Journal of Science and Sustainable Development (JSSD), 2023, 11(1), 21-32

ISSN: 2304-2702 (print); 2414-4479 (online)
DOI: https://doi.org/10.20372/au.jssd.11.1.2023.0431

RESEARCH PAPER

Antibiofilm Activity of Green Synthesized Silver Nanoparticles

Seun Owolabi Adebajo[1*], Paul Olusegun Bankole[2], Abidemi Esther Ojo[1], Aderonke Kofoworola Akintokun[1] and Jesujuwon Akinsanya[1]

[1]*Department of Microbiology, Federal University of Agriculture, Abeokuta, Ogun State, Nigeria;*
[2]*Department of Pure and Applied Botany, Federal University of Agriculture, Abeokuta, Ogun State, Nigeria*
Corresponding Author:** Email: **adebajoso@funaab.edu.ng; seunadebajo63@yahoo.com

Abstract

Numerous diseases that affect both humans and animals are due to the growth of biofilms on a variety of inanimate surfaces. This study investigated bacterial biofilms in water and poultry drinkers and their control using silver nanoparticles (AgNPs). Water and swabs of poultry drinkers were collected from five poultry houses. Isolation and characterization of bacterial isolates were carried out using standard microbiological techniques and identified bacterial isolates were screened for biofilm potential using Congo Red Agar method (CRA) and tube method (TM). Antibiotic susceptibility of the isolates was carried out using the agar well diffusion method. Fungal isolate was used to synthesize AgNPs and the synthesized AgNPs was confirmed through visual, UV-Vis and FT-IR analysis. Fifty-nine bacterial isolates were identified and the predominant bacterial isolates were: Staphylococcus spp and Citrobacter spp. CRA revealed that fifteen isolates (25.4%) were biofilm formers while TM showed only 9 isolates (15.3 %) to be strong biofilm formers. All the biofilm formers were multi drug resistant isolates except Proteus vulgaris and high resistance was observed in gentamycin. The synthesized AgNPs was brown in colour, UV-Vis spectra was observed at 410nm and FT-IR revealed the presence of functional groups that are responsible for the synthesis of silver nanoparticles. AgNPs inhibited the growth of the biofilm formers with highest and lowest inhibition zone of 2.6 cm to 0.4 cm by Proteus vulgaris and Enterobacter aerogene. AgNPs are thus a crucial bio resource due to their capacity to suppress the establishment of bacterial biofilms.

Keywords: Biofilms, Congo red agar, Tube method, silver nanoparticles

Introduction

education and just ensure that there is water availability for their animals to drink but are generally unaware of the negative effects that poor hygiene has on both their animals and the general public. The quality of water is frequently disregarded, despite the fact that it is the most important nutrient for birds and serious disease outbreaks are seen by many farmers as more spiritual developments than those that emerge from farm hygienic issues (Folorunso *et al*., 2014). The most prevalent and successful forms of life on Earth are biofilms and are responsible for driving the biogeochemical cycling of the majority of elements in water, soil, sediment and subterranean habitats (Meckenstock et al., 2015). Biofilms are pervasive, long-lasting microbial communities that may form on almost any surface in contact with water. They can shed cells continuously, which aids in the spread of germs (Liu et al., 2016).

The biofilms' ability to build an exopolysaccharides matrix, which can hold and store substances including nutrients to offer food reserves for microbial members during a starving time contributes to the bacteria improved resistance (Liu et al., 2016). It is imperative to develop a strong treatment and prevention strategy to tackle biofilms because,

according to earlier study of Abdullah et al., (2016), about 61% of human biofilm infections are zoonotic in nature. The growing resilience of biofilm to standard treatments increases the need for innovative control measures. The hunt for naturally occurring substances/chemicals capable of preventing the formation of biofilms is a viable approach to controlling biofilms (Lizana *et al*., 2013).

Nanoparticles are biologically active substances that have served as an important source of natural products for the treatment and prevention of diseases, promoting human health (Salleh et al., 2020). Green synthesis of nanoparticles (NPs) display an excellent approach whereby NPs are produced through the oxidation/reduction of metallic ions by secreted biomolecules such as enzymes, proteins, sugars and carbohydrates in order to combat pathogens (Zhang *et al*., 2016). Saif *et al.,* (2016) reported that stable and well-functionalized nanoparticles from bacteria, actinomycetes, fungus and yeasts serve as environmentally friendly and sustainable precursors. Studies have indicated that silver nanoparticles are broad spectrum antiseptic that are effective against both Gram negative and Gram positive bacteria, fungi and viruses (Sharmin et al., 2021).

The oligo-dynamic impact of silver in ionic or nanoparticle form has a broad spectrumbroad-spectrum antibacterial activity and is particularly effective against microbial colorizations linked to biomedical illnesses and the anti-microbial properties of silver are known to fight a variety of harmful microbes (Ramasamy and Lee, 2016). Typically, silver nanoparticles (AgNPs) have a diameter of 1–100 nm and contain 20–15,000 silver atoms (Han et al., 2017). Silver nanoparticles have a greater surface area to volume ratio for interactions, which makes it easier for them to penetrate and damage bacterial cells (Duran et al.,. 2016). They have also been demonstrated to destroy biofilm matrices (Sinha *et al*., 2011) and their antibacterial properties include cell barrier contact, microbial cell penetration, diffusion and adsorption (Wang et al., 2017).

