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Antimicrobial metal and metal oxide nanoparticles: application in food industry

Dipankar Halder[1,*], Atanu Mitra[2,*]

[1] Deapartment of Food Technology and Biochemical Engineering, Jadavpur University, Kolkata, India
[2] Department of Chemistry, Sree Chaitanya Collge, Habra, 24 Parganas (North), Habra, India
*corresponding author e-mail address: ***dipankar_h@ftbe.jdvu.ac.in | mitra_atanu@hotmail.com***

ABSTRACT

Among several modern applications of metal and metal oxide nanoparticles, application as antimicrobial substance especially for food industry use is one of the most promising one. In addition, to combat against the threat of antibiotic resistance inclusion of nanomaterials as potent antimicrobial agent in the field of food packaging and preservation is very necessary nowadays. Metals like silver, gold, platinum etc at their nanodimension show significant antibacterial as well as antifungal activities. Nanoparticle impregnated biocompatible matrix such as clay, silica, chitosan, gelatin, polylactic acid etc have already identified to be prospective applicant in food industry use especially in food packaging, food equipment sterilization, antifouling agent, lacquer coating, water purification etc. In addition to the bare form, bimetallic or core-shell nanoparticle are being proved to be even more active and efficient candidate for industrial end use. Metal oxides such as titania, zinc oxide, magnesium oxide etc also play key role in the fabrication of these type of nano-antimicrobial product development. Interestingly antimicrobial activity depends on several parameters including size, shape and coating of nanoparticle. Till now the mechanistic pathway of the antimicrobial action is not well known. However, several mechanisms have been proposed in literature from existing experimental evidences. In this review our basic objective is therefore, to cite and discuss the updated literature in the areas of nanomaterials applications in food industry including type, origin,mechanism of action, effectiveness and appropriate end use of these nanomaterials.

Keywords: *Antimicrobial, multi drug resistance, metal nanoparticle, metal oxide nanoparticle, nanocomposite, food industry.*

INTRODUCTION

Advancement in nanotechnology creates a tremendous scope to introduce new technologies to solve the existing problems as well as to improve the product quality in different applications fields like pharmaceuticals, cosmetics, foods etc. Despite the existence of antimicrobial agents including higher generation antibiotics, major economical setback in food supply chain worldwide is being caused due to contamination of food by pathogenic microbes, especially multi drug resistance (MDR) pathogens. Drug-resistant food borne bacteria like Campylobacter, Salmonella Typhi, nontyphoidal Salmonellae and Shigella are really serious threats to food industries now (Antibiotic resistance threats in the United States, 2013).Various food sectors including agri-food industry, food safety (issues regarding food pathogens), food preservation (shelf-life extension), packaging, water purification etc. are already benefited by use of different nanomaterials (Chaudhry et al 2008, Duffy et al 2002, Das 2009). To cite some remarkable uses of nanos, use of pesticides in the form of nanoemulsion or nanoencapsulation to cause better efficiency (Chowdappa and Gowda 2013), use of metal/metal oxide nanoparticle/ nanocluster or other quantum dots as surface plasmone resonance/ fluorescent probe to detect food pathogen in very low level (Zhang, Guo and Cui 2009), use of antimicrobial nanomaterials in food packaging etc.As microbes

are going to be more and more 'resistant',attempt to establish nanoparticle as true alternative antimicrobial agent is a genuine need in the field of food contact surface sterilization / food preservation / food packaging nowadays. At this background in the present review we have undertaken to discuss on different metal/metal oxide nanoparticles, their antimicrobial actions and potential food industry applications.

DEFINITION OF ANTIMICROBIAL AGENTS

The agent which kills microorganisms or inhibits their growth can be defined as antimicrobial agent. Antimicrobial agents may be disinfectants such as bleach, antiseptics such as alcohols or antibiotics such as neomycin. Antimicrobial agents can also be classified according to the microorganisms they act primarily against. For example, antibacterial agents are used against bacteria (such as ethanol, isopropanol, formaldehyde etc) and antifungal agents are used against fungi such as nystatin. They can also be classified according to their function. Agents that kill microbes are called microbicidal such as ozone, while those that merely inhibit their growth are called biostatic such as sodium azide. Some common antimicrobial agents with their chemical nature have been presented in Table 1 (Mccaulley et al. 2012)

Table 1. Common antimicrobial agents and their chemical nature.

	Antimicrobial Agent	Represent class	Other in same class
1.	Phenoxyethanol(PE)	Phenolics (excl parabens)	Benzyl alcohol, phenethyl alcohol
2.	Caprylyl Glycol (CG) (1,2-octanediol)	1,2-Alkanediols	1,2-pentanediol, 1,2-hexanediol, 1,2-decanediol, 3-[(2-ethylhexyal)oxyl]-1,2-propanediol (ethylhexylglycerine)
3.	Methylparaben (MP)	Parabens	Etyl, propyl, butyl, isopropyl, isobutyl, & benzyl paraben and their sodium salts
4.	Methylisothiazolinone (MIT)	Isothiazolinones	Methylchloroisothiazolinone
5.	9:1 wt ratio Benzyl Alcohol (BA) and Dehydroacetic acid (DHA)	ECOCERT approved antimicrobial agents (and blends with organic acids)	Benzoic acid & its esters & salts; salicylic acid & its salts; sorbic acid; dehydroacetic acid and its salts
6.	Chlorphesin (CP)	Halogenated aromatic compounds	Chloroxylenol; triclosan; dichlorobenzyl alcohol; climbazole; triclocarban
7.	DMDM Hydantoin (DMDMH)	Formaldehyde releasers	Imidazolidinyl urea; diazolidinyl urea; quaternium-15; methenamine
8.	Iodopropynyl butylcarbamate (IPBC)	Halogen non-aromatic compounds	2-bromo-2-nitropropane-1,3-diol; chloroacetamide; chlorobutanol; methyldibromo glutaronitrile
9.	Benzisothiazolinone (BIT)	Isothiazolinones	MIT, MCIT
10.	1:1 MIT:BIT for coating	Isothiazolinones	See above
11.	Bezalkonium Chloride	Quaternia	Benzethonium chloride, chlorhexidine, polyaminopropyl biguanide

LIMITATION OF CONVENTIONAL ANTIMICROBIAL AGENTS

Antimicrobial agents attack specific cell sites to cause microbial death or damage. Any given antimicrobial attacks one of four major cell targets: 1. the cell wall, 2. the cell membrane, 3.

biosynthesis pathways for DNA or RNA, 4. protein (enzyme) function.

