

IDENTIFICATION OF PREVALENCE OF ANTIBIOTIC RESISTANCE IN BACTERIAL ISOLATES IN URBAN RESTROOMS

Submitted in partial fulfilment of the requirements for the award of
Bachelor of Science Degree in Microbiology.

By

ISHA MAYA S.P (40300024)

DEEBAK.R. S (40300007)



DEPARTMENT OF BIOTECHNOLOGY

SCHOOL OF BIO AND CHEMICAL ENGINEERING

SATHYABAMA

**INSTITUTE OF SCIENCE AND
TECHNOLOGY (DEEMED TO BE
UNIVERSITY)**

**ACCREDITED WITH GRADE "A" BY NAAC | 12B STATUS BY
UGC | APPROVED BY AICTE**

JEPPIAR NAGAR, RAJIV GANDHI SALAI, CHENNAI – 600119

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(DEEMED TO BE UNIVERSITY)

Accredited with Grade "A" by NAAC

JEPPIAAR NAGAR, RAJIV GANDHI SALAI, CHENNAI - 600 119
www.sathyabama.ac.in

DEPARTMENT OF BIOTECHNOLOGY

BONAFIDE CERTIFICATE

This is to certify that this Project Report is the bonafide work of **ISHA MAYA S.P. (REG NO-40300024)**, **DEEBAK R.S(REG NO-40300007)** who have done the Project entitled "**IDENTIFICATION OF PREVALENCE OF ANTIBIOTIC RESISTANCE IN BACTERIAL ISOLATES FROM URBAN RESTROOMS**" under our supervision from October 2022 to April 2023.

Internal Guide
DR.S.M. INDUMATHI

External Guide
Dr.R.P.OVIYA

HEAD OF THE DEPARTMENT
DR. RAMESH KUMAR. V

Submitted for Viva voce Examination held on 08-05-2023

Internal Examiner
External Examiner

DECLARATION

I , ISHA MAYA S.P. (REG NO-40300024) hereby declare that the Project Report entitled "IDENTIFICATION OF PREVALENCE OF ANTIBIOTIC RESISTANCE IN BACTERIAL ISOLATES FROM URBAN RESTROOMS" is done by me under the guidance of Dr.R.P.Oviya at Department Of Biotechnology is submitted in partial fulfillment of requirements for the award of Bachelor Of Science in Microbiology.

DATE : 06-05-2023



PLACE : Chennai

SIGNATURE OF THE CANDIDATE

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ABSTRACT

The aim of this study was to determine the prevalence of antibiotic-resistant bacteria, especially *Staphylococcus aureus* and *Escherichia coli* in samples collected from urban restrooms, in Chennai. Samples were collected via swab methods from various regions inside urban public restrooms. Antibiotic susceptibility was determined using the disk diffusion or agar dilution methods. Additionally covalent nickel nanoparticle as well as bivalent copper nickel nanoparticles were used in determining antibacterial susceptibility. Results: 524 Bacterial Isolates were isolated totally from various public restrooms, among these 524 isolates 230 isolates were found to be *Staphylococcus sp* and the remaining 294 isolates were *Escherichia coli* commonly known as *E. coli*. Approximately 73.24% of the bacterial isolates collectively were resistant to the third-generation cephalosporin derivative antibiotics (Cefepime, Ceftazidime, Ceftriaxone, Amikacin). So, they were subjected again to both monovalent and bivalent nanoparticles of Nickel and Copper nickel respectively. Final results showed that bivalent nanoparticles proved useful against these bacteria as they showed 89.53% sensitivity. Hence proving effective against these common bacterial species.

Nickel nanoparticle and bivalent Cu-Ni nanoparticle were analyzed for its potential in targeting such MDR bacteria.

These MDR isolates were treated with Ni nanoparticle. 100 MDR isolates showed moderate mild sensitivity to Ni nanoparticle. Further we analyzed Cu-Ni bivalent nanoparticle which showed effects toxicity against these MDR isolates. Cu-Ni nanoparticles effecting is validated in 524 isolates. This binary nanoparticle can further be utilized alone or in combination with other drug for the treatment of infection caused by the MDR bacteria.

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LIST OF ABBREVIATION

MSA	Mannitol Salt Agar
EMB	Eosin Methylene Blue
MR	Methyl Red
VP	Voges Proskauer
MHA	Muller Hinton Agar
AR	Antibiotic Resistant
XDR	Extensively Drug Resistant
TDR	Total Drug Resistant
WHO	World Health Organization
NAP	Indian National Action plan
GLASS	Global Antimicrobial Surveillance System
MRSA	Methicillin Resistant Staphylococcus Aureus
NP	Nano Particle
Cu-Ni	Copper -Nickel
EPS	Exo Polysaccharide
CLED	Cystine Lactose Electrolyte Deficient Agar
CAUTI	Catheter Associated Urinary Tract Infection

CHAPTER – 1

INTRODUCTION

Infections can be treated with antibiotics, which have also saved countless lives. But whenever antibiotics are administered, they run the risk of having negative side effects and fuelling antibiotic resistance, one of the biggest risks to the general public's health. The perks of using antibiotics typically outweigh the dangers of adverse reactions or resistance to antibiotics. However, excessive antibiotic prescriptions and overuse endanger the effectiveness of these vital medications. To prevent harm from unwarranted antibiotic usage and to fight antibiotic resistance, it is crucial that we all only use antibiotics when necessary.

1.1 Introduction to Antibiotics

An antibiotic is a particular class of antimicrobial agent that works against bacteria. The use of antibiotic drugs is common in the treatment and prevention of bacterial infections since they are the most effective form of antibacterial agent. Bacterial growth may be prevented or killed by them. Antiprotozoal action is only present in a small number of antibiotics. Antibiotics are ineffective against viruses like the flu or the common cold; as a result, antiviral medicines or antivirals are used instead of antibiotics to describe medications that prevent the growth of viruses. They are also ineffective against fungi; antifungal medications are those that stop fungi from growing. Since the beginning of time, people have utilized antibiotics. Numerous allusions to the therapeutic benefits of moldy bread come from ancient Egypt, Nubia, China, Serbia, Greece, and Rome, among other cultures. John Parkinson (1567-1650) was the first to formally record the use of molds to cure illnesses. In the 20th century, antibiotics revolutionized medicine. Modern penicillin was developed in 1928 by Alexander Fleming (1881–1955), and its widespread usage was extremely helpful throughout the war. But since antibiotics are so powerful and accessible, certain bacteria have developed resistance to them due to abuse. Antimicrobial resistance is a widespread "serious threat that is no longer a

prediction for the future, it is happening right now in many parts of the world," according to the World Health Organization.

1.2 HISTORY OF ANTIBIOTICS

Prior to the early 20th century, traditional medicine was mostly used to cure infections. Over 2,000 years ago, mixtures having antibacterial characteristics that were used to treat infections were documented. Infections were treated with carefully chosen mould and plant materials by many ancient societies, including the ancient Egyptians and the ancient Greeks. Tetracycline in Nubian mummies was found to be present in large amounts during 1990s research. It was assumed that the source was the beer that was being brewed at the time. When synthetic antibiotics made from dyes were discovered, modern medicine started using them. The number of new antibiotic compounds used in medicine considerably grew in the middle of the 20th century. There were 12 new classes introduced between 1935 and 1968.

1.3 Antibiotic Resistance

Antibiotic resistance is a severe danger to our capacity to treat common infectious illnesses and is a fast-growing worldwide health concern. It is becoming more challenging to properly treat an expanding number of diseases, including pneumonia, TB, blood poisoning, gonorrhoea, and foodborne illnesses, since new mechanisms of resistance are developing and spreading globally. The problem is getting worse because of antibiotic abuse and overuse, such as buying antibiotics without a prescription or overprescribing by medical professionals and veterinarians. Antibiotics are frequently overused by the general people in nations without established treatment protocols. If we don't act right now, we could live in a post-antibiotic world when diseases that were once curable will once again be fatal.

Antibiotic resistance is a serious issue that is aggravated by the overuse and misuse of antibiotics, as well as inadequate measures for preventing and controlling infections. To address this problem, we must take proactive steps at all levels of society, from individuals to healthcare providers and policymakers. These steps may include promoting the responsible use of antibiotics, adopting better infection prevention and

control practices, and investing in research and development of new antibiotics. By working together, we can reduce the impact of antibiotic resistance and limit its spread. The development of antibiotic resistance is leading to increased healthcare costs and economic burden on families and societies. When common infections become resistant to first-line antibiotics, more expensive medicines must be used, leading to longer illness duration.

This scenario is not only financially costly, but it also puts the achievements of modern medicine at risk. Medical procedures such as organ transplantations, chemotherapy, and surgeries like caesarean sections become more dangerous without effective antibiotics to prevent and treat infections. We must take urgent action to address antibiotic resistance and preserve the effectiveness of antibiotics.

One of the most unexpected findings from the sequencing of microorganisms' genomes is that many bacteria and fungi encode a far higher number of NP pathways than they can actually produce in the lab. A large effort has been made to develop tools and techniques to activate their "cryptic" biosynthetic gene clusters (BGCs) in the hope of discovering novel chemical scaffolds with beneficial bioactivities. In general, at least three quarters of their potential NP capability is not switched on in vitro. Silent BGCs are shown to encode functioning NP biosynthesis pathways when they are triggered or produced heterologous in several studies. This implies that host organisms or environmental stimuli are what cause these chemicals to be produced.

Antimicrobial Resistance:

Antimicrobial resistance is a serious global health crisis and one of the most significant threats to human health. Some bacterial strains have developed resistance to nearly all existing antibiotics, which makes the development of new antibacterial agents essential to overcome resistant bacteria. To address this issue, the World Health Organization (WHO) published a list of antibiotic-resistant priority pathogens in 2017, which presents a significant threat to human health and urgently requires new antibiotics. The list is categorized into critical, high, and medium priority to guide and promote the research and development of new antibiotics. It's crucial to take prompt action to address this

issue to safeguard human health and wellbeing. When bacteria, fungi, parasites, and viruses develop over time in such a way that antimicrobial medications that once worked against them do not, antimicrobial resistance (AMR) results. Drug resistance causes illnesses to spread and become more challenging to cure. All nations are impacted by AMR, albeit some are more affected than others. Sub-Saharan Africa in particular might be disproportionately burdened. Furthermore, recent studies show that there is a dearth of high-quality information regarding AMR and infectious illnesses in low-income environments, which limits our understanding.

1.4 Introduction to Nano Particle [NPs]:

A relatively new area of chemical study is nanoparticle chemistry. Even though nanoparticles, mostly in the form of dust and smoke, have always existed in nature, these statements would have sounded perplexing to many scientists even 30 years ago. Long before their origins and characteristics were discovered and understood, nanoparticles were used in stained glass, paints, and building materials. For more than a century, petrochemical industries made significant profits from the widespread use of transition metal nanoparticles as heterogeneous catalysts. Despite these ubiquitous examples, nanoparticle chemistry did not develop into a rigorous academic field until the final decades of the 20th century, when the development of electron microscopy and other cutting-edge characterization methods provided researchers with the instruments necessary for studying objects with a diameter of less than one nanometer. Undoubtedly, the most researched category of nanoparticle systems is metal nanoparticles. Early efforts include Michael Faraday's 1850s synthesis of colloidal gold, which is among the first works. In 1908, Mie wrote on how light and metal nanoparticles interact. There are instances of ancient Romans creating stained glass with gold nanoparticles. The development of nanoparticle plasmonic in recent years has made it possible to precisely design plasmonic characteristics through chemical synthesis. The catalytic capabilities of metal nanoparticles have been the subject of several recent investigations. The frequency of surface plasmon resonance in gold and silver nanoparticles or luminescence polarisation in semiconductor nanorods are just two examples of the numerous instances in which a nanoparticle's form directly affects its

physical characteristics. Wang, Dong, and Buhro cover the chemistry, physics, and applications of one-dimensional nanomaterials, such as semiconductor discs and nanoplatelets, while Nasilowski, Mahler, Lhuillier, Ithurria, and Dubertret cover the chemistry, physics, and applications of two-dimensional nanomaterials, such as semiconductor discs and nanoplatelets, produced through the chemical process known as solution-liquid-solid synthesis. As emitters for screens and optical gain medium for lasers, these materials have a lot of potential. Another fascinating family of hollow nanostructures is covered by Wang, Feng, Bai, Zhang, and Yin. Hollow nanoparticles of plasmonic metals and nano shells exhibit plasmon resonance in the near-IR spectral range, making them suitable for photothermal cancer therapy. Hollow nanostructures are also being explored for drug delivery.

1.4.1 Nickel Nanoparticle:

Nickel nanoparticles are an excellent illustration of a reactive nanoparticle that is highly redox active and is significantly more inflammatory in the rat lung than NiO at the micron scale. When implanted in the lung, nickel oxide nanoparticles produce a gradual lung damage marked by fibrosis and alveolar lip proteinosis, in contrast to titanium dioxide, which had no inflammatory effects at the same instilled surface area dose, emphasising the significance of the reactive surface.

