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Isolation and Characterization of Some Clinical Bacterial Strains as a Biofilm Producers

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Abstract:

One of the most important microbial societies is the bacterial biofilm, which has an environmental and health importance. Ten bacterial isolates were studied in this research, obtained from public health laboratory, Misan province, and identified biochemically and genetically by applying of PCR technique and sequencing, belonged to the genera *Staphylococcus*, *Klebsiella*, *Proteus*, *Salmonella*, and *Pantoea* .Then examined by PCR technique for the ability to produce biofilm. All the isolates tested in the study, showed this ability through revealing the *icaA* bands (188bp) and *icaD* bands (198bp).

Key Words: Biofilm, Bacteria

I. Introduction

Biofilm is defined as a microbial communities that are attached to an abiotic or living surface and embedded in a matrix of extracellular polymeric substance that they have produced[1,2]. They are often cooperative association among several microbial groups (bacteria, fungi, algae and protozoa) as well as plants and animals [3]. It varies in thickness and complexity, depending upon where it occurs and how long it keeps developing as demonstrated in [4] with complexity ranges from single cell layers to thick microbial mats with dozen of interactive layers. The author in [5] mentioned that they can protect pathogens from disinfectants, or release microorganisms and microbial products that may affect the immunological system of the host.

There are many of medical implications for the biofilm. They accumulate on damaged tissue (such as rheumatic heart valves), hard tissues (teeth) and foreign materials (catheters, artificial hip joints) [4].

Lipophilic Corynebacteria as natural flora of human skin were examined in the study of Kwaszewska *et al.* [6] in which 75.6%of *Corynebacterium* species on human skin were able to form biofilms. While Wojtyczka *et al.* [7] evaluated the prevalence of the biofilm forming coagulase-negative Staphylococci in hospital environment as a risk factor for nosocomial infection. In addition to the study of [8] Balasubramanian *et al.*, in which they isolated and identified some species of microbial strains from the biofilm of urinary catheters, such as *Escherichia coli*, *Proteus mirabilis*, *Staphylococcus epidermis* and *Staphylococcus aureus*. Whereas the study of [9] included isolation of 60(59.4%) among the97 gram-negative bacterial isolates from diabetic foot ulcer were biofilm producer, and the most common isolate was *Proteus vulgaris* followed by *Klebsiella pneumonia*, *Escherichia coli*, *Klebsiella oxytoca*, *Acinetobacter sp* and *Pseudomonas aeruginosa* .

Gad *et al.* [10] recorded that Staphylococci which isolated from segments of catheter have a higher extent for biofilm production than that from urine and all of the biofilm producing of them were positive for *icaA* and *D* genes, indicated the important role of these genes in the *Staphylococcus* infections which associated with urinary catheterization as a virulence markers.

The genes (*icaA*, *icaB* and *D*) were detected also in the study of Prasanth and Saravanakumari [11] as responsible genes for biofilm formation, which suggested that the virulence factors which contribute to the development of infections can be demonstrated by understanding of the presence of the biofilm expression genes in the targeted organisms.

In the study on Otitis patients carried out by Abd Al-Abbas [12] all bacteria were positive to *icaA* and/or *icaD*, and some species appeared to have the *ica* gene for the first time such as *Providenca vermicola*, *Serratia marcescence*, *Proteus mirabilis*, *Bordetella trematum* and others.

The resent study aimed to isolate of biofilm forming bacterial species from hospital patients and environment with molecular diagnosis.

II. Material And Methods

Bacterial isolation and cultivation. Isolates were taken from Misan province, Public health laboratory, Microbiology unit, which belong to different swabs assembled from different hospitals.

Blood agar (LAB M, UK) Chocolate agar and MacConky agar (Salucea, Netherlands) were prepared according to the instructions of manufacturing companies as possible.

Swaps were cultured on the media and incubated at 35±2ºC for 24hr. Different colonies were sub cultured by streaking for purification [13], [14].

Morphological characterization. Selected colonies were characterized by shape, color, consistency, size, and fermentation of lactose according to [15], [16]. Gram staining was done by the following of (Syrbio, Jorden) company instructions.

Biochemical tests. Different biochemical tests were carried out according to [16]. The API 20E strep and API 20Staph strep (Biomerieux, France) were applied for more

identification according to instructions of manufacturing company.

Genetic identification.

Genomic DNA extraction. Activation for cultures was applied on nutrient broth for 18- 24 hr., centrifuged at 7000- 8000 cycle/min. for 10-15 min., then washed with distilled water at the same as preparing to the procedure of the genomic DNA mini kit (Geneaid, Taiwan) , with some changes in concentrations and time for the added materials.

