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Department of Biology
Morning study

# Antimicrobial Effect of Some Probiotic against the biofilm associated S. aureus S. epidermidis Isolated From Wound Infection

Graduation Research submitted to the Department Council of Biology / College of Science / University of Diyala as part of requirements for the degree of bachelor in Biology sciences

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بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

﴿ يَا أَيُّهَا الَّذِينَ آمَنُوا إِذَا قِيلَ لَكُمْ تَفَسَّحُوا فِي الْمَجَالِسِ فَافْسَحُوا يَفْسَحِ اللَّهُ لَكُمْ وَإِذَا قِيلَ انْشُزُوا فَانْشُزُوا يَرْفَعِ اللَّهُ الَّذِينَ آمَنُوا مِنْكُمْ وَالَّذِينَ أُوتُوا الْعِلْمَ دَرَجَاتٍ وَاللَّهُ بِمَا تَعْمَلُونَ خَبِيرٌ (١١) ﴾

صدق الله العليُّ العظيم

من سورة المجادلة

# الإهداء

• الى السراج الذي شعَّ على هذه الارض ليخرج اهلها من الظلمات الى النور
الى المعلم الاول الذي بعثه الله بالقرآن الى بني البشر رحمة لهم ..

**رسولنا الكريم (عليه الصلاة والسلام)**

• الى مضجع الانبياء والعلماء شعلة الانوار الهادية وبودقة الحضارات ومناهل العلم ..

**وطني العراق**

• الى الذي اوصاني الله به برآ وأحساناً واهدى لي سنين عمري ..

**والدي الحبيب**

• الى بحر الحب والحنان والنبض الساكن في عروقي ..

**امي الحنونة**

• الى نجوم سمائي المتلألئة وسندي في الحياة ..

**اخوتي**

• الى سند المستقبل وشمعة لعطاء وامل الغد المشرق ..

**اساتذتي وزملائي**

• الى كل قلب خفق لي حباً وخوفاً عليَّ اهدي اليهم ثمرة جهدي المتواضع هذا ..

**شكرا للجميع**

# الشكر و التقدير

الحمد لله رب العالمين والصلاة والسلام على أشرف الأنبياء والمرسلين سيدنا محمَّد وعلى آله وصحبه ومن تبعهم بإحسان إلى يوم الدين..

**واما بعد..**

فإننا نشكر الله تعالى على فضله حيث مَنَّ علينَّا بإنجاز هذا العمل بفضله، فله الحمد أولاً وآخراً ...

**ثم نشكر أولئك الأخيار الذين مدوا لنا يدَ المساعدة، خلال هذه الفترة..**

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إقرار المشرف وترشيح رئاسة قسم علوم الحياة

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اشهد بان إعداد هذا البحث الموسوم بـ( **تأثير مستخلصات بعض المعززات الحيوية على البايوفلم المرضي لبكتريا المكورات العنقودية الذهبية المسببة لالتهاب الجروح** ) الذي قدمه الطلبة **(كرار جابر براك ، حيدر صكر محمود ، علي هاشم عبد الأمير )** قد جرى تحت أشرافي في كلية العلوم / قسم علوم الحياة / جامعة ديالى ، وهو جزء من متطلبات نيل درجة البكالوريوس في علوم الحياة.

التوقيع:
الاسم :
المرتبة العلمية :
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التاريخ: / /

**..... بناء على التوصيات المتوافرة أرشح هذا البحث للمناقشة**

التوقيع:
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رئيس قسم علوم الحياة
كلية العلوم / جامعة ديالى
التاريخ: / /

إقرار لجنة المناقشة

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رئيس اللجنة

التوقيع :
الاسم :
المرتبة العلمية:
التاريخ : / / 2019

| عضو اللجنة | عضو اللجنة | عضو مشرف |
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| التوقيع: | التوقيع: | التوقيع: |
| الاسم: | الاسم: | الاسم : |
| المرتبة العلمية : | المرتبة العلمية : | المرتبة العلمية : |
| التاريخ: / / 2019 | التاريخ: / / 2019 | التاريخ : / / 2019 |

**مصادقة رئاسة قسم علوم الحياة \ كلية العلوم**

التوقيع:
الاسم:
المرتبة العلمية :
التاريخ : / / 2019

# Abstract

One of the most common etiological agent of wound infection is *Staphylococcus aureus*. Biofilm formation by *Staphylococcus aureus* increases microbial resistance to environmental stresses, such as antibiotics. This study is aimed at evaluating antimicrobial and anti-biofilm activity of some probiotic metabolites on biofilm-associated *Staphylococcus aureus*. Wound samples were collected at General Hospital of Baqubah from various age groups and from both genders during January 7th-29th, 2018. The bacterial isolates were identified based on their morphological and biochemical features. Our data showed that *Staphylococcus* was identified in (11) 64.74% of the total isolates, including (8) 47.05% as methicillin-sensitive *Staphylococcus auerus*, (2) 11.7% were methicillin-resistant *Staphylococcus aureus*, and (1) 5.88% was *Staphylococcus epidermidis* while (6) 35.29% were reported as other microbes. The sensitivity test showed that *Staphylococcus aureus* were highly resistant to the following antibiotics; Amoxillin, Penicillin G, Cefotaxim and Methicillin. Antibiotics in combination with probiotic metabolites were evaluated against the pathogenic bacteria. Cefotaxim in combination with probiotics metabolites showed a higher activity, in comparison to use antibiotic alone, against pathogenic *Staphylococci* isolated form wound infection. A co-aggregation assay was conducted and strong coaggregation with score (+4) was noticed between the clinical isolates and the tested probiotics. These findings indicate the ability of probiotics to compete with the pathogens on the site of colonization or for the source of nutrients and, eventually, lead to inhibit pathogens' capability to cause disease. In addition, The cell free metabolites of testing probiotics had a noticeable biofilm inhibitory effect on biofilm of methicillin-resistant *Staphylococcus aureus.*

**Key words:** *Staphylococcus aureus*, Biofilm formation, Antibiotics resistance, probiotics, coaggregation.

# Contents

# Introduction

# Introduction

Wounds are moist, warm and nutritional environment that helps to colonize and multiply microbes. The abundance and variety of microbes present in the wound are affected by several factors: type, depth, location and the wound quality (Robson et al, 1997). Several factors can aggravate the chronic wound infection, such as aging, obesity, smoking, malnutrition in addition to immune inhibition diseases (acquired immunodeficiency (AIDs) (Bowler and Armstrong, 2001). Wound infection arises from three main sources; the external, the internal environment, and endogeouns sources. One of the most common microbes in the outer environment that can cause wound infection is *S.aureus* (Duerden, 1994). The surrounding skin is colonized by many species of bacteria; *Staphylococcus epidermidis*, *Micrococci*, *Dipthheroids* (Dandara et al, 2006). The endogenous source of wound infection, including endothelial membranes of the gastrointestinal tract and the urogenital canal (Dandara et al, 2006).

*Staphylococcus aureus* is a spherical diameter (0.1 - 0.5) micron and varies in size based on their strains, growth phases and in various types of culture media. On the solid media take a round shape of a diameter of 1-4 mm. The colonies are vary in their color between white, yellow and gold. *S. aureus* is a major pathogen that can cause a variety of infections ranging from mild skin abscesses to serious life threatening infections such as bone infections and surgical soft tissue (Lowy, 1998). The treatment of such infections has become increasingly difficult due to the emergence of antibiotic-resistant strains of *S.aureus* (NNIS, 1996). Researchers have devoted long time to develop a safe and effective anti-*Staphylococcus* agents. Some recent laboratories have been able to reduce the production of virulence factors of *S.aureus* (Mayville and Beavis, 1999). In this study, we assumed that strains of probiotics, *Bacillus subtilis* KATMIRA1933 and *Bacillus amyloliquefciense* B-1895 with their reported antimicrobial features, could prevent the growth and biofilm formation of *Staphylococcus* species isolated from wounds. The antimicrobial potential of *Bacillus subtilis* KATMIRA1933 and *Bacillus amyloliquefciense* B-1895 isolated from yoghurt was evaluated in this work

## 2.1 Research Objectives:

Evaluation the antimicrobial activity of tested probiotics bacilli against *S.aureus* bacteria isolated from wounds infection.

# Literature review

# Literature review

## 2.1 Importance and composition of skin:

The skin represents about 16% of body weight and it is the first physical barrier of the internal organs. It prevents lossing of fluids and thus prevents dryness. In addition, acid secretions of skin limit the attack of microbial pathogens where the pH of skin becomes acidic, about (4-4.5). The skin maintains its elasticity by retaining water and salts. The melanin granules present in the skin protect the skin from harmful UV rays and reduce the risk of skin cancer (Lamber et al, 2006)

The skin is composed of a thick outer layers and a broad system of sweat glands. It also contains many cells which are sensitive to touch, pressure and temperature. The main skin layers include: epidermis, dermis, and the inner Hypodermis layer (Waugh and Grant, 2010).

