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Control of Pathogenic *Staphylococcus aureus* Growth by Using Antibiotics and Chemical Elements

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ABSTRACT

The aim of this study was to control of resistant *Staphylococcus aureus* by antibiotics with some chemical elements. MERSA strain was chosen, isolated and identified by vitek. Of 5 antibiotics chosen, the isolate was resistant to 2 antibiotics (Ampicillin/Sulbactam and Ceftazidime) moderate to 2 antibiotics (Ceftriaxone and Rifampicin) and sensitive to only one antibiotic (Ofloxacin). And of 15 chemical elements applied, *S. aureus* was resistant to 12 elements and only 3 ones gave low sensitivity with inhibition zones about 7 mm or 6 mm in the diameter to it. Different concentrations were prepared from each chemical elements and mixed with each antibiotic separately. The antibiotics to which bacteria was resistant, gave high sensitivity to it after adding the chemical elements with inhibition zones reaching 20 and 30 mm in diameter. In the moderate antibiotics, the inhibition zones were increased for one antibiotic (Ceftriaxone) and the other antibiotic (Rifampicin) increased with some elements and decreased with other elements in different concentrations. In the sensitive antibiotic (Ofloxacin), the inhibition zones were increased with some elements and decreased with the others in different concentrations.

Keywords: *S. aureus* resistance, Antibiotic resistance, Metal resistance, antimicrobial effect. markers.

1. Introduction

Antibiotic resistance is a major subset of Antimicrobial Resistance that applies specifically to bacteria which become resistant to antibiotics. Resistance in bacteria can arise naturally by genetic mutation or by one species acquiring resistance from another. It can appear spontaneously because of random mutations. Many groups of bacteria become resistant to Antibiotics, especially *Staphylococcus* genus.

Staphylococcus aureus is a major bacterial human pathogen that causes a wide variety of clinical manifestations although it is found in normal human flora, located on the skin and mucous membranes (Taylor and Unakal, 2022).

Staphylococci can be isolated in routinely used bacteriological media like nutrient agar, blood agar or specific media like mannitol salt agar (MSA) (Samanta and Bandyopadhyay, 2020).

On media, *Staphylococcus aureus* organisms can grow in up to 10% salt, and colonies are often golden or yellow. These organisms can grow aerobically or anaerobically (facultative) and at temperatures between 18 C and 40 C. Typical biochemical identification tests include catalase positive, coagulase positive and mannitol fermentation positive (Taylor and Unakal, 2022).

S. aureus expresses many potential virulence factors such as: Surface proteins that promote colonization of host tissues, Factors that probably inhibit phagocytosis (capsule, immunoglobulin binding protein A), Toxins that damage host tissues and cause disease symptoms (Foster and Baron, 1996)

The major targets for antibiotics in *Staphylococci* are the cell envelope, the ribosome and nucleic acids. Horizontally acquired resistance can occur by one of the following mechanisms:

- Enzymatic drug modification and inactivation.

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- Enzymatic modification of the drug binding site.
- Drug efflux.
- Bypass mechanisms involving acquisition of a novel drug-resistant target.
- Displacement of the drug to protect the target.
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Acquisition of resistance by mutation can result from:

- Alteration of the drug target that prevents the inhibitor from binding.
- Derepression of chromosomally encoded multidrug resistance efflux pumps.
- Multiple stepwise mutations that alter the structure and composition of the cell wall and/or membrane to reduce drug access to its target (Foster, 2017).

S. aureus can resist different groups of antibiotics like β-Lactam antibiotics, Glycopeptides Lipopeptides, Tetracyclines, Aminoglycosides, Macrolides and many other antibiotics.

Methicillin-resistant *Staphylococcus aureus* (MRSA) and Vancomycin-resistant *Staphylococcus aureus* (VRSA) are the most common resistant of *S. aureus* founded in the world. (CDC, 2011)

There are many of chemical elements using in various drugs with limit concentrations to increase their effectiveness e.g., Lithium, Calcium, Magnesium, Copper and Iron. Some elements using as a supplement to treat diseases such as using Lithium Carbonate for mental diseases and bipolar disorder.

2. Materials and Methods

I. Materials

Organisms: *S. aureus*, identified according to Vitek.

Chemical elements such as:

- Lithium carbonate Li2CO3 "Li (1)" and Lithium sulphate Li2SO4 source of Li+
- Calcium nitrate Ca (NO3)2 source of Ca+2
- Magnesium Sulphate MgSO4 source of Mg+2
- Copper sulphate CuSO4 source of Cu+2
- Cobalt chloride CoCl2 source of Co+2
- Barium chloride BaCl2 source of Ba+2

Concentrations applied (1m M, 500 µM, 250 µM, 125 µM, 50 µM, 25 µM and 10 µM).

