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# Metal ions as antibacterial agents

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## ABSTRACT

Metals like mercury, arsenic, copper and silver have been used in various forms as antimicrobials for thousands of years. The use of metals in treatment was mentioned in Ebers Papyrus (1500BC); i.e, copper to decrease inflammation & iron to overcome anemia. Copper has been registered at the U.S. Environmental Protection Agency as the earliest solid antimicrobial material. Copper is used for the treatment of different E. coli, MRSA, Pseudomonas infections. Advantage of use of silver is it has low toxicity to human's cells than bacteria.It is less susceptible to gram +ve bacteria than gram –bacteria due to its thicker cell wall. Zinc is found to be active against Streptococcus pneumonia, Campylobacter jejuni. Silver & zinc act against vibrio cholera & enterotoxic E. coli. The use of metals as antibacterial got reduce with discovery of antibiotics in twentieth century, immediately after that antibiotic resistance was seen due to transfer of antibiotic resistance genes by plasmids also known as Resistance Transfer Factors or R-factors. Metal complexes are used to show synergistic activity against bacteria's like copper & chlorhexidine on dental plaque bacteria, silver nanoparticles & cephalexin against E. coli & S. aureus.

**Keywords:** Metals, Oligodynamic effect, Copper, Silver

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## 1. INTRODUCTION

The crust of the earth & ecosphere is rich in metals. Due to the Great Oxidation Event (GOE) which occurred 2.3–2.4 billion years back, there was broad exposure of metal ions to the bacteria. Due to increase in oxygen content in atmosphere various oxidized forms of metal compounds are found in & around the earth crust. Metals like copper, iron, zinc were used for redox reactions by enzymes. Metals are vital for life process but poisonous at elevated intracellular concentrations hence cells need homeostasis mechanism to maintain intracellular concentration. Zinc & copper are closely related by mechanism of pathogen killing in eukaryotes, where oxidative stress is used to kill the engulfed microbe. Metals like gold, silver & mercury are very toxic at low concentration to bacteria.[1]

Metals were used as antimicrobial agents several years ago, use of metals in industries can cause damage to ecological system whereas its medical use is highly beneficial. Mercury, silver, copper, zinc, and arsenic were used to cure infections. Antimony and arsenic are used as pesticides, rodenticides, fungicides, growth enhancers & are also used for protozoal infections. Copper salts is used for preparing Bordeaux & Burgundy mixture to control fungal and bacterial diseases in plants & are used as growth enhancers in animals where as zinc salts can be used to cure diarrhoea. Silver is used to relief from burns. Organic mercury compounds are used to maintain integrity of eye drops. Mercury was used as disinfectant & to cure from syphilis infection. Admixture of mercury with copper, silver, tin is used in dental restoration.[2,3]

## 2. OLIGODYNAMIC EFFECT

Karl Wilhelm von Nägeli discovered the biocidal nature of heavy metals know as oligodynamic effect. Metals can show the biocidal action even at low concentration.

According to oligodynamic effect metals bind to thiol or amine moiety of cellular proteins & lead to deactivation, precipitation of proteins. Because of high attraction of metal ions by proteins it leads to increase in cellular concentrations & cell death. Microbes can develop resistance to this mechanism & it can be mediated by plasmids. Correspondingly silver reacts with sulfhydryl groups & form silver sulphides which deactivates proteins, enzymes functions & alters cell membranes. Copper ion binds to negative charge moiety & deforms the cell wall & helps in the entry of silver ion inside the cell which further associate with RNA, DNA, proteins & enzymes leading to cell injury & death. Silver act as an antiseptic at lower concentration i.e. thousand times lower than toxic concentration to humans.

***Copper***

1. Storing of water in brass vessels has antibacterial application i.e. container releases minimum quantity of copper ions into water & has capability of killing 1 million bacteria per ml.
2. Combination of $Cuso_4$ & lime has antihelminthic & antifungal applications**.**
3. Copper sulfate is used mainly to kill green algae in ponds, & reservoirs to avoid barnacle growth on boat bottoms they are painted with paint consisting of copper

***Aluminium***

1. Burow's solution containing aluminium acetate is an astringent & antiseptic.
2. Aluminium salts like aluminium chlorohydrate, & aluminium-zirconium-glycine (AZG) are used as antiperspirant, they form plugs on the surface of sweat ducts & decrease the perspiration.

***Antimony***

Paints, & plastics, containing esters of diarylstibinic acids are used as fungicides & bactericides

***Arsenic***

1. Syphilis was treated using arsenic,
2. Also used in rat poisons, weed killers, and pesticides.
3. Cases of murder due to arsenic poisoning is mentioned in history

***Bismuth***

1. Used as astringent, disinfecting & bacteriostatic agent.
2. Bismuth subgallate in dermal powders has antifungal application. Earlier bismuth was used to treat malaria & syphilis.

***Gold***

Gold is used in dental fillings & has bacteriostatic application.

