Probiotic Inhibit Biofilm Formation for Acinetobacter Baumannii

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Abstract

Acinetobacter baumannii bacteria have been classified as the most problematic in hospitals, and they need to be researched and developed significantly new antibiotics by the World Health Organization (WHO), and as an urgent public health threat by the Centers for Disease Control and Prevention (CDC). In this study, twenty-five isolates (20.83%) of these bacteria were isolated from a total of 120 samples collected from various clinical sources (burns, blood, wounds, sputum) from patients with various medical injuries from Baquba Teaching Hospital in Diyala Province for the period from (November 1, 2021 to February 1, 2022). According to the physical and microscopic diagnosis and the use of VITEK2 and molecular detection, all diagnosed isolates are A. baumannii bacteria. All MDR insulation showed complete resistance to the Colistin antibiotic. The results of the appearance detection of some factors of virulence showed that most isolates have the ability to adhere to epithelial cells and the formation of the biofilm and the formation of the biofilm is associated with resistance to antibiotic isolates (MDR) and on this basis studied (10) clinical isolates and their composition ability of the biofilm and to different degrees were seven very strong isolates and by (70%), 1 (10%) medium formation and two weakly formed isolates of the biofilm so took the ratio (20%). The susceptibility of isolates to the formation of gelatinase was high by (67%), which mean that (19) isolates was formed of gelatinase, while the enzyme lipase was 22) isolated and 88% produced by this enzyme, while the appearance detection of the capsule was the result 1 (4%). The results of the molecular detection of the gene (Bab) showed the presence of one isolate carrying this gene and a ratio of (10%). RT-PCR technology was used to measure the gene expression of the gene studied (Bab) by extracting DNA (RNA) for A.baumannii bacteria before and after treatment with probiotic, one bacterial isolates of A.baumannii was treated with two types of probiotic Lactobacillus plantarum, Lactobacillus acidophilus and note how much they affect gene expression when MIC was used (for bacteriophage CFS at a concentration of 12.5 µg/mL) the results showed an effect of Lactobacillus plantarum bacteria on the gene expression of A. baumannii causing full inhibition of Bap gene when treated LP.

Keywords: Probiotic, Bap gene, Acinetobacter bumannii, Lactobacillus plantarum

1. Introduction

Acinetobacter baumannii bacteria are negative for spherical gram stains with a compulsory anaerobic fermentation that grows at temperatures ranging from 37°C to 44°C and Coccobacillus bacillus. Because of their wide tolerance, these temperatures are widespread in different environments [1]. These bacteria have multiple virulent factors that have enabled them to resist various environmental conditions and resist antibiotics, including the formation of biofilms, antibiotic resistance, heavy metals and capsule formation, making them more dangerous to cause injury and spread in different environments [2, 3]. The ability to form biofilm is one of the most important factors of ferocity of A.baumannii bacteria, namely the formation of multi-layered bacterial communities coated exceptionally resistant to antibiotics, which play an important role in the survival of bacteria and the spread of infections [4, 5].

To inhibit the ability of bacteria to form virulent agents and their resistance to antibiotics, probiotics have been used in recent years in several areas, including their use as antimicrobial antibiotics, and Lactobacillus is one of the most common types of probiotics [6].

WHO has identified them as microorganisms, when given in sufficient quantities, give health benefits to host [7]? Lactobacillus bacteria are characterized by their ability to form antimicrobial bacteriocin and their activity against pathogenic microbes [8].

Lactobacillus bacteria also produce many antimicrobial peptides, organic acid formation and other substances [9]. Lactobacillus plantarum is a valuable type for the development of probiotics, utilizing it for bowel health, metabolic disorders, brain health [10].

These bacteria are also important for regulating human health and immune systems, lowering cholesterol levels, maintaining intestinal flora balance, and reducing the risk of tumors, where Lactobacillus plantarum bacteria produce lactic acid, bioactive antimicrobial compounds and many external saccharide to express the hostile ability against the activity of food-borne pathogen. Lactobacillus acidophilus bacteria is the second most important type of use as a probiotic, capable of producing antimicrobial metabolic materials capable of controlling or inhibiting bacteria (kill or inhibition) by producing organic acids, including mainly lactic acid production, hydrogen peroxide and production of antimicrobial proteins (bactriosinates) [8].

