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Research Article

Antibacterial Investigation of Silver Nanoparticle against *Staphylococcus, E. coli* and *Salmonella* Isolated from Selected Live Bird Markets

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ABSTRACT

An antibiogram study of newly introduced Silver Nano particles (AgNPs) was performed against the available poultry bacteria. Herein, this research involved isolation, identification and characterization of *Staphylococcus, E. coli* and *Salmonella* of different bird types from two live bird markets surrounding Bangladesh Agricultural University (BAU) campus for determining antibiogram profiles against AgNP in comparison with commercially available antibiotic disks. For this a total of 120 cloacal swab samples collected from Broiler (48), Sonali (48) and Deshi chicken (24) from Kamal Ranjit (KR) and Kewatkhali live bird market from which *Staphylococcus* (68), *E. coli* (97) and *Salmonella* (91) were isolated. Representative of these three bacterial isolates were used for antibiogram profiling. The standard disk diffusion method was employed for the antibiogram assays where the zone of inhibition surrounding the disk used as a parameter for evaluating the sensitivity of AgNP as well as commercial antibiotic disk. The isolates showed sensitivity to Ceftriaxone (26.99 ± 0.2), Ciprofloxacine (21.57 ± 0.15), Gentamycin (23.59 ± 0.2) and resistance to Amoxicillin (0.00), Tetracycline (7.23 ± 0.06) while AgNP (22.93 ± 0.38) was sensitive against all the isolates. Thus AgNP proved sensitivity to bacteria even resistant to Amoxicillin and Tetracycline. This sensitivity of AgNP against poultry bacteria holds promise for the use as antibacterial in poultry industry.

Keywords: Silver nanoparticle; Antibacterial activity; Antimicrobial resistance; Poultry bacteria; Antibiogram study

INTRODUCTION

Nanomaterials has been attracted much attention because of their enhanced functionality, durability and stability based on the purposes [1,2]. Several nanoparticles of metal, metal oxides and organic origin have been extensively used in livestock and poultry industries world-wide as drug and nutrient carriers for reducing doses of drug as well as conserving nutrient through enhancing Feed Conversion Ratio (FCR) [3]. There are numerous nanomaterial's with antimicrobial effect such as Silver (Ag), Titanium (Ti), Zinc (Zn) etc. have been used in commercial house hold sanitizers, equipment's (Freezer), cosmetics products [4,5]. However, their application as antibacterial in livestock and poultry sector has not been explored thus fur. Hence, this research focusses on exploring the antibacterial effects of Silver Nanoparticle (AgNP) [6,7] against circulating avian microflora collected from live bird markets surrounding Bangladesh Agricultural University (BAU).

The excellent enhancement in functional properties of nano structured AgNPs compared to their bulk origin has attracted their applications in various fields such as Agriculture, Biomedical and Aquaculture [8,9]. Many of this enhanced functionality are due to increased surface area, ion exchange capacity, ion absorption ability and chemical complexation [10]. Availability of functional atom on the surface is another potential reason for such enhanced functionality of nanoscale materials compared to their bulk counterpart [11]. In particular, the nano structured AgNPs has become attractive in many applications including engineering, biology and medicine because of the differences in their surface composition, variation in reactivity and different types of surface interaction sites

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[12,13]. Basically, AgNPs having positively charged surface, ligand replacement ability and oxidative dissolution capabilities facilitate their binding to the negatively charged surface of bacteria and result in an enhancement of the bactericidal effect [14]. Based on this potential effect the present program was proposed for investigating antibacterial activity of the AgNPs on the bacterial pathogen isolated from live bird market *in vitro* condition.

Recently Antimicrobial Resistance (AMR) resulting from indiscriminate uses of antibiotics has been emerged as a challenge for public health worldwide with grave consequences in many developing

countries including Bangladesh [15]. This global health threat appears as fast growing threat in the society as a consequence of the frequent use of antibiotics in animal and poultry industries [16]. Generally the commercial farmers are indiscriminately using antibiotics to their herds and flocks and selling out the poultry and its products without considering their withdrawal period [17]. As a result majority of those animal and poultry products carry significant level of antibiotic residues to the consumers [18]. Thus, these antibiotic residues of animal and poultry products are responsible for conferring resistance microflora among consumers [19,20]. Besides, majority of the treated antibiotics in feed and water are exposing to surrounding environment [21]. Such exposure of antibiotics results resistant microflora in the environment. As a consequence, majority of available antimicrobials are failed to cure subsequent infection to consumers [22]. For instance, the circulating environmental microflora developed resistance to penicillin, first and second generation cephalosporin, first, second and third generation quinolones, sulphonamides (100%) , aminoglycosides (50%), tetracycline (50%), third generation cephalosporin (30%) and forth generation quinolones (30%), and other broad spectrum antibiotics (20%) [23]. As a result, fourth generation cephalosporins, tigecycline, aztreonam, colistin, carbapenem, teicoplanin, voriconazole, and amphotericin B etc. are on field use which are categorized as reserve antimicrobials [24]. If this continues, situation will arise when bacteria would have developed resistance against all those reserve antimicrobials. Hence, many countries in the advanced world are forecasting emergence of post antibiotic era with serious consequences when patient would have been died due to ordinary septicemia [25], reason why AMR has been appeared as potential public health threat in many countries of the world [26]. Therefore, generation/introduction of new materials with antimicrobial effect is critically required as preparedness for fourth-coming post-antibiotic era [27]. With this in mind the present research is designed to investigate antibacterial effect of AgNPs against available avian microflora to tackle the said AMR issue.

