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Determining the ability of mannoprotein extracted from *Saccharomyces cerevisiae* to cure some virulence factor genes of some species of pathogenic bacteria isolated from different sources

Ashwaq Muthanna Wahib[1], Mohammed Nadhir Maaroof[2]

[1]*Ph.D. Student College of Education for Pure Science, University of Tikrit, Iraq*

[2]*College of Education for Pure Science, University of Tikrit, Iraq*

[1]*mm252930@gmail.com* [2]*dr.mohammed78@tu.edu.iq*

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Abstract

Background: Bacterial diseases play a major role in wound and burn infections and urinary tract infections, which lead to wound and burn contamination due to the change in the host defense mechanism. **Objectives**: Discovery of the ability of mannoprotein to cure the virulence factors genes of the causative agent of wound infections, burns, and urinary tract infections at the molecular level. **Materials and methods**: One hundred samples were collected from different sources of infections, and then cultured on blood agar, MacConkey agar, and mannitol agar. Bacterial isolates were identified using biochemical tests and molecular detection of these factors using specific primers - *ndvB, rsmA, fimH, ureR, ureD, and hlb*. **Results**: The *ureR, rsmA, fimH, ureD, ndvB, and hlb* genes were found in all isolates (100%). After the samples were treated with the first and second mannoprotein extracted from Saccharomyces cerevisiae, the genes responsible for the production of the virulence factors biofilm, Hemolysin, and urease were curing from appearing and the production of virulence factors was inhibited because the DNA was destroyed which indicates that Saccharomyces cerevisiae is one of the beneficial substances for the body's immunity against bacterial diseases**. Conclusion**: A difference was observed in the gene expression of the genes responsible for the production of biofilm, Hemolysin, and urease virulence factors in the bacterial isolates before and after treatment with mannoprotein extraction.

Keywords: mannoprotein, Gene expression, virulence factors, PCR, Curing gene.

Introduction:

Burns are known as fire that causes strong layers and damage to the skin and dermis tissues as a result of radiation, electron current or hot chemicals, in addition to hot gases, liquids, and vapors, which are among the most common factors that cause burns(1). Although burn care has evolved over the past decades, burn infection remains the leading cause of death among this group of patients (2). Wounds are defined as a break in the skin or mucous membrane, which facilitates the entry of microbes and bacteria, leading to infection (3). Serious wound infections lead to death or morbidity on a global scale, as multiple bacterial infections and colonization by more than one type of bacteria can cause damage to the affected tissues. The main problem in wound infections is the continuous increase in bacterial resistance to antibiotics (4). Wound injuries, especially surgical wounds, are among the most dangerous and important causes that lead to a high

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mortality rate among patients. Therefore, when damage occurs to the skin surface due to an external factor, the internal tissues will be in contact with the external environment and without protection, which provides a moist, warm, and nutrient-rich environment, which leads to the invasion of tissues by *Pseudomonas aeruginosa* bacteria. It also can invade wound surfaces by forming biofilm (5). Urinary tract infections are caused by microbial colonization and proliferation in the urinary tract (6,7), Urinary tract infection is caused by the presence of some types of pathogenic bacteria in it. It is one of the most common infections in society, ranking second after respiratory tract infection (8). These bacteria contain several virulence factors that have been responsible for causing urinary tract infections (9,10). Virulence factors are specialized structures or specific enzymes produced by pathogenic bacteria to promote their spread, contributing to their successful presentation and colonization within their host cells and thus contributing to infection (11,12). These factors are encoded by genes carried on mobile genetic elements such as plasmids, transposable elements, and phages, or carried at locations on the bacterial chromosome called pathogenicity islands (PAIS) (13). Bacteria contain several types of virulence factors such as Biofilms, Urease, and Hemolysin enzymes.

Materials and Methods

1- Culture media

1-1 The commercially prepared culture

MacConkey agar, Nutrient Agar, Mannitol Salt Agar, Sabrouraud dextrose Agar, and Brain heart infusion broth were used, as the media were prepared, and pH values were adjusted and sterilized as per the manufacturer's instructions.

1-2: Culture media prepared in the laboratory

Blood agar medium

It was prepared by adding sterilized sheep blood with a concentration of (5% v/v) to the prepared blood agar base medium according to the manufacturer's instructions (14).

Urea agar Medium

It was prepared by dissolving (2.1 g) of urea base in (95 ml) of distilled water, then sterilized by autoclave and cooling the medium after the completion of sterilization, then added (5 ml) of urea solution prepared at a concentration of 40% and sterilized by filtering, and distributed the medium on sterile test tubes It is sealed and kept in a slanted form until use (14).

2- Specimen Collection and Transportation

One hundred samples were collected from different infections (burns, wounds, and urinary tract infections) from patients at Baquba Teaching Hospital and Muqdadiyah General Hospital, Diyala, Iraq, for the period from 20/8/2022 to 20/1/2023. Samples were transported by a cork-lined box containing ice to the Microbiology Unit, Agriculture Laboratory.