Detection of genomic DNA by gel electrophoresis. According to Sambrook and Russell [17], method of electrophoresis by using 0.8% agarose was done as possible due to facilities.

Identification by Polymerase Chain Reaction (PCR). Lanes' primers [18] of 16SrDNA which were B27F (5-AGAGTTTGATCCTGGC-3) and U1492R (5-GGTTACCTTGTT ACGACTT-3) were used, with PCR program selected[19], as well as adaptation by the author [20] as shown in table (1). The Prokić *et al*. [21] amplification steps were abstracted with little changes.

Table (1): PCR amplification program, showed temperatures, times and cycles number in different steps.

Step	Temperature	Time	No. of cycle
Initial denaturation	92 °C	2 min	1
Denaturation	94 °C	30 sec	30
Annealing	51.8 °C	45 sec	
Extension	72 °C	1.5 min	
Final extension	72 °C	5 min	1

Detection of 16S rDNA by gel electrophoresis. Agarose of 2% was used as well as the steps of Sambrook and Russell [17] methods for detection of the 16S rDNA gene, with some differences required.

Purification, sequencing and manipulation of data for PCR product. The obtained product of PCR was sent for Macrogene Company laboratories/ China, for purification and sequencing. Then data were manipulated.

Detection of biofilm by PCR. The PCR method for amplification the genes of *icaA* and *icaD* to detect the biofilm (as slim formation) was done according to Arciola *et al*. [22] , with the primers used as F: 5'-TCTCTTGCAGGAGCAAT
CAA-3' and R: 5'-TCAGGCACTAACATCCAGCA-3' for *IcaA* gene, and F: 5'-ATGGTCAAGCCCAGACAGAG-3' and R: 5-'CGTGTTTTCAACATTTAATGCAA-3' for *IcaD* gene. The program was described in table (2), with reagent used; DNA template 5 µl, each primer1 µl, master mix 12.5 µl, nuclease free wate 5.5 µl, with final volume of 25 µl.

Table (2): Program use in PCR amplification for *icaA* or *icaD* gene

Steps	Temperature	Time	No . Of cycles
Initial denaturation	94˚C	5min	1
Denaturation	94˚C	30 sec	50
Annealing	55.5˚C	30 sec	
Extension	72˚C	30 sec	
Final extension	72˚C	1 min	1

Detection of PCR product by agarose gel electrophoresis. The solution, procedure and viewing were identical to those for the detection of universal *16srDNA* bands . However there are few exceptions using 100 bp DNA ladder **.**

III. RESULTS

Isolation, cultivation and morphological characterization. Ten isolates were selected and identified by morphological characterization; as found in table (3) and biochemical tests, table (4)

API 20E and API 20 STAPH systems: The results of these systems showed some resembles and differences in response to the reactions of the tests for the isolates 1-10 in table (5), and a little differences among *Staphylococcus* isolates in table (6)

Table (3): primitive identification of isolates, revealed different genus and species, with morphological characterization; gram staining and the characteristics of colonies on different media

Isolate number	Name	Gram staining	Colonies characteristics
1	*Proteus spp*	Gr –ve bacilli bacteria	MacConkey agar: non ferment lactose Blood agar : swarming phenomena
2	*Klebsiella pneumoniae*	Gr –ve bacilli bacteria	MacConkey agar: ferment lactose , large , mucoid colonies
3	*Klebsiella pneumoniae*	Gr –ve bacilli bacteria	MacConkey agar: ferment lactose , large , mucoid colonies
4	*Proteus spp*	Gr –ve bacilli bacteria	MacConkey agar: non ferment lactose Blood agar : swarming phenomena
5	*Salmonella typhi*	Gr –ve bacilli bacteria	MacConkey agar: non ferment lactose , large , gray – white , circular ,with smooth convex surface and entire edge
6	*Staphylococcus epidermidis*	Gr +ve cocci bacteria like cluster	Blood agar: small to medium, gray – white colonies, most colonies non hemolytic
7	*Pantoea*	Gr –ve bacilli bacteria	MacConkey agar: ferment lactose, circular , opaque , convex , shiny , moderate in size
8	*Pantoea*	Gr –ve bacilli bacteria	MacConkey agar: ferment lactose, circular , opaque , convex , shiny , moderate in size
9	*Staphylococcus epidermidis*	Gr +ve cocci bacteria like cluster	Blood agar: small to medium, gray – white colonies, most colonies non hemolytic
10	*Staphylococcus aureus*	Gr +vecocci bacteria like cluster	Blood agar: small to medium, white-yellowish colonies, most colonies β - hemolytic

