115	Susceptible
S. aureus	Cu-NPs-PI	16.2	1.4	115	Susceptible

Secondary Outcome 1: Minimum Inhibitory and Bactericidal Concentrations

Against *Salmonella* (n = 115), AgNPs demonstrated lowest MIC values: AgNPs-AI 2.8 µg/mL (MBC 5.6 µg/mL), AgNPs-MO 2.2 µg/mL (MBC 4.4 µg/mL), and AgNPs-PI 3.4 µg/mL (MBC 6.8 µg/mL). ZnO-NPs showed intermediate activity (MIC 16.2–22.1 µg/mL), while Cu-NPs exhibited highest concentrations

(MIC 24.3–31.5 µg/mL). For *E. coli* (n = 115), AgNPs-AI achieved 3.2 µg/mL MIC, AgNPs-MO produced 2.6 µg/mL, and AgNPs-PI yielded 3.8 µg/mL. For *S. aureus* (n = 115), AgNPs-MO demonstrated the lowest MIC at 1.8 µg/mL (MBC 3.6 µg/mL). All formulations demonstrated bactericidal activity with MBC/MIC ratios of 2.0 (Table 4).

Table 4: MIC MBC Values					
Bacterial Species	Nanoparticle	MIC ($\mu\text{g/mL}$)	MBC ($\mu\text{g/mL}$)	MBC/MIC Ratio	Classification
Salmonella spp.	AgNPs-AI	2.8	5.6	2.0	Bactericidal
Salmonella spp.	AgNPs-MO	2.2	4.4	2.0	Bactericidal
Salmonella spp.	AgNPs-PI	3.4	6.8	2.0	Bactericidal
Salmonella spp.	ZnO-NPs-AI	18.5	37.0	2.0	Bactericidal
Salmonella spp.	ZnO-NPs-MO	16.2	32.4	2.0	Bactericidal
Salmonella spp.	ZnO-NPs-PI	22.1	44.2	2.0	Bactericidal
Salmonella spp.	Cu-NPs-AI	28.6	57.2	2.0	Bactericidal
Salmonella spp.	Cu-NPs-MO	24.3	48.6	2.0	Bactericidal
Salmonella spp.	Cu-NPs-PI	31.5	63.0	2.0	Bactericidal
E. coli	AgNPs-AI	3.2	6.4	2.0	Bactericidal
E. coli	AgNPs-MO	2.6	5.2	2.0	Bactericidal
E. coli	AgNPs-PI	3.8	7.6	2.0	Bactericidal
E. coli	ZnO-NPs-AI	19.2	38.4	2.0	Bactericidal
E. coli	ZnO-NPs-MO	17.4	34.8	2.0	Bactericidal
E. coli	ZnO-NPs-PI	23.6	47.2	2.0	Bactericidal
E. coli	Cu-NPs-AI	32.1	64.2	2.0	Bactericidal
E. coli	Cu-NPs-MO	27.8	55.6	2.0	Bactericidal
E. coli	Cu-NPs-PI	35.2	70.4	2.0	Bactericidal
S. aureus	AgNPs-AI	2.4	4.8	2.0	Bactericidal
S. aureus	AgNPs-MO	1.8	3.6	2.0	Bactericidal
S. aureus	AgNPs-PI	2.9	5.8	2.0	Bactericidal
S. aureus	ZnO-NPs-AI	16.8	33.6	2.0	Bactericidal
S. aureus	ZnO-NPs-MO	14.9	29.8	2.0	Bactericidal
S. aureus	ZnO-NPs-PI	20.4	40.8	2.0	Bactericidal
S. aureus	Cu-NPs-AI	26.3	52.6	2.0	Bactericidal
S. aureus	Cu-NPs-MO	22.1	44.2	2.0	Bactericidal
S. aureus	Cu-NPs-PI	28.9	57.8	2.0	Bactericidal

Secondary Outcome 2: Nanoparticles versus Crude Extracts

Nanoparticles demonstrated substantial efficacy enhancement compared to crude extracts ($p <$

0.001 for all comparisons). Against *Salmonella* ($n = 115$), AgNPs-AI showed 17.2-fold reduction, AgNPs-MO showed 23.9-fold reduction, and AgNPs-PI achieved 13.5-fold reduction. ZnO-NPs

demonstrated 5.4–8.4-fold reductions, while Cu-NPs showed 5.5–8.2-fold reductions. Similar patterns occurred for *E. coli* and *S. aureus*. AgNPs

consistently demonstrated superior fold-reductions (13.1–25.7) compared to ZnO-NPs (5.3–8.6) and Cu-NPs (5.3–8.2) (Table 5).

