MINISTERE DE L'ENSEIGNEMENT SUPERIEUR ET DE LA RECHERCHE SCIENTIFIQUE
UNIVERSITE AKLI MOHAND OULHADJ – BOUIRA
FACULTE DES SCIENCES DE LA NATURE ET DE LA VIE ET DES SCIENCES DE LA TERRE
DEPARTEMENT DE BIOLOGIE

Réf : ……./UAMOB/F.SNV.ST/DEP.BIO/2021

MEMOIRE DE FIN D'ETUDES

EN VUE DE L'OBTENTION DU DIPLOMEMASTER

Domaine : SNV **Filière** : Science Biologique
Spécialité : Microbiologie Appliquée

Présenté par :

Hocine MOHAMMEDI &Sara KHITER

Thème

Criblage de l'activité antibactérienne de quelques molécules complexes métalliques

Soutenu le : 14 / 07 / 2021 **Devant le jury composé de :**

Nom et Prénom	*Grade*		
Dr KADRI N	*MCA*	*Univ. de Bouira*	*Président*
Dr REMINI H	*MCB*	*Univ. de Bouira*	*Examinateur*
Dr DJENADI K	*MCB*	*Univ. de Bouira*	*Promoteur*
Dr BOULOUDENINE M	*MCA*	*Univ. de Souk Ahras*	*Co-promoteur*

Année Universitaire : 2020/2021

MINISTRY OF HIGHER EDUCATION AND SCIENTIFIC RESEARCH
AKLI MOHAND OULHADJ – BOUIRA UNIVERSITY
FACULTY SCIENCES OF NATURE AND LIFE AND EARTH SCIENCES
DEPARTMENT OF BIOLOGY

REF : ……./UAMOB/F.SNV.ST/DEP.BIO/2021

MASTER'S project

SUBMITTED FOR THE DEGREE OF MASTER "SECOND CYCLE LMD"

Field: Nature and Life Sciences Branch: Biological Sciences
Specialty: Applied Microbiology

By
Hocine MOHAMMEDI & Sara KHITER

Screening for the antibacterial activity of some metallic complex molecules.

Supported : July 14th, 2021 **Ahead of the Jury:**

Last and first name	*Grade*		
Dr KADRI N	*MCA*	*Bouira university*	*Chair*
Dr REMINI H	*MCB*	*Bouira university*	*Examiner*
Dr DJENADI K	*MCB*	*Bouira university*	*Supervisor*
Dr BOULOUDENINE M	*MCA*	*Souk Ahras university*	Co-supervior

Scholar year: 2020/ 2021

Dedication

I dedicate this work

At first, to my parents, who made me what I am and who have always supported me to become the man who I am.

*To Dr **K. DJENADI**, my supervisor, the greatest discovery that I made in the university, who believed in me, who doesn't only supervised us but also trained us, supported us and taught us the rules of the game.*

*To all my teachers since the first-year class, especially Dr **REMINI. H**, Dr **BOUTHELDJA**, Mrs **MEDBOUA. C**, Mrs **TIGHIDAT. S**, Dr **KADRI. N**, Dr **DAHMOUNE. F** and others, "My success is your's too".*

To all the administrative staff of the faculty Sciences of Nature and Life and Earth Sciences Department of Biology

*To my sisters **Yasmine** and **Saida**, my brothers; **Samir**, **reda** and **lounis***

*To my brothers from other mothers and their families; **Abdellatif. A**, **Mouad. H**, **Seif eddine. H** and **Mounir. D**, you're the best brothers ever.*

*To my friends **Said. B**, **Mouloud. R (Miou)**, **Hichem. L**, **Sarah. H** and **Sarah. H**, I wish you all the best.*

*To **Ferhat**, **Abdel Djalil**, **Rayane** & all my family*

*To **Louiz** my grandmother, who steel alive in my heart. I wish that you're proud of me where you are. May god welcome you in his vast paradise.*

*To **Katia (Bob-Kizkiz)** and **Thanina D** and their mother **Samia. H**.*

*Finally, to the person who is the fuel of my success, who supported me and loved me for what I am, My dear **Thileli. M.***

Hocine. M

Dedication

First of all I dedicate this dissertation to our Almighty God, who gave me strength and knowledge for me everyday

To my grandmother Mrs.Laldja Ben Fares, who inspired me to be strong despite of many obstacles in life

To my mother Mrs.Zohra and my father Mr.Ali

who gave the little they had to ensure I would have the opportunity of an education, Their efforts and struggles have allowed me to have a key to me the mysteries of my world, and beyond, for their understanding and for their overwhelming, support morally and financially

To my brother and sister, Bouzid and Samia, who are always by my side, and all my friends for their eternal love

To all those who encouraged me to reach this goal

Sara. K

Thanks

At the end of this work, we would like to thank God the Almighty for giving us the courage and the energy to get here.

A special thank for the jury member; Dr **H. REMINI**, Dr **K. DJENADI**, Dr **M. BOULOUDENINE** and Dr **N. KADRI** for being here exanimating our work.

A special thanks to our collaborator from the high school of mines of Annaba and for all those who participated to the achievement of our work.

We thank also the laboratories managers of our faculty for their help and collaboration

Big thank for all our teachers, administrative staff and colleagues of the faculty SNV/ST

Finally, A VIP thank for the best teacher and supervisor ever, **Dr K. DJENADI**.

Hocine. M & **Sara. K**

Table list

Figure list

Abbreviation list

AMR: Anti-Microbial Resistance

ARB: Antimicrobial Resistant Bacteria

ARG: Antibacterial Resistance Gene

ATCC: American Type Culture Collection

CFU: Colony Formant Unity

DNA: Desoxyribonucleic Acid

DPPH: 2,2-**di****p**hényl 1-**p**icryl**h**ydrazyle

LB: Lauria Bertani

MBC: Minimum Bactericide Concentration

MIC: Minimum Inhibitory Concentration

MRSA: Methicillin-Resistant Staphylococcus Aureus

OD: Optical Density

ROS: Reactive Oxygen Species

VIS-UV: Visible Ultra Violet

Table of content

General Introduction

General introduction

A heavy metal is a dense metal that is (usually) toxic at low concentrations. Although the phrase "heavy metal" is common, there is no standard definition assigning metals as heavy metals. Heavy metals are present in various environmental compartments (atmosphere, water, soil, sediments and living organisms) and these forms are controlled by physical and chemical conditions as well as by biological processes [1].

The term “heavy metal” is linked in many people’s minds to metals (or their compounds) that are toxic. However, this is a feeling rather than a conclusion based on scientific evidence. Two facts should be kept in mind. The effect of any substance on a living system is always dependent on the concentration of it available to cells. Thus, there are no substances that are always toxic. What we need to evaluate toxicity are dose–response data; i.e. quantitative dose–response relationships [2]

In the beginning of the 30s. in World War I, After the use of penicillin, the first resistant bacteria emerged; in 1945, Fleming postulated the potential risks associated to the use of antibiotics; he showed that the use of a large and prolonged scale can select resistant bacteria, observing in his laboratory that bacteria sensitive to penicillin multiply in the presence of increasing concentrations of the antibiotic. During the 40s, the first report of penicillin resistance by strains of *Escherichia coli* (*E. coli*) and *Staphylococcus sp* was reported. In 1947, resistance to streptomycin among patients with tuberculosis was detected, where 80% of them relapsed within three months due to the formation of resistant bacilli. In the years 1952 and 1957, resistance to tetracycline and chloramphenicol was reported and in the decade of the 60s, β-lactamases producing strains, such as TEM and SHV of wide spectrum (detected in gram-negative bacilli) were discovered [3].

A 2014 review on tackling this phenomenon estimated the number of deaths that may occur globally due to AMR in the next 35 years could be as high as 300 million individuals and that the economic damage could amount to 60–100 trillion US dollars [4].

According to multiple sources, one of the most problems threating humanity is the bacterial resistance to antibacterial drugs which requires extensive researches of techniques and substances which could replace chemical antibacterial drugs, those to which bacteria are susceptible and have no resistance. That’s why our study is interested in metallic particles that science have proved their efficacy against bacteria, substances with which are associated other

compounds in order to obtain a metallic complex molecules. An association that makes them more effective at minimal concentrations in order to guarantee a better efficiency thus limiting the risks associated with the toxicity caused by overdosage of these molecules which are also known by their toxicity to different types of cells and tissues.

Theoretical part

First chapter

Pathogenic bacteria & Multi Drugs Resistant Bacteria

Theoretical Part

Chapter I: Pathogenic bacteria & multidrug resistant bacteria

1. Pathogenic bacteria

Microorganisms are found throughout nature and the environment. Pathogen bacteria are distributed in soil, marine and water, the intestinal tract of animals and contaminated with fecal matter or estuarine waters [5]. Infectious diseases are due to the multiplication of a pathogenic agent (bacterium, virus, parasite or fungus) in an organism, which isn't generally present before the illness, although members of the normal bacterial microbiota can cause *opportunistic* infections if the host's status suddenly changes [6].

During the past decade gram-positive bacteria have gradually emerged as the most frequent causes of nosocomial disease. These pathogens are especially difficult to treat because of their high frequency of drug-resistance traits. Methicillin-resistant strains now make up 60 to 90 percent of all isolates of coagulase-negative staphylococci, the most frequent cause of infections related to intravascular catheters and prosthetic devices [7]

S. aureus is the most frequent germ cause of skin and wound infections and bacteremia. And the second most frequent cause of lower respiratory infections in nosocomial disease [3] In the United States enterococci have become the third most common organism causing hospital-acquired infections (after *S. aureus and Escherichia coli*) such as those of wounds and the urinary tract, septicemia, and endocarditis [7]

Untreatable *Pseudomonas aeruginosa* or *P. cepacia* infections have become a tragically frequent occurrence in patients with cystic fibrosis. Acinetobacter, a common free-living microorganism and inhabitant of the human skin that is resistant to all available antibacterial agents except sulbactam, has caused fatal disease in patients in an intensive care unit[9]. Novel, plasmid-borne, extended-spectrum β-lactamases capable of inactivating antibiotics (such as ceftazidime or imipenem) specifically developed against β-lactamase-producing gram-negative bacteria have been detected in nosocomial isolates of klebsiella and in P. aeruginosa [7]

Table 1: Pathogenic bacteria, diseases they cause, toxins they secrete, infection sources and mortality rates for humans infected by microorganisms used as biological warfare agent (BWA). [**5**]

Bacteria	Disease	Toxin	Infection sources	Mortality when used as BWA
Bacillus anthracis	Anthrax	Edema factor	Milk or meat, BWA	Fatal
Brucella melitensis	Brucellosis	–	Milk or meat, BWA	Low
Campylobacter jejuni	Diarrhea dysentery	–	Dairy products, meats, mushrooms	–
Clostridium botulinum	Botulism	Neurotoxin	Food	–
Coxiella burnetti	Pneumonia	–	BWA	Low
Corynebacterium diphtheriae	Diphtheria	Diphtheria toxin	BWA	Low
Escherichia coli	Gastroenteritis	Enterotoxin	Meats, fish, milk, rice, vegetables	–
Francisella (*Pasteurella*) *tularensis*	Tularemia	–	BWA	Low
Mycobacterium tuberculosis	Tuberculosis	–	BWA	High
Rickettsia rickettsi	Rocky Mountain-spotted fever	–	BWA	High
Salmonella paratyphi	Paratyphoid	–	Fecal contamination, eggs, milk, meats	–
Salmonella typhi	Typhoid fever	–	BWA	High
Shigella dysenteriae	Bacillary dysentry	Neurotoxin	Fecal contamination	–
Staphylococcus aureus	Pneumonia	Enterotoxin	Human carriers	–
Streptococcus pneumoniae	Pneumococcal pneumoni	Erythrogenic toxin	Human carriers	–
Treponema pallidum	Syphilis	–	Infected exudate or blood	–
Vibro cholerae	Cholera	Enterotoxin	Fecal contamination	High
Yersinia pestis	Bubonic plague	Plague toxin	BWA	Fatal

[a] BWA, biological warfare agent.