***Lead***

1. Different forms of lead are used to treat various diseases i.e from constipation to communicable diseases like plague.
2. Lead arsenate as insecticides and herbicides.
3. Thiomethyl triphenyllead is used as fungicide & cotton preservative;
4. Tributyl lead acetate and ; tributyl lead imidazole used as cotton preservative

***Mercury***

1. Phenyl mercuric borate and acetate solutions i.e. 0.07% in aqueous solutions were used to clean mucous membranes. Phenylmercury salts are no longer used due to toxicity issue.
2. Dental amalgam used in fillings has bacteriostatic activity
3. Thimerosal is used as topical disinfectant & preservative.
4. Used in the treatment of syphilis.
5. Also used as insecticides and fungicides.

***Nickel***

Nickel toxicity varies in bacteria fungi, yeasts, and differs considerably.

***Silver***

1. Silver & less soluble silver compounds (silver chloride) interfere with metabolism of organism at lower concentration of 0.01–0.1 mg/L & function as germicides or bactericides
2. Atmospheric oxygen imparts bactericidal effect to metallic silver by forming silver oxide
3. Use of colloidal silver decreases bactericidal concentrations since it has higher surface area
4. Silver vessels were used to prevent contamination of drinking water
5. Silver coins or foils were placed on wounds for recovery
6. Silver sulfadiazine ointment is used for widespread burns.
7. Dispersion containing equal concentration of colloidal silver & dissolved silver ions can be used to purify drinking water at sea.
8. Implants and catheters are incorporated with silver.
9. Silver iodide is used in disinfection of surfaces.
10. Silver-impregnated wound dressings are active against antibiotic-resistant bacteria.
11. Silver nitrate has wide application as a hemostatic, antiseptic and astringent agent.
12. Use silver nitrate to protect the new born from gonorrheal neonatal ophthalmia
13. Silver has wide application in our day to day life such as it used in refrigerator doors, food containers to keep food fresh & to reduce the odors

***Thallium***

1. Thallium sulphate is used for protection of fabrics from moths, bacterial & fungal spores.
2. Thallium sulphate has been used as a depilatory agent and in the treatment of topical fungal infections, venereal disease, and tuberculosis.

***Tin***

1. Tetrabutyltin is used in paints to impart antifouling effect & also used in water recirculating systems to protect it from contamination with slime
2. Used as disinfectant & also imparts protection to textiles & wood
3. Tricyclohexyltin hydroxide is an acaricide.
4. Triphenyltin acetate & Triphenyltin hydroxide are fungicides.

***Zinc***

1. Zinc oxide is used in lotion and sunscreen
2. Zinc chloride is used in mouthwashes and deodorants
3. Zinc pyrithione has antidandruff application.
4. Zinc sulfate & Zinc iodide function as topical antiseptics.
5. Also used as algicide & mold-growth inhibitor. [4]

## 3. TRANSITION METALS

Most of the transition metals (Cu, Ag, and Au) & post transition metals (Zn, Cd, Hg) possess antimicrobial properties. They belong to d-block of periodic table. They are classified as transition elements due to partly filled d sub-shell. The following are properties of transition elements which are not found among elements of other blocks.

1. Forms coloured compounds because of *d–d* electronic transitions
2. Forms compounds with more oxidation states because of less energy difference among various oxidation states
3. Forms paramagnetic compounds because of unpaired *d* electrons. Some of representative elements also form paramagnetic compounds. E.g. oxygen

***Coloured Compounds***

Transition-metal compounds are coloured because of 2 electronic transitions

❖ *Ligand-to-metal charge-transfer (LMCT) transition*

It involves transfer of electrons from the orbit of ligand to the metal orbit. Metals with high oxidation can undergo this type of transition

Eg: Red colour of mercuric iodide is due to LMCT transition. Chromate, dichromate and permanganate are coloured to LMCT transition

❖ *Metal-to-ligand charge transfer (MLCT) transition*

It involves transfer of electrons from the orbit of metal to the ligand orbit. Metals with low oxidation can undergo this type of transition

***Oxidation States***

Transition metals forms compounds with many oxidation states which differ from each other by one example, vanadium compounds are with oxidation states from -1 [V(CO)6] , to +5 VO3−4. Representative elements of groups 13 to 18 also forms compounds with various oxidation states. The major distinction in oxidation states of transition and other elements is it contains single atom of the element & unpaired electrons.

***Physical Properties***

Transition metals possess have high density , boiling points & melting points , whereas group 12 elements possess very low, boiling points & melting points because they cannot undergo d-d bonding due to filled d subshells .Mercury is liquid at room temperature & its melting point is −38.83 °C.

***Catalytic Properties***

Transition metals possess multiple oxidation states & forms complexes easily hence due to this property it has homogeneous and heterogeneous catalytic activity and their compounds are known for their homogeneous and heterogeneous catalytic activity.

