Study of Antibacterial Properties of TiO_2, ZnO and CuO nanoparticles on bacteria

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By

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Declaration by Student

I hereby declare that the data presented in this Dissertation entitled, **"Study of Antibacterial Propeties of TiO2, Zno and CuO nanoparticles on bacteria"** is based on the results of investigations carried out by me in Marine Biotechnology at the School of Biological Sciences and Biotechnology, Goa University under the Supervision of Dr. Dhermendra Kumar Tiwari and the same has not been submitted elsewhere for the award of a degree or diploma by me. Further, I understand that Goa University or its authorities will not be responsible for the correctness of observations / experimental or other findings given the dissertation. I hereby authorize the University authorities to upload this dissertation on the dissertation repository or anywhere else as the UGC regulations demand and make it available to any one as needed.

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ABBREVIATIONS

ug- Microgram

mg- Milligram

ul- Microlitre

ml- Millilitre

rpm- Revolutions per minute

NP- Nanoparticle

E. coli- *Escherischia coli*

S. aureus- *Staphylococcus aureus*

P. aeruginosa- *Pseudomonas aeruginosa*

LB- Luria Bertani

MTT- (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium bromide)

TBARS- Thiobarbituric acid reactive substance

ROS- Reactive Oxygen Species

PBS- Phosphate Buffer Saline

RT- Room Temperature

OD- Optical Density

DMSO- Dimethyl Sulfoxide

SDS- Sodium Dodecyl Sulphate

CHAPTER 1

ABSTRACT , INTRODUCTION AND REVIEW OF LITERATURE

1.1 ABSTRACT:

With the advent of new and innovative technologies, studying the underlying mechanisms of diseases and molecular designing of novel drugs, it has become somewhat easy to tackle the diseases and their adverse effects. However, infectious diseases continue to pose a threat as a worldwide challenge. The main drawbacks of the conventional antibiotics and antimicrobial agents are multi-drug resistance and their adverse effects leading to the formation of "superbug". Drug resistance not only enforces high antibiotic dose administration but also results in toxicity, requires production of new antibiotic agents which ultimately requires significant time , labor and capital investments. Therefore, development of some non-traditional antimicrobial agents which might overcome the resistance produced by various pathogenic microorganisms against most of the commonly used antibiotics is gaining interest of the scientists. One of the most potent such antimicrobial agents include several nanoparticles which have proven their effectiveness in treatment against these resistant microbes. Nanoparticles not only combat antibiotic resistance but also act as medium of antibiotics. This review summarizes the use of certain nanoparticles as a potential treatment against certain infectious disease causing pathogenic targets.

1.2 INTRODUCTION:

The increasing resistance of microbial pathogens towards antibiotics has resulted in serious health issues in recent times.Bacteria have several mechanisms to maintain this resistance via prevention of drug penetration, changes in the target, enzymatic inactivation of drugs, and excretion of antibiotics from the cells.(Kumar et al.,2005, 26) (Blair JM et al., 2014,27) With the applications of modern science technologies such as Nanotechnology and intrinsic antimicrobial activity of metals and their oxides are becoming the new potential treatment against resistant pathogens. However, proper studies need to be done in order to accept metals and their oxides nanoparticles as alternatives to current antibiotics. (Dizaj et al., 2014, 1) .

Antimicrobial activities of various metals like Silver (Ag), Copper (cu), Gold (Au), Titanium (Ti), Zinc(Zn) each having their intrinsic antibiotic properties and characteristics have been known for centuries now. (ude.b et al., 2020, 2) Metal based nanoparticles are popular inorganic alternatives against traditional antibiotic resistance. Their mechanism of action is completely different from traditional antibiotic treatment , targeting multiple biomolecules and thus , compromising resistance development in bacteria. (Slavin et al., 2017, 3)

Nanoparticles are materials in the range of 100 nm or less exhibiting a wide range of properties including optical, electrical , catalytic, (Department of Bioengineering, Rice University, P.O. Box 1892, MS-142, Houston, TX 77251-1892; Departments of et al., 2003, 4) magnetic and biological which are surprisingly different from their bulk materials. (Gyawali et al., 2011, 5). The bactericidal properties of these metal nanoparticles is due to their size and high surface-to-volume ratio. Such characteristics allow them to interact closely with bacterial membranes, rather than the effect being solely due to the release of ions. (6) Nanoparticles
Have demonstrated broad-spectrum antimicrobial activity against both Gram-positive and Gram-negative bacteria. (Ramalingam & Das, 2016,25)

1.3 ABOUT COPPER, TITANIUM AND ZINC NANOPARTICLES:

Copper is required by microorganisms in trace quantities for their growth and functioning. However, higher concentrations can have a toxic effect. (Trevors & Cotter, 1990, 7). Copper has been reported to have antimicrobial activity against many microorganisms, including *Salmonella enterica*, *Campylobacter jejuni*, *E. coli*, *Listeria monocytogenes*, and *Staphylococcus aureus.* (8) (9) Very limited information is available on the possible antimicrobial effects of Cu and Cu-compounds. However, copper nanoparticles are known to play a dual role ; acting as an antibacterial agent and enhancing porosity of the material. As CuO is cheaper than silver,it can easily be mixed with polymers and is relatively stable in terms of both chemical and physical properties. Highly ionic nanoparticulate metal oxides, such as CuO, may be particularly valuable antimicrobial agents as they can be prepared with extremely high surface areas and unusual crystal

morphologies.Copper oxides in nanoform display enhanced activity against microbes. Hence, effects of CuO nanoparticles have been studied during the dissertation project.

Among metal- oxide antimicrobial agents, TiO2 is one the most widely studied antimicrobial agents which belongs to the transition metal series. It has features such as good physicochemical properties such as photocatalysis, non-toxicity, resistance to chemical erosion, reduced cost etc.

TiO2 nanoparticles in general present larger surface areas and they also possess great surface morphologies. These also show excellent anti-bacterial and anti-fungal properties against contaminating microbes. (Lim et al., 2021, 10). The crystal structures and the shape of TiO2 NPs are both the most important properties that affect their physicochemical properties, and therefore their antimicrobial properties.

Extensive alterations in cell wall and membrane are the main factors that explain the bactericidal activity of these TiO2 nanoparticles. Based on the type of microorganisms, cell wall composition is different for different bacteria and fungi. And since, cell wall is the first line of defense for any of these microbes, reactive oxygen species (ROS) damage the cell wall first.These include hydroxyl radicals , hydrogen peroxide, and malondialdehyde (MDA). (Gow et al., 2017, 11) The second usual target is cell membrane and these ROS usually lead to increase in cell membrane fluidity, cellular content leakage and finally cell lysis. (Khezerlou et al., 2018, 12) TiO2 nanoparticles are known to inhibit respiratory chain in microbes due to lipid peroxidation of cell membranes due to overproduction of ROS by NPs. (Staerk et al., 2017, 13) DNA is very sensitive to oxidative damage, thus damage at the molecular level affects microbe metabolism, replication , transcription and cell division. (Gogniat & Dukan, 2007, 14) It has also been observed that microorganisms are deficient in phosphorus uptake and metabolism in the presence of TiO2 NPs. (Kubacka et al., 2014, 15) TiO2 NPs are also known to oxidize signaling pathway components and also change gene expression by interfering with the transcription factors. (Apel & Hirt, 2004, 16)

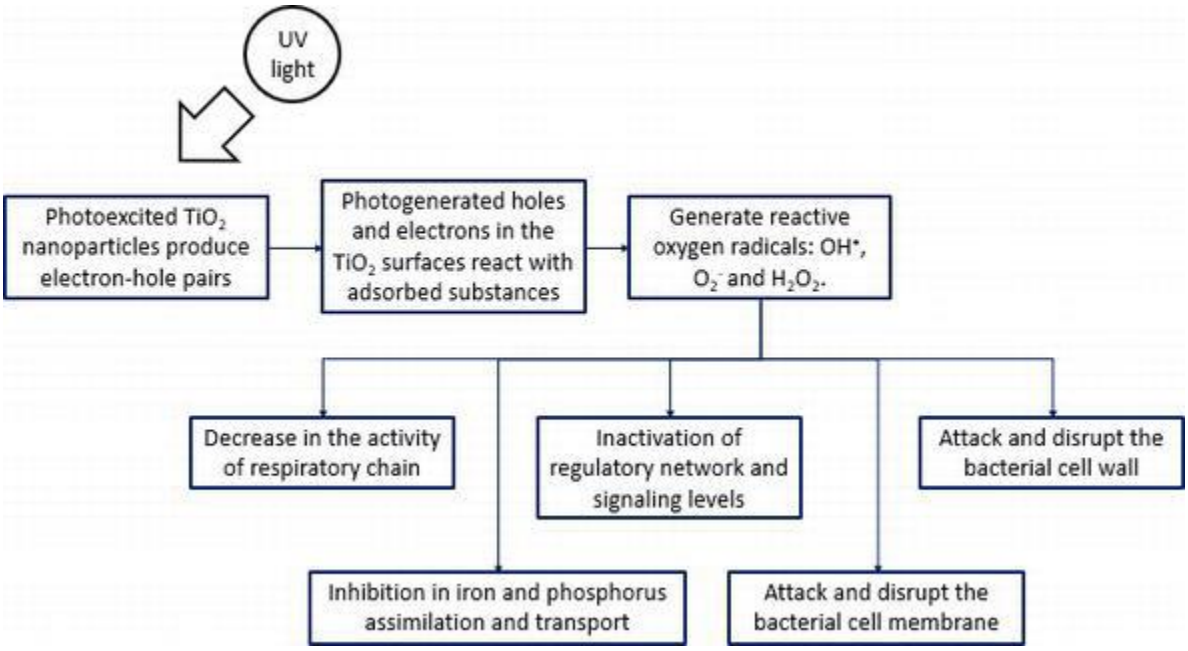

Fig.1: Scheme of main antimicrobial activity based process (Lim et al., 2021, #)

