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EVALUATING THE ROLE OF CACTUS AND CUMIN NANO-EMULSION IN CONTROLLING MULTIDRUG-RESISTANT *ENTEROCOCCUS* SPECIES IN BROILER CHICKENS

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ABSTRACT

Antimicrobial resistance (AMR) threatens human and animal health worldwide. This study examined the antibacterial properties of cactus and cumin nanoemulsions on MDR Enterococcus species isolated from broiler chicken. *Enterococcus* species was found in 30% of the samples. Cloacal swabs had a 46% incidence rate (21/50), liver samples 17% (6/35), and joint samples 20% (3/15). Enterococcus isolates included *E. faecalis* (50%) and *E. faecium* (36%), *E. hirae* (10%), and *E. durans* (3.33%). They were highly resistant against ampicillin and erythromycin (100.0%), chloramphenicol (86%), tertracycline (71%), and linezolid (62%). Nanoemulsion (NE) characterization of cactus and cumin showed optimal NE features, including a minuscule droplet size (7.03 and 11.48 nm), lowered PDI, and +13 and -17.7 zeta potential, with IC50 of 114.4 and 109.2 ug/ml. Cactus and cumin NE had MICs of 25 and 6.25 ug/ml against E. faecalis. Oxidative stress markers MDA (9.72 nmol/ml) and nitric oxide (21.5 µmol/L) are highest with *E. faecalis,* along with high superoxide dismutase (SOD) activity (60.86 U/ml). Cumin nanoemulsion (NE) significantly decreased nitric oxide (12.5 µmol/L), but increased catalase (CAT) (18.41 U/L), reduced glutathione (GSH) (9.22 U/L) and total antioxidant capacity (TAC) (0.27 mM/L). Cactus NE displayed intermediate values, with strong SOD and CAT activities supporting oxidative balance. These results indicate antioxidant responses across groups, reflecting adaptive mechanisms to oxidative stress with *E. faecalis*. NE inhibited antibiotic resistance genes expression (Erm B, van A, and Tet K), however, Tet K genes had the least impact. Cactus and cumin NE are viable solutions for safe substitution for synthetic antibacterial agents and antioxidant supplements for MDR E. faecalis infections.

Keywords: *Enterococcus faecalis*, chicken, cumin and cactus extract, nanoemulsion, MDR, gene expression, oxidative stress, Antioxidants.

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INTRODUCTION

Enterococcus species (Enterococcus spp.) are one of the most common infections in broilers, causing high mortality, growth retardation, and economic costs for antimicrobial prevention and treatment (Rehman *et al.,* 2018). They are also an emerging avian pathogen. Broiler chickens with arthritis, osteomyelitis, lameness, and endocarditis had Enterococcus spp. (Avbersek *et al.,* 2021). The most prevalent Enterococcus species associated with disease conditions in poultry are *E. cecorum*, *E. faecalis*, and *E. faecium* (Dolka *et al.,* 2017).
In poultry, *E. faecalis* causes first-week mortality, amyloid arthropathy in layers, and valvular endocarditis, salpingitis, peritonitis, and arthritis in broilers. Septicemic disease in white Peking ducklings is linked to *E. faecium* (Wodz *et al.,* 2024). *E. faecalis* and *E. faecium* are mostly found in 1-day-old chicks' intestines, while *E. durans* is in the crop. The intestines contain *E. durans* after 3-4 weeks (Ribeiro *et al.,* 2023).

Multiple virulence factors and widespread antibiotic resistance make enterococci dangerous illnesses. Molecular mechanisms of enterococcal infections include oxidative stress. *Enterococcus faecalis* produces antioxidant enzymes for the oxidative stress response, which is regulated by several transcriptional regulators. The understanding of molecular mechanisms behind the production of free radicals and the antioxidant status in *E. faecalis* might suggest new alternatives for the treatment of enterococcal infections and related diseases (Szemes *et al.,* 2010). Estimating oxidative stress and endogenous antioxidants is crucial to initiating infectious disease molecular mechanisms (Hussain *et al.,* 2022 & Soliman, 2023).

Cumin is antioxidant rich. Cumin seed extracts may improve antioxidant enzyme performance, study shows. Cumin contains phytochemicals that prevent oxidative stress when combined with E. faecalis or nanoparticles (Lee *et al.,* 2023; Mekky, 2024). Betalains, flavonoids, and polyphenols in cactus boost their antioxidant properties. These compounds reduce cellular oxidative stress by scavenging free radicals. Cactus antioxidants neutralize ROS to fight infections. This is especially important when considering *E. faecalis*, which can produce oxidative stress in host organisms (El-Mostafa *et al.,* 2014). Previous studies have revealed that cumin and cactus have antioxidant and antibacterial properties, but further research is needed on their effects on *E. faecalis* infections and redox status.

Cumin has been tested for antibacterial properties against several microbes. Cumin essential oil kills multidrug-resistant *Staphylococcus aureus*, according to research. Essential oil disrupts bacterial cell membranes, causing cell death. Cumin also disrupts quorum sensing and biofilm growth, which are essential for bacterial pathogenicity. Cumin nanoemulsions are promising antibacterial agents. Cumin nanoemulsions kill MDR bacteria by penetrating cell membranes and deforming and killing cells. Because the active components are more stable and accessible in nanoemulsion form, they are more effectively delivered to the circulation. (Sharifi *et al.,* 2021)

By damaging bacterial cell membranes and limiting biofilm formation, cactus nanoemulsions are antimicrobial. Due to higher solubility and stability, nanoemulsions of cactus extract phytochemicals are more effective. Cactus nanoemulsions kill various bacteria, including drug-resistant *Staphylococcus aureus* and *E. coli*. Cactus phytochemicals are more effective in nanoemulsions due to their higher bioavailability (Ragab *et al.,* 2023).

Chicken gut microbiota consists of a multitude of microbial symbionts

longitudinally colonizing the gastro-intestinal tract, whose interactions with the host affect well-being and performance at several levels, including nutritional, immunological, and physiological (Diaz *et al.,* 2019) that has been applied to impact broiler production. However, previous AGP usage resulted in an increasing antimicrobial resistance (AMR) among microorganisms, which is regarded as a severe health issue for both animals and people alike (Munita and Arias, 2016). Vancomycin-resistant Enterococcus (VRE) is a significant source of numerous diseases in animals (Tan *et al.,* 2017). Van A is the most prevalent phenotype of VRE and displayed greater resistance to vancomycin and teicoplanin (Ozbak, 2018). Moreover, the van A gene produces a multifunctional protein, which plays a significant role in cell division, biofilm formation, resistance, and survival in Enterococcus (Ramirez-Arcos *et al.,* 2005). Erythromycin and tetracycline are medicinal drugs used for the treatment of enterococci infections. Undoubtedly, the development of resistant bacteria is the outcome of the broad use of these antibiotics (Tian *et al.,* 2019).

