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# *Enterococcus faecium* BACTERIOCIN EFFLUX PUMP *MexA* GENE AND PROMOTE SKIN WOUND HEALING IN MICE

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**ABSTRACT**

The process involved isolating *E. faecium* from the gut of honeybees, screening the bacterium for bacteriocin-like inhibitory substance (BLIS), evaluating its impact on the expression of the *mexA* gene in multidrug-resistant (MDR) *P. aeruginosa*, and determining the role of bacteriocin in treating infected wounds in mice through histopathological examination. After evaluating the best circumstances for producing BLIS, it was discovered that glucose was a superior carbon source and yeast extract was the best source of nitrogen. The pH was found to be 5, the ideal incubation time was 72 hours, and ammonium sulfate salt was used for partial purification at 80% saturation. The identification of MDR *P. aeruginosa* isolates from pus infections was a further focus of the study. The VITEK 2 system was used to perform the identification. The results of antibiotic susceptibility tests revealed that the greatest resistance rates were found against Meropenem (83.3%) and Gentamicin (73.3%), followed by beta-lactam antibiotics (Ticarcillin, Ticarcillin/Clavulanic Acid, Piperacillin, and Aztreonam), which showed resistance in about 66.6 and 36.6% of the study isolates, respectively. Followed by Imipenem (63.3%), Ceftazidime (36.6%), and Cefepime (36.6%). The *mexA* gene was detected in all nine strains. The study also investigated the impact of the bacteriocin of the chosen strain on the expression of the *mexA* gene. An in vivo study revealed that wound healing was enhanced by treating infected wounds with *E. faecium* bacteriocin. Conclusion: Down-regulation and up-regulation in the expression of the genes following exposure to Bacteriocin indicate the potential of E. faecium as an effective antimicrobial agent against MDR *P. aeruginosa* infections.

**Keywords:** BLIS; pus; *mexA* gene; necrotic tissue; MRS Agar

## INTRODUCTION

The lactic acid bacteria (LAB) use carbohydrates as its primary product of carbon and it is classified as a Gram-positive microorganism. LAB bacteria are usually tolerating low pH levels (pH 5.5–5.8) **(George F *et al*., 2018)**. The primary LAB species found in bee products is a member of a unique LAB subclass known as fructophilic lactic acid bacteria (FLAB). One of the primary sources of FLAB strains is habitats that are high in fructose, such as the gut of honeybees and bee products **(Wang Y *et al*., 2021)**. They are a Gram-positive, catalase-negative and high tolerance for low pH **(Maeno *et al*., 2021)**. Probiotic microorganisms produce Bacteriostatic or bactericidal substances including lactic acid, hydrogen peroxide, lysozymes, and proteases these substances can cause membrane disruption (fatty acids or peptides), or enzyme inhibition, Additionally, a large number of LABs generate antibacterial peptides, such as bacteriocins **(Stephan *et al*., 2019)**. *Pseudomonas aeruginosa* is pathogenic bacteria that is characterized as Gram-negative, heterotrophic bacteria these bacteria are rod-shaped and they are motile. *P. aeruginosa* is non-spores forming. These bacteria can grow via both aerobic and anaerobic respiration **(Diggle and Whiteley, 2020)**. A multidrug-resistant pathogen can cause acute or chronic infections in immunocompromised people with cystic fibrosis, cancer, traumas, burns, and sepsis **(Jurado-Martín *et al*., 2021; Jangra and Chhillar, 2022)**. The efflux pump mechanisms are responsibility of removing antibiotics from cells, the *MexA* gene important multi-drug resistant efflux pumps gene **(Oumaima *et al*., 2020)**. For use in food technology, numerous recent studies have determined that novel antimicrobials that are effective against bacteria that are surface-fitting and resistant to multiple drugs are required. Both crude bacteriocin and bacteriocin are antibacterial proteins made by *P. aeruginosa* and MRSA **(Ahmed and Seddiq, 2018)**.
To test the lactobacilli that produce bacteriocin's antifungal activity against the yeast *Candida*. Additionally, ready-made Lactobacillus isolates and a number of commercial brands of probiotics were utilized **(Mohsin and Ali, 2021)**. Other antimicrobial activity niacin from *Lactobacillus spp* antimicrobial agents **(Abed *et al*., 2021)**.
Native drug resistance through the synthesis of bacterocin from various species o f bacteria and its application in all fields, particularly medicine **(Ahmed *et al*., 2023)**. This study will add to the body of knowledge on the use of live cells to treat bacterial skin infections by providing a foundation for future research on the use of *S. epidermidis* vital cells as probiotics. This research encourages the continued creation of novel, potentially effective anti-infective medications. According to these results, Enterococcus francium suspension may be used as a potent antimicrobial agent to treat P. aeruginosa infections that show drug resistance. The aim of this research is to detect the effect FLAB suspension in upregulation or downregulation of the efflux pump genes.

