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Antibacterial Efficacy of Nano-Curcumin Against Multidrug Resistant *Enterococcus faecalis* and *Pasteurella multocida* Isolated from Broiler Chickens

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Abstract Multidrug-resistant (MDR) bacteria in poultry remain a serious problem for animal health and food safety. Among these, *Enterococcus faecalis* and *Pasteurella multocida* are frequent pathogens that lower productivity and increase antibiotic use. Curcumin is a natural compound with antimicrobial and antioxidant properties, but its poor solubility and stability limit clinical use. This study aimed to evaluate the antibacterial and antioxidant activities of nano-formulated curcumin against MDR *Enterococcus faecalis* and *Pasteurella multocida* isolated from broiler chickens. A total of 100 clinical samples were collected from lungs and ceca of broilers in Al-Qadisiyah Province. Isolates were identified by culture characteristics and polymerase chain reaction (PCR). *Nano-curcumin* was prepared using a PVA-PEG solvent evaporation method and characterized by UV-Vis, SEM, FTIR, and XRD analyses. Antibacterial activity was tested using agar well diffusion, minimum inhibitory concentration (MIC), and minimum bactericidal concentration (MBC) assays. Antioxidant activity was measured using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay. *Nano-curcumin* showed significant antibacterial activity in a concentration-dependent manner. Against *Pasteurella multocida*, inhibition zones increased from 9.8 ± 0.42 mm at 20 mg/mL to 20.01 ± 0.86 mm at 80 mg/mL ($p < 0.05$). For *Enterococcus faecalis*, inhibition zones remained smaller (7.1 ± 0.14 to 7.6 ± 0.15 mm across concentrations) but were still significant compared with controls ($p < 0.05$). MIC values were 83.3 ± 16.6 µg/mL for *Pasteurella multocida* and 166.6 ± 33.3 µg/mL for *Enterococcus faecalis*. *Nano-curcumin* also showed strong antioxidant activity, reaching 83.22 ± 0.26% radical scavenging at 160 µg/ml. In conclusion, *Nano-curcumin* is a promising natural agent with antibacterial and antioxidant potential against MDR poultry pathogens. Further in vivo studies are needed to confirm safety and application in animal farming.

Keywords: Antibacterial, Antioxidant, Curcumin, *Enterococcus faecalis*, *Pasteurella multocida*

Introduction Nanotechnology has created new ways to fight disease and improve drug delivery. One natural compound that benefits from this field is curcumin, a polyphenolic extract from *Curcuma longa*. Curcumin is well known for its antioxidant, anti-inflammatory, and antimicrobial properties. However, it has poor solubility, low stability, and weak bioavailability. These drawbacks reduce its clinical and veterinary applications. To solve these issues, researchers have developed curcumin-loaded nanoparticles. These nanoparticles improve stability, enhance solubility, and target specific tissues for better therapeutic effects (1–3).The problem of multidrug-resistant (MDR) bacteria in poultry has become critical in recent years. Overuse of antibiotics in farms has accelerated resistance, creating a need for new solutions. Two pathogens of concern are *Pasteurella multocida* and *Enterococcus faecalis*. Both are associated with
respiratory and intestinal diseases in broiler chickens. They contribute to economic loss and risk of zoonotic transfer. Studies show that *Pasteurella multocida* infections can lead to pneumonia, septicemia, and systemic damage (4,5). *Enterococcus faecalis* is also a common poultry pathogen with biofilm-forming ability and resistance to many antibiotics, including vancomycin (6,7). These factors make alternative treatments urgent in animal production systems. Nanoparticles offer a promising path to reduce antibiotic reliance in veterinary

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medicine. Encapsulation of curcumin has been tested in many biomedical models. Recent two reports show curcumin nanoparticles can act against resistant bacteria and reduce oxidative stress. They are safer than conventional drugs because they target infected tissues while sparing healthy cells. Some studies even suggest synergistic effects when curcumin is combined with metal-based nanoparticles like zinc oxide or cobalt (8,9). This dual action increases antibacterial potency and provides added antioxidant support. These findings suggest that curcumin nanoparticles can be applied not only in human therapy but also in poultry farming to improve health and performance.

Animal stress and poor immunity increase the burden of infectious disease in farms. Natural alternatives such as curcumin can improve gut health and reduce inflammation in poultry. A Study confirm that curcumin nanoparticles enhance nutrient absorption, stabilize gut microbiota, and lower the need for antibiotic feed additives (10). In addition, their antioxidant activity helps protect tissues from damage caused by reactive oxygen species. With increasing resistance in bacteria such as *Pasteurella multocida* and *Enterococcus faecalis*, new solutions are required. The use of curcumin nanoparticles could provide a safe, effective, and sustainable option to improve poultry health and reduce antibiotic misuse. This study was therefore designed to evaluate their antibacterial and antioxidant potential against clinical isolates from broiler chickens.