Table (4): Results of manually biochemical tests for isolates, showed different responses to the tests among the different isolates

Isolate no. / Test	1	2	3	4	5	6	7	8	9	10
Indole	-	-	-	-	-	-	-	-	-	-
Urease	V	+	+	V	-	+	-	-	+	V
Citrate utilization	V	+	+	V	-	-	-	-	-	+
Voges- Proskauer	-	+	+	-	-	-	+	+	-	+
Motility	+	-	-	+	+	-	+	+	-	-
Catalase	+	+	+	+	+	+	+	+	+	+
Oxidase	-	-	-	-	-	-	-	-	-	-
Coagulase*						+			+	+
Triple sugar iron agar(TSI)	A/A no gas with H2S	A/A no gas no H2S	A/A no gas no H2S	A/A no gas with H2S	K/A No gas with H2S	A/A no gas no H2S	A/A no gas no H2S	A/A no gas no H2S	A/A no gas no H2S	A/A no gas no H2S

+: positive, -: negative, V: variable, A: acidic, K : alkaline, *: test were done to *Staphylococcus* only

Table (5): Results of biochemical tests of API 20E system for some isolates, showed the different responses to the tests among them

Isolate no. / Test	1	2	3	4	5	7	8
ONPG	-	+	+	-	-	-	+
ADH	-	-	-	-	-	-	-
LDC	-	+	+	-	+	-	-
ODC	+	-	-	+	-	-	-
CIT	+	+	+	-	-	-	-
H2S	+	-	-	+	+	-	-
URE	+	+	+	-	-	-	-
TDA	+	-	-	+	-	-	-
INO	+	-	-	-	-	-	-
VP	-	+	+	-	-	+	+
GEL	+	-	-	+	-	-	-
GLU	+	+	+	+	+	+	+
MAN	-	+	+	-	+	+	+
INO	-	+	+	-	-	+	+
SOR	-	+	+	-	+	-	-
RHA	-	+	+	-	-	+	+
SAC	-	-	+	-	-	+	+
MEL	-	-	-	-	+	-	+
AMY	-	+	+	-	-	-	-
ARA	-	+	+	-	-	+	-
OX	-	-	-	-	-	-	-

; + positive, - negative, ONPG: (Ortho NitroPhenyl-ßDGalactopyranosidase , ADH: Arginine DiHydrolase, LDC: Lysine DeCarboxylase, ODC: Ornithine DeCarboxylase , CIT: Citrate utilization , H2S: H2S production, URE: Urease, TDA: Tryptophane DeAminase, IND: Indole production, VP: acetoin production (Voges Proskauer) ,GEL: Gelatinase ,GLU: Fermentation / oxidation (Glucose) , MAN: f / o (Mannitol) , INO: f / o(Inositol), SOR: f / o (Sorbitol), RHA: f / o (Rhamnose), SAC: f / o (Saccharose), MEL: f / o (Melbiose), AMY: f/ o (Amygdalin), ARA: f/o (Arabinose), OX: Cytochrome-Oxidase

Table (6): Results of biochemical tests of API STAPH system for the suspected *Staphylococcus*, showed a little different responses

Isolate no. / Test	6	9	10
0	-	-	-
GLU	+	+	+
FRU	+	+	+
MNE	+	-	+
MAL	+	+	+
LAC	+	+	-
TRE	-	-	+
MAN	-	-	-
XLT	-	-	-
MEL	+	-	-
NIT	-	-	+
PAL	+	+	+
VP	-	-	-
RAF	-	+	-
XYL	-	-	-
SAC	+	+	+
MDG	-	-	-
NAG	-	-	+
ADH	+	+	+
URE	+	+	-
LSTR	-	-	-

; + positive, - negative, 0: negative control, GLU: Acidification of D-Glucose , FRU: Acidification of D-Fructose, MNE: Acidification of D-Mannose, MAL: Acidification of D-Maltose, LAC: Acidification of D-Lactose, TRE: Acidification of D-Trehalose, MAN: Acidification of D-Mannitol, XLT: Acidification of Xylitol, MEL: Acidification of D-Melibiose, NIT: Reduction of nitrate, PAL: Alkaline phosphatase, VP: Voges proskauer, RAF: Acidification of Rafinose, XYL: Acidification of Xylose, SAC: Acidification of Saccharose, MDG: Acidification of Methyl – α- D-Glucopyranoside, NAG: Acidification of N-acetyl- glucose amine , ADH: Arginine DiHydrolase , URE: Urase , LSTR: Lysostaphin resistance .

Genetic identification.