Antibiotics:

- Ampicillin/Sulbactam 20/10µg (A.R.E)
- Ceftazidime 30µg (A.R.E)
- Rifampicin 5µg (A.R.E)
- Ceftriaxone 30µg (A.R.E)
- Ofloxacin 5µg (A.R.E).

II. Methods

Disc diffusion method (DDM)

Agar disk-diffusion testing developed in 1940, is the official method used in many clinical microbiology laboratories for routine antimicrobial susceptibility testing.

In this well-known procedure, agar plates are inoculated with a standardized inoculum of the test microorganism. Then, filter paper discs (about 6 mm in diameter), containing the test compound at a desired concentration, are placed on the agar surface. The Petri dishes are incubated under suitable conditions (Balouiri *et al.,* 2015)

Streak Plate Method (SPM)

The streak-plate procedure is designed to isolate pure cultures of bacteria, or colonies, from mixed populations by simple mechanical separation. Single colonies are comprised of millions of cells growing in a cluster on or within an agar plate (Sanders, 2012).

Pour Plate Method (PPM)

This method often is used to count the number of microorganisms in a mixed sample, which is added to a molten agar medium prior to its solidification. The process results in colonies uniformly distributed throughout the solid medium when the appropriate sample dilution is plated (Sanders, 2012).

3. Results

The data of Figs. 1,2 showed that only Cr+6, Ba+2 and Fe+2 had an impact on the bacteria (*S. aureus*) with inhibition zone diameter (7 mm, 7 mm and 6 mm) respectively.

Concerning the antibiotics the data further, showed that Ampicillin/Sulbactam 20/10µg had no effect on the growth of *S. aureus*.

Mixing of the antibiotic with different chemical elements had a synergistic action where Cr+6 had the maximum inhibitory effect with diameter 32 mm while Ba+2 gave lower IZD with diameter 23 mm. Other elements had an intermediate inhibitory effect.

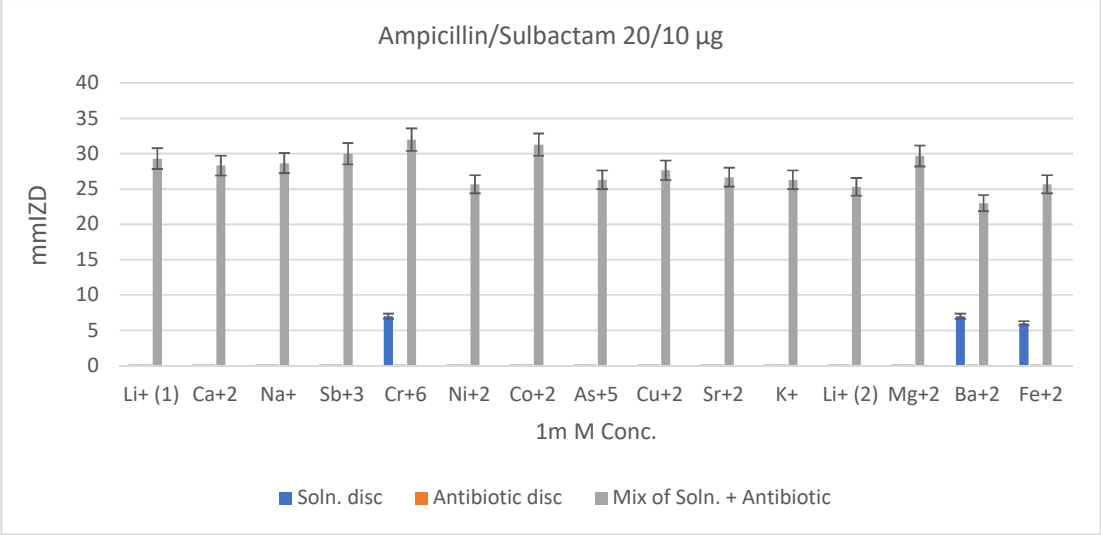

Fig. 1: Effect of different chemical elements concentration (1m M), Ampicillin/Sulbactam 20/10µg and their mixture on *S. aureus* growth.