Ex: Vanadium oxide in contact process,

Nickel in catalytic hydrogenation & Iron in Haber process.

Catalyst developed from nano material forms bonds between reactant molecules of solid surface & atoms on the surface of catalyst thereby decreasing the activation energy (weaking of bonds) & increasing the concentration of reacting molecules on the surface of catalyst. Transition metals are more efficient as catalysts because they can change their oxidation states.[5]

### 3.1 Copper

Copper is one of the oldest metals. The commencement of smelting, casting, and forging of copper is reported around 5000 BC, but, smelting of copper from the complex ores with arsenic (As) and lead (Pb), is reported around 1000 years after it , bronze was obtain by fusing copper with tin around 2500-2200 BC.

The first recorded use of copper as the bactericidal agent was mentioned in the Smith's Papyrus, which is the oldest medical document in history of mankind, it includes texts from 3200 BC.[6] Copper and its compounds were suggested as medication for epilepsy (wobbling of the limbs), treatment of burns, skin diseases and soft warts on the neck. Various forms of copper like chips, splinter, its salts & oxide was used for treatment of various diseases. Malachite is a mineral consisting of copper carbonate hydroxide also known as green pigment & used in paints ,where as chrysocolla is a mineral containing hydrated copper phyllosilicate.

During the Ancient Greek civilization copper was easily available & hence it became important medicine. It was mentioned in Corpus Hippocraticum that it is used for treatment of ulcers with varices; Corpus Hippocraticum is a compilation of Ancient Greek medical works assembled by the Alexandrian scholars in III century BC. Mixture of a dry powder from cupric oxide and copper sulphate was suggested to prevent infection from wounds. Mixture of honey & red copper oxide was suggested as an antiseptic for wounds.

Scientific research has established that copper may suppress the pathogens which are harmful to health & lives of people. Ex: methicillin-resistant Staphylococcus aureus (*MRSA*), Clostridium difficile, Escherichia coli, legionella, infection with Clostridium difficile mostly occurs at hospitals*.* Legionellosis is characterized by pneumonia caused by legionella bacteria. Copper also contains properties that can destroy the type A flu viruses. Hence it can be used as an agent for suppressing avian flu epidemy.

About 80% of the communicable diseases are spread by contact. The furniture, medical instruments used in hospitals are made of aluminium and stainless steel, which gives good impression of cleanliness & maintenance but they are basis for many harmful and lethal bacteria and viruses infections caused by methicillin-resistant Staphylococcus aureus (MRSA, Escherichia coli, Klebsiella pneumoniae, and Clostridium difficile are the most frequent infections in hospitals. These germs causing infections are due to infections of the urinary tract (25%), lower airways (23%), surgical wounds (11%), skin infections (10%), and blood flow disturbances (6%).

These Germs are carried on surfaces & in the atmosphere of the hospital for weeks or even months, where the staff, patients and visitors get in contact with the contaminated surfaces & get affected with diseases. Therefore, items like door handles light switches, trolleys, tables, beds, bed side cabinets, stairs, handrails, etc should be made from copper and its alloys in order to avoid such infections. Cloths of patients, service staff, and bed-clothes should be made from copper containing fibre to get optimal protection from these infections.

About 10 million Staphylococcus aureus germs die within 90 minutes on 1 $cm^2$ of pure copper surface. In Great Britain about 1 billion pounds were invested for the treatment of hospital-acquired infections & about 5000 patients die because of complications. Approximately15% of infections may reduce yearly by utilizing antibacterial properties of copper & there by saving about 150 million pounds a year. [7]

- **Bactericidal activity of copper surfaces**

Various bacteria , fungi, viruses are killed by copper by means of contact killing , an estimated amount include 90 types of Bacteria, 30 species of fungi, and 20 different viruses, and it can be assumed that all bacteria and viruses are susceptible to contact killing. Various possible mechanisms on copper bactericidal activity have been published. Four toxicity mechanisms in contact killing are:

1. Damage to the cell membranes i.e. outer & inner membrane
2. Generation of reactive oxygen species & causing oxidative damage
3. Inhibiting activity of essential enzymes, and
4. Breakdown of deoxyribo nucleic acid (DNA). [8,9]

Depending on the experimental system and the organism under study there will be difference in the key toxic principle. The first important aspect in contact killing is release of copper ions from the surface & the second is its contact with the bacterial surface thereby causing severe damage to the bacterial membranes.[10]

Various techniques have been used to determine the damage to the cell covering in various organisms like direct microscopic examination of cells, , staining of respiring cells, staining of cells with redox dyes or by viewing membrane depolarization. Enterococcus hirae was kept in contact with copper surface for prolong period & "Live/Dead" staining technique was used to determine the results. Staining technique uses green fluorescent SYTO9 DNA dye & it stains all cells with green colour as it is membrane permeable, whereas red fluorescent propidium iodide dye enters only damaged cells & it stains the green cellular DNA with red colour there by differentiating the damaged cell from the normal cell.[11,12]