Genomic DNA extraction and detection. Electrophoresis technique showed bands of isolated DNA for the all ten isolates as found in the figure (1).

Amplifying of the 16S rDNA gene by (PCR) Technique. With using a universal primer, results illustrated the band of 16S rDNA for each isolate of the all along with electrophoresed ladder in the region of 1500bp, as in the figure (2).

Sequencing. All the ten isolates were identified to the level of strain, as demonstrated in the table (6).

Amplifying of the 16S rDNA for *icaA* and *icaD* genes . PCR products of all isolates for *icaA* and *icaD* genes revealed bands at 188bp and 198bp (respectively) position in comparison with choosing ladder (100 – 1000 base pair) as in figures (3, 4) .

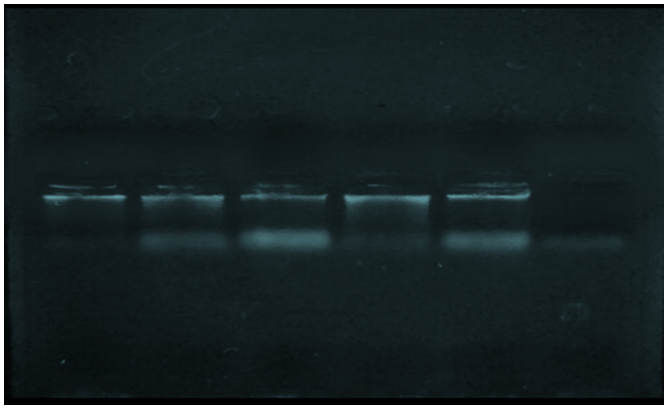

Figure (1): Gel electrophoresis, showed the isolated genomic DNA bands for different isolates, all showed the same bands.

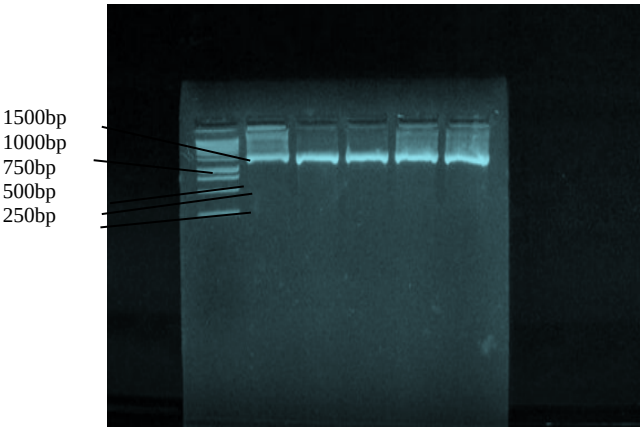

Figure (2): Gel electrophoresis for PCR technique, showed the band of 16s rDNA in different of isolates, First lane: DNA ladder of 1Kb.

Table (6): Sequencing of all isolates after data manipulation, showed different strains

