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Relationship between Ica gen and hemaaglutination in *Staphylococcus epidermidis* form biofilm

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Abstract

The research included isolated 50 *S. epidermidis* strains from clinical specimen (60% blood culture, 22% catheters urine, 12% wound and burn swab as well as 6% skin and nasal swab from hospital staff) from 427 patients at (Imam Ali, Al-Kendy, Al-Wasety, Ibn-Albalade and Baghdad) Hospital, In a period of study (February to July 2014). Identified and diagnostic these bacteria by APi 20, VIETK 2 system, as well as classical method. 34, 20, 10 and 2% respectively of the *S. epidermidis* isolates from blood culture, urine catheters, wound and burn swab as well as swab of Skin and nasal hospital staff produced biofilm phenotypically. Slime producing which detected by three phenotyping methods 60% of bacterial isolate were positive biofilm produced by microtiter plate, while 38 and 16% respectively were positive for both tube and CRA methods. The results indicated that the IcaA gene had the highest rate in blood culture (20 %) and Catheter urine specimen (10%) followed by IcaD gene which was detected in (16 %) of blood culture and 20% of Catheter urine specimen. The prevalence of IcaA gene (56%) was high versus that of IcaD gene (38%). 17 and 15 strain of *Staph. epidermidis* were biofilm-positive for Ica A and Ica D respectively by Mtp while 15, 12 respectively strain were positive to both IcaA and IcaD by tube methods. Hemagglutinating isolates were significantly more likely to be recovered in high number from blood culture(6 and 8) respectively strain comparing to catheters urine 5 and 6 strains respectively. Also this study appearance a strong correlation between hemagglutination (18 strain) and adherence to plastic (30 strain) and Ica A genes (22 strain).

Keywords: *Staph. epidermidis*, Biofilms, Ica (Intercellular adhesion), Hemagglutination.

Introduction

Staphylococcus epidermidis is the most commonly isolated bacteria from the skin as non-pathogenic in healthy people but people with compromised immunity are at risk for developing an infection (Farran *et al.,* 2013), also its a commensal and a nosocomial pathogen usually with an opportunistic role association with implanted foreign body materials (GustafPrag, 2014), it's recognized as important cause of disease in the world and generally hospital-acquired (Conlan *et al.,* 2012), through ability to form potent biofilms on adherent surfaces, thereby giving way to catheter related infections and heart valve associated infections as medical devices can get easily contaminated with these bacteria from the skin of hospital staff and visitors (Farran *et al.,* 2013). Increasing frequency of *Staph. epidermidis* as pathogen of nosocomial sepsis, and accounts for approximately 30% of all nosocomial blood stream infections (Piette, 2009) and catheter, therefore in recent years, a lot of work has been focused on this bacteria (GustafPrag, 2014), which a leading to cause biofilm-related infections, particularly, in patients with indwelling medical devices (Zhou *et al.,* 2013), biofilms play a key role in bacterial resistance against antibacterial agents an issue that causes multiple problems in medical fields, particularly with Staphylococcus biofilms that colonize medical indwelling devices (Elchinger *et al.,* 2014).

Biofilm form an important virulence factor of *Staphylococcus epidermidis*, were defined as the structural phenotype of microbial communities enclosed in the self-produced polymeric matrix (Ho and Seanghuoy, 2014), major part of the biofilm is called polysaccharide intercellular adhesion (PIA) encoded by the icaADBC genes (Ogara, 2001), among ica genes, icaA and icaD have been reported to play a significant role in biofilm formation in *S. epidermidis* and *S. aureus* (Nasr, 2012).

Different clones isolated from different clinical

sources can make a difference in the adhesion and/or ability to form biofilm (Chaieb, 2005). In recent years, new molecular techniques based on PCR for identification of virulent biofilm-forming strains, the detection of the genes governing the production of such extracellular polysaccharide and, in particular, the icaA and the icaD genes are a rapid and accurate technique for strain characterization (Muhammad, 2013).

