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INFLUENCE OF AgNPs ON FOODBORNE BACTERIA- *CAMPYLOBACTER JEJUNI*

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Abstract

Nanotechnology is likely to express new ways to combat and inhibit diseases by means of atomic scale modified form of materials. Silver nanoparticles (AgNPs) have gained much attention in recent years due to their biomedical applications, especially as antimicrobial agents. Various food- borne pathogens have been discovered as causes of food-borne sickness. Campylobacter is a main factor of foodborne gastro-intestinal disorders worldwide. Recently antibiotic resistivity of Campylobacter has turn out to be a main public health alarmed it has built an interest for emerging new antibacterial approaches for decreasing the effect of this food-borne pathogen on human health. Silver nanoparticles can be used as an alternative to antibiotic growth promoters in poultry production. AgNPs penetrate bacterial cells and interact with the cell's molecular structure, allowing them to proliferate. The efficiency of NPs is due to their Nanoscale size and large ratio of surface area to volume However, the influence of AgNPs on food-borne microorganisms is little known. The aim of present study was to investigate the effect of silver nanoparticles against the food-borne bacteria campylobacter with 20 different strains jejuni and coli.

Keywords: *Silver nanoparticles, Zinc oxides, Titanium Oxides, Minimal Inhibitory Concentration, Minimal Bactericidal Concentration.*

INTRODUCTION

Nanotechnology compacts with matter of different structures having size of the order of a billionth of a meter. Nanotechnology can be defined as any technology at the scale of nanometre (1). The objectives of nanoscience and nanotechnology were established by the physicist Richard Feynman, entitled "There's Plenty of Room at the Bottom". He explains the nanotechnology a method in which researchers would be capable to use and organize individual atoms and molecules (2).

The applications of nanotechnology are increasing rapidly in different fields and technologies. In the technological sector the application of metallic nanoparticles (NPs) has gained special interest in number of areas as nanosensors, biosensing, photonic, catalyst, optics, water treatment, biocidal activities and cell electrodes (3). For antimicrobial effect of nanoparticles, the size and shape of NPs are important and is recommended that antimicrobial activity increases when the size of NPs decrease. As compare to rod and spherical shape NPs the antimicrobial activities of triangular shape of nanoparticles are greater. In contrast of the other noble metallic nanoparticles, AgNPs have maximum interest due to their anti-inflammatory effects, chemical stability, good conductivity, and biocidal activity toward micro-organisms, fungi, as well as virus (4).

Nanoparticles are incredibly crucial matters because they exhibit unique features with a wide range of therapeutic uses (5). The subject of nanoparticles in biology is unquestionably growing (6). According to studies silver nanoparticles release free ions from their surface when they come in touch with water (7).

These free ions have a strong antibacterial impact, causing microbes to die quickly by blocking cellular respiration and causing bacterial cell membranes to degenerate (8).

Silver nanoparticles have pyrethroid- like effects on gram positive and gram negative bacteria, as well as pathogens that cause food poisoning (9). Silver nanoparticles can be employed as antimicrobial growth enhancers in poultry farming (13).

Foodborne infections are global health hazard emerged by parasites and microorganisms that mix their way into food and drink. Poultry items such as raw milk, precooked meat, fruits and vegetables are prime reservoirs of food-borne illness (14-16). Pathogenic infiltration is possible through contaminated water and undercooked food. Pathogens in food and water must be detected before they invade the body and pose a severe outbreak (17-19).

In 2010, the World Health Organisation (WHO) estimated that 600 million people around the world were infected with foodborne infection, with 420,000 fatalities (20).

In food poisoning the bacteria, *Bacillus cereus, Campylobacter jejuni, Clostridium perfringens, Escherichia coli* O157:H7, *Pseudomonas aeruginosa, Salmonella sp*, are main offenders (15, 21, 22).

The most prevalent bacterial cause of food-borne illness is *campylobacter jejuni*. It is primarily transmitted through uncooked food. The neuromuscular paralysis Guillain- Barre syndrome is linked to campylobacter infections (23-24).

Enterocolitis is a familiar problem *of campylobacter jejuni*, and is implicated in a severe inflammatory retort that can cause tissue damage and is thought to be the source of several health issues (25).

TYPES OF NANOPARTICLES

There are different types of nanomaterials like metals (Au, Ag, Cu) metal oxides (ZnO, SiO_2, Fe_2O_3, TiO_2) clay, organic and full carbon materials, other nanomaterials consisting of nano-composites and nano-encapsulates. Silver and gold nanoparticles are used as anti-inflammatory agent; Nanobiocides are one of the most common applications for improving food safety through the use of nano-sieves to kill microbes (26). ZnO and CuO nanoparticles also show antibacterial activity against microorganisms, including foodborne pathogens. Because of their antibacterial and antifungal qualities, zinc oxide and copper oxide nanoparticles are commonly used in food packaging and coatings (27). Zinc oxide (bulk, greater than 100 nm) is also widely used in food packaging and to strengthen cereal- based foods in the United States (28).