Due to paucity of information on the potential of AgNPs on biofilm formers from poultry drinkers and its bacterial identification; this study aimed to characterize bacteria in water and crevices of drinkers from poultry houses, screen isolates for biofilms, determine the antibiotic susceptibility of biofilm formers and control their growth using synthesized AgNPs.

Materials and methods

Sample collection

Fifteen samples each of water and poultry drinker swabs were collected from five poultry houses at Alabata and Odo–eran area in Abeokuta, Ogun State.

Isolation and identification of bacteria

Swabs and water samples were collected from poultry drinkers and serial dilutions were carried out using sterile saline. Aliquot of the samples were plated on Nutrient agar, MacConkey agar, Eosin methylene blue and *Salmonella shigella* agar sterile plates. The plates were incubated for 37°C for 24h and 48h, respectively and isolates were subcultured to obtain pure colonies. Pure colonies were identified using microscopic, physiological and biochemical characterization (Karim *et al*., 2018).

Production of AgNPs

Production of fungal biomass

Biomass of A. *flavus* obtained from water sample was prepared by introducing the spores into Erlenmeyer flasks containing 100 ml of sterile Potato dextrose broth, placed in an orbital shaker, incubated at 25°C and agitated at 100 rpm for 72 h. The biomass was harvested and then centrifuged at 3000 rpm for 40 mins. The filtrates obtained were discarded and the biomass (residue) was washed twice with sterile distilled water (Gaikwad and Gade, 2013).

Synthesis of AgNPs

Silver nitrate was prepared by dissolving 0.17g of silver nitrate into 1000 ml of distilled water. Biomass (10g) was inoculated into conical flasks containing 100 ml of distilled water and placed in the orbital shaker again at 100 rpm for 72 h. The mixture of the biomass and the distilled water was then centrifuged after which the resulting cell filtrate (50 ml) was mixed with 50ml of 1 mM silver nitrate solution. The flask was finally agitated in the orbital shaker for 72 h. A conical flask containing only the fungal biomass was also placed in an orbital shaker to serve as a control sample (Gaikwad and Gade, 2013).

Characterization of AgNPs

Visual observation

This was done using the development of color change as compared to the control.

UV-Vis spectroscopic analysis

The reduction of pure silver ions synthesized by fungal culture was monitored by measuring the UV- Vis spectrum of the reaction mixture in the range of 250–800 nm.

Fourier-Transform Infrared spectroscopy

The filtrate was freeze-dried and diluted with potassium bromide in the ratio of 1:100. The FTIR spectrum of sample was recorded on a FTIR instrument (model, maker and country) with diffuse reflectance mode. All measurements were carried out in the range of 4000–400 cm−1 at a resolution of 4 cm−1 (Saifuddin *et al*., 2009).

Screening of isolates for biofilm activity

Isolates from the water and poultry-drinkers were screened for the potential to produce biofilms using the congo red agar and tube assay.

Congo Red Agar (CRA) was prepared according to Thilakavathy et al. (2015) and composed of 37g/L of Brain heart infusion (BHI) broth, 10g/L agar base, 50 g/L sucrose, 1 L water and 0.8 g/L Congo red indicator. The pure isolate was inoculated on the sterile congo red agar plates and were incubated at 37 °C for 24 h. Positive results were indicated by black colored colonies which indicate biofilm producers while negative results were indicated by pink colored colonies.

Tube method

A loopful of bacterium was inoculated into tryptic broth and incubated at 37 °C for 72 h. After incubation, the broth was discarded and the test tubes were washed with phosphate buffer saline pH 7.2 and stained with safranin. The tubes were air-dried and observed for the occurrence of a visible film along the walls of the test tube which indicates the presence of biofilm. The empty tubes were graded visually as absent, moderate and strong biofilm formation respectively (Ugwoke *et al.,* 2019).

Antibiotic susceptibility tests of biofilm formers

Antibiotic susceptibility tests were carried out following the Kirby–Bauer disc diffusion method on Mueller–Hinton agar, as described by (Daniel *et al.,* 2020). The test organisms (positive biofilm formers) were swabbed each on the surface of the Mueller-Hinton agar plates. Then, a sterile forceps was used to place the Gram- negative antibiotic discs (Amoxicillin, Augmentin, Chloramphenicol, Gentamycin, Ciprofloxacin, Tarvid, Pefloxacin, Streptomycin, Sparfloxacin, Septrim) and Gram-positive antibiotics discs (Amoxicillin, Augmentin, Gentamycin, Ciprofloxacin, Erythromycin, Pefloxacin, Rocephin, Streptomycin, Septrim, Zinnacef) on the surface of the plates and were incubated at 37°C for 24 hrs. The results were interpreted according to the Clinical Laboratory Standards Institute Guidelines (CLSI, 2018).