Hemagglutinin plays a direct role in adherence to polymers and thus prosthetic-device infection or serves as an easily demonstrable marker for adherence and biofilm formation as well as essential for the pathogenesis of biomaterial-associated infections caused by *S. epidermidis* (Dietrich *et al.,* 1999), as well as PIA is necessary for the functional activity of the hemagglutinin of *S. epidermidis* (Heilmann *et al.,* 1996) .

The major objectives of this study were:

1-Isolation Staph.epidermidis bacteria from blood, catheter urine, wound and burn swab as well as skin and nasal swab from hospital staff and identification by culturing, Api E 20, Vitek 2 System as well as Bact for bacteria which isolated from blood.

2-*In vitro* study of qualitative and quantitative of biofilm which includes:-Formation of biofilm on microtiter plates, Tube method, Congo red agar, and comparative between these methods.

3-Determine the presence of the icaA and icaD in S. epidermidis from catheter urine, blood, burn and wound infections also skin and nasal swab from hospital staff were detected by PCR and specific primers.

4- Study the correlation between the presence of Ica A and biofilm production and haemagglutination assays (by solution erythrocytes).

Materials and Methods

Collection of samples: Catheter urine were taken from patients ,who suffering from indwelling bladder catheter, direct swab were taken from patients who suffering from Burn and Wound infections as well as Blood collected were taken from patients who suffering from septicemia and indicated found bacterial pathogenic by used Bact system.

Culturing of the samples.

Urine samples: Under aseptic conditions using standard bacteriological disposable plastic loop, 10µl of uncetrifuged urine were streaked on MacConkey and Blood agar plates and incubated at 37ºC for 24hrs, if no growth was detected, plates were re-incubated for another 24hrs before reported negative cultures.

Swabs: Swabs from burn and wound infection were cultured by streaking on blood and MacConkey agar, plates were incubated aerobically overnight at 37ºC, if no growth was detected, plates were re-incubated for another 24hrs before reported negative cultures.

blood culture: For blood samples, 2ml each was injected into blood culture bottles and incubated at 37°C in an automated blood culture system (BACTEC 9120), the incubation time (in hours) before the culture became positive was recorded. Each positive bottle was sub cultured onto blood and MacConkey agar plates and incubated at 37°C for 24–48hrs.

Biofilm study:

Phenotypic characterization of slime-producing bacteria: Qualitative detection of the phenotypic production of biofilm formation by all strains was studied by

Detection of Biofilm by Culturing on Congo red agar plates (CRA): The morphology of the colonies and their phenotypic changes were studied using CRA cultures which composed from: 37g/l of brain–heart infusion broth,36 g/l of sucrose,15 g/l of agar agar, 0.8g/l of Congo red ,All these ingredients were dissolving in 900 ml of D.W. excepted congo red stain and autoclaving . Congo red stain was dissolved in 100 ml of D.W and autoclave, it was added when the agar had cooled to 55°C and poured in sterile petri dishes, plates with Congo red medium were incubated aerobically for 24hrs at 37°C to obtain single bacterial colonies, CRA-positive strains appeared as black colonies with a rough, dry and crystalline consistency on CRA, while CRA-negative strains remained red, smooth colonies with a darkening at the center(chaieb, 2005).

Detection of Biofilm by Tube Method (TM): Biofilm production was investigated by the tube adherence test proposed by (Christensen *et al.,* 1985). Ten ml Trypticase soya broth with 1% glucose was inoculated with the test organism on nutrient agar individually, broth were incubated at 37 ºC for 24 hrs , then cultures were aspirated and tubes were washed with phosphate buffer saline pH 7.3. The tubes were dried and stained with 0.1% crystal violet. Excess stain was removed. Tubes were dried in inverted position. In positive biofilm formation, a visible stained film was seen along the walls and bottom of the tube.

