Therapeutic Potential of Plant Extracts Against Multidrug Resistance Poultry Bacteria

Nur-E-Alam, Md. Rayhan Ali, Md. Tarek Molla, Shahin Mahmud, Kaisar Ali Talukder and A. K. M. Mohiuddin*

Department of Biotechnology and Genetic Engineering, Mawlana Bhashani Science and Technology University, Tangail-1902, Bangladesh

Keywords: Poultry, Antibiotic resistance, Plant extract, Cross-contamination, Safe poultry source.

Abstract

Plant extracts were evaluated on bacteria isolated from poultry farm for developing substitutive therapeutic agent of antibiotics. A diverse range of bacterial load observed both in total viable count (TVC) and in total coliform count (TCC) in 30 samples randomly collected from poultry feeds, drinking water and faeces. A total of six bacterial isolates e.g. Pseudomonas spp., Aeromonas spp., Citrobacter spp., Vibrio spp., Escherichia coli and Plesiomonas spp. were found in the samples cultured in MacConkey Agar medium. Fifteen antibiotics were studied against bacterial susceptibility. All the bacterial isolates exhibited multi-antibiotic resistance (MAR) with gross resistance to erythromycin and ampicillin. E. coli had the highest MAR (53.3%), and Vibrio spp. as well as Plesiomonas spp. both had the same MAR (46.7%). Methanolic extract of Terminalia chebula and Azadirachta indica showed significant zone of inhibition against all the tested bacteria. These findings confirm the presence of multidrug resistant bacteria in poultry environment that reveals a possibility of cross-contamination to human and animals. The plant extracts could be developed into therapeutic drugs to rein antibiotic poultry resistant bacteria.

Introduction

Poultry is an agricultural term that refers to all domesticated birds kept for egg-laying and meat production (Danbappa et al. 2018). As a major source of animal protein, human depends largely on poultry (Mulder 1997). It is the second most widely-eaten meat in the world, accounting for about 38% of the world meat (Sule and Ilori 2017). In Bangladesh, a large number of people consume poultry meat and eggs to fulfill their daily protein demand (Rahman et al. 2014). Since early 1990, commercially produced poultry has been

*Author for correspondence: <akmmohiu@yahoo.com>

DOI: https://doi.org/10.3329/ptcb.v30i1.47797
growing up rapidly in Bangladesh by using improved genetics, manufactured feeds and proper management (Sultana et al. 2017). It was estimated that the poultry meat alone contributes 37% of the total meat production, and about 22-27% of the total animal protein supply in Bangladesh (Hamid et al. 2017). The poultry sector in Bangladesh is expected to employ around 11.2 million people and 2.0 million new households by the year 2020 (Rahman et al. 2017). The poultry industry in Bangladesh is obstructed by a number of constraints of which major one is the outbreak of disease causing mortality of chickens (about 30%) in every year (Sultana et al. 2017). Feed and water are the primary sources of disease causing pathogens in meat and egg producing birds (Adedeji et al. 2015).

Poultry feeds are usually food materials formulated with all nutritional materials (Okonko et al. 2010) needed for proper production of meat and eggs in birds (Chowdhury et al. 2011). Poultry feeds are often contaminated with food borne pathogen during preparation, contaminated raw materials, improper handling, etc. (Chowdhury et al. 2011, Roy et al. 2017). Different bacterial species such as, Escherichia coli, Staphylococcus aureus, Salmonella spp., Listeria spp., Streptococcus spp., Klebsiella spp., Pseudomonas spp., etc. found in the poultry feeds could cause diarrhoea, fowl cholera, salmonellosis, staphylococcosis, colibacillosis, erysipelas, listeriosis, etc. in poultry birds (Maciorowski et al. 2007). Water plays an important role in poultry metabolism makes up 55-75% of the body and elimination of waste products via urine (Jafari et al. 2006). Campylobacter spp., E. coli, Pseudomonas spp. and Salmonella spp. are the main poultry pathogens responsible for water contamination along with fecal coliform (Maes et al. 2019).