Herein, AgNPs obtained from Sigma Aldrich (Saint Louis, MO, USA) was characterized and tested for evaluating antibacterial effects against circulating poultry bacteria.The research was involved with

preparation of AgNPs impregnated disk followed by their antibiogram profiling against bacteria (poultry isolates) in comparison with commercially available antibiotics such as Ceftriaxone, Ciprofloxacine, Gentamycin, Amoxicillin and Tetracycline. The available poultry bacterial isolates i.e. *Staphylococcus aureus*, *E. coli* and *Salmonella* were isolated, identified and characterized from poultry cloacal samples collected from the live bird markets surrounding the Bangladesh Agricultural University campus [28,29].

MATERIALS AND METHODS

Chemicals and reagents

Silver nano-powder, mercaptoethanol was purchased from Sigma Aldrich (Saint Louis, MO, USA), bacterial culture medium (Nutrient broth, Nutrient agar, EMB agar, SS agar, MS agar, MH agar), antibiotic disk and blotting paper purchased from HiMedia Laboratories Pvt. Ltd., Mumbai, India, different sugar solution (dextrose, maltose, sucrose, mannitol, lactose) and gram staining materials from the laboratory stock of DMH, PCR master mixer Primer sets (16s rRNA, inV, nuc), agarose powder, DNA ladder etc. purchased from Promegra, Madison, USA.

Preparation of AgNPs impregnated blotting paper

The blotting paper cut into pieces with a diameter of 0.5 cm was used for the preparation AgNPs impregnated disk. For that as received AgNPs powder was dispersed and solubilized in mercaptoethanol by vortexingat 100 µg/ml [30]. The homogenous AgNPs solution was poured on to the predesigned blotting paper and kept at room temperature for an hour. After air drying the mercaptoethanol was evaporated out and leaving the AgNPs impregnated blotting paper.

Surface topography of AgNPs impregnated disk using Atomic Force Microscopy

The topographic investigation of AgNPs impregnated blotting paper was performed at the Department of Physics, University of Dhaka, using the Atomic force Microscope (AFM) as illustrated in Figure 1a.

Scanning was performed using contact mode at a scan rate of 10.3 µm to 3.74 µm per second for obtaining the topographic features of the used AgNPs. The dimensions of the AgNPs were measured from the height profile of the topographic images.

Isolation and identification of bacteria from poultry cloacal swab sample

Media (Nutrient broth, Nutrient agar medium, Eosin Methylene Blue medium, *Salmonella* and Shigella agar medium, Mannitol Salt agar medium, Muller Hilton agar medium) were prepared as required. Following solidification of media on petridishes microbiological sterility was ascertained and preserved for future use. Cloacal samples were chosen to be collected from two local live bird market surrounding BAU campus-KR market and Kewatkhali market, Mymensingh, Bangladesh. Samples were categorized according to the sources and bird types such as domestic chicken, sonali and broilers. Samples were collected from the cloaca and placed into nutrient broth and transferred to laboratory and kept at 37°C for 24 hrs for the propagation of bacteria as showed in Figure 1b. The propagated bacteria from nutrient broth was streaked on nutrient agar medium and

incubated at 37°C for 24 h as showed in Figure 1c. Single colony was achieved through sub culturing on the agar plates and then transferred to specific medium such as MS agar medium, EMB agar medium and SS agar medium. Following incubation at 37°C for 24 h cultural characteristic of specific bacteria were investigated. For viewing their staining characteristics colonies from specific medium were smeared on sterilized glass slides and stained with gram staining materials with standard procedure [28] as described in Figure 1d. For further characterization sugars (dextrose, maltose, mannitol, glucose and sucrose) fermentation tests were performed as illustrated in Figure 1e. Finally, molecular identification was performed with genus/species specific genes final confirmation as shown in Figure 1f. For this DNA was extracted by boiling method [29] from the single morphologic colony. Then the DNA templates along with specific primers (nuc, 16s rRNA, invA) and PCR master mixes were amplified maintaining standard protocol following visualization of the agarose gel run PCR products with UV transluminator.