In several studies, ZnO nanoparticles are reported to be non-toxic to human cells, hence they have been potentiated to be used as antimicrobial agents , toxic to microorganisms. (Colon et al., 2006, 17) Again, antibacterial activity is attributed to their small size and high surface: volume ratio. Nano-ZnO particles exhibit a variety of morphologies and antibacterial activities against a wide spectrum of bacteria. (Buzea et al2007, 18) There are various mechanisms listed in the available literature which state the probable mechanism of action of ZnO nanoparticles such as direct contact with cell walls and disrupting cell integrity, liberation of Zn2+ ions , ROS formation. (18) (19)

1.4 ABOUT BACTERIA USED IN THE CURRENT STUDY:

1. *Escherichia coli* : *E. coli* is a Gram - negative bacillus (rod -shaped) and a facultative anaerobic coliform bacteria. Most strains are harmless but some can cause serious food poisoning in their host. The strain used in the given study is pathogenic. (Singleton, 1999, 20)

2. *Staphylococcus aureus :* is a Gram- positive bacteria that are cocci shaped (spherical/ ball-like) and tend to be arranged in clusters described as grape-like fashion. Colonies are often seen as golden/yellow (aureus). These grow aerobically or anaerobically between 18-40 degrees. It is a major bacterial human pathogen. Since these microbes are normally present on human skin , it usually does not cause infection unless it enters the bloodstream. (taylor & Unakal, 2022, 21)

3. *Pseudomonas aeruginosa* : is a Gram-negative, aerobic, non-spore forming rod-shaped bacteria capable of causing infection in humans. It is a commonly found pathogen in the environment particularly in freshwater. It possesses many mechanisms of antibiotic resistance . Hence, a very good target to check against NPs antimicrobial activity. (Wilson & Pandey, 2023, 22)

AIM: **To study the cytotoxic effects of TiO2 , CuO and ZnO nanoparticles on *Escherichia coli* , *Staphylococcus aureus* and *Pseudomonas aeruginosa***

OBJECTIVES:

1. To determine the minimum inhibitory concentration (MIC) of each of the nanoparticles using the broth dilution method.

2. To plot a growth curve of bacteria after treatment with nanoparticles .

3. To perform biochemical assays like MTT assay for colorimetric analysis of bacterial cell metabolic activity.

4. To perform TBARS assay to check lipid peroxidation in bacterial cells.

CHAPTER 2

MATERIALS, INSTRUMENTATION AND METHODOLOGY

2.1 MATERIALS AND INSTRUMENTATION

The following materials were used in the study:

2.1.1) TiO_2 , CuO and ZnO nanoparticles in powdered form (received from Prof. M.P. Deshpande's Laboratory, Department of Physics, Sardar Patel University, Anand , Gujarat) ,Luria Bertani Broth (Himedia laboratories Pvt. Ltd. India),Luria Bertani Agar (Himedia laboratories Pvt. Ltd. India) ,Pathogenic bacterial cultures of *E.coli* , *S. aureus* and *P. aeruginosa (*provided by Prof. Savita Kerkar, Goa University) ,EZcount™ MTT Cell Assay Kit (Himedia laboratories Pvt. Ltd. India) ,EZAssay™ TBARS Estimation Kit for Lipid Peroxidation (Himedia laboratories Pvt. Ltd. India)

The following instruments were used in the study:

2.1.2) Shimadzu UV-Vis Spectrophotometer Model: uv-1800 ,Millipore Unit ,Horizontal Laminar Air Flow (RUSA), Shaker incubator (Rivotek) ,Water sonicator (Digital Ultrasonic Cleaner) ,Rotospin (Tarsons Test Tube rotator),T1-90E Incubator bact, iMARK microplate reader (BioRad), autoclave

2.2. METHODOLOGY

The antimicrobial efficacy of the given nanoparticles was determined by **Minimum Inhibitory Concentration(MIC)** using the broth dilution method.

2.2.1) Minimum Inhibitory Concentration is the lowest concentration of antimicrobial substance that will inhibit visible growth of a microorganism after overnight incubation. (Andrews, 2001, 24)

Nanoparticle stock solutions were made for each of the nanoparticle samples with concentration 2 mg/ml.These were then sonicated on a water sonicator for about 30 minutes. Different dilutions were made for each nanoparticle - 1000 ug/ml, 500 ug/ml, 250 ug/ml, 100 ug/ml and 25 ug/ml

using milli Q water. An identical amount of overnight grown culture of bacteria was introduced in the tubes containing 2 ml LB broth containing progressively lower concentrations of the nanoparticles.Tubes were kept for incubation at 37 degrees for 14-16 hours in a shaker incubator. Optical Density was measured at 600 nm on a UV-Vis spectrophotometer.

2.2.2) Growth Curve of Bacteria with different NPs

Nanoparticle stock solutions were taken and a working stock of 1000ug/ml was prepared using autoclaved Milli q water for each nanoparticle. These working stocks were sonicated for 30 minutes to homogenize. They were then left under UV for sterilization. 30ul of overnight grown culture of bacteria was taken in test tubes containing 2 ml LB broth . These were then treated with prepared nanoparticle concentration by adding 0.5ml to each tube. Tubes were then incubated at 37 degrees for one hour along with control. Optical Density was recorded at 600nm
at an interval of one hour till nine hours and a graph was plotted for the same.

2.2.3) Biochemical Assays

2.2.3.1 MTT Assay

MTT is a colorimetric assay based on the cleavage of the tetrazolium ring of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) by dehydrogenases in active mitochondria of living cells as an estimate of viable cell number.

1 ml overnight grown culture of bacteria was taken in individual tubes and were centrifuged at 8000 rpm for 5 minutes. After centrifugation, supernatant was discarded and the pellet was resuspended in 1X PBS and treated with individual concentrations of nanoparticles and kept for incubation at RT with continuous agitation together with control. 200ul of treated culture was

transferred to a fresh 1.5 microcentrifuge tube and centrifuged at 8000 rpm for 5 minutes and then supernatant was discarded. 200ul of MTT solution (0.5mg/ml in 1X PBS) was then added to each tube and incubated for 3 hours to allow formation of formazan crystals. The tubes were then centrifuged again at 8000 rpm for 5 minutes , supernatant was discarded and 200ul of DMSO was added to each tube and incubated for 30 minutes in dark to dissolve the crystals. The solution was then transferred to a 96-well microplate and OD was taken at 595 nm using a microplate reader.

2.2.3.2 TBARS Assay

Thiobarbituric acid reactive substance (TBARS) assay is a method to detect lipid oxidation. This assay measures malondialdehyde (MDA), which is a split product of an endoperoxide of unsaturated fatty acids resulting from oxidation of lipid substrates.
Here, lipid peroxidation is seen in the bacterial membranes on exposure to antimicrobial agents.

1 ml overnight grown culture of bacteria was taken in individual tubes and were centrifuged at 8000 rpm for 5 minutes. After centrifugation, supernatant was discarded and the pellet was resuspended in 1X PBS and treated with individual concentrations of nanoparticles and kept for incubation at RT with continuous agitation together with control. The treated cells were then lysed by using a lysis buffer (5% SDS) and centrifuged at 5000 rpm for 10 minutes at 4 degrees.
200 ul lysate was taken into a fresh 1.5 ml microcentrifuge tube and 400 ul of ice cold trichloroacetic acid was added to each tube and were then incubated on ice for 15 minutes for protein precipitation to occur. The samples were then centrifuged at 10,000 rpm for 10 minutes at 4 degrees and 200 ul of 0.67% thiobarbituric acid was then added to each tube and allowed to boil for 10 minutes in a boiling water bath. The samples were then allowed to cool and loaded onto a 96-well microplate for OD measurement at 540 nm.

