



# Silver Nanoparticles as a Promising Therapeutic Strategy for Infections Caused by Resistant Bacteria in Cattle and Birds



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Submission: June 22, 2018; Published: August 06, 2018

## Abstract

Bacterial infections, especially mastitis, colibacillosis, salmonellosis and gastrointestinal campylobacteriosis are responsible for high rates of morbidity and mortality in humans and animals worldwide, leading to high costs of treatment and loss of cattle and poultry herds on large and small farms. In addition, when these infections are caused by multidrug-resistant bacteria they present a more invasive pattern and are associated with poor treatment outcomes. In this context, nanotechnology, especially silver nanoparticles (AgNPs), arises as a promising new therapeutic option to thwart the progression of these infections. In this context, this mini-review aims to shed light on recent findings related to the promising potential of AgNPs for the veterinary clinic against bacterial infections in cattle and birds.

**Keywords:** Bacterial infections; Cattle; Birds; Multidrug-resistant bacteria; Silver nanoparticles

## Introduction

Infectious diseases such as mastitis, *colibacillosis*, *salmonellosis* and gastrointestinal *campylobacteriosis* are among the leading causes of death in bovine and birds used in the food industry. These animals are susceptible to human opportunistic bacteria and environmental pathogens [1,2]. Moreover, a great loss in the food industry have been caused by the high prevalence of these infections in several countries, posing a serious threatening challenge to public health, because of the risk of human contamination [3,4].

In recent decades, many of the infections in these animals are caused by resistant microorganisms. Bacterial resistance is an inevitable consequence of natural selection, but some factors may accelerate its emergence, including inappropriate use of antimicrobial agents in human and animal health [3-7]. The dissemination of resistant pathogens in bovines and birds has resulted in a therapeutic failure in many infectious diseases [8]. Notwithstanding the production of new antimicrobials for veterinary applications has increased in the last three decades, bacterial infections are still responsible for enormous economic losses in cattle and birds rearing [1-5,8]. Therefore, the silver nanoparticles arise as a promising alternative to the control and therapy of these bacterial infections [9]. This nanomaterial presents broad antibacterial potential for different microorganisms

and is emerging as an alternative of antimicrobial agent for bacterial infections with resistance profile [10,11]. In this way, the disclosure of studies related to the new approaches and therapeutic options for infectious diseases in veterinary medicine is needful.

## Bacterioses in bovines and birds

Bovines and birds are commonly affected by bacterioses, including mastitis, colibacillosis, salmonellosis and gastrointestinal campylobacteriosis. Byaruhanga et al. [4] conducted a study to identify disease-causing microorganisms in birds and bovines from the analysis of 836 samples obtained from these animals and found that 21.4% of the diseases were caused by bacterial infections. In the same study, bovine mastites caused by bacteria were identified as the main bacteriosis in bovines, contaminating about 43.7% of the animals diagnosed with bacterial infections. Bacterial infections are considered the primary causes of mastitis, in which the main etiological agents are *Staphylococcus aureus*, *Streptococcus agalactiae*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Corynebacterium bovis* and *Bacillus cereus* [12-14]. Kayesh et al. [15] evaluated the prevalence of bovine subclinical mastitis in 200 milk samples of dairy cows and found that 28.5% of the animals were positive for subclinical mastitis pathogens, *Staphylococcus* spp. were found in 73% of the positive samples, *Streptococcus* spp.

in 33% and *E. coli* in 7%. A similar result was reported by Moreno et al. [16] who investigated the frequency of bacteria responsible for mastitis in cows. From the analysis of 750 milk samples of cows with subclinical mastitis on farms in the state of São Paulo, Brazil, *Staphylococcus* spp. was present in 76% of the samples and *E. coli* in 7% of the samples.

The term used to define infections that are caused by *E. coli* bacteria is colibacillosis. This bacteriosis affects numerous animal species, such as calves, chickens and pigs, and is considered the main cause of bacterial infections in birds and one of the most prevalent in bovines [4]. Byaruhanga et al. [4] estimated a prevalence of 61.7% and 10.5% of the disease, among bacterioses, in birds and bovines, respectively. Most *E. coli* strains live in a commensal relationship with domestic poultry, however, some virulent strains referred to as avian pathogenic *E. coli* (APEC) induce localized and systemic infections in birds [17]. In calves, colibacillosis is characterized by two different types of diseases, and the enteric form is determined by the presence of dehydration, diarrhea and acidosis, which can evolve to death in a few days, and the systemic form characterized by a shock state of the animal, with a rapid evolution towards death [18].