3-Culturing and Identification Procedure

Samples were cultured on blood agar, MacConkey agar, and mannitol agar. Samples were incubated at 37°C for 24 h in an aerobic environment. Bacterial isolates were identified by imvic biochemical tests.

4-Virulence factors.

4-1 Phenotypic detection of virulence factors of isolated bacterial species.

The phenotypic detection of virulence factors for only Gram-negative isolated bacteria

Biofilm Formation

The tube method (TM) was used, which is based on direct observation of biofilms after growing one fresh colony from a fresh culture on (10ml) brain heart infusion broth (BHIB) and cultured for 24 hours at 37°C to measure the quality of biofilm formation. The tubes were emptied and washed with buffered saline to remove non-adherent cells. Crystal violet (0.1%) was used to dye the dried tubes. Tubes were cleansed with deionized water after the extra stain was removed, and the tubes were checked for the formation of biofilms. Positive biofilm formation was defined as the presence of a

discernible coating on the tube's bottom and wall. The experiments were carried out three times in triplicate (15).

Urease production

Urea agar medium was inoculated with a fresh colony of bacteria to be tested, then incubated at a temperature of 37 °C for 24 hours, the positive result was indicated by changing the color of the medium from yellow to pink (16).

Hemolysin Production

The ability of bacterial isolates to produce Hemolysin Enzyme was detected by planting the bacterial isolates by the plotting method on the surface of solid blood agar medium, then incubating them at a temperature of 37°C for 24 hours, after which the bacterial colonies were examined to determine the type of hemolysis (17).

Detection of virulence factor production after treatment with mannoprotein

The production of virulence factors by pathogenic bacterial isolates was detected after treatment with mannoproteins such as urease, biofilm factor, and hemolysin.

5- Activation and growth of *Saccharomyces cerevisiae* isolates:

Dry yeast isolates were activated and grown according to (18), by taking 1 g of yeast powder and inoculating it in 10 ml of liquid Sabouraud dextrose broth medium and incubating it at 30°C for half an hour. After the incubation period was over, it was inoculated on solid Sabouraud dextrose medium and then incubated for 48 hours at 30°C.

5-1 production Mannoprotein:

Yeast isolates were activated on Sabouraud dextrose liquid medium, mixed well, and incubated at 30°C in a shaking incubator for 4 days (19).

4-2 Mannoprotein extraction:

The obtained cells were separated by centrifugation at 10,000 rpm for 20 min, then the cells were suspended in phosphate buffer and the suspended cells were boiled for 5 min and then centrifuged at 3000 rpm for 10 min. Then the supernatant representing the crude mannoprotein was taken and placed in dialysis bags to obtain the mannoprotein after getting rid of the excess water by filtering it through the dialysis bags after placing the dialysis bags containing the mannoprotein in the sucrose and covering them completely to complete the filtration (19). Two isolates of *Saccharomyces cerevisiae* were extracted to extract mannoprotein from them.

5-2 Molecular detection of virulence factors of isolated bacterial species.

After conducting the phenotypic detection of the virulence factors, the species that showed a high percentage of these factors were selected to conduct the molecular detection of these factors.

DNA plasmid extraction:

Presto Mini gDNA Bacterial Extraction Kit from Geneaids company was used. The extraction process was carried out according to the steps provided by the manufacturer.

Characterization of DNA

Measuring the concentration and purity of DNA using the Nano Drop device was done.

Agarose gel electrophoresis of DNA

Mix 8 microliters of each of the DNA samples with 2 microliters of bromophenol blue dye solution (loading buffer) well, then carry the mixture of each sample on the prepared agarose gel with a concentration of 1%, then turn on the power supply and adjust the device. After the migration was over, the gel was removed from the gel plate of the electrophoresis device and examined in a dark room by exposing it to a (UV transilluminator) at a wavelength of 260 nm. The gel was photographed using the Gel documentation. The molecular size of the DNA segments was estimated by comparing the position and thickness of the bundle with the standard size guide (100 bp. (DNA). The molecular weights of the DNA were estimated depending on the distances made by these particles in the gel (20).

Polymerase Chain Reaction

Table (1) shows the virulence factors to be investigated the bacterial species they possess and their number according to the source of the isolation and the target genes.

The master reaction mixture was prepared consisting of (19 µl nuclease-free water, 2 µl premix solution, 1 µl of 10 pmol/µl of each forward primer, and 1 µl of 10 pmol/µl of each reverse primer) according to the number of samples for each bacterial species. Then the master reaction solution was distributed into 0.2 ml Eppendorf tubes with a volume of 23 µl for each sample. Then 2 µl of DNA was added to each sample for each tube to make the final volume 25 µl. Then it was introduced into the thermocycler using the appropriate program as shown in Table (2).

The primers prepared by Macrogen Company and shown in Table (3) were used and re-dissolved according to the manufacturer's instructions to obtain a stock solution for each primer at a concentration of (100 pmol/µl). The pre-mix solution prepared by Pioneer Company was also used, consisting of PCR solution, NTPS deoxynucleotide solution, and Taq DNA polymerase.