Detection of biofilm by microtiter plate method (Mtp) or spectrophotometric method: Quantitation of biofilm which measures the total biofilm biomass (bacterial cells and extracellular matrix) were performed according to (Eftekhar and Speert, 2009). The isolates were cultured in Muller-Hinton agar (Oxoid, Basingstoke, UK) for 24hrs at 35ºC and 20µl inocula of bacterial suspensions were prepared

(0.5 McFarland),and added to each well of a sterile 96-well polystyrene flat-bottom microtitre plate (Costar 3599, Corning, NY, USA), wells were filled with 180µl of trypticase soy broth (TSB) medium (Oxoid; Basingstoke, UK) supplemented with 0.25% glucose for Staphylococcus epidermidis and incubated the plates for 18hrs at 35ºC, then the wells rinsed three times with sterile saline to remove non-adherent cells, attached bacteria were fixed with methanol for 20min and dried for 30min at room temperature , then staining the bacteria with Crystal violet (0.5%) for 15min and eluted biofilm with ethanol for 30min without shaking and by microtitre plate reader (Behring EL 311, Hoechst, Akasaka, Japan) measurement the absorbance at 450 nm.

Genetic Technique: In order to detect genes related to biofilm formation, bacterial cultures were lysed, DNA extracted, and gene specific primers were used to PCR amplify DNA fragments.

The DNA extraction method After overnight culture on brain-heart infusion agar plates, one or two colonies from a *S. epidermidis* isolate were suspended in 20ml of sterile distilled water, and the suspension was then heated at 100ºC for 20 min. From this suspension, a 5-ml aliquot was directly used as template for PCR amplification.

Detection of IcaA and IcaD loci: The presences of IcaA, IcaD DNA were detected by polymerase chain reaction (Arciola, 2001), primers were synthesized by Koma Biotech Inc. (Kore).For the detection of IcaA (188-bp), the forward primer had the following sequence: 5′-TCT CTT GCA GGA GCA ATC AA; and 5′-TCA GGC ACT AAC ATC CAG CA was used as a reverse primer. The primer sequences for IcaD (198-bp) were: forward, 5′-ATG GTC AAG CCC AGA CAG AG; and reverse, 5′-CGT GTT TTC AAC ATT TAA TGC AA. The Gene Ruler 100 bp DNA ladder was used as a DNA size marker, the reaction volume was 50 µL containing PCR buffer 5µl (10mM), the forward and reverse primers (1µl each), together with 1 µl of the extracted DNA, 1µl of dNTP, 1µl of Taq DNA polymerase, and 40µL of double distilled H2O (ddH2O). A thermal step program was used, including the following parameters: incubation at 94°C for 10min, followed by 30 cycles at 94°C for 1min (denaturation), 55°C for 30sec (annealing), and 72°C for 10min after conclusion of the 30 cycles. Amplification products were analyzed using 2% agarose gel during 50min at 80V. The bands were stained with ethidium bromide (0.5µg/ml), after electrophoresis, gels were seen under ultraviolet (UV) light.

Hemagglutination assays

Erythrocytes: Human blood collected with heparin was used to retrieve erythrocytes, by adding 5 ml of blood to 45 ml of saline solution and centrifuged twice at 2500 g for 10 min. Next, added 100 µl of the pellet to 10 ml of a saline solution, obtaining a 1% erythrocyte solution which using for the hemagglutination assays.

Hemagglutination assay: Hemagglutination assay was performed as described elsewhere (Gill *et al.,* 2006) with some modifications. Briefly, *S. epidermidis* cells from an overnight culture in 37°C in trypticase soy broth were grown in fresh TSB supplemented with 0.25% glucose for 18 hrs by centrifugation were harvested and washed once with PBS containing 0.1% bovine serum albumin, The bacterial resuspended in saline, were adjusted to a McFarland standard of 3.0 in PBS with 0.1% BSA, which correlated with $\sim 10^8$ bacteria/ml.

Then 100 µl of the 1% erythrocyte solution was added to each well of (U-shaped) microtiter plates and to ensure thorough mixing of bacteria and erythrocytes, the total volume of each well was pipetted in and out with a micropipette, incubated at room temperature for 2 hrs. Hemagglutination titer were evaluated macroscopically, Erythrocytes that appeared to be negative for macroscopic hemagglutination were also evaluated microscopically. All experiments were done in duplicate with 3 repeats.