## MATERIAL AND METHODS

### Sample Collection

Twenty-five worker bees were collected during the summer foraging season and taken to the lab for testing. The bees were cleaned with 70% ethanol for 60 seconds to remove any external microbes. The worker bees were dissected in a laminar flow hood, the honey stomach and midgut were gently separated from the rest of the alimentary canal to be examined (**Zahedani and Jahantigh, 2021**)

### Isolation of *Enterococcus faecium*

Isolation was carried out with some modifications to (**Al-Ghamdi, 2018**) instructions. Each bee's nectar stomach was dissected using sterile forceps under laminar flow hood with sterile conditions. And culture on selective media MRS broth with anaerobically incubated for a period of two days at a temperature 30°C (**Leska and Moty, 2022**). Biochemical and morphological properties were adopted for bacterium identification **(Olofsson & Vásquez, 2008)**.

### Bacteriocin-like inhibitory substance (BLIS)

Culturing bacteria identification on MRS broth to obtain (BLIS) form *E. faecium* suspension was prepared with 0. 2% fructose, and the cultures were incubated anaerobically in a jar at 30°C for a period of 48 hours. Following incubation, the cultures were centrifuged at 6000 rpm for 10 min. The supernatant filtered with a pore size of 0.22 µm. Finally, the filtered suspension was stored at 4°C in (**Mourad, 2015**).

**Screening of (BLIS)**

After obtaining (BLIS) the antimicrobial activity assay by using the agar well diffusion method. Mueller Hinton agar plates were inoculated with 1.5 x $10^8$ (cell/mL) of *P. aeruginosa*. And BLIS of 100 µL of the supernatant was placed in the wells, which were 6 mm in diameter and cut with a cork borer. Then, the plates were incubated at 37 ºC for 28 hrs, and the presence of an inhibition zone was evaluated **(Ahmed & Kadhim, 2020).**

**Determination of Optimal Conditions for of Synthesis of BLIS**

After incubation, the antimicrobial activity of *E. faecium* was measured by performing an agar well diffusion assay under various conditions. Additionally, the protein concentration was determined using the Bradford assay method **(AL-Shimmary *et al*., 2020)**.

**Effect of Nitrogen Source and carbon source (sugars)**

Included (peptone water, yeast extract, Lactose, and glucose) respectively with concentrations were used,1% of each one of them added to BHI broth **(Muunim *et al*., 2019)**.

**Ammonium sulfate precipitation and dialysis**

The collected BLIS and optimim growth condation was then subjected to a partial purification step that involved infusing it with ammonium sulfate salt in the 80% saturation range while stirring continuously on ice. This was followed by an overnight incubation at 4 °C to precipitate proteins. The pellets were collected by centrifugation for 15 minutes at 4 C and 10,000 rpm. Crude bacteriocins were represented by the collected pellet. (**Yang *et al*., 2012**) Ammonium sulfate precipitate obtained from previous step was dialyzed in a dialysis tube with 3500 Mw cut off against potassiumphosphate buffer PH 7 for 24 h under cooling condition (4°C). And estimation of proteinconcentration (**Stupp and Paul, 1969**)

***Pseudomonas aeruginosa* Isolation**

Ten swab samples were collected from patients suffering from pus infections. Samples were cultured onto nutrient agar, cetrimide agar and MacConkey agar under sterile conditions. Finally, for confirming identification, VITEK-2 system was used **(MacFaddin, 2000).**