MATERIALS AND METHODS

1-Isolation and identification of Enterococcus spp. from broiler

Sampling:

A total of 100 samples were aseptically collected from cloacal swabs (n = 50), liver (n = 35), and joint (n = 15) samples. The samples were taken from lameness at four commercial chicken farms. The age of the broilers ranged from 2 to 45 days. All samples were kept in the icebox and transported to the laboratory for veterinary quality control on poultry production (RLQP) for bacterial isolation of Enterococcus spp.

Isolation and identification of Enterococcus spp.:

Isolation of Enterococci spp. was done according to Quiloan et al. (2012) using Slanetz Bartley agar, which is a selective medium used for the isolation and enumeration of Enterococci spp. The characterization of Enterococcus spp. was performed by biochemical tests including the Catalase test, Motility test, Gelatin hydrolysis test, Voges Proskauer test, litmus milk test, and Sugar fermentation test were done according to (Facklam and Teixeira *et al.,* 1998) for serotyping.

Antibiotic sensitivity

Antimicrobial susceptibility testing of enterococci to 8 different antibiotics, namely ampicillin (10 µg), vancomycin (30 µg), teicoplanin (30 µg), tetracycline (30 µg), erythromycin (30 µg), chloram-phenicol (30 µg), linezolid (30 µg), and ciprofloxacin (5 µg), was performed by disc diffusion method using Mueller–Hinton agar, and the test results were interpreted according to the Clinical and Laboratory Standards Institute (CLSI 2020) guidelines.

2- DNA and RNA Isolation for confirmation and antibiotic-resistant Genes:

The samples underwent DNA and RNA extraction using the pathoGene-spin™ DNA/RNA (Ref. Nos. 17154.2 and LOT Nos. 17152.20456).

Confirmation of Enterococcus spp. by using genus-specific primers (Ent tuf gene and 16s rRNA) using conventional PCR, the reaction mixture for each sample was: 10 µI of Cosmo master mix (Catalog No. W1020300x, Willow Fort, UK), 2 µI of forward and reverse primers Table (1), and 1 µI from nuclease-free water. Finally, add the 5 µI DNA extracted from the sample and display. The reaction was one cycle of initial denaturation at 95°C for two minutes. This is followed by 35 cycles consisting of denaturation at 95°C for 15 seconds, annealing at 55°C for 20 seconds, and extension at 72°C for one minute. Finally, there was one cycle at 72°C for ten minutes. Products for amplification Agarose gel electrophoresis were used to

examine the PCR, and ethidium bromide staining and a gel documentation system were used to visualize the results.

3- Cumin oil (10%) and cactus extract nanoemulsion (15%)

Preparation of nanoemulsion. Cumin oil 100% concentration was purchased from the National Research Center (NRC), cactus extract 100% concentration from Chemajet Company, Egypt. Tween 80 (surfactant) from Sigma-Aldrich Co., Egypt, and deionized water. 10 ml Cumin oil mixed with 10 ml tween 80 and 15 ml cactus extract mix with or 15 ml tween 80, respectively, and mix each solution with a Probe homogenizer (LK Lab, Korea) at 2500 rpm for 15 min. Then, deionized water was added slowly up to 100 ml to the mixed oil phase. Nano emulsion was performed in the Nanomaterials Research and Synthesis unit, AHRI according to El-Oksh *et al.* (2022).

Characterization of Cumin oil (10%) and cactus extract nanoemulsion (15%):

The nanoemulsion and measure electrical conductivity zeta potential (surface charge), both average particle size and distribution (polydispersity indexes PDI) of nanoemulsion using Microtrac, Wave ‖ (12.0.1.0). High-resolution transmission electron microscopy (HRTEM) observ-ations were performed with aJEM 1400F HRTEM at a beam energy of 300 keV. and using Fourier transform infrared spectro-scopy (FTIR) were analyzed fingerprints of functional groups at Research Park in Faculty of Agriculture, Cairo University.

Cytotoxicity Assay is a colorimetric assay that measures the reduction of yellow 3-(4,5-dimethythiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) by mitochon-drial succinate dehydrogenase. The MTT enters the cells and passes into the mitochondria, where it is reduced to an insoluble, coloured (dark purple) formazan product. The cells are then solubilized with an organic solvent (e.g. isopropanol) and the released, solubilized formazan reagent is measured spectrophotometrically. Read plate in ELISA Reader-measure OD in 570nm (background wavelength is 630nm) (Mosmann ,1983)

4- Determination of Minimum Inhibitory Concentration (MIC) of cactus and cumin nanoemulsions against five *E. faecalis* isolates using (MTT) assay.

The MIC of the 5 broilers chicken isolates of *E. faecalis* were tested by broth microdilution using 2 nanoemulsion. The (MTT) technique was utilized by (Raquena *et al.,* 2019). In brief, a sterile 96-well microtitration plate added 100 µl Muller Helton broth in all wells, 100 µl of stock solution of both the aqueous extract and the nanoemulsion of cactus and cumin were added to the first well. Subsequent two-fold dilutions were performed in rows on the plate, yielding concentrations from 100 ml for every extract. Added 100 µl of 1.5×106 CFU/ml concentration of bacterial inoculum at all wells. The plates were incubated to 24 hr. at (37) °C. After incubation, all wells received 10 µl from the MTT solution, and the plates were re-incubated for 4 hr. The first two rows of the plate were kept as negative (without bacterial and without plant extract inoculation inoculation) and positive control (without plant extract inoculation) rows. The MIC values were found to be the lowest active chemical concentrations at which no purple colour was seen.