To improve meat production, the poultry industry uses antibiotics for growth, and disease prevention (Glasgow et al. 2019, Mehdi et al. 2018). Antibiotics have improved poultry performance effectively (Abiala et al. 2016) but in some circumstances pathogenic bacteria like E. coli, Shigella, Salmonella, Staphylococcus, Pseudomonas showed antibiotic resistance in poultry (Kebede 2010). A large number of antimicrobials used in poultry are also essential for human medicine (Fielding et al. 2012, Agyare et al. 2018). Humans are normally exposed to antimicrobial-resistant bacteria and resistant genes of these microbes are present in human food chain. Therefore, the use of antibiotics must be reduced in poultry industry (Angulo et al. 2009). Instead of antibiotics, plant-based therapeutics can be a good choice for their safety, low toxicity, and environment friendly (Zihadi et al. 2019, Djeussi et al. 2013). The leaf extract of Azadirachta indica (local name-Neem) and dried ripe fruit of Terminalia chebula (local name-Haritaki) possesses different antimicrobial activities (Kavitha et al. 2017, Ravva and Korn 2015). The leaves of A. indica extract showed inhibitory activity against multi-drug resistant human bacterial isolates of Salmonella typhi, Shigella dysenteriae, E. coli and Vibri cholerae (Bharitkar et al. 2014) and T. chebula extract against Helicobacter pylori, Xanthomonas campestris and S. typhi (Kannan et al. 2009).
Therefore, the aim of this study was to show bacterial association in feeds, water, and chicken poultry faeces, their antibiotics susceptibility and utilization of some plant extracts to minimize the hazards and risks related to bacterial contamination in poultry.

**Materials and Methods**

A total of 30 samples were collected from poultry practicing rural community in Tangail district of Bangladesh, of which 14 were poultry feed, 9 were water and 7 were faecal samples randomly collected from poultry stores and poultry farms between May and December, 2019. The feed samples were aseptically collected in sterile polyethylene bags while water and faecal materials were aseptically collected in sterile falcon tubes which were sealed and transported to the laboratory directly. A sterile warring blender was used to homogenize 1.0g of each feed and faecal sample into 10 ml of sterile distilled deionized water (Fawole and Oso 2001) resulting 1:10 dilution. Later on, serial dilutions up to 10^6 for feed and faecal samples, and 10^3 were prepared for water.

For the determination of total viable count (TVC) and total coliform count (TCC), about 0.1ml of diluted samples were cultured in duplicate on nutrient agar and MacConkey agar media, respectively using pour plate method. These were incubated at 37°C for 18hrs in an incubator. The results of TVC were expressed as the number of organism or colony-forming units per gram (cfu/g) of feed and faeces samples and cfu/ml for water sample. The bacterial isolates were identified on the basis of morphological and biochemical tests such as Kligler iron agar (KIA) test, Motility-indole-urease (MIU) test and citrate utilization test (Holt et al. 1994).

The susceptibility of the isolates against some common antibiotics and chemotherapeutics was tested using the Kirby-Bauer disk diffusion method on Mueller-Hinton agar (Hudzicki 2009, Watts et al. 2008). A total of 15 antibiotic discs (Oxoid, UK) such as ampicillin (10 μg), azithromycin (15μg), ciprofloxacin (5μg), tetracycline (30μg), amoxicillin (10μg), nalidixic acid (30μg), and kanamycin (30μg), amikacin (30 μg), chloramphenicol (30 μg), erythromycin (15 μg), gentamicin (10 μg), levofloxacin (5μg), neomycin (30 μg), norfloxacin (10 μg), and tazobactam (110 μg) were used. The interpretation of the antibiotic susceptibility was made according to CLSI (The Clinical and Laboratory Standards Institute 2014).

Healthy and disease-free plant leaves of *Azadirachta indica* were collected directly from the plant and the dried ripe fruit of *Terminalia chebula* purchased from a local market of Tangail. The freshly collected leaves and dried ripe fruits were washed with distilled water and dried in shade for two weeks and blended into powder using mortar. About 100g powder for both *T. chebula* ripe fruit and leaves of *A. indica* were extracted with 600ml of methanol (95% for *T. chebula*), and(30, 40 and 70% methanol for *A. indica*) at 25°C for 48hrs (Sahreen et al. 2011). The crude extracts were filtered using Whatman No. 1 filter paper and evaporated by using a rotary evaporator.
Antimicrobial activity of the plant extracts was tested using disc-diffusion method. Young culture of the test organisms in 2 ml sterile Mueller Hinton broth (MHB) were made from well isolated single colony obtained from 24hrs grown cultures. The test cultures were swabbed on the top of the solidified medium and allowed to dry. The disc containing plant extract (each disc contains 30 µl of plant extract in different methanolic concentration) were placed on the plate. The plates were then incubated at 37°C for 18hrs. The diameter of the zone of inhibition around each disc was measured in mm and the mean value was calculated (Hudzicki 2009).

In this study, microbial were determined following standard formulae. Then the results were analyzed by SPSS ver. 20. Hierarchical analysis was used to estimate overall similarities of the bacterial resistance using their zones of inhibition. Statistical significance was set at a p<0.05. Microsoft Excel version 2016 was used to draw graphs wherever appropriate.

