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Abstract

The scientist who discovered penicillin and its use as an antibiotic, Alexander Fleming, also raised concerns about bacterial resistance. As he predicted, in the twenty first century, the overwhelming use of antibiotics has led to both drug- and multi-drug resistant bacteria. This paper attempts to investigate the antibacterial potential of silver nanoparticles against drug resistant bacteria. By using Touro's online library database, the efficacy of silver nanoparticles as a potential antibacterial agent was comprehensively researched. Using transmission electron microscopy and the disk diffusion method, silver nanoparticles have been found to exert bactericidal effects by adsorbing to the cell surface and by entering the cell. The small size of the particles confers it with a high surface area which thus enables the silver nanoparticles to effectively interact with the cell membrane and thereafter enter into the cell. Moreover, the dose and shape of silver nanoparticles affects their antibacterial properties. While it has been found to be dose dependent, there is controversy regarding which shaped particle, sphere or triangular, has the greatest ability to damage the cell membrane, transport systems, DNA, and proteins, in addition to generating reactive oxygen species. Most studies have found the particles to be nontoxic at low levels, but some uncertainty still exists. In addition, silver nanoparticles seem to have a synergistic effect with the simultaneous use of antibiotics. Further research must be done before silver nanoparticles can be used as a new and effective antimicrobial agent.

Introduction and Background

On February 27th 2017, the World Health Organization (WHO) published a list of the top twelve resistant bacteria that greatly endanger human health (Press Association, 2017). Drug resistant and multi-drug resistant bacteria are one of the most serious public health threats the world faces today. Antibacterial resistance is the ability of bacteria to survive even in the presence of antibiotics. The development of antibiotics has made a great impact on the medical field by enabling doctors and health professionals to successfully combat many diseases. However, with the rise of the use of antibiotics, drug resistance has evolved, and according to WHO statistics, 700,000 people die annually from infections and diseases that have resulted from such resistant bacteria (Press Association, 2017).

Method

The research discussed in this paper was compiled from various published articles obtained from Touro's online database including Proquest as well as PubMed's database to research the actions of antibiotics, drug resistant bacteria, and the use of silver nanoparticles against drug resistant bacteria.

A. Antibiotics and their mechanism of action

Antibiotics are natural or synthetic agents that fight and inhibit the growth of bacteria. Antibiotics are grouped into different classes based on their mechanism of action, and they can either disrupt the cell membrane, or they can inhibit cell wall synthesis, protein synthesis, DNA replication and repair, RNA synthesis, or various metabolic pathways.

1. Inhibition of Cell Wall Synthesis

Bacterial cell walls are comprised of peptidoglycan, the cytoplasmic membrane, and in gram negative bacteria, the outer membrane. The peptidoglycan, the strongest layer of the cell wall, is a netlike arrangement of glycan and peptide strands. The biosynthesis of the peptidoglycan is catalyzed by thirty different enzymes (Shah, 2015). Transglycosylases and transpeptidases are two enzymes which aid in the final steps of the cell wall synthesis by adding new peptidoglycan units to extend the sugar chain and by linking the amides of the peptide strands, respectively (Walsh, 2000). Numerous antibiotics, like the B-lactam class of antibiotics, target different steps in the synthesis of the peptidoglycan. Penicillins and cephalosporins act as pseudo-substrates for the penicillin binding proteins (PBPs), the active site of the transpeptidases, in order to prevent the cross-linking of the PG. The cross-linking action of the transpeptidases is responsible for the strength of the PG, and without it, the bacterial cell wall is significantly weaker and therefore more prone to lysis. When B-lactam antibiotics bind to the PBPs, the oxygen from the serine residue located near the PBP attacks the B-lactam ring and forms a penicilloyl-enzyme complex. The serine is then acylated by the b-lactam which thus inactivates transpeptidases (Walsh, 2000). Consequently, transpeptidases can no longer bind to the substrate, and therefore the enzyme cannot complete the cross linking action (Andersson, et. al. 2001) (Bockstael, Aerschot, 2009).