Damage of E. coli cell membrane up on exposure to copper surface is explained by proteomic profiling: where proteins involved in synthesis of capsule polysaccharide & cell envelope are upregulated. Subsequently, there is huge influx of copper ions into the bacterial cytoplasm. Also, these damaged E. coli cells are not capable of synthesizing glutathione, which serves as major defense mechanism against toxic heavy metal ions. E.Coli cells are more susceptible to contact killing than wild-type cells, Esp_ırito Santo et al. measured the cellular copper content in Staphylococcus using a cytoplasmic copper sensor, the amount of copper ions per cell after 5min contact with dry copper surface is $2.6 \times 10^{10}$.[13] Copper chelators such as, bathocuproine disulfonate, bicinchoninic acid or ethylenediamine tetra acetic acid extensively inhibit the process of contact killing by forming complex with copper ions.[14]

Contact killing by copper surfaces is not a simple phenomenon; it is influenced by metal surface, structure, bacteria–metal contact, corrosion phenomena, nobleness of alloying metals, the bacterial species under study. Surface roughness influences release of ions, rough surfaces release more copper ions each time than polished surfaces and it has more antibacterial activity than polished surfaces. Undoubtedly, the key toxicity mechanisms of copper and its alloys is the release of copper ions into the aqueous phase; & even in "dry" applications of bacteria to copper surfaces, there will be an aqueous phase adjacent to the bacteria. Bacteria–metal contact has key role in contact killing. bactericidal activity of copper got reduced when the metal & E. hirae are separated by inert grid ,eventhough there was no affect on release of ions from metal surface.[15]

Recent study has found that $Cu^{+}$is significantly more toxic to bacteria than $Cu^{2+}$. cupric ion react with atmospheric oxygen & get converts to cuprous ion , it also undergoes Fenton-type reaction involving generation of free radicals, particularly in lactic acid bacteria huge amount of ROS is produced i.e. hydrogen peroxide , these ROS lead to permanent cell damage by various mechanism such as by retarding cellular respiration, lipid peroxidation, & damage to the cellular proteins by oxidation. ROS production was confirmed in E. coli and Salmonella when exposed to solid copper, but to what extent ROS production is essential for contact killing remains an issue of discussion.ROS quenchers were found to hinder contact killing to some level; however killing is not impaired by anaerobic conditions which will not favour ROS production.[16]

Related to copper toxicity mechanisms in bacteria, there is difference between cells in culture and cells which are exposed to solid copper. In culture, cells are in a growing state when they are exposed to copper surface they cannot grow further, Moreover less amounts of copper enter the cell & displaces Fe-S bonds of important enzymes required for growth & maintenance. It does not involve oxidative damage & ROS production. Toxicity due to displacement of Fe- S clusters was confirmed for Ag (I), Hg (II), Cd (II), and Zn (II) at minimum concentration which is required to inhibit the cell growth, along with this toxicity of metal ions is directly proportional to their thiophilicity. Incase of inactivation of viruses various mechanism are involved, where capsid & and nucleic acid damage by copper is confirmed. This will not be dealt further as our aim is speak about bacteria.

In contact killing bacteria are starved for nutrients and are killed in the absence of growth. The most common steps in contact killing are as follows: initially there is severe damage to the cell and the cytoplasmic membranes leading to huge influx of copper ions into the cell and causing damage to DNA & intracellular components by multiple mechanisms. The significance of each toxicity mechanism and the series of events involved may vary between species and between eukaryotes, prokaryotes, and viruses. Toxicity mechanism will also vary in growing cultures & in contact killing. Similarly mechanism will also vary for biofilms, which are formed on surfaces for prolonged time and require growth and attachment of the cells to the substrate.[17]

- **Properties for metal surface to be antibacterial**

***(i) Thiophilicity and HSAB \Toxicity***

According to Pearson "hard and soft acid and base" (HSAB) concept hard ions are the one which are having high charge density (high ratio of charge to radius)and are not polarized easily, where as soft ions are the one which are having low charge density (low ratio of charge to radius) hence they are polarized easily. A characteristic feature of Soft ionic species is that it readily form compounds with sulphur, which will promote antibacterial activity & this property is called as "thiophilicity." An effort was made here to correlate existing formation constants for metal sulfides, pKS (MeS), with thiophilicity on the basis of HSAB concept. The relationship between pKS (MeS) and hard/soft species values will vary in all cases. example, Sn is hard ionic species, but it has pKS (MeS) value of 26. $Ag^{+}$, $Cu^{+}$, $Cd^{2+,}$ and $Hg^{2}$ are classified as soft species & have low pKS(MeS) values as expected & hence they are very toxic to growing bacteria. Correspondingly, $Cu^{2+}$ which is assigned as intermediate character on the HSAB scale is less toxic to bacteria than $Cu^{+}$, hence it has pKS(MeS) value of -36.2, whereas $cu^{+}$ has pKS(MeS) -47.6. Au, Pd, and Pt are of soft character; however these metals are "noble" and do not form soluble ions in aqueous media at ambient conditions due to its low solubility constants and high electrochemical standard potentials. on the whole, the mutual consideration of all these parameters may help in determining toxicity in culture or in contact killing. However solubility of metal ions, uptake by bacteria, and cell defense mechanisms can play a dominant role, depending on the experimental conditions. [18]

