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In vitro evaluation of the effects of silver nanoparticles on *Enterococcus faecalis* cells' viability*

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Enterococcus faecalis* is a component of the gut microbiota of healthy chickens. It is also an opportunistic pathogen that has a serious economic impact on poultry production. The growing presence of multidrug-resistant (MDR) enterococci in poultry environments and products represents a major public health concern, therefore finding new methods to combat MDR bacteria has become urgent. The study aimed to determine the effect of silver nanoparticles (AgNPs) on *E.

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***faecalis* viability. Three *E. faecalis* strains were treated with AgNPs at different concentrations. One experimental group was treated with 1% Virkon™ S disinfectant instead of AgNPs. The results revealed no significant decrease in the viability of *E. faecalis* due to AgNPs in contrast to the control. The largest reduction was 10-11 p.p. Virkon™ S only slightly reduced the viability of bacterial cells: to 67% (33 p.p.) in *E. faecalis* ATCC 51299 and 61% (39 p.p.) in *E. faecalis* 1D (isolate from one-day-old chicks), compared to the control (cell viability, 100%). The results showed that AgNPs exhibited little activity against *E. faecalis*. Surprisingly, in some cases, viability increased with increasing AgNP concentrations. Further analyses are needed (e.g., metabolic, proteomic) to explain the mechanisms that allow *E. faecalis* to resist AgNPs.**

KEYWORDS: silver nanoparticles / AgNPs / *Enterococcus faecalis* / bacterial viability / chicken / disinfection

Enterococcus faecalis (*E. faecalis*) is a gram positive, facultatively anaerobic bacterium. It is commonly found in the *environment* and constitutes a natural element of the microbiota of the digestive tract, reproductive tract, and the skin of mammals and birds. Like other species within the genus *Enterococcus*, this pathogen is capable of causing infections under favorable conditions [Franz *et al.* 1999].

E. faecalis is among the most frequently isolated enterococci in poultry [Dolka *et al*. 2017, Stępień-Pyśniak *et al.* 2016] and poultry meat [Chingwaru *et al.* 2003]. *E. faecalis* predominated in the intestines of one-day-old chicks, and was also found in crop [Devriese *et al.* 1994] embryonated eggs, dead-in-shell embryos, and in samples related to the poultry environment [Dolka *et al.* 2017]. *E. faecalis* made up the greatest proportion of isolates found in broilers in intensive poultry production, in retail meat, and in abattoir samples [Molechan *et al.* 2019]. It was routinely found in human sewage and broiler feces, but rarely in broiler litter [Kuntz *et al.* 2004]. Olsen *et al.* [2012a] and Rikke *et al.* [2012] suggested that both vertical and horizontal transmission of *E. faecalis* may occur in chickens. Fertner *et al.* [2011] demonstrated that a few contaminated eggs or embryos had significant potential to rapidly spread *E. faecalis* infection to almost all chickens during hatching.

Over recent years, enterococci have been increasingly problematic in poultry pathology. E. faecalis has been shown to be involved in chicken embryo mortality [Dolka *et al.* 2017, Karunarathna *et al.* 2017], yolk sac infection [Stępień-Pyśniak *et al.* 2016], pulmonary hypertension syndrome [Tankson *et al.* 2001], first-week mortality syndrome [Olsen *et al.* 2012], amyloid arthropathy [Landman *et al.* 1999]. Furthermore, *E. faecalis* is associated with valvular endocarditis, septicemia, salpingitis, peritonitis, arthritis, and combinations of some of these conditions, in broilers [Gregersen *et al.* 2010]. *E. faecalis* is not only pathogenic for animals but may also have zoonotic potential, [Olsen *et al.* 2012] and may even be given the name superbug [Hasan *et al.* 2018]. Infections caused by this bacterium are a serious health risk for people, especially as a cause of nosocomial infections [Dai *et al.* 2018, Szczypta *et al.* 2016].

Enterococcal infections constitute a significant clinical and therapeutic problem in human and veterinary medicine. E. *faecalis* exhibits an intrinsic resistance to many commonly used antibiotics [Torres *et al.* 2018]. Furthermore, enterococci have

acquired resistance to glycopeptide antibiotics, including avoparcin, which was used extensively until 2006 as a growth-promoter in food-producing animals. Enterococcal infections in poultry are usually treated with antibiotics, but many are ineffective because of multidrug resistance among bacterial strains, and even to antibiotics not approved for use in animals. There is abundant proof that multi-drug resistant (MDR) E. *faecalis*, including VRE (vancomycin-resistant enterococcus), HLAR (high-level aminoglycoside resistance), and LRE (linezolid resistant enterococci) isolates [Wang *et al.* 2015, Tamang *et al.* 2017, Kim *et al.* 2019], exist in poultry and can be transmitted to humans. Furthermore, enterococci may contaminate poultry meat and poultry meat products, and due to their resistance to high temperatures, may remain alive even after fermentation, pasteurization, or cooking [Giraffa, 2002, Martínez *et al.* 2003, Sustackova *et al.* 2004].

The emergence of MDR enterococcal infections and the failures of antibiotic therapy have raised concerns in human and veterinary medicine worldwide [Dewulf 2018]. There is an obvious need to reduce the use of antibiotics in poultry husbandry and to reduce the prevalence of bacteria in poultry production facilities and poultry food. There has been a growing scientific interest in employing silver nanoparticles (AgNPs) as alternatives to antibiotics in the poultry industry [Abd El-Ghany *et al.* 2021, Al-Sultan *et al.* 2022].

Numerous studies have proved the antimicrobial activities of silver nanoparticles (AgNPs) against bacteria, fungi, and viruses [Radzig *et al.* 2013, Bayat, *et al.* 2021, López-Martín *et al*. 2022]. Interestingly, since antiquity, silver (Ag) has been used for various medical conditions, water purification, and food preservation. It was a major therapeutic agent in the prevention of infections until the introduction of antibiotics [Alexander, 2009, Rudakiya and Pawar 2017]. Recent advances in nanotechnology have been extensively incorporated into biomedical sciences [Jian *et al.* 2020].

Nanotechnology is an interdisciplinary science involving chemistry, physics, biology, engineering, and toxicology, that has developed rapidly thanks to the ability to manufacture new materials at a nanoscale level. AgNPs are nanoscale metal structures, with the diameter of the nanoparticles being within the range of 1–100 nm. The properties of the manufactured materials depend largely on the size of the particles from which the material is made [Albrecht *et al.* 2006]. They can have different shaped particles, such as triangles, rods, or spheres. These parameters determine the ability of the material to induce bactericidal effects.

The precise AgNP mechanism is currently unresolved. It seems to involve a number of pathways and lead to bacterial cell death due to interaction with bacterial membrane proteins, intracellular proteins, phosphate residues in DNA, and interference with cell division. AgNPs may adhere to and penetrate the bacterial cell wall leading to changes in the bacterial cell wall's permeability [Rai *et al.* 2012]. Other studies have reported that AgNPs cause oxidative damage to bacteria through an increase in reactive oxygen species (ROS) production [Markowska *et al.* 2013, Bankier *et al.* 2018, Schwass *et al.* 2018, Liao *et al.* 2019].