Table (1) The virulence factors and the bacterial species they possess and their number according to the source of the isolation and the target genes.

Bacterial species	isolation source	virulence factors	Gene name
E.coli	2	Biofilm	*ndvB*
P. mirabilis	2	Urease	*ureR*
		Biofilm	*rsmA*
K.pneumoniae	2	Urease	*ureD*
		Biofilm	*fimH*
Staph. aureus	2	Hemolysin	*Hlb*

Table (2): PCR thermocycler programs

Primers	Denatur. -1	Denatur. -2	Annealing	Exten. -1	Exten. -2
ndvB	95 c^0 /3 m	95c^0/45sec	57 c^0/60 sec	72c^0/60 sec	72 c^0 /7 m.
	1 cycle	35 cycles			1 cycle
ureR	94 c^0 /2 m	94c^0/60sec	58 c^0/60 sec	72c^0/60 sec	72 c^0 /5 m.
	1 cycle	35 cycles			1 cycle
rsmA	95 c^0 /5 m	94c^0/60sec	56 c^0/60 sec	72c^0/60 sec	72 c^0 /10 m.
	1 cycle	35 cycles			1 cycle
ureD	94 c^0 /5 m	94c^0/60sec	60 c^0/45 sec	72c^0/45 sec	72 c^0 /7 m.
	1 cycle	35 cycles			1 cycle
fimH	94 c^0 /5 m	94c^0/60sec	58 c^0/60 sec	72c^0/60 sec	72 c^0 /10 m.
	1 cycle	35 cycles			1 cycle
Hlb	94 c^0 /5 m	95c^0/30sec	52 c^0/45 sec	72c^0/60 sec	72 c^0 /6 m.
		35 cycles			1 cycle

Table (3): Primers were used for PCR

Primers	Sequence	Amplicon size bp	Reference
ndvB	F:GGA CAG GGC AAG GTT TAT T R:GGT TAT ACT CAG CAG CAC TAT C	950	(21)
ureR	F:CCG GAA CAG AAG TTG TCG CTG GA R:GGG CTC TCC TAC CGA CTT GAT C	359	(22)
rsmA	F:TAG CGA GTG TTG ACG AGT GG R:AGC GAG GTG AAG AAC GAG AA	565	(23)
ureD	F:CCC GTT TTA CCC GGA AGA AG R:GGA AAG AAG ATG GCA TCC TGC	300	(22)
fimH	F:TGC TGC TGG GCT GGT CGA TG R:GGG AGG GTG ACG GTG ACA TC	550	(23)
ureE	F: ACG AAA TGA CGG TGG ATA TG R: GAC GAA GGC AAA CGT ATC A	370	(22)
Hlb	F:GTG CAC TTA CTG ACA ATA GTG C R:GTT GAT GAG TAG CTA CCT TCA GT	309	(24)

Results

phenotyping screening of virulence factors

The virulence factors of the isolated bacterial species were visually detected, and the highest percentage of production of these factors and the percentage of cell membrane factor production was (100%) in *K. pneumoniae* and *E. coli,* while *P. mirabilis* produced (71.42%), and its production percentage in *Staph. aureus* (58.33), while the percentage of production of urease enzyme in these samples was (100%) for K. *pneumoniae*, (64.28%) for *P. mirabilis*, (40%) for *E. coli*, (62.5) for *Staph. aureus*, and the percentage of production of hemolysin enzyme for *K. pneumoniae* and *E. coli* was (0) for both, while for *P. mirabilis* and *Staph. aureus* it was (100%), As shown in Figure 1 and Table 4.

Effect of mannoprotein to curing the genes responsible to encoded for virulence factors product

The study included (8) bacterial isolates from the total isolates obtained from different isolation sources. Two isolates were taken from each type of bacteria, *K.Pneumoniae*, *E.coli, and Staph. aureus* and *P.mirabilis*. These isolates were among the isolates capable of producing virulence factors (urease, biofilm, Hemolysin). A test was conducted to show the effect of the ability of mannoprotein to prevent bacteria from producing virulence factors by using three concentrations of mannoprotein 100%, 75%, and 50% in addition to using culture media specific to detection for virulence factors.

The results of our current study show that *Staph. aurase* isolates are unable to produce the virulence