## 2.2 Normal Flora of the Skin:

Normal Flora is a term that describes the bacteria and fungi that represent the resident microbes (microflora) in the each body organs such as skin, colon and vagina. These living microorganisms are producing a beneficial effect on normal, healthy conditions, but harmless influences when the immune system is suppressed. Normal flora consists of aerobic, anaerobic bacteria and yeast, which help in preventing skin infections through environmental competition with pathogenic microorganisms by dissolving fat acids to produce toxic free fatty acids. However, some pathogenic microorganisms, such as *Staphylococcus* species, pass through all of these bio-barriers and capable to colonize the skin causing skin infection (Zeeuwen et al, 2012).

## 2.3 Role of *Staphylococcus* in wound infections:

There are several diseases caused by *S.aureus* including: urinary tract infections (UTI), infection occur after the surgery, infections from contaminated medical instruments, toxic shock syndrome, systemic infections and others (Cho et al, 2010). *S. aureus* infection is accompanied by the presence of cuts, abrasions or surgical wounds in addition to the redness, swelling, pain and abscess of the skin Due to colonization of wounds tissues (Agnihotri et al, 2004; Kluytmans et.al, 1995).

## 2.4 General characteristics of *Staphylococcus*:

The German scientist Rosenbach was the first who recognizeed *S.aureus* in 1884 by colony color. The name is derived from the Latin word Aurum, which means golden color and is an important human pathogen. *Staphylococci* are rounded, smooth, transparent edges, and high- raised colonies on the surface of the culture media. They produce dyes on the solid media such as, nutrient agar and blood hemolysis on blood agar. The colony appears smooth with a glistening surface, dark and opaque appearance. It produces golden, bilious, orange pigments and some of the strains produce white pigments to the cream color. The cells of the bacteria are non-notile, non-spore forming. The optimum temperature of bacterial growth is 37 ° C. They can survive in dry pus and have a high heat rsistance of 60 °C for 30 minutes. The bacteria have the ability to live within the temperature range (12 - 40°C) and acidity 7.4 (Jawetz et al, 2013). The species called *aureus* referring to their production of yellow carotenoids staphyloxanthin (Levienson, 2014).

Staphylococci are positive to Catalase, Coagulase, Urease, DNase and Phosphate tests. The bacterial is reducing $NO_3$ into $NO_2$ (Oranusi et al, 2016). They grow on the Mannitol Salt Agar which is a selective and differential culture media for *S.aureus* which produces yellow colonies due to their fermentation of mannitol sugar. *Staphylococcus aureus* are capable to tolerate the high salinity in this media, the concentration of sodium chloride (7.5-10%). Furthermore, some strains grow on the blood agar and producing hemolysis by 94% of beta-type, which causes complete blood hemlysis (Saadi et al, 2014).

## 2.5 Enzymes produced by Staphylococcus bacteria:

All strains of *S.aureus* produce a number of enzymes that increase their ability to penetrate host tissues. Some of these enzymes are pathogenic factors facilitating infections by the most virulent strains (Levinson, 2014). The most important of these enzymes are:

**2.5.1 Catalase:** This enzyme plays an important role in protecting the bacteria from the toxic effect of $H_2O_2$, which is the oxidative end product of the metabolism of aerobic carbohydrates. This enzyme breaks hydrogen peroxide by producing oxygen and water. It has been found that when *S.aureus* grow at 25, 32 and 37 °C temperatures, catalase activity is reduced by 80-90% within one hour of inoculation (Levinson, 2014).

**2.5.2 The plasma coaglase enzyme:** A protein similar to the enzyme that coagulate plasma by a protein factor present in the serum. This interaction is an important factor indicating the pathogenic degree of *S.aureus* (Thomer et al, 2016). The main role of the plasma coagulation enzyme is not entirely known, but there is an explanation that this enzyme is responsible for the concentration of bacteria in infected tissues with septic. The bacterial fibrin formation around the infected areas protects the *Staphylococcus* from the cellular defense mechanism of the body. This allows the bacteria to multiply away from phagocytosis process. (Qin et al, 2017; Baron and Finegold, 1994).

**2.5.3 Hyalurinidase:** This is called the Spreading Factor. This enzyme is excreted by the *Staphylococcus* strains which producing other enzymes such as Lipase, Protease, and DNase. This enzyme have a lytic activity on Hyaluronic acid in the connective tissue. The action of this enzyme is to reduce the viscosity of hyaluronic acid to facilitate the penetration of tissues by *Staphylococcus* strains (Jawetz et al, 2013).

**2.5.4 DNase enzyme:** This enzyme has the ability to destroy the DNA structure as one of the pathogenic virulence factors. *S. aureus* produces two types; the heat-sensitive DNAse, which determines the pathogenesis of the bacteria, and the heat-resistant, symbolized as TNase (Bokarewa et al, 2006; Rosato et al, 2009).

**2.5.5 Staphylokinase:** This is called Fibrinolysin which produced as a non-stable enzyme. It dissolves the blood clot when added to it during 24 - 48 hours and destroy fibrin thrombosis. This enzyme is enhancing the tissues invasion by the pathogenic strains (Costa et al, 2013; Bokarewa and Tarkowski 2006)

**2.5.6 Other enzymes: Including** Lipase, Proteinase, β-Lactmase, Phosphatase, Urase.

## 2.6 Toxins produced by *Staphylococcus aureus*:

The *S. aureus* has many external toxins, which play an important role in bacterial pathogenesis. The most prominent toxins are:

**2.6.1 Hemolysin:** is a toxin that causes blood hemolysis by breaking down the cell membrane of the red blood cells, leading to blood cells degradation. Blood analysis is be many types α, β, γ ) (Brooks et al, 2010).

**2.6.2 Leucocidine:** is a multi-protein toxic compound consisting of four lukf molecules and four other luks, which act on the cytoblasmic membranes of white blood cells polymorphonucleus (PMNs), causing cellular degradation and destruction. So, it is called leukocidin (Todar, 2005).

### 2.6.3 cm Panton - valentin Leuckocidin (PVL):

Named due two scientists who discovered this protein; Panton and Valentin in 1932. This protein causes repeated injuries to skin and soft tissues, and causes holes in the white blood cell membrane and eventually, cellular break down (Levinson, 2014; Shallcross et al, 2014).

### 2.6.4 Enterotoxins:

Microbial antigens produced by *S.aureus* are linked to complex Major Histocompatibility (MHC) class II. This linked cause T-cell to stimulate and release high amounts of IL1-IL2. Also, T cells stimulate the vomiting center in the brain. These toxins including six types named A, B, C, D, E, and F, causing food poisoning, watery diarrhea and vomiting. These toxins are thermally stable, heat resistant and also resistant to the effect of digestive enzymes in the intestines. Enterotoxins are produced when *S.aureus* strains are growing in foods rich in protein and carbohydrates (Levinson, 2014).

**2.6.5 Epidermolytic or Exfoliative Eradermolytic:** these toxins separate the stratum granulosom from the desmosomes that bind the epiderm cells together causing their death and exfoliation (Amagai et al, 2000).

**2.6.6 Toxic Shock Syndrome-1 toxin (TSST-1):** is rising the temperature Pyrogenic so it's called Pyrogenic toxin C and falls within the group of antigens Sags (Al-Sulami et al, 2015).

## 2.7 *Staphylococcus aureus* antibiotic resistance:

Penicillin was one of the most common and effective antimicrobials used in medical treatment of *Staphylococcus* in the early 1940s. The antibiotic effectiveness was short-life due to the emergence of resistant strains. About 65-80% of isolated strains from wound infections showed resistance against penicillin (Wu et al, 2010; Santaji and Indrawattana 2016). The use of other beta-lactimase, such as Methicillin and Oxacillin inhibited the spread of *S.aureus* during the 1960s. Howevere, at 1975,

methicilin resistant *S.aureus* (MRSA) was appeared which showed multi-drugs resistance including; Tetracyline, Erythromycin, Clindamycin and other antibiotics (Goyal et al, 2013).

## 2.8 Probiotics:

Probiotics are microorganisms known to provide health benefits to the host when they consumed in adequent amounts (Hill et.al, 2014). Probiobiotic have been reported by Nobel laureate Elie Michnikov who consumed yogurt had lived a life Longer (Brown and Valiere, 2004). It was suggested in 1907 that the presence of gastrointestinal microbes in the food facilitates the process of altering the normal flora of our bodies and replacing pathogenic bacteria by beneficial one (Élie, 1907, re2004). The World Health Organization (WHO) in 2001 defined them as living organisms when given in sufficient quantities, they provide a health benefit to the host (Schlundt, 2012).