Results and Discussion
A diverse range of total viable counts (TVC) and total coliform counts (TCC) of bacteria were found to be associated with the samples (Table1). TVC ranged from 2.64×10⁶ to 9.76 × 10⁶ cfu/g in poultry feed, 2.6 × 10⁵ to 4.4 × 10⁵ cfu/ml in water and 9.6 × 10⁶ to 1.76 × 10⁸ cfu/g in faeces, respectively. Nasrin et al. (2007) reported that TVC of the faeces, feed and drinking water was (103.5 ± 3.62) × 10⁵, 6.5 ± 1.87) × 10⁵ cfu/g and (31.33 ± 1.12) × 10⁵cfu/ml, respectively. Three different samples of poultry faeces contained the most elevated TVC which may be due to the presence of increased number of bacterial populations in gut. On the other hand, TCC for feed, drinking water and faeces of poultry ranged from 1.1 × 10⁵ to 5.84 × 10⁶ cfu/g, 5 × 10³ to 5.6 × 10⁴ cfu/ml and 4×10⁵ to 1.92 × 10⁶ cfu/g, respectively.

Five bacterial isolates, namely E. coli, Citrobacter spp., Pseudomonas spp., Aeromonas spp. and Vibrio spp. from poultry feed samples (Layer-layer, Sonali, Layer-starter, Broiler-grower, Broiler-finisher, Broiler-starter), four (E. coli, Citrobacter spp., Pseudomonas spp. and Plesiomonas spp.) from drinking water and four (E. coli, Pseudomonas spp., Aeromonas spp. and Vibrio spp.) from poultry faeces were found in MacConkey’s agar medium (Table 2). Overall, six bacterial isolates were found in feeds, drinking water and faeces of poultry, however, E. coli and Pseudomonas spp. found in all the three samples. In case of Pseudomonas spp. similar result was also reported in feed (Okonko et al. 2010), water (Adesoji et al. 2015) and faeces (Adeleke et al. 2011) of poultry. On the other hand, the other three bacterial isolates e.g. Citrobacter spp., Aeromonas spp. and Vibrio spp. were found in any two samples, but Plesiomonas spp. found only in water (Table 2).

Biochemical tests, alternatively, confirm further the mentioned isolates of all bacteria found in three samples (Table 3). This characterization was accomplished simultaneously by observing distinct morphological characteristics and a number of biochemical tests on the basis of presence (+) or absence (-) criterion in Tables 2 and 3.
Table 1. Total viable counts (TVC) and total coliform counts (TCC) of the feed, water and poultry faeces.

| Sample                  | TVC (cfu/g) or (cfu/ml) | TCC (cfu/g) or (cfu/ml) |
|-------------------------|-------------------------|-------------------------|
| Feed-1 (Layer-layer)    | $7.20 \times 10^6$      | $1.4 \times 10^6$        |
| Feed-2 (Layer-layer)    | $4.35 \times 10^6$      | $3.60 \times 10^6$        |
| Feed-3 (Sonali)         | $4.20 \times 10^6$      | $1.85 \times 10^6$        |
| Feed-4 (Sonali)         | $3.52 \times 10^6$      | $2.60 \times 10^6$        |
| Feed-5 (Layer-starter)  | $3.40 \times 10^6$      | $2.12 \times 10^6$        |
| Feed-6 (Layer-layer)    | $3.16 \times 10^6$      | $2.05 \times 10^6$        |
| Feed-7 (Layer-layer)    | $2.76 \times 10^6$      | $1.52 \times 10^6$        |
| Feed-8 (Broiler-finisher)| $3.84 \times 10^6$    | $4.8 \times 10^5$         |
| Feed-9 (Broiler-finisher)| $5.60 \times 10^6$    | $2.56 \times 10^6$        |
| Feed-10 (Broiler-grower)| $2.64 \times 10^6$     | $1.1 \times 10^5$         |
| Feed-11 (Broiler-grower)| $5.6 \times 10^6$      | $4.56 \times 10^6$        |
| Feed-12 (Broiler-grower)| $8.96 \times 10^6$     | $3.28 \times 10^6$        |
| Feed-13 (Layer-layer)   | $9.76 \times 10^6$      | $5.84 \times 10^6$        |
| Feed-14 (Broiler-starter)| $5.44 \times 10^6$    | $8.2 \times 10^5$         |
| Water-1                 | $1.72 \times 10^6$      | $2.0 \times 10^4$         |
| Water-2                 | $1.48 \times 10^6$      | $2.8 \times 10^4$         |
| Water-3                 | $4.4 \times 10^5$       | $1.6 \times 10^4$         |
| Water-4                 | $9.2 \times 10^4$       | $5.6 \times 10^4$         |
| Water-5                 | $1.18 \times 10^5$      | $1.7 \times 10^4$         |
| Water-6                 | $9 \times 10^4$         | $2.3 \times 10^4$         |
| Water-7                 | $1.2 \times 10^5$       | $4.5 \times 10^4$         |
| Water-8                 | $3.1 \times 10^4$       | $5 \times 10^3$           |
| Water-9                 | $2.6 \times 10^4$       | $7 \times 10^3$           |
| Faeces-1                | $1.2 \times 10^8$       | $4 \times 10^6$           |
| Faeces-2                | $1.76 \times 10^8$     | $8.4 \times 10^6$         |
| Faeces-3                | $9.60 \times 10^7$      | $1.92 \times 10^7$        |
| Faeces-4                | $1.14 \times 10^8$     | $1.15 \times 10^7$        |
| Faeces-5                | $1.04 \times 10^7$     | $8.4 \times 10^6$         |
| Faeces-6                | $2.56 \times 10^7$     | $9 \times 10^6$           |
| Faeces-7                | $9.6 \times 10^6$       | $6 \times 10^6$           |
Table 2. The bacteria found in feeds, water and poultry faeces in MacConkey’s agar medium.

