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The synergistic relationship between Aminoglycoside Antibiotics and Zoledronic acid (ZOMETA) drug in their effect on Bacillus cereus and Staphylococcus aureus bacteria.

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A B S T R A C T

In this study, we investigated the synergistic relationship between some antibiotics from the aminoglycosides functional group, which were Chloramphenicol (C30µg), CEFOTETAN (CTT30µg), AMIKACIN (AK30) and CEFAMANDOLE (MA) and the chemical drug Zoledronic acid (ZOMETA 4g) which is used in Cancer treatment. This study focuses on their Effect on Gram-positive bacteria Bacillus cereus and Staphylococcus aureus using disc diffusion method for anti-susceptibility test, and spectrophotometry method for bacteria cells count. The results of the Zoledronic acid anti-susceptibility test showed that it had no effect on both bacteria. However, the antibiotic's anti-susceptibility test on B. cereus showed resistance to the antibiotic (C30) and (MA 30), also showed the highest susceptibility pattern to the antibiotic (AK 30). while the lowest susceptibility pattern was for the antibiotic (CTT 30). While on S. aureus bacteria showed resistance to antibiotic (MA 30), with the highest susceptibility pattern to antibiotic (C 30), and the lowest susceptibility pattern to antibiotic (AK 30); The results of the synergy test (the relationship between the Zoledronic acid and the antibiotic) on both B. cereus and S. aureus bacteria showed that all antibiotics have a synergistic relationship with the drug.

1 Introduction

The spread of the culture of taking antibiotics without consulting a specialist doctor, especially among people with chronic diseases, is considered one of the most dangerous things because these antibiotics have different effects on different organs of the body such as the liver, kidneys, stomach and immune system. On the other hand, antibiotics affect the natural bacteria Microbiome, which in turn leads to various problems, especially in some cancer patients, as there are some anti-cancer drugs that do not work perfectly except through the physiological interactions of these bacteria (*Blaser*, 2016). Although antibiotics should be taken when prescribed by a specialist doctor, these antibiotics can lead to disturbances in the microbial environment. This environment damaged by antibiotics may lead to infection such as Clostridium difficile and make immune cancer drugs such as immune checkpoint inhibitors work less effectively. Conversely, the incorrect use of these antibiotics may cause cancer in some tissues of the body (*Gao et al*., 2020). There is also a group of antibiotics that are considered anticancer antibiotics according to their mode of action. Anticancer antibiotics are

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chemicals produced by microorganisms that have anticancer activity (*Saeidnia*, 2015). They are mainly peptides and anthraquinones, which have a significant and effective effect on uncontrolled cell proliferation, excessive growth and spread of some malignant cancer cells. The classification of anticancer antibiotics mainly includes anthracyclines, mitomycin, bleomycin, actinomycin, and guanosine (*Cragg and Newman*, 2001). Many studies have shown that antibiotics could affect the nuclear components in cells, such as anthracycline antibiotics that interfere with the deoxyribonucleic acid structure and prevent the completion of the reaction with the enzymes that create ribonucleic acid (DNA) depending on RNA polymerase. Also, some other types work to inhibit the activity of the Topoisomerase enzyme by affecting the process of reconnecting the break created by the enzyme in the double ester bonds, which ultimately causes a break in the DNA chain (*Al-Aamri et al., 2019*). In several studies, researchers indicated that antibiotics belonging to the fluoroquinolone family, the most important of which is ciprofloxacin, have a high capacity for immunoregulatory activities by regulating the production of some cytokines and anti-inflammatory responses and preventing liver injury caused by lipopolysaccharide 30 (*Beberok et al., 2018*) and (*Assar et al., 2021*). In addition, scientific studies have confirmed that Gentamicin is one of the antibiotics belonging to the aminoglycosides (*Randjelovic et al., 2017*) and has an overlapping activity with some drugs used in the treatment of cancer, such as liver cancer, as it works directly to inhibit amino-acid Mutational Sites, which helps in the treatment of liver cancer by studying its interactive relationship with the protein TP53 (*Leelavathi*, 2024).