In appropriate and indiscriminate use of antimicrobial agents, especially antibiotics,cause microorganisms develop antimicrobial resistance (AMR) and microbes any class may develop this resistance. Microbes which are resistant to multiple antimicrobials are termed multidrug resistant (MDR) (or sometimes, superbugs). Some organisms are naturally resistant but the term most often refers to acquired resistance, which can be a result of either new mutations or transfer of
resistance genes between organisms.

In addition, when microorganisms attach to a surface and grow together as a single colony named biofilm, they are protected from cidal and static effects of antibiotics and biocides. This antibiotic resistance is easily reproduced in vitro, viz. target mutation, low cell permeability, efflux pumps and modifying enzymes showing a strong line of defense, thus explaining the level of resistance developed. Though delayed antibiotic mechanism is not the only mechanism, a reaction-diffusion interaction can also prevent the antibiotics from penetrating the biofilm matrix.

The antibiotic resistance mechanism can be understood by the following hypotheses addressing the reduced antibiotic susceptibility in biofilms:-

- Antibiotic penetration rate into the biofilms.
- Development of altered microenvironment and slow growth.
- Adaptive stress response expressed by some cells.
- A differentiated fraction of cells acting as Protective Persister State.

The increasing rates of antibiotic resistant infections are caused by antibiotic use for human and veterinary medicine. Antibiotic resistance is a serious and growing global problem: a World Health Organization (WHO) report released April 2014 stated, “this serious threat is no longer a prediction for the future, it is happening right now in every region of the world and has the potential to affect anyone, of any age, in any country”.

For these reasons, the approach to develop engineered nanoparticles as antimicrobial agent is a novel one to keep up with the constantly increasing traditional antibiotic resistance (Davies & Davies 2010).

METALS AS ANTIMICROBIAL AGENTS-THE TRADITIONAL USE

Metals have been used for treatment of different diseases including infectious ones from the ancient time. Evidences of applications have been found in Indian Ayurvedic system of medicine dating back to about 5000 years B.C. (Galib et al 2011). Even proof of use of copper and silver utensils including spoon, coin etc. as disinfectant/ preserving agent in water, milk, wine, vinegar etc during the period of Persian, Greeks, Romans and Egyptians has been demonstrated in literature. It has been documented in different literatures (Silver 2003, Chen & Schluesener 2008, Rai et al 2012, Mijnendonckx et al 2013, Chernousova and Epple 2013, Sweet, Chesser & Singleton 2012, Russell & Hugo) that silver has been used in even space shuttles as water sterilizing agent . The well-known Bordeaux mixture which was invented by French mycologist Pierre-Marie-Alexis Millardet in 1880 is nothing but a mixture of metallic (Cu and Ca) compounds. This mixture acts as fungistatic agent and thus can prevent the growth of downy mildew on grape vines. FDA has already permitted use of silver as disinfectant in packaged potable water up to a permissible limit (US Food and Drug Administration, Fed. Regist., 2009).

NANOMETAL/METAL OXIDE AS ANTIMICROBIAL AGENT –THE MODERN CONCEPT

This is already known to us that metal at the nanoscale dimension owes higher surface to volume ratio than its bulk metallic form. For this reason nanometal exhibits enhanced surface properties like catalysis. Since metal atoms interact closely with the cell surface or cell components of microorganisms and inhibit their regular growth, it is expected, therefore, in the nanoscale form such

metal can exhibit higher antimicrobial efficacy than its bulk counterpart. In addition, the antimicrobial activity depends on several factors like chemical composition of nanoparticles, concentration of nanoparticles, size and shapes of the nanoparticle, target microorganism as well its initial load (Morones et al 2005, Kim et al 2007, Pal, Tak and Song 2007, Pelgrift & Friedman 2013, Hajipour et al 2012, Rai, Yadav & Gade 2009, Lara et al 2010). Transmission electron microscope (TEM) images of various shaped nanoparticles have been illustrated in Figure1. Even antimicrobial activity of nanoparticle can be tuned by surface modification. It is also evidenced that some metal oxide nanoparticle like TiO2 may show synergistic antimicrobial activity in presence of other agent like UV-radiation (Wan et al 2011, Liu et al 2010).

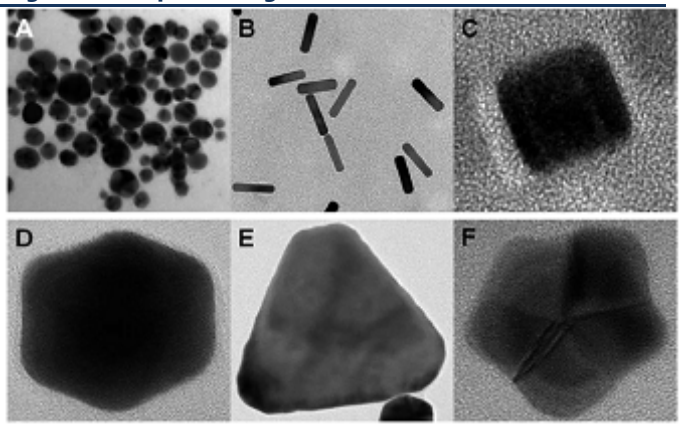

Figure 1. TEM images of some commonly used shape distributions of engineered metallic crystallites. (A) silver-spheres, (B) gold-rods, (C)cadmium-selenide squares, (D) gold-hexagons and (E) gold-triangles.(F) gold-star shapes. (Reproduced from Suresh, Pelleitier and Doktycz, Nanoscale, 2013 with permission from Royal Society of Chemistry).

MECHANISM OF ANTIMICROBIAL ACTION: CONVENTIONAL ANTIMICROBIAL VS. NANOMATERIALS

Common antimicrobial agents work either or in combination of the following mechanistic pathway: inhibition of the cell wall synthesis, inhibition of ribosome function, Inhibition of nucleic acid synthesis, Inhibition of folate metabolism, Inhibition of cell membrane function. Summary of the mode of action of different antimicrobial agents is provided in Table2.

