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Research Article
Antibacterial Efficacy of Green Silver Nanoparticles Against Bacteria Isolated from Calf Diarrhoea

[1]Gamil Sayed Gamil Zeedan, [1]Abeer Mostafa Abdalhamed, [2]Eman Shafeek Ibrahim and [1]Hanan Abdel Fattah El-Sadawy

[1]Department of Parasitology and Animal Diseases, Veterinary Research Division, National Research Centre, 33 Bohouth St., Dokki, Giza, P.O. Box 12622, Egypt
[2]Department of Microbiology, Veterinary Research Division, National Research Centre, 33 Bohouth St., Dokki, Giza, P.O. Box 12622, Egypt

Abstract

Background and Objectives: Neonatal calf diarrhoea is one of the most serious problems faced livestock, causing great economic losses in the dairy industry all over the world. Several types of bacteria causing diarrhoea are resistance to the majority of antimicrobial agents' which urgent need to search for new antimicrobial agents to treat this problem. This study aimed to evaluate the antibacterial efficacy of green Silver nanoparticles (Ag NPs) biosynthesis using natural honey against bacteria isolated from calf diarrhoea. **Materials and Methods:** A total of 290 faecal samples were collected from bovine calves suffering from diarrhoea at different governorates in Egypt; during the period from June 2017 to March, 2018. Bacteria were isolated from calf diarrhoea characterized by morphological, biochemical and polymerase chain reaction (PCR). Antibacterial activities of green Ag NPS on bacteria isolated from calf diarrhoea include multidrug-resistant bacteria (MDR) were determined by disc diffusion methods and minimal inhibition concentration (MIC). **Results:** The most bacteria isolated from calf diarrhoea were *Escherichia coli* (*E. coli*), *Salmonella* spp. and *Staphylococcus aureus* (*S. aureus*). All of these bacteria were susceptible to Ciprofloxacin, Gentamicin and Chloramphenicol but resistance to penicillin; Ampicillin and Amoxicillin. The antibacterial effects of green Ag NPS on MDR bacteria had similar or superior effects of Ciprofloxacin and Chloramphenicol. **Conclusion:** The present study proved that the green Ag NPs had marked antibacterial effect against bacteria isolated from calf diarrhoea and eco-friendly but still need deep extensive study *in vivo* before used in human or animals.

Key words: Calf diarrhoea, dairy industry, antimicrobial agents, green silver nanoparticles, *Escherichia coli*, eco-friendly, multidrug-resistant bacteria, bovine calves, natural honey

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Corresponding Author: Gamil Sayed Gamil Zeedan, Department of Parasitology and Animal Diseases, National Research Centre, 33 Bohouth Street, Post Box, 12622, Dokki, Giza, Egypt Tel: +201145535240

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Neonatal calf Diarrhoea is one of the most serious problems in dairy livestock production worldwide, it involves significant economic losses due to high morbidity, mortality as well as veterinary costs[1]. Calves diarrheal syndrome is responsible for about more than half of pre-weaning mortality in dairy calves[2]. Their etiological complexes agents are environmental, nutritional and infectious agents including bacteria, viruses, protozoa and intestinal parasites[3]. The interactions between infectious and non-infectious, predisposing factors agents were occurred[4]. *Escherichia coli*, *Salmonella* and *Cryptosporidium* commonly associated pathogens are causes calf diarrhoea[5,6]. Resistant bacteria isolated from calf diarrhoea are frequently occurred due to habitual abuse of antibiotics in livestock and human[7]. Antimicrobial drugs considered the most preferable choice for treatment of calf diarrhoea. Although, fast developing antimicrobial resistant-bacteria[8,9]. Multidrug-resistance bacteria increased attention for using alternative antimicrobial agents from natural or inorganic products[10]. Recently, silver nanoparticles have attracted attention for treating pathogenic bacteria[11]. However, the green Ag NPs production classified into three steps including; solvent selection; reducing agent and nontoxic stability substances[12]. Nanoparticles produced commercially were synthesis by physico-chemical methods that required toxic substances, high temperatures, vacuum conditions and very expensive equipment which have adverse affects on host and their environment[13]. The urgent need to optimize nanoparticles to green methods to produce green silver nanoparticles which stable at room temperature using natural honey which performed dual action induced stabilization and reduction of Ag NPs beside antimicrobial action and eco-friendly on host and environment[14]. Referring to scientific research, no published data about antimicrobial activity of green AgNPs, against different bacteria isolated from calf diarrhoea. Therefore, the present study was aimed to isolate and identify different types of bacteria cause calf diarrhoea and evaluate the antimicrobial activity of green AgNPs against multidrug-resistant bacteria.