factor hemolysin at a rate of (100%) for both yeast isolates due to the effect of mannoprotein on preventing and neutralizing the bacteria from producing the virulence factor. *K. Pneumoniae* isolates were shown to be unable to produce the virulence factor urease at a rate of (100%) for both yeast isolates due to the effect of mannoprotein on preventing and neutralizing the bacteria from producing the virulence factor urease. As for the biofilm virulence factor, the first isolate of *K. Pneumoniae* bacteria was found to be unable to produce the biofilm virulence factor for the mannoprotein extracted from both yeast isolates at all concentrations. As for the second isolate of *K. Pneumoniae* bacteria, it was unable to produce the biofilm virulence factor when treated with the mannoprotein extracted from the first yeast isolate and was able to produce biofilm for the mannoprotein of the second yeast isolate. While *E.coli*, the first isolate of bacteria was unable to produce the biofilm virulence factor for the mannoprotein extracted from both yeast isolates and for all concentrations, the second isolate of *E.coli* bacteria was able to produce the biofilm virulence factor at a rate of (50%) for the mannoprotein of the first isolate and was unable to produce biofilm for the mannoprotein of the second isolate. The results of our study also showed that the first isolate of *P. mirabilis* bacteria was unable to produce the biofilm virulence factor for the mannoprotein extracted from both yeast isolates for the first concentration only (100%) of mannoprotein, while the concentration (75%) and (50%) were ineffective in preventing the bacteria from producing the biofilm virulence factor, and thus the bacteria were able to produce the virulence factor. As for the second isolate of *P.mirabilis* bacteria, it was unable to produce the biofilm virulence factor for the mannoprotein of both yeast isolates at concentrations (100%) and (75%), while the concentration (50%) was ineffective in neutralizing the bacteria, and thus the second isolate was able to produce biofilm for the concentration (50%) only. While this bacteria produces urease, the first isolate of *P. mirabilis* bacteria was unable to produce the virulence factor urease for the mannoprotein extracted from both yeast isolates for the first concentration only (100%) of mannoprotein, while the concentration (75%) and (50%) were ineffective in preventing the bacteria from producing the virulence factor urease, and thus the bacteria were able to produce the virulence factor when treated with these two concentrations. As for the second isolate of *P.mirabilis* bacteria, it was unable to produce the virulence factor urease for the mannoprotein of both yeast isolates at concentrations (100%) and (75%), while the concentration (50%) was ineffective in neutralizing the bacteria, and thus the second isolate was able to produce urease for the concentration (50%) only.

The molecular study.

The isolates that showed high levels of virulence factor production were selected in the current study for molecular detection of the genes responsible for encoding these virulence factors. plasmid DNA of the isolates shown in Table (1) has been isolated.

DNA plasmid extraction.

The results of electrophoresis showed that all samples contained DNA Figure 2. The purity and concentration of the DNA were measured using the Nanodrop As shown in Table (5). The nucleic acid concentrations were adjusted to a concentration of (50 ng/ul) using Free nuclease water. The nucleic acid was used after adjusting the concentration in the Polymerase chain reaction Samples from (1 to 8) before treatment with mannoprotein, from (9 to 16) after the first treatment, and from (17 to 24) after the second treatment with mannoprotein.

Figure (1): Results of phenotypic detection of the production of Urease, Biofilm, and Hemolysin.

Table (4): Rate of production of virulence factors by Gram-negative bacteria

virulence factor		*E. coli* 5	*K. pneumoniae* 2	*P. mirabilis* 14	*Staph. aureus* 14
Biofilm	+	100	100	71.42	58.33
	-	0	0	28.58	41.66
Urease	+	40	100	64.28	62.5
	-	60	0	35.72	37.5
Hemolysin	+	0	0	100	100
	-	100	100	0	0

Table (5) Concentration and purity of DNA of bacterial species

Types of bacteria	Purity	Concentration ng/µl	isolation	Types of bacteria	Purity	Concentration ng/µl	isolation
K. pneumoniae	1.986	56.16	4	*P. mirabilis*	1.821	72.36	6
	1.782	84.32	5		31.18	31.18	7
	1.527	67.18	10		2.013	33.18	14
	1.605	28.13	15		1.552	47.09	16
	1.663	38.01	17		1.902	57.14	18
	1.669	83.67	20		2.105	38.26	24
Staph. Aureus	1.832	34.4	1	*E.coli*	1.747	27.22	3
	1.991	53.47	2		1.772	112.44	8
	1.563	47.23	12		1.664	88.13	9
	1.651	37.64	13		1.655	58.03	11
	1.713	66.13	19		1.906	55.27	21
	1.62	48.14	23		1.507	62.14	22

M1V1=M2V2

25*50= C*V2

1250/34.4=36.33 DNA +13.67 ddw

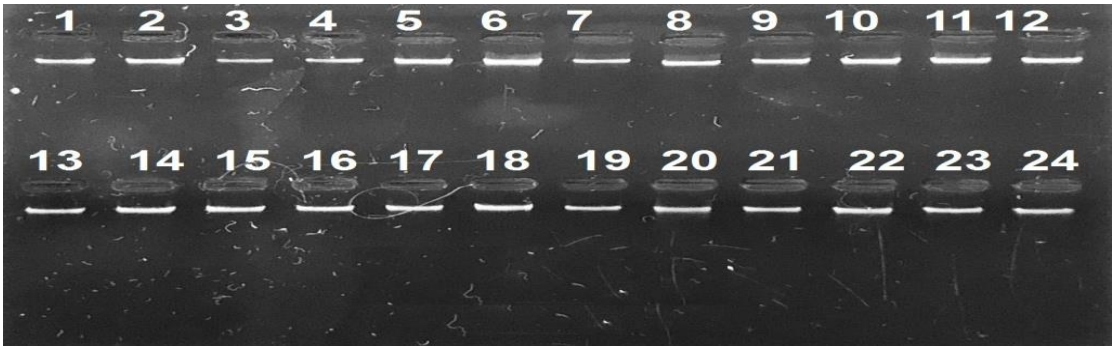

Figure 2: Results of electrophoresis of DNA plasmid on an agarose on 1% agarose gel of *E. coli, K. pneumoniae, P. mirabilis, Staph. Aureus*