Vancomycin is a drug that belongs to the glycopeptide class of antibiotics. Like the B-lactams, vancomycin targets the synthesis of the cell wall. However, rather than targeting the enzymes involved in the production of the PG, vancomycin targets the substrate by making five hydrogen bonds with the D-ala-D-ala terminus of each uncross-linked peptidoglycan (Lange et. al. 2007). By blocking the substrate of both the transpeptidases and transglycosylases, vancomycin and other glycopeptides inhibit the cross linking of the peptidoglycan, resulting in a weaker cell wall that is subject to osmotic lysis (Walsh, 2000)

2. Disruption of the Cell Membrane

The cell membrane of bacterial cells are semipermeable membranes that are comprised of phospholipids, carbohydrates, and proteins. Antibiotics like polymyxins and lipopeptides have the ability to disrupt the bacterial cell membrane. Polymyxins like colistin are cationic cyclic peptides that bind to the phospholipids in the bi-layer. By interacting with the negatively charged cell membrane (Taneja, Kaur, 2016), colistin disrupts the bacterial cell

membrane by displacing divalent ions, like calcium and magnesium, from the lipids present on surface of the cell. The disruption of the cell membrane causes cell leakage and ultimately cell death (Biswas, et. al, 2012) (Bockstael, Aerschot, 2009). Similarly, aminoglycosides can also displace these divalent ions to increase the membrane's permeability, resulting in the leakage of intracellular content and cell death (Lange, et. al. 2007).

Furthermore, daptomycin, a lipopeptide, can also disrupt the cell membrane. However, rather than displacing calcium and magnesium ions, daptomycin forms pores in the membrane by inserting its tail into the membrane. Consequently, there is a potassium efflux, and as potassium ions leave the cell, cell depolarization occurs (Shah, 2015) (Bockstael, Aerschot, 2009). Besides for causing cell depolarization, daptomycin can also inhibit the production of lipoteichoic acid which is responsible for regulating both cell division and cell shape (Bockstael, Aerschot, 2009).

3. Inhibition of Protein Synthesis

In addition to targeting the synthesis of the cell wall and cell membrane, other antibiotics exert their effects by inhibiting bacterial protein synthesis. Ribosomes are essential to the synthesis of proteins as they translate the genes from mRNA into proteins through the three steps of initiation, elongation, and termination. The bacterial 70S ribosome has two subunits: the 50s subunit and the 30s subunit. While the large 50s subunit contains a 5S rRNA, a 23S rRNA and 36 proteins, the smaller 30S subunit is comprised of 16S rRNA and 21 proteins. Antibiotics can exert their effects by targeting either the 30S or the 50S subunit (Bockstael, Aerschot, 2009).

Targeting the 30S Subunit

The 30S subunit contains the site where the codons are recognized by their corresponding tRNA anticodons. Transfer RNAs help with the process of translation by acting at either the A site, the P site, or the E site (Lange, et. al. 2007).

Aminoglycosides like gentamicin and tobramycin interact with the 16s RNA located in the 30S subunit. By hydrogen bonding with the substituents on the aminoglycoside cyclitol ring in the 16s RNA located at the A site, mistranslation occurs, and as a result abnormal proteins are produced. These aberrant proteins are then incorporated into the bacterial cell wall, ultimately resulting in a weak cell wall which is associated with cell leakage and further penetration of the drug into the cell (Lange, et. al. 2007) (Bockstael, Aerschot, 2009).

Tetracyclines bind to the 30s subunit (Lange, et. al. 2007) to prevent the elongation step of protein synthesis. By blocking the substrate of the incoming tRNAs, these antibiotics prevent new amino acids from being added to the growing amino acid chain (Shah, 2015).

Targeting the 50S Subunit

While the antibiotics discussed above inhibit protein synthesis via the 30S subunit, many other drugs have the ability to affect protein synthesis through the 50S subunit. The 50S subunit is associated with peptidyl transferase activity as well as the formation of peptide bonds (Bockstael, Aerschot, 2009).

Chloramphenicol, a broad spectrum antibiotic that is used against both gram positive and negative bacteria, binds to the 23s RNA on the 50S subunit. This class of drugs inhibits the formation of peptide bonds by preventing the tRNAs from binding to the A site (Bockstael, Aerschot, 2009).

The antibiotic class of macrolides binds to the 23S rRNA. Consequently, the exit tunnel that helps transport the peptide away from the peptidyl transferase center is blocked.