Each year there are more than 40 million hospitalizations in the United States, and about 2 million patients acquire nosocomial infections, 50 to 60 percent of which involve antibiotic-resistant bacteria. In some intensive care units, patients have a 25 to 70 percent risk of acquiring a nosocomial infection, most often one caused by resistant microorganisms. The number of deaths related to nosocomial disease is estimated at 60,000 to 70,000 per year [7]

2. Antimicrobial agents

Antimicrobial agents or Antibiotics, are chemical substances that specifically target bacteria. Because of their specificity, they differ from antiseptics, which are only used externally. It can have bacteriostatic action by preventing bacterial growth, or bactericidal action by destroying

bacteria. More than 10 000 antibiotic molecules are known, around 100 of which are frequently used in medicine [6]

3. Bacterial resistance to antimicrobial agents

Since the getting started of the antibiotherapy, resistance to these active molecules has been described, during several decades; antimicrobial resistance, an increasing menace for the effective treatment of a wide range of infections caused by bacteria, parasites, virus and fungi. AMR produces a reduced efficacy of antibacterials, antiparasitics, antivirals and antifungals; turning difficult the treatment of patients who have got this kind of microorganisms [8]

Bacterial resistance has become a serious problem due to the massive application of antibiotics, which are used prophylactically or remedially without proper medical indications; the inappropriate selection of alternate antimicrobials; and the frequent switching between antimicrobial treatments. Drug-fast and multidrug-resistant bacteria have multiple causes that can all be summarized as an interaction of intrinsic and extrinsic factors [9]

Bacterial infections are a major cause of chronic infections and mortality. Antibiotics have been the preferred treatment method for bacterial infections because of their cost-effectiveness and powerful outcomes. However, several studies have provided direct evidence that the widespread use of antibiotics has led to the emergence of multidrug-resistant bacterial strains [9].

The excessive use of antibiotics is due to the prevalence of antibiotic resistant bacteria (ARB) and antibiotic resistance genes (ARGs) in various aquatic environments; freshwater, seawater, wastewater, and even drinking water. Various ARB, including pathogenic species like *E. coli*, *Salmonella*, *Legionella* and *Pseudomonas aeruginosa* have been found colonizing in drinking water systems, as were the ARGs carried by them. The ubiquitous of ARGs and ARB in drinking water can pose health risks through cooking, bathing, and other uses, which undoubtedly undermines the drinking water biosafety [10].

Super-bacteria, which are resistant to nearly all antibiotics, have recently developed due to abuse of antibiotics. Studies have shown that these bacteria carry a super-resistance gene called NDM-1. The major groups of antibiotics that are currently in use have three bacterial targets: the cell wall synthesis, translational machinery, and DNA replication machinery. Unfortunately, bacterial resistance can develop against each of these modes of action. The mechanisms of resistance include expression of enzymes that modify or degrade antibiotics,

such as β-lactamases and aminoglycosides, modification of cell components, such as the cell wall in vancomycin resistance and ribosomes in tetracycline resistance, and expression of efflux pumps, which provide simultaneous resistance against numerous antibiotics [9]

Two basic types of resistance to antimicrobial agents are defined, intrinsic resistance and acquired resistance. The first, known also as innate or primary resistance, describes a status of general insensitivity of bacteria to a specific antimicrobial agent or class of agents. It is due commonly to the inaccessibility or the lack of target structures for certain antimicrobial agents, e.g., vancomycin resistance in Gram negative bacteria due to the inability of vancomycin to penetrate the outer membrane. It can also be due to the presence of export systems or the production of species-specific inactivating enzymes in certain bacteria [11]. Recently, bacterial strains resistant to all available antibacterial agents were identified among clinical isolates of some bacterial species [7]. Strains of methicillin-resistant S. aureus, formerly confined to large teaching hospitals, had spread by the early 1990s into smaller hospital units (where the incidence of resistance is about 20 percent of isolates) and into nursing homes. The majority of methicillin-resistant S. aureus isolates are also resistant to most other antibiotics, necessitating the use of the glycopeptide antibiotic vancomycin [7].

Vancomycin-resistant *Enterococcus faecium* (first reported from the United Kingdom and France in 1987) had been detected in several hospitals in the United States by 1989. By 1993, 14 percent of enterococcal isolates from patients in intensive care units were resistant to vancomycin (a 20-fold increase since 1987); 88 percent of the strains were also resistant to β-lactam antibiotics, aminoglycosides, fluoroquinolones, tetracycline, chloramphenicol, and teicoplanin. Of the 10,961 hospital-associated isolates of enterococci that were also tested for vancomycin susceptibility, close to 1900 were from primary bloodstream infections, and 323 of the patients (36.3 percent) died. Mortality among patients who have bloodstream infections with vancomycin-susceptible isolates was reported as 16.4 percent [7].

Genetically, the antimicrobial resistance development by either mutation, development of new resistance genes, or the acquisition of resistance genes already present in other bacteria is a complex process that involves various mechanisms. Numerous resistance genes expressing different mechanisms of resistance have been identified in various bacteria [11]. Resistance to antimicrobial drug can be divided into intrinsic resistance and acquired resistance according to the source of its genes. Intrinsic resistance is due to spontaneous mutations of existing or exogenous genes, acquired resistance results from the acquisition of new genes from other

organisms. The emergence of multidrug resistance character (MDR) in particular is a combination of the acquisition of multiple and different drug resistance genes types by the same bacteria. Generally, intrinsic resistance is less important. There are three ways by which resistance can be transferred and spread between bacteria: plasmids, transposons, and integrons [9].

The evolution of resistance development differs depending to the bacteria involved, the selective pressure imposed by the use of antimicrobial agents, and the availability and transferability of resistance genes in the gene pools accessible to the bacteria [11]. The dissemination of antimicrobial-drug resistant bacteria has become more rapid during the past decade, because of tremendously increased mobility of human populations [7]

Three methods used to overcome antibiotic resistance were the development of new drugs, high-dose administration of an antibiotic, and the combination of multiple antibiotics. However, the production of novel antibiotics could not keep up with the mutation of bacteria, and intolerable toxicity always accompanied high-dose treatment. These treatment strategies also led to antibiotic misuse and the emergence of multidrug-resistant strains. [9]

Second chapter

Biological activities of metallic complex molecules

Heavy metals are elements with a density more than 4-5 g/cm3, majority of which are harmful for the health of human being. They are mainly Pt, Pd, Ni, Hg, Cd, Zn, Pb, As, Ag, Cu, Fe and Cr. It can be released to environment from natural resources such as volcanoes or from anthropogenic activities such as factories and mines. Heavy metals are not biodegradable and can be accumulated in the nature and finally enter into the food chain. They are poisonous and lead to numerous illnesses such as cancer, ulcer, osteomalacia, aminoaciduria, and proteinuria, central and peripheral neuropathies [12].

1. Antioxidant activity

Oxygen is also a poison, so we need antioxidants to counter its toxic effects [13]. Oxygen gives birth in the body to derivatives that attack our tissues: free radicals (today we use the more general term Reactive Oxygen Species or ROS; free radicals make party of ROS). We know that free radicals are involved in aging and in many pathologies: cancer, cardiovascular diseases, the diabetes, Alzheimer's disease, Parkinson's disease, degeneration age-related macular [13].

In the body and foodstuffs, the importance of oxidation is widely recognized. Its metabolism is lethal for cells. The production of free radicals and other reactive oxygen species is the side effect of this dependence, which cause oxidative change [14]. Unfortunately, things are not as simple, biology is sometimes paradoxical. Indeed, free radicals are not necessarily harmful and antioxidants are not always beneficial, it's all about dose [13].

A. Oxygen & free radicals

A free radical is a chemical species (atom or molecule) that possesses a single electron that is not matched. This characteristic makes it unstable and gives it a high reactivity with the surrounding molecules. A free radical stabilizes at the expense of the neighboring molecule which in turn becomes a free radical and so on. The phenomenon is propagated by chain reactions. Reactive oxygen species include radical species (the superoxide anion O_2•-, the hydroxyl radical OH•) and non-subject species (hydrogen peroxide H_2O_2) [13]. When free radicals are formed excessively, they overwhelm protective enzymes such as catalase and peroxidase, superoxide dismutase and cause destructive and lethal cellular effects (*e.g.*, apoptosis) by oxidizing membrane lipids, cellular proteins, DNA and enzymes, thus shutting down cellular respiration. Furthermore, reactive oxygen species seem to influence cell signaling pathways in ways that are only now being unraveled [14].

The cells contain organelles, called mitochondria, which provide the energy needed for the cells. The mitochondria are somehow the energy plants of our cells. The more energy the cell needs for its function, the more it contains mitochondria (one muscle cell per example contains more than a thousand). In these organelles take place a series of very complicated reactions, which are perfectly known today. The balance of these reactions comes back to the burning of sugars (glucose) and fats (fatty acids) that release energy as it's shown in the *figure 1*. It is this energy that allows our muscles to contract [14].