***(ii) Electrochemical effect in contact killing***

It was found that copper alloy containing 10% silver has promoted contact killing compared to pure copper, it is because of increase in release of copper ion in presence of silver. Jing et al. has confirmed in his work that copper coated with a thin silver layer has increased bactericidal activity. studies was carried carried in humid environment to see the affect of corrosion, hence it suggests that electrochemically driven mechanisms can also enhance the release of copper or silver ions, & promotes bactericidal activity.[19]

***(iii) Oxidation***

The utmost property required for a solid metal surface to be antibacterial is it must readily oxidize under ambient conditions. Both $Cu_2O$ and CuO, can be formed under conditions encountered in the biosphere. Formation of CuO, is favoured in oxidizing conditions i.e. presence of water in air, whereas $Cu_2O$ formation is favoured in reducing conditions i.e, presence of organic matter, bacteria. Oxidizing conditions & pH values below six favor dissolution of copper oxides to aqueous $Cu^{2+}$.

The mechanism of formation of oxide & its bactericidal activity can be explained in the following steps: Initially, a layer of cuprous oxide ($Cu_2O$) is formed on a dry copper surface under ambient conditions, this is followed by a next layer which is cupric oxide (CuO) & its formation takes place in humid environment for a longer period of time. Therefore, the surface may consists of either $Cu_2O$ layers or combination $Cu_2O$/CuO double layer, depending on the atmospheric conditions & oxidation time. In the presence of chloride ions, CuO formation is favoured than $Cu_2O$. So it is assumed that chloride ions from sweat promote bactericidal activity of copper but this need to be confirmed. Incase of complexation, sulfur, ammonium, cyanide, will form complex with copper & change the kinetics of reaction very drastically making it difficult predict.

In recent studies, the antibacterial activity of $Cu_2O$ versus CuO was demonstrated. It was found that $Cu_2O$ showed fast bacterial killing compared to CuO which showed much slower bacterial killing. This was explained by relating antibacterial potency with release of metal ions from the metal surface. This suggests that the release of copper ions from copper oxides is a key parameter & is directly proportional to bactericidal activity in contact killing.20

***(iv) Solubility of oxide***

The second most parameter for metal surface to be antibacterial is its oxide must be relatively soluble to release toxic metal ions. The solvation property of metal oxides is represented by pKS[MeO/Me(OH)]. AgO and $Cu_2O$ are the most soluble oxides with pKS values of -7.7 and -9 respectively. On the other hand, the solubility of CuO is much lower and is in the range of other metal oxides.

Depending on solubility of oxide, $Cu_2O$ has higher antibacterial activity than CuO. Correspondingly Cd is predicted to be a metal with good antibacterial activity: since its oxide has a relatively high solubility with pKS [MeO/Me(OH)] value of -13.6, it does not have a noble character, it is soft ionic & has a high thiophilicity, & is toxic to bacteria . To our knowledge, studies on contact killing with cadmium are not mentioned in literature, its determination will be helpful in practical application.

Under ambient conditions, Silver does not oxidize readily since it is more noble than copper. Moreover silver oxides are formed at high pH and oxidizing conditions, thereby inducing antibacterial activity. AgO has a high solubility even greater than that of $Cu_2O$ and gives off toxic Ag+ ions. This explains the outstanding antibacterial activity of silver oxide nanoparticles.

Metallic silver surfaces are less effective in contact killing when compared to copper surface. According to Japanese test procedure JIS Z 2801:2000 for effective contact killing by metallic silver, long exposure times at elevated temperature is required i.e., 24 h at 37°C & should be carried out in aqueous environments .when silver is exposed for shorter duration (1–2 h) at room temperature it is incapable of killing bacteria or viruses. [21]

**Applications of copper**

1. Use of copper pipes in hospital to limit legionella infection
2. Use of copper compounds in agriculture to limit algae infection
3. Use of copper compounds to obtain safe & drinkable water
4. Use of copper surfaces in food industry to prevent harmful bacteria & food borne illness.
5. Use of copper in intrauterine device, & dental amalgams
6. Garments for personal & equipment protection from bacteria
7. Use of antiviral gloves in hospital & paramedics
8. Used as Mattress covers for killing dust mite & to prevent mite allergies
9. Used as antifungal socks against athlete's foot
10. Wound dressings in pressure sores & diabetic ulcers
11. Used as antiviral filters in dialysis pumps, air filters & blood banks.[22]

## 3.2 Silver

Silver and its compounds have been used as antimicrobial agents for extensive period of time in the field of medicine. Silver is active at low concentrations against bacteria & it does not cause any harm to human at such lower concentration. Silver sulfadiazine is most commonly used broad-spectrum antibiotic ointment, effective against a wide range of bacteria and some yeast.