**Antibiotic Susceptibility Testing (AST)**

The antibiotic susceptibility test was confirmed by VITEK-2 using (AST- N222) card for *P. aeruginosa*. Susceptible and resistant interpretations were automatically recorded **(Ahmed, 2018)**

**Detection of *mexA* gene**

The tested genes amplification was performed by conventional PCR and the primer sequences was taken from (Tang, H *et al*., 2022). Using 20µl volumes containing 10µl of GoTaq Green Master Mix (2X), 1µl of primer (10pmol), 6µl of nuclease-free water, and 2µl of template DNA, the PCR amplifications were carried out. PCR cycling was conducted with a PCR Express (Thermal Cycler, Thermo Fisher Scientific, USA) using following program setting: 4-minute initial denaturation at a degree of 94°C for 30 sec, annealing at 55-65°C for a duration of 30 seconds, and extension at 72°C for a duration of 30 sec. The final extension step was performed at 72°C for a period of 7 min, followed by a 10-minute at 4°C to stop the reactions. Primers used are listed in Table 1.

**Table 1** Primers that were used in PCR and real-time PCR.

| Primer Name | Sequence 5\`-3\` | Annealing Temp. (°C) | Product size (bp) | |
|---|---|---|---|---|
| *MexA-F* | F: ACCTACGAGGCCGACTACCAGA | **60** | **252** | **(Pourakbari *et al.,* 2016)** |
| *MexA-R* | R: GTTGGTCACCAGGGCGCCTTC | | | |
| **Housekeeping gene (*fbp*)** | F: CCTACCTGTTGGTCTTCGACCCG<br>R: GCTGATGTTGTCGTGGGTGAGG | **55** | **35** | **(Kasoob and Hummadi, 2022)** |

**RNA Purification**

Following the TRIzolTM Reagent protocol, RNA was isolated from the sample using the following steps: (**Faiq and Ahmed, 2023**)

A-Sample lysis: In order to prepare the pellet cells, 1.4 milliliters of the cell culture were centrifuged for two minutes at 13,000 rpm, discarding the supernatant before adding 0.75 milliliters of TRIzol™ Reagent to the pellet. Pipetting the lysate up and down multiple times resulted in homogenization.

B- For RNA Precipitation**:**

1- The aqueous phase was mixed with 0.5 mL of isopropanol, incubated for 10 minutes, and then centrifuged for 10 minutes at 12,000 rpm.

2. The precipitation of total RNA resulted in a white pellet that resembled gel at the tube's bottom.

3. Then, the supernatant was thrown away.

C- For RNA Solubility: Pellet was rehydrated in 50µl of Nuclease Free Water and incubated for 10–15 minutes at 55–60°C in a water bath or heat block.

D- cDNA Synthesis: The cDNA was synthesized using the Protoscript cDNA Synthesis Kit. The steps involved in this procedure are as follows:

1. Each extracted total RNA sample was added in five microliters to a fresh PCR tube.
2. A protoscript reaction mix with 10ul of dNTPs, buffer, and other necessary ingredients added for every sample.
3. After that, 2 ul of MuLV Enzyme were added to each sample in the reaction.
4. An aliquot of two microliter oligoT was added, bringing the volume to 20 ul.
5. Using a thermocycler, this mixture was incubated for an hour at 42 degrees Celsius. The enzyme was then inactivated by heating the mixture to 80 degrees Celsius. Nanodrop was also used to quantify the cDNA product.

**RT-qPCR Protocol**

The cDNA quantification in real-time was conducted using the GoTaq® 1-Step RT-qPCR System (Promega, USA) and the SYBR green PCR master mix. Real-time PCR was employed to analyze the expression levels of the *MexA*. For assessing the gene expression of the *mexA* gene, *fbp* gene served as a housekeeping gene. was summarized in Table 2.