MATERIALS AND METHODS

Production of Silver nanoparticles using natural honey: Fresh natural honey was purchased from Agricultural Research Centre (ARC), Cairo, Egypt. Silver nitrate (Ag NO_3) was purchased from Sigma-Aldrich (USA). Honey was used at different pH according to the method described by Koneman *et al.*[14] and Quinn *et al.*[15]. Honey weights were 20, 30 and 40 g dissolved in 80 mL, 70 and 60 mL de-ionized water, respectively. Added 15 mL from each concentration was added to 20 mL of (1 m M) $AgNO_3$ aqueous solution mixed and stirred well for 1 min at 60°C. To initiate the reduction of Ag ions, the pH was adjusted to 8.55, 9, 9.55 and 10 using Na OH. Transferred of 300 µL from each colloid solution in 96 micro-plate at different intervals, read by spectrophotometer p6 well ELISA reader at different wavelengths, 340,405, 450,490 and 630 nm.

Samples: Ethical approval for the collection of faecal swabs samples from bovine calves was taken by professional veterinarians from Egyptian Veterinary Authority. A total of 290 faecal swabs were collected from bovine calf suffering from diarrhea at different governorates in Egypt, during the period from June 2017 to March 2018, used sterile cotton swabs directly from the rectum of the diarrheic calves placed in sterile test tubes and transferred to laboratory without delay. Fecal swabs were inoculated into 10 mL of peptone water incubated at 37°C for 24 h and then inoculated into the nutrient agar (NA) incubated at 37°C for 24 h according to Clinical and Laboratory Standards Institute (CLSI)[16]. Suspected bacteria isolates were identified and characterized by Gram's stains, biochemical tests including Triple sugar iron (TSI) agar slant reaction, Voges- Proskauer (VP), Methyl-Red (MR), Indole, Urease and Citrate utilization tests.

Serological identification: Salmonella serotypes were characterized by slide agglutination test (SAT) with polyvalent and monovalent O and H antiserum according to Kauffmann *et al.*[17]. *E. coli* serotypes were identified serologically by SAT using polyvalent antiserum against *E. coli* serogroup according to Edwards and Ewing[18].

Polymerase chain reaction (PCR)

DNA extraction: Bacterial DNA was extracted according to the protocol described by Itoh *et al.*[19]. All bacteria isolates were grown in nutrient broth at 37°C for 18-24 h. About 1.5 mL of the culture was centrifuged at 5000 rpm/10 min. The bacterial pellet was boiling at 100°C for 1 min in the water bath. The disrupted bacteria re-centrifuged again and the supernatant was used as PCR temple.

Polymerase chain reaction: It was performed according to Cohen *et al.*[20], Riffon *et al.*[21] and NCCLS[22]. PCR was applied on positive bacterial isolates. In case of *S. aureus* oligonucleotide primers were used NuC F 5 -TCAGCAAATGCATCACAAACAG-3,

NuC R 5-CGTAAATGCACTTGCTTCAGG-3, mec A F 5-GGGATCATAGCGTCATTATTC-3, mecA R 5-AACGATTGTGACACGATAGC-3 which amplify at 255 and 527 bp DNA fragments for NuC and mec A genes. Also, *Salmonella* spp. a pairs of primers of fmA (F) 5 -CCT TTC TCC ATC GTCCTG AA-3, fmA (R) 5 -TGC TGT TAT CTG CCT GAC CA-3 specific for the (fim A) gene encoding for the biosynthesis of fimbriae that amplify at 85 bp. Meanwhile, *E. coli* isolates oligonucleotide primers LTI (F) 5-AGCAGGTTTCCCAC CGGATCACCA-3, LTI (R) 5-GTGCTCAGATTCTGGGTCTC-3 STa (F) 5-TTTATTTCTGTATTGTCTTT-3, STa (R) 5-ATTACAACACAG TTCACA G-3 that amplify 171 and 275 bp fragment for LTI and STa genes. PCR reaction in 25 µL PCR mixture contains the specific primers and 5 µL bacterial DNA. Also, sterile deionized water was used as negative control. The amplification was done by Thermal Cycler.

Gel electrophoresis 1.5% agarose gel containing ethidium bromide, PCR products were visualized under UV light in trans-eliminator.