Analysis of data: Statistical analysis was performed by means of SPSS 17.0 (SPSS Inc., Chicago, USA) software. Comparisons between groups were performed using the chi-square test.

Results and Discussion

Biofilm assay:

Detection of slime-production by S. epidermidis isolates

Ability of S. epidermidis to form Slime can be inferred by phenotypic characteristic when grown on Congo red agar as Seen in Figure -1 which showed Phenotypic production and non-produced slime by all investigated strains (from Blood, Catheter urine specimen, Wound and burn swab, as well as Skin and nasal hospital staff swab).

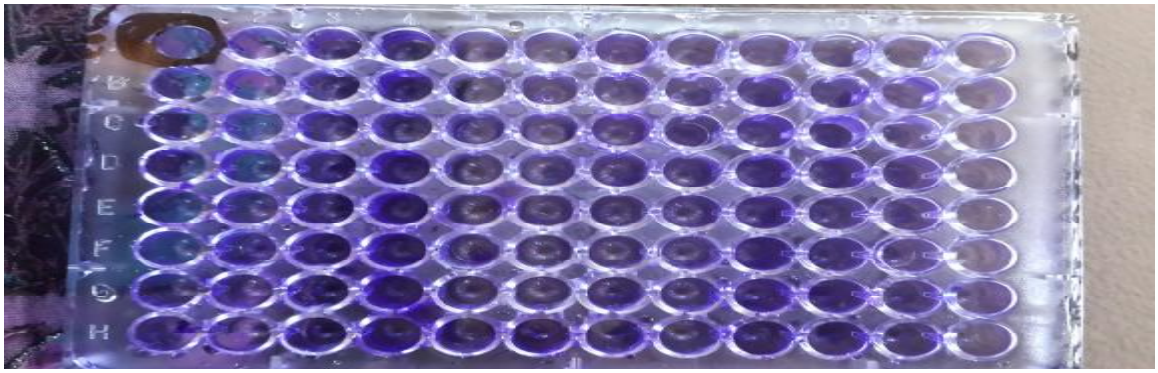
For the biofilm production By Congo red agar method, black color colonies were observed, 8 isolates gave black colors on Congo red agar while 42 isolates gave pink color indicating as non-biofilm production, also by tube method, visible thick film were obtained inside and bottom of tubes, 19 isolates were shown thick film inside the wall of the tube indicating strong biofilm production while 31 isolates were not shown biofilm formation, this results contradict with Rewatkar who showing the Congo red agar method gave 90% strong biofilm production as compared to the Tube Method (83%)(Rewatkar, 2013).

Figure (1): A- Biofilm producer and no producer on Congo-red agar B- Biofilm production by tube method and C- Tissue culture plate method for detection of biofilm production.

A-Biofilm producer and non-producer on Congo-red agar

B- Biofilm production by tube method
1- High biofilm producer 2- moderate biofilm producer 3- non biofilm producer

C- TCP method



While Ruzicka *et al.* (2004)noted that out of 147 isolates of *S. epidermidis*, CRA in 64 (43.5%) isolates and TM detected biofilm formation (53.7%) and showed for biofilm detect, TM is better than CRA, while Baqai *et al.* (2008) tested TM to formation among uropathogens, according to their results, 75% of the isolates exhibited biofilm formation. By microtiter plate assay, 60% of strains were biofilm producers and 40% of them were non or weak biofilm producers, while Mathur *et al.,* (2006) Showed that out of 152 isolates tested, the numbers of biofilm producers identified by TCP method were 53.9%, and non-biofilm producers were 46%. Microtiter plate method was more sensitive for slime layer detection as rate of production was 51.42% from all isolates when compared with 31.42% positive results in Congo red agar method (khuder, 2013). Poliana show the results of the microtitre plate test was better than the CRA test in the detection of biofilms in vitro, because of it's a higher sensitivity (100%)(Poliana 2013), both methods tissue culture plate (TCP) and CRA assay can used to identify Staphylococci biofilm-producing strains, but TCP is the gold standard (Jain and Agarwal, 2009). But when phenotyping method were compared with the P.C.R test, the results indicated that after p.c.r ,the TCP test should be the first choice because this test was more sensitive than the other methods, this result is compatibility with many study as (Jain and Agarwal, 2009; poliana, 2013).