**Table 2** The components of master mix in qRT-PCR

| Master mix components | Unit | Volume/1 µL Sample |
|---|---|---|
| qPCR Master Mix | X | 5 |
| RT mix | X | 0.25 |
| MgCl2 | | 0.25 |
| Forward primer | µM | 0.5 |
| Reverse primer | µM | 0.5 |
| Nuclease Free Water | | 2.5 |
| RNA | ng/µL | 1 |
| Total volume | 10 µL | |

**Wound induction and treatment**

Twelve mice were exposed to injury on the dorsal area using 6 mm biopsy bunch instrument to resect full thickness wound and infected locally with one drop of *P. Aeruginosa* suspension (0.5x$10^6$ cfu/ml) and divided randomly into 2 groups. The first group (infected group) leaved without any treatment and the second group (treated group) treated with *E. francium* bacitracin. Three mice were euthanized from each group at 7 and 14 day post infection and skin samples from wounded area was kept in 10% buffered formalin solution for 24 hr and send to the histology sectioning unit at the University of Baghdad-College of Veterinary Medicine. All slides were stained with hematoxylin and eosin stain (**Luna, 1968**)

**Ethical Statement**

This research was approved by the Committee of Ethical Standards in the College of Science, University of Baghdad. The study protocol and the subject information and consent form were reviewed and approved by a local ethics committee according to the document number CSEC/1022/0131 dated October 22/2022.

**RESULTS**

**Identification of Bacterial Isolates**

The isolated *E. faecium* (1-10) were characterized as Gram-positive and appeared as large and white colonies on MRS agar (Figure 1).

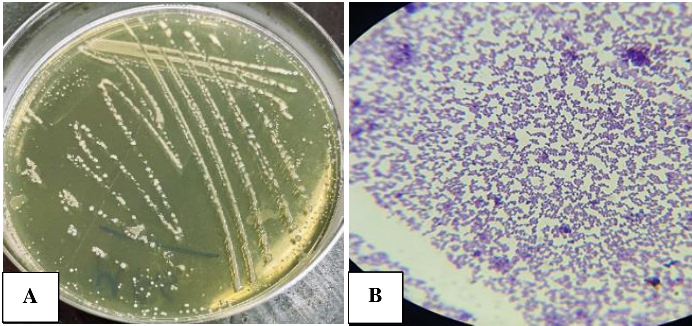


**Figure 1** *E. faecium* on (A) MRS Agar at 37°C for 24 hrs. (B) Gram staining.

The isolates of *P. aeruginosa* were identified. Figure (2) displays the colony morphology of *P. aeruginosa* isolates using selective media on Cetrimide agar. The *P. aeruginosa* isolates exhibited mucoid, smooth colonies with flat edges and raised centers. After 72 hours of incubation, these isolates demonstrated the ability to produce a distinctive blue-green pigment.

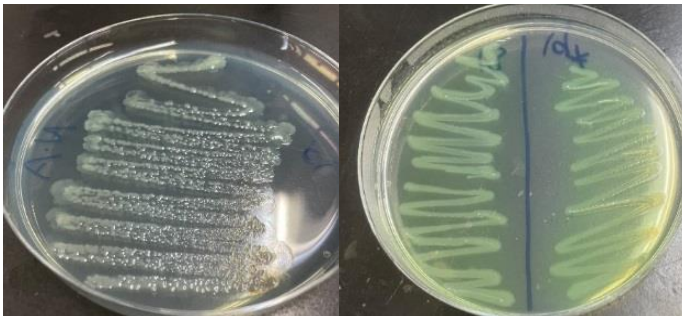

**Figure 2** *P. aeruginosa* culture media on cetrimide agar

**Screening of BLIS Activity**

*E. faecium* strain screening the best BLIS bacterocin crude antimicrobial activities' spectrum as each of them were having activity against multidrug resistant *P. aeruginosa* (Figure 3). Therefore, in addition, the results showed that antibacterial compounds were secreted into the extracellular environment during bacterial growth, as demonstrated by the clear zone observed with this method. The best activity of *E. faecium* was observed, and it was found to spread easily on a solid medium, resulting in inhibition diameters ranging from (15-20) mm for each isolate. The pH 5 of results suggest that was the best value for production when the inhibition zone's diameter (20) mm. Optimum nitrogen Source such yeast extract while glucose beast carbon source with dieter reached between (20- 21) mm. The beast isolation produced BLIS bacterocin with protein concertation 12 µg/ml.