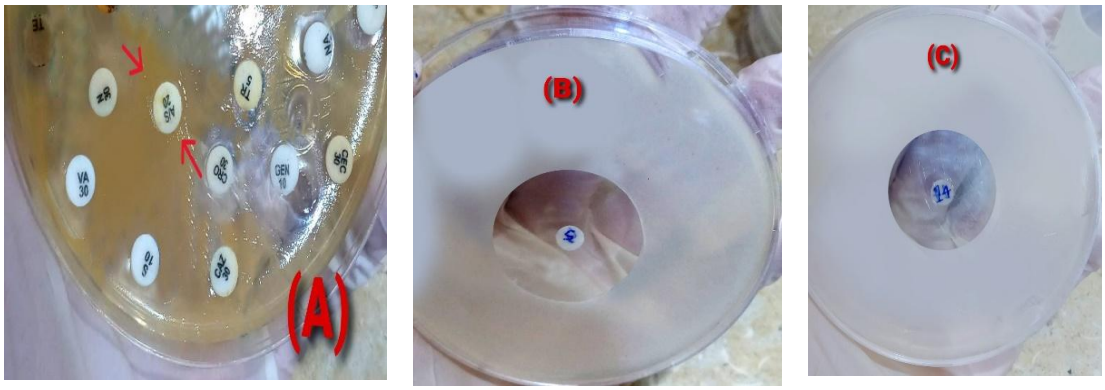

Fig. 2: Inhibition zones of Ampicillin/Sulbactam 20/10µg disc separately (A), Chromium 1m M conc. mixed with Ampicillin/Sulbactam 20/10µg (B) and Barium 1m M conc. mixed with Ampicillin/Sulbactam 20/10µg (C).

The data of Figs. 3,4 revealed that, only Cr+6, Ba+2 and Fe+2 had an impact on the bacteria (*S. aureus*) with inhibition zone diameter (7 mm, 7 mm and 6 mm) respectively.

Concerning the antibiotics the data further, showed that Ceftazidime 30µg had no effect on the growth of *S. aureus*.

Mixing of the antibiotic with different chemical elements had a synergistic action where K+ had the maximum inhibitory effect with diameter 25 mm, while Cu+2 gave the lowest IZD with diameter 14 mm. Other elements had an intermediate inhibitory effect.

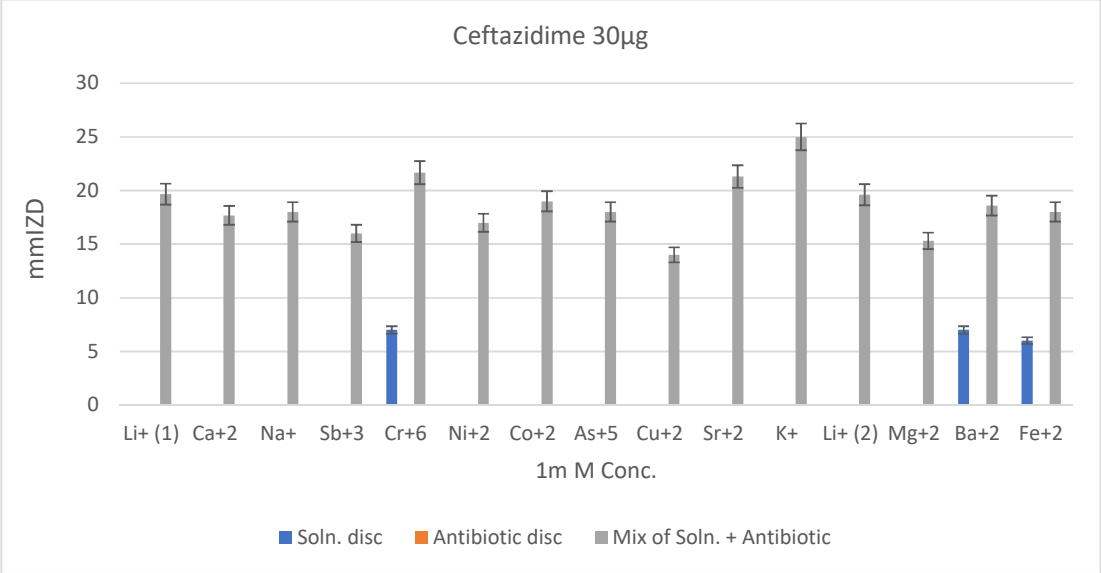

Fig. 3: Effect of different chemical elements concentration (1m M), Ceftazidime 30µg and their mixture on *S. aureus* growth.