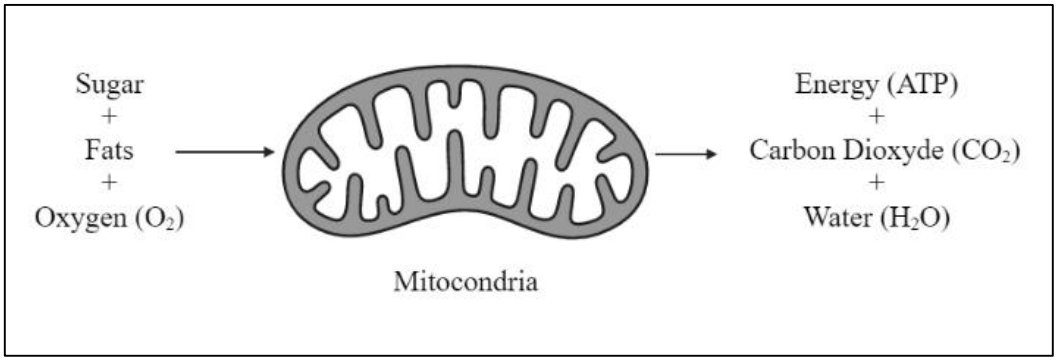

Figure 1: ATP production reaction ensured by the mithochondria [14].

Unfortunately, the chain of reactions of our mitochondria is imperfect and leads to the formation of reactive oxygen species as we could see in the *figure 2*, of which the free radicals [14].

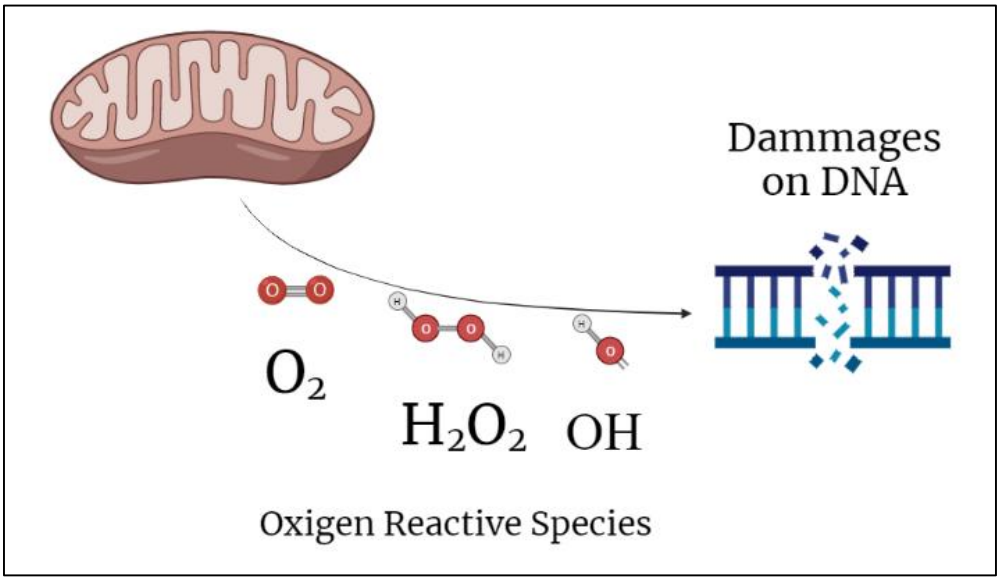

***Figure 2*:** ROS resulting from imperfect reactions of mitochondria [14].

In situations of oxidative stress, the antioxidant defense system is no longer measures to prevent cellular damage caused by reactive species of oxygen. These attack all biological materials (DNA, proteins, lipids…) [14].

- ROSs cause breaks and mutations within the DNA, initiating thus the carcinogenesis;
- they denature and inactivate proteins (oxidation of amino acids, polypeptide fragmentation…);
- they oxidize sugars (glucose);
- they degrade membrane lipids and thus alter the permeability of cell membranes.

Antioxidants are defined as the molecules that are able to prevent oxidation of other molecules. These molecules have very important role in treatment of various diseases. The role of these molecules is preventing the oxidative stresses and protecting cells by scavenging free radicals. Copper nanoparticles showed Antioxidant activity against DPPH [15].

B. Antioxidant essays

Antioxidant activities of synthesized copper complex molecules were determined by using DPPH free radical scavenging assay (Yen and Chen, 1995). Freshly prepared solution of 2,2-diphenyl -1-picrylhydrazyl (DPPH) radical was prepared in methanol (0.16 mM) and stored in the dark. Methanol solutions of the tested compounds and the standard ascorbic acid were prepared at different concentrations. Equal amounts of DPPH solution and each concentration of the tested compound were added to test tubes. Mixtures were vortexed for 1 min and incubated in the dark for 30 min at room temperature. DPPH solution without antioxidant was used as control. Absorbance measurements were determined by using a UV-visible spectrophotometer. The reduction in absorbance was determined at 517 nm. The percentage (%) of scavenging activity was determined as follow: **DPPH scavenging % = [{(AC - AT) / AC} × 100].** Where, AC = Absorbance of the control and AT = absorbance of the sample. The IC50 (50% inhibitory concentration) values were determined from the curve plotted between the concentration and DPPH scavenging %. The IC50 was defined as the concentration required to inhibit DPPH radical by 50% [15].

2. Cytotoxic activity

In immunology, cytotoxicity is the ability of an immune cell to kill or eliminate a target cell, usually by apoptotic or granulocytic mechanisms. Cytolytic activity is a property of specialized immune cells, such as natural killer (NK) cells and cytotoxic T lymphocytes, enabling surveillance and ultimate destruction of tumor cells and virus-infected cells [16].

Cytotoxicity results from interference with structures or properties that are essential for cell survival, proliferation, or function. Basal (i.e., general) cytotoxicity involves one or more of the abovementioned structures or processes when all the cell types studied show similar sensitivities [17]

A. Cytotoxicity essay

Cytotoxicity assays were among the first in vitro bioassay methods used to predict toxicity of substances to various tissues. In vitro cytotoxicity testing provides a crucial means for safety assessment and screening, and for ranking compounds. The choice of using a particular cytotoxicity assay technology may be influenced by specific research goals. As such, four main classes of assays are used to monitor the response of cultured cells after treatment with potential toxicants. These methods measure viability, cell membrane integrity, cell proliferation, and metabolic activity [17]. As an example, an essay performed by Abdullah M. Ali *& al* to investigate the cytotoxic activity of copper complex molecules as following below.

Assay performed to study copper cytotoxic activity by Abdullah M. Ali & al

Tumor cell lines were suspended in Corning® 96-well tissue culture plates at concentration of 5x105 cell/well and were incubated for 24 hours. Copper complex molecules solution were added on a 96 well plates by 3 replicates at different concentrations where media or DMSO at 0.5% solution was used as control. After 24 hours, the number of viable cells were estimated of incubation with the MTT test. Media is removed from wells and 100 µl of a fresh culture RPMI 1640 medium was added. Then, 10 µl of MTT stock solution (12 mM) was added to each well even control. Plates were then incubated at 37 °C and 5% CO_2 for 4 hours. 85 µl of the media was replaced with 50 µl DMSO and mixed with pipette then incubated at 37 °C for 10 min. With microplate reader, optical densities were determined at 590 nm. The percentage of cell viability was determined as follow: Cell viability % = [(At / Ac)] × 100%, where AT = absorbance of the sample and AC = Control absorbance [15].

3. Antimicrobial activity of heavy metals

For long times, metals have been widely used for their antimicrobial properties for thousands of years. Vessels were made of copper and silver and have been used for water disinfection and food preservation since the time of the Persian kings. This practice was adopted also by the Greeks, Phoenicians, Romans and Egyptians. In the north of America, silver coins were dropped into transport containers to preserve water, wine, milk and vinegar, and a similar

strategy was used by Japanese soldiers during the Second World War to prevent the spread of dysentery [18].

A. Copper particles

Copper particles showed a remarkable antimicrobial activity and expected to be an alternative to antimicrobial agents. They were employed as antimicrobial agents because of their small dimensions and high surface to volume ratio. They improve their antimicrobial efficiency with their ability to interact easily with other particles [15].

The antimicrobial activity of copper particles was assayed against some pathogenic microorganisms using well diffusion method. All tested species, either Gram positive Gram negative, were sensitive to copper antimicrobial activity [15], as follow in the table below.

***Table 2**:* Determination of MIC values of Cu particles [15].

Gram	Microbial species	Inhibition zone (mm)	Control	MIC value (µg/ml)
		Cu	Gentamicin	
Positive	*Staphylococcus aureus*	14	24	39
	Streptococcus faecalis	19	30	19.5
	Streptococcus mutans	16	27	19.5
Negative	*Escherichia coli*	13	30	39
	Neisseria gonorrhoeae	18	28	19.5
	Pseudomonas aeruginosa	17	31	19.5

Joseph A Lemir et al believes that in some circumstances growth inhibition and cellular death are likely to be the result of a combination of different mechanisms and that the mechanisms of toxicity differs depending on the chemistry of the metal [18].

B. Silver (Ag) antibacterial activity

Silver's antimicrobial activity has known a variety of applications because its toxicity to human cells is considerably lower than to bacteria [19]. As early as 4 000 B.C.E., it was known to the Caldeans, and was the third metal used by the Ancients, after copper and gold. Silver has been used, over these millennia, for numerous medical conditions, mostly empirically before the realization that microbes were the agents of infection [20].

In the first part of the 20th Century, it became one of the mainstays of the antibiotherapy until the introduction of antibiotics in the early 1940s. It is likely that silver nitrate also was used medically because it was mentioned in a pharmacopeia published in Rome in 69 B.C.E.

Complexes of silver and protein known as mild silver proteins also were employed. These formulations were delivered topically orally, and by injection. By 1940, at least 50 silver products were marketed in the United States [20].

According to Herodotus, the King of Persia, among his provisions took boiled water stored in flagons of silver when going to war. In 1869, Raulin has given the modern description of this effect, when he observed *Aspergillus niger* could not grow in silver vessels. The Swiss botanist Von Ngeli devised the term “oligodynamic” to describe any metal exhibiting bacteriocidal properties at minute concentrations. Copper and tin also have oligodynamic activity. Von Ngeli distinguished between "ordinary" poisoning at measurable concentrations and "oligodynamic" death. This terminology seems contributing to much confused thinking about the ways and mechanisms by which silver kills bacteria [19].

The application of silver plates to achieve better wound healing was used by the Macedonians, perhaps the first attempt to prevent or treat surgical infections. Hippocrates used silver preparations for the treatment of ulcers and to promote wound healing [20].