2. PRINCIPLE OF ANTIBIOTIC RESISTANCE IN BACTERIA:

Investigations of antibiotic resistance from an environmental perspective have provided insight on an issue that was previously thought to only affect a small group of clinically important antibiotic-resistant bacterial pathogens. It is evident that the ambient microbiota possesses a huge variety of antibiotic resistance genes, some of which are very comparable to the genes present in pathogenic microbiota, even in situations that appear to be antibiotic-free. From an anthropocentric point of view, which is concentrated on clinical elements like the effectiveness of antibiotics in eradicating illnesses and organisms that are resistant to antibiotic treatment, it is challenging to explain the function of antibiotics and antibiotic resistance in natural ecosystems. a

more comprehensive analysis of the function of antibiotics and antibiotic resistance in nature from an evolutionary and ecological perspective

2. MECHANISM OF ANTIBIOTIC RESISTANCE

It is well acknowledged that the emergence of resistance among the most significant bacterial diseases poses a danger to human health on a global scale. In addition to developing in hospitals, multidrug-resistant organisms are increasingly often found in community settings, indicating that reservoirs of antibiotic-resistant bacteria exist elsewhere. The apex of evolution and the best-known example of bacterial adaptation is the bacterial reaction to the "attack" of antibiotics. "Survival of the fittest" is a result of the enormous genetic plasticity of bacterial pathogens, which causes specific responses to lead to mutational adaptations, the acquisition of genetic material, or changes in gene expression, producing resistance to almost all antibiotics currently used in clinical practice. Therefore, it is crucial to comprehend the molecular and genetic underpinnings of resistance.

2. MUTATION AND EVOLUTION OF ANTIBIOTIC RESISTANCE:

The emergence and spread of antibiotic resistance have traditionally been attributed to a natural selection process that was influenced by the usage of antibiotics. Bacteria that must contend with antibiotic challenges adapt to become resistant, and because only the strongest survive under this intense selection pressure, resistance mechanisms and resistant clones propagate. The primary method of acquiring antibiotic resistance appears to be horizontal transfers of resistance mechanisms. Nevertheless, the environment has changed because of recent research on the hypermutability and antibiotic-induced hypermutation of bacteria. Here, we discuss the most recent information on the molecular processes underlying bacterial hypermutability and its connection to the development of antibiotic resistance. In our final section, we go through the potential that medications could not just select for antibiotic resistance.

2. ANTIMICROBIAL RESISTANCE:

Antimicrobial resistance happens when germs like bacteria and fungi develop the ability to defeat the drugs designed to kill them. That means the germs are not killed and continue to grow. Resistant infections can be difficult, and sometimes impossible, to treat. They are the main drivers in the development of drug-resistant pathogens. Lack of clean water and sanitation and inadequate infection prevention and control promotes the spread of microbes, some of which. Antimicrobial resistance is a serious global health crisis and one of the most significant threats to human health. Some bacterial strains have developed resistance to nearly all existing antibiotics, which makes the development of new antibacterial agents essential to overcome resistant bacteria. To address this issue, the World Health Organization (WHO) published a list of antibiotic-resistant priority pathogens in 2017, which present a significant threat to human health and urgently require new antibiotics. Antimicrobial resistance (AMR) is a global health and development threat. It requires urgent multisectoral action in order to achieve the Sustainable Development Goals (SDGs). WHO has declared that AMR is one of the top 10 global public health threats facing humanity. Misuse and overuse of antimicrobials can be resistant to antimicrobial treatment. The cost of AMR to the economy is significant. In addition to death and disability, prolonged illness results in longer hospital stays, the need for more expensive medicines and financial challenges for those impacted. Without effective antimicrobials, the success of modern medicine in treating infections, including during major surgery and cancer chemotherapy, would be at increased risk.

The clinical pipeline of new antimicrobials is dry. In 2019 WHO identified 32 antibiotics in clinical development that address the WHO list of priority pathogens, of which only six were classified as innovative. Furthermore, a lack of access to quality antimicrobials remains a major issue. Antibiotic shortages are affecting countries of all levels of development and especially in health-care systems.

Antibiotics are becoming increasingly ineffective as drug resistance spreads globally leading to more difficult-to-treat infections and death.

CHAPTER – 2

REVIEW OF LITERATURE

Antibiotics are powerful therapeutic drugs and microbes as infection-causing agents have greatly improved public health in many regions of the world. The huge threat to public health posed by antibiotic resistance is being brought to the fore by health organisations all over the world. Some life scientists think that the post-antibiotic era is just around the corner. Antibiotic resistance is becoming a greater danger as a result of multiple mechanisms having biological, behavioural, and societal components. This primer gives a general overview of antibiotic resistance and its growing impact on public health, as well as an explanation of the biological and behavioural factors that contribute to antibiotic resistance. It also gives examples of how health communication specialists can help prolong the effectiveness of our current antibiotics, as described by Rachel A. Nkuchia M. M'ikanatha and Andrew F. Read et al (2014).

This study's objective was to carry out a comprehensive evaluation of research that has examined the incidence of antibiotic resistance in *Escherichia coli* in various geographic locations. A systematic Medline search was used to find studies on the prevalence of *E. coli* resistance that were published between 1970 and 2006. These studies were then analysed with regard to the parameters of the study design, study population, technique of resistance detection, and prevalence of resistance. In various cultures and nations, the frequency of antibiotic resistance ranged from 0 to 100% and was quite variable. Studies from Middle and South America, Spain, Turkey, and other countries found greater rates of resistance than those from the United States and Central Europe. Additionally, a propensity for greater incidence rates. Antibacterial resistance is favoured by mutations, obtaining new genetic material, coming into contact with cells that have it, and the use of antibacterial drugs as growth promoters in animal feeds intended for human consumption. However, as proven by Vila and Pau et al (2010).

The antibiotics distributed in these nations are of poor efficacy due to medication dosage degradation or change, reduced active component concentrations, or both (Lewis et al 2013). For instance, low concentrations of the usual concentrations of

tetracycline and ampicillin have been discovered in Algeria (Reyes et al. 1997). Additionally, poor nations have found pharmaceuticals that have had their expiration dates changed or eliminated (Pecoul et al. 1999). When distributed in underdeveloped nations, several medications made in industrialised nations have been discovered to have expired (Reyes et al. 1997). Last but not least, the antibiotics supplied to these nations may have been improperly carried and kept, resulting in inactivation and low efficacy (Pecoul et al. 1999). Drug resistance is still a global setback in the management of many bacterial infectious diseases. The burden is more pronounced in developing countries where infectious diseases are rampant. It is estimated that globally 26% of death are due to infectious diseases of which 96% occur in developing countries. East Africa and Africa in general consist of low-income countries and thus bear the highest encumbrance of the impact of infectious diseases. In East African countries, like many developing countries, there is the uncontrolled use of antibiotics mainly because of limited sources. Most clinicians opt for rational treatment of infections hence minimal bacteriological samples are taken for culture and sensitivity analysis before initiating patient treatment. In addition, there is extensive over-the-counter treatment with widespread self-medication by the community. With the convenience of travel across the world, this does not only remain an East African problem but rather a worldwide drawback particularly because these countries are frequently visited by citizens from high-income countries for reasons such as tourism business and diplomatic reasons. Therefore, they can easily acquire resistant strains of microorganisms and carry these strains to their countries of origin (Wamalwa et al. 2013).

Drug resistance is rising globally for both developing and developed nations, however, it is amplified and enhanced in underdeveloped nations. Due to budgetary limitations to applying established criteria linked to susceptibility testing, as described (Pecoul et al. 1999), there is little to no infection control. The abuse of antibiotics that results from this, along with the population's lower general education level and lower quality of living, has been observed by Vila and Pau, et al. (2010).

With activity against various chromosomal and plasmid-mediated β -lactamases of both Gram-positive and Gram-negative bacteria, clavulanate is a broad-spectrum

-lactamase inhibitor. Even though clavulanate exhibits only weak antibacterial activity in vitro, mounting evidence indicates that it might still have an impact on pathogenic bacteria, regardless of whether or not they produce -lactamases. It has been demonstrated that clavulanate binds to penicillin-binding proteins (PBPs) in both Gram-positive and Gram-negative bacteria, just like other -lactams do. In *Streptococcus pneumoniae*, it was discovered to bind to PBP3 in a specific manner. It has been proposed that the augmentation of the activity of other lactams by clavulanate is due to complementary binding to other PBPs and subsequent effects on autolysis. Additionally, studies with -lactamase-producing and co-amoxiclav have shown that co-amoxiclav increases the intracellular killing abilities of human polymorphonuclear cells (PMNs) (Jane Finlay *et al.*, 2003).

Spices have been shown to possess medicinal value, in particular, antimicrobial activity. This study compares the sensitivity of some human pathogenic bacteria and yeasts to various spice extracts and commonly employed chemotherapeutic substances. Of the different spices tested only garlic and clove were found to possess antimicrobial activity. The bactericidal effect of garlic extract was apparent within 1 h of incubation and 93% killing of *Staphylococcus epidermidis* and *Salmonella typhi* was achieved within 3 h. Yeasts were totally killed in 1 h by garlic extract but in 5 h with clove. Some bacteria showing resistance to certain antibiotics were sensitive to extracts of both garlic and clove. The greater anticandidal activity was shown by garlic than by nystatin. Spices might have great potential to be used as antimicrobial agents. (P.Valentine *et al.*, 2019).

n-Alkyltrimethylammonium bromides (CnTAB) have been synthesized with n-alkyl chain lengths of between C₅ and C₂₂. Antimicrobial activity was assessed as growth inhibitory activity (MIC) towards seven representative strains of microorganisms and as bacterial activity towards *Staphylococcus aureus*, *Saccharomyces cerevisiae* and *Pseudomonas aeruginosa*. These data indicated that whilst the level of activity was generally parabolically related to n-alkyl chain length, separate dependencies existed for short ($n < 10$) and long ($n > 10$) chain substituents, with disproportionately large increases in activity, in the order of three log cycles, occurring as substituent chain lengths

increased from $n = 10$ to $n = 12$. This is interpreted in terms of distinct mechanisms of action, binding site and/or physicochemical properties for extreme members of the series (P. Gilbert et al., 2018).

Nanoparticles (NPs) are increasingly used to target bacteria as an alternative to antibiotics. Nanotechnology may be particularly advantageous in treating bacterial infections. Examples include the utilization of NPs in antibacterial coatings for implantable devices and medicinal materials to prevent infection and promote wound healing, in antibiotic delivery systems to treat disease, in bacterial detection systems to generate microbial diagnostics, and in antibacterial vaccines to control bacterial infections. The antibacterial mechanisms of NPs are poorly understood, but the currently accepted mechanisms include oxidative stress induction, metal ion release, and non-oxidative mechanisms. The multiple simultaneous mechanisms of action against microbes would require multiple simultaneous gene mutations in the same bacterial cell for antibacterial resistance to develop; therefore, it is difficult for bacterial cells to become resistant to NPs. In this review, we discuss the antibacterial mechanisms of NPs against bacteria and the factors that are involved. The limitations of current research are also discussed. Linlin Wang et al., 2017.

CHAPTER 3

AIM AND OBJECTIVES

1. AIM:

To identify the prevalence of drug resistance in bacterial isolates and identify the potential of nickel and copper as therapeutic strategies to treat drug-resistant bacteria.

2. OBJECTIVES:

- To isolate bacteria from public lavatories, especially high-risk *E.coli* by staph aureus.
- To perform biochemical characterization of the bacterial isolates.
- Bacteria isolated to the 4th generation.
- To study the resistance pattern to the 4th generation.
- To evaluate the impact of isolated bacterial species' susceptibility to silver nanoparticles.
- To evaluate the impact of an isolated bacterial species' susceptibility to a silver copper binary nanoparticle.

CHAPTER – 4

MATERIALS AND METHODS

4.1 COLLECTION OF SAMPLES:

The sample was collected in a public restroom by using a swab method in a square of 10cm x 10cm as shown in the diagram below. In this, swab method sterile, autoclaved buds were used to collect samples.

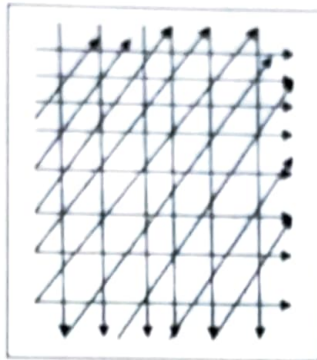


Fig 1: Swab culture method

1.1 SAMPLE PLATING:

The swab method was used during the plating of the sample.

1.2 SWAB METHOD:

- In the swab method, the following method is followed.
- Gently rub the end of the cotton swab with your sample across the agar.
- Use an 'S' pattern being careful not to rub so hard that the agar tear.
- Rotate the dish 90 degrees, and apply another 'S' pattern perpendicular to the first one.

- Replace the cover and seal the plate with masking tape or paraffin tape.
- Ensure to mark the dish with appropriate information.