CHAPTER 3

RESULTS AND DISCUSSION

3.1 Minimum Inhibitory Concentration of nanoparticles

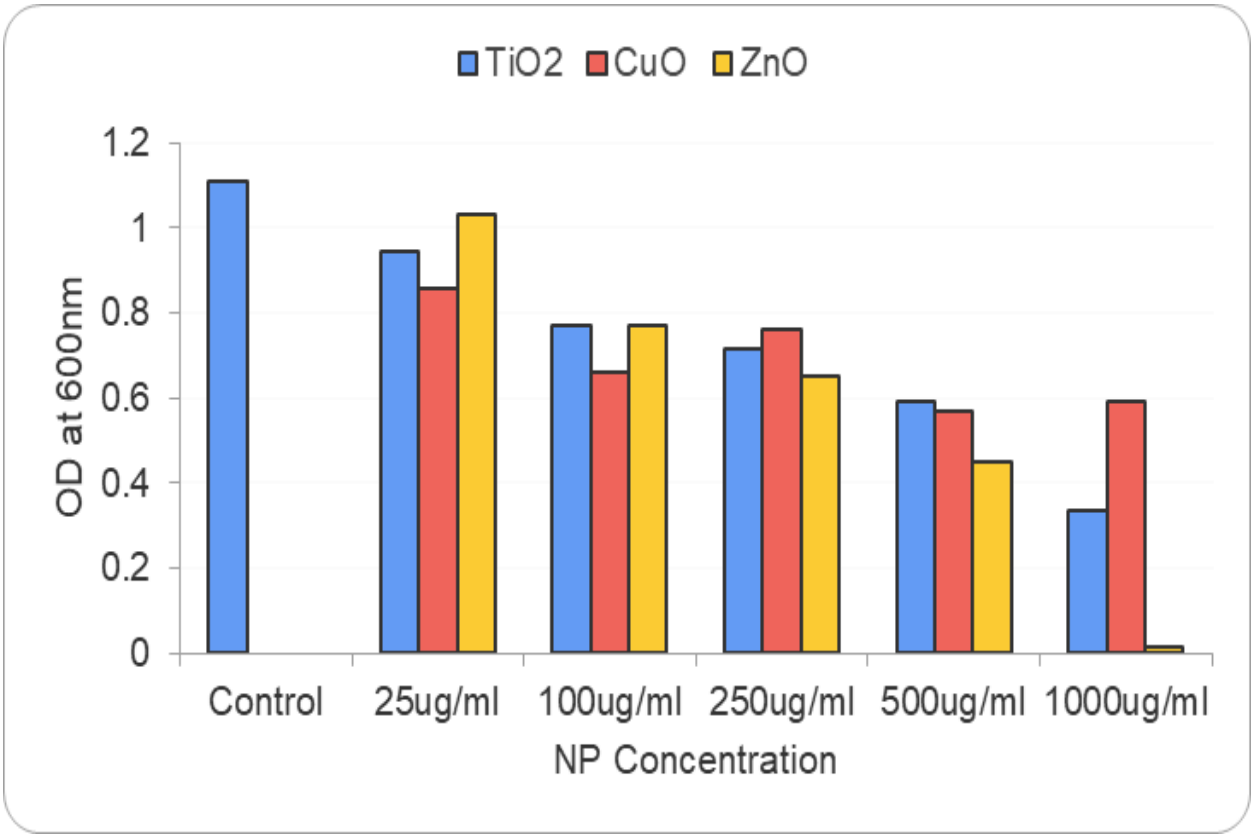

Fig. 1 Optical Density Vs NP concentration graph for *E. coli*

When different concentrations of NPs were added in the tubes containing bacterial culture and incubated for 16 hours, OD was recorded and a graph was plotted (fig.1) . From this data , it was observed that with increasing concentration of NPs , decrease in the absorbance was seen. It was then inferred that ZnO NP at the concentration of 1000 ug/ml was able to inhibit *E. coli* growth. However, TiO2 and CuO were also observed to decrease growth of bacteria but are needed at a higher concentration (>1000 ug/ml) to completely inhibit growth.

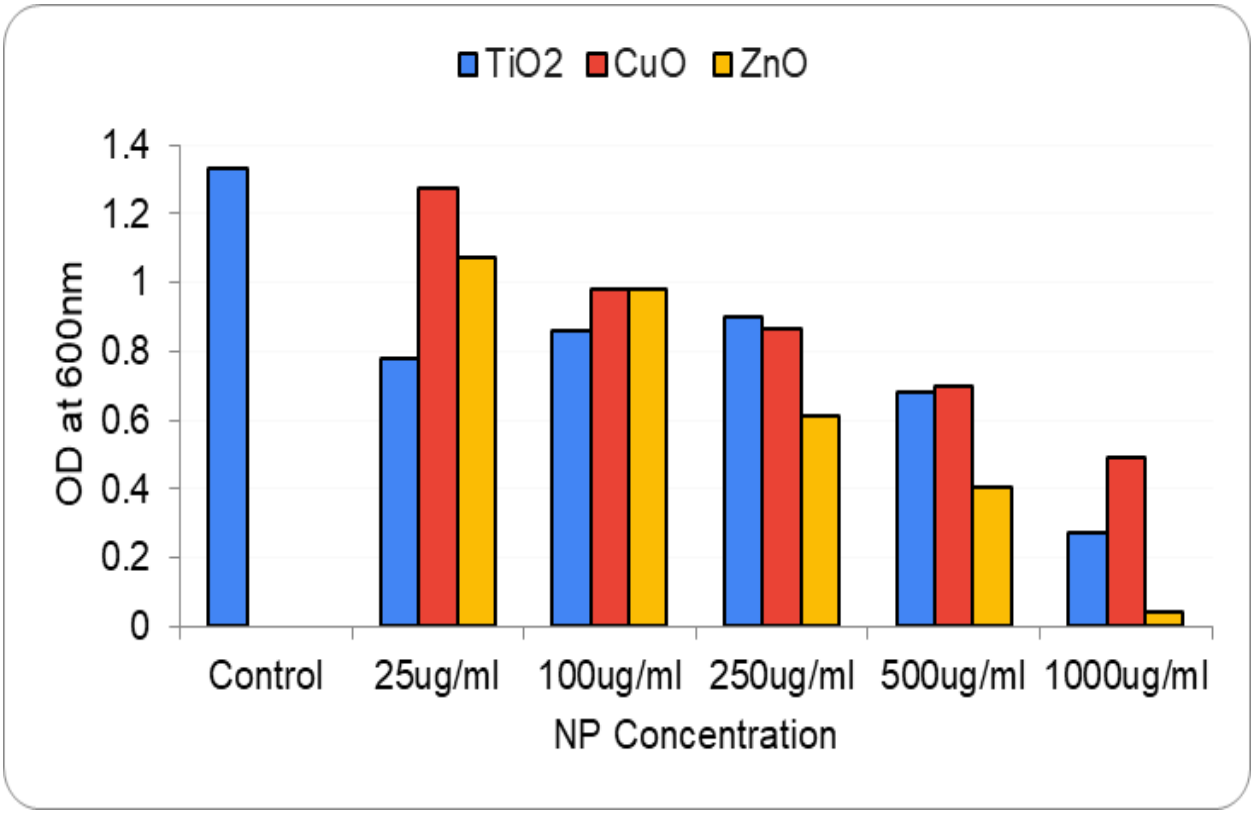

Fig.2 Optical Density Vs NP concentration graph for *S. aureus*

From this data (fig.2), it was inferred that the minimum inhibitory concentration of ZnO NP was 1000ug/ml for *S. aureus*. However, both TiO2 and CuO were seen to decrease the growth of bacteria with increasing concentration. To completely inhibit the bacteria, concentrations higher than 1000 ug/ml are required for both TiO2 and CuO NPs.