| Isolates          | Feed | Drinking water | Faeces |
|-------------------|------|----------------|--------|
| *Escherichia coli*| +    | +             | +      |
| *Citrobacter* spp.| +    | +             | -      |
| *Pseudomonas* spp.| +    | +             | +      |
| *Aeromonas* spp.  | +    | -             | +      |
| *Plesiomonas* spp.| -    | +             | -      |
| *Vibrio* spp.     | +    | -             | +      |

+ = Presence, − = Absence.

Table 3. Major biochemical tests for the bacteria isolated from feeds, drinking water and poultry faeces growing on MacConkey agar medium.

| Slant | KIA | MIU | Simon’s Citrate | Presumptive identified organisms |
|-------|-----|-----|-----------------|---------------------------------|
| K     | A   | +   | +               | +                               | *Citrobacter* spp.           |
| A     | A   | -   | +               | -                               | *Escherichia coli*           |
| K     | K   | -   | -               | +                               | *Pseudomonas* spp.           |
| K     | A   | +   | +               | +                               | *Aeromonas* spp.             |
| K     | K   | -   | +               | -                               | *Plesiomonas* spp.           |
| K     | A   | -   | +               | +                               | *Vibrio* spp.                |

K = Alkaline, A = Acidic, ‘+’ = Presence, ‘−’ = Absence.

Six bacterial isolates viz., *Pseudomonas* spp., *Aeromonas* spp., *Citrobacter* spp., *Vibrio* spp., *E. coli* and *Plesiomonas* spp. isolated from poultry chicken sources were used to test antimicrobial susceptibility against 15 commercial antibiotics. Percentage of resistance observed from the isolates were 26.7, 40, 26.7, 46.7, 53.3 and 46.7% against *Pseudomonas* spp., *Aeromonas* spp., *Citrobacter* spp., *Vibrio* spp., *E. coli* and *Plesiomonas* spp., respectively (Table 4). All the bacterial isolates showed gross resistance against erythromycin, ampicillin and gross susceptible against tazobactam, gentamicin, ciprofloxacin, amikacin, chloramphenicol (Table 4).

In this study, presence of *E. coli* in the feed, drinking water and faeces of poultry chickens was found resistant to multiple antibiotics. The pathogenic strains did not only increase the resistance against the antibiotics but also increased resistance in the endogenous flora of humans and animals (Kolář et al. 2002). *E. coli* isolates showed resistance against amoxicillin, tetracycline, erythromycin, norfloxacin, levofloxacin, ampicillin, azithromycin and nalidixic acid. Chowdhury et al. (2011) reported that *E. coli* isolates from poultry in Savar of Bangladesh were found resistant to chloramphenicol,
Therapeutic Potential of Plant Extracts Against Multidrug Resistance

ampicillin, ciprofloxacin and tetracycline. It is not clear as to why this discrepancy was found in case of ciprofloxacin and chloramphenicol. It could be due to different strains/serotypes obtained from different locations.