AgNPs exhibit a broad spectrum of activity against gram positive and gram negative bacteria, including *Enterococcus* sp. (E. *faecalis*), *Streptococcus* sp. (*S. mutans*), *Staphylococcus aureus*, coagulase-negative *Staphylococcus* (*S. epidermidis*), *Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa*, and *Enterobacter cloacae* [Sondi and Salopek-Sondi 2004, Gong *et al.* 2007, Roe *et al.* 2008, Kalishwaralal *et al.* 2010, Namasivayam *et al.* 2011, Guzman *et al.* 2012, Schwass *et al.* 2018, Kalińska *et al.* 2019, Yin *et al.* 2020]. Furthermore, AgNPs may be an option for treating infections caused by MDR bacteria, such as vancomycin-resistant *Enterococcus* spp., methicillin-resistant *E. faecium*, methicillin-resistant *S. aureus* (MRSA) - Schwass *et al.* [2018]. The graphene oxide-silver nanocomposite (GO-Ag) is a promising biocidal agent against bacteria commonly found in hospital environments, such as *E. faecalis* [de Moraes *et al.* 2015]. AgNPs exhibit antibiofilm activity against different bacterial pathogens, including *Enterococcus* sp. and *E. faecalis* [Roe *et al.* 2008, Schwass *et al.* 2018]. AgNP gel has been effective against *E. faecalis* and can be used to eliminate residual enterococcal biofilms during root canal disinfection [Arora *et al.* 2021]; however, the efficacy of AgNPs against E. faecalis biofilms may depend on the mode of application [Wu *et al.* 2014].

AgNPs have various applications in poultry production [El Sabry *et al.* 2018, King *et al.* 2018], for example, as antimicrobial agents for treating infections [Abd El-Ghany *et al.* 2021] and as growth promoting and immune-stimulating additives to improve health and bird performance [Sawosz *et al.* 2007, Fouda *et al.* 2021]. The silver-doped silica nanoparticles could be considered a reasonably safe dietary supplement for chicken broilers due to their anti-inflammatory, antimicrobial, and immune-stimulatory properties [Dosoky *et al.* 2021]. Chicks fed with AgNPs demonstrated increased body weight gain and muscle weight, improved feeding efficiency, and increased ash digestibility [Saleh i El-Magd 2018]. Most recent developments offer attractive potential benefits from nanoparticle-based poultry vaccines, 'smart drug' delivery systems and rapid pathogen detection methods [El Sabry *et al.* 2018]. There are also emerging concerns arising from dietary application of AgNPs in poultry [Loghman 2012]. Interestingly, AgNPs orally administrated to chicken hen may be translocated to their liver and subsequently transferred to egg yolk. This means that the oral administration of AgNPs through the hens' animal feed could be a source of consumer exposure to AgNPs, but it may also depends on the type of AgNPs (chemical form, shape, dimension) [Gallocchio *et al.* 2017]. Nanotechnology can be used to enhance the microbiological food safety and quality of poultry products prior to being supplied to consumers. Control strategies in the poultry industry consist of combined pre-harvest and post-harvest applications that aim to decrease foodborne pathogens on-farm and also minimize their introduction at poultry processing plants[King *et al.* 2018]. Morsy *et al.* [2014] demonstrated the activity of AgNPs incorporated into pullulan films (an edible polysaccharide polymer) to control S. aureus and Listeria monocytogenes on meat and poultry products. Other authors have highlighted the potential applications of AgNPs in disinfecting eggs and hatchers to reduce bacterial

contamination [Banach *et al.* 2016] in farms and abattoirs [Elsayed *et al.* 2020], in food packaging [Carbone *et al.* 2016, Kwon and Ko, 2022], in filters for tap water and air purification devices, and also in surface and water nano-enabled disinfection in animal husbandry, and textiles (protective clothing) [Thamilselvi and Radha 2017].

This study aimed to determine the influence of AgNPs on the viability of E. faecalis strains. In addition, one of the most commonly used disinfectants in hatcheries was tested instead of AgNPs. We also compared the Presto Blue test and XTT reagent, which were used to evaluate the viability of *E. faecalis*.

Material and methods

Bacterial cells preparation (Experiment 3)

Two reference strains of E. faecalis, ATCC 29212 and ATCC 51299, and one *E. faecalis* strain (1D) isolated from the yolk sacs of one day-old chicks were used. The bacterial strains were stored in a BHI (brain heart infusion) broth with 20% glycerol at -20°C, then thawed and rinsed with sterile distilled water to remove the glycerol. Each bacterial strain was added to 50 ml of nutrient broth medium (Bio-Rad, Warsaw, Poland) in sterile glass flasks. The flasks were placed in a rotating incubator (Stuart Shaking Incubator SI500; Cole-Parmer Ltd, Stone, Staffordshire, United Kingdom] for 24 h. at 37°C.

Preparation of bacterial treatments with AgNPs, and with 1% Virkon™ S instead of AgNPs

Three experiments were carried out following modified methods presented by Kalińska *et al.* [2019]. Physicochemical properties of tested AgNPs were similar to their results and did not differ from the data available in references provided by other authors.

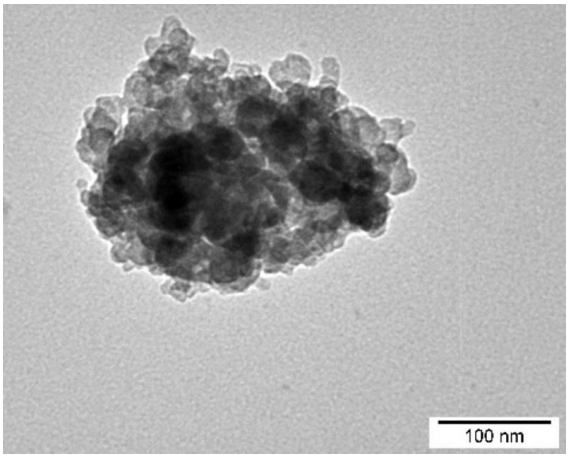
In the first experiment, a colloidal solution of AgNPs at a concentration of 50 ppm (i.e. µg/ml, mg/L, mg/kg) was used (batch number 66E/06/2017, Nano-Tech, Poland). Flasks containing 50 ml of nutrient broth (Bio-Rad, Poland) were prepared for the control (C) and experimental groups (Ag0.5, Ag1, Ag2, Ag5). The three control groups contained nutrient broth and 100 µl of bacterial suspension (*E. faecalis* ATCC 29212, ATCC 51299, and 1D). Each assay was performed three times for accuracy. In the experimental groups, 0.5 ml, 1 ml, 2 ml, and 5 ml of nutrient broth were removed from the flasks. In the next step, 0.5 ml, 1 ml, 2 ml, and 5 ml of colloidal solution containing AgNPs was added to the flasks in order to obtain the following AgNP concentrations: 0.5 ppm, 1 ppm, 2 ppm, and 5 ppm, respectively. The experimental groups were labeled as Ag0.5, Ag1, Ag2, and Ag5. Each of the flasks with designated concentrations of AgNPs was prepared in triplicate.

A further experimental group (V) contained 1% Virkon™ S (Bayer Animal Health, Germany) instead of AgNPs. Three flasks containing 0.5 g Virkon™ S and 50 mL of nutrient broth were prepared; then 100 µl of each E. faecalis strain (ATCC 29212, ATCC 51299, 1D) was added. Each assay was determined three times.

In the second experiment, different colloidal solutions of AgNPs (69E/10/2017, Nano-Tech, Poland) were used for E. faecalis ATCC 51299 and 1D. There was also the V group, which consisted of E. faecalis ATCC 51299 and 1D, and 1% Virkon™ S (instead of AgNPs).

In the third experiment, a bacterial solution of E. faecalis ATCC 29212 was treated with AgNPs (66E/06/2017, Nano-Tech, Poland), and separately with Virkon™ S (group V). Unlike the other two experiments, this third experiment used a different method for reading the results.

Example photographs of AnNPs at a concentration of 5 ppm taken with a transmission emission electroscope (TEM) are included in Photograph 1.



Photograph 1. AnNPs at a concentration of 5 ppm presented using TEM (batch 69E/10/2017).