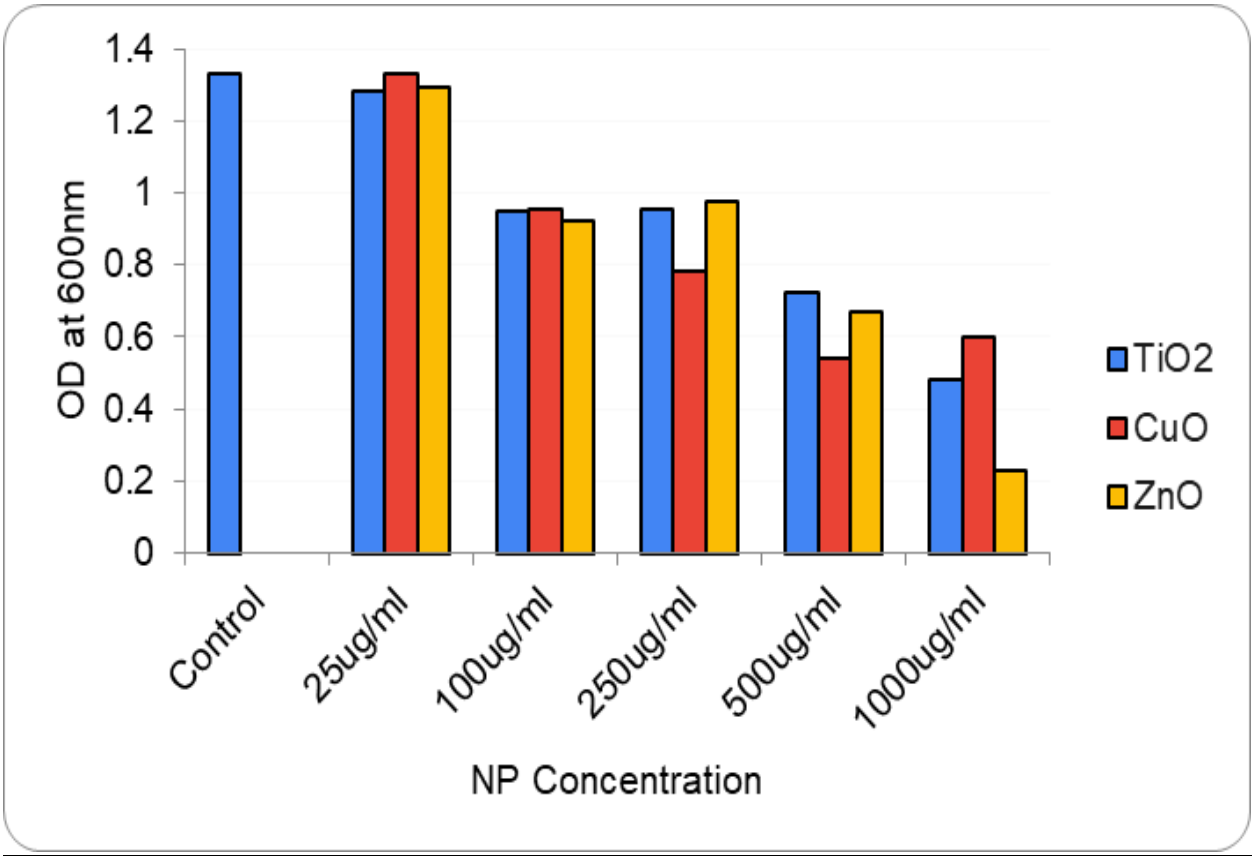

Fig.3 Optical Density Vs NP concentration graph for *P. aeruginosa*

Similarly, for *P. aeruginosa* (fig.3) it was observed that all the three nanoparticles showed decrease in the growth of bacteria with increase in concentrations. However, all of them are required in higher concentrations (>1000 ug/ml) to completely inhibit the growth.

3.2 Bacterial Growth curve on NP treatment

Bacterial growth curve represents number of live cells in bacterial population over time. There are four distinct phases in the growth curve: lag, log (exponential), stationary (plateau), death (decline). The initial lag phase is where the bacteria are not dividing but are metabolically active.

The log phase is the period of logarithmic growth of bacteria. The stationary phase is marked by the number of cells dividing equals the number of cells dying. The last phase which is the decline phase is the logarithmic death of cells.