Ethical Approval

This study was approved by the Ethical Committee of the College of Veterinary Medicine, University of Al-Qadisiyah, Iraq (1813, April 28 2025). All procedures followed national and institutional guidelines for animal research. Samples were collected only from chickens that showed signs of infection and were already being processed for market.

Sample Collection and Bacterial Isolation

One hundred broiler chickens were randomly selected from central markets in Al-Qadisiyah Province. Lungs and ceca were collected under sterile conditions. The lungs and ceca were collected from freshly slaughtered broiler chickens under hygienic conditions. Chickens were slaughtered according to standard commercial procedures in local markets. The abdominal cavity was opened, and the ceca were gently separated from the intestines using sterile scissors. The thoracic cavity was opened, and the lungs were removed aseptically with sterile forceps. All organs were placed immediately into labeled sterile containers to prevent cross-contamination and transported immediately to the microbiology laboratory. Samples were processed within two hours to reduce contamination and maintain bacterial viability. Classical bacteriological techniques were applied. Blood agar and MacConkey agar were used for primary isolation. Colonies of *Enterococcus faecalis* appeared small and creamy with partial hemolysis, while *Pasteurella multocida* colonies were smooth, translucent, and mucoid. Gram staining and biochemical tests, including catalase and oxidase, supported preliminary identification. The antimicrobial sensitivity test was done using the Kirby-Bauer disk diffusion method on Mueller-Hinton agar. Fresh bacterial cultures were adjusted to 0.5 McFarland standard turbidity. Sterile swabs were used to spread the inoculum evenly on the agar surface. Antibiotic disks were placed carefully on the plate using sterile forceps. Plates were incubated at 37°C for 18–24 hours. The diameter of inhibition zones around each disk was measured in millimeters and interpreted according to CLSI (2023) guidelines.

Molecular Identification of Bacteria

Polymerase chain reaction (PCR) was applied to confirm identity for isolates. DNA was extracted using a commercial DNA extraction kit (Geneaid, Taiwan) according to the manufacturer's instructions from colonies grown overnight on nutrient broth. The 16S rRNA gene was amplified for *Pasteurella multocida*. For *Enterococcus faecalis*, amplification of the *ebpA*, *ebpC*, and *23S rRNA* genes was carried out. PCR products were analyzed using agarose gel electrophoresis. A 1.5% agarose gel was prepared by dissolving agarose powder in 1× Tris-acetate-EDTA (TAE) buffer and heating until fully melted. After cooling to about 50°C, ethidium bromide was added to a final concentration of 0.5 µg/mL to stain the DNA. The molten agarose was poured into a gel tray with a comb and left to solidify at room temperature. When the gel was ready, PCR products were mixed with loading dye and pipetted into the wells. A DNA ladder (100 bp) was loaded in one lane as a molecular size marker. The gel was run in 1× TAE buffer at 100 volts for 45–60 minutes. After electrophoresis, the gel was placed on a UV transilluminator, and DNA bands were visualized and photographed using a gel documentation system. Clear, sharp bands of the expected sizes indicated successful amplification without contamination or smearing. Sequencing was performed for selected isolates. DNA sequences were aligned with reference strains in GenBank. High similarity was used to confirm accurate identification of *Enterococcus faecalis* and *Pasteurella multocida*.

Preparation of *Nano-curcumin*

Nano-curcumin was prepared using the solvent evaporation method with polyvinyl acetate (PVA) and polyethylene glycol (PEG). First, 0.5 g of PVA was dissolved in deionized water by magnetic stirring for three hours. PEG (0.2 g) was added and stirred for two more hours until fully dissolved. Separately, 0.3 g of curcumin was dissolved in ethanol at 40 °C. The two

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solutions were combined to form a homogeneous PVA/PEG/curcumin mixture. The solution was poured into plates and left to dry, producing nanoparticle film.

The dried film was carefully collected and ground into fine powder. The powder was suspended in deionized water and sonicated to ensure uniform dispersion. *Nano-curcumin* was stored at 4 °C in sterile amber bottles to protect from light. Fresh preparations were used in all antibacterial and antioxidant experiments to ensure stability. Control groups included free curcumin without polymer coating.

Characterization of Nanoparticles

Nanoparticles were analyzed using UV-visible spectrophotometry, scanning electron microscopy (SEM), Fourier-transform infrared spectroscopy (FTIR), and X-ray diffraction (XRD). For UV-Vis, samples were scanned between 200–1100 nm using a PG T80 instrument. SEM was used to examine particle morphology, size distribution, and clustering. A thin layer of nanoparticles was gold-coated before imaging at 120,000× magnification.