The two different batches of AgNPs used in the study differed in terms of pH and conductivity. The specifications for each batch were as follows: batch 66E / 06/2017 (used in the 1st and 3rd experiment), pH=6.98 and conductivity 24.70 µS; batch 69E / 10/2017 (used in the 2nd experiment), pH=6.89 and conductivity 23.01 µS. According to information from the Nano-Tech representative, these differences result from the water used in the production. The size of 70% of the nanoparticles present in the solution was in the range of 3 to 10 nm. No stabilizer was used for their production.

Nanoparticles and Virkon™ S activity evaluation

The Presto Blue test (Thermo Fisher Scientific, USA) was used to assess bacterial viability after exposure to either nanoparticles or Virkon™ S in the first and second experiment. Presto Blue reagent is a ready-to-use resazurin-based solution that can enter cells easily. This is a cell viability indicator that uses the reducing power of living cells. The reagent is modified by reducing the environment of the viable cells, turning from blue to pink in color and becoming highly fluorescent. The color changes

can thus be estimated using absorbance measurements. The control group (cells with 100% viability) and experimental groups had a volume of 90 μL and were located in a 96-well plate, to which 10 μL of Presto Blue reagent was added. The cell viability reagent was warmed to room temperature before use. The plate was incubated for 20 min. and then the absorbance was measured at 570 nm using a microtiter plate reader (Infinite M200, Tecan, Durham, NC, USA).

In the third experiment, a colorimetric XTT based assay (Roche Diagnostics GmbH, Germany) was used for the nonradioactive quantification of cellular proliferation and cell viability. This assay is based on the cleaving of yellow tetrazolium salt (XTT), which is then formed into an orange formazan dye by metabolically active cells. Therefore, this conversion only occurs in viable cells. This formazan dye is soluble in aqueous solutions and is directly quantified using a spectrophotometer. The control group and experimental groups had a volume of 100 μL and were located in a 96-well plate, to which 50 μL of XTT reagent was added. The plate was incubated for 4 h. and then the absorbance was measured at 450 nm using a microtiter plate reader (Infinite M200, Tecan, Durham, NC, USA).

Nanoparticles and Virkon™ S activity evaluation

The viability of bacterial cells was expressed as a percentage of the control group. The viability of the cells in the control group was presented as 100% and compared with the viability of the cells in the experimental groups. The obtained data were analyzed using one-way analysis of variance (ANOVA) in SPSS IMAGO 5.1. The differences between the groups were estimated using Duncan's test. The results were presented as average values with standard errors. Differences at $p \leq 0.05$ were considered statistically significant. Due to the number of groups, a Bonferroni correction was applied: 0.05:6=0.0083; 0.01:6=0.0016 (Tab. 1 and 2).

The effect of AgNP concentration and Virkon™ S (without AgNPs) on bacterial cell viability, as estimated using the Presto Blue Test (Experiments 1 and 2)

In the first experiment, statistically significant differences ($p \leq 0.01$) were demonstrated for all the tested *E. faecalis* strains (ATCC 29212, ATCC 51299, 1D) compared to the control group (Tab. 1).

Compared to the control group, the best results for decreasing the viability of bacteria were demonstrated for all the tested strains exposed to Virkon™ S (group V). Compared to the control group, the viability of the bacterial cells of E. faecalis ATCC 29212, ATCC 51299, and 1D strains treated with Virkon™ S decreased by 20, 33, and 39 percentage points (p.p.), respectively (Tab. 1).

In the case of the *E. faecalis* ATCC 29212 strain exposed to various concentrations of AgNPs (1 ppm, 2 ppm, 5 ppm), an increase in the viability of bacterial cells was observed to a level of 20, 3 18, and 18 p.p., respectively. However, in the case of *E. faecalis* ATCC 51299 and 1D strains, a decrease in the viability of the bacteria was noted. The greatest reduction in viability of ATCC 51299 cells was observed in the Ag2 group, showing an 11 percentage point (p.p.) decrease compared to the control.

Table 1. The comparison of the viability of various strains of Enterococcus faecalis bacteria under the influence of two commercial batches of AgNPs and Virkon™ S (without AgNPs treatment)

Group	Experiment 1 AgNPs batch 66E/06/2017 Presto Blue Test						Experiment 2 AgNPs batch 69E/10/2017 Presto Blue Test			
	E. faecalis ATCC 29212		*E. faecalis* ATCC 51299		*E. faecalis* 1D		*E. faecalis* ATCC 51299		*E. faecalis* 1D	
	LSM (%)	SE	LSM (%)	SE	LSM (%)	SE	LSM (%)	SE	LSM (%)	SE
C	100.0^{A}	2.62	100.0^{A}	3.98	100.0^{A}	1.71	100.0^{ab}	0.86	100.0^{ab}	2.25
V	79.5^{B}	6.70	66.5^{B}	1.63	60.6^{B}	0.38	107.3^{b}	1.67	101.7^{ab}	7.94
Ag0.5	99.5^{A}	2.08	94.7^{AC}	0.50	100.0^{A}	2.46	98.4^{a}	1.58	89.6^{a}	5.45
Ag1	120.0^{C}	0.98	95.3^{AC}	1.72	95.3^{A}	1.97	99.1^{ab}	0.70	104.0^{ab}	6.20
Ag2	103.2^{A}	1.84	89.3^{C}	0.94	93.6^{A}	3.65	101.3^{ab}	1.71	108.2^{ab}	0.51
Ag5	118.0^{C}	6.54	92.1^{AC}	1.45	90.5^{A}	1.75	104.5^{a}	3.49	112.3^{b}	1.64
p value	<0.001		<0.001		<0.001		0.020		0.049	

SM – least squares mean; SE – standard error.

Fig. 1. The results of the evaluation of the viability of Enterococcus faecalis, estimated using the Presto Blue test, in the research groups treated with AgNPs (groups Ag0.5, Ag1, Ag2, Ag5) or Virkon™ S (group V), and in the control group (C) (Experiment 1).

However, in the case of the 1D strain, the greatest reduction in viability was recorded in the Ag5 group, where it amounted to 10 p.p. compared to the control group (Fig. 1, Tab. 1).

In the second experiment, statistically significant differences in the viability of the *E. faecalis* ATCC 51299 ($p = 0.02$) and E. faecalis 1D ($p \leq 0.05$) strains were demonstrated (Tab. 1). For both strains in group V, bacterial viability increased by 7 and 2 p.p., respectively. However, under the influence of various concentrations of

AgNPs, both a growth and reduction in bacterial viability were observed. In the case of the *E. faecalis* ATCC 51299 strain, the greatest reduction in viability was 2 p.p. in the Ag.0.5 group, while the greatest increase in bacterial viability was 4.5 p.p. in the Ag5 group, compared to the control group. In the case of the *E. faecalis* 1D strain, the greatest reduction in viability was 10 p.p., which was the same for the Ag0.5 group, while the greatest increase in bacterial viability was 12 p.p., again in the Ag5 group, compared to the control group (Tab. 1, Fig. 2).