In order to find an alternative treatment for the periodontitis, silver was tested for antimicrobial activity in vitro killing assays conducted in phosphate buffered saline with a series of oral bacteria including gram-negative periodontal pathogens and gram-positive streptococci. At 0.5 mg/mL of concentration, it produced a 3 $\log_{10}$ reduction in colony forming units (CFU)/mL or greater against all periodontal pathogens tested including *Porphyromonas gingivalis, Prevotella intermedia, Prevotella denticola, Bacteroides forsythus, Fusobacterium nucleatum vincentii, Campylobacter gracilis, Campylobacter rectus, Eikenella corrodens,* and *Actinobacillus actinomycetemcomitans* [21].

Oligodynamic: oligos", small + "dynamis", power [19].

Periodontitis: an infectious disease of bacterial origin (1). In recent years, considerable progress has been made as to the identification of the bacterial species responsible for the disease and to their impact on systemic health [21].

***Table 3**:* Antimicrobial activity of silver nitrate against oral bacteria [21].

Oral bacteria	Antimicrobial Agent	Log_{10} Reduction in CFU/mL					
		50 µg/mL	25 µg/mL	5 µg/mL	0.5 µg/mL	0.05 µg/mL	0.005 µg/mL
P. gingivalis A7A1-28	$AgNO_3$	nd	nd	nd	4.2[a], 3.1	3.0, 3.1	0
P. gingivalis ATCC 33277	$AgNO_3$	nd	nd	4, 4.2	4, 4.2	2.9, 3.3	0.2, 0
P. denticola ATCC 33185	$AgNO_3$	nd	nd	4.1, 3.8	4.1, 3.8	nd	nd
P. intermedia ATCC 25611	$AgNO_3$	nd	nd	nd	3.8, 3.3	3.8, 3.3	0.2, 0.4
B. forsythus ATCC 43037	$AgNO_3$	nd	nd	nd	4.2, 3.2	0.3, 0	0.2
C. gracilis ATCC 33236	$AgNO_3$	nd	nd	3.7, 3.4	3.4, 3.4	nd	nd
C. rectus ATCC 33238	$AgNO_3$	nd	nd	nd	4.1	1.4	nd
E. corrodens ATCC 23834	$AgNO_3$	nd	nd	3.0, 3.4	0.8, 3.1	nd	nd
E. corrodens 558	$AgNO_3$	nd	nd	4.3, 3.1	3.9, 3.1	nd	nd
F. nucleatum ATCC 49256	$AgNO_3$	nd	nd	nd	3.4, 3.8	0, 1.6	0
A. actinomycetemcomitans ATCC 29523	$AgNO_3$	nd	nd	3.7, 3.8, 3.0, 3.9	3.0, 3.9 3.7, 3.8	nd	nd
S. gordonii 51656	$AgNO_3$	nd	0, 0	nd	nd	nd	nd
S. mitis JK 195	$AgNO_3$	0.2, 0.2	nd	0.2, 0.3	0.2, 0.3	nd	nd
S. mutans SJ32	$AgNO_3$	0, 0	nd	0, 0.1	0, 0	nd	nd
S. sobrinus DS 1	$AgNO_3$	nd	0	0.2	0	nd	nd
S. sobrinus 6175	$AgNO_3$	0, 0.6, 0	nd	0, 0, 0	0, 0, 0	nd	nd
A. viscosus 15987	$AgNO_3$	3.7	3.7	3.7, 2.7	3.7, 2.7	nd	nd

(a) numbers represent the Log_{10} Reduction in CFU/mL obtained in an independent experiment. nd=not done.

P. gingivali s= Porphyromonas gingivalis;
P. denticola = Prevotella denticola;
P. intermedia =Prevotella intermedia;
B. forsythus = Bacteroides forsythus;
C. gracilis = Campylobacter gracilis;
C. rectus = Campylobacter rectus;
E. corroders = Eikenella corrodens;
F. nucleatum = Fusobacterium nucleatum;
A. actinomycetemcomitans = Actinobacillus actinomycetemcomitans;
S. gordonii = Streptococcus gordonii;
S. mitis = Streptococcus mitis;
S. mutans = Streptococcus mutans;
S. sobrinus = Streptococcus sobrinus;
A. viscosus = Actinomyces viscosus.

C. Cerium antibacterial activity

The antibacterial activity of the tested nanoparticles was evaluated by using different methods, such as disk diffusion tests, UV–Vis measurements of the optical density (OD), the number of colony-forming units (CFUs) on solid medium, the resazurin test, and bacterial viability, using confocal microscopy. The antibacterial activity of CeO2 was evaluated by using the microdilution method, and the corresponding MIC and MBC values were evaluated against two Gram-negative and three Gram-positive pathogens [22].

The correspondent values are given in Tables 4 and 5, respectively. The cerium oxide nanoparticles demonstrated antibacterial properties against all the tested pathogens in relative low concentrations [22].

***Table 4**:* Antibacterial activity of CeO2 at 50 ug/ml and gentamicin 10 ug/ml, against pathogenic bacteria [22].

	Pathogenic bacteria	CeO_2 Zone of inhibition (mm Diameter)	Gentamicin Zone of inhibition (mm Diameter)
G-	*Escherichia coli*	9 ± 0.05	15 ± 0.43
	Salmonella typhimurium	12 ± 0.02	17 ± 0.02
G+	*Listeria monocytogenes*	10 ± 0.04	19 ± 0.01
	Staphylococcus aureus	5 ± 0.02	18 ± 0.02
	Bacillus cereus	7 ± 0.05	16 ± 0.01

***Table 5**:* MIC and MBC of CeO2/Gentamicin against pathogenic bacteria, experiments were performed in triplicates and were repeated twice [22].

Bacterial strains	CeO2 MIC (mg/ml)	Gentamicin MIC (mg/ml)	CeO2 MBC (mg/ml)
Escherichia coli	2.15	3.0	2.15
Salmonella typhimurium	1.07	0.38	1.07
Listeria monocytogenes	1.07	1.2	1.07
Staphylococcus aureus	10	0.83	10
Bacillus cereus	4.3	0.39	4.3

According to MAGERUSAN et *al*, 7the antibacterial effect of CeO2 is confirmed by the confocal microscopy. The results clearly evidence that the inhibitory effect of CeO2 is present at a lower concentration, with respect to gentamicin (the standard drug) [22].

Practical part

Practical Part

For our investigation part on the screening for the antibacterial activity of some metallic complex molecules, we selected from the laboratory of Bejaia University ten pathogenic strains between Gram positive and Gram-negative bacteria. And from Radiation Physics Laboratory "LPR" at Annaba University we selectedsix metal complex molecules classified as pollutants of our ecosystem. Our experimental study was carried out in the laboratory of microbiology, the faculty of Nature and Life Science and Earth Science and at Bouira University. We have to highlight that this work is within collaboration between Radiation Physics Laboratory "LPR" at Annaba University and Applied Biochemistry Laboratory at Béjaia University

I. Material & methods

1- Material

A. Heavy metal molecule

To study carry on the antibacterial activity of heavy metals, we selected six complex molecules including: Titane (TiO_2), Silicium (SiO_2X_1 & SiO_2X_2), MgNPs, Zinc (Zn), Silver (Ag). These molecules were provided by the Radiation Physics Laboratory "LPR" of Annaba University. We may highlight that the chosen molecules are classified as powerful pollutant of our environment and their high concentration can induce toxicity in several ecosystems including drinking water, ground water and agriculture soil. However, at low concentration these molecules demonstrate interesting activities in clinical fields including antibacterial activity, antitumor activity, and antioxydante activity. Moreover, they showed theirs efficiency in industrial fields.

B. Bacterial strains

In our investigation, we carried out numerous microbiological essays on serial bacterial strains; including: *Salmonella Sp (Laboratory strains)*, *Klebsiella pneumoniae (Laboratory strains)*, *Enterococcus faecalis* (ATCC 29212), *Pseudomonas aeruginosa* (ATCC 6633), *Acenitobacter baumannii* (610), *Methicillin-resistant Staphylococcus aureus (MRSA)*» (ATCC 43300), *Bacillus subtilis* (ATCC 6633), *Staphylococcus epidermidis* (Clinical samples), *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 6538) (Food born).

These bacterial strains are obtained from different environment including clinical and food born. We may highlight that this bacterial strains, express resistance to antibiotics molecules as illustrate in table 6. Most of them express resistance to Beta lactam antibiotic, in particular

Gram-negative bacteria. The Gram-positive bacteria they are have resistance to quinolone and macrolide antibiotics. These antibiotic classes are defined as the last therapeutic solution for clinical situation. Before use this bacteria we have to prepare the initial culture (10^8 cells/ml).

Table 6: Characteristics of tested strains.

	Strains	ATCC	Gram	Pathogenicity	Antimicrobial Resistance Statue
S_1	*Salmonella Sp*	Laboratory strain	Negative.[23]	Pathogen [24]	Ampicillin, ofloxacin [40]
S_2	*Klebsiella pneumoniae*	Laboratory strain	Negative.[31]	Important opportunistic pathogen [32]	Quinolones, piperacillin-tazobactam [41]
S_3	*Enterococcus faecalis*	ATCC 29212	Positive.[25]	Pathogen [33]	Flavomycin, avoparcin, monensi, ampicillin, streptomycin [46]
S_4	*Pseudomonas aeruginosa*	ATCC 6633	Negative.[26]	Pathogen [34]	Imipenem, amikacin [41]
S_5	*Acenitobacter baumannii*	ATCC 610	Negative.[31]	Pathogen [35]	B lactam [35] Imipenem, amikacin [41]
S_6	*Methicillin-resistant Staphylococcus aureus (MRSA)*	ATCC 43300	Positive.[29]	Pathogen [36]	Methicillin [43]
S_7	*Bacillus subtilis*	ATCC 6633	Positive.[26]	Pathogen [37]	Erytromycin, kanamycin, lipcomycin, spectinomycin, tetracycline [44]
S_8	*Staphylococcus epidermidis*	Clinical samples	Positive.[28]	Pathogen [38]	Oxacillin, erythromycin, clindamycin, mupirocin [45]
S_9	*Escherichia coli*	ATCC 25922	Negative.[27]	Pathogen [39]	b-lactames [42]
S_{10}	*Staphylococcus aureus*	ATCC 6538 - Food born-	Positive.[26]	Pathogen [38]	Methicilin, vancomycine[43]

1. Antimicrobial solution

To carry out the various antibacterial activity essays, we prepared stock solutions of each molecule at a concentration of 10 mg / l dissolved in sterile distilled water..

2. Antimicrobial activity essays

The study of the antibacterial activity of our molecules is based on the contact of bacteria and the tested metal in a culture media. Ten bacterial suspensions of the ten strains previously listed are prepared separately in test tubes. Three main tests were applied in this purpose including, well diffusion method and spots method to highlight the ability of these molecules to inhibit bacterial strains, to show which strains are susceptible and finally to measure diameter inhibition zone for each tested strain. The last essay is microtiter plate serial dilution, used to

determinate the minimum inhibitory concentration (MIC) value of tested molecules. All essays are carried under septic conditions.