Isolate	Sequence	Original strain
1	GGGGGGGCGGCGGCCTACCATGCAGTCGAGCGGTAACAGGAGAAAGCTTGCTTTCTTGCTGACGAGCGG CGGACGGGTGAGTAATGTATGGGGATCTGCCCGATAAACCCCGAAAAAATTCGGTCACGGGAGTCTAAC CCTTCCAAATGTCAAGGGTCCAATTCCCGAACTCGAGCCAGACTACGTAAAGGATAACATGTCAGCTTCT AGCGAGAAGGTCGCTCCAGGTGAACGCAGGATAGTATGAAAGGTGACCAGAACGGGGCGGGCCGAGGG GAGGGGCCTTAACGACCCTCTTCCGAGGTTTATAC	*Proteus mirabilis* strain ALK428
2	GGTGGGCGGCGCTACCATGCAAGTCGAGCGGTAGCACAGAGAGCTTGCTCTCGGGTGACGAGCGGCGGA CGGGTGAGTAATGTCTGGGAAACTGCCTGATGGAGGGGGATAACTACTGGAAACGGTAGCTAATACCGC ATAATGTCGCAAGACCAAAGTGGGGGACCTTCGGGCCTCATGCCATCAGATGTGCCCAGATGGGATTAGC TAGTAGGTGGGGTAACGGCTCACCTAGGCGACGATCCCTAGCTGGTCTGAGAGGATGACCAGCCACACTG GAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGCAAGCCT GATGCAGCCATGCCGCGTGTGTGAAGAAGGCCTTCGGGTTGTAAAGCACTTTCAGCGGGGAGGAAGGCG TTAAGGTTAATAACCTTGGCGATTGACGTTACCCGCAGAAGAAGCACCGGCTAACTCCGTGCCAGCAGCC GCGGTAATACGGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCACGCAGGCGGTCTGTCA AGTCGGATGTGAAATCCCCGGGCTCAACCTGGGAACTGCACTCGAAACTGGCAGGCTAGAGTCTTGTAGA GGGGGGTAGAATTCTAGGTGTAGCGGTGAAAAGCGTCGAGATCTGGAGGAATAACGCTGGCGGGGGAGG CCCCCTGGTCAAAAACCTGACGCTCCCGTGCGAAAACATTGTGTAGCGAGAGGGAGGAG	*Klebsiella pneumoniae* strain QLR-10
3	CNNGGAANGGGAACNNTTNTNGAGTCGAGCGGTAACAGGAGAAAGCTTGCTTTCTTGCTGACGAGCGGC GGACGGGTGAGTAATGTATGGGGATCTGCCCGATAGAGGGGGATAACTACTGGAAACGGTGGCTAATAC CGCATAATGTCTACGGACCAAAGCAGGGGCTCTTCGGACCTTGCACTATCGGATGAACCCATATGGGATT AGCTAGTAGGTGGGGTAAAGGCTCACCTAGGCGACGATCTCTAGCTGGTCTGAGAGGATGATCAGCCAC ACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGCAA GCCTGATGCAGCCATGCCGCGTGTATGAAGAAGGCCTTAGGGTTGTAAAGTACTTTCAGCGGGGAGGAA GGTGATAAGGTTAATACCCTTATCAATTGACGTTACCCGCAGAAGAAGCACCGGCTAACTCCGTGCCAGC AGCCGCGGTAATACGGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCACGCAGGCGGTCA ATTAAGTCAGATGTGAAAGCCCCGAGCTTAACTTGGGAATTGCATCTGAAACTGGTTGGCTAGAGTCTTG TAGAGGGGGGTAGAATTCCATGTGTAGCGGTGAAATGCGTAGAGATGTGGAGGAATACCGGTGGCGAAG GCGGCCCCCTGGG	*Klebsiella pneumoniae* strain NRC73
4	GNAGTTTGGGTNGTGGCCAANACTGGCNGCCCAAGCACAAAAAGCTTGCTCTCGGGTGACGAGCGGCGG ACGGGTGAGTAATGTCTGGGAAACTGCCTGATGGAGGGGGATAACTACTGGAAACGGTAGCTAATACCG CATAATGTCGCAAGACCAAAGTGGGGGACCTTCGGGCCTCATGCCATCAGATGTGCCCAGATGGGATTAG CTAGTAGGTGGGGTAACGGCTCACCTAGGCGACGATCCCTAGCTGGTCTGAGAGGATGACCAGCCACACT GGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGCAAGCC TGATGCAGCCATGCCGCGTGTGTGAAGAAGGCCTTCGGGTTGTAAAGCACTTTCAGCGGGGAGGAAGGC GTTAAGGTTAATAACCTTGGCGATTGACGTTACCCGCAGAAGAAGCACCGGCTAACTCCGTGCCAGCAGC CGCGGTAATACGGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCACGCAGGCGGTCTGTC AAGTCGGATGTGAAATCCCCGGGCTCAACCTGGGAACTGCATTCNAAACTGGCAGGCTAGAGTCTTGTAG AGGGGGGTAGAATTCCAGGTGTAGCGGTGAAATATTATAGATCTTGGAGGAATAACCCCTGGGGGGGGA AGGCCNCCN	*Proteus mirabilis* strain SM02