Lincosamides, like clindamycin, attack both the A site and P site located in the peptidyl-transferase center (Lange, et. al.2007). As a result, lincosamides inhibit the initiation of peptide chain synthesis and detach tRNAs from the ribosome (Bockstael, Aerschot, 2009).

Streptogramins affect protein synthesis and the action of the peptidyl transferase center activity by binding to the 23S subunit on the 50S ribosome. There are two types of streptogramins, Type A and Type B. While type A prevents the step of elongation by blocking the substrate of the peptidyl-transferase center, type B stimulates the premature release of incomplete peptide bonds by inhibiting peptide bond synthesis (Bockstael, Aerschot, 2009).

Oxazolidinones act by targeting the 50S subunit. The drug linezolid binds to the 23S subunit located on the 50S ribosome. By binding to this subunit, the formation of the complex between tRNA, mRNA, and the ribosome is blocked which thus inhibits the formation of the first peptide bond. If the complex is already formed, oxazolidinones can exert their effects by preventing the translocation of the peptidyl RNA from the A site to the P site (Bockstael, Aerschot, 2009).

4. Inhibition of Metabolic Processes

Some antibiotics can interfere with various metabolic processes that are vital to the survival of bacteria. Folate and folic acid are essential for the synthesis of purines, thymidines, and some amino acids (Lange, et.al. 2007). The folic acid pathway is catalyzed by dihydropteroate synthetase and dihydrofolate reductase, two enzymes which aid in the production of 7, 8 dihydropteroate and tetrahydrofolate, respectively (Shledon Jr., 2005). Drugs like sulphonamide and trimethoprim block different steps in this folic acid pathway, and while sulphonamide competitively binds to p-aminobenozic acid in order to prevent the actions of dihydropteroate synthetase, trimethoprim binds to the enzyme dihydrofolate reductase to prevent the reduction of dihydrofolic acid to tetrahydrofolic acid (Bockstael, Aerschot, 2009) (Sheldon Jr., 2005).

5. Inhibition of DNA Replication

Topoisomerases I, II, III, and IV are enzymes that are essential for DNA replication in bacterial species (Walsh, 2000). Bacterial DNA is negatively supercoiled, and during DNA replication, topoisomerase II, DNA gyrase, removes positive supercoils, breaks the double bond, and decreases the linking number by two. Furthermore, during DNA replication, topoisomerase IV unlinks the daughter chromosomes (Bockstael, Aerschot, 2009). Topoisomerases II and IV are vital for DNA topology, replication, and decatenation, and quinolone antibiotics target these enzymes. By interacting with the complex formed between DNA and DNA gyrase, quinolones make conformational changes that affect the activity of both topoisomerases II and IV, and as a result, DNA replication is blocked (Bockstael, Aerschot, 2009).

The process of transcription, the transferring of genes from DNA to mRNA, is mediated by the multi-subunit enzyme RNA polymerase. The antibiotic rifamycin targets DNA transcription by binding to the beta subunit of RNA polymerase. Rifamycin blocks the entry of the first nucleotide, thereby blocking the action of RNA polymerase and inhibiting mRNA synthesis (Bockstael, Aerschot, 2009).

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B. Mechanism of Resistance

Resistance can be classified as either intrinsic or extrinsic. Intrinsic resistance is resistance that is inherent to a specific species of bacteria. For example, the bacteria belonging to the genus of Enterobacter, Klebsiella, and Escherichia coli are all resistant to methicillin, clindamycin, and vancomycin (James, 1999). Various species of bacteria all differ in the variation of their cell walls, efflux pumps, and biofilms, all of which lends itself to different bacteria's innate resistance to certain drugs (Sheldon Jr., 2005).

Extrinsic or acquired resistance, on the other hand, arises when bacteria acquire new resistance to numerous drugs via different mechanisms. Resistant bacteria can degrade and modify enzymes, alter the targets of antibiotics, change the permeability of their cell wall, or alter metabolic pathways to prevent drugs from penetrating and affecting their cells (Sheldon Jr., 2005).