A. Well diffusion method

Heavy metal solution (50 µL) with concentration of 10mg/ml was applied into agar well (6 mm, diameter) in a Petri-dish containing 20 mL Muller-Hinton agar medium previously inoculated with 0.1 mL bacterial suspension (10^8 cells/ml). Then incubated at 37°C for 24 hours. Distilled water was used as negative control and antibiotic disc (Cefotaxim 30mg/ml) used as positive control. All analysis were performed twice time.

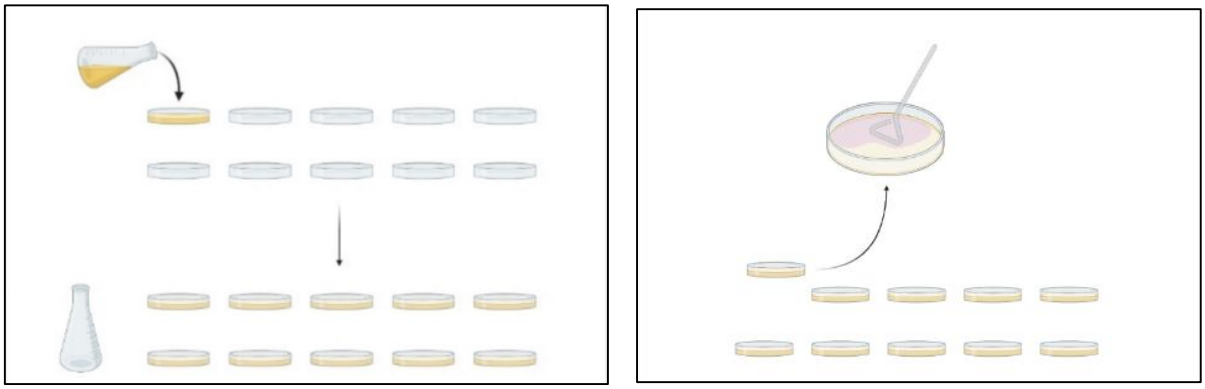

Figure 3: Preparation of plates for well method.

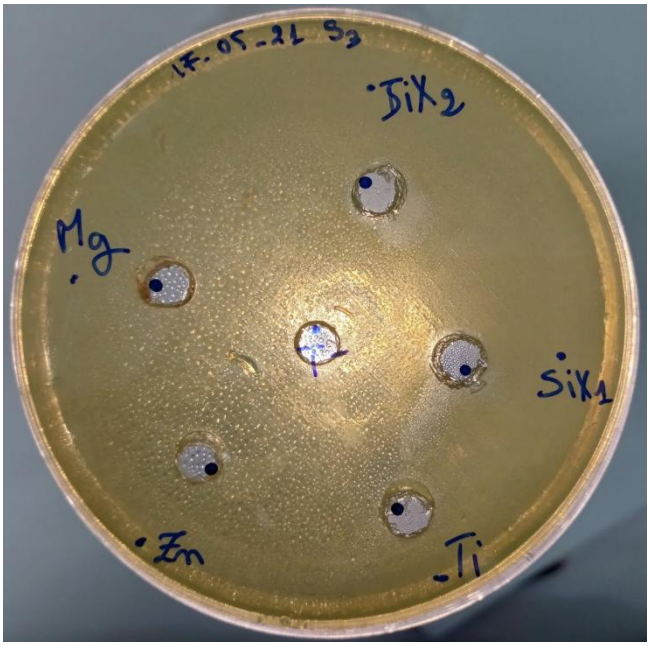

Figure 4*:* Plate prepared for well method essay.

B. Minimum inhibitory concentration (MIC)

Mostly recommended by EUCAST, MIC defined as the lowest concentration of an antibacterial agent expressed in mg/L (µg/ml) which, under strictly controlled in vitro conditions, completely prevents visible growth of the test strain of an organism. MIC was carried out according several method. In our case, we followed, dilution in agar and broth microdilution method **(EUCAST, 2014)**

C. essay on agar dilution

The minimum inhibitory concentration (MIC) is defined as the lowest concentration of the antimicrobial substance that inhibits the growth of microorganisms. Two microliters of strains inoculum containing 10^7 cells / ml are dropped on Luria Bertani (LB) medium containing the complex metallic molecules at different concentrations such as 10, 25, 50 and 100 µg / ml. Then the plate are incubated at 37 ° C. for 24 hours. Distilled water was used as a negative control. The test is repeated twice. The result was estimated by the presence or absence of growth **(EUCAST, 2014)**

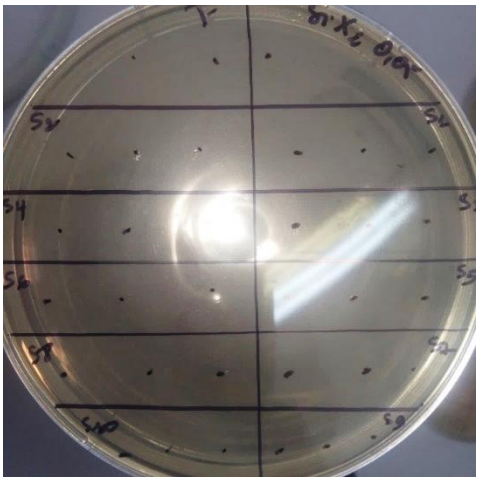

Figure 5*:* Arrangement of spots on agar

D. Minimum inhibitory concentration (MIC) essay in broth dilution

The method was described by EUCAST. For broth dilution, the antimicrobial solution is distributed into appropriate wells of microtiter plates (96-well microtiter plate format), bacteria are inoculated into a liquid growth medium in the presence of different concentrations of an antimicrobial agent. Growth is assessed after incubation for a defined period of time (16–20 hours) and the MIC value is read.

For the preparation of the microplate, we pipetted 100 µl of Luria Bertani broth into each well. Subsequently, a volume of 100 µl of the active solution is added in decreasing order of

concentration, with the exception of the last well left as a negative control. The concentration range followed is as follows: (5000, 2500, 1250, 625, 321.5, 156.25, 78.12, 39.06; 19.53 µg / ml) subsequently, the bacterial suspension is added at the rate of 20 µl in each well. The microplates are then incubated at 37 ° C. for 24 hours. The results are read under a microplate reader at a wavelength of 620nm.

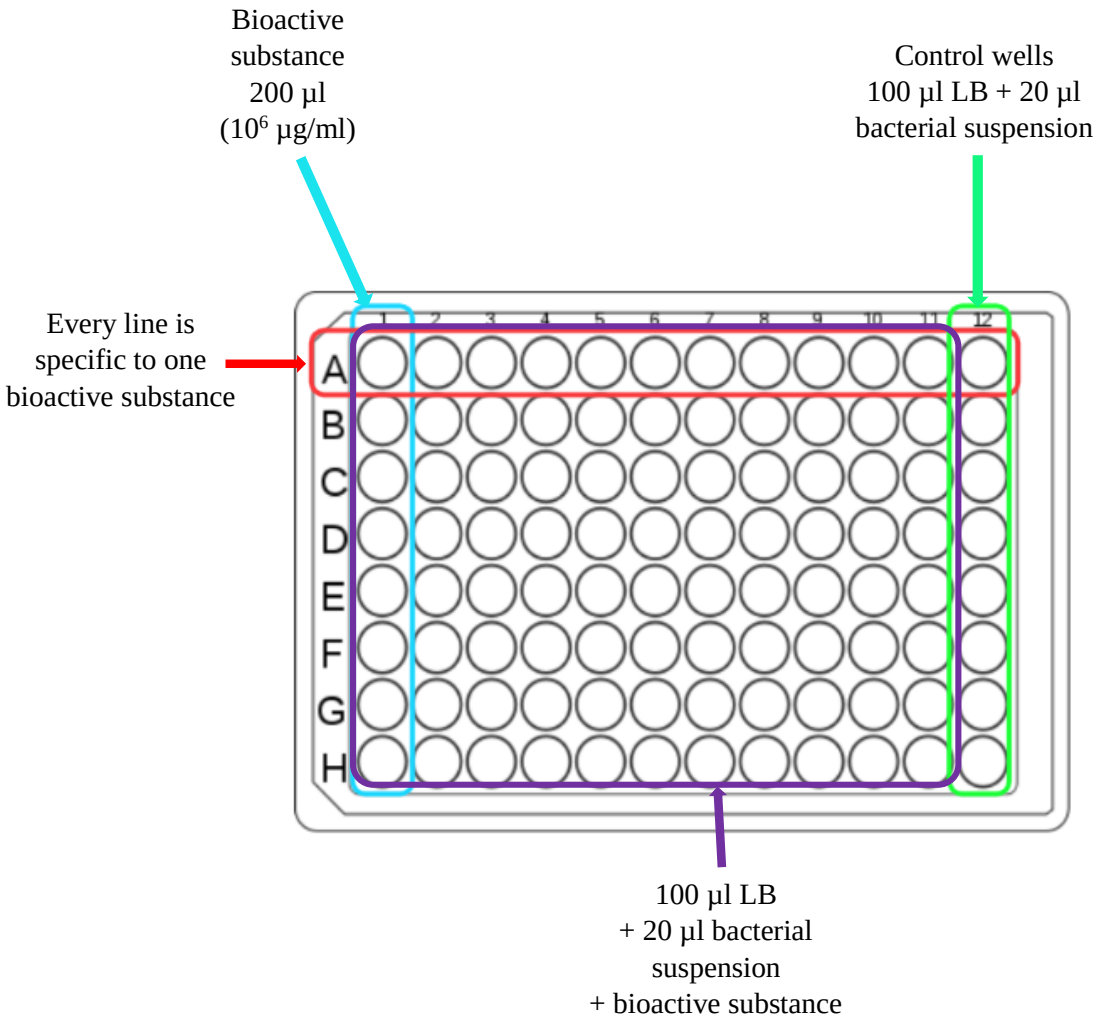

Figure 6: Microplate preparation for MIC determination.

II. Results

From all performed essay, the results reveal that metallic molecules tested including Titane (TiO_2), Silicium (SiO_2X_1 & SiO_2X_2), MgNPs, Zinc (Zn), Silver (Ag), express variables activities between Gram negatives and Gram positives bacterial groups from different sources clinical and food born.

1. In vitro antimicrobial activity of complex metallic molecules.

The obtained results relating to the study of the antibacterial activity of the complex molecules at concentrations of (10 mg / ml) tested on the ten strains, namely Gram positive and Gram negative obtained by the well method are shown in Table 07, figure n° 07 and 08.