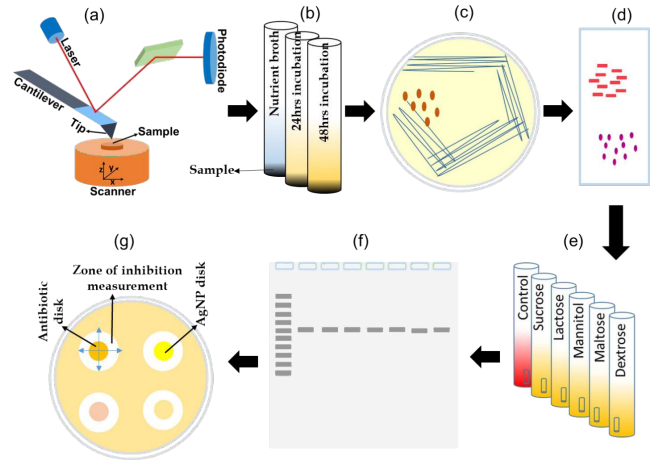

Figure 1: Schematic illustration of major tasks involved-(a) Topographic investigation of AgNP impregnated disk using AFM, (b) Cloacal swab sample incubation, (c) Bacterial culture, (d) Gram staining, (e) Biochemical test, (f) PCR documentation, and (g) Antibiogram study for the experiment.

Antibacterial investigation of AgNPs impregnated disk

The bacteria isolates were used for evaluating antibacterial activity of AgNPs in comparison with commercially available antibiotics as an illustrated task in Figure 1g. The AgNPs impregnated blotting papers along with commercially available Ceftriaxone, Ciprofloxacine, Gentamycin, Amoxicillin and Tetracycline disks were used. The antibiogram study was performed using standard disk diffusion method. Bacterial suspension prepared from MS, EMB and SS agar verified with 0.5 McFarland standards was subjected to incubation along the antibiotic and AgNPs disk on Mueller Hilton Agar at 37°C for 18-24 h and the diameter of zone of inhibition was measured in millimeter with a slide calipers. Finally the inhibitory zone was compared according with the guidelines of Clinical and Laboratory Standard Institute CLSI (2012).

Statistical analysis

The geometric means of the measured zones of inhibition (mm) for representative isolates were statistically analyzed with Origin 8 Pro and results were plotted in the following bar diagram with error bar and level of significance

RESULTS

Physical characterization of AgNPs

The 0.5 cm diameter cut pieces of blotting papers was incubated with AgNPs dispersed in mercaptoethanol for the preparation of antibacterial disk. Physical characterization of AgNPs impregnated disk was performed with AFM. Two dimensional topographic features of AFM image was given in Figure 2a (control disk without AgNPs), Figure 2b (large area of disk impregnated with AgNPs), zoomed AgNPs for profilometry analysis in Figure 2c; where, the X (350 nm) and Y (310 nm) dimensions of AgNPs clusters obtained from height profiles were presented in Figures 2e and 2f respectively, while numerous individual particles were found at its higher magnification. Nonetheless the height profile analysis at higher resolution was unachievable because of the limitation of currently used device. However, the Z dimensions (60 nm) of AgNPs obtained from three dimensional topographic images as illustrated in Figure 2d confirmed the dimensions of individual nanoparticles.

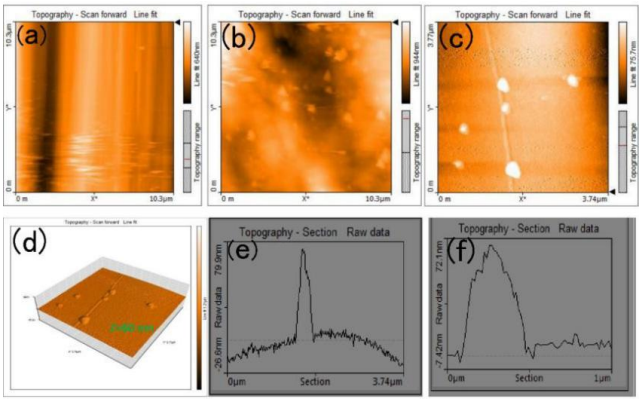

Figure 2: AFM study of AgNPs-(a) Control disk without AgNPs, (b) Disk with AgNPs, (c) Two dimensional topography of AFM image, (d) Three dimension topography of AgNPs impregnated disk, (e) Height profile of AgNPs in X axis and (f) Height profile of AgNPs in Y axis

Isolation, identification and characterization of the bacteria

Cultural characteristics revealed turbid bacterial grown on nutrient broth followed by single bacterial colony formation on nutrient agar through sub culturing which were later streaked on MS agar, EMB agar and SS agar. In case of MS agar medium colonies were arranged as bunches of grapes with a yellowish tinge showed in Figure 3a. In case of EMB agar medium colonies were found in round shaped with green metallic sheen

showed in Figure 3b. In case of SS agar round shaped blackish bacterial colonies were found, showed in Figure 3c.