## 2.9 Effect of Probiotics on *Staphylococcus aureus*:

Probiotics limit the presence of *S. aureus* bacteria by producing inhibitors such as organic acids and competitive dimensions through food competition, changing the metabolic efficiency of microorganisms and stimulating the immune system. Probiotics effect on MRSA was obvious in a study conducted on immunocompromised patients by (Warrack et al ,2014). The author found thet alimination of MRSA from 67% to 50% of treating patients with the probiotic, *Lactobacillus rhamnosus* HN001 Bio-booster, after four-hours. In a separate study of (Vuotto et al ,2014), It was reported that *Lactobacillus* spp had a significant effect to reduce the biofilm formation of MRSA isolated from oral, intestinal, vaginal wound infections. It was concluded that the using of of probiotic could inhibit the growth of pathogens and prevent their biofilm formation.

## 2.10 Biofilm formation by *Staphylococcus* species

Biofilm is defined as a mono or multy microbial community attached to a substratum, and have embedded themselves in a matrix of extracellular polymeric substance. Biofilm can exhibit an altered morphology with regard to growth, gene expression and protein production (Donlan et al, 2002). Biofilm can be formed from a single cell layer to a substantial community encased by a viscous polymeric substances (Costerton et al, 1995). The Gram-positive, *S. aureus*, is existed as opportunistic cells in humans skin and anterior nares. In human, approximately 20-

25% of Staphylococcus species have become persistently colonized on biotic and aboitic surfaces (Kluytmans et al , 1997 ; Dall et al , 2005). The staphylococci are either removed by the host innate immune response or attach to host extracellular matrix proteins and form a biofilm. The bacterial physiology is then transformed quickly into reflective of a biofilm formation. The presences of *S.aureus* in foreign body-related infections, will enhance development of biofilm formation and exhibition of antibiotic resistance, and will transform acute form infection to chronic and recurrent (Fitzpatrick et al , 2005).

*Staphylococcus aureus* can produce a multilayered biofilm embedded within a glycocalyx or slime layer with heterogeneous protein expression throughout. Early studies described the solid component of the glycocalyx as primarily composed of teichoic acids (80%) and staphylococcal and host proteins (Hussain et al, 1993). In later studies, a specific polysaccharide antigen named polysaccharide intercellular antigen (PIA) was isolated. PIA is composed of b-1,6-linked N-acetylglucosamine residues (80-85%) and an anionic fraction with a lower content of non-N-acetylated D-glucosaminyl residues that contains phosphate and ester-linked succinate (15-20%) (Mack et al, 1996).

*Staphylococcu aureus* biofilms, once established, are recalcitrant to antimicrobial treatment and the host response, and therefore are the etiological agent of many recurrent infections (Jones et al , 2001). Osteomyelitis and wound infection will be first in the context of *S. aureus* stages of colonization, infection and maturation of the biofilm as this progression of disease is mimicked in many other diseases and host sites.

*Regarding wound infection, S.aureus* biofilms have been implicated in cases of chronic infections such as diabetic foot ulcers, venous stasis ulcers and pressure sores. In fact, *S. aureus* is the most commonly isolated bacteria from such wound infections and studies involving patients with chronic venous leg ulcers found *S. aureus* positive cultures in 88-93.5% of infections (GjØdsbØl et al, 2006 ; Hansson et al, 1995; Davis et al, 2008), Observed *S. aureus* biofilm structures in a porcine wound colonization model using multiple microscopic techniques. Diabetic foot wound patients with *S. aureus* wound colonization have a 2-fold increase in healing time (Bowling et al, 2009). In addition, delayed re-epithelialization was shown to be specifically dependent on *S. aureus* biofilm development (Schierle et al,2009).

However, with modern molecular and culture independent techniques, this prevalence may change in the future.

## 2.11 Effect of probiotic on biofilm-associated *Staphylococcus* species

Cell-free supernatants (CFS) from benefecial microbes were known for decades to show inhibitory effects on the growth of pathogenic bacteria (Mariam et al, 2017 ; Melo et al , 2016). Depending on the bacterial species, highly diverse biologically active substances are secreted and can be detected in the culture supernatants. These include antimicrobial agents, such as bacteriocins (Chen et al , 2018), and metabolic products, such as organic acids ( Kim et al, 2018 ), hydrogen peroxide (Pridmore et al ,2008), or biosurfactants (Sambanthamoorthy et al,2014), all with antimicrobial and anti-biofilm activity against pathogenic bacteria, as well as immunomodulatory substances (Lee et al,2014 ; Bermudez et al,2014 ).

*Lactobacillus rhamnosus*, as probiotic bacterium culture supernatants exhibit in vitro activity against a number of Gram-positive and Gram-negative bacteria such as *Clostridium* spp, *Enterobacter* spp, and staphylococci ( Silva et al,1987). In vivo, *Lactobacillus rhamnosus* GG was shown to temporarily colonize the human gastrointestinal tract (Goldin et al,1992), with supportive effects in the case of diarrhea in children ( Szajewska et al,2011 ), against antibiotic-induced disorders in the gastrointestinal tract ( Korpela et al,2016 ). The use of *L. rhamnosus* GG as a probiotic substance has been extensively assessed regarding its safety profile and is therefore considered as relatively harmless for patients ( Snydman,2008 ). In vitro studies also provided evidence for *L. rhamnosus* GG induced effects on biofilm formation in various oral microorganisms ( Jiang et al ,2016 ). *Streptococcus salivarius* K12 produces bacteriocin-like inhibitory substances (BLIS). Among these antimicrobial peptides, the lantibiotics, salivaricin A2 and salivaricin B, showed growth inhibition on bacteria like *Streptococcus pyogenes* and *Haemophilus influenzae*, as well as *Candida albicans* (Ishijima et al ,2012 ; Fiedler et al ,2013). The preventive use of *S. salivarius* K12 leads to a significantly reduced recurrence of both bacterial and viral pharyngotonsillitis and otitis media ( Dipierro et al,2016 ).

Sensitivity of staphylococcal biofilms towards antimicrobial peptides has been described ( Mathur et al ,2018), although the majority of studies were focused on growth inhibition rather than reduction of formed biofilms. In the this study here, the effects of cell-free culture supernatants of probiotic Bacilli on planktonic growth and biofilm formation by methicillin resistant *S. aureus* and *S. epidermidis* strains were assessed.

# Materials and Methods

# Materials and Methods

## 3.1 Materials and devices:

### 3.1.1 Devices:

| | Devise | Company and origin |
|---|---|---|
| 1 | Incubator | LabTech Daihan , South Korea |
| 2 | Autoclave | LabTech Daihan , South Korea |
| 3 | Bunsen burner | EISCO , India |
| 4 | Refrigerator | BEKO RRN 2650 , Turkey |
| 5 | Light microscope | Nikon , Japan |
| 6 | Electric sensitive balance | Instrument co. ltd. , Thai |
| 7 | Vortex mixer | Gemmy Industrial Corp , Taiwan |
| 8 | Water Distillator | GFL Gesellschaft für Labortechnik , Germany |
| 9 | Centrifuge | Hettich Zentrifugen , Germany |
| 10 | Water Bath | AOHUA, China |
| 11 | pH meter | Adwa instruments, Hungary |
| 12 | Magnetic hot plate | VEVOR, China |
| 13 | Microplate Reader spectrophotometer | Diagnostic Automation, USA |

### 3.1.2 Tools :

| | Tools | Company and origin |
|---|---|---|
| 1 | Graduated glass Cylinder | Supc Orior, Germany |
| 2 | Pasteur pipette | Bioerieux, France |
| 3 | Disposable syringes | Mehec, China |
| 4 | Flasks | Mehec, China |
| 5 | Filter Paper (0.1µm) | Mehec, China |
| 6 | Forceps | Behrin, Germany |
| 7 | Petri dish | Mehec, China |
| 8 | Glass slides | Mehec, China |
| 9 | Micropipette | Brand, Germany |
| 10 | Syringe filter | Whatman, Germany |
| 11 | Swabs | Mehec, China |
| 12 | 96 well tissue culture microplate | Greiner Bio-One GmbH, Austria |

### 3.1.3 Chemicals Materials:

| | Chemicals Materials | Company and origin |
|---|---|---|
| 1 | Oxidase | BDH, England |
| 2 | Ethanol | BDH, England |
| 3 | Phosphate Buffer | BDH, England |
| 4 | Gram stain solutions | CDH, India |
| 5 | McFarland solution | Biomérieux, France |
| 6 | Catalase detector | Merk, Garmany |
| 7 | Coagulase detector | BDH, England |