Table 4. Antibiotic susceptibility profiles of the bacteria isolated from different poultry samples.

| Antibiotics      | Code | Potency (µg) | Pseudomonas spp. (%) | Aeromonas spp. (%) | Citrobacter spp. (%) | Vibrio spp. (%) | E. coli (%) | Plesiomonas spp. (%) |
|------------------|------|--------------|----------------------|--------------------|---------------------|----------------|-------------|----------------------|
| Amoxicillin      | AX   | 10           | S                    | R                  | R                   | R              | R           | R                    |
| Tetracycline     | TE   | 30           | S                    | S                  | S                   | R              | R           | R                    |
| Erythromycin     | E    | 15           | R                    | R                  | R                   | R              | R           | R                    |
| Neomycin         | N    | 30           | R                    | R                  | S                   | R              | S           | S                    |
| Norfloxacin      | NOR  | 10           | S                    | S                  | S                   | R              | R           | S                    |
| Tazobactam       | TPZ  | 110          | S                    | S                  | S                   | S              | S           | S                    |
| Kanamycin        | K    | 30           | S                    | S                  | S                   | R              | S           | R                    |
| Gentamicin       | CN   | 10           | S                    | S                  | S                   | S              | S           | S                    |
| Levofloxacin     | LEV  | 5            | R                    | S                  | S                   | S              | R           | S                    |
| Ciprofloxacin    | CIP  | 5            | S                    | S                  | S                   | S              | S           | S                    |
| Amikacin         | AK   | 30           | S                    | S                  | S                   | S              | S           | S                    |
| Ampicillin       | AM   | 10           | R                    | R                  | R                   | R              | R           | R                    |
| Azithromycin     | AZM  | 15           | S                    | R                  | R                   | R              | R           | R                    |
| Nalidixic Acid   | NA   | 30           | S                    | R                  | S                   | S              | R           | R                    |
| Chloramphenicol  | C    | 30           | S                    | S                  | S                   | S              | S           | S                    |
| MAR (%)          |      | 26.7         | 40.0                 | 26.7               | 46.7                | 53.3           | 46.7        |

R=Resistance, S= Susceptible.

*Pseudomonas* was one of the most frequently identified bacteria associated with all samples exhibited resistance to erythromycin, neomycin, ampicillin, and levofloxacin. With reference to Nigeria, *Pseudomonas* found resistant to all their test antibiotics except gentamicin (Okonko et al. 2010). Our findings showed similar consequence in regard to gentamicin also. Surprisingly, *Pseudomonas* spp. showed sensitivity against amoxicillin and azithromycin compared to other isolates which were found resistant to them.

The presence of *Vibrio* spp. in feed (Roy et al. 2017) and *Plesiomonas* spp. in water (Pilar and De Garcia 1997, Santos et al. 2015) has been re-established by our findings. The isolated *Vibrio* sp. and *Plesiomonas* spp. were found resistant to amoxicillin, tetracycline, erythromycin, kanamycin, ampicillin, and azithromycin. The observed resistance against these antibiotics that were used presumably enlightened the high usage of the drugs in the study sites. As a result, these drugs may have become seriously imperiled and ineffective. *Vibrio* spp. and *Plesiomonas* spp. displayed resistance to tetracycline and kanamycin and they both had the same MAR (46.7%).
Aeromonas spp. and Citrobacter spp. both were found in feed and faeces of poultry. These bacteria were resistant to amoxicillin, erythromycin, ampicillin, azithromycin. In another study, Aeromonas spp. was found resistant to erythromycin (Igbinosa 2014) and Citrobacter spp. showed resistance profile against tetracycline, gentamicin, nalidixic acid and chloramphenicol (Kannan et al. 2009).

The hierarchical analysis of the Gram negative bacteria isolates conjectured that the antibiotic resistance pattern of Vibrio spp. (4) and Plesiomonas spp.(6); Pseudomonas spp. and Citrobacter spp. were the most related. The other isolates, found from poultry environment were less related (Fig. 1).

![Hierarchical analysis of antibiotic resistance pattern of the Gram negative bacteria isolated from poultry environment. 1 = Pseudomonas spp., 2 = Aeromonas spp., 3 = Citrobacter spp., 4 = Vibrio spp., 5 = E. coli and 6 = Plesiomonas spp.](image)

Antibacterial activities of both 95% methanolic Terminalia chebula extract and 70% methanolic Azadirachta indica extract were significant against all the isolated bacteria that were resistant against different antibiotics. The average zone of inhibition of 95% methanolic Terminalia chebula extract and 70% methanolic Azadirachta indica extract were 11.33±1.25 and 12.33±1.25, respectively. On the other hand, 30 and 40% methanolic extract of Azadirachta indica have less significant zone of inhibition against the tested bacteria (Table 5).