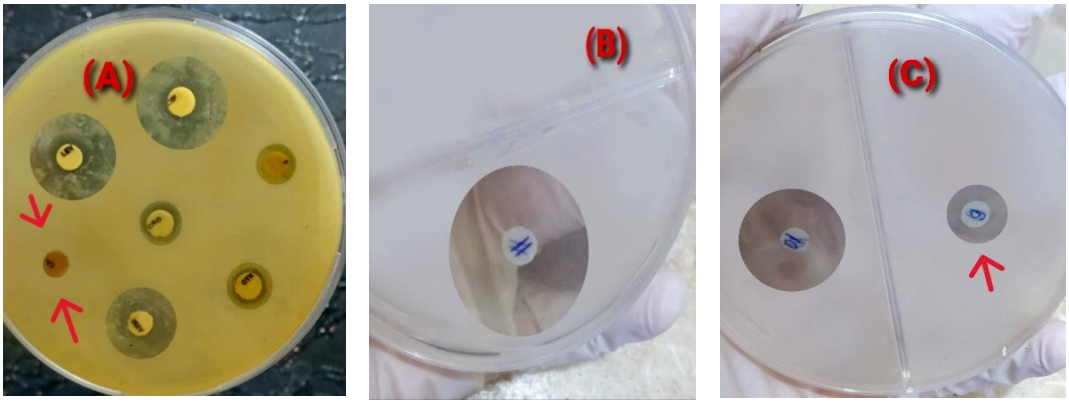

Fig. 4: Inhibition zones of Ceftazidime 30µg disc separately (A), Potassium 1m M conc. mixed with Ceftazidime 30µg (B) and Copper "no. 9" 1m M conc. mixed with Ceftazidime 30µg (C).

The data of Figs. 5,6 proved that, only Cr+6, Ba+2 and Fe+2 had an impact on the bacteria (*S. aureus*) with inhibition zone diameter (7 mm, 7 mm and 6 mm) respectively.

Concerning the antibiotics the data further, showed that Ceftriaxone 30µg had moderate effect on the growth of *S. aureus* with diameter 10 mm.

Mixing of the antibiotic with different chemical elements had a synergistic action where Li+ (2) "Lithium sulphate" had the maximum inhibitory effect with diameter 26 mm, while Na+ gave the lowest IZD with diameter 12 mm. Other elements had an intermediate inhibitory effect.

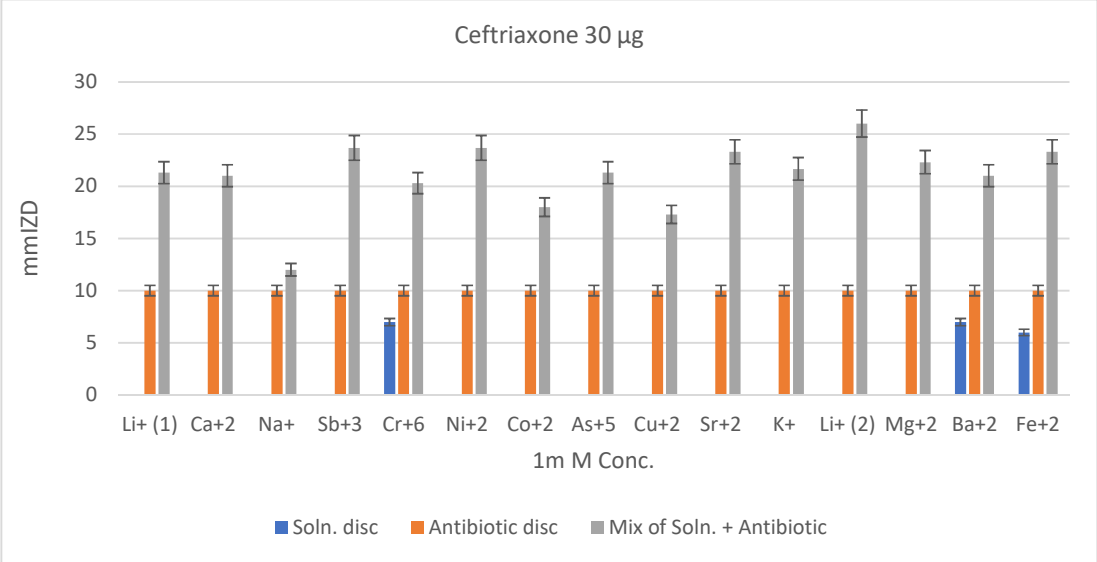

Fig. 5: Effect of different chemical elements concentration (1m M), Ceftriaxone 30µg and their mixture on *S. aureus* growth.

Fig. 6: Inhibition zones of Ceftriaxone 30µg disc separately (A), Lithium "no. 12" 1m M conc. mixed with Ceftriaxone 30µg (B) and Sodium "no. 3" 1m M conc. mixed with Ceftriaxone 30µg (C).

The data of Figs. 7,8 indicated that, Only Cr+6, Ba+2 and Fe+2 had an impact on the bacteria (*S. aureus*) with inhibition zone diameter (7 mm, 7 mm and 6 mm) respectively.

Concerning the antibiotics the data further, showed that Ofloxacin 5µg had large effect on the growth of *S. aureus* with diameter 26 mm.