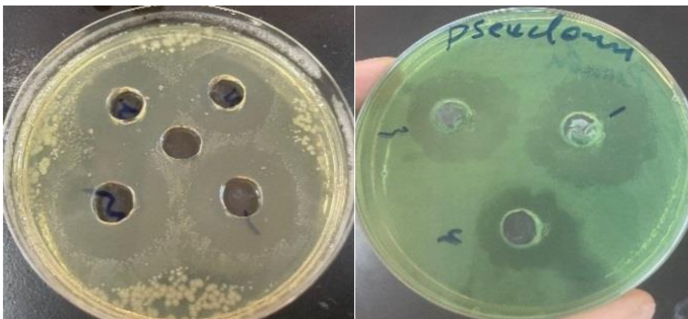

**Figure 3** Screening of *Bacteriocin* on *P. aeruginosa* on MHA anaerobic condition at 37°C.

**Antibiotics susceptibility test of *P. aeruginosa* isolates**

Antibiotic susceptibility tests were performed for isolates o by the VITEK-2 system using cards containing different antibiotics AST-N222. Results indicate variable resistance and sensitivity profiles among isolates against the antibiotics. The perverse study revealed that the greatest resistance rates found against Meropenem (83.3%) and Gentamicin (73.3%), followed by beta-lactam antibiotic A lower degree of resistance was seen toward Ciprofloxacin 10%. Nevertheless, a high level of sensitivity toward the most sensitive antibiotics were amikacin, tobramycin and colistin (100%) (Figure 4).

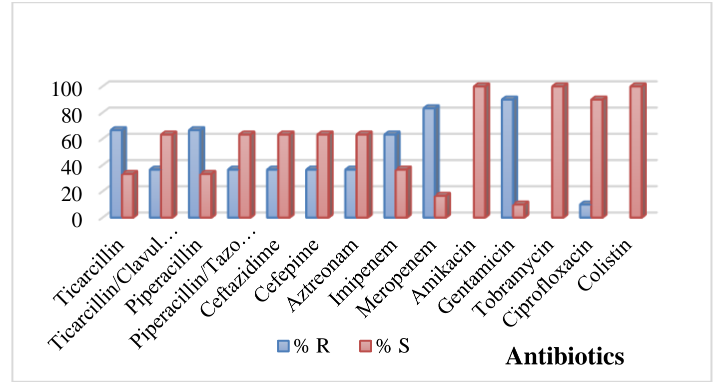


**Figure 4** Percentage of antibiotic susceptibility for *P. Aeruginosa*

**Amplification of *mexA* Gene**

The presence of *mexA* gene in five MDR *P. aeruginosa* isolates was evaluated before investigating the gene expression; the results demonstrated that the efflux pump *mexA* gene was present in all studied isolates. Gel (1.5% agarose) electrophoresis results showed the existence of a distinct and uninterrupted gene bands 252bp, 244bp and 100bp for *mexA*, which was clearly distinguished from the DNA ladder, as depicted in Figure (5). It is worth noting that there was no indication of DNA degradation, as evidenced by the absence of any smearing of the gene band.

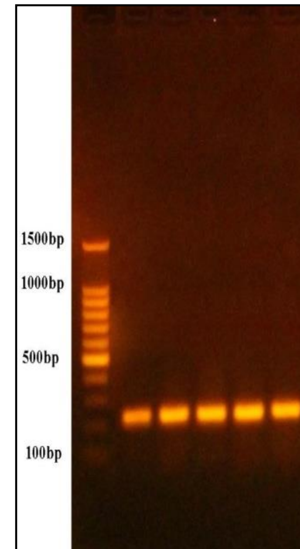


**Figure 5** Results of the amplification of *mexA* gene of *P. aeruginosa*. Bacterial DNA samples were fractionated on 1.5% agarose gel electrophoresis stained with ethidium bromide. M: 100bp ladder marker.

**Gene Expression**

The *MexA* efflux pumps gene was detected by PCR overall isolates that had shown to have the highest resistance towards antibiotics all five isolates a band. To estimate the impact BLIS *bacteriocin* of *E. faecium* at concentrations 12 µg/ml. The expression of *MexA* involving two of *P. aeruginosa* isolates was studied using the RT-qPCR technique. The results revealed a major down regulation in *MexA gene* expression after the exposure to bacteriocin suspension of *E. faecium* compared to normal gene expression in bacterial. The downregulation of the *MexA* gene expression in *P. aeruginosa* treated with bacteriocin suggests that this treatment may have a significant impact on the bacterium's multidrug resistance.