1.3 MEDIA SELECTION

Growth media are used to support the development of microorganisms as well as to study the morphology of the target organism and to identify some of its more basic traits, such as the capacity to ferment sugars like lactose and the utilisation of citrate as a carbon source. We can also identify the bacteria by using differential media. For instance, *Escherichia coli* may be detected as growth with a metallic green sheen on Eosin Methylene Blue Agar and *Salmonella* can be identified as black colonies with a centre on *Salmonella* - *Shigella* (SS) Agar

1.3.1 MANNITOL SALT AGAR:

Mannitol salt agar was chosen as the primary isolation media. This media is used to isolate *Staphylococcus aureus*. The composition of Mannitol Salt Agar is as follows-

Pancreatic Digest of Casein	5.0gm
Peptic Digest of Animal Tissue	5.0gm
Sodium Chloride	75.0gm
Beef Extract	1.0gm
D – Mannitol	10.0gm
Phenol Red	0.025gm
Agar	15.0gm
Distilled water	1000ml

Final pH 7.4 +/- 0.2 at 25 degrees Celsius.

1.4.1.2. MANNITOL SALT AGAR:



FIG 5- *Staphylococcus aureus* on MSA.

Mannitol Salt Agar contains peptones and beef extract, which supply nitrogen, vitamins, minerals and amino acids essential for growth. The 7.5% concentration of sodium chloride results in the partial or complete inhibition of bacterial organisms other than staphylococci. Sodium chloride also supplies essential electrolytes for transport and osmotic balance. Mannitol is a fermentable carbohydrate, fermentation of which leads to acid production, detected by phenol red indicator, which aids in the differentiation of staphylococcal species. Agar is the solidifying agent. Coagulase-positive staphylococci (e.g., *Staphylococcus aureus*) produce yellow colonies and a surrounding yellow medium while Coagulase negative staphylococci produce red colonies and no colour change of the phenol red indicator.

1.3.2 MACCONKEY AGAR:-

MacConkey was chosen as the secondary isolation medium. Macconkey Agar is recommended for selective isolation and differentiation of *E.coli* and other enteric bacteria.

COMPOSITION:	Gms/Litre
Ingredients	
Pancreatic digest of gelatin	17.0
Peptones (meat and casein)	3.0
Lactose monohydrate	10.0
Sodium chloride	5.0
Bile salts	1.5
Neutral red	0.030
Crystal violet	0.01
Agar	13.5
Distilled water	1000 ml

pH after sterilization (at 25 degree Celsius) 7.1 +/- 0.2.

1.4 MOTILITY TEST:

An organism's capacity to move independently is known as motility. This ability can be facilitated by either specific fibrils that generate a gliding kind of motility or by the propeller-like flagella that are only seen in bacteria. Flagella, which can have a single flagellum or many flagella and extend from the cell wall and plasma membrane, are used by motile bacteria to move. It has long been understood that motility is a crucial taxonomic tool and biological trait of microbes. In the family Enterobacteriaceae, motility is a crucial component of identification since flagella are typically seen in bacilli, however there are a few flagellated cocci. The capacity of bacteria to move has been employed as a way of categorization and differentiation of organisms since the early days of the discipline of microbiology. Most frequently, a semi-solid agar medium is used to illustrate bacterial motility. Motile bacteria's 'swarm' and produce a diffuse, spreading growth on semi-solid agar media that is visible to the unaided eye. SIM medium (Sulphide Indole Motility medium, a combined differential media that examines three separate parameters: Sulphur Reduction, Indole Production, and Motility—is the primary medium used for this purpose. These media are extremely soft, which makes it

easy for germs to move through them and cause cloudiness. Agar that is somewhat solid is poked in the centre with the inoculum. A widespread zone of growth extending from the line of injection indicates bacterial movement. While some organisms spread out over the whole medium, others appear as nodules or tiny regions that extend from the inoculation line. Only the region where they are infected, and the soft agar tube will the non- motile bacteria grow there.

Method:

A colony of a young (18 to 24 hour) culture developing on an agar medium should be touched with a straight needle.

Stab the tube once in the centre at a depth of only 1/3 to 1/2 inch. When removing the needle from the medium, make sure to keep it in the same line that it entered. For up to 7 days, incubate at 35°–37°C and check every day. Keep an eye out for a widespread growth zone that flares out from the inoculation line. In a laboratory context, it is used to separate bacteria according to their level of motility. It is done to categorise organisms according to their taxonomic group.

Testing for pathogen mobility is crucial for pathogen characterisation.

The assays are often used in Enterobacteriaceae family identification techniques. The motility test is also used to distinguish between various kinds of enterococci, which are gram-positive cocci. While *E. gallinarum* and *E. casseliflavus* /*E. flavescens* are typically motile, *Enterococcus faecium* and *E. faecalis* are not. In order to achieve an accurate diagnosis, certain organisms may not exhibit enough growth in this medium, necessitating extra follow-up testing. For comprehensive identification, it is advised to run biochemical, immunological, molecular, or mass spectrometry tests on colonies from pure cultures. If bacterial flagella are harmed by heating, shaking, or other stress, false-negative results might happen. The creature will become immobile as a result of this environmental stress. Weakly motile organisms can cause false- negative responses. It is crucial to withdraw the inoculating needle exactly along the same line that was used to inoculate the medium when using semi-solid media. Growing along the stab line may be caused by a fanning action, which might lead to a false-positive

interpretation. These biochemical tests are now routinely used in a variety of fields, including forensic science, where microbial investigation can help with the investigation of bioterrorism threats, healthcare, where they are used to help with disease diagnosis, epidemiology, where they are used to track and trace disease outbreaks, pharmaceutical, where they are used to analyse environmental microbes that may have health implications, and these tests in the fields of healthcare, epidemiology, and forensic science.

1.6 Gram Staining:

A Gram stain test looks for bacteria in areas where an infection is thought to have occurred, including the throat, lungs, genitalia, and skin wounds. Gramme stains may also be used to examine the presence of germs in bodily fluids like blood or urine. Gram-positive and Gram-negative infections are the two primary subtypes of bacteria. Based on how the bacteria respond to the Gramme stain, the several types are identified. The colour of a Gramme stain is purple. The bacteria in a sample will either stay purple or change pink or red when the dye and bacteria interact. The bacteria are Gram-positive if they continue to be purple. The bacteria are Gram-negative if they become pink or crimson. Different forms of illnesses are brought on by the two categories: Among gram-positive infections include toxic shock, strep infections, and methicillin-resistant *Staphylococcus aureus* (MRSA). *Salmonella*, pneumonia, and gonorrhoea are a few examples of gram-negative infections. The most frequent test to determine whether you have a bacterial illness is a Gramme stain. The test will indicate whether your infection is Gram-positive or Gram-negative if you do. Fungal infections can also be identified with a Gramme stain. Blood will be drawn from a vein in your arm by a medical professional. To collect a sample from the tonsils and back of the throat, your doctor will place a special swab into your mouth. If bacteria were discovered, they would likely exhibit specific characteristics that might reveal crucial details about your infection. The presence of purple-colored bacteria indicates that you most likely have a Gram-positive infection. The presence of pink or red bacteria indicates that you most likely have a Gram-negative illness.

1.7 Biochemical Test :

The crucial details required for correctly identifying the genera of distinct bacteria inside a sample might be revealed through biochemical reactions. Bacteria naturally create enormous amounts of enzymes, and it is because to these enzymes that they may be identified using biochemical techniques. Since bacteria have unique enzymatic profiles, it is typically possible to identify a bacterium by the type of enzymes that it produces. Each type of bacterial species has distinct metabolic requirements that are satisfied by various enzymes. Catalase, gelatinase, oxidase, and urease, for instance, can be used to determine the type of bacteria present. The presence of such microorganisms is necessary for the biochemical processes employed in biochemical testing.

PRINCIPLE AND INTERPRETATION:

The vital minerals, vitamins, and nitrogenous elements necessary for the development of microorganisms are provided by the pancreatic digest of gelatin and peptones (meat and casein). The carbohydrate that can be fermented is lactose monohydrate. The crystal violet and bile salts, which are inhibitory to the majority of gram-positive bacteria, are credited with the selective action of this medium. The osmotic equilibrium of the medium is kept in check by sodium chloride. *Escherichia coli* is subcultured on Macconkey Agar following enrichment in Macconkey broth. The capacity of gram-negative bacteria to digest lactose distinguishes them from other types and helps them grow effectively on the medium. Lactose-fermenting strains develop as red or pink growth and might have an acid-precipitated bile zone surrounding them.

Because lactose produces acid, the colour is red.

Final pH (at 25 degree Celsius) is 7.3 +/- 0.1.

1.7.1 CATALASE TEST:

Hydrogen peroxide (H_2O_2) is broken down into oxygen and water by catalase. A tiny inoculum of a bacterial isolate is added to a 3% hydrogen peroxide solution to determine if the isolate can generate the catalase enzyme. It is noted for the quick development of oxygen bubbles. A lack of or poor bubble generation indicates the absence of catalase. Both strict aerobes and facultative anaerobes are bacteria that produce catalase. The use of oxygen as a terminal electron acceptor allows them all to breathe.

1.7.2 TRIPLE SUGAR TEST:

Casein and meat peptones, phenol red to indicate pH, 0.1% glucose, 1% lactose, and 1% sucrose are all ingredients in triple sugar iron agar. In order to identify the generation of hydrogen sulphide, ferric or ferrous ions and sodium thiosulphate are present. The initial yellow slant that non-lactose fermenting bacteria create is caused by the glucose's generation of acid. The little glucose that is present is quickly used up. After the low concentration of glucose has been used up, oxidation metabolism continues in the slant, creating an alkaline pH from the aerobic breakdown of peptone, which causes the slant to become red. The butt remains acidic and yellow because there is no oxidative metabolism or oxygen penetration. Thus, an alkaline (K) gradient rather than an acid is produced by a non-lactose fermenting bacterium.

1.7.3 INDOLE TEST:

When tryptophanase is present, the amino acid tryptophan goes through hydrolysis and deamination. Tryptophan undergoes reductive deamination, and indole is created as a by-product via the intermediary molecule indole pyruvic acid. The tryptophanase catalyses the removal of the amino group ($-\text{NH}_2$) from the tryptophan molecule during the deamination process. Pyridoxal phosphate is needed as a cofactor for the enzyme. The catalytic process yields indole, pyruvic acid, ammonium (NH_4^+), and energy as by products.

1.7.4 CITRATE TEST:

When an organic acid like citrate is utilised as a carbon and energy source, alkaline carbonates and bicarbonates are formed, raising the pH of the medium and causing the colour shift.

Citrate transport proteins (permeases) are necessary for the utilisation of exogenous citrate. Citrate is split into oxaloacetate and acetate by citrate lyase after being taken in by the cell. Following this, the oxaloacetate is converted to pyruvate and CO₂.

Oxaloacetate + acetate equals citrate.

Pyruvate plus CO₂ equals Oxaloacetate

1.7.5 METHYL RED TEST:

As a result of using glucose as fuel, certain bacteria end up producing acids such lactic acid (LA), acetic acid (AA), and formic acid (FA). The kind of bacteria engaged in the process determine the type of acid that is generated, and this is first turned into pyruvic acid from glucose. The PH of the medium is lowered by acid production.

As a result, the methyl red's hue shifts from yellow to red, suggesting the presence of bacteria that might use the glucose present in the culture material.

1.7.6 VOGES PROSKAUER TEST:

This is a development of the methyl red test, which finds organisms capable of producing the chemical butylene. Acetoin is the reaction's intermediate product, which may be identified by using alpha-naphthol and 40% KOH. Acetoin will oxidise to diacetyl if KOH is present. In the presence of alpha naphthol, diacetyl will react with the guanidine component of peptone, resulting in a red colour that denotes a successful test.

This is done via the VP test.

2. KIRBY BAUER DISC DIFFUSION METHOD:

The Kirby Bauer Disc Diffusion technique has historically been particularly well-liked in extremely busy microbiological settings due to its simplicity and convenience of usage. It was created in the 1950s and modified by W. Kirby and A. Bauer before being standardized by the World Health Organisation (WHO) in 1961. The fact that several medications may be tested simultaneously for their effectiveness against a specific illness makes it worse. The target bacterial isolate is seeded on Muller Hinton agar plates using the Kirby Bauer Disc method, which involves inserting antimicrobial discs. The antimicrobial ingredient diffuses into the agar, where it may or may not stop the bacterial growth as seen by the appearance of a zone surrounding the germs. Early in the 1950s, several laboratories began using disc diffusion tests instead of the time-consuming broth dilution approach. Antibiotic susceptibility testing is done using Kirby Bauer assays, sometimes referred to as the Disc diffusion test. In the 1960s, there was an issue due to a lack of standardisation, and Kirby and Bauer later evaluated the description. WHO standardised the process in 1961. The original technique is currently updated and modified by CLSI, ensuring global uniformity. This test is mostly used to evaluate how susceptible or resistant aerobes or facultative anaerobes are to various antibiotic classes.

MATERIALS REQUIRED:-

- Muller – Hinton agar plates ,
- 24 hours old cultures (*S. aureus* , *E.coli*) ,
- Antibiotics ,
- Ethanol ,
- Forceps ,

Procedure :

Before doing the test, sanitise the area and turn on the hob.

To remove extra medium, a sterile cotton swab is dipped into the inoculum and pressed against the tube wall.

Rotate the plate to completely swab its whole surface area. Lawn culture or carpet culture are two names for this method.