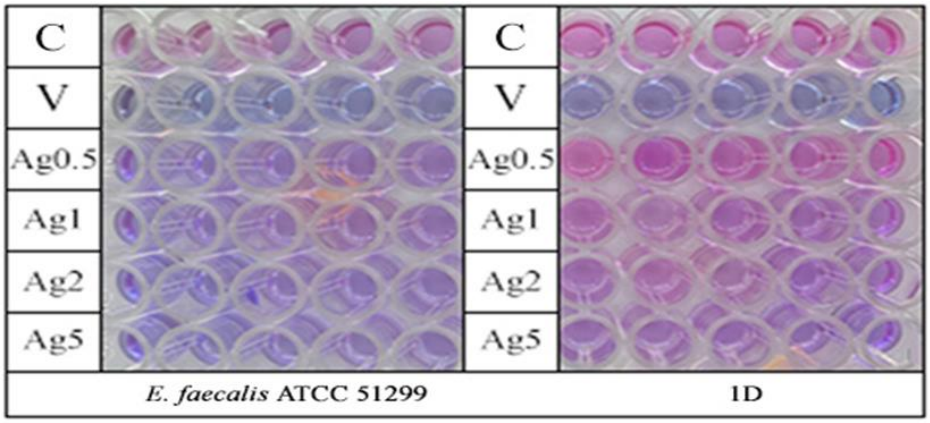


Fig. 2. The results of the evaluation of the viability of *Enterococcus faecalis*, estimated using the Presto Blue test, in the research groups treated with AgNPs (groups Ag0.5, Ag1, Ag2, Ag5) or Virkon™ S (group V), and in the control group (C) (Experiment 2).

The effect of AgNP concentration and Virkon™ S (without AgNPs) on bacterial cell viability, estimated using Presto Blue and XTT tests (Experiment 3)

In the third experiment, the viability of the E. faecalis ATCC 29212 strain was compared using the Presto Blue test and the XTT test (Tab. 2). The Presto Blue test results were the same as for the first experiment for this strain. The viability of the *E. faecalis* ATCC 29212 strain estimated using the XTT test in individual groups showed no statistically significant difference ($p>0.05$) (Tab. 2, Fig. 3). Bacterial cell viability decreased for Ag0.5 and Ag1 as measured by the XTT test. Both tests showed a decrease 5-15 p.p. in the viability of E. faecalis treated with Virkon™ S (group V).

This study investigated the impact of AgNPs (0.5 ppm, 1 ppm, 2 ppm, 5 ppm) and 1% Virkon™ S on the viability of Enterococcus faecalis strains (Tab. 1 and 2). The concentration of AgNPs needed to prevent bacteria growth may be different for each species of the same Gram-staining type [Morones *et al.* 2005]. Measuring changes in cell viability is a fundamental method for evaluating cell health, and a reliable indicator of cell viability or death.

Importantly, in our study the AgNP solutions did not considerably reduce the viability of *E. faecalis* cells compared to the non-treated control group (Tab. 2).

Table 2. The comparison of the viability of Enterococcus faecalis ATCC 29212 for each of the utilized tests (Presto Blue Test or XTT test)

Group	Experiment 3 AgNPs batch 66E/06/2017			
	Presto Blue Test *E. faecalis* ATCC 29212		XTT Test E. *faecalis* ATCC 29212	
	LSM (%)	SE	LSM (%)	SE
C	100.0^{A}	2.63	100.0	4.93
V	86.2^{A}	3.87	94.2	2.13
Ag0.5	127.1^{B}	2.98	94.4	5.12
Ag1	144.1^{BC}	7.05	96.0	5.95
Ag2	140.8^{B}	2.62	105.5	6.35
Ag5	163.8^{C}	7.68	101.3	5.09
p value	<0.001		0.575	

SM – least squares mean; SE – standard error.

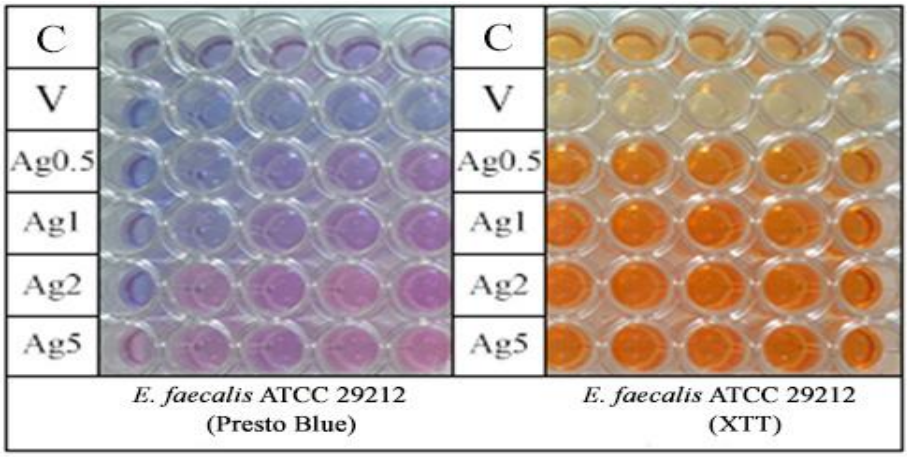


Fig. 3. The results of the evaluation of the viability of *Enterococcus faecalis*, estimated using Presto Blue and XTT tests, in the research groups treated with AgNPs (groups Ag0.5, Ag1, Ag2, Ag5) or Virkon™ S (group V), and in the control group (C) (Experiment 3).

Moreover, in some cases, the viability of *E. faecalis* increased with increasing AgNP concentrations. Surprisingly, the AgNP solution's environment might even promote the viability of bacteria. The most significant reduction in the viability of the *E. faecalis* cells under the influence of AgNPs was 10-11 percentage points compared to the control group. This reduction in viability was noted at a concentration of 2 ppm and 5 ppm AgNPs for one batch of nanoparticles (66E/ 06/2017). However, the same reduction in E. faecalis viability was obtained with a lower concentration of AgNPs (0.5 ppm), but when applied to a different batch of nanoparticles (69E/10/2017). The obtained test results indicated no biocidal effect in relation to the bacterial strains used.

These results differ from studies that generally confirm the toxicity of AgNPs for many bacteria species. In other studies, significant AgNP activity, compared to standard antibiotic drugs, has been observed for poultry pathogenic bacteria such as *E. coli, S. aureus, P. aeruginosa, and Salmonella typhi* [Lee *et al.* 2018]. Schwass *et al.* [2018] reported that AgNPs could be a useful agent against *E. faecalis*. The MIC (minimum inhibitory concentration) and MBC (minimum bactericidal concentration) of the AgNPs for *E. faecalis* were, respectively, 19.2 µg/mL (ppm), and 38.4 µg mL (ppm) [Schwass *et al.* 2018]. In a study by De Moraes *et al.* [2015], the MIC of GO-Ag for E. faecalis ATCC 29212 was 30 ppm. In the presence of GO-Ag nanocomposite, *E. faecalis* ATCC 29212 demonstrated a low MBC value of 60 ppm [de Moraes *et al.* 2015]. An antibacterial effect against *E. faecalis* (ATCC 29212) was reported by Namasivayam *et al.* [2011], who used much higher concentrations of AgNPs (20, 40, 60, 80 ppm) than those in our study. Also, another author demonstrated that AgNPs at concentrations of 75 ppm reduced the growth of different bacteria (*Pseudomonas aeruginosa, Vibrio cholera, E. coli, S. typhus*) - Morones *et al.* [2005]. Compared to the above data, it seems that the AgNP concentrations used in our study were too low. On the other hand, Kalińska *et al.* [2019] confirmed the activity of AgNPs, CuNPs (copper nanoparticles), and AgCuNPs (silver-copper nanoparticles), at the same concentrations of AgNPs as those used in our study (0.5, 1, 2, 2.5 ppm). They noted a decreased viability for *E. faecalis* and other pathogens (*E.coli, S. aureus, Enterobacter cloacae, Streptococcus agalactiae, and Candida albicans*).