For many centuries the antimicrobial property of silver was utilized by many cultures all around the world. Silver coated bottles were used by Phoenicians to store water & prevent contamination from microbes. In 1884, it was found that newborn's were infected with gonorrhoeal disease due to transmission of Neisseria gonorrhoea from infected mothers to children's during childbirth. So it became common practice to administer drops of aqueous silver nitrate to eyes to prevent the transmission & provide immunity to new borns .

Among Ionized/ non-Ionized silver forms, only ionized form possesses antibacterial activity whereas non ionized silver is inert. Silver ion is less virulent against gram positive bacteria than gram negative bacteria because of its thicker cell wall. It has thicker cell wall due to higher amount of peptidoglycan content & it also has greater entrapment efficiency than gram negative bacteria since peptidoglycan is having negative charge & silver ion is positive charge.[23]

**Mechanism of action**

***(i) Entering the cell***

Transport of silver or its ionized form $Ag^+$ across the hydrophobic cellular membrane into the interior of cells is through transmembrane protein .silver ions get access to the cytoplasm through transmembrane proteins whose putative function is to transport ions other than silver ions. Example of Transmembrane proteins is CopB-ATPase from Enterococcus hirae have been shown to transport silver ions although its putative function is a copper transporter . This proves that silver can be transported across the cell membrane by various ways even though specific silver transporters do not exist. [24]

***(ii) Protein Inactivation***

Silver's antimicrobial properties were known for centuries, but understanding of its antimicrobial mechanism has begun recently. It is believed that silver leads to deactivation of enzymes by binding to their thiol groups, & it is also going to interfere with the transmembrane energy generation & ion transport by forming stable S-Ag bonds with thiol-containing compounds in the cell membrane

It is also thought that silver can take part in catalytic oxidation reactions & result in the formation of disulfide bonds (R-S-S-R). The silver-catalyzed formation of disulfide bonds takes place between oxygen molecules in the cell and hydrogen atoms of thiol groups resulting in water as a product and the thiol groups become covalently bonded to one another through a disulfide bond (Davies and Etris, 1997). This disulfide bond formation by silver lead to change in shape of enzymes in cell & consequently affect their function.

The silver-catalyzed formation of disulfide bonds can cause changes in structure of protein and deactivation of key enzymes, that are essential for cellular respiration (Davies and it was found that activity of proteins was decreased when the cells were treated 900 ppb $Ag^+$ solution, such as 30S ribosomal subunit protein, succinyl coenzyme A synthetase, fructose bisphosphate adolase and maltose transporter (MalK).

It is hypothesized that silver ions prevent the translation of proteins by binding to the 30S ribosomal subunit, & inactivating the ribosome complex. similarly proteins that were found to be downregulated play an important role in functioning of cell such as succinyl-coenzyme A synthetase, involved in kreb's cycle, catalyzes the conversion of succinyl-CoA to succinate while phosphorylating ADP to produce ATP,enzyme fructose bisphosphate adolase take part in glycolysis cycle, it catalyzes the breakdown of fructose-1,6-bisphosphate into glyceraldehydes 3-phosphate and dihydroxyacetone phosphate , MalK is a cytoplasmic membrane-associated protein & plays an important role in the transport of maltose . thus all of these proteins play a important role in ATP and energy production for the cell, so the decreased expression of any one of these proteins can result in cell death. [25]

***(iii) DNA Association***

The other suggested mechanisms of silver antimicrobial activity was proposed by Klueh *et al.*, (2000) according to him it ($Ag^+$) denatures the DNA molecule by entering the cell and intercalating between the purine and pyrimidine base pairs & disrupting the hydrogen bonding between the two anti-parallel strands . But this need to be proved, Fox and Modak, 1974 has shown that silver ions do intercalate with DNA once they enter the cell.[26]

**Application of silver**

***(i) Silver compounds as antimicrobial agents:***

Fox and Modak (1974) demonstrated the mechanism of silver compounds in prevention of infection due to burn wound. Silver sulfadiazine topical cream was used to prevent infections in burn wounds, but it was not known if the bactericidal effect was completely due to the silver ions or if the sulfadiazine anion also contributed to the antimicrobial activity. Tests from the study concluded that sulfadiazine does not get transported into cells as much as silver. Silver ion enters cells & complex with DNA, this was determined using Silver isotopes ($^{110}Ag^+$) . Furthermore, the rate at which certain silver containing compounds release silver ions into solution was determined by incorporating silver compounds to human serum and measuring the amount of unreacted silver compound with time. It was found that silver sulfadiazine (Ag sulfadiazine) releases slowly majority of its silver ions into solution over and prolong period of time, while silver nitrate releases immediately all of its silver ions into solution. Hence, effectiveness of silver sulfadiazine's as an bactericidal agent depends its ability to dissociate in solution. If silver is used for burn wounds, burn wound needs a constant supply of silver ions prolong period of time to kill off any microbes that could probably infect the wound until it heals. In such case sliver sulfadiazine works better than silver nitrate a it releases silver ions slowly for extended period of time, moreover Compounds that release silver ions immediately would need to be applied very regularly in order to kill off invading bacteria and prevent infection, and sometimes regular application is not always possible for individuals, so compounds that release bactericidal amount silver ions steadily , such as silver sulfadiazine, are the most efficient at preventing infections from burn wound .[26]