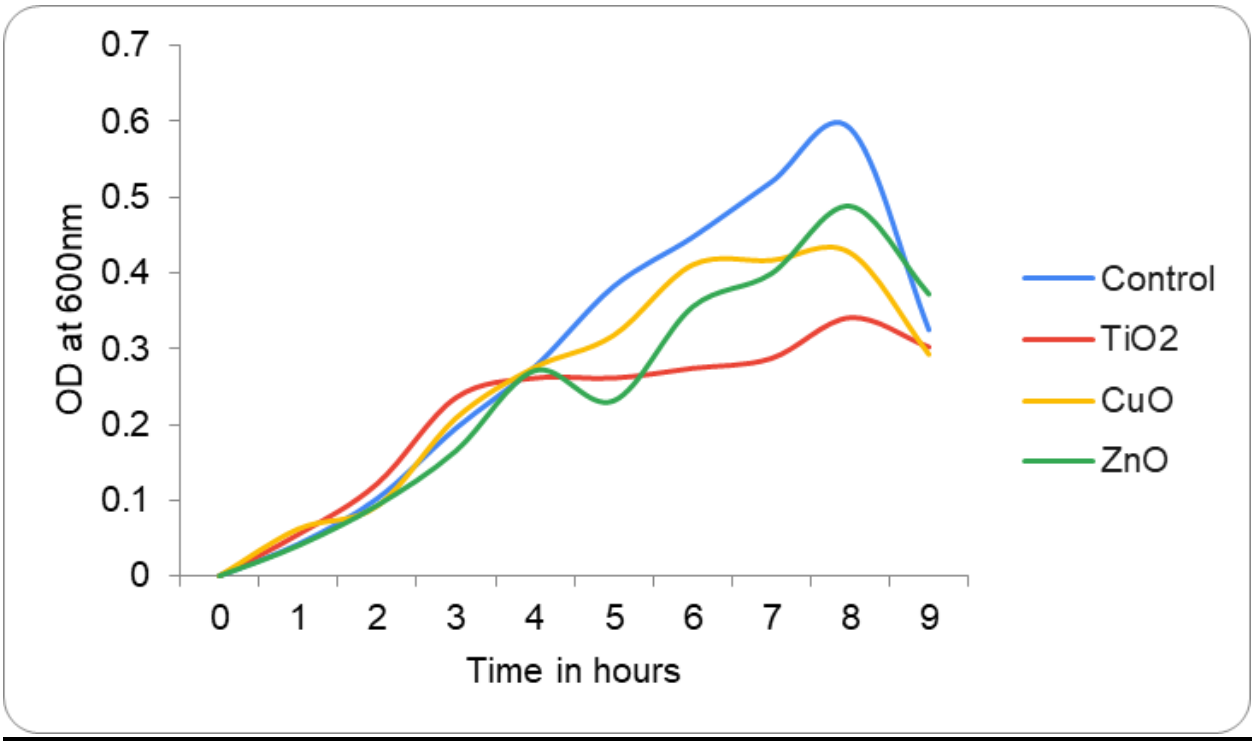

Fig.4 Growth curve of *E. coli* on NP treatment

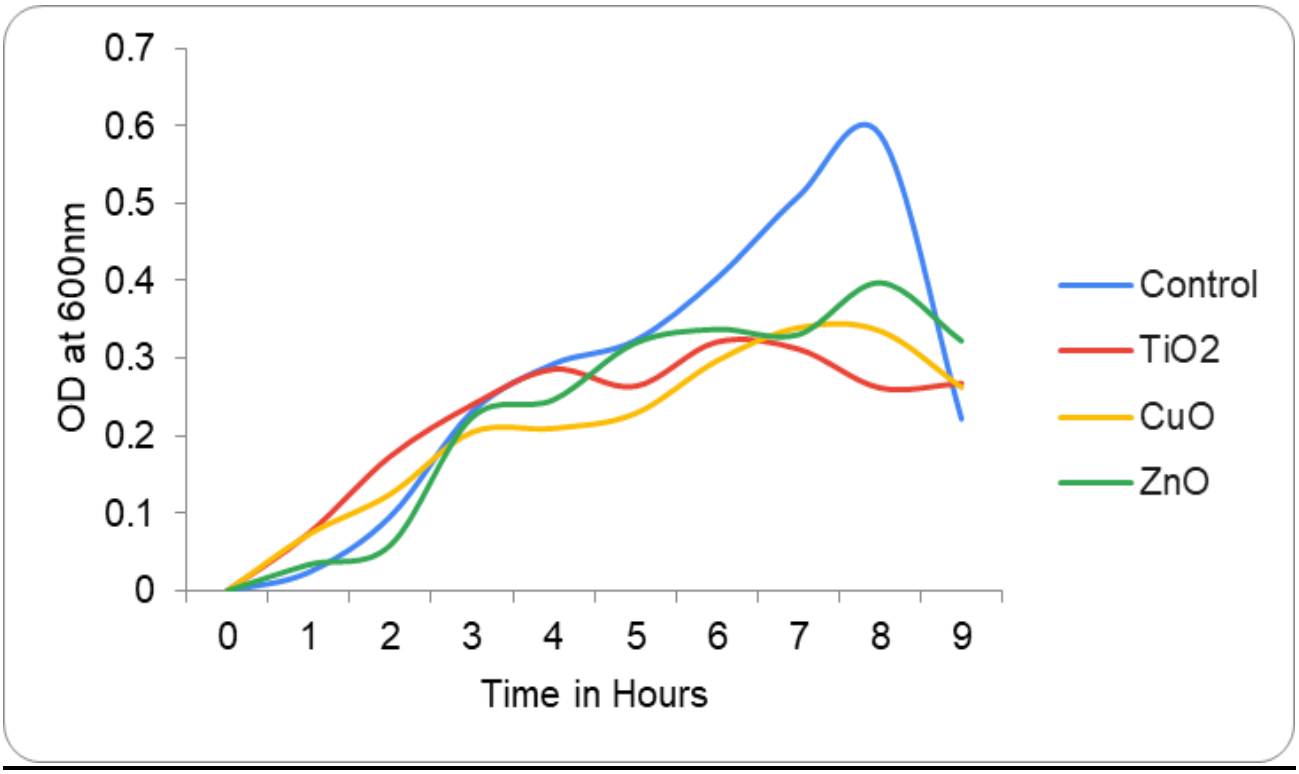

Fig. 5 Growth curve of *S. aureus* on NP treatment

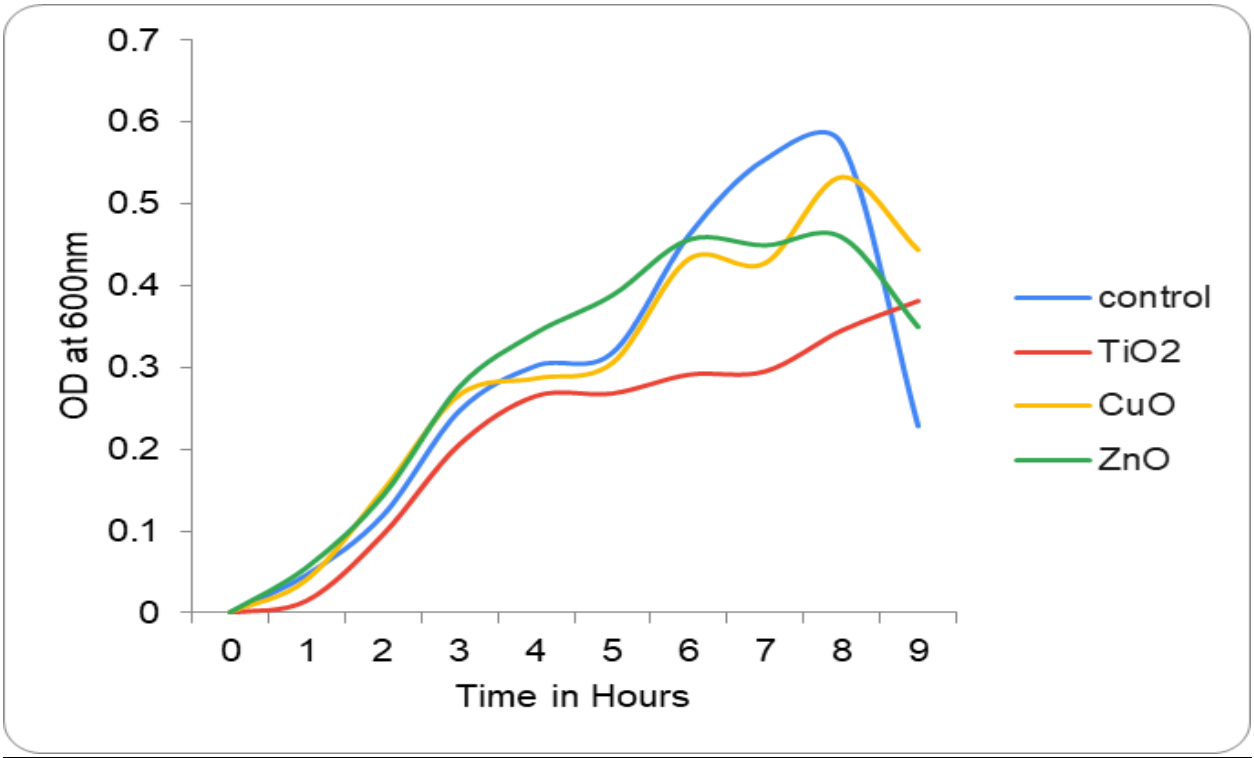

Fig. 6 Growth curve of *P. aeruginosa* on NP treatment

In Fig. 4. Fig.5 and Fig. 6 absorbance first increases , in some cases shows a plateau and then decreases which represents the normal bacterial growth curve. But from fig. 4, it can be inferred that in the presence of TiO2 NP ,somewhat stationary bacterial growth (bacteriostatic) is seen as compared to *E. coli* control over time. However, Zno and CuO NP follow the same decline trend. Similarly, in fig. 5 , CuO shows plateau growth in *S. aureus* followed by TiO2 and ZnO NPs. In fig. 6, TiO2 shows a similar trend as in fig.4 and ZnO and CuO NPs show sudden sharp decline in the growth of *P. aeruginosa* over time.

3.3 Biochemical Assays

3.3.1 MTT Assay

MTT Assay determines cell viability or metabolic activity of live cells to transform MTT dye into insoluble formazan. The quantity of formazan is presumably directly proportional to the number of viable cells which can be measured.

Fig. 7, Fig 8 and Fig.9 illustrate bar graphs for MTT Assay measurements. Fig. 7 shows the absorbance at 595 nm (y-axis) Vs NP concentration (X-axis) for *E. coli.* It was confirmed from the graph that ZnO NP at a concentration of 1000 ug/ml inhibits bacterial growth as the absorbance values decrease significantly showing lesser viability of cells.