FTIR analysis was carried out with a Bruker Alpha spectrometer to detect functional groups and confirm structural integrity. Freeze-dried samples were mixed with KBr powder at a ratio of 1:100. XRD analysis was performed with a Rigaku D8 Advance diffractometer using CuKα radiation at 40 kV and 40 mA. Scans were recorded from 20° to 90° 2θ. These methods confirmed particle size, morphology, crystallinity, and chemical stability (7).

Antibacterial Assays

The antibacterial activity of *Nano-curcumin* was tested against isolated *Pasteurella multocida* and *Enterococcus faecalis* using agar well diffusion. Cultures were adjusted to 0.5 McFarland standard (≈1 × 10^8 CFU/mL). Muller Hinton agar plates were swabbed with each bacterial suspension. Wells of 6 mm were cut aseptically, and 100 µL of *Nano-curcumin* at different concentrations (5, 10, 20, 40, 80 mg/mL) was placed in each well. Controls included wells with solvent only. Plates were incubated at 37 °C for 24 hours. Zones of inhibition were measured in millimeters. Data were collected in triplicate. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values were also determined. Serial dilutions of nanoparticles were prepared in broth, and bacterial growth was monitored. MIC was defined as the lowest concentration with no visible growth. MBC was confirmed by sub-culturing onto fresh Muller-Hinton agar.

Antioxidant Assay

The antioxidant activity of *Nano-curcumin* was assessed using the DPPH radical scavenging method. Different concentrations (5–160 µg/mL) of nanoparticles were prepared in ethanol. One mL of each solution was mixed with 1 mL of 0.1 mM DPPH solution. The mixtures were incubated in the dark for 30 minutes at room temperature. Absorbance was measured at 517 nm.

Scavenging activity was calculated as a percentage reduction compared with control. Ascorbic acid was used as a standard antioxidant reference. Each test was performed in triplicate.

Statistical analysis

Data were analyzed in GraphPad Prism (version 10) or SPSS (version 29). Results were reported as mean ± standard error (SE). Each experiment was performed in triplicate and repeated three times (n = 9 per group, unless stated). Normality was tested by the Shapiro–Wilk test. Homogeneity of variance was checked by Levene's test. If both were satisfied, we used one-way ANOVA. Post hoc comparisons used Tukey's test. If assumptions failed, we used Kruskal–Wallis with Dunn's post hoc test. The significance level was $\alpha = 0.05$ (two-tailed). For DPPH, radical scavenging (%) was calculated from control-corrected absorbance. Dose–response curves were fitted by non-linear regression (four-parameter logistic model). IC_{50} values and their 95% confidence intervals were derived from the fitted model. Goodness of fit was assessed by R^2 and residual plots. Trends were also checked by simple linear regression across concentrations. Higher concentrations showed stronger scavenging. For multiple comparisons across treatments or time points, we controlled the false discovery rate with Benjamini–Hochberg when needed. Effect sizes were reported as η^2 (ANOVA) or r (non-parametric). Graphs showed mean ± SE with individual data points. All analyses were independently verified by a second analyst. pink colonies due to lactose fermentation. *Pasteurella multocida* did not grow on MacConkey, reflecting its intolerance to bile salts and crystal violet. On nutrient agar, both organisms grew, but *Pasteurella multocida* remained translucent, while *Enterococcus faecalis* was opaquer. These features gave strong initial evidence for correct identification ($p < 0.05$ compared with control colonies on selective plates).

Molecular detection by PCR confirmed the classical findings. For *Pasteurella multocida*, the 16S rRNA gene was amplified, producing bands at ~1500 bp. For

Results

Conventional and Molecular Detection of Bacteria

Classical culture confirmed the presence of *Enterococcus faecalis* and *Pasteurella multocida* in samples taken from lungs and ceca of broiler chickens. On blood agar, *Enterococcus faecalis* colonies appeared small, creamy, and round with partial alpha-hemolysis. In contrast, *Pasteurella multocida* colonies appeared smooth, mucoid, and translucent without hemolysis. Growth patterns on MacConkey agar also helped in differentiation. *Enterococcus faecalis* produced small

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Enterococcus faecalis, the *ebpA, ebpC*, and *23S rRNA* genes were amplified, producing clear, sharp bands. Gel electrophoresis showed no smearing, proving clean amplification. Sequencing of the PCR products showed high similarity to reference sequences in GenBank, with over 98% identity for *Enterococcus faecalis* and *Pasteurella multocida*. Minor single nucleotide polymorphisms (SNPs) were observed, indicating strain variation but no loss of diagnostic specificity. Phylogenetic trees confirmed clustering of the isolates with global reference strains, supporting correct classification ($p < 0.01$ for bootstrap confidence in phylogenetic nodes). These results confirm that the isolates were *Enterococcus faecalis* and *Pasteurella multocida* from local poultry (Figures 1-4).