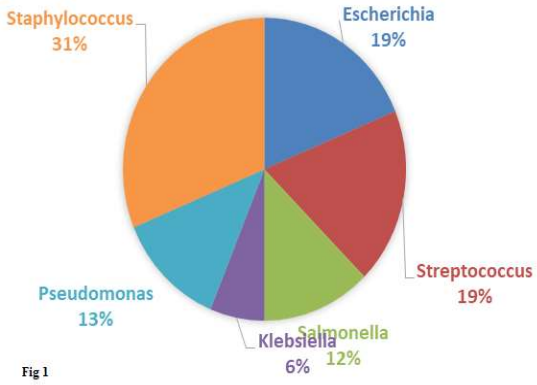
Application of silver nanoparticles (AgNPs) on biofilm formers Activity of the synthesized AgNPs on biofilms

Biosynthesized silver nanoparticles produced by A. *flavus* were assayed for antibacterial activity as suggested by Gudikandula et al. (2017) using the agar well-diffusion method. Bacterial biofilms formers (100 µl) in nutrient broth was used to prepare bacterial lawns (1 × 105 CFU/ml). Agar wells of 8 mm diameter were prepared with the help of a sterilized stainless steel cork borer. The wells were loaded with 100 µl of AgNPs and 100 µl of culture broth from A. *flavus* as control. The plates were incubated at 37 °C for 24 h and then were examined for the presence of zones of inhibition. The diameter of zones of inhibition was measured and the mean value for each organism was recorded.

A total of fifty-nine bacteria were isolated from both the water and drinkers sample. Sixteen bacterial isolates were recovered from the water samples while forty-three bacterial isolates were obtained from poultry drinkers. *Staphylococcus* spp and *Citrobacter* spp from water and poultry drinkers, respectively were the predominant isolates. *Klebsiella* spp had the least occurrence from poultry water samples (6%) while *Proteus* and *Escherichia* spp had the least occurrence from drinker samples (4%). All the bacterial isolates identified in water samples were also present in the poultry drinkers (*Pseudomonas, Streptococcus Salmonella, Staphylococcus, Klebsiella and Escherichia coli*).

Results

Identification of bacterial isolates



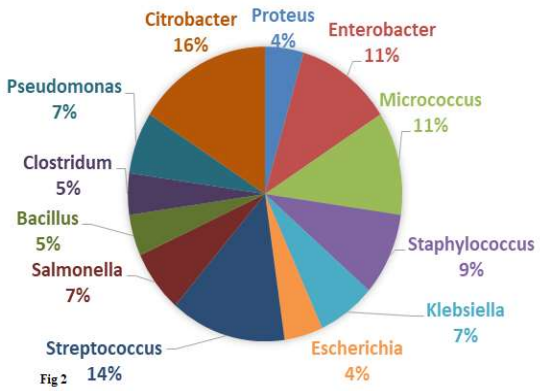


Fig 1: Percentages of different genera in water samples Fig 2: Percentages of different genera in poultry drinkers

Characterization of silver nanoparticles

The ultraviolet visible spectrophotometry showed the peak of absorbance surface plasma resonance spectrum of the solution. Visual observation of the filtrate to brown is an indication of silver nanoparticles produced after incubation as shown in Fig 3. Fig 4 shows the UV spectral of the synthesized nanoparticles. The absorption around the region of 400 nm is an indication of silver nanoparticles synthesis. The functional groups in the FTIR analysis suggested the synthesis of nanoparticles through the reduction of the silver nitrates (Fig 5).

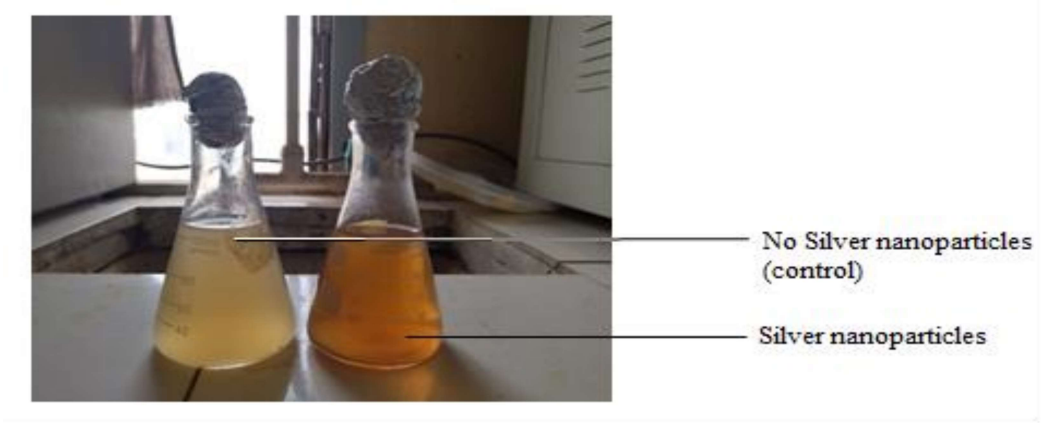


Fig 3: Colour change of the filtrate containing *Aspergillus flavus* (and AgNO3) to brown