The results indicate that samples (1 to 8) were untreated, samples (9 to 16) were treated with the first mannoprotein I extracted from the first isolate of baker's yeast, and samples (17 to 24) were treated with the second mannoprotein II extracted from the second isolate of baker's yeast. Sample (1,2) represent *Staph. aureus* bacteria before treatment, (12,13) represent *Staph. aureus* bacteria after the first treatment, and (19, 23) represent *Staph. aureus* bacteria after the second treatment. Samples (4,5) represent *K. pneumoniae* bacteria before treatment, (10,15) represent *K. pneumoniae* bacteria after the first treatment, and (17,20) represent *K. pneumoniae* bacteria after the second treatment. The numbers (6,7) represent *P. mirabilis* bacteria before treatment, (14,16) represent *P. mirabilis* bacteria after the first treatment, and (18,24) represent *P. mirabilis* bacteria after the second treatment. While samples (3,8) represent *E. coli* bacteria before treatment, (9,11) represent *E. coli* bacteria after the first treatment, and (12,22) represent *E. coli* bacteria after the second treatment.

Polymerase chain reaction.

PCR Detection of *ureR* and *rsmA* genes in *P. mirabilis*

The presence of the *ureR* gene was detected by the appearance of a band with a length of 359 base pairs, and it was found that all isolates (100%) contain this gene. The presence of the *rsmA* gene was detected by the appearance of a band with a length of 565 base pairs as shown in Figure (3), and it was found that all isolates contain the *ureR* and *rsmA* genes at a rate of (100%) each.

PCR Detection of *ureD* and *fimH* genes in *K. pneumoniae*.

The presence of the *ureD* gene was discovered through the appearance of a band of 300 base pairs and it was found that all isolates (100%) contain this gene. As for the *fimH* gene, the presence of this gene was discovered through the appearance of a band of 550 base pairs as written in Figure (3), and it was found that all isolates (100%) contain this gene.

PCR Detection of *ndvB* genes in *E.coli*.

The presence of the *ndvB* gene was detected by the appearance of a band of 950 base pairs, as shown in Figure (3). All isolates contain this gene.

PCR Detection of hlb genes in *Staph.a ureus*.

The *hlb* primer was used to detect the gene responsible for the production of the enzyme β-hemolysin in Staph. aureus bacteria. The results of electrophoresis of the polymerase chain reaction products on agarose gel showed the presence of this gene through the appearance of a band with a size of 309 base pairs as shown in Figure (3). It was found that all the isolates isolated from different sources contained this gene at a rate of (100%), but with what is called Copy number, meaning that the number of copies of the (DNA) is very small, and therefore the band appeared very weak because the primer met the (DNA) in a few cycles.

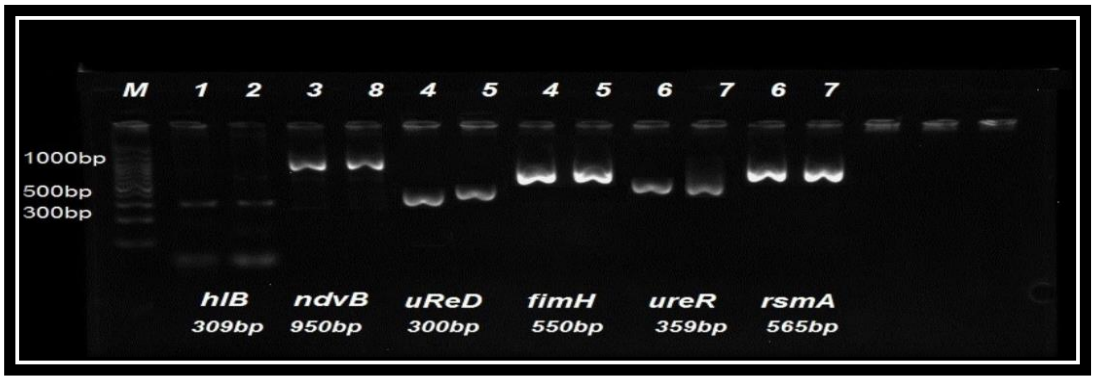

Figure 3: Results of electrophoresis on2% agarose gel *ureR*(359bp), *rsmA*(565bp), *ureD*(300bp), *fimH*(550bp), *ndvB*(950bp), *hlb*(309bp) after amplification of samples by PCR

Curing the genes responsible for the production of virulence factors after first treatment isolates with mannoprotein extracted from *Saccharomyces cerevisiae*:

PCR Detection of the ndvB gene after treatment with the first mannoprotein

The primer *ndvB* was used to detect the gene involved in the formation of biofilms in (3,8 *E. coli*) bacteria after the first treatment with mannoprotein. The results of electrophoresis of the polymerase chain reaction products on agarose gel showed the absence of this gene through the absence of a band of 950 base pairs. It was found that the isolates did not contain this gene due to their treatment with mannoprotein, which affected the production of the gene by neutralizing the appearance of the gene responsible for producing the virulence factor in the formation of biofilms in bacteria, as shown in Figure 4.