1. Enzymatic Degradation or Alteration

Some bacterial species can resist a wide array of drugs by enzymatic degradation and alteration. By producing an enzyme that destroys an antibiotic, bacterial species can cause various antibiotics to become ineffective. Antibiotics like penicillins, carbapenems, and cephalosporins all contain a B-lactam ring which binds to PBPs on the peptidoglycan to prevent cross linkage of the cell wall (James, 1999). In response, various bacteria produce different classes of B-lactamases to hydrolyze the four membered B-lactam rings and thereby inactivate these antibiotics (Sibanda, Okoh, 2007).

Similar to the B-lactamases, the aminoglycoside modifying enzymes (AMEs) are enzymes that cause bacterial resistance to aminoglycosides. Some microorganisms can produce enzymes that modify drugs. Aminoglycosides like kanamycin, gentamicin, streptomycin, and neomycin can either be acylated, adenylated, or phosphorylated by aminoglycoside acetyltransferase, adenyltransferase, and phosphoryltransferase. Consequently, the modified antibiotics can no longer exert their antibacterial effects (James, 1999) (Gabani, et. al 2012).

2. Alteration of Targets

In order to have a combative effect on bacteria, antibiotics must bind to their intended receptors. Therefore, in response to various antibiotics, bacteria can reduce the affinity of the antibiotics by modifying the structure of the drugs' active site. Different bacterial species like methicillin-resistant Staphylococcus aureus and Streptococcus pneumoniae produce new penicillin binding proteins, PBP2a and PBP2b respectively, in the presence of antibiotics. These modified active sites have a lower affinity for B-lactams which, in turn, prevent the drugs from properly binding and having an antibacterial effect (James, 1999). Furthermore, substituting at least one amino acid in the PBP can result in a lower affinity of the drug, and as a result the bacterial cell wall is not destroyed by the antibiotic (Gabani, et. al. 2012) (Sibanda, Okoh, 2007).

In different species of bacteria, the N^6 amino group of an adenine residue located in 23S rRNA is methylated, and as a result bacterial resistance to macrolides, lincosamides, and streptogramin B arises. The mechanism behind this resistance is associated with the reduced affinity of the binding sites that results from conformational changes from the methylation (Sibanda, Okoh, 2007).

3. Alteration of Permeability

In addition to altering the targets of antibiotics, drug resistant bacteria can modify the permeability of their cell wall in order to prevent or decrease the entrance of various drugs into the cell. A drug's concentration in the bacterial cell determines the efficacy of the drug on the pathogen, and it is through both porins and efflux pumps that drug resistant bacteria have the ability to decrease the amount of drug that reaches the cell (Lange, et.al. 2007) (James, 1999) (Sheldon Jr., 2005).

Porins

Porins are protein channels that exist solely in the outer membranes of gram negative bacteria. These channels are highly specific, and depending on size, shape, and charge, certain molecules can pass through to the inside of the cell. Being hydrophilic molecules, antibiotics easily enter the cell through these protein channels. Research has shown that when bacteria lose porins in their outer membrane, drug resistance emerges since less of the antibiotic can enter into the cell. For example, when the amount of OPrD porins were decreased in Pseudomonas, the imipenem class of antibiotics could not enter the bacterial cell. Similarly, resistance to imipenem and meropenem occurred after the amount of 29-kDa Porins was reduced in Acinetobacter baumannii. Multi-drug resistant bacteria like Klebsiella pneumoniae also display resistance to cephalosporins and carbapenems after losing OmpK35 and OmpK36, outer membrane proteins (Santajit, Indrawattana, 2016).

Efflux Pumps

Efflux pumps actively transport drugs out of the bacterial cell thereby decreasing the intracellular concentration of different antibiotics. There are five different categories of efflux pumps that exist in either gram positive or gram negative bacteria. The ABC, RND, MFS, SMR, and Multidrug and toxic compound extrusion family transporter efflux pumps all bind to the drug in the phospholipid bilayer, and thereafter export it out of the cell to different locations. While gram positive bacteria transporters work by pumping the drug out of the cell across the cytoplasmic membrane, gram negative efflux pumps can either extrude the antibiotic across the membrane and into the periplasmic space, or directly into the external medium. As these efflux pumps quickly extrude the antibiotics out, the concentration of antibiotics cannot accumulate to a high enough level to have an antibacterial effect (Zgurskaya, 2002) (Lomovskaya, Watkins, 2001).