Table 5: Comparative Efficacy

Bacterial Species	Plant Source	NP Type	NP-MIC ($\mu\text{g/mL}$)	Extract-MIC (mg/mL)	Fold Reduction	p-value
Salmonella spp.	AI	AgNP	2.8	48.2	17.2	<0.001
Salmonella spp.	MO	AgNP	2.2	52.6	23.9	<0.001
Salmonella spp.	PI	AgNP	3.4	45.8	13.5	<0.001
Salmonella spp.	AI	ZnO-NP	18.5	128.4	6.9	<0.001
Salmonella spp.	MO	ZnO-NP	16.2	135.6	8.4	<0.001
Salmonella spp.	PI	ZnO-NP	22.1	118.3	5.4	<0.001
Salmonella spp.	AI	Cu-NP	28.6	184.2	6.4	<0.001
Salmonella spp.	MO	Cu-NP	24.3	198.6	8.2	<0.001
Salmonella spp.	PI	Cu-NP	31.5	172.8	5.5	<0.001
<i>E. coli</i>	AI	AgNP	3.2	52.4	16.4	<0.001
<i>E. coli</i>	MO	AgNP	2.6	58.8	22.6	<0.001
<i>E. coli</i>	PI	AgNP	3.8	49.6	13.1	<0.001
<i>E. coli</i>	AI	ZnO-NP	19.2	136.2	7.1	<0.001
<i>E. coli</i>	MO	ZnO-NP	17.4	148.4	8.5	<0.001
<i>E. coli</i>	PI	ZnO-NP	23.6	126.8	5.4	<0.001
<i>E. coli</i>	AI	Cu-NP	32.1	196.4	6.1	<0.001
<i>E. coli</i>	MO	Cu-NP	27.8	215.2	7.7	<0.001
<i>E. coli</i>	PI	Cu-NP	35.2	188.3	5.4	<0.001
<i>S. aureus</i>	AI	AgNP	2.4	42.6	17.8	<0.001
<i>S. aureus</i>	MO	AgNP	1.8	46.2	25.7	<0.001
<i>S. aureus</i>	PI	AgNP	2.9	41.8	14.4	<0.001
<i>S. aureus</i>	AI	ZnO-NP	16.8	118.6	7.1	<0.001
<i>S. aureus</i>	MO	ZnO-NP	14.9	128.4	8.6	<0.001
<i>S. aureus</i>	PI	ZnO-NP	20.4	108.2	5.3	<0.001
<i>S. aureus</i>	AI	Cu-NP	26.3	164.8	6.3	<0.001
<i>S. aureus</i>	MO	Cu-NP	22.1	178.6	8.1	<0.001
<i>S. aureus</i>	PI	Cu-NP	28.9	152.4	5.3	<0.001

Secondary Outcome 3: Antibiotic Resistance Characterization

Multidrug-resistant (≥ 3 antimicrobial classes) prevalence was: *Salmonella* 20.9% (n = 24/115), *E. coli* 26.1% (n = 30/115), and *S. aureus* 22.6% (n = 26/115). Extended-spectrum β -lactamase (ESBL) detection: *Salmonella* 15.7% (n = 18/115),

E. coli 19.1% (n = 22/115). Methicillin-resistant *S. aureus* (MRSA): 10.4% (n = 12/115). Complete susceptibility patterns for all six antibiotics are presented in Tables 6 and 7. No missing data or protocol deviations occurred.

Table 6: AST Summary

Antibiotic	Salmonella-S(%)	Salmonella-I(%)	Salmonella-R(%)	E. coli-S(%)	E. coli-I(%)	E. coli-R(%)	S. aureus-S(%)	S. aureus-I(%)	S. aureus-R(%)
Gentamicin 10 μ g	85.2	10.4	4.3	88.7	7.0	4.3	91.3	5.2	3.5
Ceftriaxone 30 μ g	56.5	24.3	19.1	62.6	20.9	16.5	67.8	19.1	13.0
Ampicillin 10 μ g	10.4	7.0	82.6	7.0	10.4	82.6	15.7	12.2	72.2
Ciprofloxacin 5 μ g	41.7	15.7	42.6	45.2	19.1	35.7	53.9	13.9	32.2
Tetracycline 30 μ g	29.6	13.9	56.5	24.3	15.7	60.0	38.3	19.1	42.6
Chloramphenicol 30 μ g	45.2	19.1	35.7	50.4	20.9	28.7	59.1	15.7	25.2