PCR Detection of the *ureR* gene after treatment with the first mannoprotein

The results of electrophoresis of the polymerase chain reaction products on agarose gel in (6,7 *P. mirabilis*) showed the absence of this gene through the absence of a band of 359 base pairs as shown in Figure 4. The results showed that all isolates do not have this gene due to the effect of mannoprotein in neutralizing the virulence factor gene.

PCR Detection of *rsmA*gene after treatment with the first mannoprotein

The results showed that all isolates did not have this gene after treatment with mannoprotein compared to the same isolates that produced biofilm before treatment. Gene expression of the *rsmA* gene was absent in (6,7 *P. mirabilis*) isolates in which biofilm formation was disrupted due to treatment with mannoprotein, indicating the role of this gene in its formation as shown in Figure 4.

PCR Detection of the *ureD* gene after treatment with the first mannoprotein

All isolates (4,5 *K. pneumoniae*) were characterized by not having this gene compared to the same isolates that produced the urease enzyme before being treated with mannoprotein as shown in Figure 4. This indicates that mannoprotein has an effect in preventing bacteria from producing the urease enzyme.

PCR Detection of *fimH* gene after treatment with the first mannoprotein

The results of electrophoresis of the polymerase chain reaction products on agarose gel in (4,5 *K. pneumoniae*) showed the absence of this gene through the absence of a band of 550 base pairs as shown in Figure 4. The results showed that all isolates did not contain this gene in the bacterial isolates after treating them with mannoprotein compared to the same isolates in which the gene was detected.

PCR Detection of *hlb* gene after treatment with first mannoprotein

The *hlb* primer was used to detect the gene responsible for the production of the enzyme β-hemolysin in (1,2 *Staph. aureus*) bacteria, after the first treatment with mannoprotein. The results of electrophoresis of the polymerase chain reaction products on agarose gel showed the absence of this gene, as shown in Figure (4). It was found that all isolates isolated from different sources did not have the gene in them, as this gene was neutralized in them due to the effect of mannoprotein, which completely destroyed the DNA and prevented the appearance of the gene responsible for the appearance of the virulence factor.

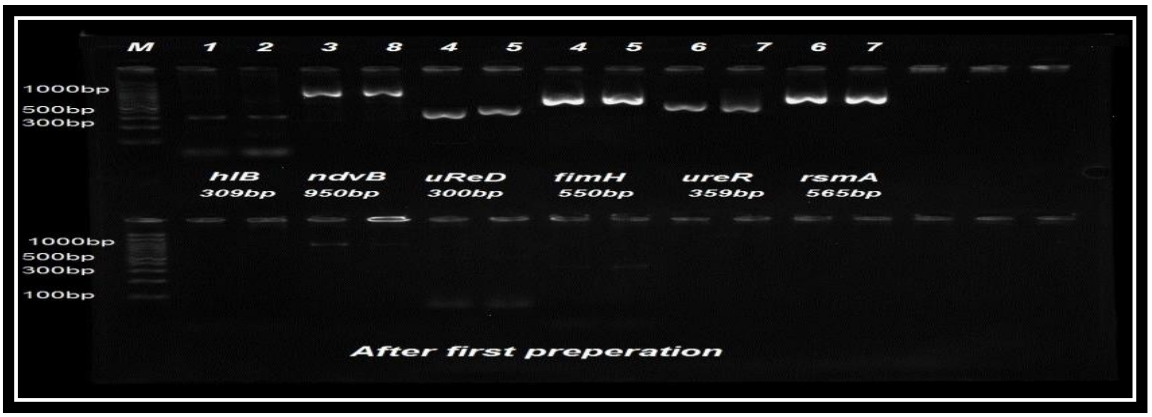

Figure 4. Results of electrophoresis on 2% agarose gel of the polymerase chain reaction (PCR) products using specific primers to the genes *ndvB, ureR, rsmA, ureD, fimH*, *hlb* of bacterial isolates after treatment with the first mannoprotein extracted from *Saccharomyces cerevisiae*.(1,2 *Staph. aureus*), (3,8 *E.coli*), (4,5 *K. pneumoniae*), (6,7 *P. mirabilis*)

Curing the genes responsible for the production of virulence factors after second treatment isolates with mannoprotein extracted from *Saccharomyces cerevisiae*:

PCR Detection of *hlb* gene after treatment with second mannoprotein

The hlb primer was used to detect the gene responsible for the production of the enzyme β-hemolysin in Staph. aureus bacteria. The results of electrophoresis of the polymerase chain reaction products on agarose gel showed the destruction of this gene (changing the properties of the DNA) by showing several areas to meet the primer (the single area was divided into several areas to meet the primer), meaning that the (DNA) appeared but affected its properties, as shown in Figure (5). It was found that all the isolates isolated from different sources had this gene neutralized due to the effect of the mannoprotein, which led to changing the properties of the (DNA) and showing several areas to meet the primer, as the single area was divided into several areas, and therefore these multiple images of the (DNA) appeared, as it affected the properties of the DNA and thus prevented the gene responsible for the appearance of the virulence factor.