Mixing of the antibiotic with different chemical elements had a synergistic action in only 3 elements, where K+ had the maximum inhibitory effect with diameter 34 mm, while had an antagonistic action in 10 elements where Cr+6 gave the lowest IZD with diameter 21 mm. Other elements had no impact.

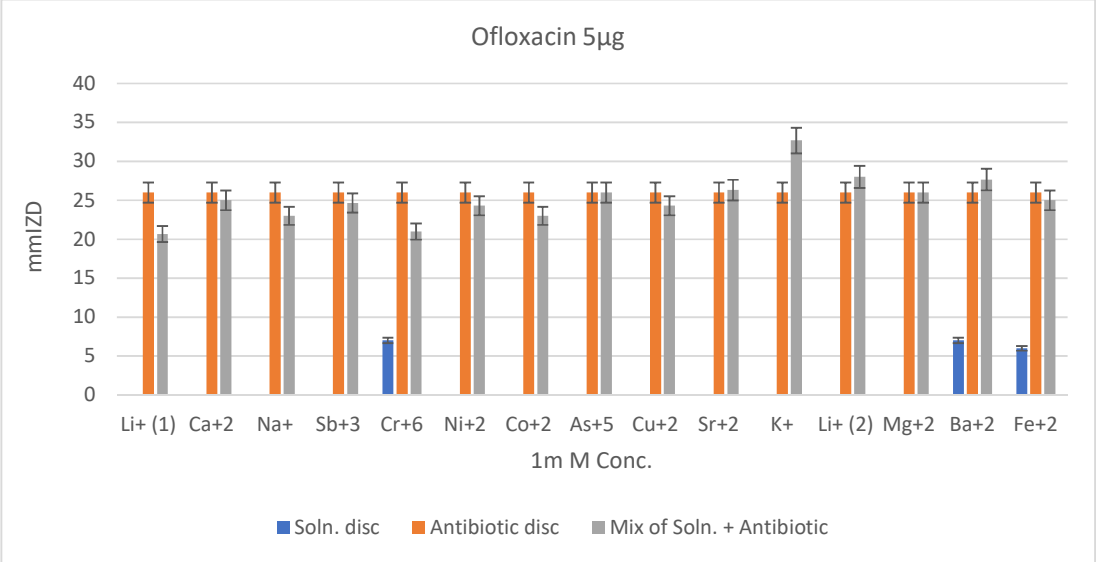

Fig. 7: Effect of different chemical elements concentration (1m M), Ofloxacin 5µg and their mixture on *S. aureus* growth.

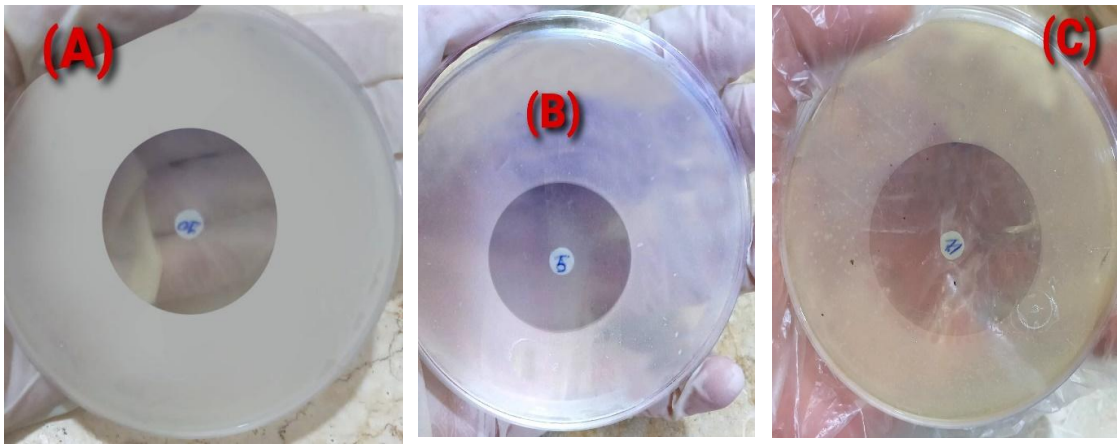

Fig. 8: Inhibition zones of Ofloxacin 5µg disc separately (A), Chromium 1m M conc. mixed with Ofloxacin 5µg (B) and Potassium 1m M conc. mixed with Ofloxacin 5µg (C).

The data of Figs. 9, 10 showed only Cr+6, Ba+2 and Fe+2 had an impact on the bacteria (*S. aureus*) with inhibition zone diameter (7 mm, 7 mm and 6 mm) respectively.