Antimicrobial susceptibility test: It was performed according to Bauer *et al.*[23]. The bacterial suspensions were streaked on Muller Hinton agar (MHA) plates with a cotton swab and with an antibiotic disc Penicillin (PEN-10), Ampicillin (AM-10), Amoxicillin (AMX), Chloramphenicol (CHL-30), Erythromycin (ERY-15), Ceftriaxone (CTX-30), Gentamicin (GN-10), Tetracycline (TET-30) and Ciprofloxacin (CIP-5) were aseptically dispenser on the surface of MHA, plate was incubated at 37°C, for 24 h. The diameters of inhibition zones around disc were measured. All the tests samples were performed in triplicates.

Modified disc diffusion methods: The antimicrobial activity of Ng NPs colloids mixtures were carried out by a modified well diffusion method according to CLSI[16]. About 100 µL of a suspension containing 10^6 CFU mL^{-1} of bacteria were spread on cultures MHA, 6 mm diameter well filled with 50 µL of different colloids, positive and control were used and incubated at 37° for 24 h. The diameters of inhibition zones around wells were measured and recorded. All the tests samples were performed in triplicates.

Minimum inhibitory concentration (MIC): This test was performed according to El-Desouky and Ammar[13]. Ag NPs colloids with honey mixture were employed two-fold serial dilutions in Muller Hinton Broth (MHB), 100 µL of tested micro-organisms added to each well. The microplates were incubated overnight at 37°C.

Statistical analysis: Data were calculated; mean with equation mean±standard deviation (SD). Significance difference between data evaluated by the student t-test at level $p<0.05$. Statistical analysis was performed according to SPSS[24]. (SPSS version 15, Windows7, Chicago).

RESULTS

Biosynthesis of silver nanoparticles (Bio-AgNPs): The color change in colloidal suspension was observed from colorless to yellow brown or deep brown color indicated formation of Ag NPs. While no color change was observed in Ag NO_3 solution or honey suspension as shown in Fig. 1.

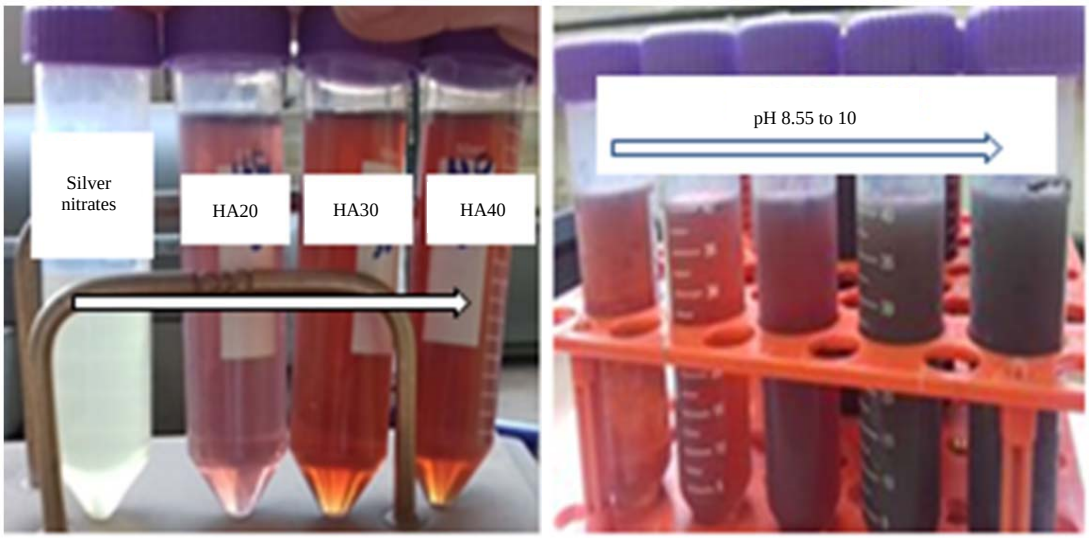

Fig. 1: Silver nanoparticles colloids suspension honey (HA) colourless left tube to golden brown, dark brown and deep dark brown right as arrow direction