Antimicrobial activity has also been discovered in a variety of metal and metal oxide nanostructures (29). Their basic physiochemical features promote the generation of reactive oxygen species in excess, resulting to oxidative stress and cell damage. Furthermore, metal ions released from outside the cell, at the cell surface, or within the cell might change cellular structure and function and it will inhibit the growth of microorganisms (30, 31). The size, shape, stability, and concentration of nanoparticles can all affect their impacts on microorganisms (32).

SILVER NANOPARTICLES

Silver nanoparticles received a lot of attention, and they have been used in a variety of fields, notably healthcare, catalysis, water treatment, biotechnology and optics (33-37).

Since the 19^{th} century, silver based compounds have been used in bactericidal applications for the treatment of burn wounds, dental work and catheters. Silver vessels were employed to keep water safe in ancient Greek and Roman civilizations (38).

AgNPs release silver ions from their surface. These free silver ions have strong antimicrobial effect, when silver ions attach to tissue proteins, structural changes occurs in the bacterial cell wall, resulting in cell apoptosis (39). The antibacterial properties of silver nanoparticles were validated by infecting E. coli cells with them. In the bacterial cell wall silver nanoparticles accumulate and forms the ''pits'' that finally lead to cell death. Sondi et al. observed that in the same bacteria, small sized particles are more efficient to show antibacterial activity due to its large surface to volume ratio in comparison with the larger particles (40). AgNPs are used in a different variety of consumer stuff such as; sprays, soaps, shampoos, socks, slippers, detergents, wet wipes, air sanitizer, pillows, respirators, toothpastes, air filters, cellular phones, coatings of refrigerators, food storage containers, vacuum cleaners, washing machines, etc (41).

To facilitate the antibacterial activity of Silver nanoparticles, AgNPs are modified by coating with stabilized polymers such as polyethylene glycol (PEG), polyvinyl alcohol (PVA), polyvinylpyrrolidone (PVP), sodium dodecyl sulfate (SDS) and Citrate. Protective polymer coatings do not increase bactericidal activity, but they do promote AgNPs contact with bacterial cells and prevents them from autoaggregating into a larger biomass that can interfere with activity (42).

Many strategies have been devised to manipulate the forms, sizes, and orientations of silver nanoparticles for specific industrial applications, based on their high surface to volume ratio and distinctive characteristics (43). Modified silver nanoroads with polyvinylpyrrolidone (PVP), polyethylene glycol (PEG) due to its safeness and low toxicity as compare to silver nitrate that shows toxicity at 2μg/ml has been used in medical application against adjuvant human immunodeficiency virus delivery (44).

The toxic efficacy of custom disinfectant, chlorhexidine in *straptococus mutants* was compared using different nanomaterials such as, silver, AgNPs, titanium oxide. Among many other nanomaterials the antibacterial activity of silver nanoparticles was greater. Much of the microbial research on synthesis of nanoparticles has used well- known model organisms like *Escherichia coli* as a Gram-negative bacterium model (45).

AgNPs are found to have synergetic activities with antibiotics, for example; erythromycin, chloramphenicol, ampicillin, penicillin G, kanamycin, amoxicillin, vancomycin and clindamycin. This synergetic effect has enhanced bactericidal property against microbial strains including *Staphylococcus aureus, Salmonella typhi, Micrococcus luteus,* and *Escherichia coli* compared to the activity of these antibiotics alone (46).

Silver nanoparticles, in instance, are demonstrated to have diverse biocidal capabilities against a variety of biological species, (47) such as *Campylobacter jejuni* and *Staphylococcus aureus* Multi Drug Resistance (MDR) (48).

FOODBORNE PATHOGEN *CAMPYLOBACTER JEJUNI*

Animals are the main cause of human illness and morbidity in industrialised countries where aquatic common. Consumption of contamination is less *Campylobacter jejuni* is the human pathogen belongs to the delta-eplison class of proteobacteria; it is the accidental visitor that has reservoirs in water and different animals (49).

It mainly causes diarrhea, fever, nausea, abdominal cramps and consistent infection with campylobacter can cause septic arthritis, bacteremia and extraintestinal manifestation (50).

Campylobacter bacteria are tiny, curved, or spiral gram-negative bacteria that thrive at 34-42°C. These bacteria require partial pressure and partial oxygen to develop in vitro. However, due to its enormous genetic, metabolic, and phenotypic variability, the genus campylobacter is found in populations with a wide range of environmental conditions (51).

