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An In vitro study of Amoxicillin/Calvulanic acid effects against *Staphylococcus aureus* and *Escherichia coli*

***Thuraya Ahmed Mohammed Kheir Allah*[1,2]**

[1]*Department of Biology, Faculty of Science and Arts in Baljurashi, Al-Baha University, Al-Baha, Saudi Arabia.*
[2]*Department of Microbiology and Molecular Biology, Faculty of science and Technology, Al Neelain University, Khartoum, Sudan.*

Abstract

This study aims to determine the effect of Amoxicillin/Calvulanic acid against *Staphylococcus aureus* and *Escherichia coli*. Isolates '*Staphylococcus aureus* and *Escherichia coli*' obtained from Biology laboratory, college sciences and art Baljurashi scientific section. Staphylococcus aureus and Escherichia coli re isolated by culturing on Nutrient Agar therefore purified, then Gram's stain tested for the shape and the reaction for two bacteria, to identify the genus and species of bacteria primary and secondary tests performed for bacterial identification. Bacteria cultured on blood Agar medium and special media "Mannitol salt agar (MSA) and Eosin methylene blue agar (EMB)", then amoxicillin / clavulanic acid 625 mg antibiotic obtained, then four concentration of amoxicillin / clavulanic acid antibiotic prepared by double fold dilution (312.5 mg/ml, 156.3 mg/ml, 78.13 mg/ml and 39.06 mg/ml) prepared. The effect of amoxicillin / clauvlanic acid antibiotic against Staphylococcus aureus and Escherichia coli estimated mean of inhibition zone by millimeter. Amoxicillin / clauvlanic acid had in vitro effects against two bacteria, but effect of this antibiotic against Staphylococcus aureus was greater than that of Escherichia coli.

Keywords: Amoxicillin, Calvulanic acid, *Staphylococcus aureus, Escherichia coli*.

Full length article **Corresponding Author*, e-mail: tkheirallah4@gmail.com

1. Introduction

Amoxicillin is a broad-spectrum beta-lactam antimicrobial originally derived from penicillin. A bactericidal agent targets and kills bacteria by inhibiting the biosynthesis of the peptidoglycan layer of the bacterial cell wall. This layer makes up the outermost portion of the cell wall and is responsible for the structural integrity of the cell. Peptidoglycan synthesis involves the facilitation of DD-transpeptidases, which are a type of penicillin-binding protein (PBP). Amoxicillin works by binding to these PBPs and inhibiting peptidoglycan synthesis, which interrupts the construction of the cell wall and ultimately leads to the destruction, or lysis, of the bacteria [1-2]. Clavulanic acid is a beta-lactamase inhibitor often used in conjunction with amoxicillin to broaden its spectrum and combat resistance. It has little to no antimicrobial activity of its own and instead works by preventing bacterial destruction of beta-lactams. Over the years, certain bacteria have evolved to develop resistance to standard beta-lactam antimicrobials through the production of enzymes called beta-lactamases. These enzymes target and hydrolyze the beta-lactam ring, which is necessary for penicillin-like antimicrobials to work. Clavulanic acid prevents this degradation by binding and deactivating the beta-lactamases, thus restoring the antimicrobial effects of amoxicillin [1-3]. Amoxicillin, Clavulanic acid. It was sold under the brand name Augmentin [4]. Antibiotics. Therapeutic Indication: Bacterial infections. Dosage Forms: Tablet, Dispersible tablet, Syrup, Suspension, Oral drops, Dry syrup, Powder, Injection. Amoxicillin-clavulanate is one of the most frequently used antimicrobials in emergency departments and primary care offices worldwide [5-6]. It is a combination of two different drugs: amoxicillin and clavulanic acid. Amoxicillin is a penicillin derivative and has similar activity against gram-positive and gram-negative bacteria, including, *Escherichia coli*, furthermore, with the addition of clavulanic acid, the spectrum increased, include all beta-lactamase-producing strains organisms and broadening the coverage to include methicillin-sensitive *Staphylococcus aureus* (MSSA), *Neisseria species*, *Proteus species*, *Pasteurella multocida*, and *Capnocytophaga* canimorsus, among others [1], [7]. According to FDA-approved indication amoxicillin clavulanic acid is use for; treatment of aspiration pneumonia, community-acquired pneumonia, acute bacterial rhinosinusitis, urinary tract infections, acute otitis media, and Skin and soft tissue infections [1], [8-15]. The effect of antibiotics on *Staphylococcus aureus*, the development of