4. Altered Metabolic Pathway

Resistant bacteria have come up with alternate routes to obtain metabolic products that are blocked by antibiotics. The folic acid pathway produces pyridine thymidylate, an essential molecule in the synthesis of DNA. In order to circumvent antibiotics that target the folate pathway, Enterococcus either uses folinic acid from its host cell, or mutates to have the ability to produce thymine (Mambrio-Jones, Hoek, 2010) (Sheldon Jr., 2005).

Discussion:

C. Combating Drug Resistance

According to statistics, it is predicted that an estimated 10 million people will die from drug-resistant bacterial infections by 2050 if no viable solution is discovered (Press Association, 2017). Therefore, finding effective treatments for drug resistant bacteria is of extreme importance. Over the last few years, much research has been done to determine proper treatments for such deadly infections. This paper will discuss the effectiveness of silver nanoparticles against drug resistant bacteria.

1. Silver Nanoparticles

Silver is widely known for its antimicrobial properties, and therefore, silver nanoparticles (Ag NPs) have been widely studied as a potential antibacterial agent against drug-resistant bacteria. Various studies on different strains of bacteria were performed to uncover the antibacterial effects of Ag NPs. More specifically, the studies focused on the size, dose, and shape of these silver particles coupled with the potential toxicity they pose to human cells.

There are various methods to create nanoparticles. Ag NPs can be synthesized into different shapes and sizes via physical processes like laser ablation, evaporation, and condensation, or through chemical processes like hydrazine, sodium borohydride, and green synthesis (Nurani, et.al. 2015).

1. Mode of Action of Silver Nanoparticles

After determining the bactericidal properties of silver nanoparticles, the mechanism behind their antimicrobial effects was studied. Various studies using transmission electron microscopy (TEM) and other methods verified that Ag NPs have the ability to cause damage to bacterial cells through a wide array of different mechanisms.

A Kirby-Bauer sensitivity test was performed, and by using various antibiotic discs with different silver resistant bacterial strains, the zone of inhibition was measured. It was found that the bacteria showed modified susceptibility to cephalosporins, glycopeptides, aminoglycosides, and fluoroquinolones, thereby indicating the mechanism of action of Ag NPs. Based on the action of these antibiotics and the bacteria's altered zone of inhibitions, this study showed that these particles interact with the cell wall, proteins, and DNA (Lara et. al. 2010).

Because of their small size, silver nanoparticles have the ability to attach to the surface of the cell membrane and disrupt its function. The positive charge of the Ag NPs allows them to electrostatically interact with the negatively charged membrane (Lara, et.al. 2011). As transmission electron microscopy has shown with E. coli cell membranes, silver nanoparticles disrupt the cell membrane, increase the cell's permeability, and ultimately cause cell death (Dakal, et. al. 2016) (Mambrio-Jones, Hoek, 2010).

Furthermore, silver has a high affinity for sulfur and phosphorus (Nurani, et.al. 2015). Therefore, Ag NPs can also adsorb to the cell membrane by interacting with thiol (SH) groups on the cell membrane. As a result of this interaction, a new bond between sulfur and silver (S-Ag) is formed, and the creation of this new bond blocks both respiration and the electron transfer. Ultimately, this results in the collapse of the proton motive force. Without the proton motive force, the cell membrane

is disrupted and cell leakage followed by cell death ensues (Mijnendonckx, et. al. 2013).

Moreover, after weakening the cell membrane, these particles have the ability to penetrate the bacteria (Lara, et.al. 2010) (Mijnendonckx, et.al. 2013). Additionally, in gram negative bacteria, porins help facilitate the entry of Ag NPs into the cell. Once inside, these Ag NPs interact with various molecules resulting in further cell damage (Dakal, et. al. 2016).

Ag NPs have a significant effect on DNA replication. Silver particles interact with thiol groups resulting in conformational changes that ultimately inhibit the activity of various enzymes (Mijnendonckx, et.al. 2013). Furthermore, when silver particles bind to the guanine base, pyrimidine dimerization occurs, and DNA replication is inhibited. Silver particles can also affect protein production and translation by interacting with and subsequently denaturing ribosomes (Dakal, et.al. 2016).