In contrast to the results of Kalińska *et al.* [2019], our results did not confirm the efficacy of the same AgNP concentrations in reducing the viability of *E. faecalis.* Other authors also noted no impact from the administration of AgNPs (5, 15, 25 ppm) on intestinal microbial composition, including the number of enterococci in chickens [Vadalasetty *et al.* 2018]. Research conducted by Chiao *et al.* [2012] partially confirmed our results. Even though their research was conducted in vivo and on different bacteria (Salmonella spp.), they stated that AgNPs alone had no anti-Salmonella activity. A dose of 3.75 ppm of AgNPs, or even ten-fold higher, did not effectively reduce systemic infection or mortality caused by Salmonella spp. in specific pathogen-free (SPF) chicks after oral administration [Chiao *et al.* 2012]. Other authors have revealed that the administration of AgNPs to the drinking water of Japanese quails significantly increased the number of bacteria (*Lactobacillus spp., Leuconostoc lactis, Actinomyces naeslundii*), but had no effect on the *E. faecium* population. Furthermore, AgNPs did not have any negative effect on enterocytes in the duodenal villi [Sawosz *et al.* 2007]. In a more recent study, Al-Sultan *et al.* [2022] showed that populations of enterococcal bacteria decreased significantly in broiler chickens on day 28 with the dietary inclusion of AgNPs, but not ($p>0.05$) on day 42 using a dose of 20 ppm. What is more, they suggested that the dietary inclusion in broilers' diets of AgNPs at more than 2.5 ppm had many negative effects, represented by the accumulation of silver residue in the broilers' meat and the possibility of transmitting nano-silver to consumers. Also, AgNPs had a cytotoxic effect on internal organs in a dose-dependent

manner in broilers and, so, might be harmful to chicken and human health [Al-Sultan *et al.* 2022].

These contradictory results may be related to variations in the AgNPs' size, shape, dose, stability, exposure time, and preparation method (electrical, electrochemical, or chemical) [Rai *et al.* 2012]. According to the literature, the bactericidal effect of AgNPs is size dependent. AgNPs of size 10-100 nm exhibit a bactericidal effect against both Gram-positive and Gram-negative bacteria. However, smaller AgNP particle sizes (~1-10 nm) enable direct interaction with bacteria (adherence to the cell wall and penetration into the bacteria's cell), which in turn improves the antibacterial activity compared to other sizes of AgNPs [Morones *et al.* 2005, Agnihotri *et al.* 2014]. In our study, the size of 70% of the nanoparticles was 3-10 nm. Therefore, we expected to see a bactericidal effect in relation to the bacterial strain used; however, we did not obtain such results.

In addition, some of the properties of the bacteria may have had an influence on the results of the experiment. Gram-positive bacteria like *E. faecalis* have a thick (30 nm) peptidoglycan layer in the cell wall that makes them less susceptible to the toxicity of AgNPs. The stronger antibacterial effect of AgNPs on Gram-negative bacteria can be attributed to the thinner (2-3 nm) peptidoglycan layer in their walls [Rai *et al.* 2012, Cavassin *et al.* 2015]. AgNPs have exhibited bactericidal activity against Gram-negative bacteria with a MIC as low as 5 ppm, compared to 25 ppm for Gram-positive [Dilshad *et al.* 2020].

In light of other studies, the bacterial viability in our experiment was not inhibited, because the AgNP concentrations used were too low. *E. faecalis* is a Gram-positive bacteria and needs a minimum concentration of 25 ppm. What is more, another work showed that AgNPs have potential bactericidal effects against *E. faecalis* at concentrations of 5,000 ppm [Krishnan *et al.* 2015]. It is probable that the concentrations used in this experiment were too low to produce the same effect.

It is worth noting that Gram-positive bacteria have high amounts of amine and carboxyl groups in their cell membranes. Some reports have indicated that this is the reason why they are more susceptible to CuNPs [Beveridge and Murray 1980, Kruk *et al.* 2015]. CuNPs could be an interesting possibility for treating bacterial diseases in poultry caused by *E. faecalis*. But it is desirable to first conduct in vitro studies with the pathogenic bacteria *E. faecalis*.

Cavassin *et al.* [2015] observed, in vitro, that the inhibitory effect of AgNPs was stronger against Gram-negative than Gram-positive bacteria, and the best result was obtained using AgNPs stabilized with citrate and chitosan. Stabilization with natural polymers, such as chitosan, which has been used in the green synthesis of AgNPs, may be a good solution. The authors suggested that the chitosan-stabilized AgNPs may enhance bactericidal properties and biocompatibility against *E. faecalis*. In another study, the combined effect of AgNPs conjugated with gentamicin and chloramphenicol was noticeable against *E. faecalis*, and was higher compared to antibiotics or AgNPs alone [Katva *et al.* 2017]. Based on the above data, we suggest that AgNPs alone - as

in our experiment - without stabilization or other additive, for example, an antibiotic, may be insufficient to produce a bactericidal effect against *E. faecalis*.

In our study, the effect of AgNPs on the viability of E. faecalis was compared to 1% Virkon™ S. The first experiment showed that the Virkon™ S decreased the viability of each of the three examined strains of *E. faecalis*. On the other hand, in the second experiment Virkon™ S did not decrease the viability of *E. faecalis* ATCC 51299 or 1D, and even slightly (7 and 2 p.p. respectively) increased viability in contrast to the control group (Tab. 1). It is hard to explain what caused this difference. It is probable that the Virkon™ S solution was relatively unstable, so the storage or preparation conditions might have had an influence. However, in the literature we find information stating that 1% Virkon™ S is effective against vegetative bacteria like Pseudomonas aeruginosa, E. coli, S. aureus, Enterococcus hirae, and Mycobacterium smegmatis [Hernndez *et al.* 2000]. Sylte *et al.* [2017] also confirmed the antibacterial activity of Virkon™ S, in which Virkon™ S fully eliminated aerobic and anaerobic bacteria from pieces of eggshells. No bacterial culture was observed after 10 min. of treatment, but a shorter incubation period of 5 min. failed to effectively eliminate bacteria from turkey eggshells. However, Virkon™ S, as well as Oxysept-333, and CLS, had significant toxic effects on embryos ($p<0.05$) after 10- and 15-min. treatments.

In our study, we compared two commercially available viability reagents. Differences in the XTT test at $p\leq0.57$ were considered statistically insignificant. In the XTT test, a decrease in the bacteria's viability was observed in the case of the Ag0.5 and Ag1 groups; and in the Presto Blue test, the obtained values for all groups with AgNPs added was above 100%. The differences between these tests could relate to the fact that Presto Blue is a resazurin based reagent, while XTT is tetrazolium based. In Lall *et al.* [2013], the paper's authors compared Presto Blue and XTT in a test using various bacteria strains (*Streptococcus mutans, Prevotella intermedia, Cutibacterium acnes* (previously named *Propionibacterium acnes*), and *Mycobacterium tuberculosis*) for drug effectiveness control. The results clearly indicated that the obtained values for bacteria viability can differ according to the growth-indicator reagents used, such as Presto Blue or XTT.

Conclusions

We found that the AgNP solutions did not considerably reduce the viability of *E. faecalis* cells compared to the non-treated bacterial cells. The highest reduction in the viability of *E. faecalis* cells under the influence of AgNPs was 10 and 11 percentage points compared to the control group. This decrease in viability was noted at different concentrations of AgNPs (0.5 ppm, 2 ppm, and 5 ppm) depending on the batch of commercial nanoparticles used. Furthermore, AgNPs increased *Enterococcus faecalis* viability in some cases.

In comparison, Virkon™ S alone decreased the viability of *E. faecalis* cells by 20, 33, and 39 (E. faecalis ATCC 29212, ATCC 51299, 1D, respectively). Our results did not confirm previous studies in which AgNPs showed strong antibacterial effects;

however, these were usually at higher concentrations than those used in our study. We suggest that AgNP-based disinfectants at the tested concentrations would not be effective at reducing *Enterococcus faecalis* in poultry environments, or preventing infections caused by this enterococcal species. More experimental studies are needed to assess the bactericidal potential of using AgNPs against enterococci. There is a need to find a new approach to AgNPs synthesis that may be more effective against this group of bacteria.