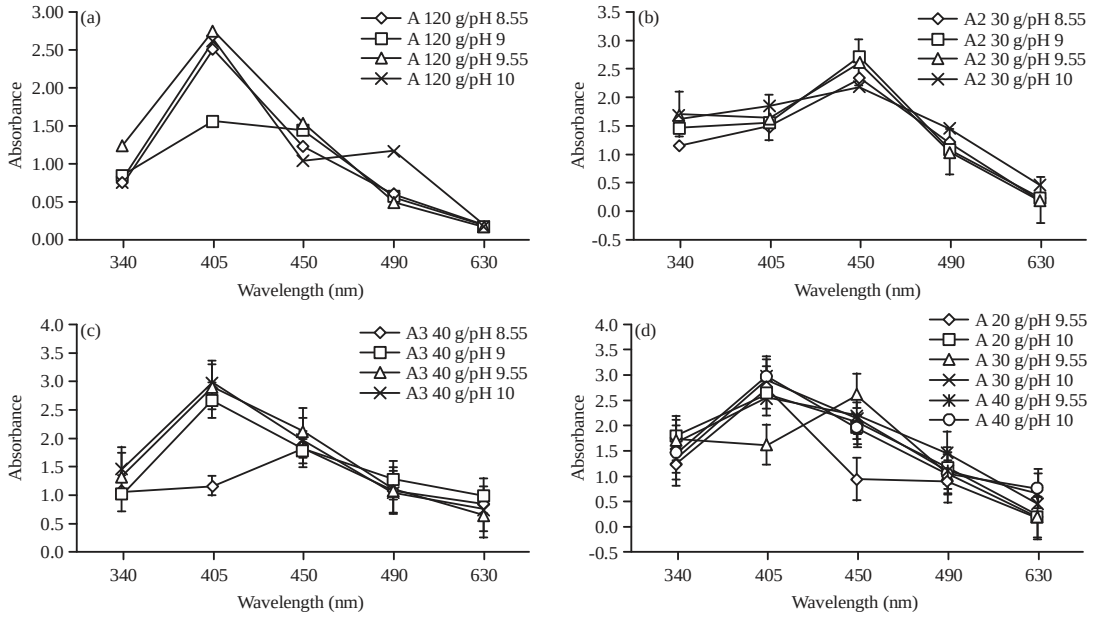

Fig. 2(a-d): UV spectrophotometer analysis (Optical density) of different Ag NPs colloids at pH 8.55-10, (a) 20 g/pH (8.55-10), (b) 30 g/pH (8.55-10), (c) 40 g/pH (8.55-10) and (d) 20-40 g/pH (9.55-10)

Table 1: Isolation bacteria (%) from fecal swabs of bovine calves related to ages

Calf ages	No. of diarrheic in calves		*S. arueus* (%)		*E. coli* (%)		*Salmonella* spp. (%)		Negative or other causes (%)	
	C. calves	B. calves	C. calves	B. calves	C. calves	B. calves	C. calves	B. calves	C. calves	B. calves
1st m	54	42	3 (5.50)	5 (11.90)	15 (27.27)	18 (42.85)	12 (22.22)	9 (21.40)	16 (29.62)	4 (9.50)
2nd m	32	33	1 (3.12)	8 (24.42)	6 (18.27)	12 (36.36)	3 (9.37)	3 (9.09)	20 (62.50)	5 (15.15)
3rd m	29	24	4 (13.79)	3 (12.50)	3 (10.34)	5 (20.83)	5 (17.24)	2 (8.33)	11 (37.90)	11 (45.83)
4th m	21	11	2 (9.52)	2 (18.20)	4 (19.04)	1 (9.09)	2 (9.52)	1 (9.09)	12 (57.14)	7 (63.85)
5th m	10	15	1 (10.00)	1 (6.66)	0 (0.00)	0 (0.00)	2 (20.00)	2 (13.33)	7 (70.00)	12 (80.00)
6th m	9	12	1 (11.11)	1 (8.33)	1 (11.11)	2 (16.66)	1 (11.11)	1 (8.33)	3 (33.33)	8 (66.60)
Total	155	135	12 (7.75)	20 (14.81)	29 (18.70)	38 (28.14)	25 (16.12)	18 (13.33)	69 (44.50)	47 (34.80)

1st m: First month of calf age, 2nd m: Second months of age, 3rd m: Third months of calf aged, 4th m: Fourth months of calf age, 5th m: Fifth months of calf age and 6th m: Sixth months of cal age, *S. aureus*: *Staphylococcus aureus*, *E. coli*: *Escherichia coli*, C. calves: Cows calves, B. calves: Buffaloes calves

UV-spectrophotometer analysis and characterization: The highest optical density (OD) of Ag NPs synthesis using different honey weights (20, 30 and 40 g) at pH, 8.55-10 were gave sharp peaks curve from wavelength ranged from 405-450 nm as in Fig. 2 (A1, A2 and A3). While, All Ag NPs colloids formation at pH 9.55 and 10 were showed similar sharp peaks ranged from 405-430 nm as in Fig. 2 (A4).