The methanolic extract of T. chebula and A. indica extracts demonstrated antibacterial activities against antibiotic resistant E. coli. This observation is in conformity with other workers (Mostafa et al. 2011, El-Moez et al. 2014). The activity of T. chebula (Kannan et al. 2009) and A. indica (Harjai et al. 2013) was evident against Pseudomonas. The antibacterial
Therapeutic Potential of Plant Extracts Against Multidrug Resistance

activity of plant extracts inhibits the growth of *Vibrio* and *Plesiomonas*. This finding was consistent with previous reports (Mostafa et al. 2011, Thakurta et al. 2007) by using *T. chebula* and *A. indica* extract against *Vibrio*. However, *Aeromonas* and *Citrobacter* spp. both have the susceptibility against the *A. indica* extract (El-Moez et al. 2014, Dhayanithi et al. 2010) as well as *T. chebula* extract.

### Table 5. Antibacterial activity of *Terminalia chebula* and *Azadirachta indica* plant extracts.

| Isolates          | 95% methanolic T. chebula extract | 70% methanolic A. indica extract | 40% methanolic A. indica extract | 30% methanolic A. indica extract |
|-------------------|----------------------------------|---------------------------------|---------------------------------|---------------------------------|
| *Pseudomonas* spp. | 11                               | 13                              | 0                               | 0                               |
| *Aeromonas* spp.  | 13                               | 12                              | 0                               | 0                               |
| *Citrobacter* spp. | 12                               | 13                              | 0                               | 0                               |
| *Vibrio* spp.     | 11                               | 12                              | 0                               | 0                               |
| *E. coli*         | 9                                | 10                              | 0                               | 0                               |
| *Plesiomonas* spp.| 12                               | 14                              | 0                               | 0                               |
| Mean ± SD         | 11.33 ± 1.25                     | 12.33 ± 1.25                    | 0                               | 0                               |

There was no significant relationship between *Terminalia chebula* and *Azadirachta indica* extract against the six bacteria isolated from different poultry sources (t value = 1.85, p = 0.138) (Table 6).

### Table 6. Statistical t value, p value and 95% confidence interval of *T. chebula* and *A. indica* extract on bacteria isolated from different poultry samples.

| R | df | F  | T value | 95% confidence interval | p value |
|---|----|----|---------|-------------------------|---------|
|   |    |    |         | Lower                  | Upper   |
| 0.679 | 1 | 3.41 | 1.85 | -0.341                  | 1.7     | 0.138 |

Poultry environment serves as a source of multidrug resistance bacteria. These bacteria not only infect the poultry but also can infect or reach into the human population through farm to fork model. The indiscriminate and overuse of antibiotics as growth promoters and prevention of diseases are behind the incidence of resistance. Therefore, an alternative approach is important to reveal the plants extract which contains the bioactive compounds. Thus, an onward action plan might be taken nationwide to monitor the use of antibiotics and the application of therapeutic use of medicinal plants will ultimately reduce resistant bacteria.
Reference

Abiala M, Olayiwola J, Babatunde O, Aiyelaagbe O and Akinyemi S (2016) Evaluation of therapeutic potentials of plant extracts against poultry bacteria threatening public health. BMC Compl. & Alt. Med. 16: 417-424. https://doi:10.1186/s12906-016-1399-z.

Adeleke EO and Omavube BO (2011) Antibiotic resistance of aerobic mesophilic bacteria isolated from poultry faeces. Res. J. Microbiol. 6(4): 356-365.

Adesoji AT, Ogunjobi AA and Olatoye IO (2015) Molecular characterization of selected multidrug resistant Pseudomonas from water distribution systems in southwestern Nigeria. Annals Clinical Microbiol. & Antimicrob.14: 39. https://doi.org/10.1186/s12941-015-0102-4.

Agyare C, Boamah VE, Zumbi CN and Osei FB (2018) Antibiotic use in poultry production and its effects on bacterial resistance. In: Antimicrobial Resistance-A global threat. Yashwant Kumar (ed.) IntechOpen. DOI: 10.5772/intechopen.79371.

Angulo FJ, Collignon P, Powers JH, Chiller TM, Aidara AK and Aarestrup FM (2009) World Health Organization ranking of antimicrobials according to their importance in human medicine: A critical step for developing risk management strategies for the use of antimicrobials in food production animals. Clin. Infec. Dis. 49(1): 132-141.