Disclosures

The authors confirm that there are no known conflicts of interest associated with this publication and that there has been no significant financial support for this work that could have influenced its outcome.

REFERENCES

1. ABD EL-GHANY W. A., SHAALAN M., SALEM H.M., 2021 - Nanoparticles applications in poultry production: an updated review. ***World's Poultry Science Journal*** 77, 1001–1025.
2. AGNIHOTRI S., MUKHERJI S., MUKHERJI S., 2014 - Size-controlled silver nanoparticles synthesized over the range 5-100 nm using the same protocol and their antibacterial efficacy. ***RSC Advances*** 4, 8, 3974-3983.
3. AKRADI LOGHMAN., 2012 - Histopathologic and apoptotic effect of nanosilver in liver of broiler chickens. ***African Journal of Biotechnology*** 11, 22, 6207-6211.
4. AL-SULTAN S.I., HEREBA A. R. T., HASSANEIN K. M. A., ABD-ALLAH S.M.S., MAHMOUD U.T., ABDEL-RAHEEM S.M., 2022 - The impact of dietary inclusion of silver nanoparticles on growth performance, intestinal morphology, caecal microflora, carcass traits and blood parameters of broiler chickens. ***Italian Journal of Animal Science*** 21, 1, 967-978.
5. ALBRECHT M.A., EVANS C.W., RASTON C.L., 2006 - Green chemistry and the health implications of nanoparticles. ***Green Chemistry*** 8, 5, 417-432.
6. ALEXANDER J.W., 2009 - History of the medical use of silver. ***Surgical Infections*** 10, 3, 289-292.
7. ARORA S.S., SHETTY R., HEMAGIRIYAPPA M.S., THAKUR S.S., MISHRA N., LOKHANDE N.M., 2021 - Comparative Evaluation of Antibacterial Efficacy of Silver and Cadmium Nanoparticles and Calcium Hydroxide against Enterococcus faecalis Biofilm. ***Journal of Contemporary Dental Practice*** 22, 12, 1438-1443.
8. BANACH M., TYMCZYNA L., CHMIELOWIEC-KORZENIOWSKA A., PULIT-PROCIAK J., 2016 - Nanosilver Biocidal Properties and Their Application in Disinfection of Hatchers in Poultry Processing Plants. ***Bioinorganic Chemistry and Applications*** 2016.
9. BANKIER C., CHEONG Y., MAHALINGAM S., EDIRISINGHE M., REN G., CLOUTMAN-GREEN, E., CIRIC L., 2018 - A comparison of methods to assess the antimicrobial activity of nanoparticle combinations on bacterial cells. ***PLoS ONE*** 13, 2.
10. BAYAT M., ZARGAR M., CHUDINOVA E., ASTARKHANOVA T., PAKINA E., 2021 - In vitro evaluation of antibacterial and antifungal activity of biogenic silver and copper nanoparticles: The first report of applying biogenic nanoparticles against pilidium concavum and pestalotia sp. fungi. ***Molecules*** 26, 17.
11. BEVERIDGE T.J., MURRAY R.G.E., 1980 - Sites of metal deposition in the cell wall of Bacillus subtilis. ***Journal of Bacteriology*** 141, 2, 876-887.
12. CARBONE M., DONIA D.T., SABBATELLA G., ANTIOCHIA R., 2016 - Silver nanoparticles in polymeric matrices for fresh food packaging. ***Journal of King Saud University - Science*** 28, 4, 273-279.

13. CAVASSIN E.D., DE FIGUEIREDO L.F.P., OTOCH J.P., SECKLER M.M., DE OLIVEIRA R.A., FRANCO F.F., … COSTA S.F., 2015 - Comparison of methods to detect the in vitro activity of silver nanoparticles (AgNP) against multidrug resistant bacteria. ***Journal of Nanobiotechnology*** 13, 1, 1-16.
14. CHIAO S.H., LIN S.H., SHEN C.I., LIAO J.W., BAU I. J., WEI J. C., … SU H. L., 2012 - Efficacy and safety of nanohybrids comprising silver nanoparticles and silicate clay for controlling Salmonella infection. ***International Journal of Nanomedicine*** 7, 2421-2432.
15. CHINGWARU W., MPUCHANE S.F., GASHE B. A., 2003 - Enterococcus faecalis and Enterococcus faecium isolates from milk, beef, and chicken and their antibiotic resistance. ***Journal of Food Protection*** 66, 6, 931-936.
16. DAI D., WANG H., XU X., CHEN C., SONG C., JIANG D., … ZENG H., 2018 - The emergence of multi-resistant enterococcus faecalis clonal complex, CC4, causing nosocomial infections. ***Journal of Medical Microbiology*** 67, 8, 1069-1077.
17. DE MORAES A.C.M., LIMA B.A., DE FARIA A.F., BROCCHI M., ALVES O.L., 2015 - Graphene oxide-silver nanocomposite as a promising biocidal agent against methicillin-resistant Staphylococcus aureus. ***International Journal of Nanomedicine*** 10, 6847-6861.
18. DEVRIESE L.A., HOMMEZ J., POT B., HAESEBROUCK F., 1994 - Identification and composition of the streptococcal and enterococcal flora of tonsils, intestines and faeces of pigs. ***Journal of Applied Bacteriology*** 77, 1, 31-36.
19. DEWULF, J., 2018. *8 myths on antibiotic resistance disproved*. Jeroen Dewulf.
20. DILSHAD E., BIBI M., SHEIKH N.A., TAMRIN K.F., MANSOOR Q., MAQBOOL Q., NAWAZ M., 2020 - Synthesis of functional silver nanoparticles and microparticles with modifiers and evaluation of their antimicrobial, anticancer, and antioxidant activity. ***Journal of Functional Biomaterials*** 11, 4, 76.
21. DOLKA B., GOŁĘBIEWSKA-KOSAKOWSKA M., KRAJEWSKI K., KWIECIŃSKI P., NOWAK T., SZUBSTARSKI J., … SZELESZCZUK P., 2017 - Occurrence of Enterococcus spp. in poultry in Poland based on 2014-2015 data. ***Medycyna Weterynaryjna*** 73, 4, 220-224.
22. DOSOKY W.M., FOUDA M.M.G., ALWAN A.B., ABDELSALAM N.R., TAHA A.E., GHAREEB R.Y., … KHAFAGA A.F., 2021 - Dietary supplementation of silver-silica nanoparticles promotes histological, immunological, ultrastructural, and performance parameters of broiler chickens. ***Scientific Reports*** 11, 1.
23. EL SABRY M.I., MCMILLIN K.W., SABLIOV C.M., 2018 - Nanotechnology Considerations for Poultry and Livestock Production Systems - A Review. ***Annals of Animal Science*** 18, 2, 319-334.
24. ELSAYED M.M., ELGOHARY F.A., ZAKARIA A.I., ELKENANY R.M., EL-KHATEEB A.Y., 2020 - Novel eradication methods for Staphylococcus aureus biofilm in poultry farms and abattoirs using disinfectants loaded onto silver and copper nanoparticles. ***Environmental Science and Pollution Research*** 27, 24, 30716-30728.
25. FERTNER M.E., OLSEN R.H., BISGAARD M., CHRISTENSEN H., 2011 - Transmission and genetic diversity of Enterococcus faecalis among layer chickens during hatch. ***Acta Veterinaria Scandinavica*** 53, 1, 56.
26. FOUDA M.M.G., DOSOKY W.M., RADWAN N.S., ABDELSALAM N.R., TAHA A.E., KHAFAGA A.F., 2021 - Oral administration of silver nanoparticles-adorned starch as a growth promotor in poultry: Immunological and histopathological study. ***International Journal of Biological Macromolecules*** 187, 830-839.
27. FRANZ C. M.A.P., HOLZAPFEL W.H., STILES M.E., 1999 - Enterococci at the crossroads of food safety? ***International Journal of Food Microbiology*** 47, 1-2, 1-24.
28. GALLOCCHIO F., BIANCOTTO G., CIBIN V., LOSASSO C., BELLUCO S., PETERS R., … RICCI A., 2017 - Transfer Study of Silver Nanoparticles in Poultry Production. ***Journal of Agricultural and Food Chemistry*** 65, 18, 3767-3774.