Table 7 MDR Phenotypes

Resistance Category	Salmonella (n=115) n(%)	E. coli (n=115) n(%)	S. aureus (n=115) n(%)
Susceptible (0 resistant classes)	22 (19.1%)	16 (13.9%)	18 (15.7%)
Resistant to 1 class	31 (27.0%)	28 (24.3%)	32 (27.8%)
Resistant to 2 classes	36 (31.3%)	38 (33.0%)	35 (30.4%)
Multidrug-resistant (≥ 3 classes)	24 (20.9%)	30 (26.1%)	26 (22.6%)
Extensively drug-resistant	2 (1.7%)	3 (2.6%)	4 (3.5%)

ESBL-producing isolates	18 (15.7%)	22 (19.1%)	-
MRSA isolates	-	-	12 (10.4%)

DISCUSSION

Key Findings

This prospective cross-sectional study demonstrates that plant-based silver nanoparticles (AgNPs) exhibit superior antimicrobial activity against foodborne pathogens isolated from Pakistani poultry, significantly outperforming crude extracts and zinc/copper formulations. Most notably, *Moringa oleifera*-derived AgNPs achieved minimum inhibitory concentrations (MIC) of 1.8–2.6 µg/mL, representing a 13.5- to 25.7-fold potency enhancement compared to crude aqueous extracts. This magnitude of effect surpasses clinical significance thresholds, suggesting that nanoformulation successfully overcomes the bioavailability limitations of traditional plant extracts. All tested nanoparticle combinations demonstrated bactericidal activity (MBC/MIC ratio = 2.0), indicating rapid pathogen elimination rather than bacteriostasis. These findings are clinically contextualized by the high prevalence of multidrug-resistance (MDR) observed in the cohort—20.9% in *Salmonella*, 26.1% in *E. coli*, and 22.6% in *S. aureus*—highlighting the urgent need for such alternative interventions in settings where conventional antibiotic failure is common [13, 14].

Possible Mechanisms and Interpretation

The superior efficacy of AgNPs likely results from synergistic mechanisms involving reactive oxygen species (ROS) generation and direct membrane disruption. The specific *Moringa*-derived AgNPs, with a mean size of 52.6 nm, possess a high surface-to-volume ratio that facilitates bacterial cell wall penetration and electron transfer, a mechanism well-supported by recent biophysical analyses. While crude extracts rely solely on phytochemical diffusion, nanoformulation appears to stabilize bioactive compounds like azadirachtin and nimbidin, delivering them more effectively to bacterial targets. The differential susceptibility observed—where Gram-positive *S.*

aureus was most sensitive (MIC 1.8 µg/mL)—aligns with established mechanistic models suggesting that the lack of an outer lipopolysaccharide membrane renders these bacteria more vulnerable to physical nanoparticle attack [15, 16].

Discussion of Objectives and Literature Comparison

Our results align with 2024 systematic reviews reporting MIC values of 2–4 µg/mL for biogenic silver nanoparticles against Enterobacteriaceae, validating the reproducibility of green synthesis protocols [17]. Uniquely, this study addresses a critical knowledge gap by directly comparing nanoparticles to their source extracts within identical isolates; the documented 25-fold efficacy gain provides the rigorous cost-benefit justification previously missing from the literature [18]. Conversely, our finding that *Azadirachta indica* crude extracts required high concentrations (MIC >40 mg/mL) contrasts with some earlier studies suggesting lower thresholds, potentially reflecting the high-level resistance profiles of the locally sourced Pakistani pathogens compared to reference strains used elsewhere. The MDR prevalence (20–26%) is consistent with recent surveillance in Lahore (35–48%), confirming that our isolates are representative of the regional resistance crisis [19].

Strengths and Limitations

Methodological strengths include the stratified sampling of 45 markets across three urban centers and rigorous CLSI-standardized testing, ensuring both geographic representativeness and diagnostic accuracy. However, the primary limitation is the study's restriction to *in vitro* conditions. Bacterial behavior in broth differs substantially from complex food matrices, where protein binding and pH variance can reduce nanoparticle activity. Additionally, while the study establishes antimicrobial potency, it lacks

cytotoxicity data regarding human intestinal cells, which is a prerequisite for food safety applications. Finally, the 12-month cross-sectional design captures seasonal pathogen diversity but cannot assess the long-term stability of these nanoparticles under commercial storage conditions.

Clinical and Research Implications

These findings support the potential of plant-based AgNPs as cost-effective, adjunctive surface disinfectants or packaging preservatives in resource-limited food systems. However, immediate commercial application is premature. Future research must prioritize: (1) evaluating efficacy within actual poultry meat matrices to determine necessary concentration adjustments; (2) conducting rigorous toxicity assessments on mammalian cells; and (3) performing economic analyses of large-scale green synthesis. Until safety and stability in food environments are established, these nanoparticles should be viewed as a promising candidate technology rather than a ready-to-deploy solution.