5	NNTAANNNNTCNTGCNTAAACCTGTCNGTTTAGCGAAAGACNAGAAGCTTGTTGCTTCTTCGCTGACGAGT GGCGGACGGGTGAGTAATGTCTGGGAAACTGCCTGATGGAGGGGGATAACTACTGGAAACGGTGGCTAAT ACCGCATAACGTCGCAAGACCAAAGAGGGGGACCTTCTGGCCTCTTGCATTCATATGTGCCCCTATGCCAT TATGTTGTTGTGGAGGTGACCGCTCTTACCAAGCAAACGATACGTGACCGACCTGAAAGGGTGATCGGCCA AACTGGAATTGAGACACCGTCCGGACTCCTACGGGAGGAATCAGTAGGGAATCTTCCGCAATGGGCGAAA GCCTGACGGATCAACGCCGCGTGAGTGAGGAGGGTCTTCAGATCGTAAAACTCTGATATTAGTGAAGAAC AAACGTGTATGTACTGTGCCCGTCTTGACGGTATCTTAATTTGAAAGCTACTGCTAACTTACGTGCCACAGC CGCGTGAATACGTAGGTGGCAAGCGTCATCCGGAATTATTGGGCGTATATCGCGCTTATGCGATTTTTTTTG AGACCGATGTGAAACGCCCAC	*Salmonella typhimurium* strain S1.I
6	TACATGCAAGTCGAGCGAACAGACGAGGAGCTTGCTCCTTTGACGTTAGCGGCGGACGGGTGAGTAACAC GTAGGTAACCTACCTATAAGACTGGGATAACTTCGGGAAACCGGAGCTAATACCGGATAATATTTCGAACC GCATGGTTCGATAGTGAAAGATGGCTTTGCTATCACTTATAGATGGACCTGCGCCGTATTAGCTAGTTGGT AAGGTAACGGCTTACCAAGGCAACGATACGTAGCCGACCTGAGAGGGTGATCGGCCACACTGGAACTGAG ACACGGTCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGGCGAAAGCCTGACGGAGCA ACGCCGCGTGAGTGATGAAGGTCTTCGGATCGTAAAACTCTGTTATTAGGGAAGAACAAACGTGTAAGTA ACTGTGCACGTCTTGACGGTACCTAATCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACG TAGGTGGCAAGCGTTATCCGGAATTATTGGGCGTAAAGCGCGCGTAGGCGGTTTTTTAAGTCTGATGTGAA AGCCCACGGCTCAACCGTGGAGGGTCATTGGATACTGGAATACTTGAGTGCAGAAGAGGAGAGTGGAATT CCATGTGTAGCGGTGATATGCGCAGAGATATGAAGGAAACACCAGTGGCGAATGCGACTTTCTGCTCTGTT ACTGACGCTGATGCGCAAAAGCGTGAGAATCAAACCGTATTAGATACCGTGCCTCCCCACTCCTTAACCTA TGAGCGCTACGCGTGGGGGGTTCCCTCCCCTTTTGGCCTCAACTAATCCATTATCCAACTCCGCCTGGCGAG GACAACCCCCAGGGTTTAAACTCAAAAGTATTAAAAGGGGCCCCCCCCCCCACCTGTTGGCCCAATTAGTTT TAAATTAAAACTTACCCCCAAAAACCTTACCCAAAGTCTTGAACATCCTTTGGATCCCCTCCTGGAATTTTT AAAATTTTCCCCCCTCCCGGGGGAACAAAAAGCAAACAGGCTTGGCGCCCCTTGTTTTTTCCTTCCCCCAA AAAAACCGAGGGTGTATTAGGGATGGGGGAAAAAA	*Staphylococcus epidermidis* strain 14F
7	GGNNNGGNGNGNGGNCTTNATNNCTTACNANNTCGAAGCGGTAGCACAGAGAGCTTGCTCTCGGGTGACG AGTGGCGGACGGGTGAGTAATGTCTGGGAAACTGCCTGATGGAGGGGGATAACTACTGGAAACGGTAGCT AATACCGCATAACGTCGCAAGACCAAAGAGGGGGACCTTCGGGCCTCTTGCCATCAGATGTGCCCAGATG GGATTAGCTAGTAGGTGGGGTAACGGCTCACCTAGGCGACGATCCCTAGCTGGTCTGAGAGGATGACCAG CCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCG CAAGCCTGATGCAGCCATGCCGCGTGTATGAAGAAGGCCTTCGGGTTGTAAAGTACTTTCAGCGGGGAGG AAGGTGTTAAGGTTAATAACCTTGTCGATTGACGTTACCCGCAGAAGAAGCACCGGCTAACTCCGTGCCAG CAGCCGCGGTAATACGGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCACGCAGGCGGTCT GTCAAGTCGGATGTGAAATCCCCGGGCTCAACCTGGGAACTGCATTCGAAACTGGCAGGCTGGAGTCTTGT AGAGGGGGGTAGAATTCCAGGTGTAGCGGTGAAATGCGTAGAGATCTGGAGGAATACCGGTGGCGAAGG CGGCCCCCTGGACAAAAG	*Pantoea agglomerans* strain QTYC45b