For the medium to properly absorb the inoculum, give the plates five minutes to dry.

Before picking up antibiotic discs, first sanitise the forceps with alcohol. Discs ought to be spaced apart by 24 mm. To prevent misplacement, lightly touch each disc with the

forceps to make sure it is in contact well. Turn the plate over and incubate it for 24 hours

at 37 °C.

KIRBY BAUER DISC DIFFUSION TECHNIQUE:

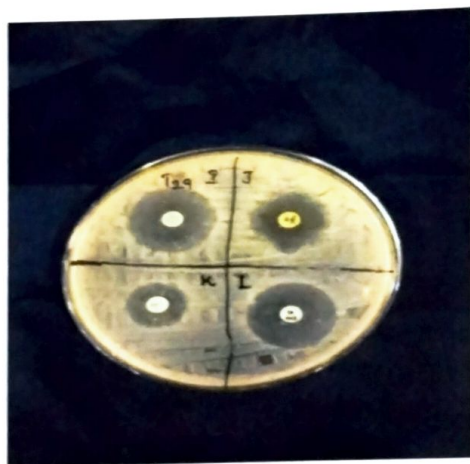


FIG 2 - ZONE OF INHIBITION

Early in the 1950s, several laboratories began using disc diffusion tests instead of the time-consuming broth dilution approach. Antibiotic susceptibility testing is done using Kirby Bauer assays, sometimes referred to as the Disc diffusion test. In the 1960s, there was an issue due to a lack of standardisation, and Kirby and Bauer later

evaluated the description. WHO standardised the process in 1961. The original technique is currently updated and modified by CLSI, ensuring global uniformity. This test is mostly used to evaluate how susceptible or resistant aerobes or facultative anaerobes are to various antibiotic classes. The objective of this test is it aids physicians to assist in selecting treatment options. The growth of organisms determines the ability of antibiotics to inhibit the organisms. The test organism must be cultured in broth for an entire night before being compared to the 0.5 McFarland Turbidity standard. Because it does not block sulphonamides and provides repeatability with the medium's composition and pH, Mueller-Hinton agar must be employed. When put onto Petri plates, the agar should be 4mm thick.

Materials Required

- Mueller- Hinton agar
- Antibiotic discs
- Cotton swabs
- Petri dishes
- 0.5 McFarland Turbidity standard
- Inoculum
- Forceps
- Metric ruler or caliper

Procedure to be followed:

Before doing the test, sanitise the area and turn on the hob.

To remove extra medium, a sterile cotton swab is dipped into the inoculum and pressed against the tube wall.

Rotate the plate to fully swab its whole surface area. Lawn culture or carpet culture are two names for this method.

For the medium to effectively absorb the inoculum, let the plates dry for five minutes to dry.

Before picking up antibiotic discs, first sanitise the forceps with alcohol.

Discs ought to be spaced apart by 24 mm.

To prevent misplacement, lightly touch each disc with the forceps to make sure it is in contact well.

Turn the plate over and incubate it for 24 hours at 37 °C.

Result Interpretation:

Use a metric ruler to measure the zone of inhibition and factor in the diameter of the disc after the 24-hour incubation period.

To report the outcome, compare it to the CLSI standards.

Susceptible (S), Intermediate (I), or Resistant (R) are the results' respective designations.

Advantages of Kirby Bauer Disc diffusion Technique are The results of this test are used to choose the best medications to treat an illness.

It can be helpful for choosing the right antibacterial agents and for monitoring antimicrobials. It may be performed without specific equipment and is interpretable by all medical staff. This test is less expensive to run.

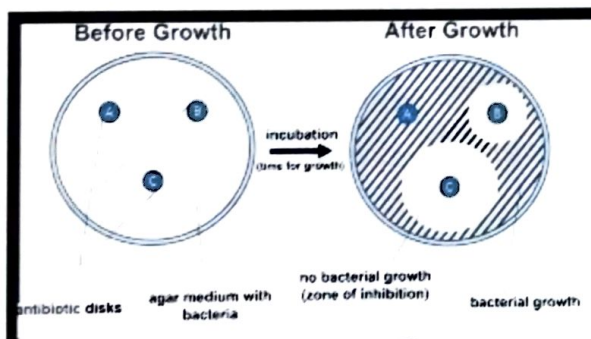


FIG 1- MULTIDRUG RESISTANCE

2.1 MEDIA USED IN KIRBY BAUER DISC DIFFUSION TECHNIQUE:

2.1.1 Mueller Hinton Agar:

In microbiology, Mueller Hinton agar is a type of growth media used to cultivate bacterial isolates and test them for antibiotic resistance. Microbiologists at Harvard University, John Howard Mueller and Jane Hinton, invented this medium for the first time in 1941. But in addition to agar to harden the combination, Mueller Hinton agar also contains beef extract, acid hydrolysate of casein, starch, and other ingredients. Mueller Hinton agar's make-up might differ depending on the supplier and the application, but in general, the medium is nutrient-rich and devoid of inhibitors that can obstruct bacterial growth. The disc diffusion method, a straightforward and well-liked technique for evaluating the antibiotic susceptibility of bacterial isolates, frequently employs Mueller Hinton agar. By placing tiny discs impregnated with various antibiotics on the agar surface, the zone of inhibition surrounding each disc is evaluated to ascertain the bacterial isolates resistance to that antibiotic. Due to its low concentration of calcium and magnesium ions, which might interfere with some antibiotics' effectiveness, Mueller Hinton agar is particularly helpful for evaluating a variety of medicines. As an alternative to the germ tube test, MH agar may be employed in the laboratory for the quick presumptive identification of *C. albicans*. The growth properties of bacterial isolates and how they react to drugs can both be influenced by the Mueller Hinton agar's composition. For instance, changes in the pH of the medium might impact how well some antibiotics work, and the presence of certain nutrients can encourage the development of a particular bacterial species. For precise microbiological experiments, Mueller Hinton agar must be carefully chosen and prepared. The creation of antibiotics and the investigation of antibiotic resistance have both benefited greatly from the use of Mueller Hinton agar.

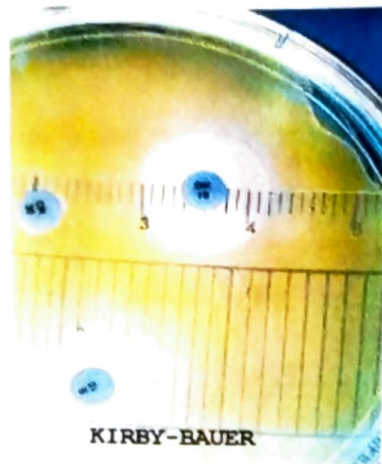


FIG 3-MEASUREMENT OF ZONE OF INHIBITION.

2.1.2 Composition of Mueller Hinton Agar:

Mueller–Hinton agar is a microbiological growth medium that is commonly used for antibiotic susceptibility testing, specifically disk diffusion tests. It is also used to isolate and maintain *Neisseria* and *Moraxella* species. It typically contains:

- 2.0 g beef extract
- 17.5 g casein hydrolysate
- 1.5 g starch
- 17.0 g agar
- 1 liter of distilled water.
- pH adjusted to neutral at 25 °C

Susceptibility tests on *Campylobacter* and *Streptococcus* species may also include the addition of nicotinamide adenine dinucleotide and 5% sheep's blood. It excels as an

antibiotic due to a few characteristics. It is a nonselective, nondifferential medium first and foremost. As a result, practically any organism that is plated on it will grow. Starch is also a component. Toxins generated by bacteria are known to be absorbed by starch, preventing them from interfering with antibiotics. It is a loose agar, secondly. Unlike most other plates, this enables greater antibiotic diffusion. A truer zone of inhibition results from improved diffusion. Mueller-Hinton agar was co-developed by a microbiologist.

John Howard Mueller and a veterinary scientist Jane Hinton at Harvard University as a culture for gonococcus and meningococcus. They co-published the method in 1941.

Mueller Hinton Agar is mostly used for assessing the

susceptibility of microbes to various antibiotics. The Bauer Kirby technique now uses it as the de facto standard medium, and the NCCLS specifies its performance. It may be utilised to grow Neisseria. It is outlined in the FDA Bacteriological Analytical Manual for food testing and typical techniques used on facultative anaerobic and aerobic microorganisms. The reason why MHA is used in antimicrobial susceptibility testing are It is a non-differential and non-selective medium. This implies that nearly every creature plated on this surface will flourish. It has starch in it. Toxins generated by bacteria are known to be absorbed by starch, preventing them from interfering with antibiotics. Additionally, it controls how quickly the medicines diffuse through the agar. It is a granular agar. Unlike most other plates, this enables greater antibiotic diffusion. A truer zone of inhibition results from improved diffusion. For susceptibility testing, MHA has adequate batch-to-batch repeatability. Sulfonamide, trimethoprim, and tetracycline inhibitor concentrations are low in MHA (i.e., thymidine and thymine inhibitor concentrations are low in MHA). Mueller Hinton Agar has the least amount of thymine/thymidine and para-aminobenzoic acid (PABA), respectively.

2.1.3 Preparation Of MHA:

38 grams of the medium should be dissolved in 1 litre of distilled water. For the medium to fully dissolve, heat it while stirring often and bring it to a boil for one minute 15 minutes at 121 °C in the autoclave. Until room temperature, cool. Mueller Hinton Agar that has cooled down should be added to sterile petri dishes on a flat, horizontal surface to ensure equal depth. Let the food reach room temperature. At 25°C, make sure the pH is 7.3 ± 0.1. Keep the plates between 2 and 8 °C.

2.1.4 Limitations of MHA:

The size of the inoculum, the pace of development, the composition of the medium, and the pH all have an impact on the outcomes. In order to guarantee

accurate findings, strict procedure adherence is necessary. Long incubation periods needed by sluggish growth may lead to drug inactivation. The results of the tests with *P. aeruginosa* isolates using aminoglycoside, tetracycline, and colistin are affected by variations in the concentration of divalent cations, particularly calcium and magnesium.

3. ANTIBIOTICS USED:

There are numerous techniques for testing an antibacterial's susceptibility.

However, Kirby Bauer Disc Diffusion was employed.

In this method the following antibiotics were used. These are Cephalosporins Derivatives.

- Cefepime
- Ceftazidime
- Ceftriaxone
- Amikacin

3.1 Cefepime

The rise of resistant organisms, such as certain *Enterobacteriaceae* that manufacture inducible and extended spectrum beta-lactamases, has been problematic as a result of the popularity of several third-generation cephalosporins. Cefepime has a number of benefits over earlier medications, including a twice-daily dose regimen, improved efficacy against *Enterobacteriaceae* and gram-negative pathogens. Comparative and noncomparative trials in the United States and Europe have proven cefepime's clinical effectiveness. Lower respiratory tract infections, urinary tract infections, skin and skin structure infections, and severe infections, including those with concurrent bacteraemia, have all responded well to cefepime with twice-daily doses. When given three times a day to febrile neutropenic patients, cefepime is equivalent to ceftazidime in terms of clinical and bacteriologic response rates. Cefepime works against forms that have resistance to other medications. Cefepime still has action against *E. cloacae* and *E. coli* strains resistant to other cephalosporins as well as against many *P. aeruginosa* bacteria resistant to ceftazidime, according to a number of investigations. The treatment of many patients receiving single-agent third-generation therapy is complicated because cefepime has a low level of cross-resistance with third-generation cephalosporins, a low propensity for selecting resistant mutants, and a low potential for inducing bacterial resistance. Considering the possibility for resistance, action against resistant organisms, and cost, cefepime should be used instead of ceftazidime.

3.2 Ceftazidime :

'Third generation' cephalosporin ceftazidime is a brand-new antibiotic used intravenously or intramuscularly. Like other third generation cephalosporins, it exhibits a wide range of in vitro activity against Gram-positive and Gram Negative aerobic bacteria. It is especially effective against *Enterobacteriaceae* (including strains that are beta-lactamase positive), and it is resistant to hydrolysis by the majority of beta-lactamases. In vitro ceftazidime is now the most effective cephalosporin against

Pseudomonas aeruginosa, however it is less effective than first and second generation cephalosporins against *Staphylococcus aureus*. Cefazidime appears to be equally effective to 'standard' comparative medications in treating complicated and/or chronic urinary tract infections in elderly and disabled patients, although only larger comparative trials are likely to detect any statistically significant differences in clinical efficacy that may exist between cefazidime and other antibiotics. In immunocompromised individuals, however, the coadministration of an antibiotic with better efficiency against Gram-positive bacteria should be taken into consideration. Cefazidime may be just as effective as aminoglycosides in treating intra-abdominal, obstetric and gynaecological, skin and soft tissue infections, according to the results of a small number of comparison trials. To clarify the comparative efficacy in these situations as well as in septicaemia/bacteraemia, meningitis, and bone and joint infections, more clinical experience is required, in particular a small number of well-planned comparative investigations.