However, development of resistance by microbes against common antimicrobial agents is a burgeoning threat now in biological sciences.

Based on the chemical structure as well as mechanism of action of an antimicrobial substance, specific microorganism modifies itself in such a way that it can avoid inhibitory effect of the substance and thus the microbe gets resistant to it. We have already shown different modes of action of antimicrobial substances including antibiotics. Unfortunately, bacteria may develop resistance against each of those modes of action. Studies have shown that bacteria may show resistance against antibiotics aminoglycosides and Tetracyclines by degrading the antibiotic enzymatically and by modifying ribosomes, respectively. Researchers have also shown that multidrug resistant microbes may even activate different efflux pumps to get rid of those antibiotics (Nikaido 2009).

Fortunately, the limitations of the conventional antimicrobial agents as discussed above may be outweighed by employing metal/metal oxide nanoparticles as alternative antimicrobial agent. The main advantages of the nanoparticles over the conventional antimicrobial agents as established by different studies so far are of two types. Firstly, nanoparticles are broad spectrum and may act against an wide spectrum of bacteria, algae,fungi , virus and even biofilm. Secondly, mechanism of action for nanoparticles include mainly contact to bacterial cell wall and thus the above mentioned resistance mechanisms against antibiotics are not applicable here. That means nanoparticles may be less susceptible to the buildup of resistance than the conventional antimicrobial substances.

Though a variety of investigations have been conducted to underline the mechanism of action of the nanoparticles, yet the actual mode of action is yet under cloud. A number of factors viz. role of nanoparticle type, their shape and size,biofilm formation rate, the process of synthesis and growth conditions can be assumed to be influencing the actual mechanism, but the main constraint remains the behavior of different strains of microorganisms to the nanoparticles. (Nair et al

2009, Pal, Tak and Song 2007, Gupta 1998, Martínez-Castañón et al 2008)

Following four principal possible mechanisms have been suggested in literature (Emamifar et al 2010, Emamifar et al 2011, Morones et al 2005). The first one is that the metal nanoparticle can attach to the bacterial cell wall and disturb its power function. As a result plasmolysis (separation of cytoplasm from bacterial cell wall) and inhibition of synthesis of bacterial cell wall occur. Secondly, metal nanoparticle can strongly interact with the sulphur-phosphorus containing compounds present inside (DNA, proteins) and outside of cell and affect the metabolism (or normal function) of the cell which ultimately leads to cell death. The third one is that the metal nanoparticle releases metal ions which can interact with DNA and cellular protein and hence DNA loses its replication ability and cellular proteins become inactivated.

Oxidative stress induced by reactive oxygen species (ROS) generation in nanoparticles systems is thought to be the fourth mechanism of their antibacterial activity. In particular, many previous studies have explored the photogeneration of ROS on the surfaces of metal-oxide nanoparticles. However, other possible mechanistic routes have also been proposed by different authors which are depicted in the Figure 2.

Table 2.mode of action of different conventional antimicrobial agents.

Antimicrobials	Cidal nature	Mechanism of action	Citations
Penicillins	Bactericidal	Inhibition of cell wall synthesis	Kennedy, Bek and Griffin 2005; Walsh , 2003.
Cephalosporins	Bactericidal	Inhibition of cell wall synthesis	
Carbanepems	Bactericidal	Inhibition of cell wall synthesis	
Polypeptide antibiotics	Bactericidal	Inhibition of cell wall synthesis	
Quinolones	Bactericidal	Inhibition of DNA synthesis	
Metronidazole	Bactericidal	Inhibition of DNA synthesis	
Rifamycins	Bactericidal	Inhibition of RNA transcription	
Lincosamides	Bactericidal	Inhibition of protein synthesis	
Aminoglycosides	Bactericidal	Inhibition of protein synthesis	
Macrolides	Bacteriostatic	Inhibition of protein synthesis	
Tetracyclines	Bacteriostatic	Inhibition of protein synthesis	
Chloramphenicol	Bacteriostatic	Inhibition of protein synthesis	
Sulfonamides	Bacteriostatic	Competitive inhibition	

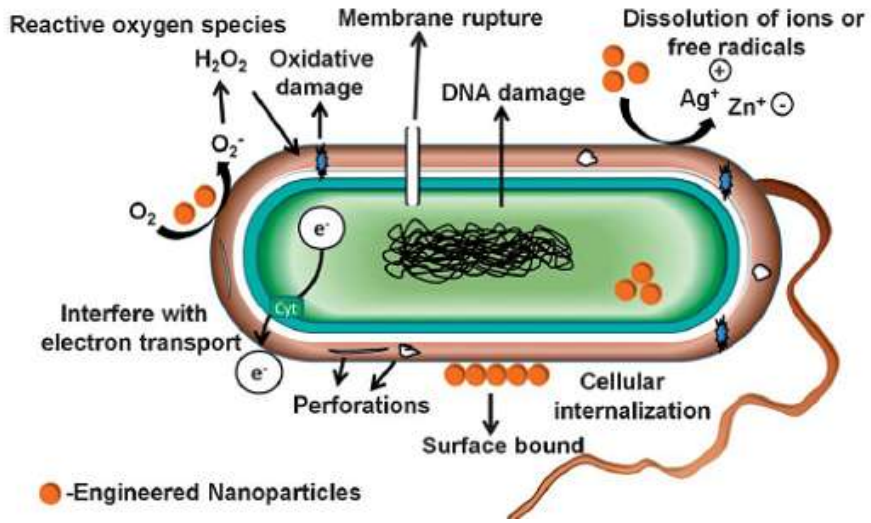

Figure 2. Illustration of potential interactions and modes of toxicity when engineered nanoparticles interact with bacterial cells. Various nanoparticle forms might render.bacterial toxicity through one or several of these mechanisms. DNA: deoxyribonucleic acid; Cty: cytochromes (Reproduced from Suresh, Pelleitier and Doktycz, Nanoscale, 2013 with permission from Royal Society of Chemistry).

TECHNIQUES FOR ASSESSMENT OF ANTIMICROBIAL ACTIVITIES

Different experimental techniques have been used to assess the antimicrobial activity of nanoparticle, but many of those techniques are unsound. As a result, to get reliable results often multiple techniques are used in single study. Moreover, the responses of Gram-positive and Gram-negative bacteria or fungi or biofilm are different towards antimicrobial nanoparticles, so naturally they demand different assay techniques (Ncube, Afolayan and Okoh 2008). The technique chosen for a study depends, in part, on the type of data needed.