Bacterial isolation: The percentages of *E. coli*, *Salmonella* spp. and *S. arueus* isolated from faecal swabs from cow's calf suffering from diarrhoea were 18.7, 16.12 and 7.75%, respectively. Also, bacteria isolated from buffaloes calf suffering from diarrhoea were 28.14, 14.81 and 13.33% for *E. coli*, *S. arueus* and *Salmonella* spp., respectively as shown in Table 1.

Characterization of bacteria isolated from calf diarrhea: Characterization and identification of bacteria isolated from fecal swabs by morphological (Gram's stain) and biochemical methods. *E. coli* isolates were positive for catalase test, TSI, motility; indole, MR and negative for coagulation test, vogues proskauer, citrate, urease, oxidase and hydrogen sulphide. While *Salmonella* spp. were negative for TSI, indole, VP and citrate utilization. Also *S. aureus* produced acid without gas, coagulation plasma, catalase and methyl red but negative reaction to Indole, citrate, urease and oxidase as in Table 2.

PCR results: The bacteria isolates were identified with PCR with oligonucleotide primers nuc or mec A genes at 527 and 255 bp for detection of *S. aureus* (Fig. 3) and LT and ST genes

Table 2: Characterization of isolated bacteria by phenotype and biochemical tests

Coagulate test	Hemolysis test	Catalase test	TSI			Mot	In	MR	VP	Cit	Ur	Ox	H_2S	Identification isolates
			Glu	Lac	Suc									
-	-	+	+	+	+	+	+	+	-	-	-	-	-	*E. coli*
+	-	+	-	-	-	+	-	+	-	-	+	+	+	*Salmonella* spp.
+	+	+	+	+	+	-	-	+	+	-	-	-	+	*S. arueus*

+: Positive reaction, -: Negative reaction, TSI: Triple sugar iron, Mot: Motility, In: Indole, MR: Methyl red, VP: Voges proskauer, Cit: Citrate, Ur: Urease, Oxi: Oxidase, Cat: Catalase, H2S: Hydrogen sulphide

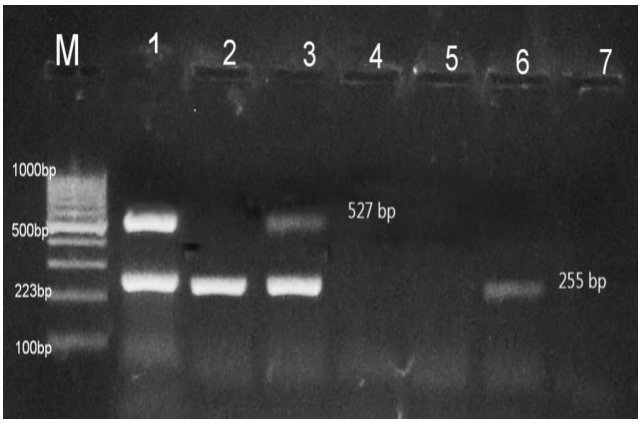

Fig. 3: Agarose gel electrophoresis for PCR product to nuc gene and mec A genes at 527 and 255 bp for detection of *S. aureus*

Lane M, (100 bp marker), Lane 1 and 3: Positive results at 527 and 255 bp. While Lane 2 and 6 positive for 255 bp only and Lane 4 and 5: negative samples. While Lane 7: Control negative

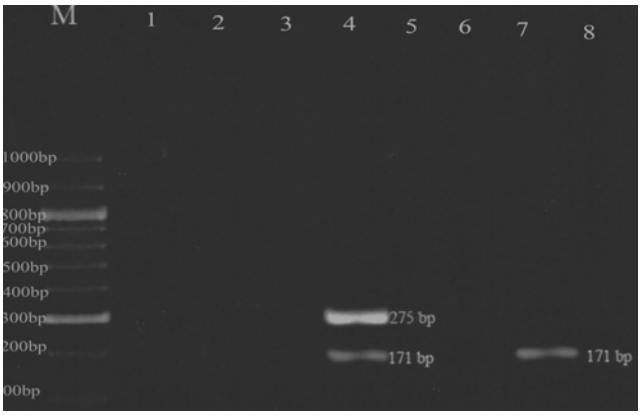

Fig. 4: PCR amplification of LT1 and ST genes of *E. coli* showing positive amplicons at 171 and 275 bp DNA size