We noticed that all the metallic complex molecules express an antibacterial activity against Gram positive and negative bacterial strains tested in this essay, where six of them; *A. baumanii, MRSA, B. subtilis, S. epidemiadis, E. coli and S. aureus* were resistant to Cefotaxime used as control. The TiO_2 express activities against all tested strains expect MRSA and Bacillus cereus. The MgNPs, SiO_2 (X_1 and X_2) and ZnO_2 have low effect on all tested strains. However, Ag express an interesting effect on tested strains, with higher effect against Gram positives strains (Table 01).

Table 7: Results noted for well method.

Tested trains	Zone inhibition diameter (mm)							
	Mg	**Zn**	**SiX1**	**SiX2**	**Ti**	**Ag1**	**Ag2**	**Control**
Salmonella Sp	7	7	8	7	8	**10**	7	8
Klebsiella pneumoniae	7	8	7	8	7	**10**	8	6
Enterococcus faecalis	7	6	8	7	7	**10**	**10**	**10**
Pseudomonas aeruginosa	7	6	8	6	**9**	**11**	8	7
Acenitobacter baumannii	6	6	6	7	7	2	2	0
Methicillin-resistant Staphylococcus aureus (MRSA)	7	6	6	6	6.5	2	2	0
Bacillus subtilis	6	6	6.5	6.5	6	2.5	2.5	0
Staphylococcus epidermidis	6	6	7	7	7	1.5	2	0
Escherichia coli	6	7	8	7	8	**20**	**12**	0
Staphylococcus aureus	7	7	7	**9**	8	2.2	**30**	0

Control: Cefotaxime (CTX) 30mg
0 mm: no inhibition

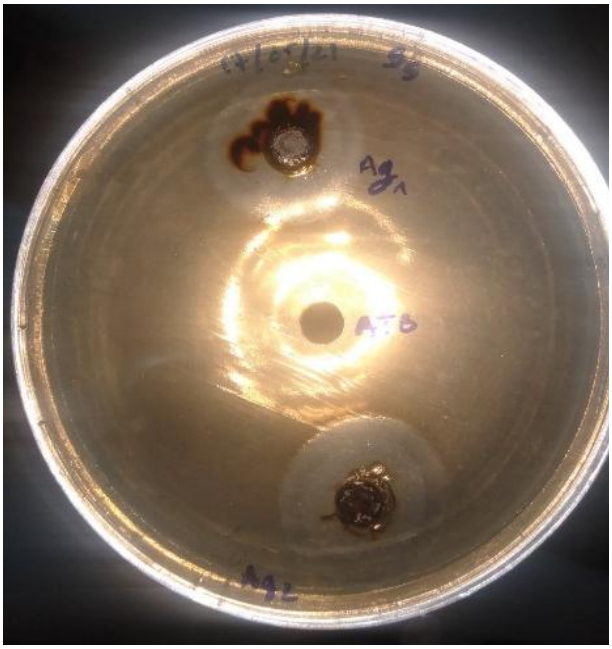

Figure 7: Inhibition zone of Ag1 & Ag2 on an agar culture of *Acenitobacter. baumannii*

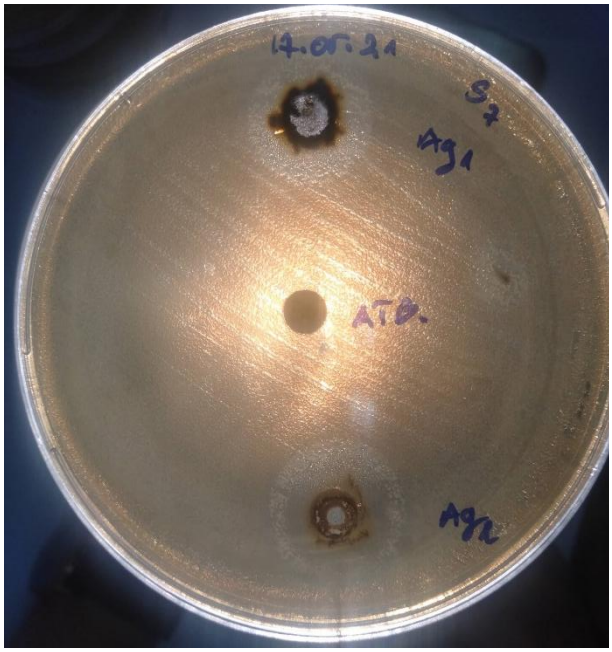

Figure 8: Inhibition zone of Ag1 & Ag2 on an agar culture of *Bacillus subtilis*

2. Spot method results

Table 8: Results noted for spots method.

Molecules	Concentrations (Mg/ml)	S_1	S_2	S_3	S_4	S_5	S_6	S_7	S_8	S_9	S_{10}
MF	0.01	+	+	+	+	+	+	+	+	+	+
	0.025	+	+	+	+	+	+	+	+	+	+
	0.05	++	++	++	++	++	++	++	++	++	++
	0.1	++	++	++	++	++	++	++	++	++	++
	T^-	-	-	-	-	-	-	-	-	-	-
Zn	0.01	+	+	+	+	+	+	+	+	+	+
	0.025	+	+	+	+	+	+	+	+	ND	+
	0.05	++	++	++	++	++	++	++	++	++	++
	0.1	+	++	+	+	+	+	+	+	+	+
	T^-	-	-	-	-	-	-	-	-	-	-
SiX_1	0.01	++	++	++	++	+	+	+	+	+	+
	0.025	+	+	+	+	+	+	+	+	+	+
	0.05	+	+	+	+	+	+	+	+	+	+
	0.1	++	++	++	++	+	+	++	+	+	+
	T-	-	-	-	-	-	-	-	-	-	-
SiX_2	0.01	++	++	++	+	+	+	+	+	+	+
	0.025	++	++	++	++	+	+	+	+	+	+
	0.05	-	-	-	-	-	-	-	-	-	-
	0.1	-	-	-	-	-	-	-	-	-	++
	T^-	-	-	-	-	-	-	-	-	-	-
Ti	0.01	+	+	+	+	+	+	+	+	+	+
	0.025	+	+	+	+	+	+	+	+	+	+
	0.05	+	+	+	+	+	+	+	+	+	+
	0.1	+	+	+	+	+	+	+	+	+	+
	T^-	-	-	-	-	-	-	-	-	-	-
Ag2	**0.01**	-	-	-	-	-	-	-	-	-	-
	0.025	-	-	-	-	-	-	-	-	-	-
	0.05	-	-	-	-	-	-	-	-	-	-
	T^-	-	-	-	-	-	-	-	-	-	-
Witness	/	-	-	-	-	-	-	-	-	-	-

(+): Growth (++): Growth with diffusion (-): No growth

S_1: Salmonella Sp
S_2: Klebsiella pneumoniae
S_3: Enterococcus faecalis
S_4: Pseudomonas aeruginosa
S_5: Acenitobacter baumannii
S_6: Methicillin-resistant Staphylococcus aureus (MRSA)
S_7 Bacillus subtilis
S_8: Staphylococcus epidermidis
S_9: Escherichia coli
S_{10}: Staphylococcus aureus

In this essay, we could confirm the sensibility of the tested bacteria to our molecules and by which concentration they are affected (first determination of CMI). In the two plates of Magnesium with **0.01** mg/ml and **0.025** mg/ml we noticed a week growth of all the strains, but on the other plates of **0.05** mg/ml and **0.1** mg/ml of concentration, bacteria were strongly growth even the important concentration of the Mg.

Zinc and titanium complex molecules haven't shown bacterial growth inhibition, where we observed a week growth of the ten strains for all the concentrations of Titanium and the concentrations **0.01** mg/ml, **0.025** mg/ml and **0.1** mg/ml of zinc. But, at **0.05** of concentration, all the strains tested haven't been affected by zinc particles and have strongly grown.

At high concentration, **SiX_2** inhibit bacterial growth, where, after 24 hours of incubation, no growth was observed for the concentrations 0.05 mg/ml and 0.1 mg/ml for all bacterial strains, except for *S. aureus*, which were inhibited for 0.05 mg/ml but strongly grew for 0.1 mg/ml of concentration.

Among the studied molecules, silver performed a high antibacterial activity; its results showed that no bacterial growth was noted for his different concentrations.

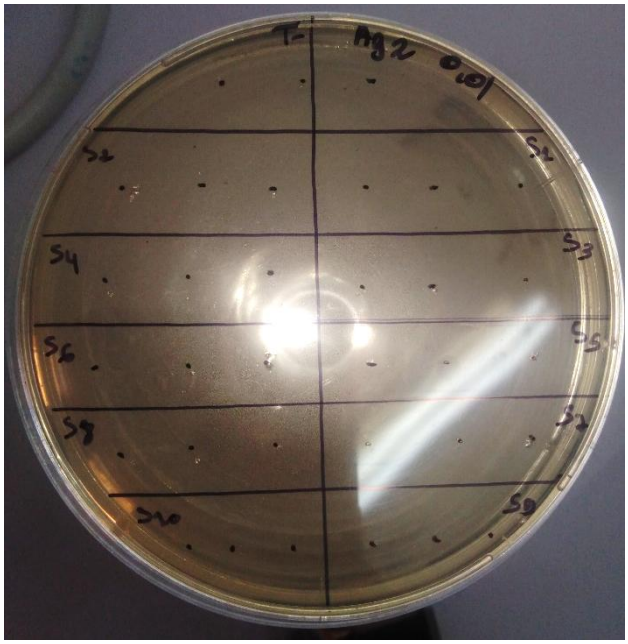

Figure 9: Spots arrangement on plate for spot essay.

3. Microplate essay results

The obtained results are summarized in the table 9, which reveal that the metallic compound have variable activities on the tested strains.

From the MIC essays, we found that there is correlation between the obtained results from broth and solid MICs. The strains *Acenitobacter baumannii* S5 have low CMI with Ti and SiX_2, respectively 78.12µg/ml and 19.53µg/ml. While with Ag molecule, the MIC is high 1250µg/ml. concerning the strains *Bacillus cereus* S7, the MIC with SiX_2 is 652µg/ml but with Ag is higher 2500µg/ml. On the opposite of strain *Escherichia coli* S9 that have a CMI of 39.06µg/ml.