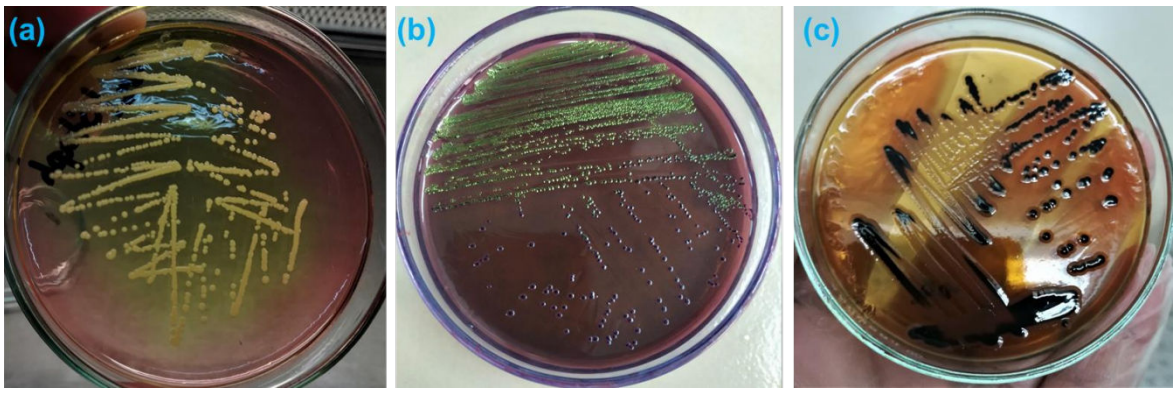

Figure 3: Results showing pure colony isoltion of bacteria in (a) MS agar medium (b) EMB agar medium, and (c) SS agar medium

Morphologically characterized bacterial isolates with Gram staining revealed a round bunched purple color colony as showed in Figure 4a when stained from MS agar, while rod shaped pink color colonies were found in both cases when stained with bacteria from EMB and SS agar in Figures 4b and Figure 4c respectively.

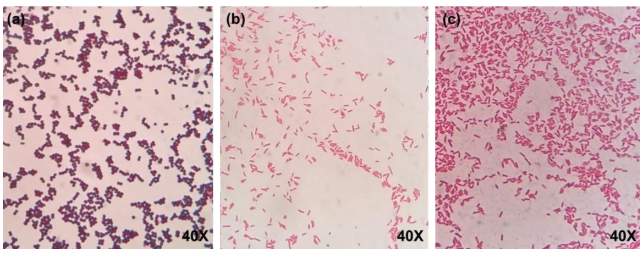

Figure 4: Image showed the Gram staining characteristic of pure colony isolates from (a) MS agar, (b) EMB agar, (c) SS agar

The sugars fermentation tests exhibited orange colour production without gas formation when inoculums was transferred from MS agar as showed in Figure 5a. Whereas, similar color changes occurred with the production of gases in cases of isolates from EMB agar as showed in Figure 5b, but color changes along with gases occurred in only three sugars (except Lactose and Sucrose) were formed when isolates were taken from SS agar showed in Figure 5c.

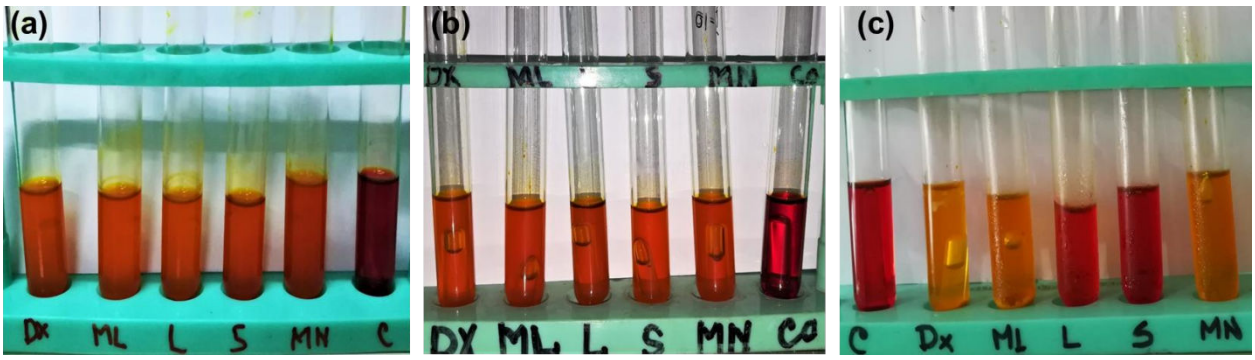

Figure 5: Images showing sugar fermentation test for inoculums from (a) MS agar, (b) EMB agar, (c) SS agar

The molecular characterization revealed the band size of 585 bp of PCR products shown on gel documentation when incorporated with 16s rRNA confirmed E. coli isolates as shown in Figure 6a. Whereas invar was amplified for Salmonella sp. and the band size was 211 bp shown in Figure 6b. In case of *Staphylococcus aureus* nuc was employed and the band was 279 bp shown in Figure 6c.