Lane M: DNA marker (M l00 bp ladder), Lane 1, 2, 3 and 6: Negative samples, Lane 4: Positive at 171 and 275, Lane 7: Positive samples at 171 bp only and Lane 8: Negative control

of *E. coli* shown at 171 and 275 bp (Fig. 4). While, primers of fimA gene at 85 bp were detected the *Salmonella* species as in Fig. 5.

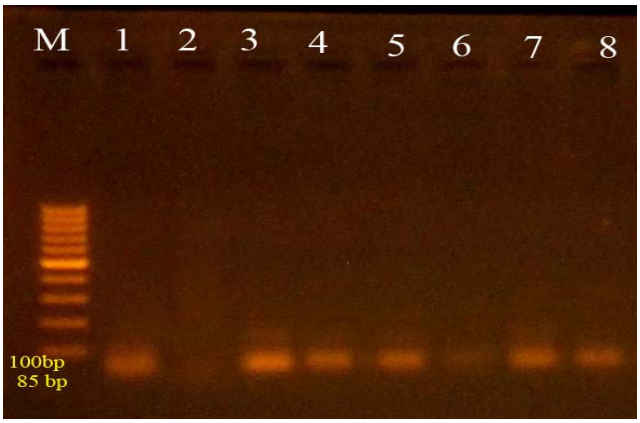

Fig. 5: DNA amplification PCR product for fimA gene at 85 bp for detection of *Salmonella* spp.

Lane M: 100 bp marker, Lane 1: Control positive, Lane 2: Control negative, Lane 3, 4, 5, 6, 7 and 8: Positive samples

Antibiotic susceptibility testing: The multi-drug resistant bacteria isolated from calf diarrhea were detected by disc diffusion methods. Bacteria exhibited resistant more than two antibiotics considered multidrug resistant bacteria. *E. coli* isolates were sensitive to Ciprofloxacin, Gentamicin and Chloramphenicol but resistances to penicillin (94.12%); ampicillin (95.0%) and amoxicillin (97%). The majority of the *Salmonella* spp. isolates were showed resistant to Amoxicillin (95.34%), Ampicillin (93.02%) and penicillin (90.69%). While the *S. aureus* isolates were resistant to amoxicillin (93.75%), ampicillin (90.6%) and penicillin (87.5%) as in Table 3.

Antimicrobial effect of Ag NPs against bacteria causing in dairy calves: The highest inhibitory effect of Ag NPs/h 40 g/pH 10 against *S. aueus* was 28±0.96 nm followed by Ag NPs/h 20 g pH 10 was 24±0.96 nm. While moderate inhibitory effects on *Salmonella* and *E. coli* of Ag NPs/h 40 g/pH 10 was 19±0.12 nm diameters using modified disc diffusion method. The MIC of Ag NPs/honey weights 20 and 40 g at pH 10 were 1.56 mg mL^{-1} against *Salmonella* and *S. aureus*. When Ag NPs concentration increased no bacterial growth was observed. While, MIC of Ag NPs/h 40 g/pH 8.55 and Ag NPS/h 40 g/pH 9 were 12.5-25 mg mL^{-1} concentration against *E. coli* as in Table 4.

Table 3: Antibiotics susceptibility test for MDR bacteria isolated from calve diarrhea

	E. coli (N = 67) (%)		*Salmonella* spp. (n = 43) (%)		*S. arueus* (n = 32) (%)	
Antibiotics	R	S	R	S	R	S
PEN-10	63 (94.12)	4 (5.98)	39 (90.69)	4 (9.31)	28 (87.50)	4 (12.50)
AM-10	64 (95.52)	3 (4.48)	40 (93.02)	3 (6.97)	29 (90.60)	3 (9.37)
AMX	65 (97.00)	2 (3.00)	41 (95.34)	2 (4.65)	30 (93.75)	2 (6.25)
CHL-30	39 (58.20)	28 (41.79)	31 (72.03)	12 (27.90)	15 (46.80)	17 (53.12)
ERY-15	47 (70.14)	20 (29.85)	31 (72.03)	12 (27.90)	22 (58.75)	10 (31.25)
CTX-30	45 (67.14)	12 (17.91)	18 (41.86)	25 (58.13)	20 (62.50)	12 (37.50)
GN-10	43 (64.14)	24 (35.82)	18 (41.86)	25 (58.13)	30 (93.75)	2 (6.25)
TET-30	50 (74.62)	17 (35.37)	32 (74.41)	11 (25.28)	12 (37.30)	20 (62.70)
CIP-5	18 (26.86)	49 (73.14)	12 (27.90)	31 (72.093)	3 (9.40)	29 (90.60)