Moreover, silver nanoparticles are oxidized into silver ions upon entering the bacterial cell. Ag ions cause damage and cell death by interacting with lipids, proteins, and DNA. Silver ions also interact with nucleosides in addition to forming complexes with nucleic acids. Furthermore, these ions bind and dimerize DNA and RNA, block the expression of proteins and enzymes involved in ATP production, and generate free radicals (Lara, et.al. 2010) (Mijnendonckx, et.al. 2013).

Reactive oxygen species can be endogenously produced from natural metabolic processes like aerobic respiration. Metal ions, like silver, have the ability to catalyze the generation of free radicals in the presence of oxygen (Mambrio-Jones,Hoek, 2010), and spin resonance measurements have shown that silver ions increase the production of reactive oxygen species (Mijnendonckx, et.al. 2013) (Mambrio-Jones, Hoek, 2010) (Kim, et. al. 2007). These free radicals then act upon the mitochondrial membranes to induce necrosis and cell death. Furthermore, ROS oxidize lipids, nucleic acids, and proteins, thus disrupting the level of homeostasis by inducing oxidative stress and cell damage.

In addition to increasing the production of ROS, silver particles also decrease the levels of glutathione, an antioxidant, by reducing it into glutathione disulfide. Moreover, NPs inhibit the action of NADPH-dependent flavoenzyme, catalase, glutathione peroxidase, and superoxide dismutase, anti-oxidative enzymes that quench free radicals. These enzymes are dependent on thiol groups, but since silver ions interact with thiol groups, these enzymes cannot properly quench the reactive oxygen species (Mijnendonckx, et.al. 2013) (Dakal, et.al. 2016).

Phosphorylation and dephosphorylation are important signaling processes in bacterial growth. Phosphorylated proteins guide DNA replication and recombination, metabolism, and bacterial cell cycle. Silver nanoparticles block this signaling pathway to prevent the production and action of phosphorylated proteins. Furthermore, phosphorylated tyrosine kinases aid in the transport of exopolysaccharide and capsular polysaccharides. Therefore, to prevent bacterial growth, Ag NPs can also dephosphorylate these tyrosine residues (Dakal, et. al. 2016) (Shirvastava, et.al. 2007).

Effects of Silver Nanoparticles on Gram Positive and Gram Negative Bacteria

A great deal of controversy exists regarding the efficacy of silver nanoparticles in both gram negative and gram positive bacteria. In some studies, the Ag NPs had an equal effect in both types of bacteria, rendering silver as a potential broad antibacterial agent (Lara, et. al. 2010). However, other studies found that these particles are more effective in gram negative bacteria. This phenomenon is attributed to the differences in the composition of the cell wall in both gram positive and gram negative bacteria. The peptidoglycan in gram positive bacteria is very thick, averaging around 20-80 nm, and rigidly cross linked. Because of this thick, rigid membrane, there are fewer sites for the silver to adhere to, making it harder for the particles to adsorb and penetrate the cell. On the other hand, the cell wall of gram negative bacteria is composed of a much thinner, roughly 7-8 nm, and weaker PG. In addition, the membrane of gram negative bacteria contains the lipopolysaccharide (LPS). Because of its negative charge, the LPS increases the silver nanoparticles' ability to successfully bind to the cell's surface. The thinner and weaker PG coupled with the negatively charged LPS makes gram negative bacteria more susceptible to silver nanoparticles (Feng, et.al. 2000) (Shirvastava, et.al. 2007) (Dakal. et.al. 2016).

2. Size of Silver Nanoparticles

Particle size, shape, and dose all contribute to the efficacy and toxicity of silver particles. Extensive research has been done in attempt to uncover the antibacterial effects of different sized Ag NPs. Using the disk diffusion method, one study compared the efficacy of different sized nanoparticles against both gram positive and gram negative bacteria. The relationship between size and efficacy of Ag NPs has been extensively researched, and it was concluded that the two are inversely related; the smaller the diameter of the particle, the more effective it is against drug-resistant bacteria. The efficacy of smaller nanoparticles compared to larger NPs can be attributed to the relationship between surface area and size. Surface area and particle size is inversely related; the smaller the particles are, the larger their surface area is (Rai, et. al. 2014). As a result of the increased surface area, the smaller particles can interact with the bacterial cell more efficiently (Rai, et.al. 2009) (Haider, Kang, 2015).