3.3 Ceftriaxone :

With an extended half-life and a suggested once-daily administration regimen, ceftriaxone is a new "third generation" semisynthetic cephalosporin. It is given intravenously or intramuscularly and has a wide range of action against both Gram-positive and Gram-negative aerobic bacteria as well as certain anaerobic bacteria. Ceftriaxone has usually more antibacterial action against Gram-negative bacteria than the "first" and "second generation" cephalosporins, but less antibacterial activity against numerous Gram-positive bacteria than the previous generations of cephalosporins. Even though ceftriaxone has modest action against *Pseudomonas aeruginosa*, based on the available data, it cannot be suggested as the only antibiotic treatment for pseudomonal infections.

Ceftriaxone has shown success in treating infections brought on by additional "difficult" species such as multidrug-resistant *Enterobacteriaceae*. Ceftriaxone was successful in treating bacterial septicaemia, skin, soft tissue, bone, and joint infections,

complex and simple urinary tract infections, lower respiratory tract infections, and paediatric meningitis caused by susceptible microorganisms. Once-daily treatment seems effective in treating the majority of these illnesses. Results from a small number of patients with ear, nose, and throat infections, intra-abdominal infections, obstetric and gynaecological infections, and adult meningitis were likewise positive, but due to the limited experience, judgements about the drug's effectiveness in these indications are not yet achievable. Ceftriaxone has been demonstrated to be extremely successful when compared to conventional treatment for gonorrhoea caused by both non-penicillinase-producing and penicillinase-producing strains of *Neisseria gonorrhoeae*. The comparative effectiveness of ceftriaxone and other antibacterial in treating various infections and providing perioperative prophylaxis for patients having surgery has been evaluated in a few short trials. These comparative studies found few significant differences in response rates between therapeutic groups, but larger, well-designed studies are required to more clearly compare ceftriaxone's effectiveness to other antimicrobials, particularly aminoglycosides and other "third generation" cephalosporins, and to confirm the drug's apparent lack of serious side effects. Ceftriaxone will provide a significant alternative if more widespread use demonstrates its safety and effectiveness, especially for the treatment of serious infections caused by multidrug-resistant Gram-negative bacteria and in circumstances where the drug's prolonged half-life may provide valuable cost and convenience advantages.

3.4 Amikacin:

Since the 1940s, a class of antibiotics known as aminoglycosides has been used to predominantly treat a wide range of bacterial infections. Enzymatic modification by aminoglycoside-modifying enzymes, which are split into acetyl- transferases, phosphotransferases, and nucleotidyltransferases, is the main method of resistance against these antibiotics. In the 1970s, fresh semi- synthetic aminoglycosides were created to address this issue. Amikacin, the most often used semi-synthetic aminoglycoside, is resistant to the majority of aminoglycoside modifying enzymes. Amikacin was created by acylating the C1 amino group of the deoxystreptamine moiety

of kanamycin A with the 1 (-)-amino-hydroxy butyryl side chain. Aminoglycoside 6'-N-acetyltransferase type Ib [AAC(6')-Ib], an enzyme whose gene is located in integrons, transposons, plasmids, and chromosomes of Gram-negative bacteria, is the primary amikacin resistance mechanism observed in clinics. Finding methods to counteract the effects of AAC (6')-Ib and prolong the shelf life of amikacin is the topic of several research projects. It was discovered that in bacteria carrying the aac (6')-Ib gene, small compounds and complexes ionophore-Zn²⁺ or Cu²⁺ might impede the acetylation pathway and cause phenotypic conversion to susceptibility. Plazomicin, a novel semi-synthetic aminoglycoside, is now being developed and will help to rekindle interest in this class of antibiotics.

4. NANOFLUID PREPARATION FROM NANOPARTICLES:

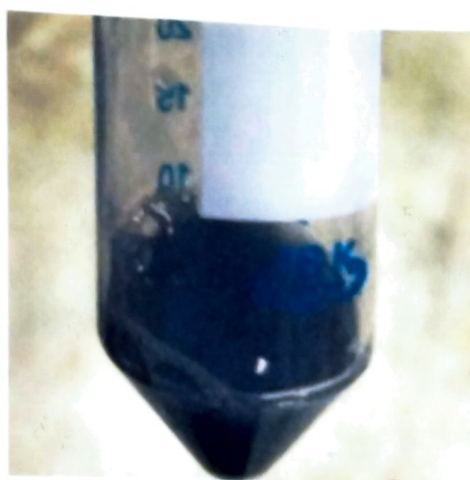


FIG 4- PREPARATION OF NANOPARTICLE

By spreading nanoparticles in the base fluid, nanofluids are created. For nanofluid to be used, good dispersion is necessary. As a result, surfactants are occasionally utilized to increase the stability of nanofluids. In addition, the surface of the scattered particles may be modified, and strong forces may be applied to the clusters of the dispersed nanoparticles, which may improve the stability of nanofluids. Physical one-step and

two-step procedures are the two main types of nanofluid preparation. Another new approach for creating nanofluids is the chemical process. The commonly used method in nanofluid preparation is the Two-step Method

Two- Step Method:

This is the most cost-effective way to prepare nanofluid on a wide scale. In a two-step procedure, the nanoparticles are first produced using a variety of techniques, and then they are distributed into the base liquid to create the required nanofluid. This production method is cheap and widespread. Nanoparticle aggregation is the two-step method's principal downside. The usage of surfactant is due to instability. This is how nanofluids are made commercially. This approach is preferred by the majority of researchers when creating nanofluids for study. Al_2O_3 /water nanofluid was made using a two-step technique by Zhu et al.

4.1 Sonication:

Better dispersion is achieved using the sonication approach than magnetic stirring, which also has a lower chance of particle agglomeration. Sonication uses ultrasonic waves to pass through a nanofluid. To create a more homogeneous suspension, sonication is helpful. Sonication outperforms magnetic stirring for uniform dispersion. There are two different kinds of sonicates: probe type and bath type. Better results are obtained with probe type sonication than with bath type sonication. When sonication is used, the ultrasonic waves cause the clumped nanoparticles to vibrate. The cavitation bubbles that are created during sonication expand till a critical condition. Hot spots are produced when there is a local temperature and pressure combination that is extremely high. The crucial state is when the hotspots are created. These heated areas disintegrate the agglomerated particles. If the concentration of nanoparticles rises, so does the nanofluid's thermal conductivity. The majority of the researchers' models for effective thermal conductivity are based on the concentration of nanoparticles. Researchers demonstrated both the linear and non-linear connection

between thermal conductivity and nanoparticle concentration. In the instance of the $\text{Fe}_2\text{O}_3/\text{water}$ nanofluid, Zhu et al. discovered a non-linear connection. CuO nanofluid was examined by Barbés et al., who discovered a linear connection. Ding et al. discovered a nonlinear rise in the thermal conductivity of MWCNT nanofluid; Xie et al. discovered a nonlinear connection in the case of glycol based CNT nanofluids.

4.2 Stability of Nanofluid:

For nanofluids to have the same thermophysical characteristics, stability is crucial. Electrical double layer repulsive force and Van der Waals attractive force are connected to the stability of nanofluid. To produce a stable nanofluid, the Electrical Double Layer Repulsive Force (EDLRF) must be greater than the Vander Waals attractive forces. Nanoparticle clustering is caused by the van der Waals attractive interactions that exist between them. If this force is strong, nanoparticles are separated from the base fluid and clustered nanoparticles fall to the bottom of the vessel under the influence of gravity. EDLRF, on the other hand, works in opposition to Van der Waals attraction, which keeps the particles apart.

4.3 Electron Microscopy Method :

Nowadays, the majority of researchers use electron microscopy in addition to other methods. Transmission Electron Microscopy (TEM) apparatus, which takes electron micrographs, is the advanced equipment required for this technique. This gadget takes pictures with a high resolution. If clusters of nanoparticles are found in the picture, sedimentation may occur. By using TEM, Zhu et al. [28] assessed the stability of Cu nanofluid. The similar approach was utilised by Jalal et al. [40] to assess the stability of $\text{ZnO}/\text{glycerol}$ nanofluid.

4.4 Antimicrobial activity of Nanofluids:

A major risk to human health is the sharp rise of pathogenic bacterial antibiotic resistance. According to a recent report by the Centres for Disease Control and Prevention, the world is about to enter a "post-antibiotic era" in which bacterial illnesses will be the leading cause of death rather than cancer. Nanoparticles (NPs) have recently come to light as innovative methods for fighting severe bacterial diseases. Strategies based on nanoparticles can get around problems that standard antimicrobials have, such as antibiotic resistance. Several nanoparticle-based strategies to treat bacterial infections have been emphasised in this tutorial review, giving important insight into the design factors that are essential in producing antimicrobial nanotherapeutics.

5.NICKEL:

Due to the ease with which their form, size, and chemical characteristics may be changed, nanoparticle research is becoming increasingly popular. Nanoparticles exhibit quantum phenomena and have surprising optical features. Nickel is occasionally found free in nature but is most frequently found in ores. In order to create corrosion-resistant alloys, it can be alloyed with iron, tungsten, molybdenum, chromium, and other metals. It is employed in many applications because it is electrically conductive. The forms of nickel nanoparticles include passivated, ultrahigh purity, high purity, coated, and dispersed nanofluids.

6.NICKEL -COPPER BIVALENT :

A simple chemical process was used to create nickel and copper nanoparticles, which were then tested for antibacterial properties. Copper and Nickel nanoparticles were discovered to be 24.00 nm and 13.13 nm in size, respectively, after being synthesised. The nickel and copper nanoparticles' crystal system is shown by the XRD examination. While nickel nanoparticles were monoclinic, copper nanoparticles were

discovered to be orthorhombic. Investigations were done on the antibacterial properties of nickel and copper nanoparticles dissolved in DMSO. Using the agar diffusion cup plate method, a comparative sensitivity test of these synthesised nanoparticles was conducted against three pathogenic microorganisms (Gram negative bacteria), namely *Escherichia coli*, *Klebsiella pneumoniae*, and *Pneumonia Typhus*. At 100 g/ml, copper and nickel nanoparticles demonstrated notable sensitivity against all test microorganisms. Copper nanoparticles were discovered to have a higher zone of inhibition (ZOI) than Nickel nanoparticles.

7. Antibacterial Resistivity determination of Nanoparticles:

The antibacterial activity of nickel as monovalent and nickel-copper as bivalent nanoparticles against pathogenic microorganisms was examined using the disc diffusion technique. These bacterial strains were cultivated in Luria UK broth for 24 hours using fresh cultures. Each bacterial culture was spread out across a layer of Mueller Hinton agar plates. On sterile, blank paper discs, four different concentrations of Ni and Ni-Cu nanoparticles were applied, and the combination was then incubated. The zone generation was seen after a 24- hour incubation period. The experiments went through several versions.

CHAPTER – 5

RESULTS & DISCUSSION

1. Prevalence of Bacterial isolates:

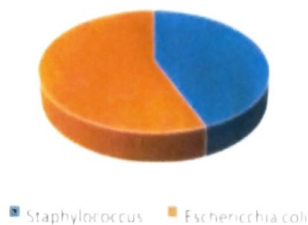
Numerous public bathrooms were used to obtain bacterial isolates. *Escherichia coli*, *Staphylococcus aureus*, and *Staphylococcus epidermis* were a few of the 70 bacterial species found in the diverse public lavatory settings along OMR, Chennai. Out of all the locations of the public restroom that were analysed, the bathroom floor and side tiles contained the most isolates ($n = 78\%$), whilst the wash basin had the fewest ($n = 22\%$). 524 Bacterial Isolates were isolated totally from various public restrooms. Among these 524 isolates 230 isolates were found to be *Staphylococcus sp* and the remaining 294 isolates were *Escherichia coli* commonly known as E.coli.

The following table represents the microbial population in a public lavatory at OMR, Chennai on a random day:

TABLE 5.1

Name of the Microorganism	No.of isolates obtained	Percentage
<i>Staphylococcus</i>	230	43.89%
<i>Escherichia coli</i>	294	56.10%

Microorganism load observed



Used organisms *staphylococcus* (230) and *E coli* (294) were obtained

1. Identification of Bacteria based on morphology:

The isolates were organized based on their colour and morphological characters.

TABLE 5.2

Microorganism	Colony Morphology
<i>Staphylococcus</i> sp	Golden coloured colonies on MSA Agar
<i>Escherichia coli</i>	Pink colored, Circular, slightly raised, Smooth colonies on MacConkey agar

3 . Biochemical Identification of Bacterial isolates:

Methods for sugar fermentation, microscopy, biochemistry, and colony morphology on various culture mediums. During this experiment, bacteria from the *Staphylococcus aureus* and *Escherichia coli* species were among those isolated. Lactose fermentation revealed *Escherichia coli* isolates as yellow colonies on CLED agar. Growing colonies had rounded, irregular edges, and were wet in some places but dry in others. Citrate-negative *E. coli* isolates might be identified by their green colour and the absence of citrate in their metabolism. Tryptophan in the media is broken down by the enzyme tryptophanase to produce indole. The success of the experiment was determined by adding Kovac's reagent, which interacts with indole to give a vivid pink colour.