PEN-10: Penicillin, AM-10: Ampicillin, AMX: Amoxicillin, CHL-30: Chloramphenicol, ERY-15: Erythromycin, CTX-30: Ceftriaxone, GN-10: Gentamicin, TET-30: Tetracycline, CIP-5: Ciprofloxacin

Table 4: Determination antibacterial of AgNPs against MDR bacteria by modified agar well diffusion methods (Mean±SD) and MIC

Ag NPS colloids with	Inhibition zone diameter Mean±SD			MIC		
different h and pH	*S. aureus*	*E. coli*	Sal. spp.	*S. aureus*	*E. coli*	Sal. spp.
Ag NPS h 20 g/pH 8.5	9±0.65	6±0.97	12±0.24	12.50	3.25	3.125
h20 g/pH 9	13±0.63	4±0.25	13±0.42	6.25	3.25	6.500
h 20 g/pH 9.55	14±0.25	5±.45	14±0.45	6.25	3.25	6.500
h 20 g/pH 10	24±0.96	12±0.56	15±0.56	1.56	3.25	6.250
Ag NPS/h 30 g/pH 8.55	10±0.59	6±0.25	11±0.48	12.50	6.25	6.250
h 30 g/pH 9	9±0.28	5±0.27	14±0.26	6.25	6.25	6.250
h 30 g/pH 9.55	12±0.45	6±0.29	13±0.45	6.25	6.25	12.500
h 30 g/pH 10	18±0.46	15±0.45	16±0.48	6.25	6.25	6.250
Ag NPS/h 40 g/pH 8.55	11±0.58	13±0.49	11±0.95	12.50	12.50	12.500
h 40 g/pH 9	7±0.56	5±0.48	14±0.47	12.50	25.00	6.250
h 40 g/pH 9.55	16±0.28	7±0.45	13±0.15	3.25	25.00	6.250
h 40 g/pH 10	28±0.85	19±0.89	19±0.12	1.56	6.25	1.560
CHL	10±0.89	19±0.56	18±0.45	6.25	1.56	1.560
CIP	17±0.95	20±0.89	22±0.58	1.56	1.56	1.560

Ag NPs h 20 g/pH 8.55: Ag NPs formations using honey weight 20 g at pH 8.55, 9, 9.55 and 10. Ag NPs h30 g/pH 8.5, 9, 9.55 and 10. Ag NPs formations using honey weight 30 g. Ag NPs h 40 g/pH: Ag NPs formations using honey weight 40 g at pH 8.55, 9, 9.55 and 10, CHL-5: Chloramphenicol, CIP: Ciprofloxacin, SD: Standard division

DISCUSSION

Neonatal calf diarrhoea is a major health problem causes by a variety of bacteria occur in different calf ages, seasons, management and herd size[25,26]. The bacteria resist to antibiotics due to habitual abuses in different disease stage to various species of animals. This situation is need develop new antimicrobial agent to overcome this problem. Silver nanoparticles unique physical and chemical properties was attract attention as new and rapidly evolving research of nanotechnolog[27].

The present study demonstrated that the natural honey induced rapid reduction of Ag ions increased in colloidal suspension which leading to change color from colorless to yellow or deep dark brown as a results of excitation of Surface Plasmon Resonance (SPR). Figure 1 and these results are in agreed with Dehkordi *et al.*[12]. The SPR of Ag NPs produced sharp peaks centred at around 405-450 nm as in Fig. 2 (A1, A2, A3 and A4) and these results are in agreement with Hosny *et al.*[28].

UV visible spectroscopy is primary confirmation of the presence of silver nanoparticles at absorbance peaks 410-425 nm is used to identify the possible bio-molecular responsible for the reduction of the Ag+ ions and escaping of the bio-reduced of silver nanoparticles synthesized using honey[29,30].