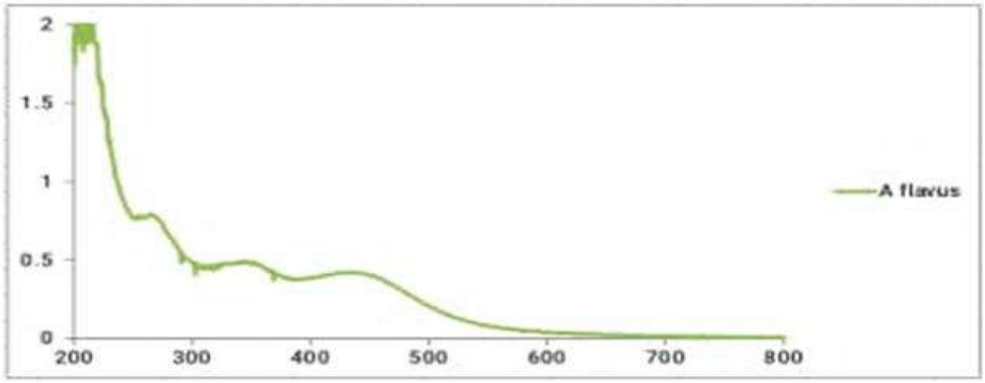


Fig 4: UV-spectra of the synthesized AgNPs of A. flavus

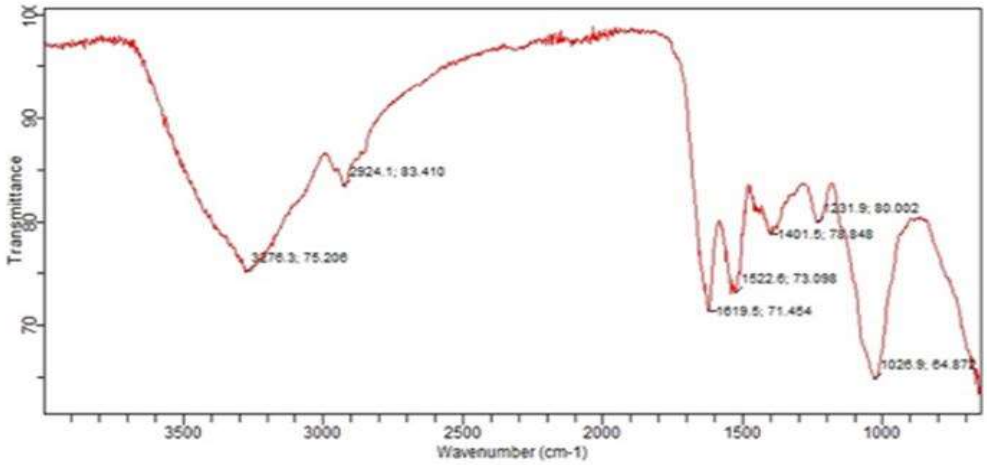


Fig 5: FTIR spectrum of silver nanoparticles synthesized from *Aspergillus flavus*

Congo red screening method for biofilm formation

Among the fifty-nine bacterial isolates, 15 bacterial isolates (25.4%) were positive while 44 (74.6%) tested negative as non-biofilm formers. The highest diameter of zone was observed from Streptococcus *pyogens* and the lowest diameter was observed from *Citrobacter freundii* (Table 1).

Table 1: Congo red agar assay

S/N	Bacterial isolates	Diameter of zone in cm
1	*Proteus vulgaris*	2.0±0.2
2	*Klebsiella oxytoca*	5.6±1.2
3	*Staphylococcus aureus*	4.6±1.5
4	*Pseudomonas aeruginosa*	1.7±0.4
5	*Salmonella typhi*	3.8±0.6
6	*Citrobacter freundii*	1.0±0.0
7	*Klebsiella pneumonia*	5.8±0.4
8	*Staphylococcus aureus*	6.2±0.3
9	*Enterobacter cloacae*	5.7±0.4
10	*Enterobacter aerogene*	2.5±0.2
11	*Escherichia coli*	2.1±0.6
12	*Klebsiella pneumonia*	3.9±1.2
13	*Streptococcus pyogens*	7.1±0.9
14	*Micrococcus luteus*	2.4±0.8
15	*Streptococcus pyogens*	3.0±1.0

Tube method

Strong biofilm producers could be easily detected using this method but it was difficult to differentiate between moderate and weak biofilm producers due to the variations in the results obtained visually. Nine isolates (15.3 %) tested positive as strong biofilm formers, twenty-four isolates (40.7%) were moderately positive and twenty-six bacterial isolates (44%) were non-biofilm formers. Most of the strong biofilm formers were Gram negative bacteria (Table 2).