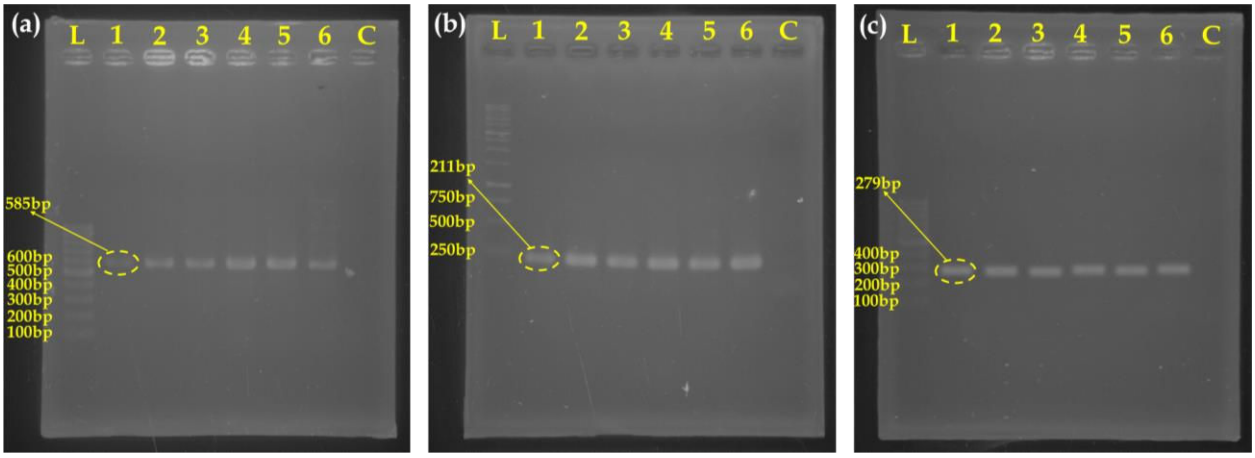

Figure 6: Result images of PCR amplification and molecular characterization of isolates for (a) *E. coli*,(b) *Salmonella sp*., (c) *Staphylococcus aureus*

A total of 120 nos. samples were collected from KR and Kewatkhali live bird market for investigating and subjected to cultural, staining, biochemical and molecular confirmation. The number of positive samples with known isolates from the samples were illustrated in the Table 1.

Table 1: Numbers of positive cases from the collected samples.

Bird type	Sample no.	K.R. market (Nos. of positive case)			Sample no.	Kewatkhali market (Nos. of positive case)		
		E.coli	*Salmonella sp.*	*Staphylococusaureus*	*E.coli*	*Salmonella sp.*	*Staphylococcus aureus*	
Broiler	24	20	23	14	24	22	20	16
Sonali	24	18	20	17	24	20	17	14
Domestic birds	12	10	6	4	12	7	5	3

A total of 60 samples tested from K.R market among which 48 for E. coli, 49 for Salmonella and 35 for Staphylococcus aureus were positive. While from Kewatkhali market among 60 samples 49 for E. coli, 42 for Salmonella and 33 for Staphylococcus aureus were positive.

Antibacterial effects of AgNPs and commercially available antibiotics on the isolated bacteria

The characterized *Staphylococcus aureus, E. coli and Salmonella sp.* isolates from each bird types of both markets were subjected to antibiogram study. The results obtained from measuring the zone of inhibitions produced against representative of each isolates were shown in Figure 7. The *Salmonella* isolates showed zone of inhibition against AgNPs, Ceftriaxone, Ciprofloxacine, Gentamycin whereas such zone was absent to Amoxycillin and Tetracycline showed in Figure 7a. Likewise, for *E. coli* isolates, the inhibitory zone was observed against AgNPs, Ceftriaxone, Ciprofloxacine, Gentamycin whereas such zone of inhibition was absent in case of Amoxycillin and Tetracycline showed in Figure 7b.Whereas of *Staphylococcus aureus* isolates showed zone of inhibition to AgNPs as well as all antibiotics tested except Amoxycillin in Figure 7c.