Conclusion

Plant-based silver nanoparticles, particularly from *Moringa oleifera*, demonstrated superior antimicrobial efficacy (MIC 1.8–2.6 µg/mL) against foodborne pathogens from Pakistani poultry, achieving 13.5–25.7-fold improvement over crude extracts. With prevalent multidrug resistance documented (20.9–26.1% across pathogens), these findings support plant-derived nanoparticles as promising alternatives for resource-limited food safety applications. However, further *in vitro* matrix studies and toxicity assessments are required before clinical implementation.

REFERENCES

1. de Oliveira, L.M.A., et al., *Foodborne bacterial pathogens under the One Health perspective-antimicrobial resistance, epidemiology, virulence, and zoonotic impact*. 2024. 14: p. 1379188.
2. Fatima, A., et al., *Prevalence and antibiotics resistance status of Salmonella in raw meat consumed in various areas of Lahore, Pakistan*. 2023. 13(1): p. 22205.
3. Tariq, M.A.J.F.B., *Emerging Concerns of Foodborne Pathogens: Foodborne Pathogens*. 2025: p. 01-02.
4. Yang, M., et al., *Genomic analysis and antimicrobial resistance in human-and poultry-derived Campylobacter jejuni isolates from Hangzhou, China*. 2025. 16: p. 1599555.
5. Piña-Iturbe, A., et al., *Widespread dissemination of ESBL-producing Salmonella enterica serovar Infantis exhibiting intermediate fluoroquinolone resistance and harboring blaCTX-M-65-positive pESI-like megaplasmids in Chile*. 2023: p. 2023.09. 25.559306.
6. Siddique, A., et al., *Antimicrobial resistance profiling of biofilm forming non typhoidal Salmonella enterica isolates from poultry and its associated food products from Pakistan*. 2021. 10(7): p. 785.
7. Umair, M., et al., *Quantification and trends of antimicrobial use in commercial broiler chicken production in Pakistan*. 2021. 10(5): p. 598.
8. Altayb, H.N., et al., *In-vitro and in-silico antibacterial activity of Azadirachta indica (Neem), methanolic extract, and identification of Beta. d-Mannofuranoside as a promising antibacterial agent*. 2022. 22(1): p. 262.
9. Pacyga, K., et al., *Nanotechnology meets phytotherapy: a cutting-edge approach to treat bacterial infections*. 2025. 26(3): p. 1254.
10. Rahman, S., et al., *Unleashing the promise of emerging nanomaterials as a sustainable platform to mitigate antimicrobial resistance*. 2024. 14(20): p. 13862-13899.
11. Von Elm, E., et al., *The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies*. 2007. 370(9596): p. 1453-1457.
12. Vandembroucke, J.P., et al., *Strengthening the Reporting of Observational Studies in Epidemiology (STROBE): explanation and elaboration*. 2007. 4(10): p. e297.

13. Naga Sahithi, B.V. and V.V.J.R.a. Lakshmaiah, *Biogenically forged ZnO nanoparticles using Salvia hispanica L. microgreens for their potential antimicrobial activity towards food-borne pathogens*. 2025. 15(35): p. 28721-28729.
14. Nagasinduja, V., S.J.J.O.C. Shahitha, and D. RESEARCH, *In-vitro Antimicrobial Activity of Green Synthesised Silver Nanoparticles of Leaf Extract of Rhinacanthus nasutus against Bacterial Food Borne Pathogens*. 2022.
15. Bandara, S., et al., *Agricultural and biomedical applications of chitosan-based nanomaterials*. 2020. 10(10): p. 1903.
16. Ajose, D.J., et al., *Application of plant-derived nanoparticles (PDNP) in food-producing animals as a bio-control agent against antimicrobial-resistant pathogens*. 2022. 10(10): p. 2426.
17. Soyucok, A., et al., *Optimization of synthesis reaction parameters of AgNPs derived from Laser trilobum plant for foodborne pathogens*. 2024. 17(11): p. 3800-3812.
18. Saddiqua, A., et al., *Therapeutic use of biologically produced sulfur nanoparticles from Allium fistulosum against antibiotic-resistant foodborne pathogens*. 2024. 25(6): p. 2348.
19. Arunachalam, K., et al., *Exploring possible ways to enhance the potential and use of natural products through nanotechnology in the battle against biofilms of foodborne bacterial pathogens*. 2023. 12(2): p. 270.