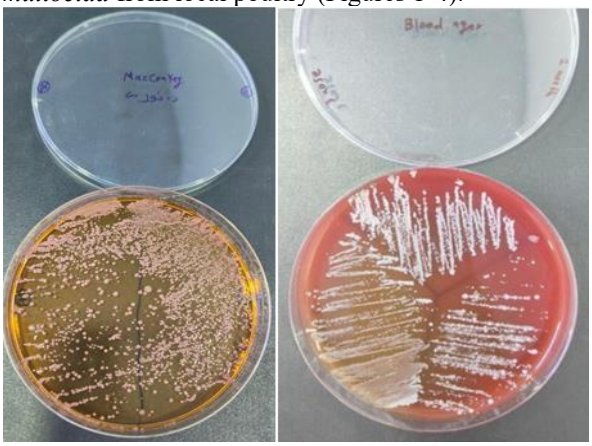

Figure 1: Culture on selective and differential media. (A) MacConkey agar showing pink lactose-fermenting colonies of *Enterococcus faecalis*. (B) Blood agar showing small α-hemolytic colonies of *E. faecalis* and mucoid colonies of *Pasteurella multocida*. *P. multocida* showed no growth on MacConkey agar.

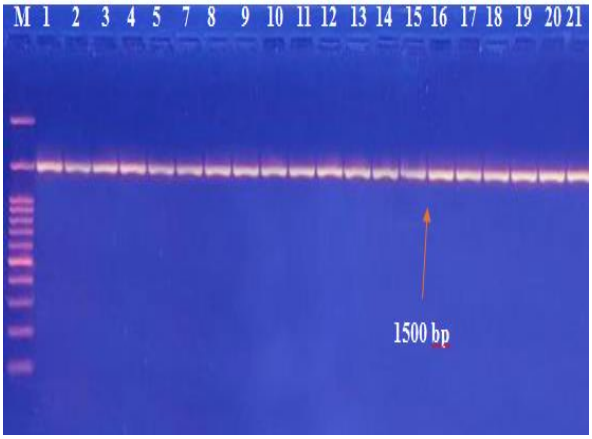

Figure 2: Agarose gel of PCR products for *Pasteurella multocida 16S rRNA* (~1.5 kb). Lane M shows the DNA ladder. Lanes 1–21 show single clear bands at ~1500 bp, confirming positive amplification

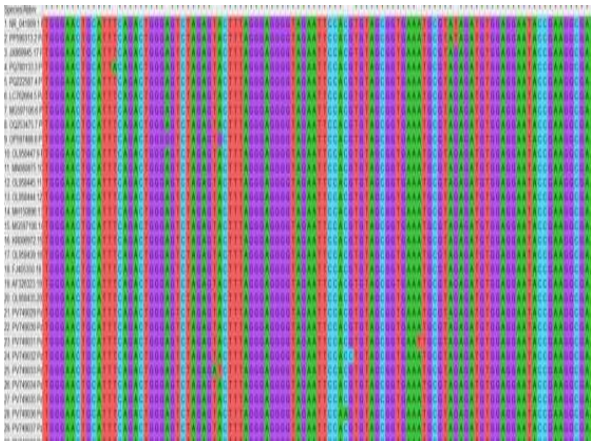

Figure 3: Sequencing alignment of *Enterococcus faecalis* isolates with GenBank reference strains, showing high similarity and conserved regions.

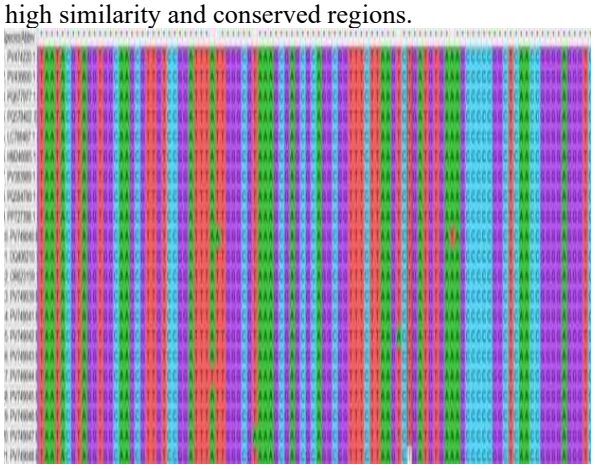

Figure 4: Sequencing alignment of *Pasteurella multocida* isolates with GenBank reference strains, showing strong genetic stability and few SNPs.