Concerning the antibiotics the data further, showed that Rifampicin 5µg had moderate effect on the growth of *S. aureus* with diameter 13 mm.

Mixing of the antibiotic with different chemical elements had an antagonistic action in 12 elements where the inhibition zone of mix was lower than which of antibiotic separately and had a little synergistic action in only 3 element which shown that Fe+2 had the maximum inhibitory effect with diameter 16 mm, while Ba+2 gave the lowest IZD with diameter 10 mm.

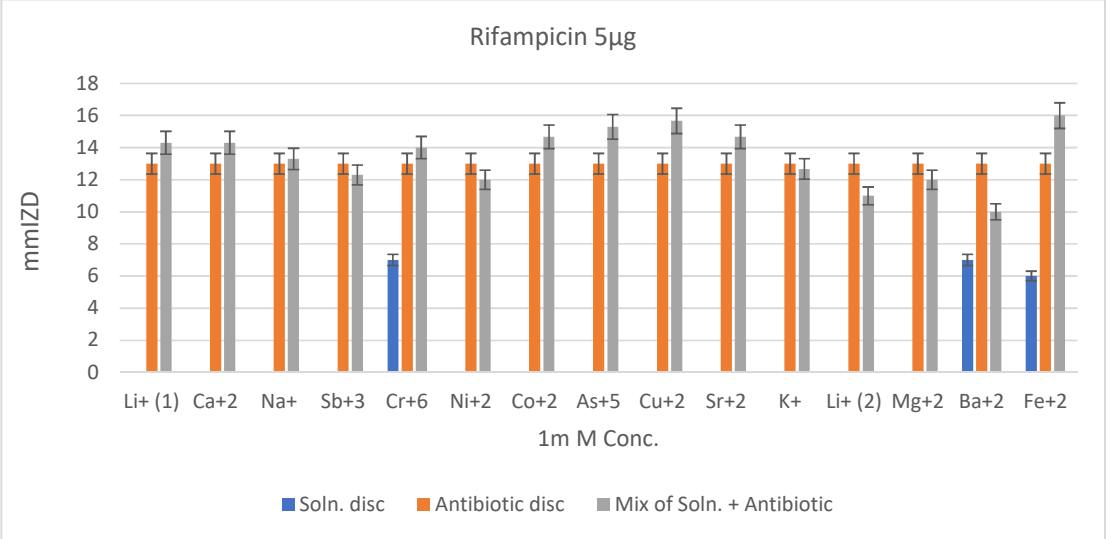

Fig. 9: Effect of different chemical elements concentration (1m M), Rifampicin 5µg and their mixture on *S. aureus* growth.

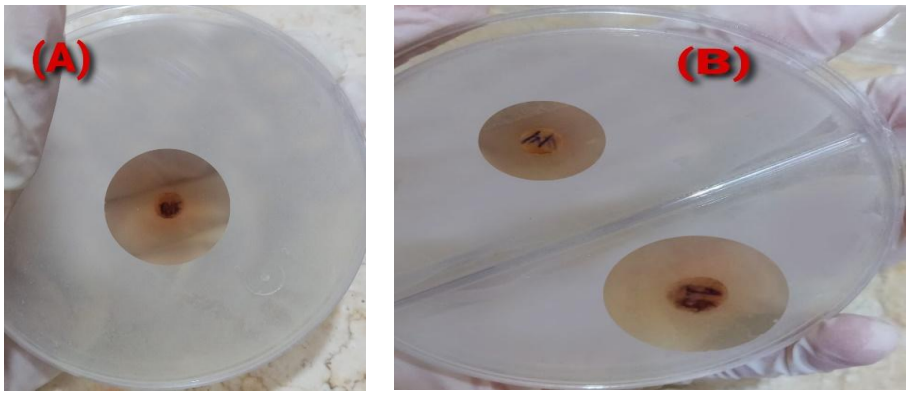

Fig. 10: Inhibition zones of Rifampicin 5µg disc separately (A), Barium "no. 14" 1m M conc. mixed with Rifampicin 5µg and Ferrous "no. 15" 1m M conc. mixed with Rifampicin 5µg (B).

4. Discussion

Inhibition zones decrease with decreasing the concentration in all antibiotics and chemical elements. All antibiotics which bacteria resistant to such as Ampicillin/Sulbactam and Ceftazidime, gave large inhibition zones reaching 20 and 30 mm in diameter after mixing with different chemical elements. In the moderate and sensitive antibiotics, the inhibition zones increased in some of them like Ceftriaxone, decreased in others and increased with some chemical elements and decreased with other elements in the other antibiotics ex. Rifampicin and Ofloxacin.