### 3.1.4 Media:

| | Media | Company , origin , size and prepare |
|---|---|---|
| 1 | **Blood agar** | Saluca Dutch technology in life science, Netherlands Prepared with pH 7.4 ± 0.2, size 500 g, dissolve 28 g in 1000 ml of distilled water and boil until the media melts and sterilize at 121 ° C, Pres. 15 lb, for 15 minutes and mix well before casting. |
| 2 | **De Man, Rogosa and Sharpe agar (MRS) agar** | MAST Group, USA Size 500 g, dissolve 62 g in 1000 ml of distilled water and boil until the media melts and sterilize with a degree at 121 ° C. Pres. 15 lb, for 15 minutes and mix well before casting. |
| 3 | **Manitol salt agar** | HiMedia, India Size 500 g, dissolve 111.02 g in 1000 ml of distilled water and boil until the media melts and sterilize with a degree at 121 ° C. Pres. 15 lb for 15 minutes and mix well before casting. |
| 4 | **DNase Agar** | HiMedia, India Size 500 g, dissolve 39 g in 1000 ml of distilled water and boil until the media melts and sterilize with a degree at 121 ° C Pres. 15 pounds for 15 minutes and mix well before casting. |
| 5 | **Agar powder** | HiMedia, India |
| 6 | **Muller Hinton** | HIMEDIA ref M173-500G, India Size 500 g, dissolve 38 g in 1000 ml of distilled water and boil until the media melts and sterilize with a 120 ° C rack. Pres. 15 lb for 15 minutes and mix well before casting. |

### 3.1.5 Antibiotics : Standard Chart for Antibiotic susceptibility testing :

| | Antibiotic | symbol | concentration | Diameter of the inhibition zone in millimeters | | |
|---|---|---|---|---|---|---|
| | | | | R | I | S |
| 1 | Amoxillin | AX | 10 µg | ≤28 | - | ≥29 |
| 2 | Penicillin G | P | 10 IU | ≤28 | - | ≥29 |
| 3 | Cefotaxime | CTX | 30 µg | ≤14 | 15-17 | ≥18 |
| 4 | Methicillin | ME | 5 µg | ≤9 | 10-13 | ≥14 |
| 5 | Oxacillin | OX | 1 µg | ≤10 | 11-12 | ≥13 |

## 3.2 Working methods:

### 3.2.1 samples collect:

Samples were collected from the General Teaching Hospital of Baqubah of different ages and both sexes for the period between the January 7th - 29th, 2018. The swabs were transferred to the microbiology labs at the Department of Biotechnology, college of Science, Diyala University and inoculated on their suitable culture media.

### 3.2.2 Method of preparing medias:

1. The powder of the culture media was weighted with a sensitive balance according to the instructions mentioned on the culture media box. Methods of preparation for each media were reported as in 3.1.4.
2. The Distilled water was added to the powder of culture media that has been weighed. the contents were mixed well, heated and stirred well for the initial dissolution of the media.
3. The media were transferred to the autoclave for sterilization.
4. The culture media were kept in the dark and cool place if not used directly until pouring in petri-dishes.

### 3.2.3 Methods for preparation of solutions, reagents and enzymes:

Many reagents and solvents were prepared in this study and were sterilized using autoclave at 121 ° C for 15 minutes at 15 baung / ang 2. The heat-affected materials and solutions were sterilized using Millipore filters with a diameter of 0.22 µm. The glass material was sterilized by Oven at 180 °C for 2 hours.

### 3.2.3.1 Gram's stain solution:

The solutions described by Cowan and Steel (1967), consisting of the solutions; violet crystals, the iodine solution, the absolute alcohol and the safranin dye. It was used for staining of the glass slides prepared from the bacterial growth to observe their characteristics and microscopic features and classified them into Gram positive and Gram negative categories.

### 3.2.3.2 Coagulase Reagent:

This reagent is used for identification of coagulase enzyme production *S.aureus*. Aliquots, 0.5 ml of the diluted vial was mixed with a quantity of microbial colonies and incubated for 1-4 hours at 37°C. The presence of coagulation indicates the positive result (Geo et al, 2007).

### 3.2.3.3 Catalases enzyme:

Prepared as hydrogen peroxide 3% in distilled water and store in a freezer in a dark container. Catalases enzyme used to detect the ability of the bacterial isolates to produce an enzyme splitting $H_2O_2$ to the $H_2O$ and $O_2$ (Colle et al, 1996).

### 3.2.3.4 Oxidase:

Instantly prepare 1 ml of Tetra Methyl-Para- Phenylene diamino dihydrochloride in 90 mL of distilled water and then complete the volume to 100 mL. This reagent is used to detect the ability of bacteria to produce the oxidase enzyme (Koneman et al, 1997).

## 3.2.4 Diagnosis of Isolation Bacteria:

The bacteria were identified based on their cultural characteristics; microbiological, and biochemical as followings:

### 3.2.4.1 Culture Appearance Characteristics:

A. Blood Agar: The growing bacterial isolates were identified based on their phenotipic characteristics in terms of the size, color of colonies, as well as their ability to hemolyse red blood cells on this culture media.
B. Mannitol Salt Agar (MSA): A selective culture media to isolate *S.aureus* that has the ability to grow at 7.5 to 10% concentration of NaCl. MSA is used to differentiate between *S.aureus,* which ferment mannitol sugar from other non-fermented species. The color of the media changes from red to yellow due to the presence of a methyl red reagent (Brooks et al, 2010).

**3.2.4.2 Microbial characteristics:** Bacterial isolates were subjected to microscopic examination. A bacterial coloniies were stained with Gram staining techniques and examined under the ocular lens to distinguish the shape, size and the collection of the cells as well as their reaction to Gram stains; Gram positive or Gram-negative.

### 3.2.4.3 Biochemical tests:

A. **Catalase production test:** A (18-24) hours bacterial growth was transferred to a clean and dry glass slide using wooden sticks, and a drop of 3% hydrogen peroxide was added. The result is positive, when the air bubbles appeared on the slide's surface. The bubbles are an indication of the destruction of toxic hydrogen peroxide and the release of oxygen and water (Harley and Prescott, 2002).

B. **Coagulase production test: This test was done in two ways:**

1. Slide Coagulase test: Presence of blood clotting is an indication of coagulase production by *S.aureus*. The test was performed by adding a drop of normal saline solution on a clean glass slide, and a colony of *S.aureus* transferred and well mixed with a drop of plasma. The formation of clot refers to a positive result which occured within 15 seconds. The Clamping factor binds to the microbial cell converter the fibrinogen to the fibrin (Forbes et.al, 2007).
2. Tube coagulation test: A number of bacterial colonies were transferred to a test tube containing plasma and incubated for (1-4) hours. After incubation, coagulation (clot) formation is a sign of coagulase production by *S.aureus* (Brooks et al, 2010).

### 3.2.5 Antibiotic Sensitivity Test:

The standard Kerby & Bauer method was used for identification of bacterial sensitivity to antibiotics (Vandepitteet et al, 1991). Briefly, After bacterial growth on appropriate culture media, 3-5 colonies of *S.aureus* are suspended into 3-5 ml of phosphate buffer saline. The turbidity of bacterial suspension was adjusted to $5\times10^5$ CFU/mL using McFarland tube No. 5. To facilitate the turbidity comparison, a white sheet was placed beneath the tubes. A Sterile swab saturated with bacterial suspension was streaked over the Mueller Hinton agar plate in 3 directions. The entire surface of the dish was covered by bacterial cells. A sterile forceps was used

to pick up the antibiotic disc and placed on the surface of the Mueller Hinton agar plate. The antibiotic discs were placed 15 mm from the edge of the dish and 25 mm between each disc was left. the agar plate was left for 30 minutes until the antibiotic was diffused from the disc into the surrounding agar surface and then dishes was incubated in an air incubator a under 35-37 °C for 16 - 18 hours. After incubation, the inhibitory zones were measured and the results were expressed as sensitive (S), resistant (R) or intermediate (I) according to the standard chart for antibiotic susceptibility testing (NCCLs, 2017).

### 3.2.6 Co-aggregation test:

A Co-aggregation test is performed to assess the ability of the *Bacillus* strains; *B. subtilis* KATMIRA1993 and *B. amyloliquefaciens* B-1895 to co-aggregate with *S. aureus.* The method described by (Cisar et al ,1979) with some modifications. Bacterial cells were grown from plankton cells incubated at 37 ° C with centrifuge (4480 g, 15 min, 23 °C) and washed with PBS twice. After the second wash, the cells collected and re-suspended again with PBS. The optical density ($OD_{600}$ ) was adjusted to 0.25.Then, 100 µL of *S. aureus* were mixed with 100 µL of each *Bacillus* strain into a 96 wells microplates. From each bacterial species, 200 µL of each bacterial suspension (as a monoculture) was used as a controls. Samples of 10 µL were withdrawn after 2 and 24 hour of incubation for Gram-staining and observed microscopically for coaggregation (Fig. 5). Each experiment was performed in triplicate.