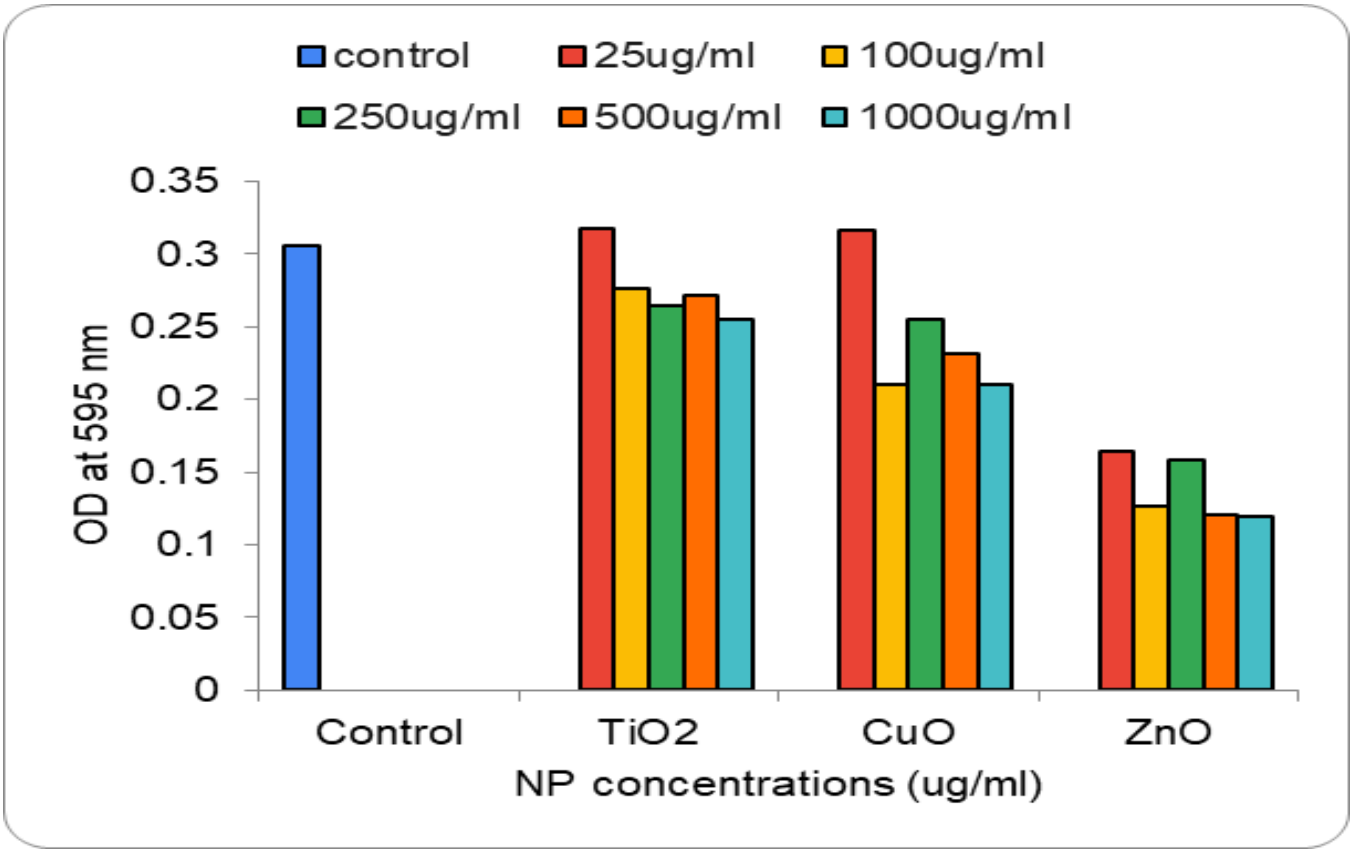

Fig. 7 MTT Assay graph for *E. coli*

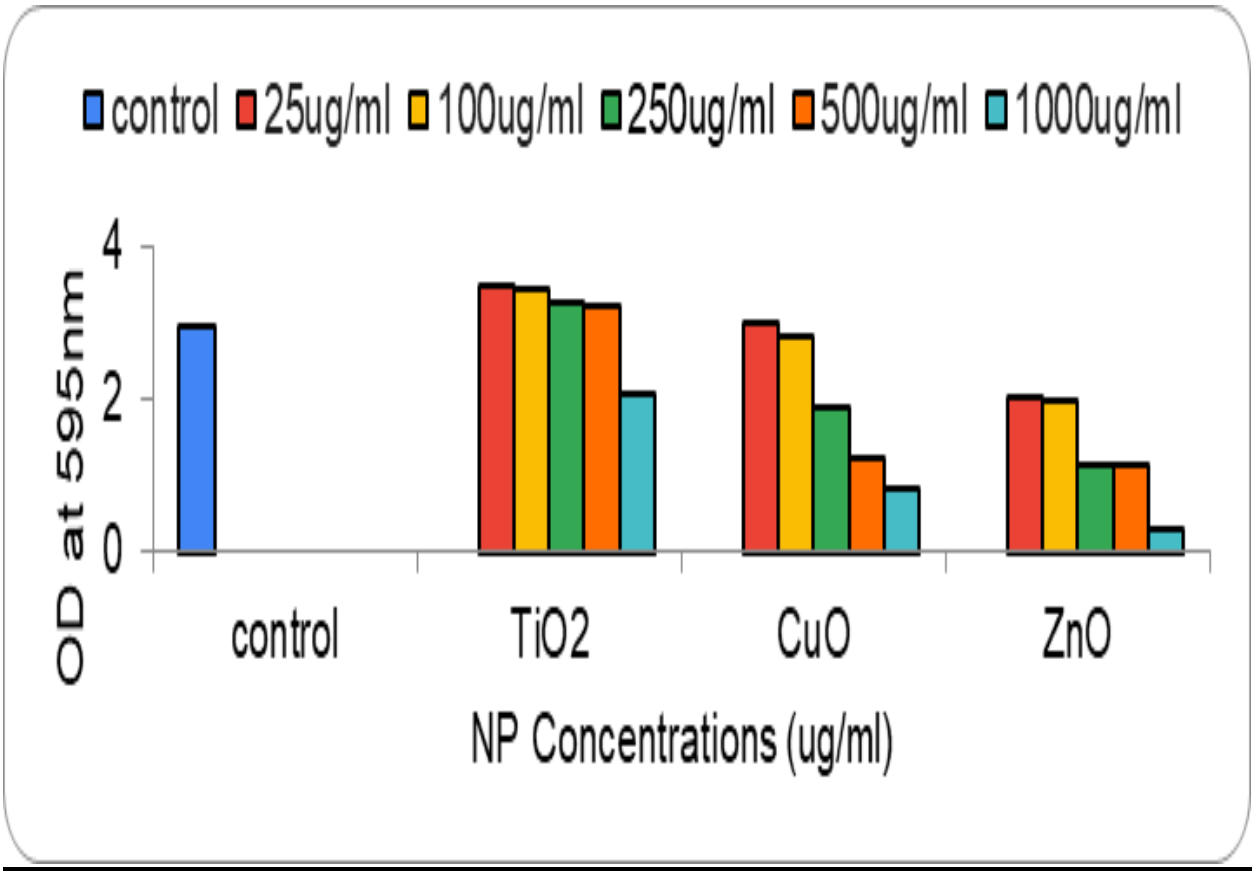

Fig. 8 MTT Assay graph for *S. aureus*

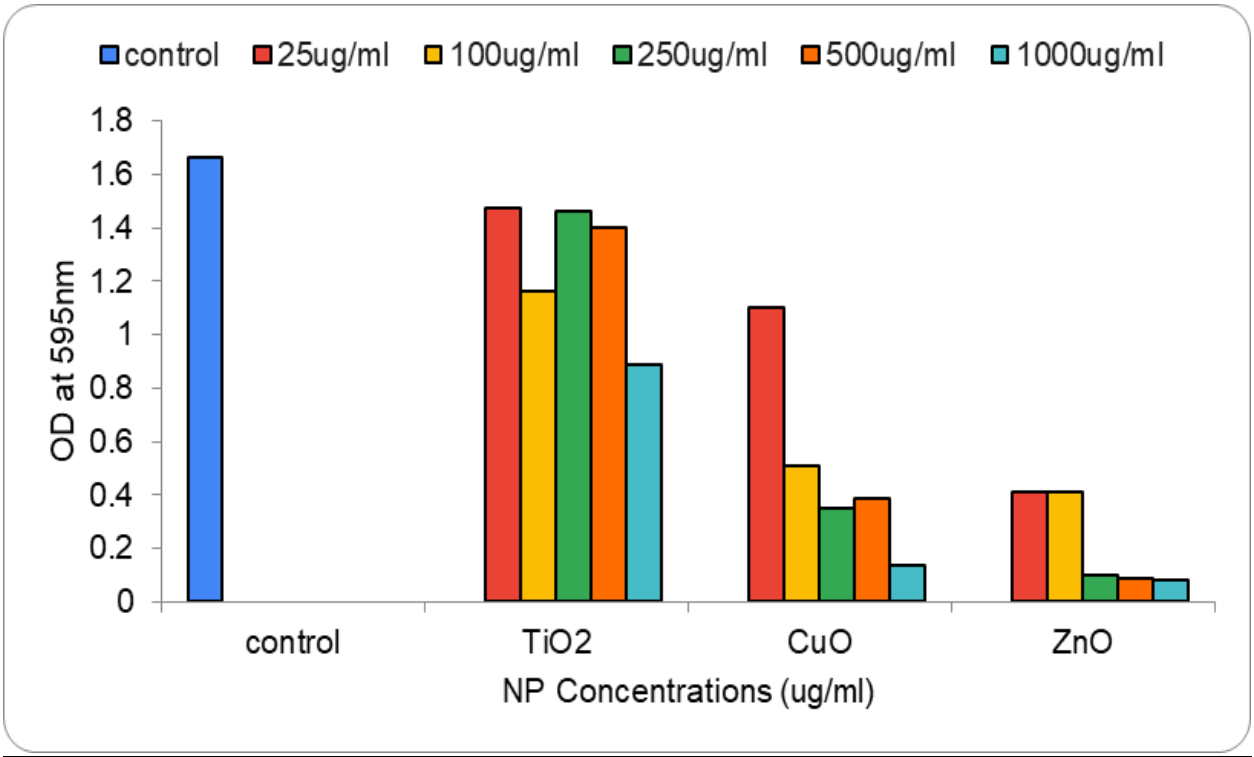

Fig. 9 MTT Assay graph for *P. aeruginosa*

In fig 8. Both CuO and ZnO NP show significant decrease in the absorbance values of *S. aureus* indicating lesser viability. In fig. 9, clear inhibition of bacteria is seen in the case of ZnO and CuO NPs at concentrations between 250 ug/ml and 1000 ug/ml indicating their efficacy as potential drugs.

3.3.2 TBARS Assay

This Assay determines the amount of thiobarbituric acid reactive substances such as malondialdehyde on exposure of microbial membranes to antimicrobial agents which cause lipid peroxidation. Greater the lipid peroxidation, lesser the cell viability.

Fig. 10, fig 11 and fig. 12 illustrate bar graphs for TBARS Assay measurements.