S. no	Probable Organism	Oxidation Reduction	Cell Arrangement	Indole	Methyl Red	Voges Proskauer	Citrate Utilization on test	TSI Test	Catalase test	Motile or not
T1	Staphylo coccus	+	Cocci	+	-	+	-	+	+	+

T2	Staphylo coccus									
T3	Staphylo coccus	+	Cocci	+	-	+	-	+	+	+
T4	Staphylo coccus	+	Cocci	+	-	+	-	+	+	+
T5	Staphylo coccus	+	Cocci	+	-	+	-	+	+	+
T6	Staphylo coccus	+	Cocci	+	-	+	-	+	+	+
T7	Staphylo coccus	+	Cocci	+	-	+	-	+	+	+
T8	Staphylo coccus	+	Cocci	+	-	+	-	+	+	+

T9	Staphylo coccus	+	Cocci	+	-	+	-	+	+	+
T10	Staphylo coccus	+	Cocci	+	-	+	-	+	+	+
T11	Staphylo coccus	+	Cocci	+	-	+	-	+	+	+
T12	Staphylo coccus	+	Cocci	+	-	+	-	+	+	+
T13	Staphylococcus s	+	Cocci	+	-	+	-	+	+	+
T14	Staphylo coccus	+	Cocci	+	-	+	-	+	+	+
T15	Staphylo coccus	+	Cocci	+	-	+	-	+	+	+
T16	Staphylo coccus	+	Cocci	+	-	+	-	+	+	+
T17	Staphylo coccus	+	Cocci	+	-	+	-	+	+	+
T18	Staphylo coccus	+	Cocci	+	-	+	-	+	+	+
T19	Staphylo coccus	+	Cocci	+	-	+	-	+	+	+

T31	Staphylo coccus	+	Cocci	+	-	+	-	+	+	+
T32	Staphylo coccus	+	Cocci	+	-	+	-	+	+	+
T33	Staphylo coccus	+	Cocci	+	-	+	-	+	+	+
T34	Staphylo coccus	+	Cocci	+	-	+	-	+	+	+
T35	Staphylo coccus	+	Cocci	+	-	+	-	+	+	+
T36	Staphylo coccus	+	Cocci	+	-	+	-	+	+	+
T37	Staphylo coccus	+	Cocci	+	-	+	-	+	+	+
T38	Staphylo coccus	+	Cocci	+	-	+	-	+	+	+
T39	Staphylo coccus	+	Cocci	+	-	+	-	+	+	+

T40	Staphylo coccus	+	Cocci	+	-	+	-	+	+	+
T41	Staphylo coccus	+	Cocci	+	-	+	-	+	+	+
T42	Staphylo coccus	+	Cocci	+	-	+	-	+	+	+
T43	Staphylo coccus	+	Cocci	+	-	+	-	+	+	+
T44	Staphylo coccus	+	Cocci	+	-	+	-	+	+	+
T45	Staphylo coccus	+	Cocci	+	-	+	-	+	+	+
T46	Staphylo coccus	+	Cocci	+	-	+	-	+	+	+
T47	Staphylo coccus	+	Cocci	+	-	+	-	+	+	+
T48	Staphylo coccus	+	Cocci	+	-	+	-	+	+	+

T49	Staphylo coccus	+	Cocci	+	-	+	-	+	+	+
T50	Staphylo coccus	+	Cocci	+	-	+	-	+	+	+

T20	Staphylococcus	+	Cocci	+	-	+	-	+	+	+
T21	Staphylococcus	+	Cocci	+	-	+	-	+	+	+
T22	Staphylococcus	+	Cocci	+	-	+	-	+	+	+
T23	Staphylococcus	+	Cocci	+	-	+	-	+	+	+
T24	Staphylococcus	+	Cocci	+	-	+	-	+	+	+
T25	Staphylococcus	+	Cocci	+	-	+	-	+	+	+
T26	Staphylococcus	+	Cocci	+	-	+	-	+	+	+
T27	Staphylococcus	+	Cocci	+	-	+	-	+	+	+
T28	Staphylococcus	+	Cocci	+	-	+	-	+	+	+

T29	Staphylococcus	+	Cocci	+	-	+	-	+	+	+
T30	Staphylococcus	+	Cocci	+	-	+	-	+	+	+

Escherichia coli

S no	Probable Organism	Gram Reaction	Cell Arrangement	Indole	Methyl Red	Voges prausker	Citrate Utilization on test	TSI	Catalase test	Motility Test
T250	Escherichia coli	+	Cocci	+	-	+	-	+	+	+
T251	Escherichia coli	+	Cocci	+	-	+	-	+	+	+
T252	Escherichia coli	+	Cocci	+	-	+	-	+	+	+
T234	Escherichia coli	+	Cocci	+	-	+	-	+	+	+
T235	Escherichia coli	+	Cocci	+	-	+	-	+	+	+
T236	Escherichia coli	+	Cocci	+	-	+	-	+	+	+
T236	Escherichia coli	+	Cocci	+	-	+	-	+	+	+
T257	Escherichia coli	+	Cocci	+	-	+	-	+	+	+
T258	Escherichia coli	+	Cocci	+	-	+	-	+	+	+
T259	Escherichia coli	+	Cocci	+	-	+	-	+	+	+
T260	Escherichia coli	+	Cocci	+	-	+	-	+	+	+
T261	Escherichia coli	+	Cocci	+	-	+	-	+	+	+
T262	Escherichia coli	-	Rod	+	-	-	-	+	-	+
T263	Escherichia coli	-	Rod	+	-	-	-	+	-	+

T255	<i>Escherichia coli</i>	-	Rod	+	-	-	-	+	-	+
T256	<i>Escherichia coli</i>	-	Rod	+	-	-	-	+	-	+
T257	<i>Escherichia coli</i>	-	Rod	+	-	-	-	+	-	+
T258	<i>Escherichia coli</i>	-	Rod	+	-	-	-	+	-	+
T259	<i>Escherichia coli</i>	-	Rod	+	-	-	-	+	-	+
T260	<i>Escherichia coli</i>	-	Rod	+	-	-	-	+	-	+



Fig no 6- TRIPLE SUGAR TEST



Fig No 7- CITRATE TEST



Fig No 8- METHYL RED TEST.



Fig No. 3 Indole production test

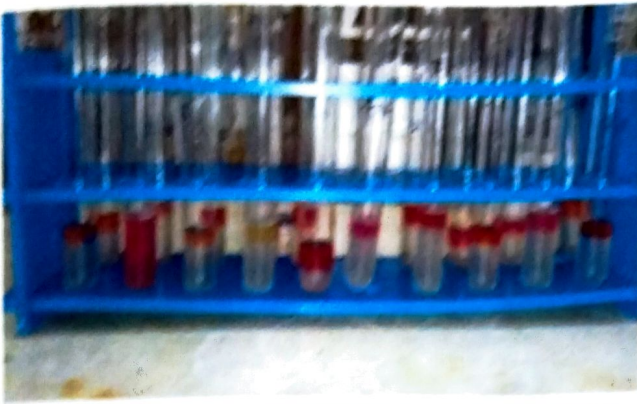


Fig No. 4 Indole test



Fig No. 5 Catalase test

Table 4.1

Date	Time	Tidegauge		Littorina		Littorina		Littorina	
		Time of initiation (min)		Time of initiation (min)		Time of initiation (min)		Time of initiation (min)	
1	1	1	1	1	1	20	0	12	0
	2	1	1	1	1	18	0	9	0
2	3	1	1	1	1	15	0	10	0
	4	1	1	1	1	12	0	15	0
3	5	1	1	1	1	10	0	10	1
	6	1	1	1	1	17	0	17	0
4	7	1	1	1	1	13	0	14	0
	8	1	1	1	1	15	0	12	0
5	9	1	1	1	1	12	0	6	0
	10	1	1	1	1	19	0	18	0
6	11	1	1	1	1	13	0	14	0
	12	1	1	1	1	0	0	11	0
7	13	1	1	1	1	0	0	10	0
	14	1	1	1	1	0	0	9	0

115	Staphylo coccus aureus	18	R	18	R	11	I	13	S
116	Staphylo coccus aureus	19	R	16	R	14	R	14	S
117	Staphylo coccus aureus	15	R	17	R	17	R	16	S
118	Staphylo coccus aureus	15	R	8	S	18	R	18	S
119	Staphylo coccus aureus	23	R	8	S	16	R	20	S
120	Staphylo coccus aureus	10	I	8	S	12	S	7	R

Escherichia coli

Sample no	Organism name	Ceftriaxime	S/R/I	Cefepime	S/R/I	Ceftazidime	S/R/I	Ampicillin	S/R/I
T300	Escherichia coli	9	S	20	R	18	R	13	I
T301	Escherichia coli	18	R	14	R	16	R	15	R
T302	Escherichia coli	13	R	14	R	15	R	15	R
T303	Escherichia coli	10	S	18	R	15	R	8	S
T304	Escherichia coli	10	S	3	S	17	R	9	S
T305	Escherichia coli	13	R	18	R	18	R	14	R
T306	Escherichia coli	10	S	19	R	12	I	16	R
T307	Escherichia coli	13	R	23	R	12	I	17	R
T308	Escherichia coli	17	R	23	R	12	I	9	S
T309	Escherichia coli	18	R	18	R	14	R	9	S
T310	Escherichia coli	19	R	19	R	16	R	12	R
T311	Escherichia coli	20	R	15	R	18	R	14	R
T312	Escherichia coli	17	R	16	R	3	S	16	R
T313	Escherichia coli	19	R	10	S	15	R	20	R

1314	Escherichia coli	17	R	10	S	2	S	14	I
1315	Escherichia coli	17	R	10	S	2	S	14	I
1316	Escherichia coli	18	R	14	R	17	R	16	R
1317	Escherichia coli	18	R	14	R	16	R	3	S

1318	Escherichia coli	18	R	19	R	2	S	12	R
1319	Escherichia coli	17	R	20	R	18	R	16	R
1320	Escherichia coli	15	R	20	R	13	S	17	R

Antibacterial Activity in Monovalent Nickel (Ni) Nanoparticle

Table 5.5-

Sample no	NPs used	D ilution				Zone of S/I/R				inhibition (mm)			
T1	Nickel	20	40	60	80	5	5	7	10	R	R	R	R
T2	Nickel	20	40	60	80	4	5	8	9	R	I	R	R
T3	Nickel	20	40	60	80	7	6	7	10	R	R	R	R
T4	Nickel	20	40	60	80	6	6	8	8	R	R	R	R
T5	Nickel	20	40	60	80	5	5	9	9	R	S	I	S
T6	Nickel	20	40	60	80	4	6	8	9	R	S	R	R
T7	Nickel	20	40	60	80	5	5	7	10	I	R	R	R
T8	Nickel	20	40	60	80	4	5	8	8	R	R	R	R
T9	Nickel	20	40	60	80	5	6	9	8	R	R	S	R
T10	Nickel	20	40	60	80	3	6	7	8	R	R	S	R
T11	Nickel	20	40	60	80	3	5	7	9	R	I	R	S
T12	Nickel	20	40	60	80	4	6	6	9	R	R	I	R
T13	Nickel	20	40	60	80	3	5	7	10	R	R	R	S
T14	Nickel	20	40	60	80	2	6	8	11	R	R	R	R
T15	Nickel	20	40	60	80	3	6	7	12	R	I	R	R
T16	Nickel	20	40	60	80	4	5	8	10	S	R	R	S
T17	Nickel	20	40	60	80	3	5	7	13	S	R	R	S
T18	Nickel	20	40	60	80	4	6	7	12	S	R	R	S
T19	Nickel	20	40	60	80	2	6	7	10	I	R	R	R
T20	Nickel	20	40	60	80	2	5	8	10	R	I	R	R
T21	Nickel	20	40	60	80	3	5	7	9	R	R	S	R
T22	Nickel	20	40	60	80	2	5	6	9	R	I	S	R
T23	Nickel	20	40	60	80	3	6	7	9	R	R	R	R

T24	Nickel	20	40	60	80	2	5	8	10	R	R	R	S
T25	Nickel	20	40	60	80	2	6	7	12	R	R	S	P
T26	Nickel	20	40	60	80	1	5	7	12	I	I	S	S
T27	Nickel	20	40	60	80	2	6	8	9	R		R	R
T28	Nickel	20	40	60	80	3	7	9	9	R	I	I	R
T29	Nickel	20	40	60	80	3	5	7	9	R	R	R	S
T30	Nickel	20	40	60	80	4	6	7	10	R	R	R	S
T31	Nickel	20	40	60	80	3	6	6	10	R	R	R	S
T32	Nickel	20	40	60	80	2	4	7	17	R	R	R	R
T33	Nickel	20	40	60	80	2	5	8	17	R	I	R	R
T34	Nickel	20	40	60	80	3	5	7	13	R	R	R	R
T35	Nickel	20	40	60	80	1	6	8	13	S	R	R	R
T36	Nickel	20	40	60	80	1	5	7	13	S	R	R	R
T37	Nickel	20	40	60	80	2	5	7	10	S	S	S	S
T38	Nickel	20	40	60	80	3	6	7	12	I	S	S	S
T39	Nickel	20	40	60	80	3	6	8	12	I	S	R	S
T40	Nickel	20	40	60	80	2	5	8	10	R	R	I	R
T41	Nickel	20	40	60	80	1	6	7	10	R	R	R	S
T42	Nickel	20	40	60	80	1	6	7	9	R	R	R	S
T43	Nickel	20	40	60	80	2	7	8	9	R	R	R	R
T44	Nickel	20	40	60	80	3	6	9	9	R	R	R	R
T45	Nickel	20	40	60	80	3	5	8	10	R	R	R	S
T46	Nickel	20	40	60	80	2	4	7	12	R	R	R	S
T47	Nickel	20	40	60	80	2	6	7	12	R	R	R	R
T48	Nickel	20	40	60	80	3	5	8	9	S	R	R	S
T49	Nickel	20	40	60	80	4	6	9	9	S	S	S	S
T50	Nickel	20	40	60	80	4	4	7	9	R	R	S	S
T51	Nickel	20	40	60	80	4	6	7	10	I	R	R	S
T52	Nickel	20	40	60	80	4	6	6	10	R	R	I	R