In our current study, our aim is to find out the effect of probiotic bacteria, especially Lactobacillus plantarum and Lactobacillus acidophilus on the effectiveness of some of the resistant genes and the tolerance found in the A.baumannii bacteria.

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2. Materials and Methods

Samples collecting

120 samples were collected from various sources, including blood, wounds, burns and sputum, from Baquba Teaching Hospital and advisory clinics in Diyala province, Republic of Iraq. Samples were collected from patients who were lying and not lying in hospital from 1st of November 2020 to the beginning of February, 2022 for both gender and at different ages.

A total of 25 isolates were obtained from A. baumannii bacteria, including 12 isolates from burns, 8 were taken from the blood, 4 isolates were obtained from wounds, and one isolate from the sputum.

Isolates diagnosis

All isolates were diagnosed with microscopy, cultural diagnosis, and biochemical tests. The effect probiotic (Lactobacillus plantarum, Lactobacillus acidophilus) was studied on Bap gene expression f using RT-PCR technology.

Biofilm formation By Microtitre plates method

According to Stepanović et al. [11], Tang et al. [12] in detecting the composition of the biofilm in bacterial isolates using the microtitre method.

DNA extraction

DNA extracted for A.baumanni insulation as reported from the manufacturer's instructions and using the Genomic DNA extraction kit, which was prepared by the American company USA Promega for DNA extraction of negative bacteria.

PCR test and electrophoresis

After DNA extraction of A.baumannii bacteria, Bab gene, was investigated and the presence of this gene was detected as an important factor in the severity of A.baumannii. The final size of the PCR 20 microliter all the components of the mixture were mixed together in the Ebbendorf tube by vortex. The components were placed in the thermal cycle system, which was programmed for each starter individually depending on its source and as shown in the table (1). After that, 5 microliters were taken from the multiplier output and transferred to the agarose gel for electrophoresis.

Table (1): PCR interaction program used in the study.

N.Cycle	Extension	Annealing	Denaturation	Genes
30	72 °C/ 40 sec	55 °C/35 sec	95 °C/30 sec	16SrRNA
30	72 °C /60 sec	55 °C /60 sec	95 °C/30 sec	Bab

Bacterial RNA extraction

The ribosomic RNA of bacterial insulation was extracted in accordance with the manufacturer's instructions and using the Genomic RNA extraction kit prepared by Promega-USA for extraction RNA negative bacteria.

Gene expression

Real time PCR was used in gene expression experiments of Bab gene and 16SrRNA was used as a control housekeeping gene source, and one isolate was chosen further after it was proven to have a Bap gene through PCR technology, after it was exposed to MIC concentration of probiotics.

RNA was extracted before and after the treatment with probiotics, and then several (One Step qRT-PCR) were used for the purpose of testing gene expression.

The components of the interaction were mixed in table (4) using a micro pipette and then centrifuged for one minute, after which the tubes were transferred to an RT-PCR device and programmed according to the optimal conditions of each gene as in table 5, the process was repeated twice for one isolate and each material according to the amount of expression obtained by each gene according to the following equations:

Folding = 2-ΔΔCt, ΔΔCT= ΔCT treated - ΔCT control

CT Control = CT (before treatment) – CT 16SrRNA Δ

CT = CT target gen – CT 16SrRNAΔ (Rao et al., 2013).

Table (2): Promoters sequences used in the study and the expected output size of each promoter.

Source	Size bp))	MT	Promoter sequences	Name of gene
Higgins et al.(2004)	150	55°C	F: CAG CTC GTG TCG TGA GAT GT R: CGT AAG GGCCAT GAT GAC TT	16SrRNA
Badmasti et al. (2016)	1449	55 °C	F: ATG CCT GAG ATA CAA ATT AT R: GTC AAT CGT AAA GGT AAC G	Bap

Table (3): Components of Real Time PCR reaction.