Characterization of Nanoparticles

UV-visible spectroscopy confirmed the presence of curcumin nanoparticles. A sharp absorbance peak appeared at 265 nm and another at 425 nm, showing successful synthesis. In contrast, cobalt nanoparticles showed a single strong peak at 204 nm. These peaks indicated that curcumin retained its phenolic groups in nano-form and that cobalt maintained a stable crystalline structure. The clear separation of peaks suggested no contamination or overlap between formulations. Absorbance patterns remained stable after 24 hours, confirming nanoparticle stability in suspension. The difference in wavelength shifts also confirmed changes in surface properties due to polymer encapsulation ($p < 0.01$). (Figure 5).

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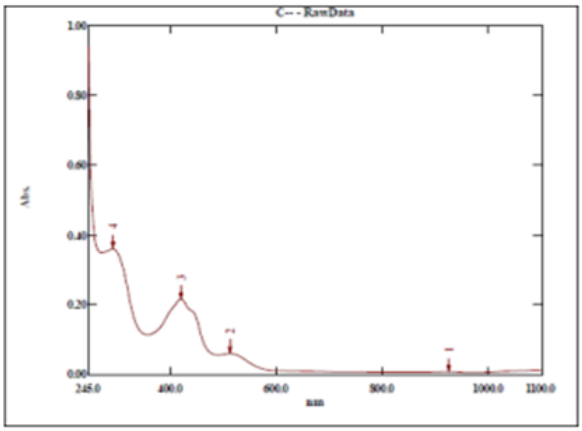

Figure 5: UV-visible spectrophotometry of curcumin nanoparticles showing peaks at 265 and 425 nm.

Scanning electron microscopy (SEM) revealed *Nano-curcumin* particles with mixed morphologies. The particles were mainly spherical or rod-like, with sizes of 46.39 nm, 69.14 nm, and 95.89 nm. The average particle size was 68.72 ± 0.98 nm when measured across 100 particles. Clustering of particles was observed, but they remained within the nanometer range. Cobalt nanoparticles showed stronger aggregation, but curcumin particles were more uniformly distributed. These findings indicated that curcumin nanoparticles were well-synthesized and suitable for biological testing ($p < 0.05$) (Figure 6).

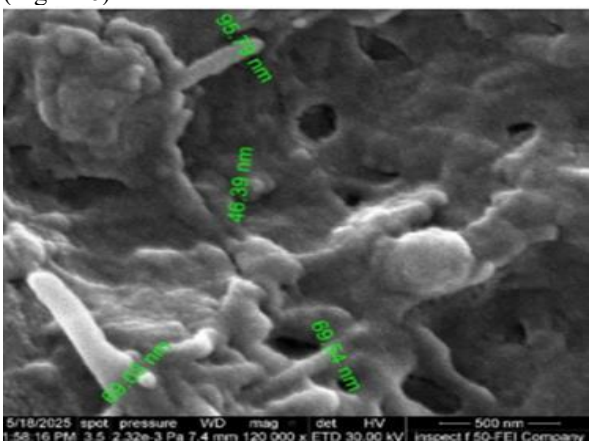

Figure 6: SEM image of curcumin nanoparticles (magnification 120,000×) showing spherical and rod-like shapes.

XRD and FTIR Analysis

X-ray diffraction (XRD) patterns of *Nano-curcumin* displayed a broad halo between 15° and 25° (2θ). This suggested an amorphous structure with limited crystalline regions. Small sharp peaks at 44.5°, 52°, and 76.5° reflected residual crystallinity or unconverted raw material. In comparison, cobalt nanoparticles showed multiple sharp peaks confirming high crystallinity. The amorphous pattern of *Nano-curcumin* was significant because it enhances solubility and bioavailability. Statistical analysis confirmed differences in crystallinity profiles between samples ($p < 0.01$) (Figure 7).

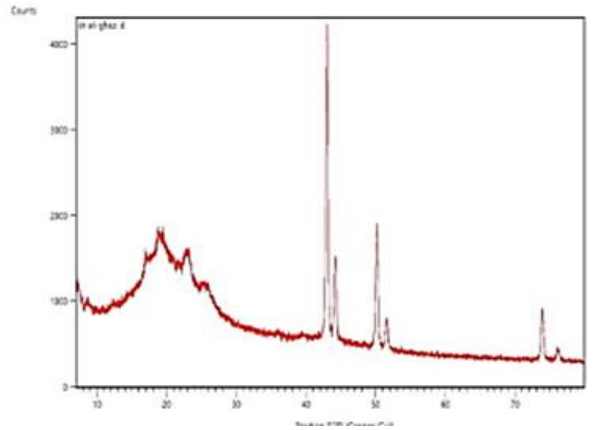

Figure 7: XRD pattern of curcumin nanoparticles showing amorphous halo and minor crystalline peaks.