Table 2: Tube assay for detection of strong biofilm formers

S/N	Bacterial isolates	Results
1	*Proteus vulgaris*	Positive
2	*Klebsiella oxytoca*	Positive
3	*Staphylococcus aureus*	Positive
4	*Escherichia coli*	Positive
5	*Pseudomonas aeruginosa*	Positive
6	*Klebsiella pneumonia*	Positive
7	*Salmonella typhi*	Positive
8	*Enterobacter aerogene*	Positive
9	*Streptococcus pyogens*	Positive

Antibiotic susceptibility test

Results of the antibiotic susceptibility pattern of the bacterial isolates (Table 3) demonstrated that all the biofilm formers were multidrug resistant and were resistant to the six classes of antibiotics except for *Proteus vulgaris. Staphylococcus aureus, Escherichia coli* and *Klebsiella pneumonia* depicted highest resistance potential to the antibiotics.

Table 3: Antibiotic susceptibility pattern of bacterial isolates

Biofilm formers	**Gram positive antibiotics**									
	Am	**Aug**	**Gen**	**Cp**	**Ery**	**Pef**	**Roc**	**Str**	**Sep**	**Zin**
Streptococcus pyogens	R	R	R	S	R	S	S	S	S	R
Staphylococcus aureus	S	R	R	R	R	R	R	R	R	R
	Gram negative antibiotics									
	Am	**Aug**	**Chl**	**Gen**	**Cpr**	**Tar**	**Pef**	**Str**	**Spa**	**Sep**
Proteus vulgaris	S	S	S	S	S	S	S	S	S	S
Salmonella typhi	R	S	R	S	S	R	S	S	S	R
Klebsiella oxytoca	R	R	R	R	S	R	R	S	S	S
Escherichia coli	R	R	S	R	S	R	R	S	R	R
Klebsiella pneumonia	R	R	R	R	S	R	R	S	S	R
Enterobacter aerogene	S	S	S	R	R	R	R	R	S	R
Pseudomonas aeruginosa	S	R	S	R	S	S	R	R	R	R

Antibacterial activity of the synthesized AgNPs on biofilm formers

Table 4 shows the ability of AgNPs on biofilm isolates and results revealed that the silver nanoparticles synthesized from Aspergillus *flavus* has an inhibitory effect on *Klebsiella oxytoca, Pseudomonas aeruginosa, Escherichia coli, Salmonella typhi, Enterobacter aerogene* and *Streptococcus pyogens* at varying levels. Maximum inhibitory zone of 1.9 cm was observed on Proteus vulgaris and minimum inhibitory zone of 0.4 cm was observed on *Enterobacter aerogene*. However, the AgNPs did not inhibit the growth of Staphylococcus *aureus and Klebsiella* pneumonia.

Table 4: Antibacterial activity of synthesized AgNPs by *Aspergillus flavus* on biofilm formers

Isolates	Zone of Inhibition (cm)
Proteus vulgaris	2.6±0.3
Klebsiella oxytoca	0.8±0.0
Staphylococcus aureus	Resistant (no zone)
Pseudomonas aeruginosa	1.2±0.2
Escherichia coli	0.6±0.0
Klebsiella pneumonia	Resistant (no zone)
Streptococcus pyogens	0.5±0.0
Enterobacter aerogene	0.4±0.0
Salmonella	1.9±0.4

Discussion

Poor farming management, overcrowding, unsanitary conditions, insufficient ventilation and poor feed quality may cause diseases in poultry houses (Karkhanis *et al.,* 2021). Favorable growth and proliferation of organisms may be fostered when water is refilled without cleaning the water drinkers properly (Folorunso *et al.,* 2014). This study revealed that unhygienic poultry drinkers and water are vehicles for the introduction of multi-drug resistant isolates. The presence of bacteria in the water and drinker sample provided to the birds revealed poor sanitation and renders the chicken a carrier of the bacterium, which might be consumed by humans who take chicken or poultry meat. Different methods for handling and distributing water, feed ingredients, levels of hygiene, biosafety precautions and management techniques for raising chickens may all play a role in the variations in the dispersion of microbes from water and poultry drinkers (Islam *et al.,* 2017).

E. coli, Proteus, Salmonella, Pseudomonas, Staphylococcus and *Streptococcus* etc. identified in this study had been reported in the previous studies of Islam *et al., (*2017). These organisms can harm both humans and animals and their presence indicated a potential health risk.

It was evident that silver nanoparticles had formed in the medium when the color of fungal cell filtrates with silver ions changed to an intense brown. This was primarily because the nanoparticles' surface plasmon vibrations had

been excited. The color of the cell filtrate did not alter in controls (those without silver ions) under the same incubation conditions. The reduction of silver nitrate solution to silver nanoparticles is confirmed by the unique and solitary surface plasmon resonance (SPR) band at 410 nm. Studies by Othman *et al., (*2019) have recorded similar observations and Kalimuthu *et al., (*2008) submitted that fungi secrete several proteins into the medium that may be crucial to the stabilization and reduction of silver ions in the form of nanoparticles.