The final size of one sample (10 µm)	Name of materials
5µ	q PCR Master Mix)SYBR)
0.25µ	RT Mix
0.25µ	MgCl2
0.5µ	Forward primer
0.5µ	Rever primer
1µ	RNA
2.5	Nuclease Free Water

Table (4): Programming in-Step Qantatifi Art-Qabkar

Cycle	Time	Temperature	Step
1	15 mins	37 °C	RT.Enzyme Activation
	5 mins	95 °C	Initial Denaturation
40	20 sec	95 °C	Denaturation
	60sec	55 °C	Annealing: 16SrRNA

		55 °C	Bab
	20 sec	72 °C	Extension
1	07:00	72 °C	Final extension

3. Results and Discussion

Microscopic diagnosis of A. baumannii bacteria

Microscopy showed that (25) isolates from A.baumannii bacteria were negative for the gram stains, generally shaped by bi-cocci shaped like bacilli [13].

Morphological detection

The morphological detection was detected by culturing the 25 isolates on the medium of the MacConkey and showed small forms of white mucous not fermented for lactose sugar, but on the medium of the blood agar were pale pink shapes that are not analyzed for blood [14]. All isolated was grow at 44°C and showed that these isolates are able to grow in these grades because they are a characteristic of A. baumannii bacteria than other species [13].

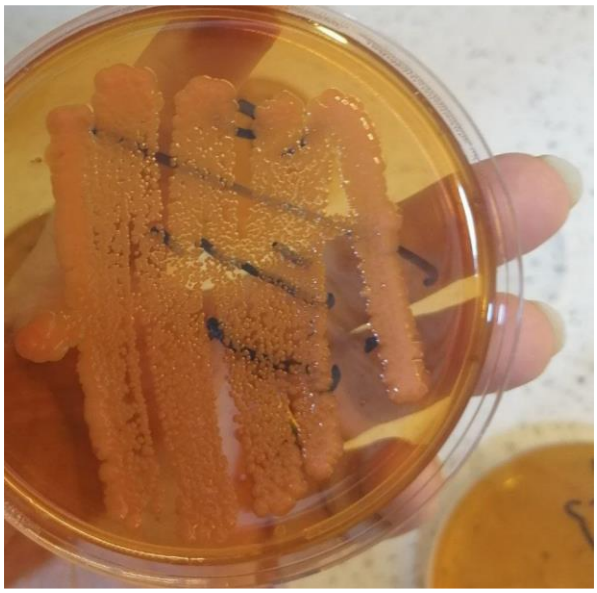

Figure (1): Shows the shape of the colonies of A.baumannii bacteria on the medium of MacConkey.

Diagnosis based on biochemical tests

All isolates gave mixed results for the lipase test, and negative results for the urease test for not transform the medium, which confirms that the bacteria are not urea-producing and mixed results of gelatinase tests included (84%) positive for testing and (16%) non-gelatinase-producing. While oxidase test was negative, and not producing hydrogen sulfide gas, these results matched many studies, including Al-Musawi [15].

Detection of the susceptibility of A.baumannii and formation

MTP method was used to detect the production of the biofilm of A.baumannii bacteria and to identify biofilm levels by using a wavelength (630) nm optical spectrometer to calculate the visual density resulting from the adhesion of bacteria cells to the walls of the microtiter plate.

The results of our study showed that all A.baumannii isolates are able to form biofilms but at different levels. The results of the ELAESA reading device were very strong (70%), (10%) medium, (20%) weak composition of biofilm as shown and this classification was explained according to table (5) [12]. There was no isolate in the absence of biofilm because the formation of biofilm is a characteristic of A. baumannii bacteria and is an important factor in which bacteria can resist conditions in hospital environments. The isolates studied were (10) MDR and the formation of the biofilm is linked to the fact that isolation is MDR. According to the study by Al-Shamiri et al. [16], MDR is an important factor contributing to the chronic infection of A. baumannii bacteria and the formation of biofilm on living and non-living surfaces [17]. The study conducted in Mexico by Avila-Novoa et al. [18] found that (86.6%) of the isolates made up of biofilm by a weak percentage, and that (13.3%) is not composed of it and thus the study conducted with our current study varies.