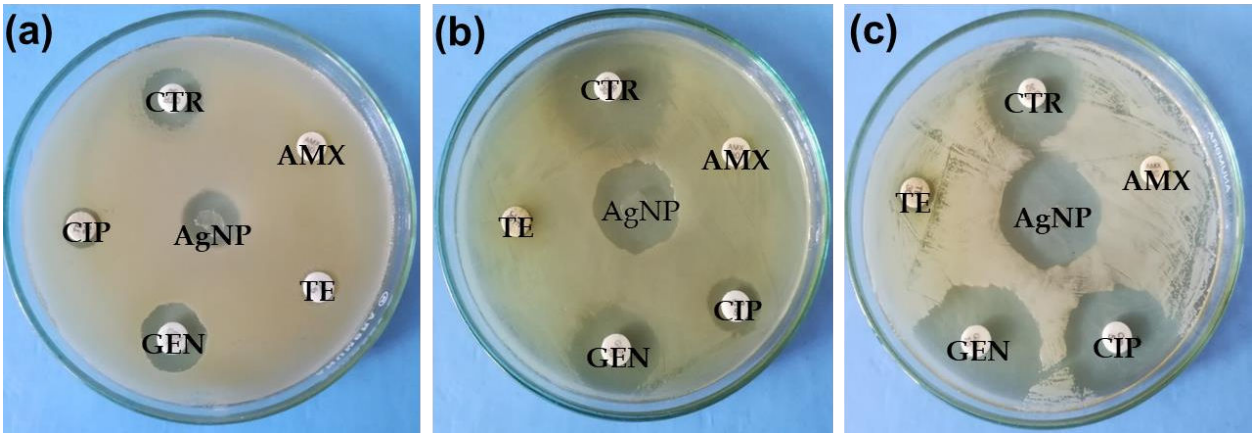

Figure 7: Images showing zone of inhibition for (a) *Salmonella sp*., (b) *E. coli*, (c) *Staphylococcus aureus*

Antibiogram profiles for those tested antibiotics and AgNPs were achieved by measuring the specific zone of inhibition against representative of those three isolates from each markets irrespective of bird types and the results were presented later in the graphs.

Staphylococcus aureus revealed a significant ($p<0.05$) level of sensitivity with an inhibitory zone against AgNP (17.11 mm), Ceftriaxone (19.37 mm), Ciprofloxacin (19.45 mm), Gentamycin (18.44 mm) whereas such zone of inhibition was absent for all the isolates against amoxycillin while a non significant zone of inhibition was measured incase of Tetracycline as showed in Figure 8.

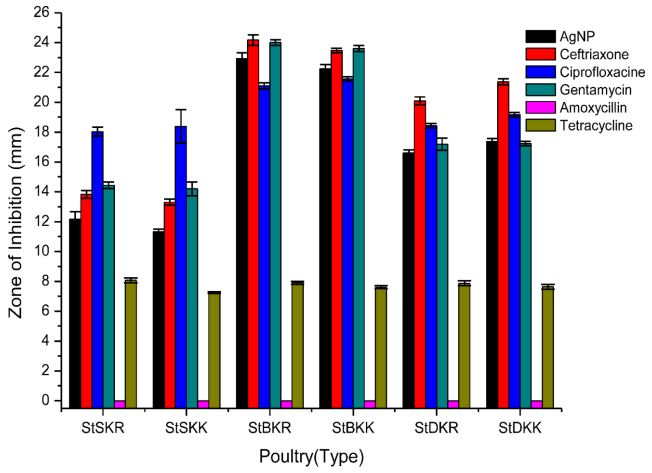

Figure 8: Graphical representation of zone of inhibition for the representative isolates of *Staphylococcus aureus* where X-axis represents the used antibiotics with AgNPs and Y-axis represents diameters of zone of inhibition (mm)

Abbreviation: StS stands for *S. aureus from sonali*, StB stands for *S. aureus from* broiler, StD stands for *S. aureus* from domestic chicken, KR stands for KR market samples and KK stands for Kewatkhali market samples

E. coli isolates also revealed a significant ($p<0.05$) level of sensitivity with inhibitory zone of 18.62 mm for AgNP, 21.83 mm for Ceftriaxone, 14.21 mm for Ciprofloxacin, 16.22 mm for Gentamycin whereas such zone of inhition was absent for all the isolates against amoxycillin while such zone of inhibition was non significant incase of Tetracycline as showed in Figure 9.