2 Materials and Methods

Some isolated and identified bacteria samples were worked on from patients visiting Sabha Medical Hospital and their identification was confirmed by the Department of Microbiology Faculty of Science, Sabha University. They are Gram positive bacteria (*Bacillus subtilis, Staphylococcus aureus*), Growth of bacteria samples: Muller Hinton Broth and Muller Hinton Agar were used to grow the samples, where a swab was taken from the preserved samples using the loop and was suspended in this medium for activation. It was incubated in the incubator at 37 °C for 12 hours. Susceptibility Test: The sensitivity test was conducted by diffusion method according to the method (Kirby & Bauer, 1966) following the instructions of the National Committee for Clinical Laboratory Standards (NCCLS). Sterile filter papers were saturated with the concentrations of the prepared compound so that their volume did not exceed 50 µl, which is the maximum capacity of the filter paper was then placed by sterile forceps on the solid Mueller medium MHA on which the bacterial samples were grown by the sweep method. The plates were incubated at 37°C, and the results were taken the next day. 3 replicates were taken for each bacterial isolate (*Gajic et al.*, 2022).

Bacterial count test:

In this test, serial dilutions were made of the sample of bacteria that had been grown for an overnight, then the tubes were injected with the compound and antibiotic so that to the final concentration and a control sample was placed that did not contain the drug, then the Measure the samples using a spectrophotometer after an hour, then incubate the samples and measure them again after 24 hours, and compare the bacterial cell counts to the control sample according to the standard equation.

1 Results and discussion:

3.1 Susceptibility test results:

Results the results of the antibiotic sensitivity test on B. cereus bacteria showed that it was resistant to all antibiotics C, MA and showed the highest susceptibility pattern to the antibiotic AK, which was 20 mm, and the lowest sensitivity pattern to the antibiotic CTT, which was 19 mm, as shown in Table () and Image (). These results differ from what was reached by (Frenzel et al., 2015), as his study, which was conducted on the relationship of B. cereus bacteria with antibiotics, showed that the bacteria are usually highly resistant to the antibiotic amikamycin AK and gentamicin N, due to the ability of these bacteria to form a semi-spore-like coating that prevents the passage and interaction of some types of antibiotics with them. It also differs from what was indicated by (Fiedler et al., 2019), in his study that focused on the effect of a large group of antibiotics on the forementioned bacteria, as his results showed high sensitivity to the antibiotic cephalosporin C and resistance to amikamycin AK. As for the bacteria S. aureus, it was resistant to the antibiotic MA, while the antibiotics C, CTT, AK, were (19, 20, 25) mm,

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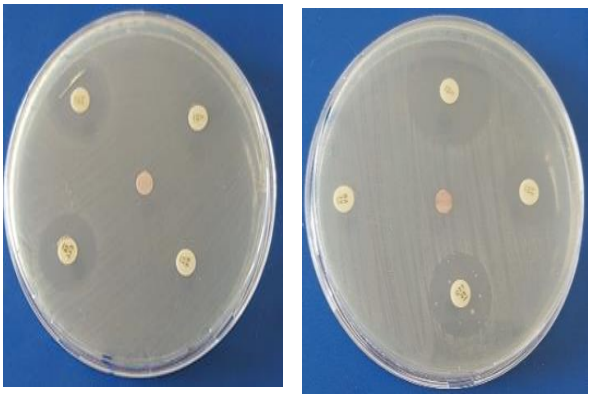
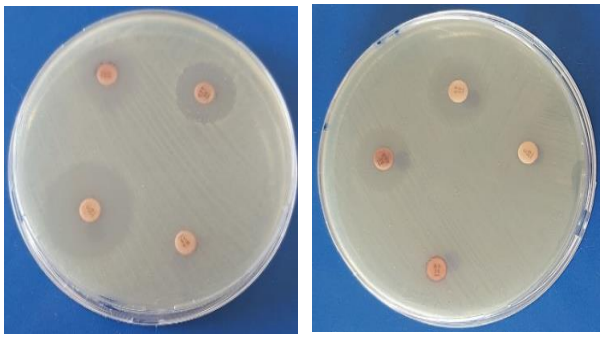
respectively, as shown in the table () and the picture (), which differs from what was reached by (DAVID J., et., al., 2011). The results of his studies, which were conducted on the relationship between bacteria and the antibiotics of Beta-lactam, proved that they were sensitive to all antibiotics except vancomycin, and were also sensitive to the synergistic interaction between the two groups of beta-lactam and aminoglycosides antibiotics.