Bharitkar YP, Bathini S, Ojha D, Ghosh S, Mukherjee H, Kuotsu K, Chattopadhyay D and Mondal NB (2014) Antibacterial and antiviral evaluation of sulfonoquinovosyl-diacylglyceride: A glycolipid isolated from Azadirachta indica leaves. Letters in Appl. Microbiol. 58(2): 184-189.

Chowdhury A, Iqbal A, Uddin MG and Uddin M (2011) Study on isolation and identification of Salmonella and Escherichia coli from different poultry feeds of Savar Region of Dhaka, Bangladesh. J. Scientific Res. 3(2): 403-411.

Clinical and Laboratory Standards Institute (CLSI) (2014) Performance standards for antimicrobial susceptibility testing; Twenty-fourth informational supplement. CLSI document M100-S24. 34: 47-135.

Danbappa AA, Alhassan KA and Shah MM (2018) Isolation and identification of microbial contaminants associated with commercial poultry feeds. J. Appl. & Adv. Res. 3(5): 142-147.

Dhayanithi NB, Ajith Kumar TT and Kathiresan K (2010) Effect of neem extract against the bacteria isolated from marine fish. J. Environ. Biol. 31(4): 409-412.

Djeussi DE, Noumedem JA, Seukep JA, Fankam AG, Voukeng IK, Tankeo SB, Nkute AH and Kuete V (2013) Antibacterial activities of selected edible plants extracts against multidrug-resistant Gram negative bacteria. BMC Compl. & Alt. Med.13(1): 164-271.

El-Moez SI, Omaara ST, Amer HA and Zaki FN (2014) Antimicrobial activities of neem extract (Azadirachta indica) against microbial pathogens of animal origin. Global Veterinaria. 12(2): 250-256.

Fawole MO and Oso BA (2001) Laboratory manual of Microbiology: Revised edition Spectrum Books Ltd. Ibadan.

Fielding BC, Mnabisa A, Gouws PA and Morris T (2012) Antimicrobial-resistant Klebsiella species isolated from free-range chicken samples in an informal settlement. Archives of Med. Sci. 8(1): 39-42.
Glasgow L, Forde M, Brow D, Mahoney C, Fletcher S and Rodrigo S (2019) Antibiotic use in poultry production in Grenada. Vet. Med. Inter. 4: 1-7. https://doi:10.1155/2019/6785195.

Hamid MA, Rahman MA, Ahmed S and Hossain KM (2017) Status of poultry industry in Bangladesh and the role of private sector for its development. Asian J. Poultry Sci. 11(1): 1-13.

Harjai K, Bala A, Gupta RK and Sharma R (2013) Leaf extract of Azadirachta indica (Neem): A potential antibiofilm agent for Pseudomonas aeruginosa. Pathogens and Dis. 69(1): 62-65.

Holt JG, Krieg NR, Sneath PH and Staley JT (1994) Bergey’s Manual of Determinative Bacteriology. 9th Ed, William & Wilkins, Baltimore, USA.

Hudzicki J (2009) Kirby-Bauer disk diffusion susceptibility test protocol. American Society for Microbiology.

Igbinosa IH (2014) Antibiogram profiling and pathogenic status of Aeromonas species recovered from chicken. Saudi J. Biol. Sci. 21(5): 481-485.

Jafari RA, Fazlara A and Govahi M (2006) An investigation into Salmonella and fecal coliform contamination of drinking water in broiler farms in Iran. Int. J. Poultry Sci. 5(5): 491-493.

Kannan P, Ramadevi SR and Hopper W (2009) Antibacterial activity of Terminalia chebula fruit extract. African J. Microbiol. Res. 3(4): 180-184.

Kavitha M, Raja M, Kamaraj C, Karthik RR, Balasubramaniam V, Balasubramani G and Perumal P (2017) In vitro antimicrobial activity of Azadirachta indica (leaves) against fish pathogenic bacteria isolated from naturally infected Dawkinsia filamentosa (Blackspot barb). Medicinal and Aroma. Plants. 6(3): 294-300.

Kebede F (2010) Pseudomonas infection in chickens. J. Veterinary Medicine and Animal Health. 2(4): 55-58.

Kolár M, Pautček R, Bardoň J, Vagnerova I, Typovska H, Valka I and Doškař J (2002) Occurrence of antibiotic-resistant bacterial strains isolated in poultry. Veterinary Medicine - Czech. 47(2-3): 52-59.

Maciorowski KG, Herrera P, Jones FT, Pillai SD and Ricke SC (2007) Effects on poultry and livestock of feed contamination with bacteria and fungi. Animal Feed Sci. & Tech. 133(1-2): 109-136.