Due to the difference in the percentages of the composition of biofilms due to multiple factors, the most important of which is the difference in temperature and pH, interference with other micro-organisms at the site of injury, nature of the nutritious medium in terms of the presence of sucrose, calcium and phosphate, type of nutrient surface charge and chemical composition as well as the use of disinfectants [19].

The ability to form biofilm may affect antibiotic sensitivity even when the dose given is within the sensitive range [20].

Table (5): A.baumanni bacteria are classified according to the strength of biofilm formation.

Non-biofilm	OD ≤ ODc
Moderately biofilm producer	ODc < OD ≤ 2 x ODc
Strong biofilm producer	2xODc<OD

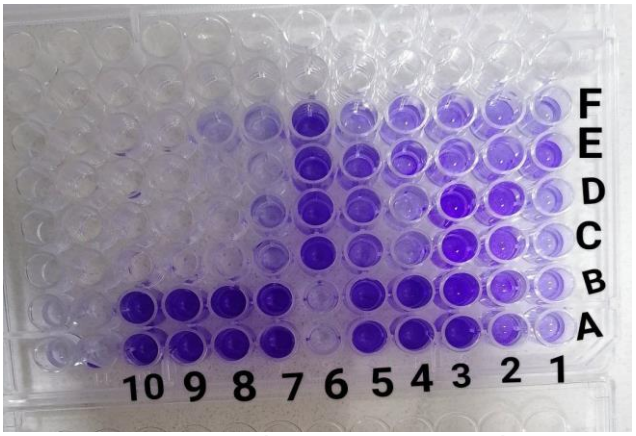

Figure (2): Testing biofilms in the method of microtitre plate, color concentration represents the production force of biofilms (the greater the concentration of color the stronger the concentration of biofilm).

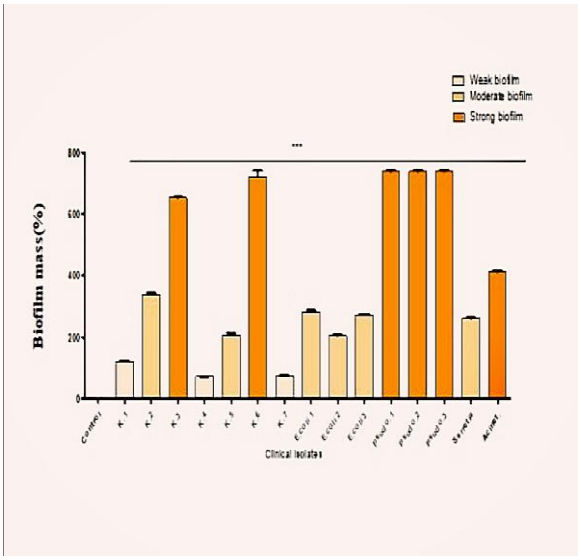

Figure (3): Chart showing levels of biofilm formation of A.baumannii insolates.

Molecular detection of Bab gene

To detect Bap virulence gene in A.baumannii bacteria, technique (PCR) has been used. The bundle that form by molecular size (1500) base pair compared to the volumetric index used through the use of a specialized starter to detect its presence in (Figure 5).

Molecular detection results showed that 1 (10%) was a carrier of the Bap gene, which coded the protein associated with the formation of the biofilm (Bap), which mediates the formation of biofilm in these bacteria as the presence of this protein is important in the process of adhesion cells to surfaces and medical materials consisting of polystyrene [19].

This protein also reduces the spread of antibiotics among living cells, causing biofilm formation and thus resistance to isolates to harsh environmental conditions such as the hospital environment [21].

The formation of polysaccharide genes does not require the presence of algC gene [22]. The different composition of the biofilm at multiple levels within the morphological study and the low presence of the Bap gene are due to the presence of other genes involved in biofilm formation, including OmpA, csuE, blaPER [23].