### 3.2.7 Antibiotic in combination with *Bacillus* CFS

Previously explained in the Kerby & Bauer method for identification bacterial sensitivity to antibiotics, the bacterial suspension in PBS was adjusted to $5\times10^5$ CFU/mL using McFarland tube No. 5. Then, a swab saturated with bacterial suspension was streaked over the Mueller Hinton agar plate in 3 directions. The entire surface of the dish was covered by bacterial cells. A sterile forceps was used to pick up the antibiotic discs (Antibiotic disc, Antibiotic disc saturated with Bacillus CFSs, blank disk saturated with Bacillus CFS only, and blank disc as control). All of discs were placed on the surface of the Mueller Hinton agar plate. The antibiotic discs were placed 15 mm from the edge of the dish and 25 mm between each disc was left. the agar plate was left for 30 minutes until the antibiotic was diffused from the disc into the surrounding agar surface and then dishes was incubated in an air incubator a under 35-37 °C for 16 - 18 hours. After incubation, the inhibitory zones

around each disc were measured and the results were expressed as sensitive (S), resistant (R) or intermediate (I) according to the standard chart for antibiotic susceptibility testing (NCCLs, 2017).

### 3.2.8 Determination of minimum biofilm inhibitory concentrations (MICs-B)

The MIC-B of bacilli CFS determination was performed following the method of (Sutyak et al , 2008) with minor modifications. Briefly, *B. subtilis* KATMIRA1933 and *B. amyloliquefacience* B-1895 CFSs were two-fold diluted with an appropriate volume of fresh TSB supplemented with 1% Glucose (TSBG1%) in a 96-well tissue culture plate (manufacture). The final volume of antimicrobial agents diluted into the TSB was 100 µL into each well. The overnight cell culture of staphylococci species at $3\pm2x10^{9}$ CFU/mL was diluted in TSBG1% to a final concentration of $5x10^{6}$ CFU $mL^{-1}$ (the number of bacterial cells was determined using plate counting method). From the diluted bacterial cells, 100 µL were added to the wells containing pre-determined concentrations of CFS. Plates were incubated under aerobic conditions at 37 °C for 24-36 hr. The MIC-B was determined as the lowest concentration of CFS, at which more than 90 % of *Staphylococcus* biofilm was inhibited without influencing the growth of non-adherent cells. Determination of biofilm inhibition by staining with crystal violate After a 24 hr incubation, the non-adsorbed cells were removed from each well by aspiration with micropipette and the numbers of cells were adjusted using spectrophotometer ($OD_{600}$). The wells were then gently washed three times with 200 µL of TSB. The biofilm was fixed by heating at 60 °C for 60 minutes and stained with crystal violet (CV) according to (Borucki et al , 2003) with minor modifications. Into the treated wells, 50 µl of 0.2% CV was added over the biofilm and left at room temperature for 25 min. Each well was then washed thrice with 200 µl of distilled water and left for 10 minutes to dry at incubator 37 °C. For solubilizing the CV-stained biofilm, 200 µL of 95% (v/v) ethanol/water was added into each well. An absorbance measurement was made using an Diagnostic automation Absorbance reader at 630 nm (Diagnostic automation, Inc, Calfornia, USA).

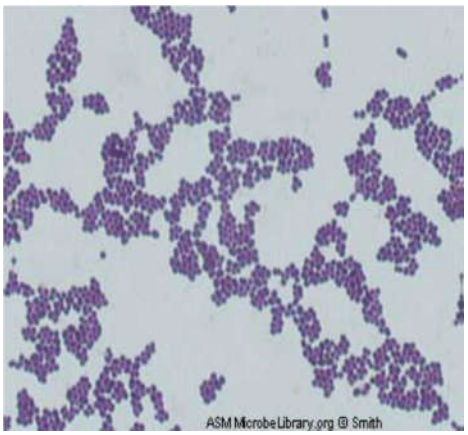
# Results and Discussion

# Result and Discussion

## 4.1 Bacterial Identification:

The biochemical identification tests showed a total of 10 (58.8%) of 17 isolates were *S. aureus*. The growing bacterial colonies turn the color of Manitol salt agar media yellow in case of *S. aureus* but still redish-pink in case of *S. epidermidis* (Fig 1) . The results of the microscopic examination showed that the isolating cells have a spherical shape, clusters arrangement, and Gram-positive, non-spores forming, as shown in Figure (1).


A- staphylococci species on Manitol Salt agar




B - Gram positive *Staphylococcus aureus*

D - Staphylococci on DNase agar

**Figure 1: Initial identification of Staphylococcus**

**Table (1) The frequency of bacterial species isolated from both genders.**

| Type of bacterial infection | Statistics | Sex | | Summation |
|---|---|---|---|---|
| | | Male | Female | |
| *Staphylococcus aurease* | number | 3 | 5 | 8 |
| | % | 17.65 | 29.41 | 47.05 |
| Methicillin-resistant *Staphylococcus aureus* (MRSA) | number | 0 | 2 | 2 |
| | % | 0 | 11.76 | 11.76 |
| *Staphylococcus epidermidis* | number | 1 | 0 | 1 |
| | % | 5.88 | 0 | 5.88 |
| Other non-Staphylococci | number | 4 | 2 | 6 |
| | % | 23.53 | 11.76 | 35.29 |
| Total Summation | number | 8 | 9 | 17 |
| | % | 47.05 | 52.95 | 100% |

Table 1 showed that 8 of methicillin sensitive *S.aureus* were isolated; 5 (29.41%) from female and 3 (17.65%) from male. In addition, 2 (11.76%) of methicillin resistant *S.aureus* were isolated from female only and 1 (5.88%) was *Staphylococcus epidermidis* while (6) 35.29% were reported as other microbes. The total bacterial isolates were 9 (52.97%) from female and 8 (47.05%) from male.

In comparison to previous studies, our study was in agreement with Abdullah (2018) who found that *S. aureus* was the most predominant isolated bacteria from wound infection samples. Same study found that the isolation presentages of methicillin-resistant *S. aureus* was significantly lower than that of methicillin-sensitive *S. aureus* at 20%, 80%, respectively. The ratio appeared to be as similar as in Abdullah (2018) study. Eventhogh, a lower number of MRSA were isolated, the urgent strategies have to be taken to avoid distribution such multi-drug resistant bacteria. Our data illustrated that distribution wound infection associated *S. aureus* was in female 9 (52.97%) more than in male 8 (47.08%).

## 4.2 Antibiotic susceptibility of Staphylococci species:

The effect of antibiotics was investigated for *S. aureus* isolates of 8 isolates and S. epidermis 1 isolate by using sensitivity drug test to antibiotics (Amoxillin, penicillin, Cefotaxime, Methicillin). The results of the present

study show that all isolates are resistant to methicillin and vary in resistance to other antibiotics as shown in figure ( 2 ).

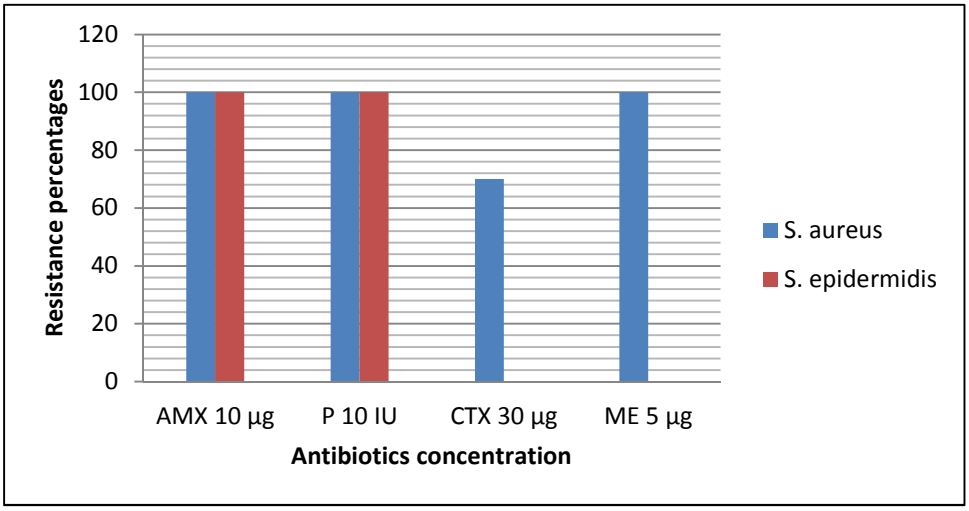



**Figure 2: Antibiotic susceptibility test; Resistance percentages to antibiotics.**

The results of figure 2 ilustrated the antibiotic resistance of *S. aureus* isolated from wound infection. All isolates of *S. aureus* were 100% resistant to amoxicillin 10 µg, penicillin 10 IU and methicillin 5 µg but 70% resistance to cefotaxime 30 µg. The isolated *S. epidermidis* was resistant to amoxicillin 10 µg and penicillin 10 IU but sensitive to methicillin 5 µg and cefotaxime 30 µg. Our data were comparable to the study of Abdullah (2018) who found that agree for the All isolated *S. aureus* were resistant to penicillin G and cefotaxime, but sensitive to methicillin. A concentration of methicillin (higher than 5 µg) was used in the work of Abdullah (2018).