β-Lactam antibiotics (ex. Penicillin and Ampicillin) are bactericidal agents that interrupt bacterial cell-wall formation as a result of covalent binding to essential penicillin-binding proteins (PBPs), enzymes that are involved in the terminal steps of peptidoglycan cross-linking in both Gram-negative and Gram-positive bacteria. (Bush and Bradford, 2016)

Cephalosporins (like Ceftazidime and Ceftriaxone) are a large group of bactericidal antimicrobials that work via their beta-lactam rings. The beta-lactam rings bind to the penicillin-binding protein and inhibit its normal activity. Unable to synthesize a cell wall, the bacteria die. (Bui and Preuss, 2023)

Quinolone antibiotics (such as Ofloxacin and Ciprofloxacin) inhibit DNA synthesis by targeting two essential type II topoisomerases, DNA gyrase and topoisomerase IV (Topo IV). Both targets allow one double-stranded DNA molecule to pass through another, followed by religation of the original

strand, thereby changing the linking number of DNA by two in each enzymatic step. (Fàbrega *et al.,* 2008)

Rifampin specifically inhibits bacterial RNA polymerase, the enzyme responsible for DNA transcription, by forming a stable drug-enzyme complex with a binding constant of 10(-9) M at 37 C. (Wehrli, 1983)

Metals have long been used as antimicrobials; for example, copper plates were used to sterilize drinking water and, recently, coat door handles. Silver has also been widely used in recent times in several fields, such as dental implants, catheters and burn wounds. With the advent of nanotechnology, metal nanoparticles have become the latest research focus. Although metallic nanoparticles can be inert in nature, it is believed that following immersion in an aqueous solution, free metal ions and reactive oxygen species (ROS) generated on the particle surface exert antimicrobial effects. Nanoparticles can kill bacterial cells non-specifically by targeting multiple components. In a study applied to determine the impact of copper, cobalt, zinc and silver nanoparticles on the growth of *S. aureus*, *S. epidermidis* and *E. coli* strains which shown that metals can target specific cellular components and, therefore, are likely to enhance pathogen clearance when combined. Silver ions have been reported to initiate cell death through association with disulphide bonds in enzymes, thus interfering with normal metabolic processes. Furthermore, silver binds with phosphorus in bacterial DNA, thus impairing DNA replication and protein expression. Copper, on the other hand, can not only disrupt DNA and cell membrane by ROS production but also alter metabolic pathways. Cobalt has been shown to disrupt iron homeostasis through interaction with Fe–S clusters which are critical co-factors for respiration and DNA repair. While the mode of action of some metal ions has been extensively studied, to the authors' knowledge, the combined mode of action has yet to be researched. A varied chemical reactivity of metal ions with bacterial cells and their unique cellular targets explains the difference in the antimicrobial efficacy between different combinations (Raja *et al.,* 2023).

Unlike the other nanoparticles used in this study, the cobalt nanoparticles were partially passivated at the time of manufacture to avoid surface oxidation. Therefore, the activity loss could be partially due to the protective coating on the cobalt nanoparticles. Even though there is a lack of data showing the antimicrobial properties of cobalt or cobalt oxide nanoparticles, metallic cobalt has been shown to demonstrate antimicrobial action against *S. aureus* and *E. coli*. With the exception of cobalt and zinc, all nanoparticles and their combinations showed a rapid and effective antimicrobial activity. Despite the rapid actions of treatment groups, no significant difference was seen between copper and Cobalt or silver and cobalt after 24 h, indicating no synergistic or antagonistic effects of the combination metals. The lack of antimicrobial activity exhibited by cobalt treatment groups and lack of significance between copper and Cobalt or silver and Ag and cobalt groups suggests that the reduction in bacterial load seen in Cu + Co and Ag + Co treatment groups is attributable to the actions of copper and silver alone (Raja *et al.,* 2023)

To date, the mechanisms of nickel toxicity in microorganisms and higher eukaryotes are poorly understood. In a recent study, the authors summarize nickel homeostasis processes used by microorganisms and highlight in vivo and in vitro effects of exposure to elevated concentrations of nickel. On the basis of this evidence, we propose four mechanisms of nickel toxicity: (1) nickel replaces the essential metal of metalloproteins, (2) nickel binds to catalytic residues of non-metalloenzymes; (3) nickel binds outside the catalytic site of an enzyme to inhibit allosterically and (4) nickel indirectly causes oxidative stress (Macomber and Hausinger, 2011).