Optical density of cell suspensions

One of the easy techniques to estimate the cell density in a bacterial suspension is to measure the optical density, or turbidity, of the cell suspension and correlating that optical density to cell concentration. This technique can estimate the rate of proliferation by measuring cell density across a range of time points. In general with the help of a spectrophotometer initially a standard curve measuring the optical density against different concentration of bacterial suspension is constructed. The different concentration of bacterial suspension is achieved by serial dilution of a particular bacteria cell suspension. Each diluted bacteria suspension can be spread onto Agar plate and the number of colony forming units (CFUs) present in each sample can be accurately counted after an appropriate incubation time and then concentration of bacterial suspension of experimental sample can be determined by matching the experimental turbidity with the cell density of standard curve and can be correlated with the CFUs. Like bacteria cell bodies, nanoparticles are also capable of scattering spectrophotometer light. To resolve this issue, authors of some studies have measured the optical densities of a standard curve of nanoparticles alone and then subtracted those values from the optical densities of the suspensions of a combination of cells and nanoparticles.

Determination of Minimum Inhibitory Concentration (MIC)

MIC technique is extensively used in the comparative testing of new antimicrobial agents. MIC can be defined as the lowest concentration of an antimicrobial agent which inhibits the visible growth of a microorganism. Initially the serial dilution technique is applied for the antimicrobial agent using separate dilution vessels. An equal volume of inoculums made of test organism is added to each vessel then. The inoculated vessel is then incubated at a temperature which is appropriate for the test organism. After the incubation period, dilution vessels are checked for any microbial growth (indicated by turbidity and/or pellet formation at the bottom). The concentration of the antimicrobial agent at the last vessel in the series which does not exhibit any growth is indicated as MIC of that agent.

Cell counting instruments

Devices used to quantify the number of cells in a liquid suspension, including Coulter counters, can also be used to determine bacteria populations. In contrast to optical density measurements which do not provide information on the size of the cells and particles present, a Coulter counter provides more useable data.

Spread-plate colony counts

Viable CFUs present after exposure to nanoparticles can also be determined by spreading bacteria suspensions on an agar plate. In the so called spread-plate technique, cell suspension samples are serially diluted and a small volume of sample is then spread across the surface of an agar plate. CFUs are counted after an incubation period. Taking the dilution process into consideration, calculations are then performed to determine the cell density in the original sample. When compared to plates spread from samples that did not contain nanoparticles, the percent reduction in viable CFUs can be determined. A small volume of bacteria suspension is spread on the agar plate and incubated. CFUs are counted after suitable colonydevelopment. A reduced number of CFUs on an agar plate with incorporated nanoparticles indicates that the nanoparticles have an antibacterial effect.

Crystal violet staining

As a bacteria colony takes hold in a host, a biofilm may be formed. Crystal violet (hexamethyl

pararosaniline chloride) can be used to evaluate the amount of biofilm formed by staining thethick peptidoglycan layer of Gram-positive bacteria, the thin peptidoglycan layer of Gram-negative bacteria, and components of the extracellular biofilm. When exposed to a crystal violet stain solution, the amount of stain absorbed by the biofilm is generally proportional to the quantity of biofilm present. A solvent can then be used to remove the absorbed crystal violet, and the extent to which the solvent changes color due to the presence of the crystal violet stain can be measured with a spectrophotometer. This color change is proportional to the quantity of biofilm present. In this way, biofilm formation in the presence of nanoparticles can be compared to the control biofilm.

Agar Well Diffusion Method

Agar well diffusion assay may be employed to examine susceptibility or resistance of a bacterial strain to an antibacterial agent. Initially, a bacterial suspension is spread onto the surface of the solidified agar medium contained in a petri plate. Then an antimicrobial agent is applied to a well that is cut into the agar medium. The agent will thus tend to diffuse uniformly through the agar around the well. If bacterial growth is observed right up to the well, then the bacterial strain is deemed to be resistant to the antibacterial agent. On the other hand if there is a clear zone around the well, then the bacteria have been adversely affected by the antibacterial agent. The size of the inhibition zone (sometimes called as diameter of inhibition zone) can be measured and related to standards, in order to determine whether the bacterial strain is sensitive to the antibacterial agent.Alternatively, disks of an absorbent material soaked with the antibacterial agent of interest may directly be placed onto the agar surface. The agent will then diffuse out of the disk into the agar medium around the disk. This version of agar diffusion is known as the Kirby-Bauer disk-diffusion assay.

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METAL AND METAL OXIDE AS ANTIMICROBIAL AGENT

In this section, antimicrobial property of some common metal and metal oxide nanoparticles are discussed individually.

Metal nanoparticles

Silver (Ag)

As already discussed, silver, in bulk form has been in use as antibacterial agent since ancient ages, which encouraged investigation of the activity of silver nanostructures as natural antibacterial metals (Sahayaraj and Rajesh 2011).

1) Silver nanopartcles (AgNPs) may attach to the surface of the cell membrane disturbing permeability and respiration functions of the cell. Bactericidal activity depends upon the surface area of the particles. For instance smaller AgNPs having the large surface area available for interaction would give more bactericidal effect than the larger AgNPs. How the silver nanoparticles get adhered to the bacterial cell may be evidenced through TEM image as illustrated in Figure 3.

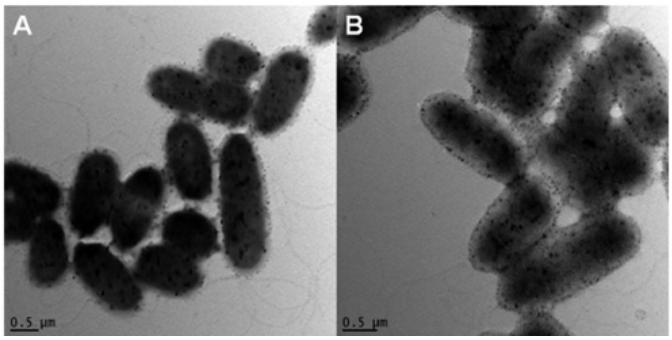

Figure 3. TEM images of engineered silver nano-particles interacting with the bacterium E.coli. The nanoparticle range in size from 2-70nm and were prepared biogenically. (Reproduced from Suresh, Pelleitier and Doktycz, Nanoscale, 2013 with permission from Royal Society of Chemistry).