Several campylobacter species have been documented to cause diarrhea, including *Campylobacter jejuni, Campylobacter coli, and Campylobacter ureolyticus.* C.jejuni is the most commonly isolated species from humans and commercial poultry, followed by *Campylobacter coli*. In countries like south Africa and Thailand where *Campylobacter coli* was a predominant specie isolated from retail poultry, the percentage of *Campylobacter coli* to *Campylobacter jejuni* was significantly different (52).

Contaminated poultry products might lead to infection with campylobacter. Chickens and other bird species are thought to have *campylobacter jejuni* as a symbiotic organism. In contrast, when chickens are infected with *C.jejuni*, it might cause diarrhea. Humans have a stronger symptomatic response to campylobacter infection than chickens (53-54).

SILVER NANOPARTICLES AND *CAMPYLOBACTER JEJUNI*

Antimicrobial drugs of various types are used to prevent and control illnesses in broiler production. Antimicrobials can impact the host intestinal flora by lowering intestinal bacteria colonization, suppressing harmful microorganism growth, and boosting the immune system, therefore preventing illnesses and improving animal performance (55- 57).

Antibiotic resistance develops in bacteria as a result of excessive use of antimicrobial agents (antibiotics), which is damaging to animal and human health (58-60). For example, ciprofloxacin resistance in Campylobacter jejuni identified from Danish broiler meat increase from 0% in 2009 to 17% in 2010 (61). Since 2006, the European Union has made it illegal to use any antibiotics as a growth enhancer (56, 62).

Treatment becomes more challenging as the incidence of illnesses caused by multidrug-resistant campylobacter strains climbs (63). As a result, innovative antibiotic options for the management of campylobacter are needed. New technologies are being developed to improve existing antimicrobials in order to overcome antimicrobial resistance. Nanotechnology offers a new idea for both patients and professionals to deal with drug resistance bacteria (64-65).

Recent research focused on antibacterial materials such as diverse natural (oils, acids), inorganic antimicrobial agents such as metals (Ag, Au, Cu), and metal oxides (ZnO, SiO2, Fe2O3, TiO2). Silver is one of the most promising metal nanoparticles and is used in a variety of nanotechnology products. Because of their antibacterial qualities, several consumer items now incorporate silver nanoparticles (AgNPs) (66-69).

Silver is well known for antimicrobial activity. Amongst the other metallic nanoparticles silver nanoparticles are most vital and fascinating. SNPs have been used for numerous applications including, as antibacterial agent, in pharmaceutical industry, cosmetics, optical sensors, orthopeadics, and drug delivery (70). SNPs shows biocidal activity in contradiction of gram positive and gram negative microorganisms plus food borne pathogens like *Campylobacter jejuni, Escherichia coli 015: HZ, Salmonella, Staphylococcus aureus, Listeria monocytogens* (71).

Despite substantial research into silver nanoparticles antimicrobial impact, the mechanism of antibacterial activity specific to bacteriostatic or bactericidal activity remains unknown. Silver nanoparticles can discharge Ag ions from their surface when exposed to water, according to studies. A free silver ion has a strong antibacterial action that kills bacteria by interrupting bacterial cell membrane faction and preventing cellular respiration. When silver ion interacts to tissue proteins, structural changes in bacterial cell membrane occur, resulting in cell death. The bacterial electron transport chains' essential protein complexes are found on the cell's outer membrane (39).

AgNPs have the capability to act against 650 strains of spoilage and disease- causing microbes (72). They can display the antimicrobial activity even in concentration as low as 10 parts per million (73).

Food-borne pathogens are the most common cause of zoonotic illnesses, and they have a significant influence in mortality and prevalence in developing nations, costing billions of dollars to treat (15, 20, 22).
Campylobacter is the most common cause of bacterial food poisoning in the globe. Patients may develop mild to severe disease, with GI symptoms such as diarrhea, stomach cramps, nausea, and fever among the most common (74).

Poultry products such as undercooked meat, raw milk, fruits and vegetables are main source of food-borne infections. Antimicrobial resistance in microorganisms from dairy products, such as campylobacter, has become a major public health concern in both industrialized and developing countries in recent years. A growing numbers of campylobacter isolates have developed resistance to a variety of antibiotics, including fuoroquinolones, aminoglycosides, macrolides, and beta- lactams (75-76).
According to a 2005 WHO report, 1.8 million people died from diarrhea caused by foodborne infections (77). Due to their high surface area/ volume ratio and higher reactivity, nanoparticles format antibacterial compositions have previously been shown to be effective bactericidal materials (78).