### 4.3 Antibiotic combination with CFS of tested Bacilli

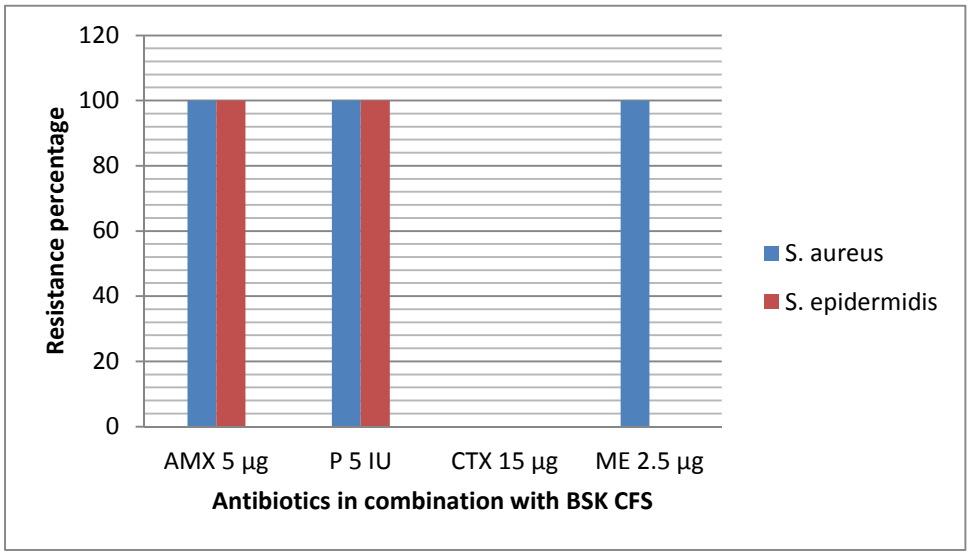



**Figure 3: Antibiotic susceptibility test; Resistance percentages to antibiotics in combnation with CFS of *B. subtilis* KATMIRA1933**

Figure 3 showed the resistance percentages of *Staphylococcus* species to antibiotics in combination with CFSs of *B. subtilis* KATMIRA1933. Saturation each antibiotic disc with 20 µL of CFS of tested Bacilli, caused a two-folds dilutions of the antibiotic concentration. All cefotaxime resistant strains became sensitive when cefotaxime 15 µg (after dilution) was combined with CFS of overnight growth of *B. subtilis* KATMIRA1933. Neither CFS of *B. subtilis* KATMIRA1933 alone nor cefotaxime individualy had antimicrobial effect on growth of *S. aureus* (MRSA or MSSA) (Data not shown). A synergistic activity was obvious when antibiotic was combined with tested Bacilli. *B. subtilis* KATMIRA1933 reported to have antimicrobial activity against wide range of microbial pathogens such as *Listeria monocytogenes* and *Gardnerella vaginalis* (Algburi et al, 2015).

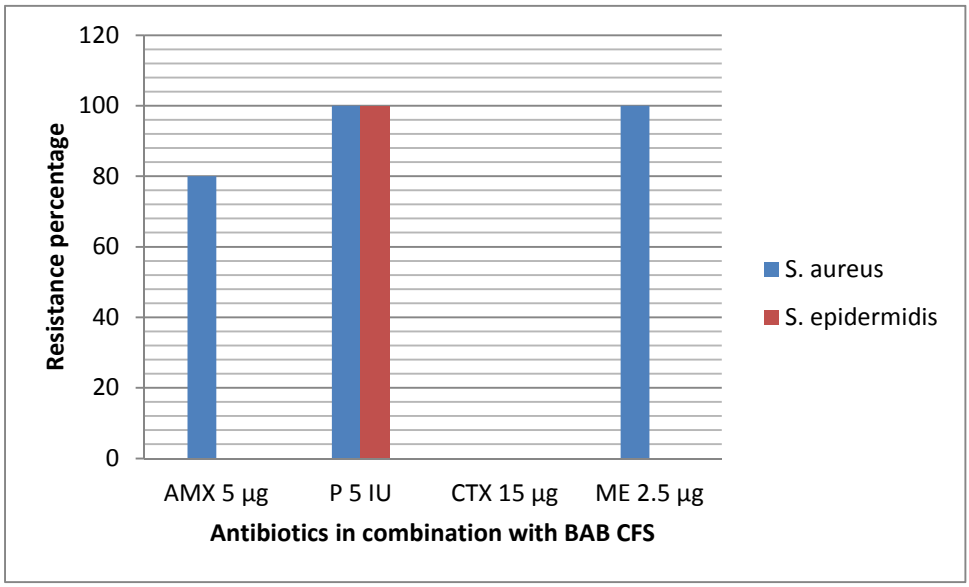



**Figure 4: Antibiotic susceptibility test; Resistance percentages to antibiotics in combnation with CFS of *B. amyloliquefaciens* B-1895**

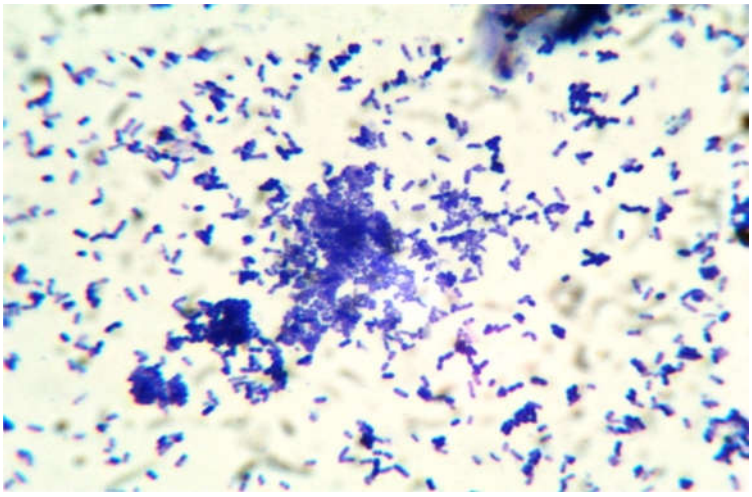
Figure 4 showed the resistance percentages of *Staphylococcus* species to antibiotics in combination with CFS of *B. amyloliquefaciens* B-1895. Saturation each antibiotic disc with 20 µL of CFS of tested Bacilli, caused a two-folds dilutions of the antibiotic concentration. All cefotaxime resistant strains became sensitive when cefotaxime 15 µg (after dilution) was combined with CFS of overnight growth of *B. amyloliquefaciens* B-1895*B.* In addition, 100% of *S. aureus* which was resistant to amoxicillin 10 µg when it was used alone while 20% of isolates became sensitive when amoxicillin 5 µg (after dilution with CFS) was combined with CFS of *B. amyloliquefaciens* B-1895. Neither CFS of *B. amyloliquefaciens* B-1895 alone nor using cefotaxime, amoxicillin individualy had antimicrobial effect on growth of *S. aureus* (MRSA or MSSA) (Data not shown). A synergistic activity was obvious when antibiotics, cefotaxime, amoxicillin, were combined with tested Bacilli. *B.*

*amyloliquefaciens* B-1895 reported to have antimicrobial activity against wide range of microbial pathogens such as *Listeria monocytogenes* and *Gardnerella vaginalis* (Algburi et al, 2015).