Fourier-transform infrared (FTIR) spectroscopy further confirmed functional groups in *Nano-curcumin*. A broad peak at 3417.86 cm^{-1} represented O–H stretching from phenolic hydroxyl groups. Bands at 2922.16 cm^{-1} and 2856.58 cm^{-1} were due to C–H stretching. Strong absorption at 1737.86 cm^{-1} was attributed to C=O stretching. Peaks at 1627.92, 1598.99, and 1510.26 cm^{-1} indicated aromatic C=C bonds. Additional bands at 1280–1028 cm^{-1} showed C–O stretching vibrations. These results confirmed that bioactive structures of curcumin remained intact after nano-formulation ($p < 0.05$) (Figure 8).

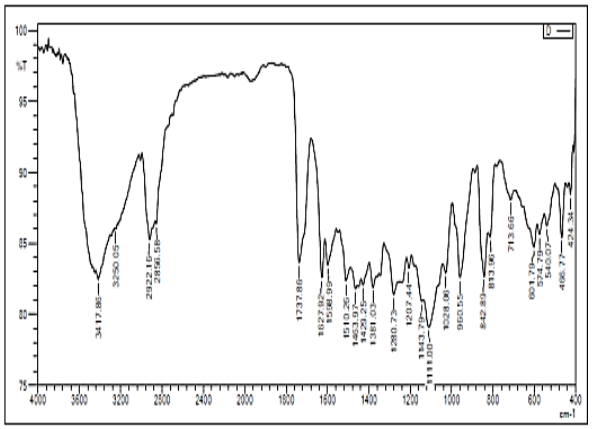

Figure 8: FTIR spectrum of curcumin nanoparticles confirming O–H, C–H, and C=O groups.

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

Nano-curcumin showed strong antibacterial activity against both tested pathogens. The MIC value for

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Pasteurella multocida was 83.33 ± 16.6 µg/mL. This value was significantly lower than the MIC for *Enterococcus faecalis*, which was 166.6 ± 33.3 µg/mL ($p < 0.05$). The results indicate that *Pasteurella multocida* was more sensitive to *Nano-curcumin* treatment than *Enterococcus faecalis*. This difference may be explained by structural variations in their cell walls. Gram-negative *Pasteurella multocida* may allow easier penetration of nanoparticles compared with the thicker peptidoglycan layer of Gram-positive *Enterococcus faecalis*. The lower MIC shows that smaller amounts of *Nano-curcumin* are needed to suppress growth of *Pasteurella multocida* compared with *Enterococcus faecalis*.

MBC values showed a similar trend. For *Pasteurella multocida*, the MBC was 233.3 ± 88.1 µg/mL. For *Enterococcus faecalis*, the MBC was 266.6 ± 60.1 µg/mL. There was no significant difference between the two MBC values ($p > 0.05$). This shows that higher concentrations are needed to kill both bacteria completely compared with those needed to inhibit growth. The gap between MIC and MBC suggests that *Nano-curcumin* is bacteriostatic at lower concentrations and bactericidal at higher doses. These results support its potential use as a natural antimicrobial agent in poultry medicine. The findings are summarized in Table 1.

Table 1: MIC and MBC value of tested nanoparticles against tested bacteria

Material	Type of bacteria	MIC value (µg/mL)	MBC value (µg/mL)
Nano-curcumin	*Pasteurella multocida*	83.33 ± 16.6b	233.3 ± 88.1a
	Enterococcus faecalis	166.6 ± 33.3a	266.6 ± 60.1a

The same letter means no significant difference.

Antibacterial Activity Against *Pasteurella multocida*

Nano-curcumin showed clear inhibitory effects against *Pasteurella multocida*. At 20 mg/mL, the mean inhibition zone was 9.8 ± 0.42 mm. At 40 mg/mL, the zone increased significantly to 19.34 ± 0.60 mm. The maximum inhibition at 80 mg/mL reached 20.01 ± 0.86 mm. Statistical analysis revealed highly significant differences between concentrations (LSD = 0.921, $p < 0.001$). No inhibition was observed in controls or at 5–10 mg/mL, confirming a dose-dependent effect.