AgNPs show powerful antimicrobial properties against different bacterial species, including multidrug resistance (MDR) strains (79). Due to the need of providing alternatives to the resistance that many pathogenic microbes demonstrate to most commonly used antibiotics, the use of silver nanoparticles as antibacterial agents has grown in popularity in recent years in the medical industry (80).

To facilitate contact with the environment, silver nanoparticles can be coated. Coating silver nanoparticles with glutathione GSH boosts their solubility and capacity to interact with their surroundings in this way (81). Silver nanoparticles disrupt bacteria's cellular signaling by altering the phosphotyrosine profile of putative bacterial peptides, eventually halting their growth (82).

The use of an Ag ion biocide to reduce the level of food borne disease campylobacter in poultry transportation packing proved to be very successful (83). Stabilized silver nanoparticles are capable to inhibit the growth of campylobacter (84).

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) are two widely used indicators of synthesized nanomaterials' relative antibacterial activity. However, it is crucial to recognise the limits of such methodologies, as well as the fact that validated of these techniques for nanomaterial assessment is still required (85).

Table I: Strains of campylobacter

Isolation source	Strains designation	Specie	Isolation source	Strain designation	Specie	Reference
Chicken Food Chain (FC)	FC1	*C. jejuni*	Clinical (C)	C1	*C. jejuni*	-
	FC2	*C. jejuni*		C2	*C. jejuni*	(85)
	FC3	*C.jejuni*		C3	*C.jejuni*	
	FC4	*C. jejuni*		C4	*C. jejuni*	
	FC5	*C. coli*		C5	*C. coli*	
	FC6	*C. coli*		C6	*C. coli*	
	FC7	*C. coli*		C7	*C. coli*	
	FC8	*C. coli*		C8	*C. coli*	
	FC9	*C. jejuni*		C9	*C. coli*	
	FC10	*C. jejuni*		C10	*C. jejuni*	
	FC11	*C. jejuni*		C11	*C. jejuni*	
	FC12	*C. jejuni*		C12	*C. jejuni*	
	FC13	*C. jejuni*		C13	*C. jejuni*	
	FC14	*C. coli*		C14	*C. jejuni*	
	FC15	*C. jejuni*		C15	*C. jejuni*	
	FC16	*C. coli*		C16	*C. coli*	
	FC17	*C. coli*		C17	*C. coli*	
	FC18	*C. coli*		C18	*C. coli*	
	FC19	*C. jejuni*		C19	*C. coli*	
	FC20	*C. jejuni*		C20	*C. coli*	

Table II: Influence of Glutathione- Stabilized AgNPs against Campylobacter strains determined using microtiter drop plate method

Food chain strains	MIC μg/ml	MBC μg/ml	Clinical Strains	MIC μg/ml	MBC μg/ml
FC1	19.7	19.7	C1	9.85	19.7
FC2	9.85	9.85	C2	19.7	39.4
FC3	9.85	9.85	C3	9.85	39.4
FC4	9.85	9.85	C4	19.7	19.7
FC5	39.4	39.4	C5	19.7	19.7
FC6	4.92	9.85	C6	9.85	19.7
FC7	4.92	9.85	C7	9.85	19.7
FC8	9.85	19.7	C8	19.7	19.7
FC9	19.7	19.7	C9	19.7	19.7
FC10	19.7	39.4	C10	19.7	19.7
FC11	39.4	39.4	C11	19.7	39.4
FC12	19.7	39.4	C12	19.7	19.7
FC13	19.7	39.4	C13	19.7	19.7
FC14	19.7	39.4	C14	19.7	39.4
FC15	19.7	39.4	C15	19.7	19.7
FC16	19.7	19.7	C16	19.7	39.4
FC17	19.7	39.4	C17	39.4	39.4
FC18	19.7	19.7	C18	39.4	39.4
FC19	9.85	19.7	C19	19.7	39.4
FC20	19.7	39.4	C20	39.4	39.4

CONCLUSION

In conclusion, this review suggests that silver nanoparticles could be employed as an antibacterial against campylobacter. AgNPs' antimicrobial uses in the food and biomedical industries have grown in recent decades as a result of their broad spectrum of activity against a variety of spoilage and pathogenic microbes for which traditional antimicrobials have proven difficult to use. However, many researchers have looked at how different concentrations, sizes, and forms of AgNPs affect different microorganisms. In general, the benefits of silver's antibacterial effect must be evaluated against the possibility of tissue damage due to silver's cytotoxic tendency. The antibacterial effect of nanotechnology against important diseases of concern to the poultry sector implies that the poultry business can benefit from nanotechnology. The efficiency of NPs against these important infections should be factored into the development of future targeted applications including crates, conveyor belts, and packaging.

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