resistance to many antibiotics by *S.aureus* has involved acquisition of determinants by horizontal gene transfer of mobile genetic elements; these determinants may have evolved in antibiotic producers to protect them from potentially inhibitory molecules, or in their competitors. Resistance also can emerge by mutations that alter the drug binding sites on molecular targets and by increasing expression of endogenous efflux pumps. The development of resistance by mutation in principle reduces by using combinations of inhibitors that target different sites or for two or more mutations to be required for resistance to pass the breakpoint [16]. Penicillin-resistant strains of *Staphylococcus aureus* emerged shortly after the introduction of the antibiotic in the early 1940s expressed a β-lactamase that hydrolyzed the critical β-lactam bond and destroyed the drug's antibacterial activity. Substitutions of the natural aminoadipoyl chain of penicillin with bulkier moieties created semisynthetic variants that were not substrates for β-lactamase. Methicillin was the first but had the disadvantage of being acid labile. It superseded by the acid-stable isoxazoyl penicillin oxacillin [17]. The effect of antibiotics on *Escherichia coli* is a bactericidal action of penicillin and of other inhibitors of cell wall peptidoglycan synthesis such as vancomycin and cycloserine, is secondary or tertiary to their ability to inhibit specific reactions in the assembly of an osmotically protective cell wall. Examples given of the inhibition of these reactions, which results in inhibition of cell growth (bacteriostatic action) in the absence of either cellular lysis or rapid loss of viability.in some instances, inhibitory concentrations of these drugs are, in effect, sub lethal, penicillin specifically inhibits the synthesis of the “basal structure” of the cell wall. The major portion of wall synthesis not affected by penicillin, but the abnormal structure formed lacks the mechanical strength of the normal cell wall [18].

2. Materials and Methods

Collected isolates *Staphylococcus aureus* and *Escherichia coli* cultured on Nutrient Agar for purification. For bacterial identification. Purified bacteria cultured on blood agar medium to test blood hemolysis, and special media ‘Mannitol salt agar (MSA), and Eosin methylene blue agar (EMB)" as differential, and special media, incubated aerobically at 37ºC 24 - 48 hour. Colonies on these media examined macroscopically for cultural characteristics, also microscopically for Gram’s stain’s reaction, as well as the shape of the microorganism, Pure culture used for more bacteriological primary tests; (growth aerobically, catalase test, oxidase test and glucose fermentation test) to identify the bacterial genus, and secondary tests; (coagulase test, Voges-Proskauer (VP), Methyl red test) to specify the species. Amoxicillin / clavulanic acid 625 mg antibiotic obtained, then four concentration of amoxicillin / clavulanic acid antibiotic prepared by double fold dilution (312.5 mg/ml, 156.3 mg/ml, 78.13 mg/ml and 39.06 mg/ml) prepared. The effect of amoxicillin / clauvlanic acid antibiotic against *Staphylococcus aureus* and *Escherichia coli* estimated mean of inhibition zone by millimeter, and the results recorded.