2) It is also possible that AgNPs not only interact with the surface of membrane, but can also penetrate inside the bacteria.

3) AgNPs synthesized using disaccharides, maltose and lactose, have a higher antibacterial activity than those synthesized using monosaccharides, glucose and galactose.

4) SDS, Tween 80 has the capability to modify the antibacterial activity.

Several investigations have shown that silver nanoparticles may be used as effective antimicrobial agents against the genera like *Escherichia*, *Enterococcus*, *Staphylococcus*, *Pseudomonas*, *Bacillus*,*Shigella* and*Salmonella* etc. (Halder et al 2011, Kumar et al 2014) In addition silver nanoparticles have been found by researchers to be well active against fungi like *Candida albicans*(Eby et al 2009, Egger et al 2009, Kim et al 2008, Kim et al 2009), *Aspergillus niger*(Egger et al 2009,Sanchez-Valdes et al 2009), yeast isolated from Bovine mastitis (Kim et al 2007) as well as against algae like*Chlamydomonas reinhardtii*(Navarro et al 2008). Silver nanoparticles are reported to be potent agent against biofilm of *Candida albicans*, too.

Interestingly studies have shown also that silver nanoparticles may show fare inhibitory effect against HIV and monkey pox virus (Lara et al 2010, Galdiero et al 2011)

It is worth mentioning here that silver nanoparticles are equally effective against antibiotic resistant strains of the microorganisms. However, Gram-negative bacteria have been found to be more susceptible to silver nanoparticles than Gram-positive bacteria .This may be due to the fact that silver ions released from the surface of the nanoparticles may find higher resistance during the passage through the peptidoglycan rich relatively thicker outer membrane of Gram positive bacteria (Choi et al 2008, Dibrov et al 2002, Lok et al 2007). Mechanism of antimicrobial activity of silver nanoparticle against different microorganisms with reference to its size has been demonstrated in Table3.

Due to its broad spectrum antimicrobial activity, silver nanoparticles have found diversified applications in food and allied industries substituting different conventional antimicrobial agents. Example of applications includes silver nanoparticles impregnated plastics for food storage containers or surfaces of other food contact materials, water purification system etc (Azeredo 2009, Brody et al 2008, Duncan 2011).

Copper (Cu)

Copper has been stated to possess broad spectrum antimicrobial properties in various investigations. Like silver nanoparticle, copper nanoparticle also kills cells by diverse mechanisms, such as disruption of membrane, blocking biochemical pathways, complex formation with proteins, and DNA damage (Cioffi et al 2005, Ruparelia et al 2008, Santo et al 2007, Babushkina et al 2010, Raffi et al 2010). In studies, it has been stated that, Gram positive bacteria strains like *S. aureus*, *B. subtilis* were more sensitive to copper nanoparticles than the Gram negative, *E.coli*(Dinda et al 2015). The studies show that the ultimate cell death of microbes was occurred by copper nanoparticle mediated damage of cell membrane leading to the leakage of cytoplasomic content (loss of intracellular K^+).The presence of greater number of amine and carboxyl group on the cell surface of Gram- positive bacteria may cause higher susceptibility towards Copper nanoparticle (Ruparelia et al 2008, Yoon et al 2007).Another possible explanation for the higher resistance of *E.coli* to the nanoparticle is that the outer membrane of Gram-negative bacteria such as *E.coli* is predominantly constructed from tightly packed lipopolysaccharide (LPS) molecules, which provide an effective resistive barrier against copper nanoparticle (Yoon et al 2007)

In most microorganisms, except viruses, there is an integrated set of proteins that delivers Copper to specific sub-cellular compartments and Copper containing proteins without releasing free Cu ions. Viruses lack DNA repair mechanisms, permeability barriers, intra and extracellular sequestration of metals by cell envelope, active metal transport membrane efflux pumps and enzymatic metal detoxification mechanism, as found in bacterial cells. These reduced capabilities of viruses explain their high vulnerability and susceptibility to copper. Investigations demonstrate the importance of role of copper ions in antibacterial mechanism (Thompson et al 2014). Though it is not clear whether the antibacterial property is due to the copper ions released from the nanoparticle's surfaces, or the nanoparticles themselves are responsible.

Gold (Au)

Several studies demonstrated that size and shape dependent optical properties of gold nanomaterials may be exploited for photothermal lysis of microbes in non-invasive pathway (Ray et al 2013). In this simple approach gold nanoparticle acts as a "light-directed nano-heater". The

underlying mechanism involves the conversion of light absorbed by the nanoparticles into heat by rapid electron-phonon relaxation followed by phonon-phonon relaxation. Since wavelength of the absorbed light is dependent on the size and shape of the gold nanoparticle, generation of heat may be regulated by tuning the size and shape of the microbes conjugated gold nanoparticle. Studies exhibit that damage of microbe cell in this process occurs due to various thermal effects including denaturation of proteins/enzymes, induction of heat-shock proteins, metabolic signaling disruption, endothelial swelling, microthrombosis, etc.

Beside photothermal destruction of cell, gold nanoparticle also exhibits similar cell lysis mechanism as discussed earlier (Chandran et al 2014).

Several reports exists on gold nanoparticles non covalently bound with antibiotics and exhibiting enhanced bactericidal activity (Zhou et al 2012, Wang et al 2011, Amin et 2009, Norman 2008, Bowman et al 2008, Sametband et al 2011). These specific antibiotics contain free amino acids, which have a strong affinity to gold nanoparticles surfaces. Rai et al (suggested that the particle structure acts to amplify the cell membrane damage initiated by antibiotics, increases the cell penetration into Gram negative bacteria and disrupts bacterial DNA

Metal oxide nanoparticles

Aluminum Oxide (Al_2O_3)

In an investigation to study antimicrobial sensitivity of Escherichia coli to alumina nanoparticles, alumina NPs over a wide concentration range (10-1000 µg/mL) demonstrated a mild to moderate growth-inhibitory effect on *E. coli* with negligible dependence on the concentration. Alumina is thermodynamically stable over a wide temperature range and has a corundum-like structure, with oxygen atoms adopting hexagonal close packing and Al^{3+} ions filling two thirds of the octahedral sites in the lattice. Alumina may act as direct antioxidant, thus blocking the oxygen stress induced cell death process(Ansari et al 2014, Sadiq et al 2009).