5- Expression of antibacterial resistance genes by real-time PCR

The expression levels of enterococcus antibiotic resistance genes (van A, Tet K, and *Erm* B) as shown in Table (1), five *E. faecalis* isolates were determined by qRT-PCR utilizing a HERA SYBR® Green RT-qPCR Kit (Willow fort) and a real-time PCR detection system (Applied Biosystems). Amplification was performed using 10 µL reaction volumes containing 0.5 µL of each primer and RT enzyme

Mix, SYBR mix 5 µL, 1 µL of RNA, and 2.5 µL nuclease-free water. The subsequent conditions for thermal cycling were applied: reverse transcription at 55°C for 15 minutes, followed by activation at 95°C for 5 min, 40 cycles of denaturation at 95°C for 10 seconds, annealing (62°C for 16srRNA and 60°C for (*van* A, *Tet* K, and *Erm* B) for 30 seconds, and extension at 60°C for 30 seconds.

Table (1): All Primers used in this study (conventional and gene expression)

Primer name	Forward and Reserve	Annealing Temperatures	Amplified region	References
Housekeeping gene *16s* rRNA	F: CCGAGTGCTTGCACTCAATTGG R: CTCTTATGCCATGCGGCA TAAAC	62	(137 pb)	Bolhari et.,2018)
Ent *tuf* gene	F: TACTGACAAACCATTCATGATG R: AACTTCGTCACCAACGCGAAC	55	(112pb)	(Ke et al. 1999)
***Van* A gene**	F: GCCGGAAAAAGGCTCTGAA R: TTTTTGCCGTTGTTTCCTGTATCC	60	(90 pb)	(He *et al.,* 2020)
***Tet* K gene**	F: GATAGGAACAGCAGTATATGGAA R: AGATCCTACTCCTTGTACTAACCT	60	(164 pb)	(Wajda et al 2022)
***Erm* (B) gene**	F:ACTACTTAGGATGATGTCGTGGAA R: CCCTGAACAATTGGTGGCATA	60	(188 pb)	

6- Estimation of Oxidative Stress and Antioxidant Enzymes:

The homogenates of *E. faecalis* alone or treated with natural nanoemulsions (Cumin-Nps and/or Cactus-Nps) were centrifuged, and the cell free supernatants from MTT assay were collected. The supernatants were used for oxidative stress; malondialdehyde (MDA), Nitric oxide (NO) and the activities of the antioxidant enzymes; superoxide dismutase (SOD), catalase (CAT), glutathione reduced (GSH) and total antioxidant capacity (TAC). Parameters were measured using kits (Bio diagnostics, Egypt) according to the manufacturer's instructions references based on Okhawa *et al.,* (1979); Montgomery & Dymock (1961); Nishikimi *et al.,* (1972); Aebi (1984); Eutler *et al.,* (1963) and Koracevic *et al.,* (2001), respectively.

7- Statistical analysis:

The statistical analysis and imaging were carried out using Microsoft Excel software. Using the 2−ΔΔCT method, the target genes' relative expression was evaluated in the control group. The average CT values of the target genes were deducted from those of the endogenous control gene 16srRNA to obtain the ΔCT values (Livak, Schmittgen, 2001). Oxidative stress and antioxidant enzymes data were analyzed statistically via one-way ANOVA test and then Duncan's multiple range test. The results have been given as (mean ± SE) using SPSS 22.

RESULTS

1-Isolation and sensitivity tests: Among 100 samples collected from cloacal swabs (n = 50), liver (n = 35) and joints (n = 15), the prevalence ratio of Enterococcus spp. in all examined samples in broilers of different ages was 30% (30/100) (Table 2). Forty-two percent (21/50) of cloacal swab samples, seventeen percent (6/35) of liver samples, and twenty percent (3/15) of joint samples containing Enterococcus isolates, which were identified into four species. The predominant species were

Enterococcus faecalis 15 (50 %), followed by *Enterococcus faecium* 11 (36%), *Enterococcus hirae* 3 (10%), and *Enterococcus durans* 1 (3.33%).

The highest resistance rate was observed to Ampicillin (100%) and erythromycin (100%), followed by Chloramphenicol (86%), tetracycline (70%), linezolid (60%), Ciprofloxacin (20%), teicopio (13%), and vancomycin (10%) for isolates were presented in Table (3).

Table 2: Prevalence and serotyping of 30 selective strains Enterococcus spp. isolated from the diseased broiler chickens.

Enterococcus serotype	Source			
	cloacal swabs	liver	Joint	total
E. faecalis	12	2	1	50 % (15/30)
E. faecium	6	4	1	36 % (11/30)
E. hirae	2	_	1	10 % (3/30)
E. durans	1	_	_	3.33 % (1/30)
Total	42% (21/50)	17% (6/35)	20% (3/15)	30%(30/100)

%: was calculated according to the corresponding number (No.) of isolates
The distribution of antimicrobial resistance to eight antimicrobial agents in the 30 Enterococcus spp.

2- Identification of *Enterococci* spp. using genus-specific primers for (tuf) gene

All detected enterococcus isolates were amplified by PCR using the *Enterococcus tuf* gene. After ethidium bromide staining and electrophoresis, amplicons were found in agarose gels (112 bp)

Table 3: Antibiotic resistance profile of isolates of Enterococcus spp.

Antibiotic Profile	Resistance (R)	Intermediate (I)	Sensitive (S)
Ampicillin	30\30 (100%)	-	-
Chloramphincol	26\30 (86%)	-	4\30 (14%)
Ciprofloxacin	6\30 (20%)	16\30 (53%)	8\30 (27%)
Erythromycin	30\30 (100%)	-	-
Tetracycline	21\30 (70%)	5\30 (17%)	4\30 (13%)
Vancomycin	3\30 (10%)	1\30 (3%)	26\30 (87%)
linezolid	18\30 (60%)	-	12\30 (40%)
Teicopio	4\30 (13%)	-	26\30 (87%)

3- Characterization of cumin and cactus nanoemulsions

Cactus and Cumin nanoemulsions had a narrow size distribution of 7.03±0.309 nm and 11.48±0.37 nm respectively. HRTEM analysis confirmed the absence of aggregation and the presence of size uniformity and spherical morphology with polydispersity index and zeta potential of 0.9 and +16.6 mV; 0.6 and -17mV; respectively (Figure 1A-B).

Cytotoxicity Assay:

Cactus and Cumin microemulsions exhibited concentration-dependent effects on cell viability. IC50 was determined to be 114.4 and 109.2 ug/ml, respectively as shown in Fig. (2A).