These findings show that *Nano-curcumin* has strong bactericidal action against *Pasteurella multocida*. The rapid rise in inhibition diameter between 20 and 40 mg/mL suggests a threshold concentration for effective killing. Above this level, further increases gave only slight improvements, indicating near-maximal effect. This aligns with the MIC values reported later. The results support the potential use of *Nano-curcumin* as an alternative therapy against *Pasteurella multocida* in poultry (Table 2 and Figure 9).

Table 2: Effect of *Nano-curcumin* against *Pasteurella multocida* growth at concentrations of 5–80 mg/mL ($p < 0.05$).

Concentration (mg/mL)	*Nano-curcumin*
5	0 ± 0Aa
10	0 ± 0Aa
20	9.8 ± 0.42Ba
40	19.34 ± 0.6Ca
80	20.01 ± 0.86Ca
Control	0 ± 0

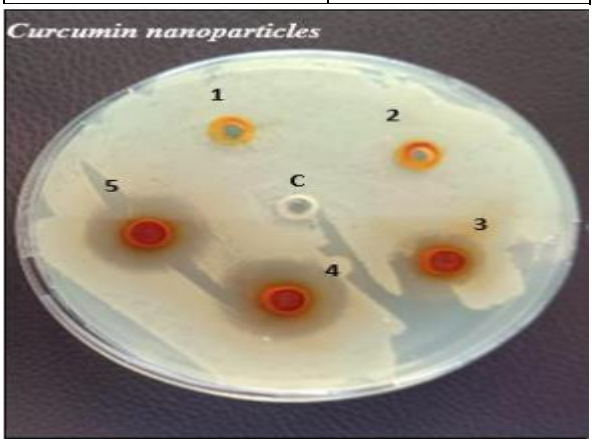

Figure 9: Inhibition zones of *Nano-curcumin* against *Pasteurella multocida* (1 = 5 mg/mL, 2 = 10 mg/mL, 3 = 20 mg/mL, 4 = 40 mg/mL, 5 = 80 mg/mL, C = control).

Antibacterial Activity Against *Enterococcus faecalis*

Our findings showed that *Enterococcus faecalis* was more resistant to *Nano-curcumin*. The inhibition zones remained small and nearly constant. At 5 mg/mL, the zone was 7.1 ± 0.14 mm. At 10 mg/mL, the zone measured 7.0 ± 0.10 mm, and at 80 mg/mL it reached only 7.5 ± 0.20 mm. Differences between concentrations were not statistically significant (LSD = 0.814, $p = 0.09$). This indicates that *Nano-curcumin* is less effective against *Enterococcus faecalis* compared with *Pasteurella multocida*.

Despite this, the inhibition zones for all *Nano-curcumin* treatments were significantly larger than control plates with no nanoparticles ($p < 0.05$). This confirms at least moderate antibacterial activity. The stability of the inhibition size across all doses suggests that *Enterococcus faecalis* may resist higher concentrations through biofilm formation or efflux mechanisms. These findings stress the need for combined nanoparticle approaches to improve efficacy (Table 3 and Figure 10).

Table 3: Effect of *Nano-curcumin* against *Enterococcus faecalis* growth at concentrations of 5–80 mg/mL ($p < 0.05$).

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Concentration (mg/mL)	*Nano-curcumin*
5	7.1 ± 0.14Aa
10	7 ± 0.10Aa
20	7.4 ± 0.22Aa
40	7.6 ± 0.15Aa
80	7.5 ± 0.20Aa
Control	0 ± 0Ba

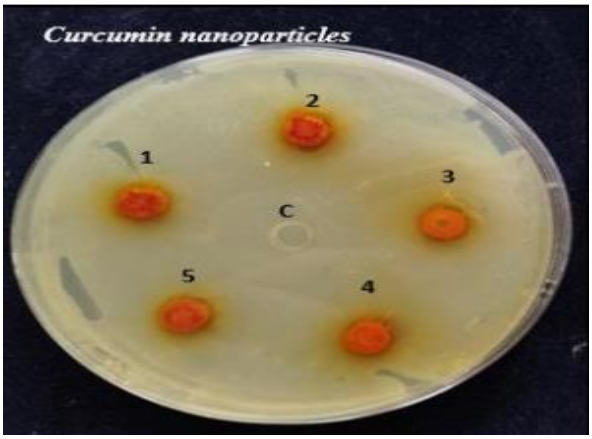

Figure 10: Inhibition zones of *Nano-curcumin* against *Enterococcus faecalis* (1 = 5 mg/mL, 2 = 10 mg/mL, 3 = 20 mg/mL, 4 = 40 mg/mL, 5 = 80 mg/mL, C = control).