Cupric and Cuprous Oxide (CuO and Cu_2O)

The oxides of Copper have been widely in use due to its captivating properties. A unique property of crossing the cell membrane can potentially be attributed to the engineered cupric oxide nanoparticles through such bacterial pores(Ren et al 2009, Erbe et al 2013, Jadhav et al 2011) Another study comparing the antibacterial activity of CuO, ZnO, and NiO against *E.coli, B. subtilis, S. aureus* speculated that CuO have much dominant antimicrobial effect than ZnO and NiO (order: CuO > ZnO > NiO). (Baek and An 2011).

Iron Oxide (Fe_2O_3)

Iron oxides are of particular interest not only due to their super paramagnetic properties that allows such particles to be directed in situ with a magnetic field but also as an antimicrobial agent. Experiments conducted to check the activity of Fe_2O_3 nanoparticles to reduce *S. aureus* viability revealed that at conc. of 3mg/ml,the engineered nanoparticles were able to reduce the cell viability at 4,12 and, 24 hrs as compared to control cultures. The antibacterial activity of Fe_2O_3 nanoparticles has been related to cell permeability and generation of Reactive Oxygen Species (ROS)(Chatterjee, Bandyopadhyay and Sarkar 2011, Agarwala, Choudhury and Yadav 2014, Tran et al 2010).

Titanium Dioxide (TiO_2)

TiO_2 has been widely applied as surface coatings with photocatalytic disinfecting properties. (Allahverdiyev et al 2011, Liu et al 2010). This antimicrobial activity may be attributed due to photo-induced super hydrophilicity and generation of ROS by TiO2 nanoparticle. In addition, when phospholipids present in the microbial cell membrane undergo peroxidation promoted by TiO_2 nanoparticle photocatalysis, cell integrity is lost(Maness et al 1999). TiO_2 photocatalysts can be used as effective biofilm disinfectant in food processing industries (Chorianopoulos et al 2011, Pham, McDowell and Wikins et al 1995). Aqueous suspensions containing TiO_2 can effectively kill bacteria (*Escherichia coli)* and viruses under UV light (Wei et al 1994, Ireland et al 1993). Several studies suggested that photocatalytic activity of nanostructured TiO_2 may reduce the time of disinfection, eradicating pathogenic

microorganisms in food contact surfaces and thus enhance food safety (Chorianopoulos et al 2011). To bypass the disadvantage of using UV radiation, visible light absorbing photocatalysts with Ag/AgBr/TiO_2 has been developed for the purpose of killing bacteria like *S. aureus* and *E. coli*(Hu et al 2006)

Zinc Oxide (ZnO)

Compared to organic materials, inorganic materials such as ZnO possess superior durability, greater selectivity, and heat resistance Moreover, zinc is a mineral element essential to human health and ZnO is a form in the daily supplement for zinc. ZnO nanoparticles also have good biocompatibility to human cells.

Many studies have shown that someNPs made of metal oxides, such as ZnO NP, have selective toxicity to bacteria and only exhibit minimal effect on human cells, which recommend their prospective uses in agricultural and food industries (Jones et al 2008, Huang et al 2008, Zhang et al 2007). The antimicrobial activity of zinc oxide nanoparticles have been studied against the food related bacteria *Bacillus subtilis*, *Escherichia* coli and *Pseudomonas fluorescens*(Reddy et al 2014, Jiang, Mashayekhi and Xing 2009). ZnO NP could potentially be used as an effective antibacterial agent to protect agricultural and food safety from foodborne pathogens, especially E. coli O157:H7 (Liu et al 2009). ZnO NPs possess antimicrobial activities against *Listeria*. There are also other studies confirming the strong antimicrobial activity of ZnO nanoparticles wherein the nanoparticles could completely damage the food-borne bacteria *Salmonella typhimurium* and *Staphylococcus aureus* (Liu et al 2009, Chakraborti et al 2014). The above findings suggest that ZnO nanoparticles can find applications in food systems and can be used to inhibit growth of pathogenic bacteria. A comprehensive literature involving antimicrobial activity of different metal and metal oxide nanoparticles analyzed by different evaluation techniques has been represented in Table4.

Nanocomposites

Nanoparticles of Ag , TiO_2 , ZnO , MgO , CaO etc. have been doped in various food packaging materials to enhance shelf life and safety of packaged food from microorganisms (Chaudhry, Castle and Watkins 2010,). Researchers are developing 'smart packaging' systems involving nanomaterials which would be able to indicate if the food inside becomes contaminated as well as self-repair the hazardous condition. Many researchers have shown that (Rhim and Wang 2014, Rhim et al 2014) Nanosilver based composite films exhibit increased oxygen / moisture barrier properties, higher mechanical strength, enhanced thermal stability and strong antibacterial activity compared to their native polymers / conventional composites. Different nanosilver-composite films along with their characteristics and possible future applications have been represented in Table5.

Table 3. Mechanism of antimicrobial activity of silver nanoparticle against different microorganisms with reference to its size.

Microorganisms (MO)	Type of MO	Size of Silver nanoparticle (AgNP)	Mechanism of antimicrobial action	Citations
Acinetobacter baumannii	Bacteria Gram (-)	2-5 nm (70-75%), 5-100 nm (25-30%).	Alteration of cell wall and cytoplasm	Łysakowska et al. 2015
Aspergillus niger	Fungus	22-52 nm	Damage by interacting with electron phosphorous and sulphur containing compounds such as DNA	Kathiravan et al. 2015
Bacillus cereus	Bacteria Gram (+)	1-20 nm	Silver nanoparticles combined with respiratory enzyme, protease enzyme and DNAs of	Lokina et al. 2014