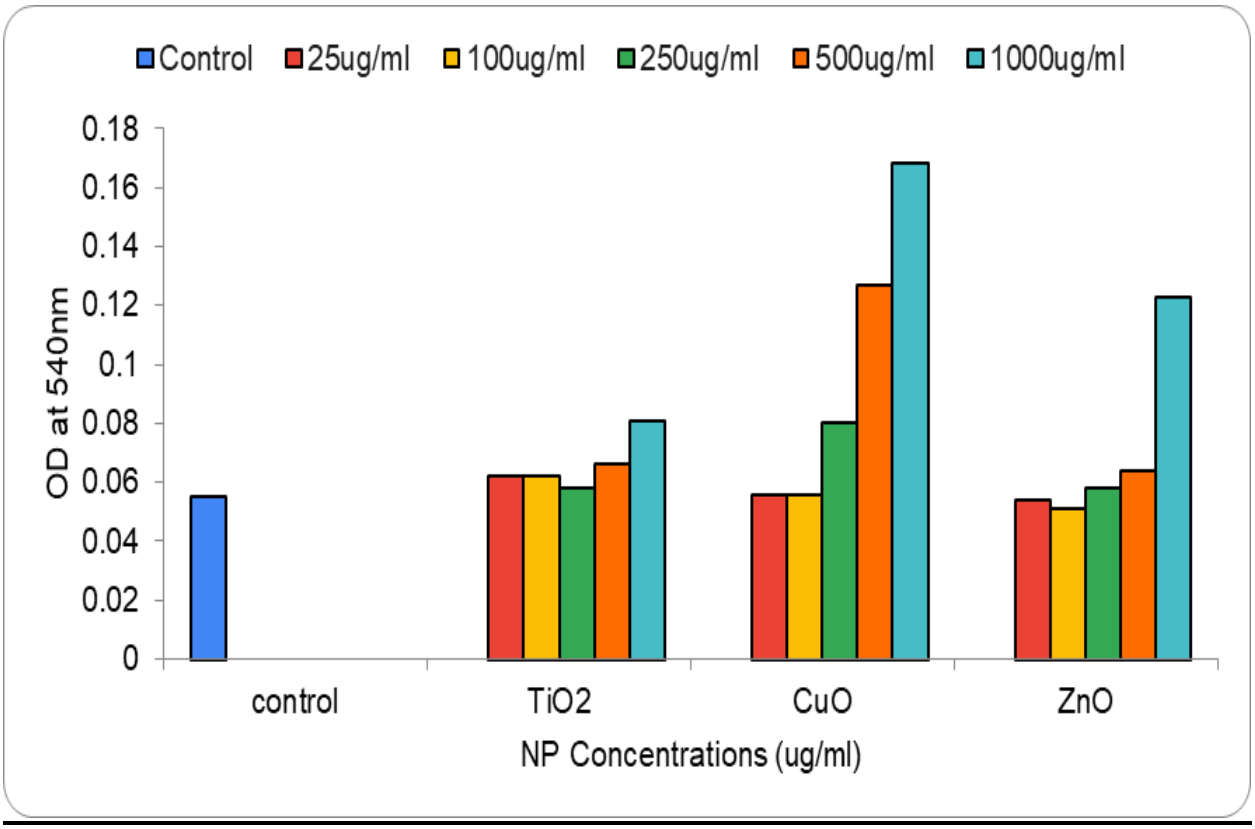

Fig. 10 TBARS Assay Graph for *E. coli*

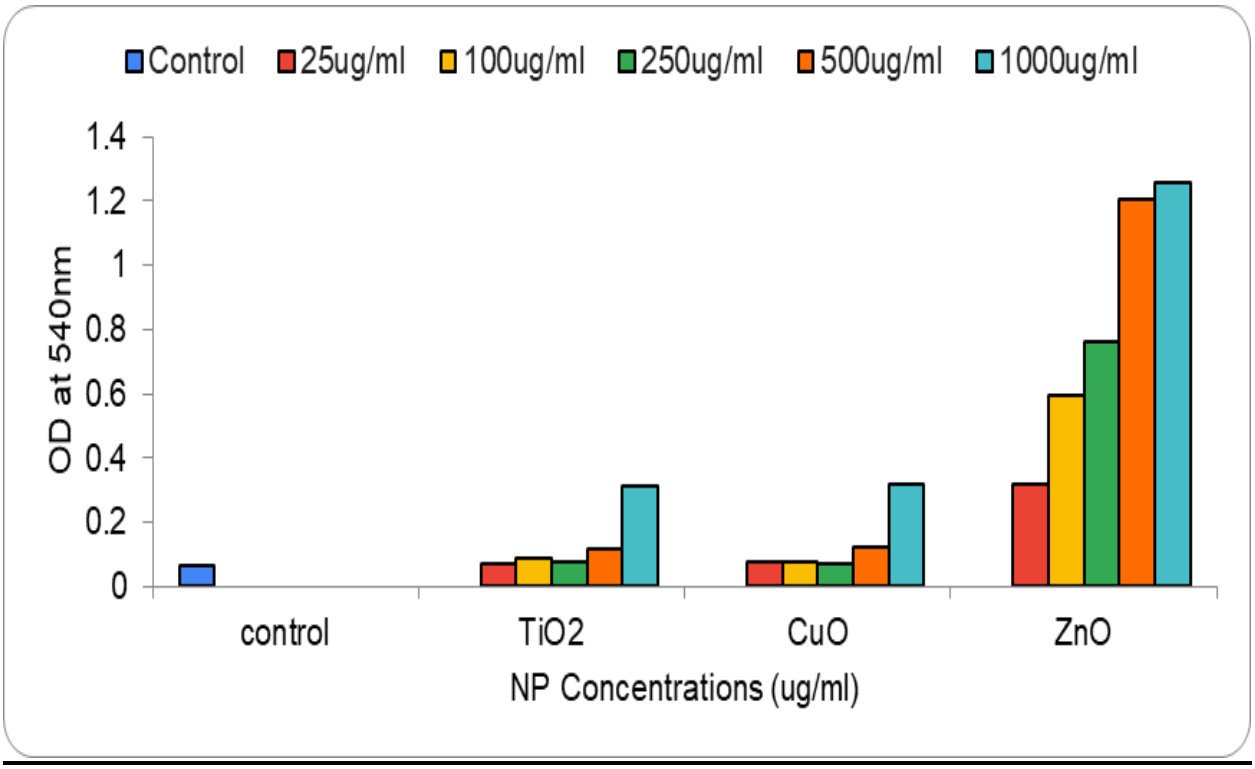

Fig. 11 TBARS Assay Graph for *S. aureus*

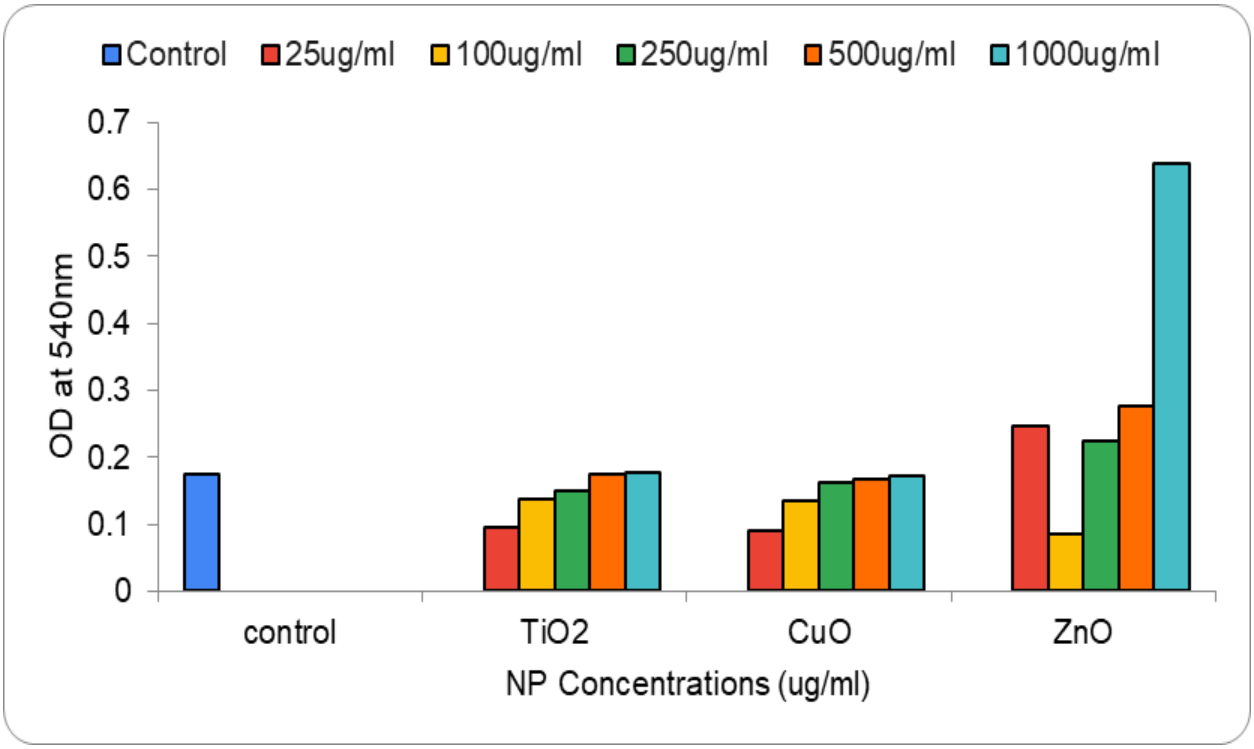

Fig. 12 TBARS Assay Graph for *P. aeruginosa*

In fig. 10, greater absorbance is seen in case of CuO and ZnO NPs at concentrations higher than 500 ug/ml indicating greater peroxidation of membrane lipids in *E. coli.* Thus, this means that the viability of cells is decreasing with increasing concentration of NPs. In fig. 11 higher lipid peroxidation is seen in ZnO NPs which indicates greater activity of these NPs as antibacterial drug alternatives. Similarly, in fig. 12 , ZnO NPs show better activity than other nanoparticles.

3.4 DISCUSSION

The problem of antibiotic resistance poses a global problem especially in the public health sector. In order to combat this problem, metal and metal oxide nanoparticles can be potential drug candidates since bacteria are unable to get resistant to these due to their extensive physicochemical properties. Present study was an attempt to study the cytotoxic nature of these nanoparticles and their potential application as bactericidal agents.

In this study, it was inferred that Zinc Oxide Nanoparticles in powdered form at a concentration of about 1000 ug/ml were most efficient to inhibit Gram Negative bacterial strains *E. coli* and *P. aeruginosa* and Gram Positive *S. aureus* bacteria. TiO2 and CuO Nps at a concentration higher than 1000 ug/ml are suggested to produce a pronounced antimicrobial effect . Moreover, antibacterial effect is also attributed to NPs size, here powdered Nps were used.

No significant toxicity was observed at NPs concentration less than 250 ug/ml. Bacteria showed viability even after exposure to NP at concentration of 25 ug/ml. The studies from broth dilution and growth curve indicated that nanotoxicity of NPs increased with increasing concentrations and thus, inhibitory effect on bacteria.

In growth curve study, it was observed that TiO2 NPs at a concentration of 1000 ug/ml were able to produce a bacteriostatic response in *E. coli* , *S. aureus* and *P. aeruginosa* whereas Zno and CuO produced a bactericidal effect. The control in each curve shows a partly sigmoidal pattern indicating standard growth. However, slight distortion shows an unusual growth pattern as compared to control which could be indicative of manual error, environmental or instrument error.

In MTT assay study, it was confirmed that ZnO NPs have the most profound antibacterial effect on the bacteria at concentrations greater than 500 ug/ml followed by CuO NPs. In TBARS assay study, again, it was confirmed that ZnO NPs are most efficient in killing bacteria and thus offer to be a potential drug alternative in the coming years.

CHAPTER 4

SUMMARY AND FUTURE PROSPECTS

The current study was aimed towards finding the possible ways to combat the problem of multidrug resistance in bacteria due to use of antibiotic drugs as a method to prevent infection. The whole study was to check the cytotoxic effects of NPs on common pathogenic bacterial cultures . The NPs used in this study were TiO2 , CuO and ZnO NPs in powdered form. The bacteria used in this study were pathogenic *E. coli* , *S. aureus* and *P. aeruginosa* .