3. Results and discussion

Staphylococcus aureus on nutrient agar gave white to yellow colonies, while on blood agar gave beta hemolytic, on mannitol salt agar gave yellow colonies due to mannitol fermentation. *Escherichia coli* on nutrient agar gave white colonies, gave beta hemolytic on blood agar, EMB agar gave metallic green colonies. *Staph.aureus* colonies picked from nutrient agar were Gram-positive oval single pairs tetra and in clusters, while *E. coli* was Gram-negative rod. Primary tests for both bacteria; grown aerobically, catalase test was positive, oxidase test was negative and glucose fermented, oxidation fermentation medium gave fermentation., then S.aureus identified by performing coagulase test and, Methyl red (MR) _Voges-Proskauer (VP) tests which were negative and positive respectively while E.coli gave negative and positive results for MR- VP tests respectively. Different concentrations for amoxicillin/Clavulanic acid (312.5 mg/ml, 156.3 mg/ml, 78.13 mg/ml and 39.06 mg/ml), against *Staphylococcus aureus* had a lethal significant effect on its linear growth as explained in table (1), and figure (2 and 3). Amoxicillin/Clavulanic acid at different concentrations (312.5 mg/ml, 156.3 mg/ml, 78.13 mg/ml and 39.06 mg/ml), against *Escherichia coli* had a lethal significant effect on its linear growth as explained in table (2), and figure (4 and 5).
B-lactam antibiotics prescribed for treating human. In addition, due to their broad spectrum of uses, there are few side effects associated with this activity. Amoxicillin/clavulanic acid approved for medical use in the United States in 1984. The World Health Organization classifies amoxicillin/clavulanic-acid as critically important for human medicine. It is available as a generic medication. In 2020, it was the 107th most commonly prescribed medication. Studies conducted in vitro; several studies have confirmed that β-lactams have antibacterial activity. In this study against *Staphylococcus aureus* and *Escherichia coli*, the effect of a broad-spectrum antibiotic commonly used (amoxicillin-clavulanic acid). The inhibition zone showed different results for antibiotic concentrations; the reason of diffusions of the diluted antibiotic with the increase in the diffusion of the antibiotic in the medium, its concentration that decreased and its concentration becomes so low that it is no longer able to prevent the growth of bacteria. The osmotic property of the cell Bacterial, which is the movement of solute particles from the area of high concentration to the area of low concentration; if the concentration of solute is high outside the bacterial cell, this leads to the entry of solute into the cell, which leads to the explosion and decomposition of the cell. This experiment explained amoxicillin-clavulanic acid strongly affected microorganism "*Staphylococcus aureus* and *Escherichia coli*". The same effects of amoxacillin/clavulanic acid agree with. Previous studies have shown that clavulanic acid not only irreversibly inactivates beta-lac-tamase but also exhibits synergism with amoxicillin against amoxicillin-resistant bacteria [19-22]. The rate of amoxicillin resistance in Saudi Arabia is markedly higher than those reported in the United States and Europe [23-24]. This study agrees with that the isolated strains of *E. coli*, and staphylococcus aureus showed increased susceptibility with amoxicillin/clauvlanic acid antibiotic [25],[26].

Table 1. The Effects of Amoxicillin/ Clavulanic Acid against *Staphylococcus aureus*.

Concentration of Antibiotic (mg/ml)	Linear growth	Reduction
312.5	40.3[a]	55.2[a]
156.3	43.0[a]	52.2[a]
78.13	40.3[a]	55.1[a]
39.06	40.0[a]	55.5[a]
L.S.D at 5%	14.05	15.62

Table 2. The Effects of Amoxicillin/ Clavulanic Acid against *Escherichia coli*.

Concentration of Antibiotic (mg/ml)	Liner growth	Reduction
312.5	25.0[a]	72.2[b]
156.3	26.0[a]	71.7[b]
78.13	23.6[a]	73.6[b]
39.06	17.0[b]	81.1[a]
L.S.D at 5%	5.60	6.21