***(ii) Various forms of silver***

1. Use of antibacterial silver zeolite. Zeolite is a porous matrix of sodium aluminosilicate consisting of

micropores to huge amount of silver ions binds. This zeolite is placed in cationic solution, so that Silver ions are released from the matrix in exchange to cations present in the solution and the amount released is proportional to the cation concentration in the solution. It is believed that silver zeolite can be incorporated into dental materials, even in periodontal pockets which is used in anaerobic conditions. Use of Silver nanoparticles in wound dressing, In in-vitro studies silver impregnated wound dressings were found to consistently kill Pseudomonas aeruginosa cultures completely and were able to kill Staphylococcus aureus cultures with >99.99% efficiency. [27]

2. Use of Silver coated endotracheal tubes by individuals showed decreased lung colonization by Pseudomonas aeruginosa . use of silver coatings on inner side of endotracheal tube lead to delay in growth of bacteria
3. Use of masks coated with silver nanoparticle to prevent infection, & there was no skin irritation from it, there were able to reduce viable E. coli and S. aureus cells by 100% after incubation
4. AGC glass is silver coated glass & the company reports that glass is capable of killing 99.9% of bacteria that come in contact with it.
5. Silver coated plastic or paper food wraps, are used to deter microbial growth and to maintain quality of food. [28]

## 4. HEAVY METALS

These are the metals with high densities, atomic numbers or atomic weights. But there is an exception where some of the post-transition heavy metals like zinc, cadmium, and lead have some of the properties of light metals like soft nature, having lower melting points, and forming colourless complexes.

### *Physical properties of heavy metals*

1. They have higher density & tensile strength.
2. They are hard & have low thermal expansivity.
3. They have low to high melting points.

### *Chemical properties of heavy metals*

1. Sulphides & hydroxides are insoluble.
2. Forms coloured complexes.
3. Its salts give coloured solutions in water.
4. They are less reactive & less found in earth crust.
5. Mostly belong to group 3 to 16 of periodic table.
6. Consists of micronutrients like iron,cobalt,nickel,copper & zinc. [29]

### Heavy metals toxicity mechanism

Heavy metals have been used as antimicrobial agents for long period of time in history prior to the discovery of antibiotics. At present it used in surface coatings & devices which are placed internally. Recent study has shown that some metals have synergistic activity with antibiotics (Cu with polycide) & are able to kill multi-drug resistant bacteria, and can disrupt biofilms. General toxicity mechanism is given, although there will difference depending on the metal & the type of bacteria it is going to affect.[30]

#### *(i) Oxidative Stress*

In bacterial cells, iron is necessary for enzymatic activity as it serves as cofactor , but it also used by cells for reactive oxygen species (ROS) generation by Fenton reaction that cause damage to biological macromolecules . Normally in cells, only 20 μm of $Fe^{2+}$ is present for Fenton reaction, & the rest is used for coordination interactions with ligands . When extracellular Fe, Cu, or Ni enters the cell by other means, an increase in ROS and radical production is seen. Thiols will also reduce metals covalently, thereby leading to ROS generation. Antioxidants are depleted, In order to buffer the redox state within the cell and the cell becomes more susceptible to ROS from metabolism byproducts . Hence, there is correlation between cellular toxicity & metal's solubility and reduction potential . [31]

#### *(ii) Protein Activity Interference*

To make an enzyme inactive only a few amino acid alterations near the metal-binding region is required. Metal ions frequently oxidize residues resulting in formation of carbonyl groups & proteins with these groups are assigned for intracellular degradation. For example a family of Fe-S dehydratases in bacteria is very susceptible to such ROS-mediated oxidation. In addition, metal cations oxidize thiol and disulfide groups that are important for substrate binding. It has been proven that metal cations displaces wild-type cofactors in enzymes by exhibiting ionic or molecular mimicry i.e. metals are structurally similar to co-factors but physiologically troublesome. Example substitution of iron with gallium in ribonucleotide reductase, the first enzyme for DNA replication. Ga & Fe has similar ionic radius, but gallium does not have other oxidation state that is required for enzyme activity. Moreover, it also inhibits transcription of pvds (Fe-responsive transcription regulator) in Pseudomonas aeruginosa, thereby generating a positive feedback cycle that is toxic to multidrug-resistant organisms. By using this displacement/ substitution Fenton-inactive metals like Ag can release coordinated Fe into the cytoplasm and contribute to ROS generation. [32]