The functional groups that might have been involved in the production and stability of the produced silver nanoparticles were found in the FTIR spectroscopy spectrum. Also discernible were O-H stretching vibrations in flavonoids, alcohols and phenolic compounds; C-H stretching vibrations of methylene, methyl and methoxyl groups at 2924; absorption peaks at 1026 cm1 that may have been caused by C-N aromatic and aliphatic amine stretching vibrations and presence of sulphonamide at 1231cm-1. Sharp peak at 3276cm-1 strongly shows that silver ions are bound to the hydroxyl (-OH) group and is believed to be overlapping -NH stretching vibration, a common occurrence in proteins (Mishra et al., 2015). These findings imply that the hydroxyl/carbonyl groups might be in charge of the synthesis and stabilization of silver nanoparticles (Mohanta et al., 2019). AgNPs are produced via the reduction of silver nitrates, which is carried out by the hydroxyl and amine groups of OH/NH2, C-H Alkene, N-H Bend, C-O Alcohol, and S=O Sulfones.

The biosynthesis of AgNPs occurred through the mediation by A. flavus and it exerted an antimicrobial action against harmful microorganisms depending on the microbe type and its biological constituents (Shrivastava et al., 2007). The increased effectiveness of nanoparticles in entering bacterial cells was due to their smaller size. Numerous studies have demonstrated the effectiveness of silver nanoparticles as antibacterial agents against microorganisms (Muddassir et al., 2022).

Proteus, Klebsiella, Staphylococcus, E. coli, Enterobacter, Pseudomonas, Salmonella and Streptococcus species were found to exhibit considerable tendency to form biofilms by CRA and TM. Seven isolates from the biofilm formers were Gram negative while two species were Gram positive.

Reddy et al., (2017) noted that in order to increase the sensitivity for biofilm detection, multiple screening assays should be employed to screen isolates for biofilm formation. The Congo red agar method was discovered to be quicker and easier to perform than other phenotypic approaches. Our findings agree with those of Manandhar et al., (2018) who found significant differences between the results of CRA and TM, where CRA recorded two isolates as positive and TM showed eleven isolates out of 78 as strong biofilm formers. However, our results do not agree with Fatima et al., (2011) who submitted that 87.6% of isolates to be strong biofilm producers.

Results on antibiotic susceptibility showed that 100% of the Gram-positive bacteria were resistant to Erythromycin, Gentamycin, Augmentin, and Zinnacef, while only 55.6% were resistant to Amoxicillin and Gentamycin, 22.2% to Ciprofloxacin, 66.7% to Augmentin and Pefloxacin, and 71.4% to Tarvid. The potential for multidrug resistance of the bacteria to eight of the nine antibiotics aggravated the issue of antibiotic resistance worldwide and had major health implications for the use of antibiotics in poultry (Eja et al., 2012). This outcome is consistent with research by Mohammed and Dubie (2022), who both observed high levels of bacterial drug resistance. Proteus vulgaris was the only biofilm-forming bacterium that did not exhibit multiple resistance to the tested antibiotics. Ciprofloxacin had high sensitivity when compared to the other tested drugs, which is consistent with the conclusions of Abebaw et al., (2018).

The antibacterial activity of AgNPs toward microbes can be understood using a variety of modes of action. One of these methods is that AgNPs may adhere to the negative charge on the surface of microorganisms, changing the

characteristics of the cell wall, cell membrane and affecting respiration, permeability, electron transport and osmoregulation (Marambio-Jones and Hoek, 2010). According to AshaRani et al., (2009), after piercing the microbial cell wall, AgNPs can interact with cell components such as DNA and proteins.

AgNPs capacity to dislodge biofilms and bacteria may be a result of their ability to penetrate bacterial cell walls, alter their structural makeup as a result of their nanoscale size and disrupt cellular membranes, which will ultimately cause organelles rupture and cellular lysis (Yassin et al., 2022). In the report by Muddassir et al., (2022), measurements of inhibition zones were demonstrated to be a valid method for determining the inhibition effects of silver nanoparticles on harmful bacteria. Lower dose of AgNPs could be responsible for its inability to inhibit the growth of Staphylococcus aureus and Klebsiella pneumonia and we suggest an increase in the dosage of AgNPs application for future research. The effectiveness of silver nanoparticles against biofilms produced by P. aeruginosa and E. coli was demonstrated by Gurunathan et al., (2014). Goswami et al., (2015) also discussed the removal of biofilms using silver nanoparticles and discovered that 89% of S. aureus and 75% of E. coli biofilm formation was inhibited by silver nanoparticles. This was supported by Franci et al., (2015) who discovered that silver nanoparticles significantly inhibited the growth of bacteria and biofilms.

Conclusion

The study revealed the presence of multidrug-resistant (MDR) isolates in poultry drinkers and water. Green synthesis of AgNPs by Aspergillus flavus was also accomplished and the biosynthesized AgNPs had antibacterial efficacy on biofilm-forming organisms. Increment dose of AgNPs and conventional antibiotic combinations may be investigated as potential substitutes for antimicrobial drugs for removal of biofilms caused by Klebsiella pneumonia and Staphylococcus aureus etc. Moreover, the implications of multidrug resistant bacteria in chicken products should be made clear to livestock and poultry owners as well as enforcement of strict cleanliness and sanitation standards.

References

Abdullahi, U. F., Igwenagu, E., Muazu, A., Aliyu, S. and Umar, M. I. 2016. Intrigues of biofilm: A perspective in veterinary medicine. Vet. Wor. 9(1), 12-18

Abebaw, A., Tesera, H., Belachew, T. and Mihiretie, G. 2018. The bacterial profile and antibiotic susceptibility pattern among patients with suspected bloodstream infections, Gondar, North-West Ethiopia. Path. Lab. Med. Int. 10, 1-7.