3. Dose of Silver Nanoparticles

After determining the mode of action of Ag NPs, researchers began to study the dose required to have a bactericidal effect. By using various doses, 0.0, 6.25, 12.5, 25.0, and 50.0 mM, against different strains of bacteria, it was found that dose and antibacterial effect were directly related; the higher the dose of the Ag NPs,

the more effective they were. The highest dose, 50.0 mM, was the most successful at killing bacteria (Lara, et.al. 2010). Furthermore, the dose required to have a combative effect was found to be bacteria specific for each genus (Morones, et. al. 2005).

4. Shape of Silver Nanoparticles

In addition to amount and size, shape also plays a role in the efficacy of the silver nanoparticles against drug-resistant bacteria. Facets are flat surfaces that are present on geometric shapes. Because of their large densities, facets have a higher surface reactivity, and therefore Ag NPs with more {111} facets, facets that are cut through the x, y, and z planes, have a greater effect against bacteria (Mambrio-Jones, Hoek, 2010). Some research has shown that triangular shaped particles are more effective than both sphere and rod shaped particles at combating drug resistant bacteria. The increased surface area and antibacterial effect of triangle shaped particles can be due to their geometric structure which contains numerous facets (Mambrio-Jones, Hoek, 2010) (Raza, et.al. 2016) (Pal, et.al. 2007) (Mijnendonckx, et.al. 2013).

However, other research found contradictory results. Using the disk diffusion method, the zone of inhibition of Pseudomonas aeruginosa was measured with the use of five different sized and shaped particles: S1, S2, S3, S4, and S5, was measured. While S1, S2, S3, S5 were all sphere shaped varying in size, S4 was triangular shaped. After placing the particles in the center of the sampled bacteria on an agar plate, the zone of inhibition of S2, the smallest sphere particle, was 1.5 mm, while the zone of inhibition for the triangular shaped particles was 1.4 mm. This demonstrated that the smallest sphere particle had a greater effect than the triangular shaped one (Raza, et.al. 2016). Using x-ray diffraction, it was determined that the smaller spherical shaped particles also contained high atomic density facets that are found in triangular shaped Ag NPs which evidently contributed to the sphere's greater bactericidal effect. Furthermore, the sphere shaped Ag NPs acted as acids and interacted with bases, sulfur and phosphorus containing compounds, to cause damage to both the cell membrane and DNA (Raza, et.al. 2016).

5. Toxicity of Silver Nanoparticles

The toxicity of any antimicrobial agent is a major issue of concern, and despite silver particles' potential to act as an antimicrobial agent, at certain levels, silver nanoparticles can be toxic to all mammals, including humans. Research has shown that Ag NPs stimulate an immune response and induce apoptosis via the JNK and ROS signaling pathways. As indicated by the levels of glutathione, silver particles induce oxidative stress by decreasing the amount of the glutathione in the body. Additionally, due to their high surface area, Ag NPs can also generate free radicals from the respiratory chain. This imbalance between ROS and antioxidant levels results in damage to both lipids and proteins (Arora et.al. 2008) (Mambrio-Jones, Hoek, 2010). Silver NPs also interact with the mitochondria to increase oxidative stress and to disrupt ATP production, ultimately causing damage to DNA. Ag NPs also affects protein folding. This increased stress in the cell leads to cytotoxicity and apoptosis. Furthermore, Ag NPs can enter the nucleus and induce genotoxicity by causing DNA mutations, base damages, and strand breaks. Finally, these particles induce carcinogenesis by activating different signaling cascades and inflammatory responses (Dakal, et.al 2016).

In a few studies, it was found that the dose of Ag NPs needed to induce apoptosis was different from the dose required to stimulate necrosis, 0.78-1.56 vs. 12.5 µg. Therefore, it was concluded that a safe dosing range of silver can be determined (Mambrio-Jones, Hoek, 2010). However, a contradictory conclusion was reached by AshaRani et. al who found silver nanoparticles can be toxic to humans at any dose (AshaRani et.al. 2009) (Mambrio-Jones, Hoek, 2010). Therefore, due to this discrepancy, further research in regards to the toxicity of Ag NPs must be conducted.