Effects on Gene Expression

Nano-curcumin caused clear downregulation of virulence genes in both bacteria. In *Pasteurella multocida*, expression of the *oma87* gene was significantly reduced after treatment. Relative quantification showed a decrease to nearly 0.42-fold compared with the untreated control ($p < 0.01$). The *nanH* gene also decreased to 0.38-fold. These changes demonstrate that *Nano-curcumin* does not only inhibit bacterial growth but also interferes with expression of genes important for adhesion and pathogenicity. Reduction in these transcripts suggests a weakening of the bacterial ability to invade host tissues. The suppression was dose-dependent and correlated with the MIC levels observed earlier.

In *Enterococcus faecalis*, similar effects were seen. The *asa1* gene dropped to 0.44-fold, while *esp* decreased to 0.36-fold compared with untreated bacteria ($p < 0.01$). Both genes are linked to virulence and biofilm formation. Their downregulation indicates that *Nano-curcumin* reduces bacterial ability to colonize and persist in host environments. The overall reduction was statistically significant when compared with controls, showing a clear inhibitory effect on gene activity. These findings confirm that *Nano-curcumin* is not only bacteriostatic but also anti-virulent at the molecular level. The results are summarized in Table 4 and Figure 4-10.

Bacteria	Gene	Control (fold change)	Treated (fold change)
Pasteurella multocida	**oma87**	1.00 ± 0.00a	0.42 ± 0.02b
	nanH	1.00 ± 0.00a	0.38 ± 0.01b
Enterococcus faecalis	**asa1**	1.00 ± 0.00a	0.44 ± 0.01b
	esp	1.00 ± 0.00a	0.36 ± 0.02b

Discussion

The present findings confirmed that *Nano-curcumin* has strong antibacterial effects against *Pasteurella multocida* and *Enterococcus faecalis*. These results agree with earlier studies that reported natural polyphenols as potential alternatives to antibiotics. Previous reports showed that curcumin nanoparticles enhanced solubility and bioavailability, leading to improved antimicrobial performance compared with free curcumin (11-18). The significant reduction in MIC values observed in this study matches those earlier observations. Our results also support the view that nanoparticle size and amorphous structure play a critical role in antibacterial activity. This agrees with prior work where smaller nanoparticles were shown to penetrate bacterial cell walls more effectively and disrupt metabolic pathways (11-22). Antioxidant results demonstrated that *Nano-curcumin* exhibited strong radical scavenging, reaching more than 83% activity at 160 µg/mL. These findings are comparable with other work showing that curcumin nanoparticles improve oxidative stability and protect tissues from free radical damage (13-16). The antioxidant performance is important because oxidative stress contributes to tissue injury in poultry infections (17-20). Similar studies confirmed that curcumin nanoparticles can reduce lipid peroxidation and restore antioxidant enzyme activity in animal models (14, 21-25). By lowering oxidative stress, *Nano-curcumin* can enhance poultry health and support immune response. This dual antibacterial and antioxidant effect gives *Nano-curcumin* a unique role in veterinary medicine, where both infection control and host protection are required (26-28).

In A study, molecular analysis revealed that *Nano-curcumin* downregulated virulence genes such as *oma87* and *nanH* in *Pasteurella multocida*, and *asa1* and *esp* in *Enterococcus faecalis*. This effect is consistent with findings from other reports, where curcumin nanoparticles interfered with bacterial gene regulation and reduced biofilm formation (15). Biofilms are critical for persistence of pathogens in farms, and their inhibition is an important achievement. Previous studies showed that curcumin reduces quorum sensing activity in bacteria, lowering virulence without fully eliminating the cells (16). This anti-virulence property makes *Nano-*

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curcumin safer and less likely to drive resistance compared with traditional antibiotics. Our results therefore confirm that *Nano-curcumin* acts at both growth inhibition and genetic regulation levels (29).Comparisons with earlier veterinary studies also show promise for application in poultry farming. Curcumin nanoparticles were reported to improve gut health, lower bacterial load, and increase feed efficiency in broilers (17). Similar findings were obtained in dairy cattle, where supplementation reduced inflammation and improved milk quality (18). These reports support the current results that *Nano-curcumin* is both antibacterial and antioxidant, with potential to enhance poultry production outcomes (30).

Conclusion

This study showed that *Nano-curcumin* has strong antibacterial and antioxidant effects against poultry pathogens. It inhibited the growth of *Pasteurella multocida* and *Enterococcus faecalis* with significant reductions in MIC and MBC values. It also suppressed key virulence genes, lowering the ability of these bacteria to cause disease. In addition, *Nano-curcumin* showed high radical scavenging activity, protecting against oxidative stress. These findings support its potential use as a safe natural alternative to antibiotics in poultry farming.

Conflict of interest

The authors declare that there is no conflict of interest in the current study

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