Table 9: obtained results from MIC in brot**ND**: non determine

	S1	***S2***	***S3***	***S4***	***S5***	**S6**	***S7***	***S8***	***S9***	***S10***
Ti	>19.53µg/ml	>19.53µg/ml	>19.53µg/ml	>19.53µg/ml	**78.12µg/ml**	ND	>19.53µg/ml	>19.53µg/ml	>19.53µg/ml	>19.53µg/ml
SI X1	>19.53µg/ml	>19.53µg/ml	>19.53µg/ml	>19.53µg/ml	>19.53µg/ml	ND	>19.53µg/ml	>19.53µg/ml	>19.53µg/ml	>19.53µg/ml
SI X2	>19.53µg/ml	>19.53µg/ml	>19.53µg/ml	>19.53µg/ml	**19.53µg/ml**	ND	**652 µg/ml**	>19.53µg/ml	>19.53µg/ml	>19.53µg/ml
Zn	>19.53µg/ml	>19.53µg/ml	>19.53µg/ml	>19.53µg/ml	>19.53µg/ml	ND	>19.53µg/ml	>19.53µg/ml	>19.53µg/ml	>19.53µg/ml
Mg	>19.53µg/ml	>19.53µg/ml	>19.53µg/ml	>19.53µg/ml	>19.53µg/ml	ND	>19.53µg/ml	>19.53µg/ml	>19.53µg/ml	>19.53µg/ml
Ag	>19.53µg/ml	>19.53µg/ml	>19.53µg/ml	>19.53µg/ml	**1250 µg/ml**	ND	**2500 µg/ml**	>19.53µg/ml	**39.06 µg/ml**	>19.53µg/ml

III. Disscussion

Investigation on heavy metal activity has captivated the attention of numerous scientists around the world. This motivated us to work on this type of molecules and carry on a screening of their antimicrobial, namely: Titane (TiO_2), Silicium (SiO_2X_1 & SiO_2X_2), Mg, Zinc (Zn), Silver (Ag) on pathogenic germs either of the Gram positive or Gram-negative group or of different origin. The activity of these molecules is highly variable between the strains tested.

The TiO_2express activities against all tested strains expect MRSA and Bacillus cereus. The Mg, SiO2 (X_1 and X_2) and Zn have low effect on all tested strains. However, Ag express an interesting effect on tested strains, with higher effect against Gram positives strains. The activity of Ag NPs was confirmed with MIC essay (Table 02). The tested strains express a CMI of 100µg/ml. The strange results was with SiO_2 (X_2), with high concentrations including 100µg/ml and 50 µg/ml there was no strains growth, while the strains are resistance on 25 and 10 µg/ml concentration.

Our obtained results corroborate with those obtained with other investigation in the same fields. Djebbou and his collaborator, reveal that silver nanoparticles exhibit high antibacterial activity against Gram-positive bacteria such as *Staphylococcus aureus* and Gram negative such as *Escherichia coli, Klebsiella pneumoniae, Salmonella spp.* Among metals, silver (AgNPs) have emerged and gained much interest over the last decade because to their interesting properties including good conductivity, chemical stability, catalytic and antimicrobial activities. The high quality and monodispersed AgNPs contribute to the size-dependent nanomedicine applications [47].

The high antimicrobial activities of silver is mainly related to the morphological change of cell surface inducing a damage in bacterial cell membranes that could induce a leakage of cytoplasmic contents in damaged cells. Moreover, the increased cell membrane permeability can serve as an evidence for the formation of AgNPs “pits” inmorphological changes of cell surface. Following the microscopy observation obtained by Annaba team, reveal an interesting difference between Gram-positive *S. aureus* and Gram-negative *E. coli, Klebsiella pneumonia* and *Salmonella*. This may be attributed to the alteration of the cells’ peptidoglycan layer between the four different indexed pathogenic bacteria ‘already mentioned’. Besides, Gram-positive cell envelope consists of lipoteichoic acid containing thick peptidoglycan layer and cell membrane [47].

Microbial activities of other metallic compound is related to theirs chemical structure, we may give the example of Zn. Wang and coworker revealed that chemical structure of metallic compound including the crystallographic orientation and type of surface plane can influence antibacterial efficiency of ZnO_2 nanowires. They showed that randomly oriented ZnO_2 nanowires were more efficient in killing *E. coli* than regularly oriented ones. This is probably due to different spatial arrangements of ZnO_2 [48].

The obtained results proved that we may use metallic complex molecule in clinical essays but we have first to investigate on theirs toxicities.

Conclusion

Essays performed during this investigation have successfully showed good promising results to discover alternative treatments for the classic antibiotherapy, we could confirm the potentiality of the complex metallic antibacterial activity according to silver and Silicium results, for which all the tested bacteria were susceptible. For the other molecules, microdilution should be realized for low concentrations than those we used previously.

This paper results open a new era for another new substance in the biotherapy, to be adapted as new treatment against bacterial infections, more intensive essays must be applied in order to determinate all the characteristics of this molecules. The action mode of metallic complex molecule must be investigated and their MBC must be determined, the cytotoxicity activity also is one of the most necessary steps for this wide study, in order to evaluate at which concentrations our molecules can only eliminate cancer cells and what are their limits after which it can be toxic for human safe cells.

Bibliographic references

[1]. JEAN-LUC, JEFFERSON, MORTATTI, AND PROBST. Characteristics of heavy metals and their evaluation in suspended sediments from Piracicaba river basin (São Paulo, Brazil). **[Online]**. (2010). *Revista Brasileira de Geociências.* Volume 40. Number °3. pp. 375-379. ISSN 0375-7536. https://oatao.univ-toulouse.fr/5442/

[2]. APPENROT, K-J. Definition of "Heavy Metals" and Their Role in Biological Systems. **In:** *Soil Heavy Metals, Soil Biology.* Volume 19. . Berlin Heidelberg: © Springer-Verlag. (2009). P. 19–29.

[3]. CASTRO-GUTIÉRREZ.L.T., LÓPEZ-VELANDIA. D. P., .PRADA-QUIROGA.C.F., TORRES-CAYCEDO.M.I. Antibiotic Resistance: Origins, evolution and healthcare-associated infections. *Scielo (scientific electronic library online).* **[Online].** (2018). Volume 34, Number °2, p. 494-505. http://dx.doi.org/10.14482/sun.34.2.615.32

[5]. ATANASOV, P., IVNITSKI, D., IHAB, A, H., WILKINS, E. Biosensors for detection of pathogenic bacteria **[Online]**. **IN:** *Biosensors & Bioelectronics*. Volume 14 issue 7. Department of Chemical and Nuclear Engineering, University of New Mexico, Albuquerque, NM 87131, USA: Elsevier Science S.A. (1999). P. 599–624. https://doi.org/10.1016/S0956-5663(99)00037-8 (ISSN: 0956-5663)

[6]. COSSART, P. *The New Microbiology: From Microbiomes to CRISPR. Washington, DC 20036-2904,* USA: ASM Press, (2018). ISBN 9781683670100

[8]. CASTRO-GUTIÉRREZ.L.T., LÓPEZ-VELANDIA. D. P., .PRADA-QUIROGA.C.F., TORRES-CAYCEDO.M.I. Antibiotic Resistance: Origins, evolution and healthcare-associated infections. *Scielo (scientific electronic library online).* **[Online].** (2018). Volume 34, Number °2, p. 494-505. http://dx.doi.org/10.14482/sun.34.2.615.32

[9]. Chen Hu, C., Shao, L., Wang, L. The antimicrobial activity of nanoparticles: present situation and prospects for the future. *International journal of nanomedecine.* **[Online].** (2017). Volume 12. P. 1227–1249. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5317269/

[10]. LIN, W., WAN, K., YE, C., YU, X., ZENG, J., & ZHANG, M. Co-selection and stability of bacterial antibiotic resistance by arsenic pollution accidents in source water. *Environnent International*. **[Online]**. (2020). Volume 135. P. 15. https://doi.org/10.1016/j.envint.2019.105351

[11]. ROBERTS, M. C., SCHINK, A.-K., SCHWARZ, S., VAN DUIJKEREN, E., & WANG, Y. Mechanisms of Bacterial Resistance to Antimicrobial Agents. **[Online].** (2018). *Microbiology Spectrum.* Volume 6. Number °2. P. 33. https://www.researchgate.net/publication/322463803_Mechanisms_of_Bacterial_Resistance_to_Antimicrobial_Agents

[12]. SHAHIDI, F., & ZHONG, Y. Measurement of antioxidant activity. *Journal of Functional Foods, Memorial University of Newfoundland.* **[Online].** (2015). Volume 18. Part B. p. 757-781. http://dx.doi.org/10.1016/j.jff.2015.01.047

[13]. Guide des aliments antioxydants

[14]. Antolovich, E., McDonald.M., Prenzler, P. D., Patsalides. S., & Robards, K. Methods for testing antioxidant activity. *The Analyst*. **[Online].** (2001). Volume 127. Number °1. P. 183–198. https://doi.org/10.1039/B009171P

[15]. M.A. Synthesis. Characterization and biological activities of copper nanoparticles. *The Egyptian Society of Experimental Biology of Egypt*. **[Online]**. 2019. Volume 15. Number °2. P. 393 – 402. http://egyseb.net/?mno=77143

[16]. Cytotoxicity. (2015). *Encyclopedia of Immunotoxicology, 250–250.* doi:10.1007/978-3-642-54596-2_200356

[17]. Tolosa, L., Donato, M. T., & Gómez-Lechón, M. J. (2014). *General Cytotoxicity Assessment by Means of the MTT Assay. Protocols in In Vitro Hepatocyte Research, 333–348.* doi:10.1007/978-1-4939-2074-7_26

[18]. HARRISON, J. J., LEMIRE, J. A., & TURNER, R. J. Antimicrobial activity of metals: mechanisms, molecular targets and applications. *Antibiotic alternatives*. **[Online]**. (2013). Volume 11. Number °6. P. 371–384. https://doi.org/10.1038/nrmicro3028

[19]. CLEMENT, J. L., & JARRETT, P. S. (1994). Antibacterial Silver. *Metal-Based Drugs*. **[Online]**. (1994). Volume 1. Number (5-6). P. 467–482. https://doi.org/10.1155/MBD.1994.467

[20]. J. Wesley Alexander. History of the Medical Use of Silver. *Surgical infections*. **[Online].** Volume 10, Number °3, p. 6. https://doi.org/10.1089/sur.2008.9941

[21]. BUXTON D., FRIDEN P., ROTHSTEIN D., SPACCIAPOLI P. Antimicrobial activity of silver nitrate against periodontal pathogens. *Journal of periodontal research*. [Online]. (2001). Volume 36. Number °2. P. 108-113. https://doi.org/10.1034/j.1600-0765.2001.360207.x