FT-IR Analysis of Cactus and cumin, oil and its nanoemulsions

The process of interpreting FTIR spectra entails the examination of the peaks in the spectrum to determine the molecular structure and functional groups of the compound. The following are several critical factors that will assist in interpreting an FTIR spectrum:

Around 4000-2500 cm-1, there is a region called the functional group region. In cactus and cumin extracts and their nanoemulsions, you can see peaks at 2926.48 cm-1 for OH, NH, CH, and other functional groups. In cumin extract, however, peaks at 2924.66 and 2871.31 cm-1 don't appear in the nanoemulsions. Between 2500 and 2000 cm-1, there is a region that contains **triple bonds* (C≡C, C≡N). In both the nanoemulsions and the nanoemulsions, with the exception of cumin extract, where peaks at 2070.40-2078.47 cm-1 are present.

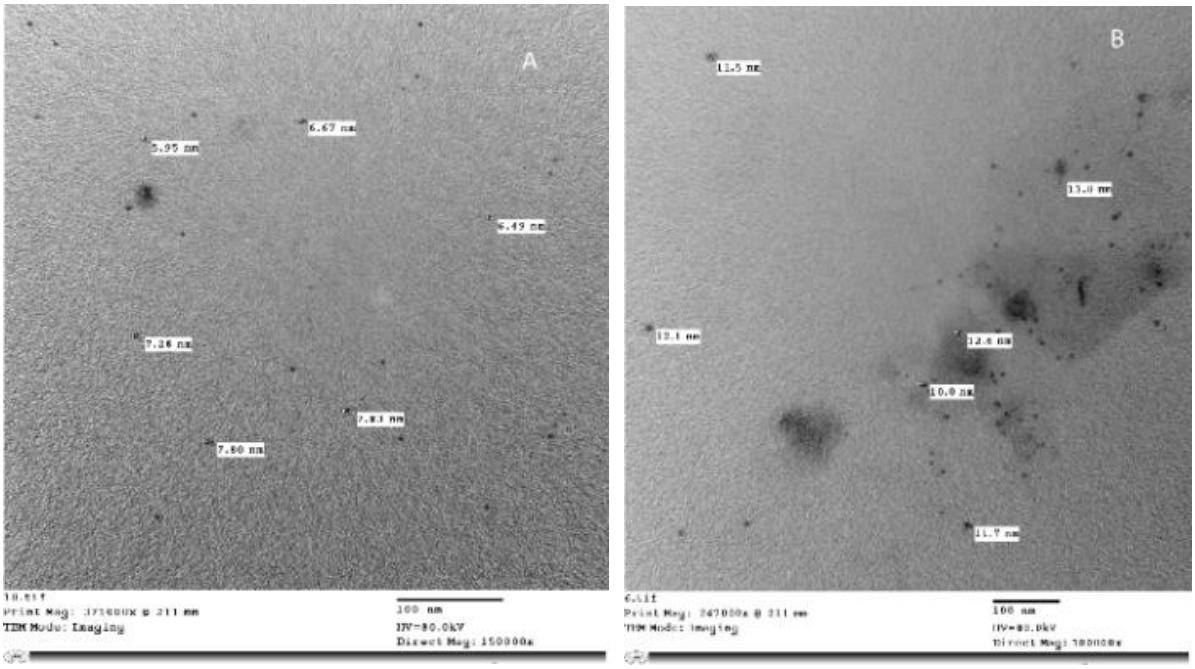

Fig. (1) HR TEM revealed that droplets size (**A)** Cactus extract nanoemulsion was 7.03±0.309 nm and (B) cumin oil nanoemulsion was 11.48±0.370 nm, there was no aggregation, size homogeneity and spherical nature.

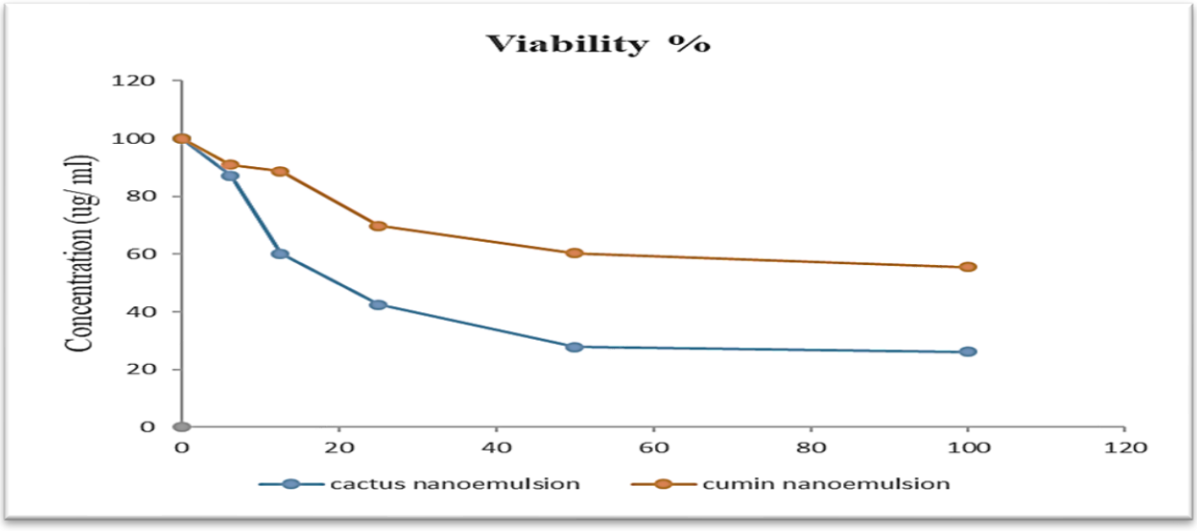

Fig. 2 (A): Cell viability % of Cactus and cumin nanoemulsions effect on Vero cells IC50 was determined to be 114.4 and 109.2 ug/ml respectively.

**Single bonds* and *bending vibrations* (C-C, C-O, C-N) fall within the range of 1300–500 cm-1. Peaks at 1448.55, 1380.64, 1300.88, 1128.53, 882.48, 832.11, 601.31, and 537.04 cm-1 were seen in cumin, but in its nanoemulsion, peaks at 1453.03, 1358.46, 1250.89, 1101.58, and 592.11 cm-1 were observed. The cactus nanoemulsions showed four new peaks at 1461.68, 1353.82, 1251.60, and 1096.93 cm-1. The cactus extract and nanoemulsion displayed a single peak at 579.61 cm-1, as shown in Fig. 2 (B).