**Table 3** Mean (± SD) fold of change of *mex A* gene expression in *P. aeruginosa* Before and After treatment with bacteriocin.

| Gene Type | Mean ± SD Gene Expression Fold of Change | | Sig. | *p* value |
|---|---|---|---|---|
| | **Before** | **After** | | |
| *mex A* | 1.0 | 0.56 ± 0.04 | ** | <0.0001 |

NS: Non-significant, **: $p < 0.01$, SD: Standard Deviation

**Histopathological Evaluation**

At day 7 post infection the infected group showed severe destruction in the skin tissue with necrotic debris and loose collagen fibers (Figure 6a) with severe

hemorrhage in the dermis and subcutaneous tissue, in addition present of abscess in the subcutaneous tissue (Figure 6b), while at 14 day post infection healed skin incomplete regeneration of the epidermal layer under the necrotic tissue (Figure 7a) and thin layer of regenerated epithelia with papillary like projection revealed to epithelia hyperplasia (Figure 7b).

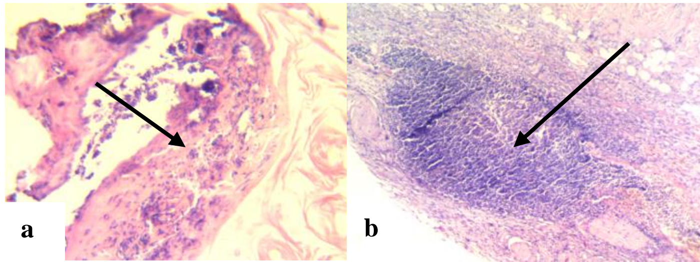


**Figure 6** Infected group after 7 days showed (a) necrotic tissue (arrow) replaced the epithelial layer (400x). (b) large abscess (arrow) in the subcutaneous region (100x).

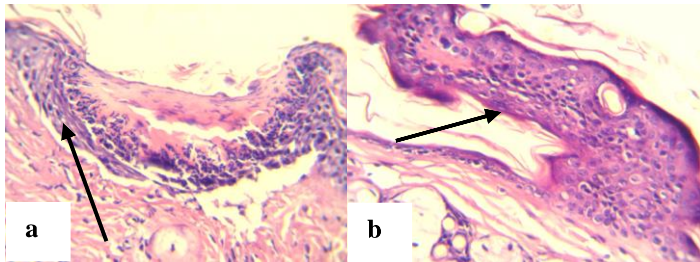


**Figure 7** Infected group after 14 days post injury showed (a) incomplete regeneration of the epidermal layer (arrow) the necrotic tissue (200x). (b) thin layer of regenerated epithelia with papillary like projection (arrow) revealed to epithelia hyperplasia (200x).

The group treated with bacteriocins at day 7 post infection showed incomplete regeneration of the epidermal epithelia of necrotic skin with proliferation of irregular collagen fiber and newly blood vessels formation in the dermis (Figure 8a) in addition to large abscess in the subcutaneous tissue (Figure 8b). At 14 day the skin showed complete regeneration of dermal epithelia under necrotic tissue with irregular collagen fiber proliferated in the dermis layer (Figure 9a) with focal sub epidermal aggregation of MNCs (Figure 9b).

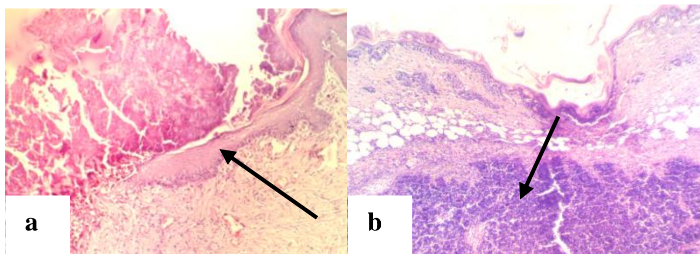


**Figure 8** Treated group 7-day post infection showed (a) incomplete regenerated epithelia (arrow) of the epidermis under the necrotic tissue with proliferation of irregular collagen fiber and newly blood vessels formation in the dermis (100x). (b) large abscess (arrow) in the subcutaneous tissue (100x).