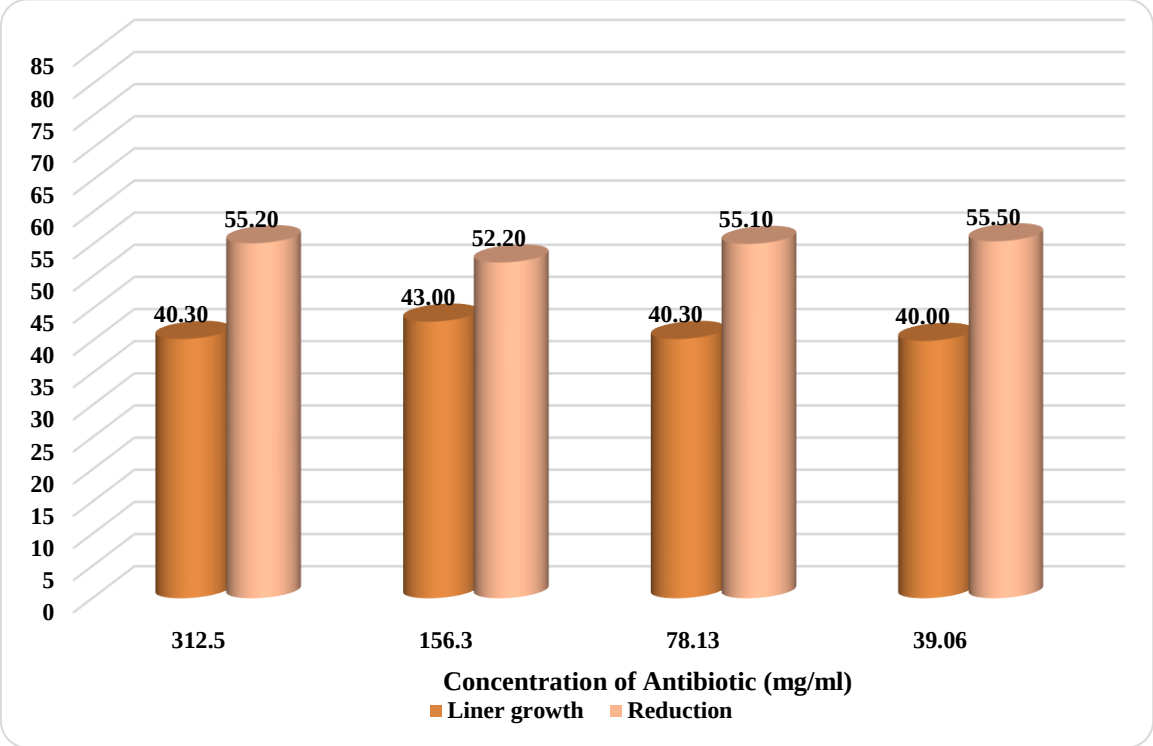

Figure 1. The effects of Amoxicillin / Clavulanic Acid against *Staphylococcus aureus*.

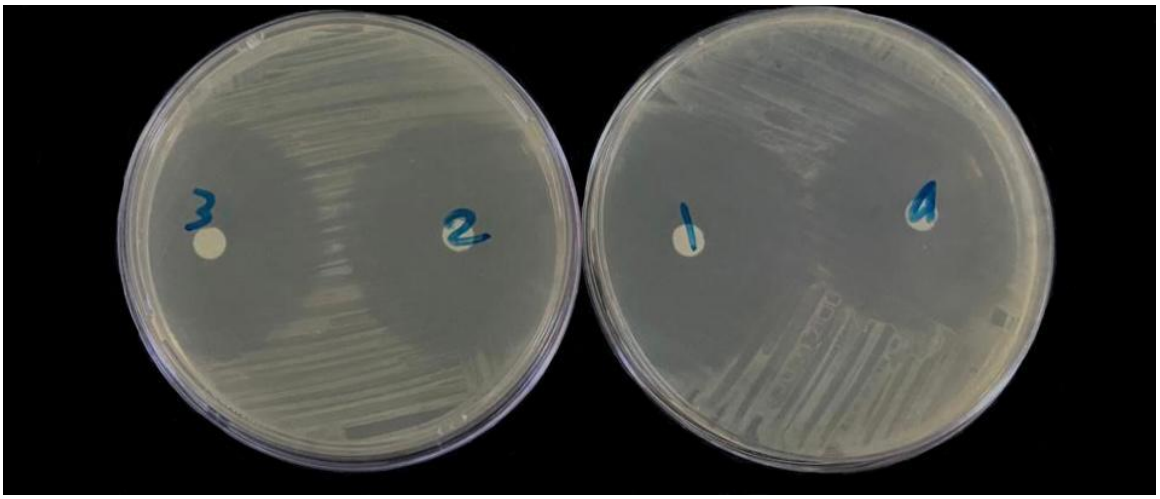

Figure 2. The effects of different concentrations of Amoxicillin/ Clavulanic Acid against *Staphylococcus aureus*.