PCR Detection of ndvB gene after treatment with second mannoprotein

The primer ndvB was used to detect the gene contributing to the formation of biofilms in E. coli bacteria after treating it with the second treatment of mannoprotein. The results of electrophoresis of the polymerase chain reaction products on agarose gel showed the destruction of this gene through the absence of a band of 950 base pairs as shown in Figure (5). It was found that the isolates did not contain this gene due to their treatment with mannoprotein, which affected the production of the gene by changing the properties of the (DNA) and showing several areas to meet the primer, as one area was divided into several areas, and therefore these multiple images of (DNA) appeared, which neutralized the appearance of the gene responsible for producing the virulence factor in the formation of biofilms in bacteria.

PCR Detection of *ureR* gene after treatment with second mannoprotein

The results of electrophoresis of the polymerase chain reaction products on agarose gel showed that this gene did not appear through the absence of a band of 359 base pairs as shown in Figure (5). The results showed that all isolates do not have this gene

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due to the effect of the mannoprotein in neutralizing the virulence factor gene from appearing in the bacteria, as the DNA appeared in several areas to meet the primer, as one area was divided into several areas, which means that the mannoprotein affected the properties of the DNA and led to its destruction.

PCR Detection of *rsmA*gene after treatment with the second mannoprotein

The results of electrophoresis of the polymerase chain reaction products on agarose gel showed that this gene did not appear in the bacterial isolates under study through the absence of a band of 565 base pairs as shown in Figure (5). The results showed that all isolates had DNA degradation that prevented them from expressing the gene. Gene expression of the rsmA gene was absent in *P.mirabilis* isolates in which the formation of biofilms was disrupted due to treatment with mannoprotein, which indicates the role of this gene in their formation.

PCR Detection of *ureD* gene after treatment with the second mannoprotein

The primer ureD was used to detect the gene responsible for the production of the enzyme Urease in *K. pneumoniae* bacteria. After it was treated with the second treatment of mannoprotein, the results of electrophoresis of the polymerase chain reaction products on agarose gel showed that this gene did not appear through the absence of a band of 300 base pairs as shown in Figure (5), due to the appearance of several fragmented regions of the DNA due to the effect of mannoprotein that led to its destruction.

PCR Detection of *fimH* gene after treatment with second mannoprotein

The *fimH* primer was used to detect the gene responsible for the production of the adhesive subunit in *K. pneumoniae* bacteria after treatment with the second treatment. The results of electrophoresis of the polymerase chain reaction products on agarose gel showed the appearance of DNA damage that led to a change in its characteristics in expressing the G, as shown in Figure (5).

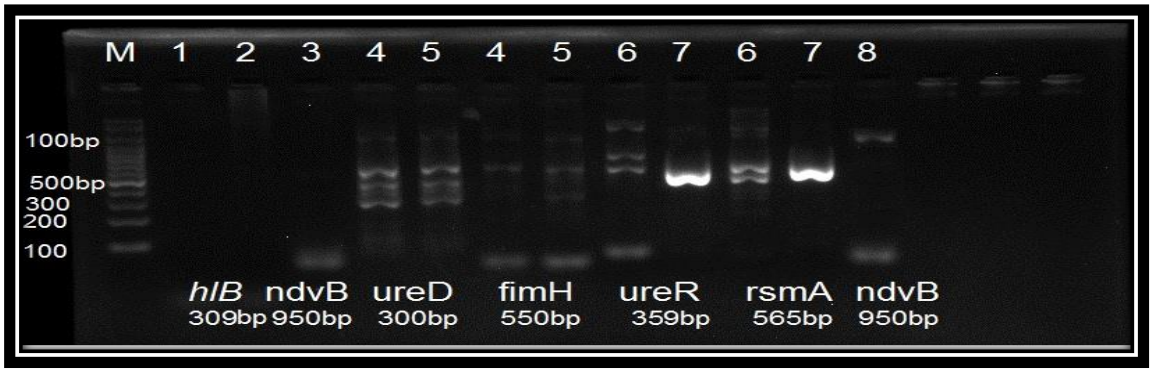

Figure (5) Electrophoresis on a 2% agarose gel of the polymerase chain reaction (PCR) products using primers specific to the genes *hlb, ndvB, ureR, rsmA, ureD, fimH* for bacterial isolates after being treated with the second treatment of mannoprotein extracted from the second yeast isolate.