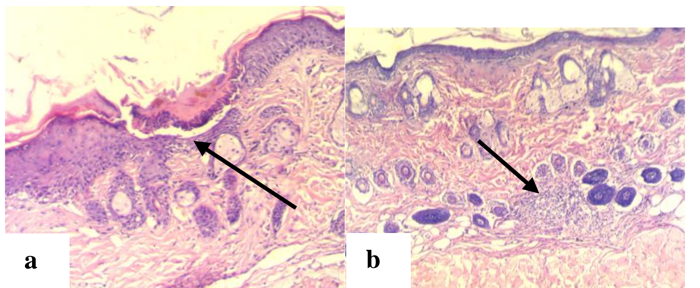


**Figure 9** Treated group 14-day post infection showed (a) complete regeneration of epidermal epithelia (arrow) under necrotic tissue with irregular collagen fiber proliferated in the dermis layer (100x). (b) focal sub epidermal aggregation of MNCs (arrow) (100x).

## DISCUSSION

Carbohydrates are necessary nutrition for microorganisms to grow. Bacteria can take in and utilize a variety of carbohydrates as building blocks for their synthesis, including supplies of nitrogen for maximum growth and stimulated synthesis. Different nitrogen sources have different effects on BLIS activity depending on their kinds and concentrations in the medium. (**Nasser and Abdulrazaq, 2022**). *Actobacillus* isolates were obtained from vaginal samples and put through a screening program to find out how well they worked as antagonists against *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumonia*, and *Proteus spp*. isolates were chosen from the first round of screening. (**Tareq and Luti, 2022**).

Using the technique outlined by Hiba et al., the antibacterial activity of many species of *L. crispatus* cells within gel formula preparation was examined against *Pseudomonas aeruginosa* and *E. coli* (**Rasheed and Alaubydi, 2020**). Alternative research when compared to other methods, the well diffusion assay (WDA) approach yields more pure bacteriocin (MRSAcin) when used against resistance P. acnes because of its broad inhibitory zone (**Muna *et al*., 2018**).

The results were consistent with (**Elzeini, 2021**) in which FLAB was isolated from the digestive system of bee. All isolates were confirmed and diagnosed using the VITEK-2 system from the 10 clinical isolates named (P1-P10). The results of the VITEK-2, probability of about 95%-99% belong to the genus *Pseudomonas aeruginosa* and compares with CLSI to detection antibiotic sensitivity test according (**Al-Awadi and Alwan, 2014).** The other result agrees with **Ahmed *et al*., (2022)** where antimicrobial activity of bacteriocin Bacillin and S-Pyocin antimicrobial activity production *P. aeruginosa* antifungal activity were reported According to the results which correlate with (**Sara *et al*., 2023**) the antimicrobial activity sliver nanoparticles as antimicrobial agent and optimization on different condition, while different incubation periods, the production of bacteriocin from MRSA produce was observed. It was noticed that the highest production appeared at 72 hours, which produced the largest inhibition zone, measuring 20 mm. However, after 48 and 17 hours, bacteriocin activity decreased, with the lowest inhibition zone measuring 10 mm. However, another study that uses bacteriocin looks at how salmonella spp. bacteria isolated from various food products may be used to produce bacteriocin, and how crude bacteriocin works against gram positive and negative bacterial isolates from tap water in various parts of Iraq's Basra city (**Ahmed *et al*., 2022**). Furthermore a collection of substances used in medicine to treat *L. tropica* and *L. donvani* On *L. tropica* promastigotes, staphylococcin derived from staphylococcus aureus exhibited inhibitory action (**Ahmed *et al*., 2018**). These results are consistent with (**Ahmed & AL-Shimmary, 2018**), the observations found the highest activity from *E. faecalis* was observed after a three-day incubation period.

Many factors underlie their antimicrobial activity, including the fact that, in contrast to antibiotics, they contain distinct functional groups and that, as a result, bacteria are less resistant to bacteriocin and plant antimicrobials (**Ahmed *et al*., 2021**).