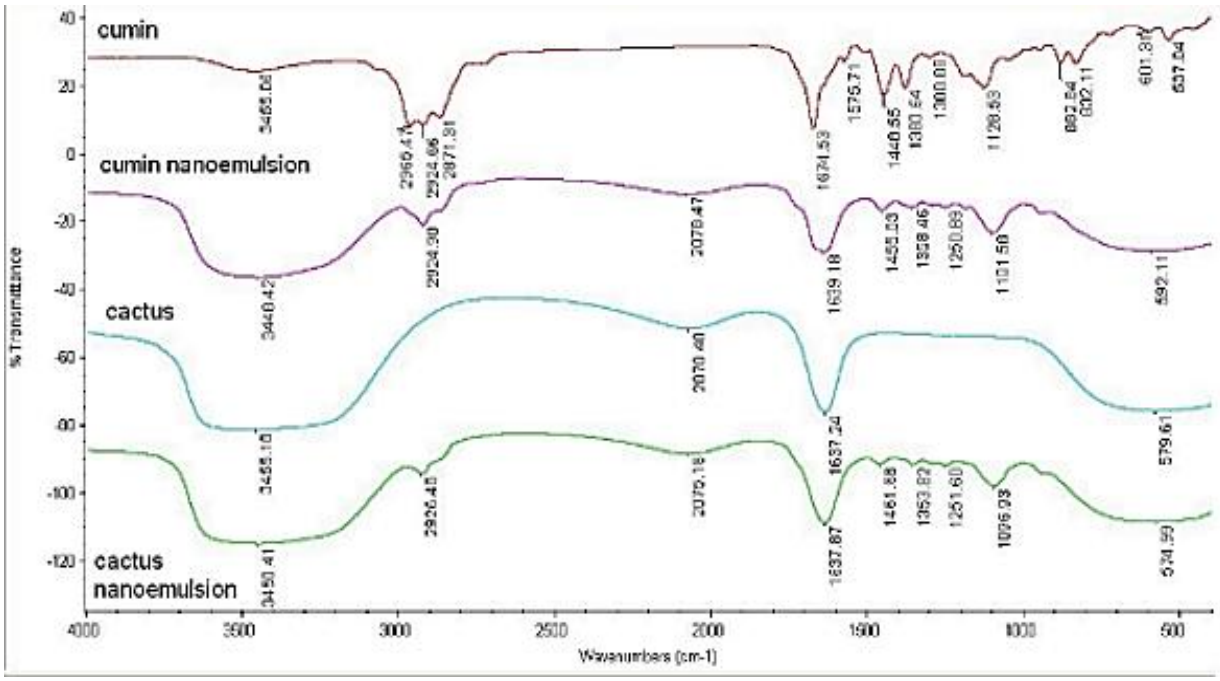

Fig. 2 (B): FT-IR Analysis of Cactus and cumin, oil and its nanoemulsions for functional groups

4- Minimum Inhibitory Concentration (MIC) of cactus and cumin nano-emulsions against five isolates of *E. faecalis*:

Cumin nanoemulsion had a more antibacterial effect against different isolates of E. faecalis with MIC values of (6.25 µg/ml, and 3.12 µg/ml) respectively, while the Cactus Nanoemulsion showed MIC values of 12.5 and 25 µg/ml for the same isolates as shown in Fig. (3).

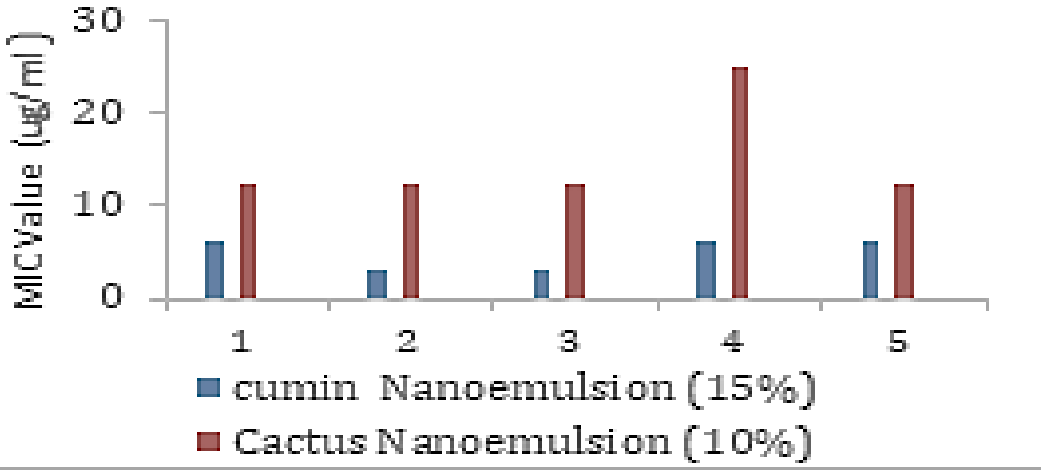

Fig. (3) Minimum Inhibitory Concentration (MIC) results of cactus and cumin nanoemulsions against five isolates of *E. faecalis*

5-Results of the cactus and cumin nanoemulsions MIC values Effect (6.25 µg/ml) on *E. faecalis* cells are shown by the TEM pictures:

TEM photos reveal that all untreated E. faecalis cells preserved their characteristic round morphology and had smooth, intact surfaces. After 24 hours, the cactus or cumin nanoemulsion infiltrated *E. faecalis* bacteria, we can view the structural picture of the degradation of the cell wall and identify *E. faecalis* cells treated with cactus or cumin nanoemulsion. Bacterial cells underwent rupture, lost their structural integrity, and dissolved entirely after 24 hours, as shown in Fig.(4).

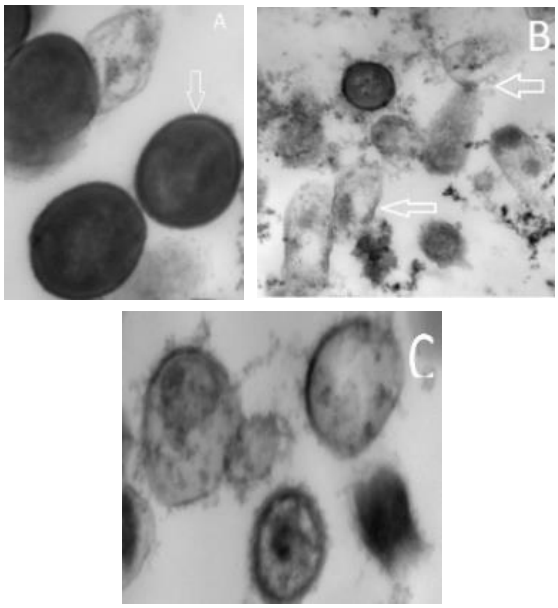

Fig.(4): Under the transmission electron microscopy (TEM) (A) *E. faecalis* bacteria morphology(B) release of *E. faecalis* bacteria cell content due to cactus nanoemulsions effect after 24 hrs (C) Effect of cumin nanoemulsions by *E. faecalis* bacteria after 24 hrs.