Microorganisms (MO)	Type of MO	Size of Silver nanoparticle (AgNP)	Mechanism of antimicrobial action	Citations
			bacteria to cause suffocation, indigestion and inhibition of cell replication respectively	
Bacillus subtilis	Bacteria Gram (+)	2-4 nm	Release of Ag+ electrostatic interaction, cell wall damage and rupture	Ruparelia et al. 2008
Candida albicans	Fungal biofilm		Alteration of cell membrane permeability and interaction with macromolecules like proteins and DNA thereby affecting the replication machinery and cellular processes	Gupta et al. 2014
Citrobacter koseri	Bacteria Gram (-)	1-20 nm	Silver nanoparticles combined with respiratory enzyme, protease enzyme and DNAs of bacteria to cause suffocation, indigestion and inhibition of cell replication respectively	Lokina et al. 2014
Escherichia coli	Bacteria Gram (-)	12 nm	Alteration of membrane permeability and respiration	Sondi, Salopek-Sondi, 2004
Klebsiella pneumoniae	Bacteria Gram (-)	30-55nm	Alteration of membrane	Naraginti, Sivakumar,2014
Listeria monocytogenes	Bacteria Gram (+)	5-15 nm	Morphological changes, separation of the cytoplasmic membrane from the cell wall, plasmolysis	Tamayo et al. 2014
Micrococcus luteus	Bacteria Gram(+)	15–25 nm	Alteration of membrane	Manjumeena et al. 2014
Propionibacterium acnes	Bacteria Gram (+)	20-70 nm	interacting with the microbial cell wall or plasma membrane	Velmurugan et al. 2015
Proteus mirabilis	Bacteria Gram (-)	20-70 nm	Alteration of cell membrane damage and DNA denaturation.	Muhsin et al. 2014
Pseudomonas aeruginosa	Bacteria Gram (-)	8-20 nm	Irreversible damage on bacterial cells; Alteration of membrane permeability and respiration	Dhas et al. 2014
Pseudomonas aeruginosa	Bacterial biofilm	9.3 ± 1.1 nm	Interference or inhibition and/or regulation of exo-polysaccharides (EPS)	Velázquez-Velázquez et al 2015
Pseudomonas putida	Bacteria Gram (-)	10 nm	Cell membrane damage and bactericidal effect	Gajjar et al. 2009
Salmonella typhi	Bacteria Gram (-)	10-25 nm	Inhibition of bacterial DNA replication, bacterial cytoplasm membranes damage, modification of intracellular ATP levels	Shameli et al. 2012
Staphylococcus aureus	Bacteria Gram (+)	10-25 nm	Irreversible damage on bacterial cells	Shameli et al. 2012

Microorganisms (MO)	Type of MO	Size of Silver nanoparticle (AgNP)	Mechanism of antimicrobial action	Citations
Staphylococcus epidermidis	Bacteria Gram (+)	7-20 nm	Inhibition of bacterial DNA replication, bacterial cytoplasm membranes damage, modification of intracellular ATP levels	Jain et al. 2009
Trichosporon asahii	Fungus	5-20 nm.	Damaged the cell wall and cell membrane, penetrated inside the cells	Xia et al. 2014
Vibrio cholerae	Bacteria Gram (-)	21±18 nm	Alteration of membrane permeability and respiration	Morones et al. (2005)

Table 4. Antimicrobial activity of different metal and metal oxide nanoparticles analyzed by different evaluation techniques.

Type of NP	MO	Methods	Antimicrobial activity	Citations
TiO_2, SiO_2 , ZnO	B. subtilis, E. coli	Colony count	Toxicity decreased from ZnO to TiO_2 to SiO_2	Adams et al., 2006
MgO, TiO_2, Al_2O_3, CuO, CeO_2, ZnO	S. aureus	Microdilution	Al_2O_3 and ZnO had antibacterial activity, while other nanoparticles did not exhibit inhibiting effect	Jones et al., 2008
Ag, ZnO, Au	S. mutans	Microdilution	AgNP were the most effective.	Hernández-Sierra et al., 2008
Ag, Cu	E. coli, B. subtilis, S. aureus	Disk diffusion, Microdilution	*E. coli* and *S. aureus* were more sensitive to AgNP, while to CuNP.	Ruparelia et al., 2008
Ag, Au	E. coli, S. aureus	Microdilution	AgNP had higher antibacterial activity than AuNP.	Amin et al., 2009
Ag, CuO, ZnO	P. putida	Colony count	AgNP had higher antibacterial effect than CuO and ZnO nanoparticles.	Gajjar et al., 2009
Fe, Cu	S. aureus	Colony count	Cu nanoparticles had higher antibacterial activity than Fe nanoparticles.	Babushkina et al., 2010
Ag, Au	E. coli, S. aureus	Well diffusion	AgNP had higher antibacterial activity against *E. coli* than S. *aureus*. AuNP had activity only against *E. coli*, which was lower than in AgNP.	Mubarak et al., 2011
Ag, Au	E. coli, S. aureus	Disc diffusion	AgNP had higher antibacterial activity against both *E. coli* and *S. aureus*.	Ahmad et al 2013
Ag, Au	E. coli, P. aeruginosa, E. aerogenes, S. aureus, B. cereus	Disc diffusion	AgNP had higher antibacterial activity against *P. aeruginosaE. aerogenes* and *S. aureus* whereas AuNP had higher antibacterial activity against *E. coli* and *B. cereus*	Basavegowda et al. 2014

Type of NP	MO	Methods	Antimicrobial activity	Citations
Ag, Au, Au–Ag alloy	S. aureus, E. coli, S. typhi, P. aeruginosa	Disk diffusion	AgNPs exhibited very good antimicrobial effect when compared to AuNPs. Au–Ag alloy NPs exhibited higher antimicrobial nature when compared to monometallic of Au and Ag NPs	Yallappa et al. 2015

Table 5. Examples of nanosilver-composite films, their characteristics and possible future applications.