8	NNTCNNNNTNNCNNCNTNGACTTTNCGAGCGGTAACAGGAGAAAGCTTGCTTTCTTGCTGACGAGCGGCG GACGGGTGAGTAATGTATGGGGATCTGCCCGATAGAGGGGGATAACTACTGGAAACGGTGGCTAATACCG CATGACGTCTACGGACCAAAGCAGGGGCTCTTCGGACCTTGCGCTATCGGATGAACCCATATGGGATTAGC TAGTAGGTGGGGTAATGGCTCACCTAGGCGACGATCTCTAGCTGGTCTGAGAGGATGATCAGCCACACTGG GACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGA TGCAGCCATGCCGCGGGGGATGAAGAAGGCCTTAGGGTTGTAAAGAACTTTCAGCGAGGAGGAAAAGGAT AAAGCTTTTACCCCTTGTGTTTGAGTTTTCCAGAGGGAAAAAGTTTTTCCTAAAAAGCCTCGCGAGAACTC GGAGAAAGCGTGGTGTTTTCTCAGATATTCCCGAAAATACTCCCCCCGCGTCCACCGCCCTGTGGGGGGGG CCTATGTAATATTTTTAAGAAACTCAGACCTCTCGCCCTCCGAAACTCGGGGAGGGAGAGTATTTATGACT TTNNTCTCA	*Pantoea gaviniae* strain: LMG 25382
9	GNNGNCGGGGTGCTATACATGCAGTCGAGCGAACAGACGAGGAGCTTGCTCCTTTGACGTTAGCGGCGGA CGGGTGAGTAACACGTAGGTAACCTACCTATAAGACTGGGATAACTTCGGGAAACCGGAGCTAATACCGG ATAATATTTCGAACCGCATGGTTCGATAGTGAAAGATGGCTTTGCTATCACTTATAGATGGACCTGCGCCG TATTAGCTAGTTGGTAAGGTAACGGCTTACCAAGGCAACGATACGTAGCCGACCTGAGAGGGTGATCGGC CACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGGCGA AAGCCTGACGGAGCAACGCCGCGTGAGTGATGAAGGTCTTCGGATCGTAAAACTCTGTTATTAGGGAAGA ACAAACGTGTAAGTAACTGTGCACGTCTTGACGGTACCTAATCAGAAAGCCACGGCTAACTACGTGCCAGC AGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGAATTATTGGGCGTAAAGCGCGCGTAGGCGGTTTTT TAAGTCTGATGTGAAAGCCCACGGCTCAACCGTGGAGGGTCATTGGAAACTGGAAAACTTGAGTGCAGAA GAGGAAAGTGGAATTCCATGTGTAGCGGTGAAATGCGCAGAGATATGGAGGAACACCA	*Staphylococcus epidermidis* strain 7N-3A
10	GNNNNGCGGNTGCTATACATGCAGTCGAGCGAACGGACGAGAAGCTTGCTTCTCTGATGTTAGCGGCGGA CGGGTGAGTAACACGTGGATAACCTACCTATAAGACTGGGATAACTTCGGGAAACCGGAGCTAATACCGG ATAATATTTTGAACCGCATGGTTCAAAAGTGAAAGACGGTCTTGCTGTCACTTATAGATGGATCCGCGCTG CATTAGCTAGTTGGTAAGGTAACGGCTTACCAAGGCAACGATGCATAGCCGACCTGAGAGGGTGATCGGC CACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGGCGA AAGCCTGACGGAGCAACGCCGCGTGAGTGATGAAGGTCTTCGGATCGTAAAACTCTGTTATTAGGGAAGA ACATATGTGTAAGTAACTGTGCACATCTTGACGGTACCTAATCAGAAAGCCACGGCTAACTACGTGCCAGC AGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGAATTATTGGGCGTAAAGCGCGCGTAGGCGGTTTTT TAAGTCTGATGTGAAAGCCCACGGCTCAACCGTGGAGGGTCATTGGAAACTGGAAAACTTGAGTGCAGAA GAGGAAAGTGGAATTCCATGTGTAGCGGTGAAATGCGCAGAGATATGGAGGAACACCAGTGGCGAAGGC GACTTTCTGGTCTGTAA	*Staphylococcus aureus* strain DF8TA