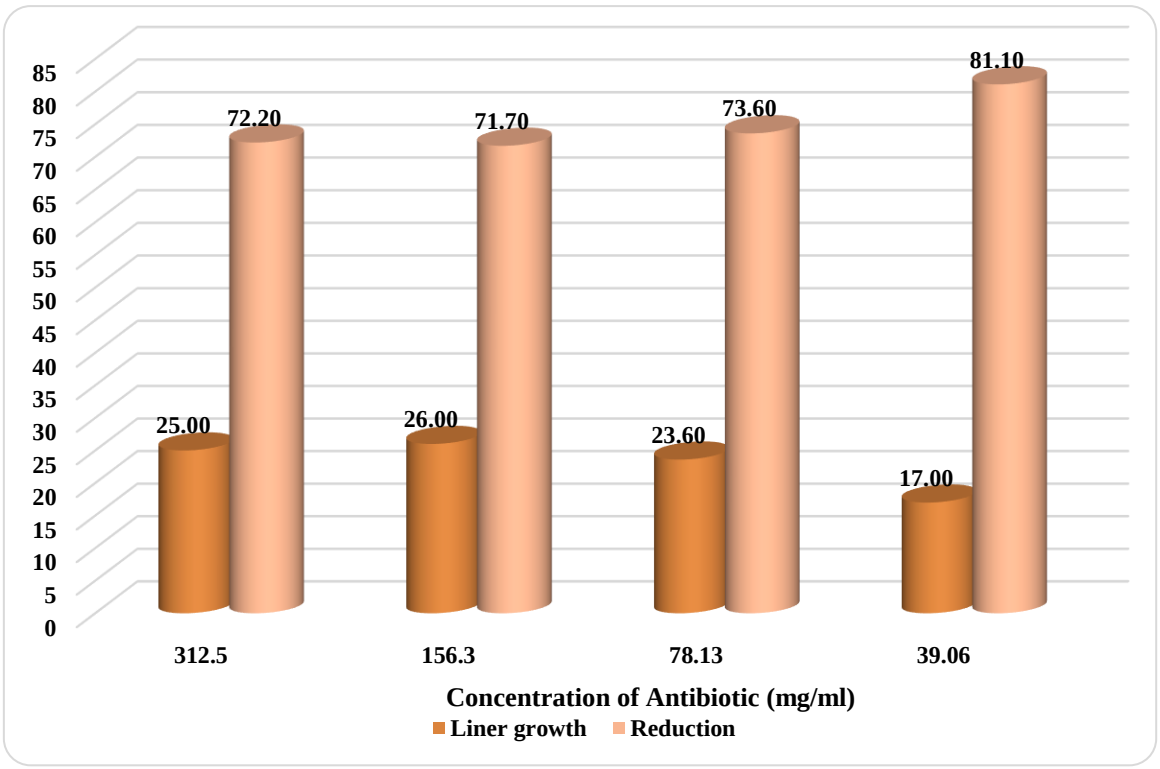

Figure 3. The effects of Amoxicillin / Clavulanic Acid against *Escherichia coli*

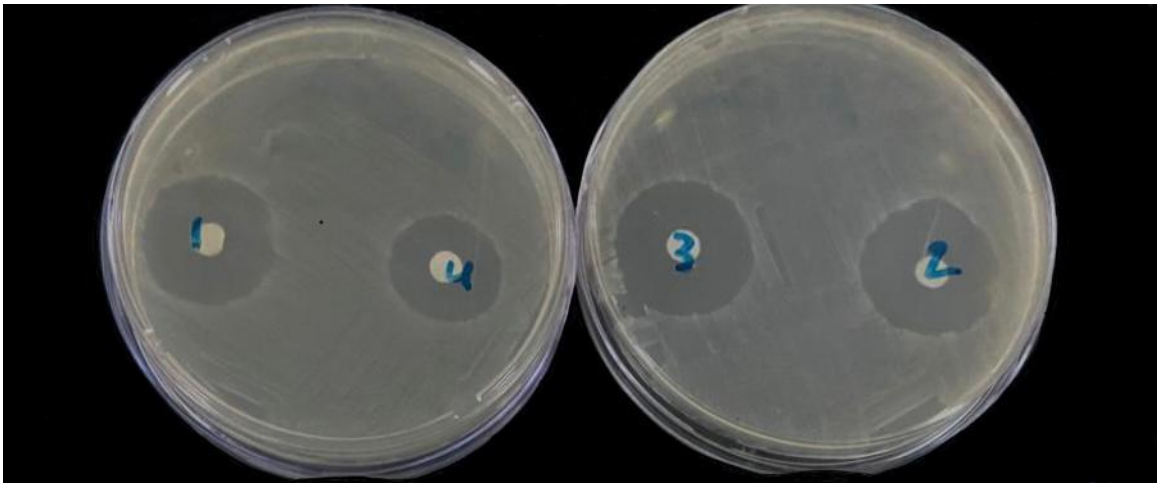

Figure 4. The effects of different concentrations of Amoxicillin/ Clavulanic Acid against *Escherichia coli*.

4. Conclusion

This study concludes that amoxicillin/clauvlanic acid antibiotic against Staphylococcus aureus and Escherichia coli had a lethal effects against both bacteria, but its effects of against Staphylococcus aureus was greater than that of Escherichia coli.

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