The agar well diffusion assay was used to measure the antibacterial activities of the *bacteriocin*, which produced a 23 mm inhibition zone diameter at a pH of 5 (**Kasimin *et al.*, 2022**). Other findings that are consistent with this one demonstrates that FLAB strains were isolated from bees and chosen for their strong antimicrobial action against pathogenic bacteria and the optimum condition was at pH 8, the lowest inhibition zone is 15.12 mm (**Phumisantiphong *et al*., 2017**). On other hand result agree with (**Lepecka, *et al*., 2021**) with the antimicrobial activity of both CFS and crude enterocin was constant at pH levels between 4 and 8. With different incubation times, the production of bacteriocin was observed. It was noticed that the highest production appeared at 72 hours, which produced the largest inhibition zone, measuring 20 mm. But after 48 and 17 hours, bacteriocin activity decreased, with the lowest inhibition zone measuring 10 mm. The result agrees with (**Kishk, R.M., *et al*, 2020).** Antibiotics susceptibility test of *P. aeruginosa* isolates showed greatest resistance rates against Meropenem and Gentamicin followed by beta-lactam antibiotic Clavulanic Acid. Efflux pump genes such as *mexA* play a crucial role in removing antibiotics from bacterial cells, thereby conferring resistance to antibiotics (**Swade *et al*., 2022**). Hence, the downregulation of this gene will lead to a decrease in the efflux pump's activity, which in turn could increase the susceptibility of *P. aeruginosa* to antibiotics. The results reveals that infections caused by multidrug-resistant bacteria may be treated with bacteriocin. The effectiveness and safety of this treatment approach the results of the effect of *bacteriocin E. faecium* on the gene expression of virulence gene in *P. aeruginosa* is unique and preformed for the first time locally. The slow healing process in infected group may be related to *P. aeruginosa* which cause suppurative reaction **(Ahmed & Al-Awadi, 2020**). Since neutrophils are major inflammatory cells deal with *Pseudomonas* clearance (**Heilbronner *et al.*, 2021**), this bacterium may protect itself by their lipopolysaccharide and hide from the immune system inside neutrophils which keep recruitment and form abscess **(Ahmed *et al*., 2018)**. In addition, necrotic lesions may contribute with *P. aeruginosa* virulence factors such as phospholipase C, exotoxin A which has necrotizing effects at bacterial colonization site as a part of the process of colonization (**Harbi and Al-Awadi, 2021**).

In this study, skin treated with bacteriocin showed butter healing than infected group and this may be due to one of the possible bacteriocin activity against other bacteria such as pore formation in the cell membrane, and impaired the synthesis of cell wall or impaired the replication and translation of nucleic acid (**Heilbronner *et al*., 2021**). A cream formula contained the bacteriocin was prepared which already examined *in vitro* and *in vivo* Results established that treatment at the onset time was more effective and the time of healing was

decreased (**Rasheed *et al.*, 2020**). These bacteria have been proven to affect life in inhibiting the expression of vital virulence factors, including *mexA* gene expression for the efflux pump, in *P. aeruginosa.* This inhibitory effect on efflux pump gene expression may represent a potential strategy for controlling *P. aeruginosa* infections.

## CONCLUSION

Development of new potential anti-infective drugs these findings suggest that B has BLIS he potential to serve as an effective antimicrobial agent against *P. aeruginosa* infections that display resistance to multiple drugs. These bacteria have been proven to be effective in inhibiting the expression of vital virulence factors, including the *MexA* gene expression for the efflux pump, in *P. aeruginosa.* This inhibitory effect on efflux pump gene expression may represent a potential strategy for controlling *P. aeruginosa* infections.

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**Data Availability:** The datasets generated during and/or analyzed during this study are available from the corresponding author upon reasonable request.

**Code availability:** Not applicable.

**Conflict of interest:** The authors have no competing interests to declare that are relevant to the content of this article.

**Author Contribution:** Mais Emad Ahmed carried out the experiment and wrote the manuscript with input from another authors. Ahmed Qassim Al-Awadi supervise the project and conceived the original idea designed the model and the computational framework and analysed the data.

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