6- Oxidative Stress and Antioxidant Enzymes results:

Results showed that there were no significant differences between groups in Malondialdehyde (MDA) value, where *E.* faecalis treated with Cumin nanoemulsions showed the lowest level of MDA (9.39±0.59 nmol/ml); whereas there is a significant difference in the level of nitric oxide (NO) between groups, where the lowest level with E. faecalis + Cumin (12.5±0.97 µmol/L). Superoxide-dismutase (SOD) significantly decrease with both cumin and cactus NE treated groups (39.17±0.83 & 39.31±1.32 U/ml), respectively. There is no significant difference in the level of catalase (CAT), but the natural NE showed the highest value especially with cumin NE (18.41 ± 1.5 U/ml). The level of GSH is significantly increased with E. faecalis treated with natural nanoemulsions comparing with it alone, but cumin showed the highest value (9.22 ± 0.02 U/L). Total antioxidant capacity (TAC) showed the highest values with both Cumin and Cactus nanoemulsion (0.27 ± 0.09 & 0.11 ± 0.06 mM/L). Cumin NE showed the highest level of antioxidant enzymes; CAT (18.4 ± 1.5 U/L), GSH (9.22 ± 0.02 U/L) and TAC (0.27 ± 0.09 mM/L) whereas; it showed lowest level of oxidative stress; MDA (9.39 ± 0.59 nMol/ml) and NO (12.5 ± 0.97 µmol/L) comparing with Cactus- NE as shown in Table (4).

7- Results of expression of antimicrobial resistance genes in five *Enterococcus faecalis* isolates treated with cumin, cactus nanoemulsion

The results of quantitative gene expression real-time PCR compared the expression of three antibiotic resistance genes (*van* A, *Tet* K, and *Erm* B) in enterococcus spp. The treated isolates with cactus and cumin nanoemulsions. Three genes showed varying degrees of decrease in expression ($p < 0.05$); the *Erm* (B) gene was more affected by nanomaterials, followed by the effect of *Van* A, then *Tet* K genes had the lowest effect by nanomaterials, Fig (5).

Table 4: Oxidative stress and antioxidants enzymes biomarkers of five Enterococcus faecalis isolates treated with Cumin or Cactus nanoemulsions

Groups	MDA (nmol/ml)	NO (µmol/L)	SOD (U/ml)	CAT (U/L)	GSH (U/L)	TAC (mM/L)
E. faecalis	9.72 ± 0.77 [a]	21.5 ± 1.6 [a]	60.86 ±1.23 [a]	14.98 ± 1.11[a]	6.93 ± 0.21 [c]	0.09 ± 0.05 [a]
E. *faecalis* + Cumin nanoemulsion	9.39 ± 0.59 [a]	12.5 ± 0.97 [c]	39.17±0.83 [b]	18.41 ± 1.5 [a]	9.22 ± 0.02 [a]	0.27 ± 0.09 [a]
E. *faecalis* + Cactus nanoemulsion	9.52 ± 0.35 [a]	15.9 ± 0.37 [b]	39.31±1.32 [b]	15.34 ± 1.06[a]	8.79 ± 0.08 [b]	0.11 ± 0.06 [a]
Sig.	N.S.	**	***	N.S.	***	N.S.

MDA; Malondialdehyde, NO; Nitric Oxide, SOD; superoxide dismutase, CAT; Catalase, GSH; Glutathione reduced, TAC; Total antioxidants capacity. N.S.; non-significant. Data are expressed as Mean ± SE, n = (5); (a:c), different superscript letters in the same column means significantly ($P \leq 0.05$).

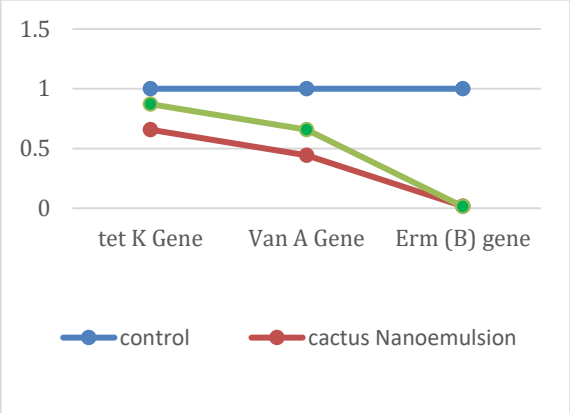

Fig. (5) Relative gene van A, Tet K, and Erm B expression of enterococcus treated with cumin, cactus nanoemulsion, and control (Fold change). Values are expressed as the mean ±SD ($p < 0.05$).

DISCUSSION

The poultry industry's integrated production, processing, and distribution processes can vertically transfer antimicrobial-resistant germs from breeding chicks to their progeny (Seo *et al.,* 2018). Another study found that post-hatching young birds had multidrug-resistant bacteria (Moreno *et al.,* 2019).

Noh et al. (2020) found that *E. faecalis* isolated from broiler breeders was highly resistant to various antimicrobials and was considered reservoirs for the transmission of resistant isolates throughout the poultry industry. This difference in resistance rate may be due to the misuse of antimicrobials used for disease prevention or therapy in each farm.

This study revealed 31.1% Enterococci, which matches Mwikuma et al. (2023) in Zambia. Aslantaş *et al.* (2019) reported 78.1% from cloacal swab samples, whereas Hammam et al. (2023) reported 61%. More was found in 6 of 300 (2%) broiler cloacal samples (Unal *et al.,* 2020).

Experience and technical details during cloacal swab collection and laboratory analysis may explain discrepancies in isolation rates between our study and comparative studies. Enterococcus spp. was identified from broiler chickens at 46% of cloacal swabs, 17% of liver samples, and 20% of joint samples, according to Hammam et al. (2023), 61.8% from liver, spleen, and heart, 57.3% from intestine, and 10% from joint. Our results were lower than Velkers *et al.* (2011), who recovered 75% Enterococcus from the liver. We identified 20% less Enterococcus from joints than they did (60%).