29. GIRAFFA G., 2002 - Enterococci from foods. ***FEMS Microbiology Reviews*** 26*,* 2, 163-171.
30. GONG, J., SI, W., FORSTER, R. J., HUANG, R., YU, H., YIN, Y., … HAN, Y., 2007. 16S rRNA gene-based analysis of mucosa-associated bacterial community and phylogeny in the chicken gastrointestinal tracts: From crops to ceca. ***FEMS Microbiology Ecology*** 59*,* 1, 147–157.
31. GREGERSEN R.H., PETERSEN A., CHRISTENSEN H., BISGAARD M., 2010 - Multilocus sequence typing of Enterococcus faecalis isolates demonstrating different lesion types in broiler breeders. ***Avian Pathology*** 39, 6, 435-440.
32. GUZMAN M., DILLE J., GODET S., 2012 - Synthesis and antibacterial activity of silver nanoparticles against gram-positive and gram-negative bacteria. ***Nanomedicine: Nanotechnology, Biology, and Medicine*** 8, 1, 37-45.
33. HASAN K.A., ALI S.A., REHMAN M., BIN-ASIF H., ZAHID S., 2018 - The unravelled Enterococcus faecalis zoonotic superbugs: Emerging multiple resistant and virulent lineages isolated from poultry environment. ***Zoonoses and Public Health*** 65, 8, 921-935.
34. HERNNDEZ A., MARTRÓ E., MATAS L., MARTÍN M., AUSINA V., 2000 - Assessment of in-vitro efficacy of 1% Virkonρ against bacteria, fungi, viruses and spores by means of AFNOR guidelines. ***Journal of Hospital Infection*** 46, 3, 203-209.
35. JIAN W., HUI D., LAU D., 2020 - Nanoengineering in biomedicine: Current development and future perspectives. ***Nanotechnology Reviews*** 9, 1, 700-715.
36. KALIŃSKA A., JAWORSKI S., WIERZBICKI M., GOŁĘBIEWSKI M., 2019 - Silver and copper nanoparticles - an alternative in future mastitis treatment and prevention? ***International Journal of Molecular Sciences*** 20, 7.
37. KALISHWARALAL K., BARATHMANIKANTH S., PANDIAN S.R.K., DEEPAK V., GURUNATHAN S., 2010 - Silver nanoparticles impede the biofilm formation by Pseudomonas aeruginosa and Staphylococcus epidermidis. ***Colloids and Surfaces B: Biointerfaces*** 79, 2, 340-344.
38. KARUNARATHNA R., POPOWICH S., WAWRYK M., CHOW-LOCKERBIE B., AHMED K.A., YU C., … GOMIS S., 2017 - Increased Incidence of Enterococcal infection in nonviable broiler chicken embryos in western Canadian hatcheries as detected by matrix-assisted laser desorption/ ionization-time-of-flight mass spectrometry. ***Avian Diseases*** 61, 4, 472-480.
39. KATVA S., DAS S., MOTI H.S., JYOTI A., KAUSHIK S., 2017 - Antibacterial synergy of silver nanoparticles with gentamicin and chloramphenicol against Enterococcus faecalis. ***Pharmacognosy Magazine*** 13, 52, S828-S833.
40. KIM Y., BIN SEO K.W., SON S.H., NOH E.B., LEE Y.J., 2019 - Genetic characterization of high-level aminoglycoside-resistant Enterococcus faecalis and Enterococcus faecium isolated from retail chicken meat. ***Poultry Science*** 98, 11, 5981-5988.
41. KING T., OSMOND-MCLEOD M.J., DUFFY L.L. 2018 - Nanotechnology in the food sector and potential applications for the poultry industry. ***Trends in Food Science and Technology*** 72, 62-73.
42. KRISHNAN R., ARUMUGAM V., VASAVIAH S., 2015 - The MIC and MBC of silver nanoparticles against Enterococcus faecalis - A facultative anaerobe. ***Journal of Nanomedicine & Nanotechnology*** 06, 03.
43. KRUK T., SZCZEPANOWICZ K., STEFAŃSKA J., SOCHA R.P., WARSZYŃSKI P., 2015 -Synthesis and antimicrobial activity of monodisperse copper nanoparticles. ***Colloids and Surfaces B: Biointerfaces*** 128, 17-22.
44. KUNTZ R.L., HARTEL P.G., RODGERS K., SEGARS W.I., 2004 - Presence of Enterococcus faecalis in broiler litter and wild bird feces for bacterial source tracking. ***Water Research*** 38*,* 16, 3551-3557.
45. KWON S., KO S., 2022 - Colorimetric freshness indicator based on cellulose nanocrystal–silver nanoparticle composite for intelligent food packaging. ***Polymers*** 14, 17, 3695.
46. LALL N., HENLEY-SMITH C.J., DE CANHA M.N., OOSTHUIZEN C.B., BERRINGTON

D., 2013 - Viability reagent, prestoblue, in comparison with other available reagents, utilized in cytotoxicity and antimicrobial assays. ***International Journal of Microbiology*** 2013.