AshaRani, P.V., LowKah, G., Hande, M.P. and Valiyaveetti, S. 2009. Cytotoxicity and genotoxicity of silver nanoparticles in human cells. ACS Nan. 3, 279–290.

Clinical and Laboratory Standards Institute (CLSI) 2018. Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals. 4th ed. CLSI: Wayne, PA, USA.

Daniel, W.W., Paul, G.M., Robert, M. W., Lilly, C. B., Helena, A. N. and Philip, N. N. 2020. Antibiotic and disinfectant susceptibility patterns of bacteria isolated from farmed fish in Kirinyaga County, Kenya. Int. J. Microbiol. 1-8.

Duran, N., Durán, M., Jesus, M. B., Seabra, A. B., Favaro, W. J. and Nakazato, G. 2016. Silver nanoparticles: A new view on mechanistic aspects on antimicrobial activity. Nanomed. Nanotechnol. Biol. Med. 12, 789–799.

Eja, M. E., Samuel-Udoekong, N., Ikpeme, E. M., Enyi-Idoh, K. H., Lennox, J. A. and Etim, K. D. 2012. Antibiogram studies and extended spectrum beta-lactamase activity profile of Salmonella-like species isolated from poultry soil of the University of Uyo, Nigeria. Malays. J. Microbiol. 8(4), 280-284.

Fatima, K., Indu, S., Meher, R., Tariq, M. and Sharma, S. C. 2011. Detection of biofilm formation in Staphylococcus aureus. Trends Med. Res. 6(2), 116-123.

Folorunso, O.R., Kayode, S. and Onibon, V. O. 2014. Poultry farm hygiene:

microbiological quality assessment of drinking water used in layer chickens managed under the battery cage and deep litter systems at three poultry farms in southwestern Nigeria. Pak. J. Biol. Sci. 17(1), 74-79.

Franci, G., Falanga, A., Galdiero, S., Palomba, L., Rai, L., Morelli, G. and Galdiero, M. 2015. Silver nanoparticles as potential antibacterial agents. Mol. 20(5), 8856–8874.

Gaikwad, A.K. and Gade, M. K. 2013. Rapid synthesis of silver nanoparticles from Fusarium oxysporum by optimizing physicocultural conditions. Sci. World J. 1-13.Goswami, S., Sahareen, T., Singh, M. and Kumar, S. 2015. Role of biogenic silver nanoparticles in disruption of cell-cell adhesion in Staphylococcus aureus and Escherichia coli biofilm. J. Ind. Eng. Chem. 26, 73-80

Gudikandula, K., Vadapally, M. A and Singara, C. 2017. Biogenic synthesis of silver nanoparticles from white rot fungi: their characterization and antibacterial studies. Open Nan. 2, 64–78.

Gurunathan, S., Han, J. W., Kwon, D. N. and Kim, J. H. 2014. Enhanced antibacterial and anti-biofilm activities of silver nanoparticles against Gram-negative and gram-positive bacteria. Nan. Res. Lett.. 9(1), 1-7.

Han, C., Romero, N., Fischer, S., Dookran, J., Berger, A. and Doiron, A. 2017. Recent developments in the use of nanoparticles for treatment of biofilms. Nanotech. Rev. 6(5), 383-404.

Islam, M. S, Khanam, S. and Mohanta, M. K. 2017. Isolation, characterization and identification of bacterial isolates from the poultry environment at Rajshahi Metropolis, Bangladesh. J. Ent. Zool. Stud. 5(4), 918-926.

Kalimuthu, K., Babu, R. S., Venkataraman, D., Bilal, M. and Gurunathan, S. 2008. Biosynthesis of silver nanocrystals by Bacillus licheniformis. Coll. Surf.B: Biointer. 65(1), 150–158

Karim, E., Dhar, K. and Hossain, T. 2018. Decolorization of textile reactive dyes by bacterial monoculture and consortium screened from textile dyeing effluent. J Gen. Eng. Biotechnol. 16(2), 375-380.

Lizana, J.A., Lopez, S., Marchal, A., Serrano, U., Velasco, D. and Espinosa-Urgel M. 2013. Use of plant extracts to block bacterial biofilm formation," in High School Students for Agricultural Science Research, Proceedings of the 3rd Congress PIIISA, Pp. 43– 50.

Manandhar, S., Singh, A., Varma, A., Pandey, S. and Shrivastava, N. 2018. Evaluation of methods to detect in vitro biofilm formation by Staphylococcal clinical isolates. BMC Res Not. 11, 1-6

Marambio-Jones, C. and Hoek, E.M. 2010. A review of the antibacterial effects of silver nanomaterials and potential implications for human health and the environment. J Nan. Res.12, 1531–1551.