Pic. (1) Results of antibiotic susceptibility testing and ZOMETA on S. *aureus* on the left, and B. *cereus* on the right

3.2 Synergistic relationship test:

The results of the synergistic test on B. cereus bacteria also showed that the antibiotics MA, C had a synergistic relationship with the drug ZOMETA, while the antibiotics CTT, AK had an antagonistic relationship with it (as shown in the table and picture). As for S. aureus bacteria, the antibiotic MA had a synergistic relationship with the drug,



Pic (2) Results of the susceptibility test for synergistic reaction with ZOMETA 4g on S. *aureus* on the left, and B. *cereus* on the right

Table (1) Results of the susceptibility test for Zoledronic acid on bacteria

S. *aureus*	B. *cereus*
R	R

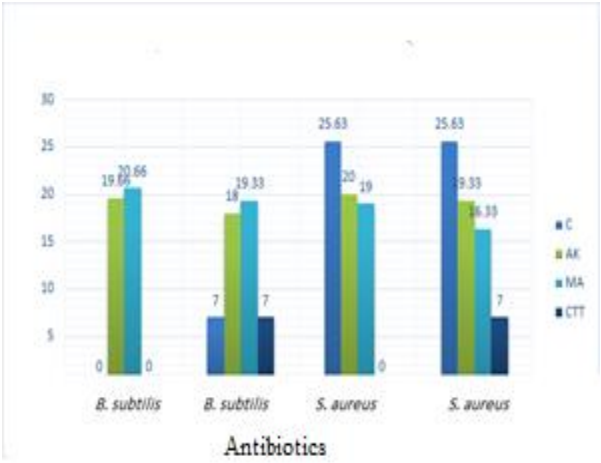


Fig1: Average for antibiotic and drug susceptibility testing, as well as synergistic testing (where * indicates synergistic testing)

Table (2) Results of antibiotic susceptibility tests and synergistic interaction with the drug Zoledronic acid.

mean SD±				antibiotics
synergism susceptibility test		Antibiotics susceptibility test		
S. aureus	*B. cereus*	*S. aureus*	*B. cereus*	
25.63 ±0.37	7 ±1	25.63 ±0.37	R	**C**
19.33 ±0.47	18 ±0.18	20 ±0.81	19.66 ±0.36	**CTT**
16.33 ±1.27	19.33 ±0.47	19 ±0.81	20.6 ±0.47	**AK**
7 ±0.1	7 ±0.1	R	R	**MA**

3.3 Actual bacterial cell count results:

The bacterial cell count was measured using a spectrophotometer, where the results of the bacterial cell count for Gram-positive bacteria B. cereus and S. aureus showed after one hour of incubation, where it was noted that no growth occurred in the samples of antibiotics (CTT ,AK) for the sample of bacteria B. cereus, also no growth for CTT, AK, C antibiotics in S.*aureus* sample,

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this is an evidence of the synergistic interaction between the mentioned antibiotics and the drug, and to confirm whether this effect is an inhibitory or bactericidal effect, the samples were incubated for 24 hour, where the results shown that the synergistic interaction between C and MA antibiotics and the drug has an inhibitory effect on both bacteria, as we notice the occurrence of bacterial growth, even if it was weak, but it is interpreted that the effect on bacteria gradually decreased over time, which helped reduce the inhibitory effect on bacteria, but the synergistic effect was between the antibiotics CTT and AK and the drug, where it was noted that there was no bacterial growth as shown in the table (3).

Table (3) Results of bacterial cell count after 24h of sysregistic test:

After 24h				
S. *aureus*	**B. *cereus***	**antibiotics**	**Control**	
			S. *aureus*	**B. *cereus***
4.336×10^5	1.102×10^5	C	6.172×10^6	7.162×10^6
0	0	CTT		
0	0	AK		
5.06×10^4	4.982×10^5	MA		

Conflict of interest: The authors declare that there are no conflicts of interest

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