Four Enterococcus species were recovered from broiler chicks in this investigation. The most common species was *E. faecalis* (50%)

due to the age of the birds examined, followed by *E. faecium* (36%), *E. hirae* (10.67%), and *E. durans* (3.33%), which was similar with Semedo-Lemsaddek *et al.* (2021). *E. faecium* was the most common species in broiler cloacal samples at 60.4%, (Unal *et al.,* 2017). *E. faecalis* was 37.9% prevalent compared to 10.5% for *E. faecium* (Mwikuma *et al.,* 2023). Aslantaş *et al*. (2019) found 87.8% *E. faecalis*, 28 (8.3%) *E. faecium*, and 8 (2.4%) *E. durans*. (04%), was identified most often by Liu et al. (2013). Changes in poultry type, chick supply, sampling methods, geographical disparities, study time, and isolation and identification processes may explain species levels throughout investigations.

Conventional polymerase chain reaction was used to validate all enterococcus isolates to quickly and accurately identify various bacterial populations.

Translation by the *Tuf* gene delivers aminoacylate tRNA to the ribosome. This gene is a good diagnostic target due to its ubiquitous presence in bacteria and strong nucleotide level conservation. Ke et al. (1999) found an amplification rate of (112 bp).

In this study, all isolates were resistant to ampicillin, erythromycin, and chloramphenicol, while other studies showed that *E. faecalis* isolates were more resistant to erythromycin, tetracycline, and ciprofloxacin but less resistant to chloramphenicol (Noh *et al.,* 2020). *E. faecalis* isolates showed high resistance to erythromycin (51.3%) and tetracycline (69.7%), medium to low resistance to ciprofloxacin (25%), and no resistance to ampicillin (Alzahrani *et al.,* 2022).

Our data demonstrated poor teicoplanin and vancomycin resistance of 13% and 10%, respectively, while another research found strong resistance of 75.0% and 87.5%, respectively (Ahmed *et al.,* 2020). In contrast, Stępień-Pyśniak et al. (2021) and Alzahrani et al. (2022) found intermediate resistance to vancomycin (2.6% and 3.3%, respectively), making it the preferred antibiotic for treating multidrug-resistant Enterococcus spp. infections in humans. Our study differs from others because commercial broiler farms use excessive and misuse antimicrobials, which can lead to resistance. The integrated broiler operation system can also vertically transmit *E. faecalis* isolates from broiler breeding chickens to retail chicken meat (Ha *et al.,* 2018).

Since bacterial antibiotic resistance is rising, herbal alternatives have been studied (Prabhakar, *et al.,* 2010). Antimicrobial plants like cumin (NP) have hydrophobic essential oils. Thus, they can break down bacterial cell wall and mitochondrial lipids and damage bacterial structures (Cowan,1999). Bacterial antibiotic resistance decreased in this research. Cactus essential oils may act non-specifically against the bacterial cell membrane, increasing antibiotic absorption or providing a multi-target impact (Yap *et al.,* 2014). This investigation validated this for *Erm* B and Van A genes (Sharifi. *et al.,* 2021). However, quantitative PCR test indicated that cumin and cactus did not substantially impact Tet K gene expression compared to the control (Shariffi *et al.,* 2021). Cactus and cumin synergistically increased vancomycin and erythromycin's antibacterial activity against (van A and *Erm* B genes), restoring antibiotic susceptibility in resistant bacteria. Gram-positive bacteria are commonly resistant to tetracycline due to the gene *tet* K. These energy-dependent membrane-associated proteins limit cell tetracycline accumulation (Speer *et al.,* 1992). Future research should use different-sized cumin and cactus nanoparticles to minimize resistance.

The improved cumin nanoemulsion formulation with 85 nm droplets, 0.021-0.1 PDI, and -18.5 mV has potential, according to Jabbar et al. (2024). *E. coli* and *S. aureus*

are strongly inhibited by cumin oil nanoemulsion. The negative zeta potential (−19.1 to −11.1) indicates that sonication interval affects nanoemulsion (NE) stability at all levels and with varied cumin oil and Tween 80 concentrations Nano-sized oil-loaded capsules with a clear core–shell structure showed homogeneous black cumin essential oil nanoemulsion dispensability in TEM. Inner light droplet color and external light dark sphere define the NE's O/W. Sharif et al. (2017) observed OSA starch-stabilized black cumin essential oil nanoemulsions killed Gram-positive bacteria. These nanoemulsions displayed nanosize distributions, with mean droplet diameters of less than 200 nm, and zeta potentials higher than -30, demonstrating substantial electrostatic repulsion between oil droplets and Gram-positive pathogenic bacteria antibacterial effectiveness.

Ranjbar et al. (2023) found a significant OH peak at 3369 cm−1 in the ATR-FTIR spectra of C. cyminum essential oil. Sp3's CH stretching vibration in alkanes is indicated by bands at 2960, 2925, and 2870 cm−1. Also, the bands at 2819 and 2721 cm−1 show C-H aldehyde. C=O stretching vibrations in aldehyde and ketones in EO are shown by strong peaks at 1702 and 1673 cm−1. These notable peaks showed higher C. cyminum EO aldehydes. The C=C skeletal vibration of aromatic compounds may explain the 1575 and 1461 cm−1 bands. The 1074 cm−1 band resulted from C-O stretching vibration. The band at 986 cm−1 represents C-H bending absorption, whereas the peak at 815 cm−1 represents benzene ring C-H vibration absorption. The 687 cm−1 band is caused by alkene vibration absorption.

Oxidative stress arises when ROS generation exceeds the body's detoxification capabilities. Cellular damage from this syndrome is linked to several illnesses. By neutralizing ROS, antioxidant enzymes like SOD and CAT reduce oxidative stress. Our results indicate the connection between oxidative stress and antioxidant enzyme levels *in E. faecalis*, especially when treated with Cumin or Cactus nanoemulsions, suggests therapeutic uses for these natural substances. Cumin and Cactus nanoemulsions reduced MDA, NO, SOD, and increased CAT, GSH, and TAC in *E. faecalis.*

In recent research, oxidative stress of *E. faecalis*, a robust pathogen that forms biofilms and causes chronic infections, were affected by cumin-NPs and Cactus-NPs. Nanoparticles can damage bacterial cell membranes and disturb biological processes, reducing bacterial growth and biofilm formation (Miglani and Tani-Ishii, 2021; Franzolin *et al.,* 2022; Banerjee, 2022).