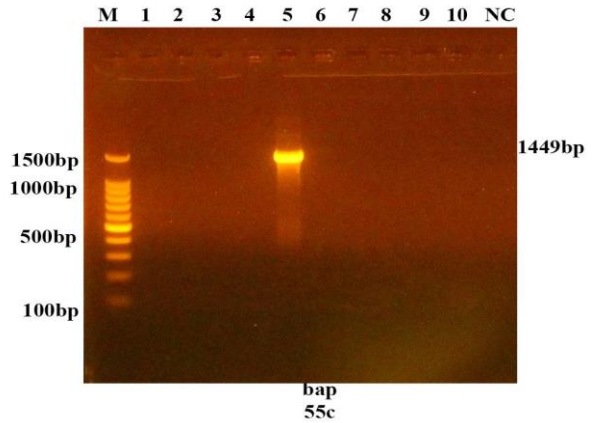

Figure (4): Results of the electrophoresis of Bap gene on the agarose gel 2% using ethidium bromide stain track M represents the volumetric index.

Inhibition by probiotic

Use two types of probiotics, which are commensal bacteria in the intestinal mucosa, and the effect of these bacteria on one isolate of the bacteria A.baumannii. These two types of Lactobacillus were suggested because of their ability to reduce pH, reduce inflammation and their ability to release antimicrobial compounds [24]. A series of probiotics (Lactobacillus plantarum, Lactobacillus acidophilus) have been made.

The method of bilateral dilution was used in the nutrient medium and the results of the study found that the average value of the minimum inhibitory concentration MIC for probiotics was 12.5 microgram/milliliter.

The results showed Lactobacillus plantarum has a high inhibition capacity for Bap, especially when used at a concentration of (12.5) µg/milliliter where biofilm did not appear in the reaction chart (RT-PCR) of the treatment bacteria when tested in the 16SrRNA gene, which is the gene detecting A.baumannii bacteria, confirming the presence of bacteria and the absence of biofilm (Figure 7). Probiotics provides an effective strategy for disease resistance. Probiotic bacteria also play an important role in the balance of host energy, as well as effectively modify intestinal bacteria [25].

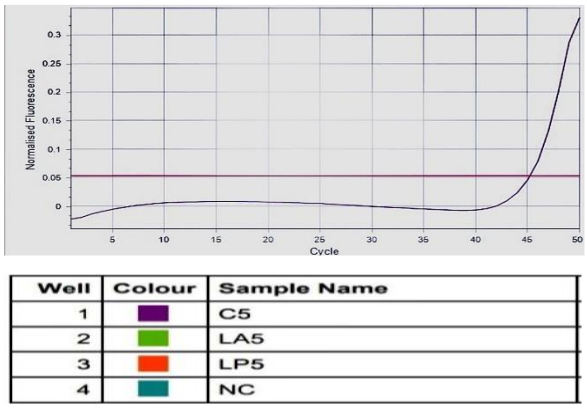

Figure (5): RT-PCR reaction chart demonstrates the complete inhibition of L.plantarum bacteria, L.acidophilus on the gene expression of Bap gene.

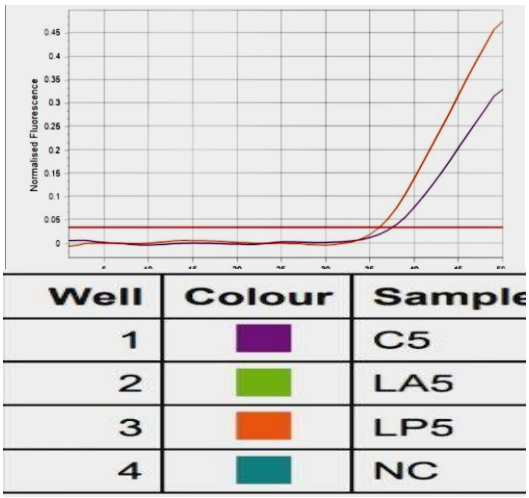

Figure (6): RT-PCR interaction chart shows detection of the presence of a 16SrRNA gene.

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