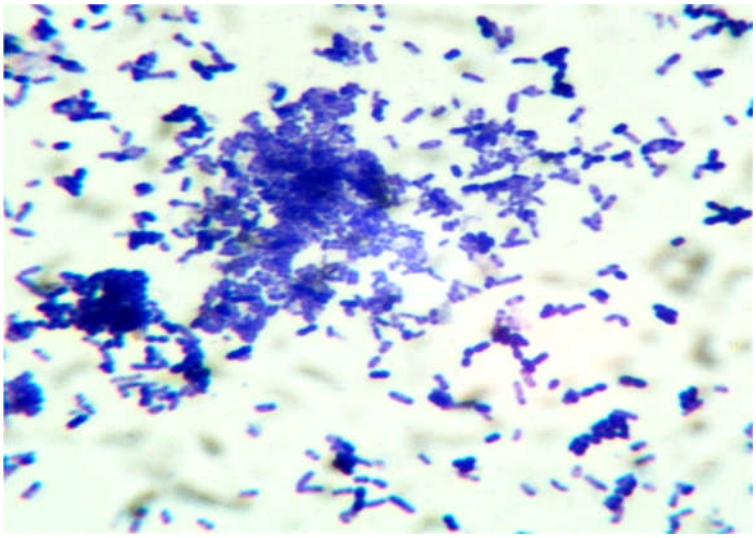
## 4.4 Auto-aggregation and co-aggregation of tested bacilli

In our study, auto-aggregation of tested bacilli and their co-aggregation with Staphylococcus were noticed on microscopic slide which was stained by Gram staining technique (figure 5). The results were expressed as 0, +, ++, +++, ++++ based on the strength of auto-aggregation and co-aggregation. An auto-ggregation was reported between the same species of tested bacteria; Staphylococcus (++), *B. subtilis* KATMIRA1993 (++) and *B. amyloliquefaciens* (+++). Both *B. subtilis* KATMIRA1993 and *B. amyloliquefaciens* were strongly coaggregated (++++ and +++, respectively) with *S. aureus*. in the figure 5, the tested probiotics as Gram positive bacilli attract *S. aureus*, the Gram positive cocci.

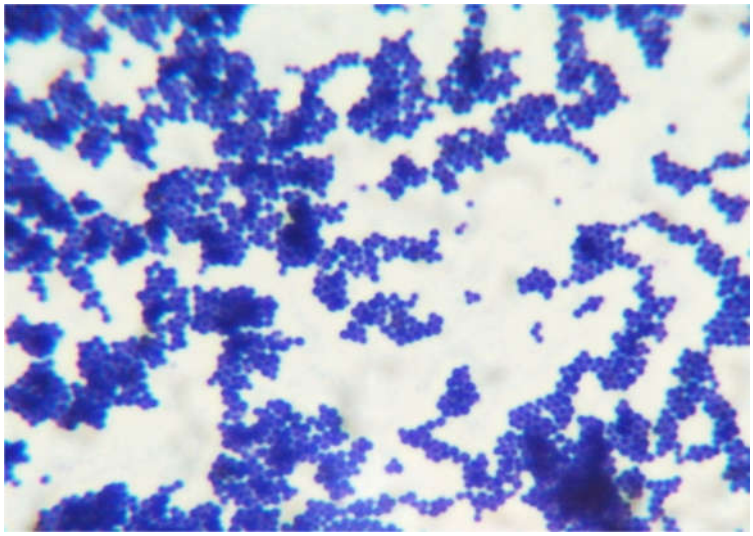
The ability of probiotics to co-aggregate and auto-aggregate is considered an advantageous characteristic feature. Their adhesion to a pathogenic organism can facilitate the elimination of the organism from the body and its ability to self-aggregate gives it an advantage in a competitive environment. The probiotic employed in this study were chosen as common organisms found in products of consumption. After 2 h, *B. amyloliquefaciens* B-1895 adhered to *s. aureus*. Both *Bacillus* strains were found to have auto-aggregating abilities after 2 hours. *B. amyloliquefaciens* B-1895 showed greater instances of auto-aggregation than *B. subtilis* KATMIRA1933, at a more significant level, during 24 h.



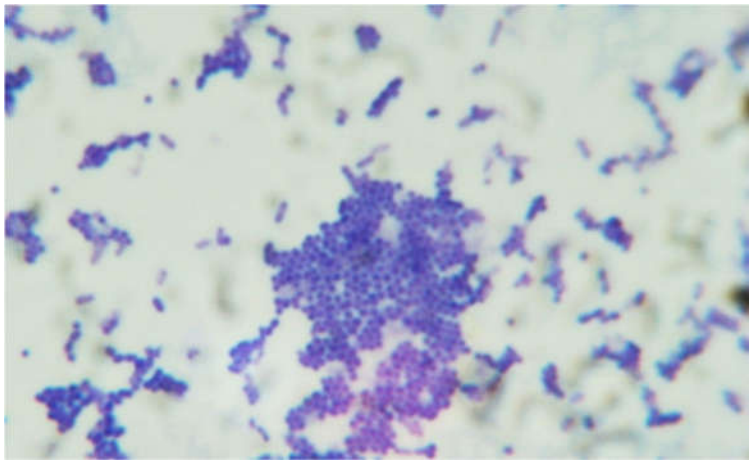
A) Auto-aggregation of *B. subtilis KATMIRA1933*



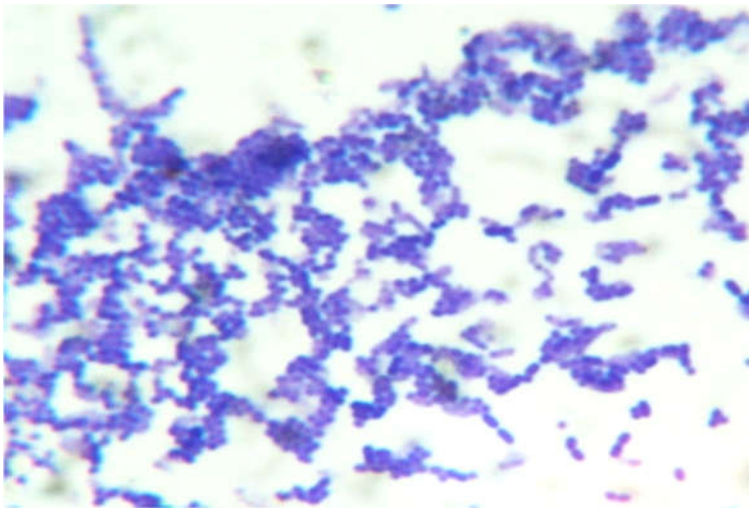
B) Auto-aggregation of *B. amyloliquefaciens* B-1895



C) Auto-aggregation of *S. aureus*



D) Co-aggregation of *B. subtilis KATMIRA1933* with *S. aureus*



E) Co-aggregation of *B. amyloliquefaciens* B-1895 with *S. aureus*

**Figure 5: Auto-aggregation and co-aggregation of testesd bacilli.**

## 4.5 Anti-biofilm activity of the CFS of studied bacilli:

In this work, The antibacterial activity of CFSs of two studied Bacilli was evaluated; *Bacillus subtilis* KATMIRA1933 and *Bacillus amyloliquefacience B-1895* against biofilm-associated *S. aureus* isolated from wound infection. Compared to the control, 25% and 50% of the CFS of *B. subtilis* KATMIRA1933 effectively prevented 55±0.7% and 61±0.5% respectively, of biofilm-associated MRSA isolates in addition to 57±0.2% and 60±0.6%, respectively of MSSA biofilm was inhibited. Regarding bacterial growth, MRSA viability were 95±0.4% and 60±0.5% while MSSA viability were 96±0.8% and 56±0.6% when same concentrations (25% and 50%) of CFS of *B. subtilis* KATMIRA 1933 were applied, respectively as shown (figure 6). In a separate experiment, the biofilm inhibitory effect of the CFS of *B. amyloliquefacience* B-1895 was evaluated against isolated Staphylococci. Our data showed that MRSA biofilm inhibition, compared to the control group, was 48±0.5% and 55±0.7% while MSSA biofilm prevention was 45±0.8% and 59±0.4% when 25% and 50% of the CFS of *B. amyloliquefacience* B-1895 were used, respectively. In regards to cell viability, 25% and 50% of the CFS of *B. amyloliquefacience* B-1895 caused slight inhibition (85±0.3%, 58±0.7% respectively) for MRSA isolates and (91±0.4, 53±0.5) for MSSA (see Fgure 7). In conclusion, no significance differences were noticed in biofilm prevented activity between CFS of *B. subtilis* KATMIRA1933 and *B. amyloliquefacience* B-1895 against MRSA or MSSA.