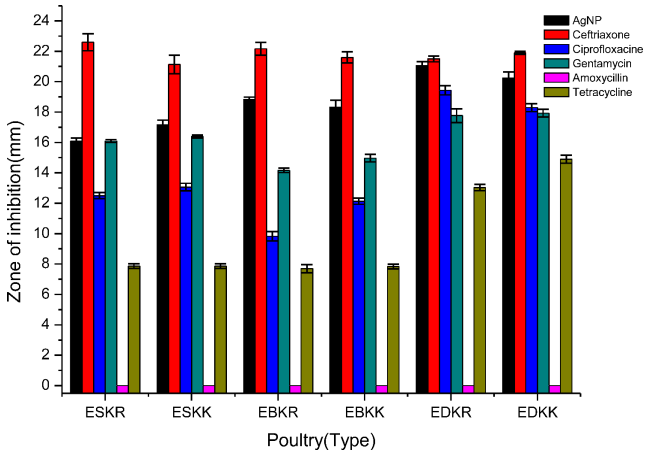

Figure 9: Graphical representation of zone of inhibition for the representative isolates of *E. coli* where X-axis represents the used antibiotics with AgNP and Y-axis represents diameters of zone of inhibition (mm).

Abbreviations: ES=*E. coli* from sonali, EB=*E. coli* from broiler, ED=*E. coli* from domestic chicken; KR= KR market samples, KK=Kewatkhali market samples.

Salmonella sp. revealed a significant ($p<0.05$) level of sensitivity with inhibitory zone 9.61 mm, 26.03 mm and 13.3 mm for AgNP, Ceftriaxone, Ciprofloxacin and Gentamycin respectively. Whereas such zone of inhibition was absent for all the isolates against amoxycillin while such zone of inhibition incase of Tetracycline was non significant as shown in Figure 10.

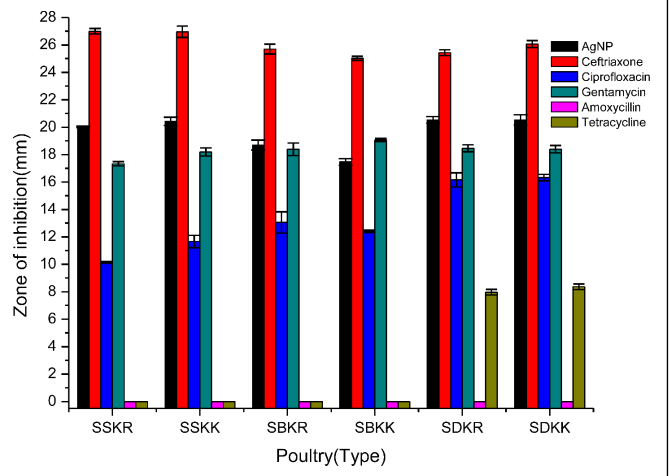

Figure 10: Graphical representation of zone of inhibition for the representative isolates of *Salmonella* where X-axis represents the used antibiotics with AgNP and Y-axis represents diameters of zone of inhibition (mm).

Abbreviation: SS=*Salmonella sp*from sonali, SB= *Salmonella sp*from broiler, SD=*Salmonella sp*from domestic chicken; KR= KR market samples, KK= Kewatkhali market sample

DISCUSSION

This study introduced Silver Nanoparticles (AgNPs) as a new antibacterial agent against commonly found poultry bacteria. For this cut pieces of blotting paper disks were prepared from mercaptoethanol dissolved AgNP powder [30-32]. The antibiogram assay was performed by disk diffusion method using the

impregnated blotting papers as antibacterial disk against the poultry bacteria. The antibacterial activity of AgNPs [33,34] was evaluated in comparison with commonly available antibiotic disk such as Ceftriaxone, Ciprofloxacin, Gentamycin, Amoxicillin, Tetracycline. The antibacterial sensitivity was determined by measuring the diameter of zones of inhibition of the surrounding disk. Thus the research involved isolation, identification and characterization of poultry bacteria with antibacterial resistance and evaluation of their sensitivity against AgNPs.

The bacteria was isolated from two different live bird markets with the aim of achieving various types of bacteria with resistance to various antibiotics. Besides, the samples from different bird types such as deshi, sonali and broilers were used for demostrating the influence of rearing practices on the resistant pattern as well as evaluating the antibacterial activity of AgNPs on them [35-37]. The samples from both markets were investigated for isolating *Staphylococcus aureus*, *E. coli* and *Salmonella* as representatives of commonly available poultry bacteria. A total of 120 samples from both markets were tested among which 68 samples were positive for *Staphylococcus aureus*, 97 for E.coli and 91 for *Salmonella*. The negative samples for those bacteria could be due to treatment of higher antibiotic prior marketting of the birds [38]. The prevelence of bacteria in both markets did not show significant variation which could be due to the birds of both markets were from similar sources. There was insignificant variations in the prevelence of the isolated bacteria among various bird types except the deshi chicken where *Staphylococcus aureus* and *Salmonella* were significantly lower than the other two commercial bird types. Unhygienic and confined rearing practice of commercial birds could be responsible for such variation in incidence of bacteria [39].