, **bp: base pairs**

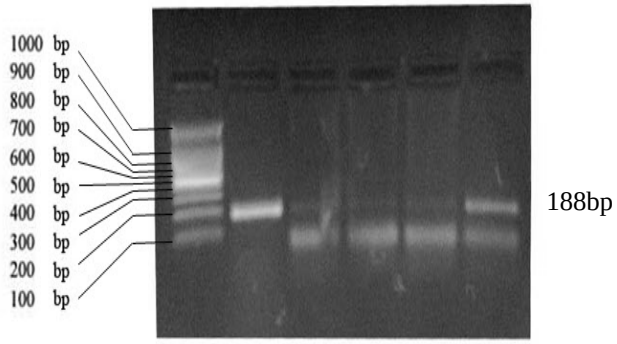

Figure (3): Agarose gel electrophoresis showed PCR product of *icaA* gene for isolates. First left Lane: (100bp-1000bp) DNA ladder , other Lanes: *icaA* bands (188bp) of different isolates.

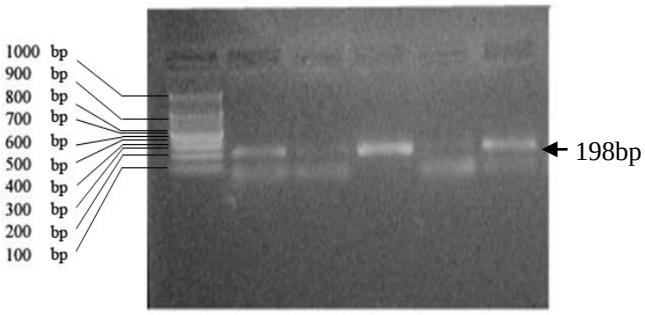

Figure (4): Agarose gel electrophoresis showed PCR product of *icaD* gene for isolates. First left Lane: (100bp-1000bp) DNA ladder, other Lanes: *icaD* bands (198bp) of different isolates.

IV. Discussion

Biofilm may be occurred by many of bacteria. In our study, Ten isolates were obtained, agreed with the study of Alhilfi *et al* [23] in which biochemical test couldn't reach to the final identification of some isolates, even for the species, whereas in molecular methods, all isolates reached to the strain level, suggested that may be belonged to the high sensitivity and accuracy for the molecular tests. In this study, after the final identification, the isolates belonged to the genus *Staphylococcus*, *Klebsiella*, *Proteus*, *Salmonella*, and *Pantoea* . and were isolated from hospital environment origin, either from facilities and devices or from injuries and infections of patients, that is in resembling with different studies, for example Wojtyczka *et al* [7] isolated *Staphylococcus epidermidis* strain from hospital environment. Bellifa *et al* [24] isolated *Klebsiella pneumoniae* from urinary catheter and endotracheal tubes during various services at the university hospital of Tlemcen, whereas *Proteus mirabilis* had been isolated from patients with indwelling bladder catheters in the study of Salih and AL-Ani[25]. Zubair *et al*[9] isolated *E coli* ,*K pneumoniae* and *P vulgaris* from diabetic foot ulcer patients.While Abdallah *et al* [26] obtained *Proteus* spp from urinary tract infection patients. Also different types of microorganisms produced biofilm such as *E coli,S aureus* isolated from intrauterine devices and its associated infection in vagina and cervix [27].

In study carried out by Ben Abdallah *et al*[28] 20 *Salmonella* strains were isolated from poultry meat and human,clinical isolates were delivered from laboratory of microbiology,university hospital Fattouma Bourguiba,Tunisia,80% of human isolates produce astrong biofilm,and only strain *Salmonella enteritidis* isolated from blood,urin and pus were positive for *sef* gene giving a617 bp band, whereas *Citrobacter freundii* was isolated from patients with urinary tract infections[29].

Van Rostenberghe [30] reviewed that *Pantoea* infections are uncommon in humans and most reports have involved adults or children after thorn injuries, reported the first clinical picture of systemic *Pantoea* spp .infection in neonates in intensive care unit caused by infected parenteral nutrition solutions.

Regarding to molecular study identification, *icaAD* genes which play an important role in biofilm formation, were considered in this study, and appeared in all the ten isolates, this came closely to the results of the authors in Terki *et al* [31] in which all bacteria in the study revealed these genes, like *Staphylococcus* spp., whereas other studies searched for another genes like *icaB,C,R* genes as recorded by the study of Solati *et al* [32] in regard with *Pantoea*. Concerning with *Proteus*, However; we have met with the work of Abd Al-Abbas [12] in which all bacteria were positive to *icaA* and/or *icaD*.

In respect to the site of ica*AD*, recent study showed these genes in the band 188bpand198bp along with ladder respectively, in which they identical to many studies such as Mirzaee *et al* [33], Seif EL-Din *et al* [34], EL-Amin *et al* [35] and disagree with others [7].

The agreement some times and the reverse in the others for the origin of isolates, genes responsible, and the sites of these genes, might be belonged to differences of strains, ecology surrounded and incidence of the genes mutation or else.

Conclusion

The public health laboratory of Misan province, received a variety of microbes from different hospitals and patients. Our study selected randomly ten isolates, which were genetically identified, and investigated for their ability to produce the biofilm through the appearing of *icaA* and *icaD genes*. However; this may increases the risk of these germs in the infection, which should be considered during the treatments of the diseases.

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