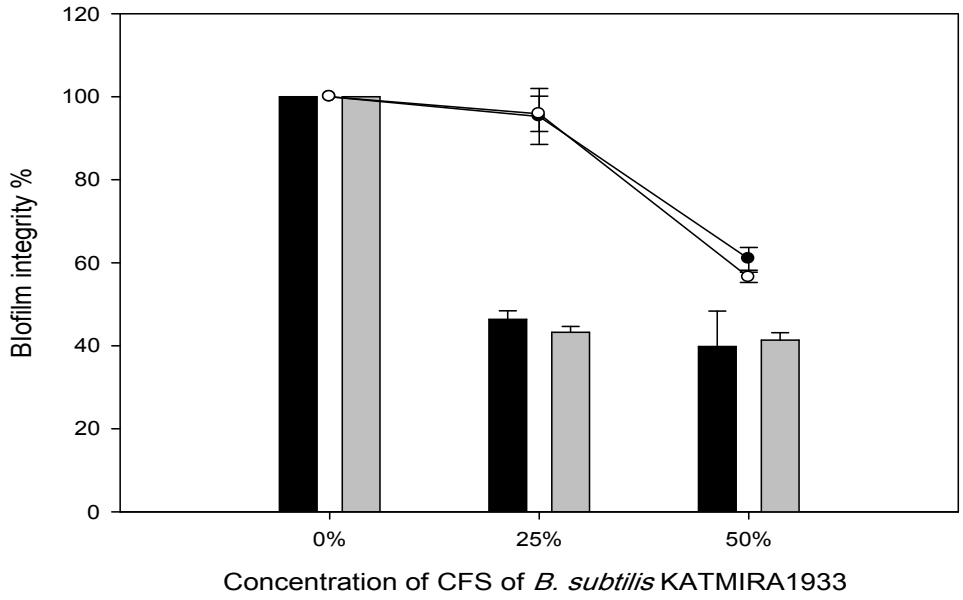



**Figure 6: Anti-biofilm activity of CFS of *B. subtilis* KATMIRA1933 against isolated MRSA and MSSA**

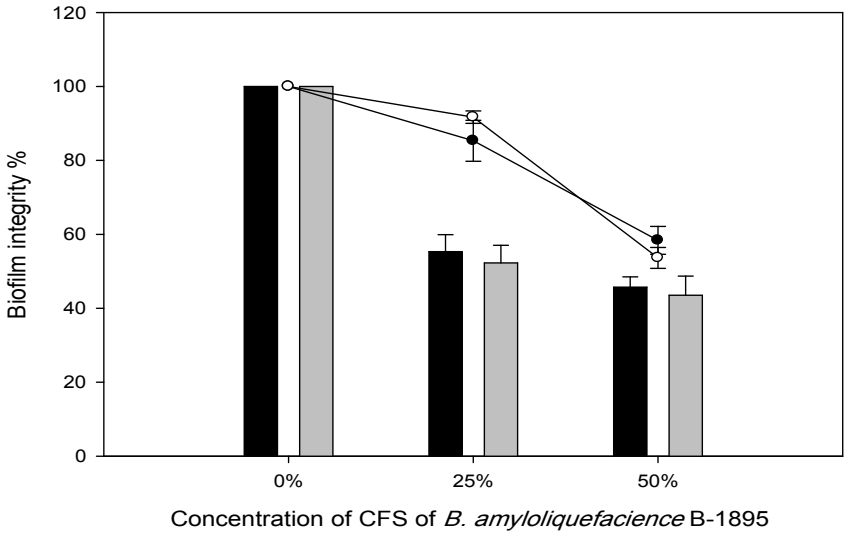



**Figure 7: Antibiofilm activity of CFS of *B. amyloliquefacience* B-1895 against isolated MRSA and MSSA**

Several studies have been reported indicating the anti-biofilm activity of the tested bacilli against pathogenic bacteria. The anti-biofilm potential of studied bacilli was assessed due to their safety to human cells in addition to their antimicrobial selectivity (Sutyak et al, 2008), (Van et al, 2012) reported that *B. subtilis* KATMIRA1933 had antibacterial and anti-biofilm activity against *L. monocytogenes* and *G. vaginalis* via production of an antimicrobial cyclic peptide, subtilosin A. The antibacterial potential of this *B. subtilis* KATMIRA1933 cyclic peptide was concentration-depended manner.

# Conclusions and Recommendations

# Conclusions and Recommendations

## 5.1 Conclusions:

1. A higher percentage of *S.aureus*-associated wound, in female more than male, is reported in this study. This could be reffered to several points including: the lack of attention to the personal cleanliness of women, with regard to genital infections, in addition to using of contaminated tools and instruments in surgical wounds .
2. The isolation of *S. epidermidis* refferes to the importance of commensal bacteria as wound contaminant and could cause wound infection.
3. Identification of methicilin resistant *S. aureus* (MRSA) in wound infection referes to the dangerous level of wound infection with multidrug resistance bacteria. This results should bring the attention of researchers to find an alternative, effective and safe antimicrobials to encounter bacterial resistance to antibiotics.
4. Antibiotic in combination with CFS of *Bacillus subtilis* KATMIRA1933 and *B. amyloliquefacience* B-1895 showed a synergistic activity against isolated Staphylococci.
5. Both *B. subtilis* KATMIRA1933 and *B. amyloliquefacience* B-1895 were strongly auto-aggregated and co-aggregated with isolated *S. aureus*. Bacilli formed of clusters surrounding *S. aureus*, thus competing on the sites of adhesion to the body tissues and preventing them from causing infection.
6. CFS of *B. subtilis* KATMIRA1933 and *B. amyloliquefacience* B-1895 showed no anti-microbial but anti-biofilm against MRSA and MSSA isolated from wound infection.

## 5.2 Recommendations:

1. In general, we recommend the health care and the use of sterile tools and instruments in hospitals to save the millions live of patients.
2. Find an effective treatment that helps to encounter the antibiotic resistance mechanisms of pathogenic bacteria.
3. Based on our findings, We recommend conducting the extensive and serious studies on the therapeutic role of benifecial bacteria (probiotics) which can be used as effective and safe alternatives to avoid the dangerous side effects of using antibiotics.

# Resources

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# الخلاصة

واحدة من اكثر العوامل المتسببة اصابات الجرح هي المكورات العنقودية الذهبية. تشكيل البايو فلم من قبل المكورات العنقودية الذهبية يزيد من المقاومة المايكروبية للضغوط البيئية ، مثل المضادات الحيوية . تهدف هذه الدراسة إلى تقييم نشاط المضادات الميكروبات و مُضادات البايوفلم لبعض نواتج المعززات الحيوية على البايوفلم المرتبط بالمكورات العنقودية الذهبية . تم جمع عينات الجروح في المستشفى العام في بعقوبة من مختلف الفئات العمرية ومن كلا الجنسين ما بين السابع من شهر كانون الثاني حتى التاسع والعشرون من نفس الشهر لسنة 2018 . تم تشخيص العزلات البكتيرية بناءً على خصائصها المظهرية والكيموحيوية . أظهرت بياناتنا أنه تم تشخيص المكورات العنقودية Staphylococcus aureus في (11) 64.74 ٪ من مجموع العزلات ، بما في ذلك (8) 47.05 ٪ من المكورات العنقودية الحساسة للميثيسيلين ، (2) 11.7 ٪ من المكورات العنقودية الذهبية المقاومة للميثيسيلين ، (1) 5.88 ٪ كانت من المكورات العنقودية الجلديةStaphylococcus epidermidis ، بينما(6) 35.29 ٪ تم تشخيصها على أنها ميكروبات أخرى. اظهر اختبار الحساسية للمكورات العنقودية الذهبية انها كانت شديدة المقاومة للمضادات الحيوية التالية Amoxillin ، Penicillin G ، Cefotaxim ، Methicillin . تم تقييم مزيج من المضادات الحيوية مع نواتج المعززات الحيوية ضد البكتيريا المسببة للأمراض. أظهر مزيج Cefotaxim مع نواتج المعززات الحيوية نشاطًا أعلى ، مقارنةً باستخدام المضادات الحيوية وحدها ، ضد المكورات العنقودية الممرضة المعزولة المعزولة من أصابة الجروح .

تم إجراء اختبار التلازن أو التجمعCo-aggregation ولوحظ تلازناً قويا مع درجة (+4) بين العزلات السريرية والمعززات الحيوية المختبرة . تشير هذه النتائج إلى قدرة المعززات الحيوية على مُنافسة مسببات الأمراض على مكان التصاقها وتغذيتها وبالتالي تثبيط قدرتها على تكوين البايوفلم المرضي و إحداث الإصابة . بالإضافة إلى ذلك ، فإن العمليات الايضية للمعززات الحيوية أمتلكت تأثير مثبط بارز على المكورات الذهبيه الحاوية على البابوفلم و المقاومه للميثِسلين.

**الكلمات المفتاحية:** المكورات العنقودية الذهبية Staphylococcus aureus ، تشكيل بيوفيلم Biofilm formation ، مقاومة المضادات الحيوية Antibiotics resistance ، المعززات الحيويةprobiotics ،فحص التلازن Co-aggregation .

وزارة التعليم والبحث العلمي

جامعة ديالى – كلية العلوم

قسم علوم الحياة

الدراسة الصباحية

# تأثير بعض المعززات الحيوية على البايوفلم المرضي لبكتريا المكورات العنقودية الذهبية و البشروية المسببة لالتهاب الجروح

بحث تخرج مقدم إلى

مجلس قسم علوم الحياة / كلية العلوم / جامعة ديالى

وهو جزء من متطلبات نيل درجة البكالوريوس في علوم الحياة

من قبل

كرار جابر براك

حيدر صكر محمود علي هاشم عبد الأمير

بأشراف

د. عمار رياض قاسم

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