47. LANDMAN W.J.M., MEKKES D.R., CHAMANZA R., DOORNENBAL P., GRUYS E., 1999 - Arthropathic and amyloidogenic Enterococcus faecalis infections in brown layers: A study on infection routes. ***Avian Pathology*** 28, 6, 545-557.
48. LEE K.D., KUPPUSAMY P., KIM D.H., GOVINDAN N., MANIAM G.P., CHOI K.C., 2018 - Forage crop lolium multiflorum assisted synthesis of AgNPs and their bioactivities against poultry pathogenic bacteria in in vitro. ***Indian Journal of Microbiology*** 58*,* 4, 507-514.
49. LIAO C., LI Y., TJONG S.C., 2019 - Bactericidal and cytotoxic properties of silver nanoparticles. ***International Journal of Molecular Sciences*** 20*,* 2.
50. LÓPEZ-MARTÍN R., RODRIGO I., BALLESTA C., ARIAS A., MAS A., SANTOS BURGOS B., … BINNS C., 2022 - Effectiveness of Silver nanoparticles deposited in facemask material for neutralising viruses. ***Nanomaterials*** 12, 15, 2662.
51. MARKOWSKA K., GRUDNIAK A.M., WOLSKA K. I., 2013 - Silver nanoparticles as an alternative strategy against bacterial biofilms. ***Acta Biochimica Polonica*** 60, 4, 523-530.
52. MARTÍNEZ S., LÓPEZ M., BERNARDO A., 2003 - Thermal inactivation of Enterococcus faecium: Effect of growth temperature and physiological state of microbial cells. ***Letters in Applied Microbiology*** 37, 6, 475-481.
53. MOLECHAN C., AMOAKO D.G., ABIA A.L.K., SOMBORO A.M., BESTER L.A., ESSACK S. Y., 2019 - Molecular epidemiology of antibiotic-resistant Enterococcus spp. from the farm-to-fork continuum in intensive poultry production in KwaZulu-Natal, South Africa. ***Science of the Total Environment*** 692, 868-878.
54. MORONES J.R., ELECHIGUERRA J.L., CAMACHO A., HOLT K., KOURI J. B., RAMÍREZ J.T., YACAMAN M.J., 2005 - The bactericidal effect of silver nanoparticles. ***Nanotechnology*** 16*,* 10, 2346-2353.
55. MORSY M.K., KHALAF H.H., SHAROBA A.M., EL-TANAHI H.H., CUTTER C.N., 2014 - Incorporation of essential oils and nanoparticles in pullulan films to control foodborne pathogens on meat and poultry products. ***Journal of Food Science*** 79, 4.
56. NAMASIVAYAM S.K.R., GANESH S., AVIMANYU., 2011 - Evaluation of anti-bacterial activity of silver nanoparticles synthesized from Candida glabrata and Fusarium oxysporum. ***Internationa Joutna of Medicobiological Research*** 1, 3, 130-136.
57. OLSEN R.H., FRANTZEN C., CHRISTENSEN H., BISGAARD M., 2012 - An investigation on first-week mortality in layers. ***Avian Diseases*** 56, 1, 51-57.
58. OLSEN R.H., SCHØNHEYDER H.C., CHRISTENSEN H., BISGAARD M., 2012 - Enterococcus faecalis of human and poultry origin share virulence genes supporting the zoonotic potential of E. faecalis. ***Zoonoses and Public Health*** 59, 4, 256-263.
59. OLSEN, RIKKE HEIDEMANN, CHRISTENSEN, H., AND BISGAARD, M., 2012. Transmission and genetic diversity of Enterococcus faecalis during hatch of broiler chicks. ***Veterinary Microbiology*** 160*,* 1-2, 214-221.
60. RADZIG M.A., NADTOCHENKO V.A., KOKSHAROVA O.A., KIWI J., LIPASOVA V.A., KHMEL I.A., 2013 - Antibacterial effects of silver nanoparticles on gram-negative bacteria: Influence on the growth and biofilms formation, mechanisms of action. ***Colloids and Surfaces B: Biointerfaces*** 102, 300-306.
61. RAI M.K., DESHMUKH S.D., INGLE A.P., GADE A.K., 2012 - Silver nanoparticles: The powerful nanoweapon against multidrug-resistant bacteria. ***Journal of Applied Microbiology*** 112, 5, 841-852.
62. ROE D., KARANDIKAR B., BONN-SAVAGE N., GIBBINS B., ROULLET J., BAPTISTE., 2008 - Antimicrobial surface functionalization of plastic catheters by silver nanoparticles. ***Journal of Antimicrobial Chemotherapy*** 61, 4, 869-876.

63. RUDAKIYA D.M., PAWAR K., 2017 - Bactericidal potential of silver nanoparticles synthesized using cell-free extract of Comamonas acidovorans: in vitro and in silico approaches. ***3 Biotech*** 7, 2, 1-12.
64. SALEH A.A., EL-MAGD M.A., 2018 - Beneficial effects of dietary silver nanoparticles and silver nitrate on broiler nutrition. ***Environmental Science and Pollution Research*** 25*,* 27, 27031-27038.
65. SAWOSZ E., BINEK M., GRODZIK M., ZIELINSKA M., SYSA P., SZMIDT M., … CHWALIBOG A., 2007 - Influence of hydrocolloidal silver nanoparticles on gastrointestinal microflora and morphology of enterocytes of quails. ***Archives of Animal Nutrition*** 61*,* 6, 444-451.
66. SCHWASS D.R., LYONS K.M., LOVE R., TOMPKINS G.R., MELEDANDRI C.J., 2018 - Antimicrobial activity of a colloidal AgNP suspension demonstrated in vitro against monoculture biofilms: toward a novel tooth disinfectant for treating dental caries. ***Advances in Dental Research*** 29*,* 1, 117-123.
67. SONDI I., SALOPEK-SONDI B., 2004 - Silver nanoparticles as antimicrobial agent: A case study on E. coli as a model for Gram-negative bacteria. ***Journal of Colloid and Interface Science*** 275, 1, 177-182.
68. STĘPIEŃ-PYŚNIAK D., MAREK A., BANACH T., ADASZEK Ł., PYZIK E., WILCZYŃSKI J., WINIARCZYK S. 2016 - Prevalence and antibiotic resistance of enterococcus strains isolated from poultry. ***Acta Veterinaria Hungarica*** 64, 2, 148-163.
69. SUSTACKOVA A., NÁPRAVNÍKOVÁ E., SCHLEGELOVÁ J., 2004 - Antimicrobial resistance of enterococcus spp. isolates from raw beef and meat products. ***Folia Microbiologica*** 49, 4, 411-417.
70. SYLTE M.J., CHANDRA L.C., LOOFT T., 2017 - Evaluation of disinfectants and antiseptics to eliminate bacteria from the surface of Turkey eggs and hatch gnotobiotic poults. ***Poultry Science*** 96, 7, 2412-2420.
71. SZCZYPTA A., TALAGA K., BULANDA M., 2016 - Vancomycin-resistant enterococci as etiologic agents in healthcare-associatedinfections – pathogenicity and control methods. ***Hygeia Public Health*** 51, 2, 134-140.
72. TAMANG M.D., MOON D.C., KIM S.R., KANG H.Y., LEE K., NAM H.M., … LIM S.K., 2017 - Detection of novel oxazolidinone and phenicol resistance gene optrA in enterococcal isolates from food animals and animal carcasses. ***Veterinary Microbiology*** 201, 252-256.
73. TANKSON J.D., THAXTON J.P., VIZZIER-THAXTON Y., 2001 - Pulmonary hypertension syndrome in broilers caused by Enterococcus faecalis. ***Infection and Immunity*** 69, 10, 6318-6322.
74. THAMILSELVI V., RADHA K.V., 2017 - A Review on the diverse application of silver nanoparticle. ***IOSR Journal of Pharmacy (IOSRPHR)*** 07*,* 01, 21-27.
75. TORRES C., ALONSO C.A., RUIZ-RIPA L., LEÓN-SAMPEDRO R., DEL CAMPO R., COQUE T. M., 2018 - Antimicrobial resistance in Enterococcus spp. of animal origin. ***Microbiology Spectrum*** 6*,* 4.
76. VADALASETTY K.P., LAURIDSEN C., ENGBERG R.M., VADALASETTY R., KUTWIN M., CHWALIBOG A., SAWOSZ E., 2018 - Influence of silver nanoparticles on growth and health of broiler chickens after infection with Campylobacter jejuni. ***BMC Veterinary Research*** 14, 1, 1-11.
77. WANG Y., LV Y., CAI J., SCHWARZ S., CUI L., HU Z., … SHEN J., 2015 - A novel gene, optrA, that confers transferable resistance to oxazolidinones and phenicols and its presence in Enterococcus faecalis and Enterococcus faecium of human and animal origin. ***Journal of Antimicrobial Chemotherapy*** 70, 8, 2182-2190.
78. WU D., FAN W., KISHEN A., GUTMANN J.L., FAN B., 2014 - Evaluation of the antibacterial efficacy of silver nanoparticles against Enterococcus faecalis biofilm. ***Journal of Endodontics*** 40*,* 2, 285-290.
79. YIN I. X., ZHAO I.S., MEI M.L., LI Q., YU O.Y., CHU, C.H., 2020 - Use of silver nanomaterials for caries prevention: A concise review. **1***5 International Journal of Nanomedicine* Dove Press.