#### *(iii) Nutrient Assimilation Interference*

Metals can be transported across the cell by fast, unspecific, MIT transporters, or specific, ABC transporters and P-type transporters. So cations bind to these transpoters & competitively inhibit accumulation of essential ions in the cell. Example, Cr (IV) reduces intracellular S concentration, by exhibiting molecular mimicry & thereby causing deficiency in this essential bio element.[33]

#### *(iv) Membrane Impairment*

It has been hypothesized copper and cadmium can cause lipid peroxidation by increasing mutations that increase the number of unsaturated bonds in fatty acids, affecting the integrity of the cytoplasmic membrane. Another method of metal toxicity is through dissipation of the chemiosmotic force or siphoning electrons from the ETC. Silver in Vibrio cholera have been found to introduce "leakage" of protons across the membrane, while other metals are reduced by the quinone molecule and steal electrons away from respiration.

#### *(v) Genotoxicity*

ROS formation by Fenton reaction leads to cell death by inhibiting DNA replication. Genotoxicity refers to the use of mutagens like Cr(IV) ( very potent mutagen) & other cations that cause DNA damage . Moreover, copper has been linked to the damage of extracellular DNA subsequent to cell lysis, and this might inhibit post-mortem horizontal gene transfer of resistance by means of transformation .[34]

## 5. METAL COMPLEX AS ANTIMICROBIAL AGENTS

**Properties required for active metal complex**

1. Initially, a biologically active metal complex should have adequately high thermodynamic stability to transport the metal to the active site. The metal complex should be hydrolytically stable.
2. The reaction kinetics with which the metal ion undergoes association or disassociation is of great significance.
3. The molecular weight of the metal complex is also important, low molecular weight compounds with neutral charge and minimum water solubility are soluble in every medium and can pass through biological membranes by passive diffusion.

- In general, drug combinations have confirmed to be an important characteristic of antimicrobial treatment due to following reasons:

(i) They amplify activity by use of compounds with synergistic or additive activity;

(ii) They prevent drug resistance;

(iii) They reduce necessary doses consequently leading to reduction in both cost and the likelihood of toxic side effects;

(iv) Amplify the spectrum of activity

- The pharmacological activity of metal complexes is extremely dependent on the nature of the metal ions and the donor sequence of the ligands because various ligands posess various biological properties. Five main factors to be considered:

1. The chelate effect i.e., such as the quinolones containing bidentate ligands exhibit higher antimicrobial efficiency towards complexes with monodentate ligands
2. Nature of the ligands
3. Whole charge of the complex; commonly the effectiveness of antimicrobials decreases in the following order

   Cationic > Neutral > Anionic complex

4. In case of ionic complexes, the nature of the counter ion plays critical role
5. Nuclearity of the metal center in the metal complex; mononuclear centers are less active than binuclear centres.

***Examples***

Among the most commonly used antibacterial agents in the world, N-Substituted sulfonamides are one of them. They are mostly used due to its low cost, low toxicity, and outstanding bactericidal activity. Sulfonamides are significant class of medicinal compounds which are widely used as antibacterial agent. It interferes with PABA (p-amino benzoic acid) in the synthesis of tetrahydrofolic acid, which is a crucial growth factor required by bacteria to aid metabolism.In 2006 , synthesis, characterization and relative biological study of a series of antibacterial copper complexes with heterocyclic sulfonamides (L) were studied. It reported that the complexes with five-membered heterocyclic rings were more active than the free sulphonamides.

Transition metal complexes of [M(L)2] type and those containing monodentate phosphines of [M(L)2(PPh3)] type where (M= Ni, Co, Cu and Zn; L = cyclohexylamine-Ndithiocarbamate;PPh3 = triphenylphosphine) have been synthesized. Coordination of metals was determined by carrying spectral studies of all compounds & it revealed that the coordination of metals occurs through the sulphur atom of the dithiocarbamate ligand in a bidentate fashion.

In vitro antimicrobial activity of ligand and their metal complexes were carried out against bacteria - Escherichia coli, Staphylococcus aureus,Salmonella typhi, Enterococcus faecalis, Pseudomonas aeruginosa and fungi Aspergillus flavus, Aspergillus carbonarius, Aspergillus niger and Aspergillus fumigates, Where the metal complexes showed higher antimicrobial activity than the parent ligands.[35]

## 6. CONCLUSION

The use of antibiotics against bacterial infections is well & good until the bacteria does not develop resistance to it . Due to rise in MDR bacteria metal complexation (Synergistic activity of silver nanoparticles with cephalexin against E.coli & S.aureus) serves as better alternative because

1. It offer synergistic or additive activity
2. prevent drug resistant
3. Decreases necessary doses
4. Raise spectrum of activity

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