Nanosilver composite film	Characteristics highlight of the film	Future application of the film	Citations
Agar-AgNPs	-Water contact angle, tensile strength, and modulus decreased slightly, but elongation at break increased after AgNPs incorporation -Strong antibacterial activity against *Listeria monocytogenes* and *Escherichia coli*	Could be applied to the active food packaging by controlling the food-borne pathogens	Shankar, Rhim 2015
Chitosan/Cellulose-AgNPs	-Significantly improved antimicrobial activities	Potentially used to prepare better bio-fouling resistant degradable membranes	Lin et al. 2015
Quaternized chitosan-AgNPs	-Surface enhanced Raman scattering (SERS) active substrate to determine tricyclazole and Sudan-I.	Used as a SERS substrate for substrate for detection of food contaminants.	Chen et al. 2015
PVA/CTS hydrogel-AgNP	-AgNPs enhanced the stretching of the polymeric backbone chains, resulting in the higher elongation at break (EAB) and slightly lower tensile strength (TS) of the composite film	Could be utilized as a biomaterial in medical applications such as wound dressing.	Nguyen and Liu 2014
κ-Carrageenan-AgNPs	-Tensile strength (TS) of the nanocomposite films increased by 14–26% and water vapor permeability (WVP) decreased by 12–27% -Nanocomposite film showed strong antimicrobial activity against Gram-negative bacteria	High potential for the application as an active packaging to secure food safety and to prolong the shelf-life of packaged foods.	Rhim, Wang 2014
Agar-AgNPs	-Thermal stability of the agar-AgNPs composite films was increased by the inclusion of metallic silver -Water vapor barrier properties and surface hydrophobicity of the films also increased slightly with the increase in AgNPs content -Agar-AgNPs films exhibited distinctive antimicrobial activity against both Gram-positive (*Listeria monocytogenes*) and Gram-negative (*Escherichia coli O157:H7*) bacterial pathogens.	Expected to be used as an active packaging application such as an antimicrobial food packaging or a biomedical application such as wound dressings.	Rhim et al. 2014
Gelatin-AgNPs	-The incorporation of AgNPs slightly	Expected to have high	Kanmani,

Nanosilver composite film	Characteristics highlight of the film	Future application of the film	Citations
	affected the physical and mechanical properties of the films -Increase in the concentration of AgNPs resulted in a substantial decrease in water vapour permeability (WVP) and tensile strength (TS) of the gelatin films.	potential as an active food packaging system to maintain food safety and to extend the shelf-life of packaged foods.	Rhim 2013
Cellulose nanocrystal-AgNPs	-Hydrophilic nature of s-CNC/Ag based films highlighting that the wettability properties are strongly influenced by the cellulose nanocrystal nature	"Smart paper", opening the way toward new perspectives in different domains with a large number of applications, use in wound dressing, body wall repairs, tissue scaffolds, or even antimicrobial filters	Fortunati et al. 2014
Chitosan-AgNPs	-Increase of the refractive index	Can be used for Polymeric optical waveguide **(**POW) and optical sensors fabrication using substrates, which are significantly cheaper than quartz.	Mironenko et al 2014
Soy protein isolate- AgNPs	-Effective antimicrobial activity against both Gram-positive and Gram-negative bacteria	May have the great potential for the application in food industry and biomedical fields.	Zhao et al. 2013
Agar-AgNPs	-Significant increase in water vapor barrier properties and surface hydrophobicity were observed with increase in the concentration of AgNPs without reduction in the mechanical strength - Exhibited strong antimicrobial activity against both Gram-positive (*Listeria monocytogenes*) and Gram-negative (*Escherichia coli* O157:H7) bacterial pathogens	High potentials for using as an active packaging application such as an antimicrobial food packaging and a biomedical application such as wound dressings	Rhim et al. 2013
Hydroxypropyl methylcellulose (HPMC)-AgNPs	-Decrease observed in the WVP values for the HPMC/AgNPs system -Bactericidal potential of the HPMC/AgNPs films against some bacteria	HPMC/AgNPs nanocomposites can be used in food packaging as active antimicrobial internal coatings	Moura et al. 2012
Polyoxometalate– AgNPs	-Composite films exhibits the electroreduction toward O_2 -Composite films are effective in prohibiting the growth of *E. coli*	-Potential application in fuel cells and also can be used as a promising kind of antibacterial coating	Gao et al. 2011
AgNPs embedded in agar–agar matrix	-Ag/agar film exhibits good mechanical stability -Antimicrobial activity of the film is found to be in the order of *C. albicans*>*E. coli*>*S. aureus*	Not mentioned	Ghosh et al. 2010

SAFETY ISSUE

Like any other technology nanotechnology has both its merit and demerit. The main concern with nanomaterial is that the consequences of its regular use is still little known, (Nickols-Richardson 2007). The nanoparticles unlike their macroscale counterparts have an enhanced capacity for migration, absorption and interaction with cellular components (Li and Huang 2008). The proposition in this respect is that the meritorious properties shown only by the nanomaterial, not by micro even macro-scale material might result in unpredictable safety problem and risk, in turn. Health risks due to nanoparticles arise due to their physico-chemical nature, degree and duration of exposure to the nanoparticles. Various studies revealed that ingestion, dermal contact and inhalation are the potential exposure routes for nanoparticles used in food packaging systems. The possible danger may be compared with that associated with the useful products such as cholofluorocarbons, asbestos and DDT(Balbus et al 2005). The risk is not associated with human health only it may be mobile or persistent in the environment (Balbus et al 2005) as a pollutant, too. In addition, this new type of tiny pollutant cannot be removed using traditional method (filtration, centrifugation, settling etc) generally used for its larger counterpart. The antimicrobial nanoparticles, especially silver (Luoma et al 2008), titanium dioxide, and zinc oxide etc. which have increasingly been in use in food packaging and food contact materials are harmful for environment. (Miller & Senjen 2008, Shatkin, 2008).The risk of bacterial and antibiotic resistance in human body due to random use of nano silver is still to be well evaluated.

A collective attempt has to be taken to introduce internationally acceptable regulation or policy designated specifically for nanotechnology applications in foods to stimulate and accelerate the growth and development of safe and secure food-nanotechnology.

CONCLUSIONS

In the present literature we have highlighted the current use and future prospect of different nanoscale metals as well as metal oxides in food industry as antimicrobial agent. The possible antimicrobial mechanism of this nanoparticle has been presented from existing literature and it shows less possibility to develop microbial resistivity against metal and metal oxides nanoparticles as compared to conventional antimicrobial agents. We have also produced an exhaustive list of metal and metal oxide nanoparticles studied by different research groups. One of the most promising future applications of nanoparticles is its use in food packaging material. We have reviewed the applications of composite packaging materials incorporating nanoparticles. Therefore the present article may be a valuable document for future reference in the areas like mechanistic action of metal and metal oxides nanoparticles on different microbes, activity-characteristic relationship, possible food industry application and safety issues.

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Conflicts of Interest

The authors declare no conflict of interest.