Maes S, Vackier T, Huu SN, Heyndrickx M, Steenackers H, Sampers I, Raes K, Verplaetse A and De Reu K (2019) Occurrence and characterization of biofilms in drinking water systems of broiler houses. BMC Microbiol. 19: 77-91.

Mehdi Y, Létourneau-Montminy MP, Gaucher ML, Chorfi Y, Suresh G, Rouissi T, Brar SK, Côté C, Ramirez AA and Godbout S (2018) Use of antibiotics in broiler production: Global impacts and alternatives. Animal Nutrition 4(2): 170-178.

Mostafa MG, Rahman M and Karim MM (2011) Antimicrobial activity of Terminalia chebula. Int. J. Medicinal and Aroma. Plants 1(2): 175-179.

Mulder RW (1997) Safe poultry meat production in the next century. Acta Veterinaria Hungarica. 45(3): 307-315.

Nasrin MS, Islam MJ, Nazir KH MnH, Choudhury KA and Rahman MT (2007) Identification of bacteria and determination of their load in adult layer and its environment. J. Bangladesh Society for Agri. Sci. Tech. 4: 69-72.
Okonko IO, Nkang AO, Fajobi EA, Mejeha OK, Udeze AO, Motayo BO, Ogun AA, Ogunnusi TA and Babalola TA (2010) Incidence of multi-drug resistant (MDR) organisms in some poultry feeds sold in Calabar Metropolis, Nigeria. Elec. J. Environ., Agri. Food Chem. 9(3): 514-532.

Pilar HS and De Garcia RR (1997) Prevalence of Plesiomonas shigelloides in aquatic environments. Int. J. Environ. Health Res. 7(2): 115-120.

Rahman MA, Kamal SH, Salam AB and Salam A (2014) Assessment of the quality of the poultry feed and its effect in poultry products in Bangladesh. J. Bangladesh Chem. Society 27(1 & 2): 1-9.

Rahman MS, Jang DH and Yu CJ (2017) Poultry industry of Bangladesh: Entering a new phase. Korean J. Agri. Sci. 44(2): 272-282.

Ravva SV and Korn A (2015) Effect of Neem (Azadirachta indica) on the survival of Escherichia coli O157: H7 in dairy manure. Int. J. Environ. Res. and Public Health. 12(7): 7794-7803.

Roy CR, Ahmed T and Uddin MA (2017) Microbiological analysis of poultry feeds along with the demonstration of the antibiotic susceptibility of the isolates and the antibacterial activity of the feeds. Bangladesh J. Microbiol. 34(2): 103-107.

Sahreen S, Khan MR and Khan RA (2011) Hepatoprotective effects of methanol extract of Carissa opaca leaves on CCl 4-induced damage in rat. BMC Complement Altern. Med. 11:48. doi: 10.1186/1472-6882-11-48.

Santos JA, Rodríguez-Calleja JM, Otero A and García-López ML (2015) Plesiomonas. In: Molecular Medical Microbiology, Yi-Wei Tang, Max Sussman, Joseph Schwartzman (Edited) (pp. 1111-1123). 2nd Edition, Academic Press.

Sule IO and Ilori IO (2017) Microbiological assessment of poultry feeds within Ilorin, Nigeria. Notulae Scientia Biol. 9(1): 34-39.

Sultana N, Haque MA, Rahman MM, Akter MR, Begum MD, Fakhruzzaman M, Akter Y and Amin MN (2017) Microbiological quality of commercially available poultry feeds sold in Bangladesh. Asian J. Medical and Biol. Res.3(1): 52-60.

Thakurta P, Bhowmik P, Mukherjee S, Hajra TK, Patra A and Bag PK (2007) Antibacterial, antisecretory and antihemorrhagic activity of Azadirachta indica used to treat cholera and diarrhea in India. J. Ethnopharma. 111(3): 607-612.

Watts JL, Shryock TR, Apley M, Brown SD, Gray JT, Heine H, Hunter RP, Mevius DJ, Paich MG, Silley P and Zurenko GE (2008) Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals; approved standard-3rd edition, Science Open, Inc. Burlington, USA.

Zihadi MA, Rahman M, Talukder S, Hasan MM, Nahar S and Sikder MH (2019) Antibacterial efficacy of ethanolic extract of Camellia sinensis and Azadirachta indica leaves on methicillin-resistant Staphylococcus aureus and shiga-toxigenic Escherichia coli. J. Advanced Veterinary and Animal Res. 6(2): 247-252.

(Manuscript received on 23 May, 2020; revised on 27 May, 2020)