A study applied on *S. aureus* and Chromium shown that chromium (III) complexes are bactericidal for *S. aureus* and *E. coli*. The chromium (III) complexes are capable of producing plasmidic DNA photodamage with high efficiency of photocleavage. chromium (III) complexes containing amino acids as ligands have antibacterial and antifungal activities, and also containing oxalohydrazide and quinoxaline as ligands would have microbiological activity, reducing virulence and antibiotic resistance, and the authors suggest that the mechanism of antibacterial activity of these complexes would be due to oxidative DNA damage. When ciprofloxacin was combined with [Cr(phen)3]3+ for the inhibition of *Staphylococcus aureus* and *Escherichia coli*, an important synergistic effect was observed (Páez *et al.,* 2013)

The biocidal effect of copper on a wide range of pathogens, such as bacteria, fungi, and viruses, has been demonstrated by many laboratory studies. The effectiveness of copper extends to MRSA and VRE. Apart from being an effective antimicrobial agent, exposure to copper has been correlated with

enhanced wound healing. The substitution of copper alloys on surfaces within a clinical setting has been demonstrated to significantly reduce bacterial colonization, and more recently, copper-infused fabrics have also been studied. The Egyptians were the first to mention the antimicrobial effects of copper in 2600 BC. They used copper vessels to sanitize drinking water and also to treat chest wounds. In papyrus circa 1500 BC, it was stated that various adaptations of copper were used to treat infections, scalds, and itching (Arendsen *et al.,* 2019).

In other study, barium zirconate titanate nanoparticle has been synthesized and tested for antibacterial activity. Results showed that the desired nano-powders had satisfactory antibacterial properties with slightly hemolytic activity which probably make them a candidate as potential antibacterial agents in DD systems. In the recent decade, some nanoparticles have been introduced that showed antibacterial and anti-cancer properties and consequently studied for their potential as antibacterial agents (Mohseni *et al.,* 2015)

There are many elements used by bacteria in their biological activities, but also have antimicrobial effect if using with large concentrations, such as Calcium, Potassium, Magnesium, Lithium and many other elements.

Treatment with LiCl reduced the severity of corneal disease by reducing corneal inflammatory response and bacterial burden. Moreover, LiCl increased anti-inflammatory cytokine interleukin-10 levels, decreased proinflammatory cytokine tumor necrosis factor-α levels, and enhanced apoptosis of infiltrating macrophages and neutrophils in the *Pseudomonas aeruginosa* (PA)-infected mouse corneas. In vitro studies further confirmed that LiCl elevated anti-inflammatory cytokine expression but reduced proinflammatory cytokine production, as well as promoted cell apoptosis in murine macrophages and neutrophils. This study demonstrated a protective role of LiCl in PA keratitis. LiCl promotes host resistance against PA infection by suppressing inflammatory responses, enhancing inflammatory cell apoptosis, and promoting bacterial clearance (Chen *et al.,* 2013)

Magnesium is a vital mineral, which is not consumed to a sufficient quantity. Addition of magnesium to food matrices, for instance, to dairy products has several added benefits. First, the antibacterial effect of Mg2+ ions enable development of the safer and healthier food. Second, improvements in the technological properties of the magnesium supplemented food enables shorter production time and high protein content of the food products. Finally, enrichment of the food with Mg2+ ions provide a new source for the delivery of this vital mineral to humans (Demishtein *et al.,* 2019)

Magnesium oxide nanoparticle (nMgO) is a light metal based antimicrobial nanoparticle. In a study applied showed that Magnesium oxide nanoparticles (nMgO) exhibited inhibitory and bactericidal/fungicidal effects on the prevalent pathogenic bacteria and yeasts. The mechanisms of the antibacterial activity of Mg alloy have been summarized from two viewpoints: the alkaline environment and Mg ions released by degradation. And the antibacterial mechanism is attributed to the synergetic effect of them, but the high alkalinity plays a major role in the antibacterial process. (Thi Nguyen *et al.,* 2018)

For iron, several bacterial ferrous iron transport systems have been described; however, only the Feo system appears to be widely distributed and is exclusively dedicated to the transport of iron. In recent years, many studies have explored the role of the FeoB and FeoA proteins in ferrous iron transport and their contribution toward bacterial virulence. The three-dimensional structures for the Feo proteins have recently been determined and provide insight into the molecular details of the transport system. The Feo system has been clearly shown to be important for the virulence of pathogenic species as well as for the survival of commensal bacteria. As such it is an interesting target for the development of new antimicrobial components (Lau *et al.,* 2016)

4. Conclusion

From the previous data we conclude that when mixed antibiotic discs with different chemical elements applied to inhibit the growth of *S. aureus,* they given a synergistic effect with most of antibiotics used more than the antibiotic discs and chemical elements separately. That is because the mixture of both impacted not only one factor of bacterial growth but also affected many biological activities of bacteria which enhanced the effectiveness of mixture to prevent the bacteria growth rapidly and easily.

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