Various concentrations of these NPs were taken and tested on each bacterial culture to check for bactericidal and bacteriostatic effects. MIC concentration of ZnO NPs was found to be 1000 ug/ml whereas for CuO and TiO2 was found to be > 1000 ug/ml . Zinc NPs were found to produce an enhanced antibacterial activity whereas TiO2 was observed to produce a bacteriostatic effect as observed from growth curves.

Future prospects this study entails include the use of metal nanoparticles such as ZnO , TiO2 and Cuo alone or in combination to enhance antibiotic activity against multi drug resistant bacteria. NPs as drug candidates against various pathogenic bacteria pose a better approach in treatment against such infectious pathogens. Moreover, recent studies also suggest the use of these NPs in drug delivery systems which will serve the purpose of desired drug delivery and biocidal effect.

CHAPTER 5

BIBLIOGRAPHY

REFERENCES

1. Antimicrobial activity of copper surfaces against suspensions of Salmonella enterica and Campylobacter jejuni. (2004, April 30). *BMC Microbiology*, *4*(2004), 19. https://doi.org/10.1186%2F1471-2180-4-19
2. Antimicrobial activity of lactic acid and copper on growth of Salmonella and Escherichia coli O157:H7 in laboratory medium and carrot juice. (2008, July 1). *Food chem*, *109*(1), 137-143. https://doi.org/10.1016/j.foodchem.2007.12.035
3. Apel, K., & hirt, H. (2004). Reactive oxygen species: metabolism, oxidative stress, and signal transduction. *Annual Review of Plant Biology*, *55*(-), 373-99. https://doi.org/10.1146/annurev.arplant.55.031903.141701
4. Buzea, C., Pacheco, I. I., & Robbie, K. (2007, December). Nanomaterials and nanoparticles: sources and toxicity. *Biointerphases*, *2*(4), 17-71. https://doi.org/10.1116/1.2815690
5. Colon, G., Ward, B. C., & Webster, T. J. (2006, September). Increased osteoblast and decreased Staphylococcus epidermidis functions on nanophase ZnO and TiO2. *Journal of biomedical materials research. Part A*, *78*(3), 595-604. https://doi.org/10.1002/jbm.a.30789
6. Comparative eco-toxicity of nanoscale TiO2, SiO2, and ZnO water suspensions. (2006). *Water research*, *40*(19), 3527-32. https://doi.org/10.1016/j.watres.2006.08.004
7. Department of Bioengineering, Rice University, P.O. Box 1892, MS-142, Houston, TX 77251-1892; Departments of, Diagnostic Imaging Physics and ‡Veterinary Medicine, University of Texas M. D. Anderson Cancer Center, Houston, TX, Sershen, S. R., & Rivera, B. (2003, november 3). Nanoshell-mediated near-infrared thermal therapy of tumors under magnetic resonance guidance. *Proceedings of the National Academy of Sciences of the United States of America*, *Volume 100(23)*(2003 Nov 11), 13549–13554. https://doi.org/10.1073%2Fpnas.2232479100
8. Dizaj, S. M., Lotfipour, F., Jalali, M. B., Zarrinton, M. H., & Drug Applied Research Center and Faculty of Pharmacy, Tabriz University of Medical Sciences, Tabriz, Iran b Biotechnology Research Center and Faculty of Pharmacy, Tabriz University of Medical Sciences, Tabriz, Iran. (2014, August 16 August 2014). Antimicrobial activity of the metals

and metal oxide nanoparticles. *Materials Science and Engineering: C*, *44*(1 november 2014), 278-284. https://doi.org/10.1016/j.msec.2014.08.031

9. Gogniat, G., & Dukan, S. (2007). TiO2 photocatalysis causes DNA damage via fenton reaction-generated hydroxyl radicals during the recovery period. *Applied and environmental microbiology*, *73*(23), 3. https://doi.org/10.1128/aem.01079-07
10. Gow, N. A., Latge, J. P., & Munro, C. A. (2017, may). The Fungal Cell Wall: Structure, Biosynthesis, and Function. *Microbiology Spectrum*, -(may 5), 3. https://doi.org/10.1128/microbiolspec.funk-0035-2016
11. Gyawali, R., Ibrahim, S. A., & AbuHasfa, S. H. (2011, October 23). Antimicrobial Activity of Copper Alone and in Combination with Lactic Acid against Escherichia coli O157:H7 in Laboratory Medium and on the Surface of Lettuce and Tomatoes. *Journal of Pathogens*, *2011*(2011), 650968. https://doi.org/10.4061%2F2011%2F650968
12. Khezerlou, A., Sani, M. A., & Ehsani, A. (2018, October). Nanoparticles and their antimicrobial properties against pathogens including bacteria, fungi, parasites and viruses. *Microbial pathogenesis*, -(123), 505-526. https://doi.org/10.1016/j.micpath.2018.08.008
13. Kubacka, A., Rojo, D., & Ferrer, M. (2014). Understanding the antimicrobial mechanism of TiO_2-based nanocomposite films in a pathogenic bacterium. *Scientific Reports*, *4*(4134), -. https://doi.org/10.1038/srep04134
14. Lim, S. E. H., Mareș, M., Lai, K.-S., & Cristina, R.-T. (Eds.). (2021). *Antimicrobial Resistance: A One Health Perspective*. IntechOpen. 10.5772/intechopen.90891

    14. Lim, S. E. H., Mareș, M., Lai, K.-S., & Cristina, R.-T. (Eds.). (2021). *Antimicrobial Resistance: A One Health Perspective*. IntechOpen.
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16. School of Aerospace, Automotive Design and Engineering, University of Hertfordshire, Hatfield AL10 9AB, UK, Queen Mary University of London, Department of Materials, London E1 2AT, UK, & Intrinsiq Materials Ltd., Farnborough, Hants GU14 0LX, UK. (2009, June). Characterisation of copper oxide nanoparticles for antimicrobial applications.

International journal of antimicrobial agents, *33*(6), 587-590. https://doi.org/10.1016/j.ijantimicag.2008.12.004

17.Singleton, P. (1999). *Bacteria in biology, biotechnology, and medicine*. Wiley.

18. Slavin, Y. L., Asnis, J., Hafeli, U. O., & Bach, H. (2017, october 3). Metal nanoparticles: understanding the mechanisms behind antibacterial activity. *Journal of Nanobiotechnology*, *15*(2017), 65. https://jnanobiotechnology.biomedcentral.com/articles/10.1186/s12951-017-0308-z

19. Staerk, C., Groupe d'Etude des interactions Hôte-Pathogène (EA 3142), UNIV. Angers, UNIV. Brest, Université Bretagne - Loire, Angers, France., Groupe d'Etude des interactions Hôte-Pathogène (EA 3142), UNIV. Angers, UNIV. Brest, Université Bretagne - Loire, Angers, France; Laboratoire de Parasitologie Mycologie, Centre Hospitalier Universitaire, Angers, France., Groupe d'Etude des interactions Hôte-Pathogène (EA 3142), UNIV. Angers, UNIV. Brest, Université Bretagne - Loire, Angers, France. Electronic address: maxime.fleury@univ-angers.fr. (2017, September). Microbial antioxidant defense enzymes. *Microbial Pathogenesis*, -(-), -. https://doi.org/10.1016/j.micpath.2017.06.015

20. Taylor, T. A., & Unakal, C. G. (2022). *Staphylococcus Aureus*. Statpearls. -

21. Toxicological impact studies based on Escherichia coli bacteria in ultrafine ZnO nanoparticles colloidal medium. (2006). *Nano Letters*, *6*(4), 866-70. https://doi.org/10.1021/nl052326h

22. Trevors, J. T., & Cotter, C. M. (1990, october 1). Copper toxicity and uptake in microorganisms. *Journal of industrial microbiology*, *6*(2), 77-84. https://doi.org/10.1007/BF01576426

23. Wilson, M. G., & Pandey, S. (2023). *Pseudomonas Aeruginosa*. Statpearls. https://www.ncbi.nlm.nih.gov/books/NBK557831/

24. Andrews, J. M. (2001). Determination of minimum inhibitory concentrations. *The journal of antimicrobial chemotherapy*, *48*(1), 5-16. https://doi.org/10.1093/jac/48.suppl_1.5

25. Ramalingam, B., & Das, S. K. (2016). Antibacterial Effects of Biosynthesized Silver Nanoparticles on Surface Ultrastructure and Nanomechanical Properties of Gram-

Negative Bacteria viz. Escherichia coli and Pseudomonas aeruginosa. *ACS applied materials and interfaces*, *8*(7), 4963-76. https://doi.org/10.1021/acsami.6b00161

26. Kumar A, Schweizer HP. Bacterial resistance to antibiotics: active efflux and reduced uptake. Adv Drug Deliv Rev (2005) 57(10):1486–513. doi:10.1016/j.addr.2005.04.004

27.Blair JM, Richmond GE, Piddock LJ. Multidrug efflux pumps in Gram-negative bacteria their role in antibiotic resistance. Future Microbiol (2014) 9(10):1165–77. doi:10.2217/fmb.14.66