[22]. MAGERUȘAN, L., SOCACIU, C., MESAROS. A., POP. O. L., VODNAR. D. C., SUHAROSCHI. R., TĂBĂRAN, F. Cerium Oxide Nanoparticles and Their Efficient Antibacterial Application in Vitro against Gram-Positive and Gram-Negative Pathogens. *Nanomaterials.* **[Online]**. (2020). Volume 10. Number °8. P. 1614. https://pubmed.ncbi.nlm.nih.gov/32824660/

[23] Ben Salem Imen., Mzoughi Ridha and Aouni Mahjoub, Laboratory Typing Methods for Diagnostic of Salmonella Strains, the “Old” Organism That Continued Challenges [online], disponible in https://www.researchgate.net/publication/221922714 .2012

[24] Michael Hensel, Salmonella Pathogenicity Island 2Molecular Microbiology, 2000, vol;36

[25] Dikdik kurnia., Putri Rachmawati and Mieke H Satari, Antibacterial of Dibenzo-p-Dioxi-2,8-Dicarboxylic Acid Against Pathogenic Oral Bacteria E.faecalis ATCC 29212 peptide Phromones: Quorum Sensing of in vitro and in silico Study, 2020, vol; 14

[26] Seung-Yong Shin., Hak-Ryul Kim and Sun-Chul Kang, Antibacterian Activity of varios hydroxyl fatty acids bioconveted by Pseudomonas aeroginosa PR3, Jornal of Applied Biological Chemistry, 2004, vol;14

[27] Sana Mukhtar., Ifra Ghori, Antibacterial activity of aqueous and ethanolic extracts of garlic, cinnamon and turmeric against *escherichia coli* atcc 25922 *and*
bacillus subtilis dsm 3256, Internationel Jornal of applied biology and pharmaceutical technology, 2012, vol; 3, p.131-136

[28] Samuel Yang., Padmini Ramachandran and Richaed E.Rothan, Rapid PCR-Based Diagnosis of Septic Antritis by Early Gram-Type Classification and Pathogen Identification, Jornal of clinical Microbiology,2008, vol;46, p.1386-1390

[29] Rui Ding., Xue-Chang Wu., Chao-Dong, Yi Teng.,Ou Li., Zha-Jun Zhan, Yu-Hua Zhao, Isolation and identification of lipopeptide antibiotics from Paenibacillus elgii B69 with inhibitory activity against methicillin-resistant Staphylococcus aureus, The Jornal of Microbiology, 2011, vom ;49, p.942-949

[30] P.Nordmane., L.Poirel . Résistances aux antibiotiques émergentes et importantes chez les bactéries Gram négatif : épidémiologie, aspects théoriques et détection ;vol.10, 2014

[31] Mariana Albano., Melissa J Karau., Audrey N Schuetz and Robin Patel, Comparison of agar dilution to broth microdilution for testing in vitro activity of cefiderocol against gram-negative bacilli, Jornal of clinical microbiology,2020, vol;59

[32] Carsten Struven., Karen Angeliki Krogfelt, Pathogenic potential of environmental Klebsiella pneumonia isolates, Enviromental microbiology,2004, vol;6, p.584-590

[33] Wattana Panphut., Tanakwan Budsabun and Pakkakul Sangsuriya, IN vitro antimicrobial activity of piper retrofactum fruit extracts against microbial pathogens causing infections in human and animals, International jourmal of microbiology, 2020

[34] Souren Paul., RC Dubey., DK Maheswari and Chul Kang, Trachspermum ammi (L.) fruit essential oil influencing on membrane permeability and surface characteristics in inhibiting food-borne pathogens, Food control, 2011, vol; 22, p.725-731

[35] Anton Y Peleg., Harald Seifert and David L Paterson, Acenotobacter baumannii. Emergence of a Successful Pathogen, Clinical microbiology reviews,2008, vol;21, p.538-582

[36] Mohd Shoeb., Mohammad Mobin., Mohd Ahmar Rauf, Mohammad Owais and Alim H Naqvi, In vitro and in vivi Antimicrobial evaluation of grapheme-polyindole (Gr@Pln) nanocomposite against Methicillin-Resistant Staphylococcus aureus pathogen , 2018, vol;3, p.9431-9440

[37] El-Shanshoury, A. E.-R. R., ElSilk, S. E., & Ebeid, M. E. (2011). *Extracellular Biosynthesis of Silver Nanoparticles Using Escherichia coli ATCC 8739, Bacillus subtilis ATCC 6633, and Streptococcus thermophilus ESh1 and Their Antimicrobial Activities. ISRN Nanotechnology, 2011, 1–7.* doi:10.5402/2011/385480

[38] J Lourtet-Hascoet., A Bicart-See., MP Félicé, G Giordano and E Bonnet, Staphylococcus lugdunesis, a serious pathogen in periprsthetic joint infections: comparison to Staphylococcus epidermidis, International Journal of infectious diseases,2016, vol;51, p.56-61

[39] Lin Chen and Hongshun Yang, Metabolic characterisation of eight Escherichia coli strains including˜Big Six″ and acidic responses of selected strains revealed by NMR spectroscopy, Food Microbiology,2020, vol;88

[40] Risky Aprillian., Dadik Rahardjo and Sztiawan Koesdarrto, Evaluation of Salmonella sp contamination and its antibiotics resistance patterns isolated isolated from broiler meat sold at wet market in center of Surabaya,2015, vol;5, n.6

[41] DMarchaim., R Zaidenstein., T Lazarovitch., Y Karpuch., T Ziv and M Weinberger, Epidemiology of bacteremia episodes in a single center: increase in Gram-negative isilares, antibiotics resistance, and patient age, European journal of clinical microbiology & infectious diseqses, 2008, vol; 27, p.1045-1051

[42] Solmaz Matinfar and Saeed Javedansirat, Phylogenetic and antibiotics resistance in extended-spectrum B-lactamase (ESBL) Uropathogenic Escheria coli :An update review, 2021, vol; 23

[43] Louis B. Rice MD,Antimicrobial resistance in gram-positive bacteria, American Journal of Infection control, 2006, vol;34,p.11-19

[44] Anne-Marie Guérout-Fleury and Patrick Stagier, Antibiotic-resistance cassettes for bacillus subtilis, 1995, vol;167, p.335-336

[45] Susana Delgado.,Rebeca Arroyo.,et al, Staphylococcus epidermidis strains isolated from breast milk of women suffering infectious mastitis: potential virulence traits ans resistance to antibiotics,2008, vol;9, p.1-11

[46] Patrick Butaye.,Luc A.Devriese and Freddy, Differences in Antibiotic resistance patterns of *Enterococcus faecalis* and *Enterococcus faecium* strains isolated from farm and pet animals, 2001, vol; 45, p.1374-1378.

[47] Djeddou Karima, Bouloudenine Manel · Soualah Alila Hana · Bououdina Mohamed , Formation of Silver Nanoparticles by a Novel Irradiation Method Without a Reducing Agent and Their Impact on Four Pathogenic Bacterial Strains. Journal of Inorganic and Organometallic Polymers and Materials. 2020. https://doi.org/10.1007/s10904-020-01466-0.

[48] Wang X, Yang F, Yang W, Yang X. A study on the antibacterial activity of one-dimensional ZnO nanowire arrays: effects of the orientation and plane surface. Chem Commun. 2007;42:4419–21.

Abstract

The antibacterial drugs resistance is one of the major problems threating humanity in our days, numerous disciplines are used to find an alternative treatment for bacterial infections because of the emergence of the bacterial resistance. That's why our study is interesting in the investigation of the activity antibacterial of complex molecules of some heavy metals; Zinc (Zn), Titanium (Ti), Magnesium (Mg), Silicium (Si) and Silver (Ag). For this investigation, Agar well method and spot essay were used at first to evaluate effects of different concentrations of each molecule on ten bacterial strains tested. After, Microdilution and electronic microscopy were performed to determinate the values and mechanisms of action for each metallic complex molecule. Results noted for the set of these techniques were very promising for another era bacterial infections treatment more efficient than actual antibiotherapy and theoretically less susceptible to get bacterial resistance mechanisms.

Keywords : Metallic Complex molecules, heavy metals, antibacterial activity, CMI.

Résumé

L'antibiorésistance est l'un des problèmes majeurs menaçant l'humanité de nos jours, de nombreuses disciplines sont menées à trouver un traitement alternatif pour les infections bactériennes en raison de l'émergence de la résistance bactérienne. C'est pourquoi notre étude s'est intéressé à l'étude de l'activité antibactérienne de molécules complexes de certains métaux lourds ; Zinc (Zn), Titane (Ti), Magnésium (Mg), Silicium (Si) et Argent (Ag). Pour cette enquête, la méthode des puits d'agar et des spots sur gélose ont d'abord été utilisés pour évaluer les effets de différentes concentrations de chaque molécule sur dix souches bactériennes testées. Ensuite, une microdilution et une microscopie électronique ont été réalisées pour déterminer les valeurs et les mécanismes d'action de chaque molécule complexe métallique. Les résultats notés pour l'ensemble de ces techniques étaient très prometteurs pour un traitement des infections bactériennes d'une autre époque plus efficace que l'antibiothérapie actuelle et théoriquement moins susceptible d'obtenir des mécanismes de résistance bactérienne.

Mots clés : Molécules complexes métalliques, Métaux lourds, CMI.

ملخص

تعد مقاومة الأدوية المضادة للبكتيريا من المشاكل الرئيسية التي تهدد البشرية في أيامنا هذه، حيث يتم استخدام العديد من التخصصات لإيجاد علاج بديل للعدوى البكتيرية بسبب ظهور المقاومة البكتيرية. هذا هو السبب في أن دراستنا مثيرة للاهتمام في التحقيق في النشاط المضاد للبكتيريا للجزيئات المعقدة لبعض المعادن الثقيلة؛ الزنك(Zn) ، التيتانيوم (Ti) ، المغنيسيوم (Mg) ، السليسيوم (Si) والفضة (Ag).في هذا التحقيق، تم استخدام طريقة آجار جيدًا والمقالة الموضعية في البداية لتقييم تأثيرات التركيزات المختلفة لكل جزيء على عشر سلالات بكتيرية تم اختبارها. بعد ذلك، تم إجراء التخفيف الدقيق والفحص المجهري الإلكتروني لتحديد قيم وآليات العمل لكل جزيء معقد معدني. كانت النتائج التي لوحظت لمجموعة من هذه التقنيات واعدة للغاية بالنسبة لعصر آخر من علاج الالتهابات البكتيرية أكثر كفاءة من العلاج بالمضادات الحيوية الفعلية ومن الناحية النظرية أقل عرضة للحصول على آليات المقاومة البكتيرية.

الكلمات المفتاحية: جزيئات المعادن المركبة، المعادن الثقيلة، الحد الأدنى للتركيز.