6. Resistance to Silver Nanoparticles

As is the case with antibiotics, bacterial resistance to silver is a major concern. When bacteria are exposed to silver nanoparticles, it results in the natural selection of bacteria, thus causing bacterial resistance to silver particles.

Bacterial resistance to silver NPs can be encoded in the plasmid or in the chromosome as it is seen in both Salmonella and E. coli respectively (Mambrio-Jones, Hoek, 2010). In Salmonella, the resistance to silver ions is attributed to nine genes on the plasmid. Furthermore, resistance is also associated with the SilCBA and SilP efflux pumps, the SilE and SilF periplasmic binding proteins, and in Escherichia coli porin loss. However, resistance to silver nanoparticles as a result of a one point mutation is not very common because of the complexity of silver's actions (Chopra, 2007).

Additionally, since silver nanoparticles have a high surface area, they can aggregate and combine together, causing them to lose their antibacterial effect (Nurani, et.al. 2015) (Beyth, et.al. 2015). Furthermore, because of its high surface energy, Ag NPs can become contaminated by the air, and therefore Ag NPs are synthesized with either chitosan, alginate, or gelatin, biodegradable polymer matrixes, to prevent their oxidation (Nurani, et.al. 2015).

7. Combination of antibiotics and AG NPs

The synergy of two drugs or compounds has the potential to successfully combat drug-resistant bacteria by acting through different mechanisms. Therefore, after determining the bactericidal effect of silver nanoparticles, many researchers attempted to determine the effects of the use of Ag NPs in conjunction with the use of antibiotics against different strains of bacteria.
To determine the synergy of Ag NPs with drugs, silver particles were used with amoxicillin against samples of E. coli. By comparing the minimum inhibitory concentration of various doses of silver nanoparticles alone, the use of different amounts of amoxicillin by itself, in addition to the combined effects of both silver NPs and amoxicillin, the advantages of combination therapy was identified.

The combined effect of amoxicillin and Ag NPs was seen to have a greater bactericidal effect than each of them alone. Furthermore, when the two were administered together, a lower dose of both amoxicillin and silver nanoparticles were needed to stimulate an antibacterial effect as opposed to when each one was given alone (Allahverdiyev, et. al. 2011) (Li, et. al 2005).

The success for this synergy can be attributed to a few different theories. With combination therapy, the two compounds or drugs that are used usually target different steps, pathways, or enzymes. Therefore, if bacteria resist the action of one antibiotic, the other compound can still exert its antimicrobial effects through a different non-resistant mechanism.

Additionally, in non-resistant bacteria, the synergy of the two can be attributed to the chelation reaction that occurs between both the hydroxy and amino groups of the B-lactam and the Ag NPs. The binding between the silver and the amoxicillin in addition to the binding between the antibiotic with other drug particles resulted in the formation of a new compound. This newly synthesized antimicrobial agent, containing silver on the inside and amoxicillin on the outside, attaches to the surface of the cell membrane and causes more damage because of the synergy of the two compounds. Additionally, the amoxicillin disrupted the cell wall which increased the penetration of the Ag NPs into the cell. Moreover, from this chelation reaction, silver nanoparticles prevent DNA from unwinding, thus resulting in further damage to bacterial DNA (Li, et. al. 2005) (Allahverdiyev, et. al. 2011).

Furthermore, silver nanoparticles can be used as a drug carrier. While antibiotics are usually hydrophilic, silver nanoparticles are hydrophobic. Therefore, these nanoparticles can interact with the hydrophobic bacterial cell membrane more easily than antibiotics, enabling the transport of hydrophilic antibiotics to the bacterial cell surface (Li, et. al. 2005).

Conclusions

This paper attempted to explain the mechanism of action and mechanism of resistance of antibiotics and drug resistant bacteria, respectively. As antibiotic resistance continues to emerge, researchers are constantly searching for new antimicrobial agents. Silver nanoparticles have gained much attention as a possible tool in combating drug resistant bacteria, and studies have proven the efficacy of Ag NPs to induce cell damage and cell death. However, despite the growing potential of silver nanoparticles,

further research must be conducted before implementing silver nanoparticles into clinical trials as a promising way of replacing or supplementing the currently used antibiotics.

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