E. coli isolated from faecal swabs were positive for TSI, motility; indole; unease, citrate utilization, MR and coagulation plasma. *Salmonella* spp. isolated from calf diarrhoea were negative for TSI, indole, MR and citrate utilization. While, *S. aureus* produces acid without gas and positive reaction for coagulase, catalase and methyl red tests but negative reaction to Indole and Voges Proskauer test. There is no doubt that the nearly similar of all bacteria isolated from faecal swabs have several genetic materials which might be responsible for biochemical character according to Vijayaraghavan *et al.*[30]. The PCR is accurate and rapid technique for identification and detection of *E. coli*, *Salmonella* and *S. aureus* as in Fig. 3-5, those results are in agreement with Guerra *et al.*[31].

The frequency of *E. coli* was 18.7% followed by *Salmonella* spp. was 16.12% and *S. aureus* was 7.25% from cow's calf diarrhoea. While, the highest percentage of *E. coli*, *S. aureus* and *Salmonella* spp. isolated from buffalos calves were 28.14, 14.18 and 13.33%, respectively as in Table 1 similar finding by Paul *et al*.[32]. Found that the cultures swabs from diarrheic calves revealed that the predominant isolate was *E. coli* 52.5% and *Salmonella* spp. 5%. *E. coli* had a nearly similar result with the findings of Hashish *et al*.[33].

Salmonella isolates serotypes were varied from one area to another may be due to difference in hygienic measures, environmental and individual cure[34]. The prevalence of Salmonella was similar to those of other previous studies in Egypt[35,36]. The most common sources of infection are contaminated food and water with faecal materials of human, rodents, birds and other animals as a common source of salmonella infection in bovine calves[37].

E. coli isolates were highly resisted to Penicillin (94.12%); Ampicillin (95.0%) and Amoxicillin (97). Similarly, *Salmonella* spp. resist to Amoxicillin (95.34), Ampicillin (93.02%) and penicillin (90.69%). While the percent of *S. aureus* resist to Amoxicillin (93.75%), Ampicillin (90.6%) and Penicillin (87.5%). The majority of *S. aureus*, *Salmonella* spp. and *E. coli* were resist to different type of antibiotics may be due to the random or long-term drug abuses in different animal diseases stages or due to developed multi drug-resistant bacteria mutant genes similar results are agree with several reports by El-Shehedi *et al*.[38] and Younis *et al*.[39].

Ag NPs synthesis using different honey concentrate had antibacterial effects against *S. aueus*, *Sallmonella* spp. and also against *E. coli*. Explanation of antibacterial effect of Ag NPs may be due to smaller size of NPs, can easily reach the core content of the bacterium and incubated several parts in surface area, providing greater contact with the bacterium. The highest antibacterial effect of green Ag NPs against *S. aureus* followed by *Salmonella* spp. and *E. coli* may be due to biocides effect of Ag NPs which leading to disruption of cell-wall causes cell death, In addition, attached AgNPs to bacterial cells wall pits surface, accumulation of the Ag NPs on the cell surface and free radicals causes cells deaths. The MIC values of Ag NPs were exhibited the superiority of antibacterial activity against Gram-negative and Gram-positive bacteria including antibiotic-resistant isolated from calf diarrhoea, explanation attachment of NPs to the surface of cell membrane lead to altering function, such as permeability and respiration. Another possibility is that NPs may penetrate inside the cell and causes damage DNA and proteins[40,41]. Green Ag NPs doesn't cause any harmful for individual or environment; it can be eco-friendly. Finally, the green AgNPs colloidal mixture exhibited promising antibacterial effect superior to antibiotics on MDR bacteria isolated from calf diarrhoea.

CONCLUSION

The most bacteria isolated from bovine calf diarrhea were *S. aureus*, *E. coli* and *Salmonella* spp. The isolated bacteria were identified by morphological, biochemical, serological and PCR. The Ag NPs were bio-synthesis through the green methodology using natural honey have promising antibacterial effects against MDR bacteria isolated from calf diarrhea nearly similar or superior to Ciprofloxacin and Chloramphenicol.

SIGNIFICANCE STATEMENT

This study discovered that the green silver nanoparticles bio-synthesis and potent antibacterial activities against multidrug-resistant bacteria isolated from calf diarrhoea. Ag NPs have antibacterial effect against MDR bacteria and overcome this problem and this study will help the researchers to uncover the critical areas of biosynthesis of Ag NPs that many researchers were not able to explore by new eco-friendly production.

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