Meckenstock, R.U., Elsner, M., Griebler, C., Lueders, T., Stumpp, C., Aamand, J., Agathos,S. N., Albrechtsen, H., Bastiaens, L., Bjerg, P. L., Boon, N., Dejonghe, W., Huang, W. E., Schmidt, S. I., Smolders, E., Sorensen, S. R., Springael, D. and Breukelen, B. M. 2015. Biodegradation: Updating the concepts of control for microbial cleanup in contaminated aquifers. Environ. Sci. Technol. 49(12), 7073-7081.

Mishra, P., Kumar, S., Kumar, N. G. and Parida, K. 2015. Biomimetic synthesis, characterization and mechanism of formation of stable silver nanoparticles using Averrhoa carambola L. leaf extract. Mat. Lett. 160, 566–571.

Mohammed, Y. and Dubie T. 2022. Isolation, identification and antimicrobial susceptibility profile of Salmonella isolated from poultry farms in Addis Ababa, Ethiopia. Vet Med Sci 8, 1166–1173. Mohanta, Y. K, Biswas, K., Jena, S. K., Hashem, A., Abdallah EF, Mohanta TK. 2019. Anti-Biofilm and antibacterial activities of silver nanoparticles synthesized by the reducing activity of phytoconstituents present in the Indian medicinal plants. Front. Microbiol. 11, 1-39.

Muddassir, M., Raza, M., Munir, S., Basirat, A., Ahmed, M., Butt, M. S, Dar, O. A., Ahmed, S. S., Shamim, S. and Naqvi, S.

Z. 2022. Antibacterial efficacy of silver nanoparticles (AgNPs) against metallo-β-lactamase and extended spectrum β-lactamase producing clinically procured isolates of Pseudomonas aeruginosa. Sci. Rep. 12, 1-6

Othman, A., Elsayed, M. A., Al-Balakocy, N. G., Hassan, M. M, Elshafei, A. M. 2019. Biosynthesis and characterization of silver nanoparticles induced by fungal proteins and its application in different biological activities. J. Gen. Eng. Biotechnol. 17, 1-8

Ramasamy, M. and Lee, J. 2016. Recent nanotechnology approaches for prevention and treatment of biofilm-associated infections on medical devices. BioMed. Res. Int. 1-17.

Reddy, K. R. 2017. Tube adherence test as a screening tool for detection of biofilm formation among Staphylococcus aureus. Int. J. Curr. Microbiol. App. Sci 6(8), 1325-1329

Rendueles, O., Kaplan, J. B. and Ghigo, J. M. 2012. Antibiofilm polysaccharides. Env. Microbiol. 15, 334-346.

Saif, S., Tahir, A. and Chen, Y. 2016. Green synthesis of iron nanoparticles and their environmental applications and implications. Nanomat. 6(11), 1-26.

Saifuddin, N., Wang, W. C. and NurYasumira, A. A. 2009. Rapid biosynthesis of silver nanoparticles using culture supernatant of bacteria with microwave irradiation. Eur. J. Chem. 6, 61–70.

Salleh, A., Naomi, R., Utami, N. D., Mohammad, A.W., Mahmoudi, E., Mustafa, N. and Fauzi, M.B. 2020. The potential of silver nanoparticles for antiviral and antibacterial applications: a mechanism of action. Nanomat 10(8), 1-20

Sharmin, S., Rahaman, M., Sarkar, C., Atolani, O., Islam, M. T. and Adeyemi, O. S. 2021. Nanoparticles as antimicrobial and antiviral agents: A literature-based perspective study. Hel. 7(3), 1-9

Shrivastava, S., Bera, T., Roy, A., Singh, G., Ramachandrarao, P. and Dash, D. 2007. Characterization of enhanced antibacterial effects of novel silver nanoparticles. Nan 18(22), 1-9

Sinha, R., Karan, R., Sinha, A.and Khare, S. K. 2011. Interaction and nanotoxic effect of ZnO and Ag nanoparticles on mesophilic and halophilic bacterial cells. Biores Technol 102(2), 1516–1520.

Thilakavathy, P., Vasantha, R. M, Jagatheeswari, P. A., Charles, J., Dhanalakshmi, V., Lallitha, S., Rajendran, T. and Divya, B. 2015. Evaluation of ica gene in comparison with phenotypic methods for detection of biofilm production by coagulase negative Staphylococci in a tertiary care hospital. J. Cli. Diag. Res. 9(8), 16-19.

Ugwoke, M. I., Machido, D. A. and Tijjani, M. B. 2019. Evaluation of the biofilm forming capacities of bacterial isolates recovered in raw and treated effluent from wastewater treatment plant of Ahmadu Bello University Zaria. Nigeria. J. Appl. Sci. Environ. Managmt 23(10), 1783-1786.

Yassin, M. T., Mostafa, A. F., Al-Askar, A. A., Al-Otibi, F. O. 2022. Facile green synthesis of silver nanoparticles using aqueous leaf extract of Origanum majorana with potential bioactivity against multidrug resistant bacterial strains. Cryst. 12, 1-18

Zhang, X., Liu, Z., Shen, W. and Gurunathan, S. 2016. Silver nanoparticles: synthesis, characterization, properties, applications and therapeutic approaches. Int. J. Mol. Sci. 17(9), 1-34.