The isolation and identification of the bacteria were performed using cultural, gram staining and sugar fermentation tests. MS agar, EMB agar, SS agar were used as selective medium for *Staphylococcus aureus, E. coli* and *Salmonella sp.* respectively. In MS agar medium colonies were arranged as bunches of grapes with a yellowish tinge in the medium which reduced mannitol salt of the medium and thus suggestive for the growth of *Staphylococcus aureus* [40]. In case of EMB agar medium colonies were

found round shaped with green metallic sheen which is a characteristic feature for the growth of *E. coli* [41]. While in SS agar round shaped blackish bacterial colonies were found that indicated the presence of *Salmonella* [42]. On gram staining bacteria isolated from MS agar, round purple color bacteria in bunched grape-like cluster paired in chain were observed that confirm the gram positive *Staphylococcus aureus* [43]. While staining from EMB agar revealed single short rod shaped pink color bacteria confirmed gram negative *E. coli* [44]. Besides, staining from SS agar, a distinct short pink color rod were found indicating gram negative *Salmonella sp.* [45]. In addition, sugar fermentation test were also employed for further confirmation of the isolates by observing changes of color and production of gases. Isolates from MS agar exhibited pinkish color turned to orange color but absence of gas in the Durham's tube suggesting the presence of *Staphylococcus aureus* [46]. Whereas, changes of pink to orange with the production of gases in cases of isolates from EMB agar suggested the presence of *E. coli* [47] but color changes occurred in only three sugars [except Lactose and Sucrose] along with gases were formed when isolates were taken from SS agar was suggestive for presence *Salmonella sp.* [48]. Furthermore molecular characterizations of the isolated bacteria were performed using amplification of DNA extracted from those characterized bacteria with their gene specific primers for confirming their genus/species accurately. Herein, 16s rRNA, invA and nuc primers were used for amplifying the DNA templates obtained from the isolates of EMB, SS and MS agar respectively. The amplicon of 585 bp on gel documentation confirmed the presence of E. coli in EMB agar [49], while 211bp and 279 bpamplicon confirmed the presence of *Salmonella* [50] and *Staphylococcus aureus* [51, 52] respectively.

The AgNP impregnated disk used in this study was confirmed with topographic view and height profile analysis of AFM image. Where clusters of AgNP with dimension around 60 nm was observed on the disk surface. Even though, the X, Y dimensions of individual AgNP at the surface were not clearly investigated during profile analysis. The higher resolution of AFM images clearly showed the firmly attached individual nanoparticles on the surface. However, the size of individual AgNP was confirmed from the three dimensional topographic views of the scanned images. It was well known that the

nanoparticles tend to agglomerate readily because of their surface functional groups [53-65]. Thus the topographic features showed aggregates of particles rather than individual particles on the disk surface.

CONCLUSION

The antibiogram profile of identified bacteria showed significant variations in sensitivity against commonly available antibiotics while such variation was absent against the newly introduced AgNP. The AgNP was effective for all the isolates from both the markets irrespective of their bird types. Whereas the isolates showed sensitivity to Ceftriaxone, Ciprofloxacine and Gentamycin and resistance for Amoxicillin and Tetracycline for all cases. Such differences in AMR are obvious because of the indicriminate and inappropriate use of antibiotics in the poultry industries. This issues has recently been considered as fast growing challenges in the society. Therefore, emergence of new antibacterial for poultry bacteria are of great significance in curving such deadly situation in the society. The sensitivity of AgNP introduced in this study holds signifiance promise for such purpose. The overall zone of inhibition for various kinds of antibiotics ≤ 15mm is known to be resistant. Thus zone of inhibitions surrounding the AgNP disk appeared for all the bacteria were used for determining its sensitivity in this research. Herein for all cases AgNP showed diameter of zone of inhibition ≥ 15mm proving their sensitivity against all the isolates. Although antimicrobial acctivity of AgNPs has been introduced in many commercial household products and cosmetics compounds. However, their application in poultry was not explored yet. Therefore, this research for the first time employed AgNPs against poultry microbes *in vitro* where it was found effective for inhibiting the growth of *Staphylococcus aureus, E. coli* and *Salmonella* effectively.

The excellent sensitivity of this *in vitro* study supported that the AgNPs can be used as a potential antibacterial agent for curving the population of resistant bacteria in environment especially by reducing the use of commercial antibiotics in poultry farming. However further investigations are required for developing an eco-friendly biosynthesize process of AgNPs for ensuring the biocompatibility issues as requires for their *in vivo* applications against poultry bacterial diseases. Thus, this new antibacterial could

be an alternative of antibiotic for treating bacterial diseases and thereby minimizing economic losses due to bacteria in poultry industry.

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