T53	Nickel	20	40	60	80	3	5	7	17	R	S	R	R
T54	Nickel	20	40	60	80	3	5	8	17	R	S	R	R
T55	Nickel	20	40	60	80	2	5	7	12	R	S	R	R

T56	Nickel	20	40	60	80	2	6	8	10	R	R	S	S
T57	Nickel	20	40	60	80	2	6	7	10	R	R	S	R
T58	Nickel	20	40	60	80	3	6	8	9	R	S	R	S
T59	Nickel	20	40	60	80	3	6	7	9	R	R	I	R
T60	Nickel	20	40	60	80	3	5	7	9	I	S	R	R
T61	Nickel	20	40	60	80	3	5	8	10	R	R	R	S
T62	Nickel	20	40	60	80	2	4	8	12	R	R	R	S
T63	Nickel	20	40	60	80	2	3	7	12	R	R	R	S
T64	Nickel	20	40	60	80	2	6	7	9	R	R	R	R
T65	Nickel	20	40	60	80	3	6	8	9	R	R	R	R
T66	Nickel	20	40	60	80	1	5	9	9	R	S	R	R
T67	Nickel	20	40	60	80	2	5	7	10	R	S	R	R
T68	Nickel	20	40	60	80	2	6	7	10	R	S	S	R
T69	Nickel	20	40	60	80	1	6	6	17	S	S	S	S
T70	Nickel	20	40	60	80	2	5	7	17	S	R	R	R
T71	Nickel	20	40	60	80	2	5	8	12	I	R	S	R
T72	Nickel	20	40	60	80	3	6	7	10	R	R	S	R
T73	Nickel	20	40	60	80	1	5	7	10	R	R	S	R
T74	Nickel	20	40	60	80	2	6	8	9	R	R	R	R
T75	Nickel	20	40	60	80	2	6	7	9	R	I	R	S
T76	Nickel	20	40	60	80	1	6	7	9	R	R	R	R
T77	Nickel	20	40	60	80	2	6	8	10	R	R	S	S
T78	Nickel	20	40	60	80	3	6	9	12	R	I	S	R
T79	Nickel	20	40	60	80	2	6	7	12	R	R	S	R
T80	Nickel	20	40	60	80	3	5	7	9	S	R	S	S

T81	Nickel	20	40	60	80	3	5	6	9	S	R	S	S
T82	Nickel	20	40	60	80	3	5	7	9	S	R	S	S
T83	Nickel	20	40	60	80	1	5	8	10	I	R	R	R
T84	Nickel	20	40	60	80	2	5	7	12	R	R	R	R
T85	Nickel	20	40	60	80	3	5	8	10	R	R	R	R
T86	Nickel	20	40	60	80	2	5	7	10	R	R	R	R
T87	Nickel	20	40	60	80	3	6	8	9	R	R	R	R

T88	Nickel	20	40	60	80	2	5	7	9	R	I	R	S
T89	Nickel	20	40	60	80	1	3	7	9	R	R	S	R
T90	Nickel	20	40	60	80	1	5	8	10	R	R	R	S
T91	Nickel	20	40	60	80	1	4	9	12	R	R	S	R
T92	Nickel	20	40	60	80	2	4	7	12	S	R	R	R
T93	Nickel	20	40	60	80	3	4	8	9	S	I	R	R
T94	Nickel	20	40	60	80	4	4	7	9	S	R	S	R
T95	Nickel	20	40	60	80	2	4	7	12	I	R	S	S
T96	Nickel	20	40	60	80	3	5	8	10	R	R	S	R
T97	Nickel	20	40	60	80	3	4	9	10	R	R	R	S
T98	Nickel	20	40	60	80	2	4	7	9	R	R	S	R
T99	Nickel	20	40	60	80	2	5	7	9	R	R	R	R
T100	Nickel	20	40	60	80	2	4	6	9	R	R	S	S
T400	Nickel	20	40	60	80	3	6	9	12	R	I	S	S
T401	Nickel	20	40	60	80	3	5	7	9	S	R	S	S
T402	Nickel	20	40	60	80	3	5	6	9	S	R	S	S
T403	Nickel	20	40	60	80	3	5	7	9	S	R	S	S
T404	Nickel	20	40	60	80	1	5	8	10	I	R	R	R
T405	Nickel	20	40	60	80	2	5	7	12	R	R	R	R
T406	Nickel	20	40	60	80	3	5	8	10	R	R	R	R
T407	Nickel	20	40	60	80	2	5	7	10	R	R	R	R

T408	Nickel	20	40	60	80	3	6	8	9	R	R	R	R
T409	Nickel	20	40	60	80	2	5	7	9	R	I	R	S
T410	Nickel	20	40	60	80	1	3	7	9	R	R	S	R
T411	Nickel	20	40	60	80	1	5	8	10	R	R	R	S
T412	Nickel	20	40	60	80	1	4	9	12	R	R	S	R
T423	Nickel	20	40	60	80	2	4	7	12	S	R	R	R
T414	Nickel	20	40	60	80	3	4	8	9	S	I	R	R
T415	Nickel	20	40	60	80	4	4	7	9	S	R	S	R
T416	Nickel	20	40	60	80	2	4	7	12	I	R	S	S
T417	Nickel	20	40	60	80	3	5	8	10	R	R	S	R
T418	Nickel	20	40	60	80	3	6	9	12	R	I	S	R

T419	Nickel	20	40	60	80	2	6	7	12	R	R	S	R
T420	Nickel	20	40	60	80	3	5	7	9	S	R	S	S
T421	Nickel	20	40	60	80	3	5	6	9	S	R	S	S
T422	Nickel	20	40	60	80	3	5	7	9	S	R	S	S
T423	Nickel	20	40	60	80	1	5	8	10	I	R	R	R
T424	Nickel	20	40	60	80	2	5	7	12	R	R	R	R
T425	Nickel	20	40	60	80	3	5	8	10	R	R	R	R
T426	Nickel	20	40	60	80	2	5	7	10	R	R	R	R
T427	Nickel	20	40	60	80	3	6	8	9	R	R	R	R
T428	Nickel	20	40	60	80	2	5	7	9	R	I	R	S
T429	Nickel	20	40	60	80	1	3	7	9	R	R	S	R
T430	Nickel	20	40	60	80	1	5	8	10	R	R	R	S
T431	Nickel	20	40	60	80	1	4	9	12	R	R	S	R
T432	Nickel	20	40	60	80	2	4	7	12	S	R	R	R
T433	Nickel	20	40	60	80	3	4	8	9	S	I	R	R
T434	Nickel	20	40	60	80	4	4	7	9	S	R	S	R
T435	Nickel	20	40	60	80	2	4	7	12	I	R	S	S

T436	Nickel	20	40	60	80	3	5	8	10	R	R	S	R
T437	Nickel	20	40	60	80	3	6	9	12	R	I	S	R
T438	Nickel	20	40	60	80	2	6	7	12	R	R	S	R
T439	Nickel	20	40	60	80	3	5	7	9	S	R	S	S
T440	Nickel	20	40	60	80	3	5	6	9	S	R	S	S
T441	Nickel	20	40	60	80	3	5	7	9	S	R	S	S
T442	Nickel	20	40	60	80	1	5	8	10	I	R	R	R
T443	Nickel	20	40	60	80	2	5	7	12	R	R	R	R
T444	Nickel	20	40	60	80	3	5	8	10	R	R	R	R
T445	Nickel	20	40	60	80	2	5	7	10	R	R	R	R
T446	Nickel	20	40	60	80	3	6	8	9	R	R	R	R
T447	Nickel	20	40	60	80	2	5	7	9	R	I	R	S
T448	Nickel	20	40	60	80	1	3	7	9	R	R	S	R
T449	Nickel	20	40	60	80	1	5	8	10	R	R	R	S
T450	Nickel	20	40	60	80	1	4	9	12	R	R	S	R

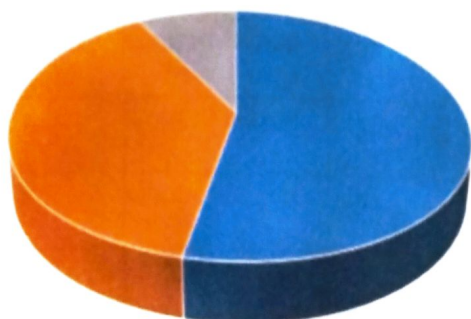
T451	Nickel	20	40	60	80	2	4	7	12	S	R	R	R
T452	Nickel	20	40	60	80	3	4	8	9	S	I	R	R
T453	Nickel	20	40	60	80	4	4	7	9	S	R	S	R
T454	Nickel	20	40	60	80	2	4	7	12	I	R	S	S
T455	Nickel	20	40	60	80	3	5	8	10	R	R	S	R
T456	Nickel	20	40	60	80	3	6	9	12	R	I	S	R
T457	Nickel	20	40	60	80	2	6	7	12	R	R	S	R
T458	Nickel	20	40	60	80	3	5	7	9	S	R	S	S
T459	Nickel	20	40	60	80	3	5	6	9	S	R	S	S
T460	Nickel	20	40	60	80	3	5	7	9	S	R	S	S
T461	Nickel	20	40	60	80	1	5	8	10	I	R	R	R
T462	Nickel	20	40	60	80	2	5	7	12	R	R	R	R
T463	Nickel	20	40	60	80	3	5	8	10	R	R	R	R

T464	Nickel	20	40	60	80	2	5	7	10	R	R	R	R
T465	Nickel	20	40	60	80	3	6	8	9	R	R	R	R
T466	Nickel	20	40	60	80	2	5	7	9	R	I	R	S
T467	Nickel	20	40	60	80	1	3	7	9	R	R	S	R
T468	Nickel	20	40	60	80	1	5	8	10	R	R	R	S
T469	Nickel	20	40	60	80	1	4	9	12	R	R	S	R
T470	Nickel	20	40	60	80	2	4	7	12	S	R	R	R
T471	Nickel	20	40	60	80	3	4	8	9	S	I	R	R
T472	Nickel	20	40	60	80	4	4	7	9	S	R	S	R
T473	Nickel	20	40	60	80	2	4	7	12	I	R	S	S
T474	Nickel	20	40	60	80	3	5	8	10	R	R	S	R
T475	Nickel	20	40	60	80	3	6	9	12	R	I	S	R
T476	Nickel	20	40	60	80	2	6	7	12	R	R	S	R
T477	Nickel	20	40	60	80	3	5	7	9	S	R	S	S
T478	Nickel	20	40	60	80	3	5	6	9	S	R	S	S
T479	Nickel	20	40	60	80	3	5	7	9	S	R	S	S
T480	Nickel	20	40	60	80	1	5	8	10	I	R	R	R
T481	Nickel	20	40	60	80	2	5	7	12	R	R	R	R
T482	Nickel	20	40	60	80	3	5	8	10	R	R	R	R
T483	Nickel	20	40	60	80	2	5	7	10	R	R	R	R
T484	Nickel	20	40	60	80	3	6	8	9	R	R	R	R
T485	Nickel	20	40	60	80	2	5	7	9	R	I	R	S
T486	Nickel	20	40	60	80	1	3	7	9	R	R	S	R
T487	Nickel	20	40	60	80	1	5	8	10	R	R	R	S
T488	Nickel	20	40	60	80	1	4	9	12	R	R	S	R
T489	Nickel	20	40	60	80	2	4	7	12	S	R	R	R
T490	Nickel	20	40	60	80	3	4	8	9	S	I	R	R
T491	Nickel	20	40	60	80	4	4	7	9	S	R	S	R
T492	Nickel	20	40	60	80	2	4	7	12	I	R	S	S

T493	Nickel	20	40	60	80	3	5	8	10	R	R	S	R
T494	Nickel	20	40	60	80	3	6	9	12	R	I	S	R
T495	Nickel	20	40	60	80	2	6	7	12	R	R	S	R
T496	Nickel	20	40	60	80	3	5	7	9	S	R	S	S
T497	Nickel	20	40	60	80	3	5	6	9	S	R	S	S
T498	Nickel	20	40	60	80	3	5	7	9	S	R	S	S
T499	Nickel	20	40	60	80	1	5	8	10	I	R	R	R
T500	Nickel	20	40	60	80	2	5	7	12	R	R	R	R