Other less prevalent bacterioses that cause serious hazards to veterinary medicine, food industry and human health are salmonellosis and gastrointestinal campylobacteriosis [19-23]. Salmonellosis is characterized by several symptoms, including fever, gastroenteritis and bacteremia. Direct contact and ingestion of infected animals can lead to salmonellosis in humans. The main *Salmonella enterica* serotypes responsible for infections in birds, bovines and humans, are Typhimurium, Enteritidis, Newport and Heidelberg [19,20]. On the other hand, the gastrointestinal Campylobacteriosis is caused by *Campylobacter* spp., and the main specie of this genus which is responsible for 90% of the cases of the disease is the *Campylobacter jejuni*. Generally, bacteria coexist in a commensal relationship in the gastrointestinal tract of several animal species, such as birds and bovines. However, in young animals and humans, they can cause enteritis, dehydration, mucoid diarrhea and malnutrition, leading to death within a few days. In addition, the infection may leave sequel in adult bovine, such as miscarriage and infertility. In humans, campylobacteriosis can cause severe sequel in the immune system, including reactive arthritis and Guillain-Barré syndrome [21-23].

### Infections in bovines and birds caused by resistant bacteria

Studies reported a worrying prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) and multidrug-resistant *E. coli* (MDR) in bovine and avian infections [2,24-26]. Noel et al. [2] showed a prevalence of 26.8% of MRSA among the 207 isolates of *Staphylococcus* spp. from samples obtained from cows with subclinical mastitis. Regarding resistance in *E. coli* isolates, studies demonstrated differences between the incidences of *E. coli* MDR strains associated with subclinical mastitis in different countries.

This fact can be evidenced by the low incidence of this disease in Switzerland and high incidence in France [4]. However, in countries with a high prevalence of these strains, the data are alarming [26]. Rangel and Marin [26] and Su et al. [3], in studies conducted in Brazil and Taiwan, respectively, demonstrated a prevalence of about 70% of *E. coli* MDR among strains of this species, isolated from cows with subclinical mastitis. In the same way, Vounba et al. [27] investigated the susceptibility profile of 57 *E. coli* isolates obtained from chickens with colibacillosis and observed a prevalence of 86.2% of *E. coli* MDR strains among the isolates analyzed. In addition, studies have demonstrated the dissemination of *E. coli* MDR strains from bovine and avian colibacillosis to humans and domestic animals, in regions of high human density where farms and food industries [1].

In the case of salmonellosis, there is a worldwide spread of MDR strains from *Salmonella enterica*. *Salmonella typhimurium* is the most prevalent serotype of *Salmonella* MDR in isolates from humans and animals in the United States [19,20]. The most commonly reported MDR phenotype in *Salmonella typhimurium* isolates was the resistance to oxytetracycline, ampicillin, streptomycin, sulfatrimetropin and chloramphenicol (ACSSUT), which was detected in 1.5%, 16.2% and 10.9% of the tested chicken isolates, cattle and pigs, respectively [28]. On the other hand, in the gastrointestinal campylobacteriosis the incidence of *Campylobacter* spp. MDR in birds and bovines is still low in most continents, but the emergence of macrolide-resistant strains, such as azithromycin and erythromycin, in these animals has worried health agencies and the food industry, especially in Europe, given the fact that these antimicrobials are drugs of choice in the treatment of gastrointestinal campylobacteriosis in humans. Studies demonstrated an exponential increase in macrolide resistance in *E. coli*. Isolated from chickens, indicating an increase from 5.2% to 11.4% between the years 2011 to 2014, while in *C. jejuni* isolates the prevalence of resistance to macrolides remains below 4% [21-23]. The dissemination of these pathogens has caused enormous economic losses to farmers and the food industry, posing a serious threat to public health, due to the risk of human contamination [1-4,19-23]. In this way, it is pivotal the research for new molecules with antibacterial activity to reduce the morbimortality rates in cattle and birds caused by these bacterioses.

### Silver nanoparticles

Nanotechnology is a research area dedicated to the manipulation and application of nanometric scale structures. In this science, the nanoparticles stand out for being easily obtained and to possess multidisciplinary applications in the fields of medicine, cosmetics, environmental remediation and biomedical devices [29]. Nanoparticles are classified as organic when carbon is the main constituent of their structure and inorganic when the elements such as ZnO, Au, Ag, Cu, Al, Co, Fe, Ni are part of the structure of this nanomaterial [30]. Currently, the potential of inorganic nanoparticles, especially silver nanoparticles (AgNPs), has been widely explored due to their chemical, physical and biological properties [31]. The AgNPs present important physico-

chemical characteristics such as high thermal and electrical conductivity, chemical stability, catalytic activity and non-linear optical behavior [32]. These properties make these particles an important alternative for therapeutic purposes in the human and veterinary medicine [10]. Studies have shown the potential applications of this nanomaterial in biomedical electronic devices, such as biosensors for gene therapy, in cancer therapy and for the treatment of microbial infections in humans and animals, particularly those caused by resistant bacteria [10,11,33-36].