Discussion

The results of the current study were consistent with the results of another study in Japan, which showed that the highest percentage was recorded for male patients more than females (25). The main reason for the high prevalence of Gram-negative species is that most species belong to the Enterobacteriaceae which present in the form of a normal flora that transforms under abnormal conditions into pathogenic bacteria, especially with the possession of many factors that enable them to attach to receptors on the inner epithelial cells (26). The exchange of plasmids to receive antimicrobials, and the changing local microbial environment also highlights the biological activities (27).

In this study. *E. coli* and *K. pneumoniae* showed their ability to produce a biofilm was (100%) and *P. mirabilis* was (71.42%), The results were consistent with a study indicating that *P. mirabilis* isolates were biofilm producers at a rate of (75%) (28). Also, *E. coli* bacteria were productive at a rate of (100%), It differed from the results of the study of Raya *et al.* (2019) that the production of this factor was (46.2%) (29). As for *K. pneumoniae* bacteria, its percentage also reached (100%) The results of our study were also consistent with the results of Haghighifar *et al*., (2021), which showed the ability of these bacteria to form biofilm by 100% (30). As for the urease enzyme, the results of the current study showed that *P. mirabilis* isolates were able to produce urease enzyme at a rate of (64.28%). The results did not agree with the results of other studies that showed the ability of *P. mirabilis* isolates to produce urease enzyme at a rate of (100%) (31,32). While *E. coli* bacteria can produce this enzyme by (40%). Also, *K. pneumoniae* isolates produced the urease enzyme by (100%). The results of our study were close to the results of Haghighifar *et al*., (2021), as *K. pneumoniae* could produce the urease enzyme by (100%) (30). The *fimH* gene contributes to the production of the type I adhesive subunit, which mediates adhesion to many epithelial cells, especially bladder epithelial cells, causing infection and biofilm formation (32). In our study, this gene was found in all isolates (100%) and these results were close to a recent study in Egypt (23). *P. mirabilis* showed the ability to form biofilms for all isolates isolated from patients. These results were consistent with the results of (30) in Iran but did not agree with the study of (28) in Diwaniyah Governorate. The *rsmA* gene contributes to the production of a regulatory protein that regulates biofilm formation in *P.mirabilis* (34). Shokouhfardetal., 2022 reported that the expression of the *rsmA* gene was decreased in *P.mirabilis* isolates in which biofilm formation was impaired, suggesting a role for this gene in its formation (35). After treating the samples in which the genes responsible for the production of the virulence factors, biofilm, and urease, were molecularly detected with mannoprotein extracted from *S.cerevisiae*, the genes in them were affected. After molecular detection, the genes responsible for the virulence factors did not appear, the genes did not appear in them due to their being affected by the mannoprotein substance that treated them, which led to the genes being curing from producing the virulence factor due to the destruction of the DNA in them. This indicates that the mannoprotein affects the genes responsible for producing the virulence factor, and this is very important at present due to the multiple resistance of bacteria towards antibiotics due to the continuous use of antibiotics against bacteria, which led to bacteria being resistant to commonly used antibiotics. Therefore, it is necessary to use antibiotics extracted from readily available natural materials. Our current study is also in agreement with the study of the researcher who indicated that mannoprotein extracted from *S.cerevisiae*, which has activity against pathogenic bacteria, can be used as an inhibitor of *Staph. aureus*. Mannoprotein also accelerates the detachment of mature staphylococcal biofilms, which were previously formed under optimal conditions. It was found that the anti-biofilm effect of mannoprotein reflects its effect on the hydrophobic cell surface

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(36). The results of our current study were consistent with the results of a study that indicated that adding mannoproteins to bacteria has a positive effect on the growth of many species of bacteria (37).

Conclusion

1- The *ndvB* gene contributes to biofilm formation in *Escherichia coli* and is found in burn and wound patients.

2- The *fimH* gene contributes to biofilm formation in *K. pneumoniae*.

3- Mannoprotein extracted from *S.cerevisiae* inhibits genes responsible for the production of virulence factors in bacterial isolates.

4- The *uerD* gene responsible for the production of Urease in *K. pneumoniae*.

5- After treating the bacterial isolates with mannoprotein, the DNA in the bacterial isolates was destroyed, and thus the bacteria were unable to produce virulence factors due to the effect of the protein on them.

A difference was observed in the gene expression of the genes responsible for the production of biofilm, Hemolysin, and urease virulence factors (*ureR, rsmA, ureD, fimH, ndvB, Hlb*) in the bacterial isolates current study before and after treatment with mannoprotein extraction. Before treatment, the genes were found in all isolates at a rate of (100%), but after treating the isolates with mannoprotein, the DNA was destroyed and thus the gene expression did not occur.

Ethical approval

The study was carried out in conformity with the ethical standards outlined in the Helsinki Declaration. Before a sample was taken, it was done with the patient's verbal and analytical consent. A local ethics committee examined and approved the study protocol, subject information, and permission form**.**

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Conflicts of interest

There are no conflicts of interest.

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