Cumin extracts increase SOD and CAT activities in biological systems. Cumin-NPs may affect *E. faecalis'* antioxidant defense systems as a stress response to nanoparticle-induced oxidative damage (Hariprasath *et al.,* 2023). Due to its high vitamin (A, C, and E), mineral, and phenolic content, cactus is also antioxidant. These components boost the endogenous antioxidant defense system by increasing SOD and CAT synthesis, which protects against oxidative damage from *E. faecalis* and AgNPs (Abd El-Galil & Alkot, 2022; Mekky *et al.,* 2024). Cactus can considerably lower oxidative stress indicators and increase antioxidant enzyme activity, suggesting it may protect cells from oxidative stress (Abd El-Galil & Alkot, 2022; Lee *et al.,* 2022). These reported compatible with our results that cumin and Cactus nanoemulsions reduced oxidative stress and increased antioxidant enzyme activity, although their effects on *E. faecalis* remain unclear. Cumin and Cactus nanoparticles' antibacterial effect may cause bacteria oxidative stress while boosting the host's antioxidant defenses.

CONCLUSION:

The combination of *Enterococcus faecalis* with Cumin or Cactus nanoemul-

sions presents a promising avenue for enhancing antibacterial and antioxidant defenses and reducing oxidative stress. Both natural compounds exhibit significant antibacterial and antioxidant properties, which can be beneficial in therapeutic contexts, particularly in conditions associated with oxidative stress. However, direct comparisons of their effects on *E. faecalis* are lacking in the current *literature*. Further research is warranted to fully elucidate the specific differences in oxidative stress responses when treated with these nanoemulsions and the mechanisms of action and optimize the therapeutic applications of these interventions.

Article Information

ANIMAL ETHICS:
The Institutional Animal Care and Use Committee (ARC-IACUC) at the Agricultural Research Center has approved the protocol (ARC-AHRI-10024) after reviewing the ethical consideration of animal use and Animal Welfare that enforces good quality science and the human treatment of animals.

Data availability: YES
All authors declared that the materials described in the manuscript, including all relevant raw data, will be freely available to any researcher wishing to use them for non-commercial purposes, without breaching participant confidentiality.

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Authors contribution. All authors were responsible for designing the experiment. Dalia Elmasry conducted the synthesis and characterization of nanomaterials, while Mai and Shaimaa were in charge of the bacteriology part. Eman as responsible for the biochemistry section, Mohammed was responsible for the biotechnology and gene expression section. Each author contributed to their unique section of the work. All authors have reviewed and endorsed the final manuscript.

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تقييم دور المستحلبات النانومترية للصبار والكمون في السيطرة على سلالات الانتيروكوكس المقاومة للأدوية المتعددة في دجاج التسمين

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تعد مقاومة مضادات الميكروبات (AMR) تهديد لصحة الانسان والحيوانات في جميع أنحاء العالم. هذه الدراسة فحصت الخصائص المضادة للبكتيريا لمستحلبات النانومترية من الصبار والكمون على سلالات الإنتيروكوكوس المقاومة للأدوية المتعددة المعزولة من الدجاج االتسمين. تم العثور على أنواع الإنتيروكوكوس في ٣٠٪ من العينات وكانت نسبة الإصابة في المسحات البرازية ٤٦٪ (٥٠/٢١)، وفي عينات الكبد ١٧٪ (٣٥/٦)، وفي عينات المفاصل ٢٠٪ (١٥/٣). شملت عزلات الإنتيروكوكوس *E. faecalis* (50%) و*E. faecium* (36%)، و*E. hirae* (10%)، و *E. durans* (3.33%) .حيث كانت مقاومة بشكل كبير للأمبيسيلين والإريثروميسين (١٠٠,٠٪)، والكلورامفينيكول (٨٦٪)، والتتراسيكلين (٧١٪)، واللينزوليد (٦٢٪). أظهرت خصائص المستحلبات النانومترية (NE) للصبار والكمون ميزات NE المثلى، بما في ذلك حجم قطيرات صغير للغاية (٧,٠٣ و ١١,٤٨ نانومتر)، وانخفاضPDI ، وقياسات زيتا +١٣ و -١٧,٧، مع IC50 بقيمة ١١٤,٤ و ١٠٩,٢ ميكروغرام/مل. كان لمستحلب النانو من الصبار والكمون تركيزات مثبطة (MIC) تبلغ ٢٥ و٦,٢٥ ميكروغرام/مل ضد .*E. faecalis* . وقد اظهرت بكتيريا *Enterococcus faecalis* زياده فى مؤشر الإجهاد التأكسدي المالون الدهيد (٩,٧٢ نانومول/مل) وأكسيد النيتريك (٢١,٥ ميكرومول/لتر) ، إلى جانب ارتفاع نشاط إنزيم السوبر أكسيد ديسموتاز (SOD) (٦٠,٨٦ وحدة/مل). أظهر المستحلب النانونى للكمون انخفاضًا ملحوظًا في مستويات أكسيد النيتريك (١٢,٥ ميكرومول/لتر) وزيادة في نشاط الكاتالاز (١٨,٤١ وحدة/لتر) والجلوتاثيون المختزل (٩,٢٢ وحدة/لتر) وايضا مضادات الأكسدة الكليه (٠,٢٧ ملي مول/لتر). كما اظهر مستحلب الصبار النانونى قيمًا متوسطة مع نشاط قوي لكل من انزيمى السوبر اكسيد ديسموتيز والكتاليز مما دعم التوازن الاجهاد التأكسدي ومضادات الأكسدة. وتشير هذه النتائج إلى استجابة انزيمات مضادات الأكسدة عبر المجموعات ضد الإجهاد التأكسدي المصاحب لهذه البكتيريا. *E. faecalis*مثبط لتعبير جينات مقاومة المضادات الحيوية (Erm B، van A، و(Tet K، ومع ذلك، كان لجينات Tet K أقل تأثير. تمثل المستحلبات النانومترية للصبار والكمون حل قابل للتطبيق كبديل آمن للعوامل المضادة للبكتيريا الاصطناعية والمكملات المضادة للأكسدة لعلاج عدوى *E. faecalis* المقاومة للأدوية المتعددة .

كلمات الدالة: انتيروكوكوس، الدجاج، المستحلبات النانومترية لكمون وللصبار ، مقاومة متعددة للأدوية، التعبير الجيني، الإجهاد التأكسدي؛ مضادات الأكسده.