### Silver nanoparticles in the treatment of bacterial infections in bovines and birds

AgNPs have stood out because they have a potential broad spectrum antibacterial activity against different microorganisms and present different mechanisms of action which can decrease the bacteria resistance development [9,37]. AgNPs can develop their antibacterial activity by altering the cell wall and cytoplasm, modifying ATP levels, modifying permeability and cell membrane respiration, inhibiting bacterial DNA replication, and generating free radicals, such as reactive oxygen species (ROS) [9-11]. This antimicrobial property has been evaluated and studied in several fields and there is an expansion tendency for studies and application of AgNPs in the meat and poultry industries [25,35]. Saied et al. [36] analyzed the antibacterial activity of AgNPs against seven strains of *Staphylococcus aureus* isolated from cows with subclinical mastitis. The authors determined the minimum inhibitory concentration (MIC) ranging from 1.25 to 10µg/ml and found that the mean time of antibacterial action of AgNPs against *S. aureus* strains was seven minutes. In another study, Kazemi et al. [38] evaluated the effect of AgNPs on 50 strains of *S. aureus* resistant to erythromycin, gentamicin, streptomycin and doxycycline isolated from bovine mastitis and MICs were determined at concentrations between 50 and 100µg/ml.

In a recent study, Yuan et al. [14] evaluated the efficacy of AgNPs against strains of *P. aeruginosa* and *S. aureus* MDR isolated from milk samples produced by goats with mastitis. The MICs of the AgNPs found for *P. aeruginosa* and *S. aureus* were 1 and 2µg/ml and the minimum bactericidal concentrations (MBC) were 2 and 4µg/ml, respectively. The authors observed that the antibacterial activity of AgNPs can be attributed to the generation of free radicals, especially to ROS generation. These studies supporting the promising use of AgNPs as antibacterial agents in the treatment of bacterial mastitis.

Kar et al. [25] studied the AgNPs antibacterial property against strains of *E. coli* MDR and Extended-spectrum beta-lactamase (ESBL)-producing *E. coli* isolated from cattle mastitis and infections in birds and obtained MICs ranging from 450 to 640mmol/l. Similarly, Smekalova et al. [37] evaluated the antibacterial activity of AgNPs alone and combined with commercial antimicrobials against bacteria with resistance profile isolated from animals. The obtained MICs for AgNPs ranged from 6.3 to 100µg/ml. With regard to combined therapy, AgNPs showed a synergistic

effect with gentamicin against *S. aureus*, *E. coli* and *Actinobacillus pleuropneumoniae* and between AgNPs and penicillin G against *Actinobacillus pleuropneumoniae*, *A. pleuropneumoniae* and *Pasteurella multocida*.

Mohamed et al. [39] evaluated the antibacterial potential of AgNPs alone and in combination with antibiotics against strains of *E. coli* and *Salmonella* resistant. AgNPs showed MIC of 0.85µg/ml for all strains analyzed. Synergistic effect of AgNPs was observed with ciprofloxacin, amoxicillin, gentamicin, cefotaxime and neomycin for resistant strains of *E. coli*. These studies concluded that AgNPs can be applied as main antimicrobial compound or as adjuvant to improve the treatment of bacterial diseases in cattle, such as colibacillosis, salmonellosis and mastitis. Omara et al. [20] studied the antibacterial activity of AgNPs for tetracycline, tobramycin, oxytetracycline, imipenem and gentamicin against resistant species of *Salmonella* and *Shigella* isolates obtained from birds with purulent dysentery and diarrhea. AgNPs showed MIC and MBC ranging from 8 to 16µg/ml against these strains. Duffy et al. [35] evaluated the in vitro activity of AgNPs against *Salmonella* and *Campylobacter* isolated from birds. MICs of AgNPs ranged from 12.5 to 25µg/ml and MBC from 50 to 100µg/ml for *Salmonella* strains, whereas for *Campylobacter* strains the MICs ranged from 6.25 to 3125µg/ml and MBC from 3.12 to 6.25µg/ml. Taken together, these results strongly suggest that the application of nanotechnology through the use of AgNPs consists in a new promising strategy for the treatment of bacterial diseases in birds, such as colibacillosis, shigellosis and campylobacteriosis, strengthening the benefits brought by nanoscience to the poultry industry.

### Conclusion

The food industry and farmers are strongly affected by bacterioses in poultry and cattle, due to the high costs of antibiotic therapy and high levels of morbimortality of these animals. Moreover, the bacterioses can be caused by MDR strains, increasing the risk of therapeutic failure. The use of nanotechnology in veterinary medicine comes out with the aim to reduce the dissemination of MDR strains and consequently reduce cases of bacterioses in food-producing animals by controlling and treating these diseases. Considering the analysis of the present study, it can be stated that silver nanoparticles are promising nanotechnological strategies to the control of bacterioses in poultry and cattle. However, the mechanisms of action and the clinical use of silver nanoparticles in the treatment and control of bacterioses in animals are not fully elucidated. In this view, it is clear the need for further in vivo studies of silver nanoparticles that can complement the in vitro studies previously reported.

### Acknowledgement

SD Costa-Junior and LAA Campos thank the National Council for Scientific and Technological Development (CNPq) for a MSc and PhD scholarships, respectively. This study was funded by Foundation of Support for Science and Technology of the State of Pernambuco (FACEPE) [APQ-0814-4.03/17].

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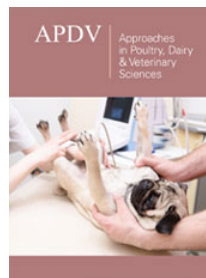
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