Samples T1 to T50 are *Staphylococcus aureus* and Samples T51 to T100 are *Escherichia coli*
 the terms S, R and I refer to Sensitive, Resistance and Intermediate respectively

ANTIBACTERIAL RESISTIVITY OF
STAPHYLOCOCCUS ON NICKEL



■ RESISTANCE ■ SENSITIVE ■ INTERMEDIATE

Antibacterial Resistivity of Nickel-Copper nanoparticle:

Table 5.6- *Staphylococcus Sp*

Sample no	NPs used	Dilution	Zone of inhibition (mm)	S/I/R
T1	Copper-Nickel	50µl	18	S
T2	Copper-Nickel	50µl	22	S
T3	Copper-Nickel	50µl	19	S
T4	Copper-Nickel	50µl	20	S
T5	Copper-Nickel	50µl	13	R
T6	Copper-Nickel	50µl	16	S
T7	Copper-Nickel	50µl	17	S
T8	Copper-Nickel	50µl	17	S
T9	Copper-Nickel	50µl	16	S
T10	Copper-Nickel	50µl	18	S
T11	Copper-Nickel	50µl	20	S
T12	Copper-Nickel	50µl	20	S
T13	Copper-Nickel	50µl	16	S
T14	Copper-Nickel	50µl	18	S
T15	Copper-Nickel	50µl	20	S
T16	Copper-Nickel	50µl	24	S
T17	Copper-Nickel	50µl	26	S

T18	Copper-Nickel	50μl	19	S
T19	Copper-Nickel	50μl	20	S
T20	Copper-Nickel	50μl	25	S
T21	Copper-Nickel	50μl	23	S
T22	Copper-Nickel	50μl	25	S
T23	Copper-Nickel	50μl	22	S
T24	Copper-Nickel	50μl	24	S
T25	Copper-Nickel	50μl	23	S
T26	Copper-Nickel	50μl	18	S
T27	Copper-Nickel	50μl	17	R
T28	Copper-Nickel	50μl	16	R
T29	Copper-Nickel	50μl	18	S
T30	Copper-Nickel	50μl	20	S
T31	Copper-Nickel	50μl	20	S
T32	Copper-Nickel	50μl	16	S
T33	Copper-Nickel	50μl	18	S
T34	Copper-Nickel	50μl	20	S
T35	Copper-Nickel	50μl	24	S
T36	Copper-Nickel	50μl	26	S
T37	Copper-Nickel	50μl	19	S
T38	Copper-Nickel	50μl	20	S
T39	Copper-Nickel	50μl	25	S
T40	Copper-Nickel	50μl	23	S
T41	Copper-Nickel	50μl	25	S
T42	Copper-Nickel	50μl	22	S
T43	Copper-Nickel	50μl	24	S
T44	Copper-Nickel	50μl	23	S
T45	Copper-Nickel	50μl	18	R
T46	Copper-Nickel	50μl	17	R

T47	Copper-Nickel	50µl	16	R
T48	Copper-Nickel	50µl	18	R
T49	Copper-Nickel	50µl	20	S
T50	Copper-Nickel	50µl	20	S
T51	Copper-Nickel	50µl	16	R
T52	Copper-Nickel	50µl	18	R
T53	Copper-Nickel	50µl	20	S
T54	Copper-Nickel	50µl	24	S
T55	Copper-Nickel	50µl	26	S
T56	Copper-Nickel	50µl	19	S
T57	Copper-Nickel	50µl	20	S
T58	Copper-Nickel	50µl	25	S
T59	Copper-Nickel	50µl	23	S
T60	Copper-Nickel	50µl	25	S
T61	Copper-Nickel	50µl	17	S
T62	Copper-Nickel	50µl	16	R
T63	Copper-Nickel	50µl	18	S
T64	Copper-Nickel	50µl	20	S
T65	Copper-Nickel	50µl	20	S
T66	Copper-Nickel	50µl	16	S
T67	Copper-Nickel	50µl	18	S
T68	Copper-Nickel	50µl	20	S
T69	Copper-Nickel	50µl	24	S
T70	Copper-Nickel	50µl	26	S
T71	Copper-Nickel	50µl	19	S
T72	Copper-Nickel	50µl	17	R
T73	Copper-Nickel	50µl	16	S
T74	Copper-Nickel	50µl	18	S
T75	Copper-Nickel	50µl	20	S

T76	Copper-Nickel	50µl	20	S
T77	Copper-Nickel	50µl	16	R
T78	Copper-Nickel	50µl	18	S
T79	Copper-Nickel	50µl	20	S
T80	Copper-Nickel	50µl	24	S
T81	Copper-Nickel	50µl	26	S
T82	Copper-Nickel	50µl	19	S
T83	Copper-Nickel	50µl	20	S
T84	Copper-Nickel	50µl	25	S
T85	Copper-Nickel	50µl	23	S
T86	Copper-Nickel	50µl	18	S
T87	Copper-Nickel	50µl	20	S
T88	Copper-Nickel	50µl	24	S
T89	Copper-Nickel	50µl	26	S
T90	Copper-Nickel	50µl	17	S
T91	Copper-Nickel	50µl	16	S
T92	Copper-Nickel	50µl	18	S
T93	Copper-Nickel	50µl	20	R
T94	Copper-Nickel	50µl	20	S
T95	Copper-Nickel	50µl	16	S
T96	Copper-Nickel	50µl	18	S
T97	Copper-Nickel	50µl	20	S
T98	Copper-Nickel	50µl	24	R
T99	Copper-Nickel	50µl	17	S
T100	Copper-Nickel	50µl	16	S

T400	Copper-Nickel	50µl	16	R
T401	Copper-Nickel	50µl	18	S
T402	Copper-Nickel	50µl	20	S
T403	Copper-Nickel	50µl	24	S
T404	Copper-Nickel	50µl	26	S
T405	Copper-Nickel	50µl	19	S

T406	Copper-Nickel	50µl	20	S
T407	Copper-Nickel	50µl	25	S
T408	Copper-Nickel	50µl	23	S
T409	Copper-Nickel	50µl	16	R
T410	Copper-Nickel	50µl	18	S
T411	Copper-Nickel	50µl	20	S
T412	Copper-Nickel	50µl	24	S
T423	Copper-Nickel	50µl	26	S
T414	Copper-Nickel	50µl	19	S
T415	Copper-Nickel	50µl	20	S
T416	Copper-Nickel	50µl	18	S
T417	Copper-Nickel	50µl	20	S
T418	Copper-Nickel	50µl	20	S
T419	Copper-Nickel	50µl	18	S
T420	Copper-Nickel	50µl	20	S
T421	Copper-Nickel	50µl	20	S
T422	Copper-Nickel	50µl	16	R
T423	Copper-Nickel	50µl	18	S
T424	Copper-Nickel	50µl	20	S
T425	Copper-Nickel	50µl	24	S
T426	Copper-Nickel	50µl	26	S
T427	Copper-Nickel	50µl	19	S

T428	Copper-Nickel	50µl	20	S
T429	Copper-Nickel	50µl	25	S
T430	Copper-Nickel	50µl	23	S
T431	Copper-Nickel	50µl	18	S
T432	Copper-Nickel	50µl	18	S
T433	Copper-Nickel	50µl	20	S
T434	Copper-Nickel	50µl	20	S
T435	Copper-Nickel	50µl	16	R
T436	Copper-Nickel	50µl	18	S
T437	Copper-Nickel	50µl	20	S

T438	Copper-Nickel	50µl	24	S
T439	Copper-Nickel	50µl	26	S
T440	Copper-Nickel	50µl	19	S
T441	Copper-Nickel	50µl	20	S
T442	Copper-Nickel	50µl	25	S
T443	Copper-Nickel	50µl	23	S
T444	Copper-Nickel	50µl	18	S
T445	Copper-Nickel	50µl	18	S
T446	Copper-Nickel	50µl	20	S
T447	Copper-Nickel	50µl	20	S
T448	Copper-Nickel	50µl	16	R
T449	Copper-Nickel	50µl	18	S
T450	Copper-Nickel	50µl	20	S
T451	Copper-Nickel	50µl	18	S
T452	Copper-Nickel	50µl	20	S
T453	Copper-Nickel	50µl	20	S
T454	Copper-Nickel	50µl	16	R
T455	Copper-Nickel	50µl	18	S

T456	Copper-Nickel	50µl	20	S
T457	Copper-Nickel	50µl	24	S
T458	Copper-Nickel	50µl	26	S
T459	Copper-Nickel	50µl	19	S
T460	Copper-Nickel	50µl	20	S
T461	Copper-Nickel	50µl	25	S
T462	Copper-Nickel	50µl	18	S
T463	Copper-Nickel	50µl	20	S
T464	Copper-Nickel	50µl	20	S
T465	Copper-Nickel	50µl	16	R
T466	Copper-Nickel	50µl	18	S
T467	Copper-Nickel	50µl	20	S
T468	Copper-Nickel	50µl	24	S
T469	Copper-Nickel	50µl	26	S

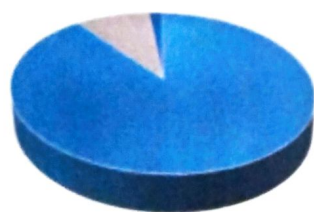
T470	Copper-Nickel	50µl	19	S
T471	Copper-Nickel	50µl	20	S
T472	Copper-Nickel	50µl	25	S
T473	Copper-Nickel	50µl	23	S
T474	Copper-Nickel	50µl	18	S
T475	Copper-Nickel	50µl	18	S
T476	Copper-Nickel	50µl	20	S
T477	Copper-Nickel	50µl	20	S
T478	Copper-Nickel	50µl	18	S
T479	Copper-Nickel	50µl	20	S
T480	Copper-Nickel	50µl	20	S
T481	Copper-Nickel	50µl	16	R
T482	Copper-Nickel	50µl	18	S
T483	Copper-Nickel	50µl	18	S

T484	Copper-Nickel	50µl		
T485	Copper-Nickel	50µl	20	S
T486	Copper-Nickel	50µl	20	S
T487	Copper-Nickel	50µl	18	R
T488	Copper-Nickel	50µl	18	S
T489	Copper-Nickel	50µl	18	S
T490	Copper-Nickel	50µl	20	S
T491	Copper-Nickel	50µl	20	S
T492	Copper-Nickel	50µl	16	R
T493	Copper-Nickel	50µl	18	S
T494	Copper-Nickel	50µl	20	S
T495	Copper-Nickel	50µl	24	S
T496	Copper-Nickel	50µl	26	S
T497	Copper-Nickel	50µl	19	S
T498	Copper-Nickel	50µl	20	S
T499	Copper-Nickel	50µl	25	S
T500	Copper-Nickel	50µl	23	S
			18	S

The samples T1 to T50 are *Staphylococcus aureus* & samples T300 to T350 are *Escherichia coli*.

Here the terms S/R/I indicate sensitive, Resistance and Intermediate respectively.

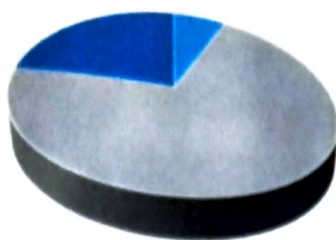
Antibacterial resistance of Ni-Cu Nanoparticle on *Staphylococcus aureus*



■ Resistance ■ Sensitive ■ Intermediate

FIG NO 12

Antibacterial Resistance of Escherichia coli on Nickel-Copper Nanoparticle



■ Resistance ■ Sensitive ■ Intermediate

FIG NO 13

CHAPTER – 6

SUMMARY

The core purpose of this project is to identify the prevalence of antibiotic resistance bacteria found in urban public restrooms of OMR, Chennai city using Swab method. The samples are then streaked in Mannitol salt Agar medium (MSA) for the isolation of *Staphylococcus aureus* and MacConkey Agar for *Escherichia coli* bacterium.

The isolated organisms are then subjected into various tests for the confirmation processes. The methods used are common Gram staining to check whether the obtained organism is Gram positive or Gram negative. Further the organisms undergo Biochemical test for the biochemical analyzation of the said bacterium. The tests done are the Indole test, Catalase test, Methyl Red test, Voges Proskauer test, etc.

The organisms are then subjected to antibacterial susceptibility tests using Kirby Bauer disc diffusion technique in order to check the resistance against fourth generation Cephalosporin derivative antibiotics. They are ceftazidime, Cefepime, Ceftriaxone and Amikacin. Approximately 80% of isolated bacteria were resistant to the antibiotics which was an outcome of their multi drug resistant nature.

Since Nickel nanoparticles moderate sensitivity to MDR isolates while all these isolates were showing resistance to Cephalosporins derivatives, hence we chose Cu-Ni nano particle, and it proved our hypothesis that bivalent nanoparticle in bacterial isolates proving its therapeutic potential against MDR isolates. This further study must be carried out.

On the contrary to previous attempts, the bivalent Nanoparticles of Nickel and Copper showed tremendous results with serious sensitivity rates on the samples of *Staphylococcus* and *E. coli*. It is concluded that the bivalent nickel- copper nanoparticles can be used alone or in addition to other antibiotic agents for the treatment of bacterial infections in the case where they are multi drug resistant.

CHAPTER 7

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