Table (1): Biofilm produced by three phenotype method according to source of *Staph. epidermidis.*

Source of *Staph. epidermidis*	No. of isolate	Biofilm producer	CRA	Tube method	M.T.P	No. of biofilm produced (٪)
Blood	30(60)	17(34)	5	6	17	13(26)
Catheter urine	11(22)	10(20)	1	10	7	1 (2)
Wound and burn swab	6(12)	5(10)	1	3	5	1 (2)
Skin and nasal swab	3(6)	1(2)	1	0	1	2(4)
Total	50(100)	33(66)	8(16)	19(38)	30(60)	17(34)

In this study showed 16% of the *S. epidermidis* isolates produced black colonies on the Congo red agar (CRA)(Figure 1) which similarity with results of Mertens (2013), But Hassan *et al.,* (2011) showed their 52% *Staph. epidermidis* isolated from catheter urine ability to formed biofilms by C.R.A, while Gamal *et al.,* (2009) show 88.6% of *S. epidermidis* were biofilm producers, as the results of Arslan and Ozkarde (2007) explained that CRA method demonstrated positive results in 38.5% of staphylococci isolated from clinical specimens. *S. epidermidis* isolates, which were gained from blood by (34%), (20%) of the isolates from urine catheters, wound and burn swab produced biofilm phenotypically by 10 % also 2% from swab of skin and nasal hospital staff, while (26, 2 and 2) % respectively of the *S. epidermidis* isolates from blood culture, urine catheters and wound and burn swab as well as (4%) from swab of skin and nasal hospital staff produced no biofilm phenotypically. Biofilm production is considered to be less in case of samples collected from healthy skin when compared to those collected from people associated with infections(Arciola, 2001) as study reported that 44.2% of the samples that were collected from patients were strong biofilm-producers compared to 0% of the samples that were isolated from healthy volunteers (Mateo, 2007).

In recent years, *S. epidermidis* has been recognized as a major nosocomial pathogen, mainly due to its ability to adhere and produce biofilms which considered virulence marker for *S. epidermidis* (Arciola, 2001). This conflicts with the results of Al-Omare *et al.,* (2013) were showen 66.6% of *Staph. epidermidis* has ability to produce biofilm, while 33.3% non-biofilm producing.

Table(2) showed prevalence of IcaA, IcaD genes were 56%, 38% respectively , The results indicated that the IcaA gene had the highest rate in blood culture (20 %) and catheter urine specimen (10%) followed by IcaD gene which was detected in 16 % of blood culture while 8% of Catheter urine specimen. The prevalence of IcaA gene (56%) was highly versus that of IcaD gene (34%). Several studies have shown formation of biofilm in staphylococci causing catheter associated and nosocomial infections is associated with the presence of both icaA and icaD genes (Satorres, 2007), co-expression of these genes are necessary for the full phenotypic expression of biofilm in clinical staphylococcal isolates (Oliveira, 2013). In *S. epidermidis* detection Ica locus is a good predictor of biofilm formation for distinguishing blood and catheter related infecting organisms from contaminating bacteria (Aricola *etal.,* 2005). Satorres (2007) reported that 42.2% of *Staph. epidermidis* isolated from blood and intravascular catheter were positive for IcaA and Ica D genes.

In Eftekhar (2009) study showed 30% of the samples collected from patients were ica positive compared to 8% of the samples collected from healthy individuals, In another study, 53% of the samples isolated from patients were ica positive compared to 0% of samples collected from healthy volunteers (El-Din, 2000).

Table (2): Presence and absence of each Ica gene in clinical and non-clinical strains.

Sources of *S. epidermidis* strain	Number of isolate	IcaA +(%)	IcaA _(%)	IcaD +(%)	IcaD _(%)
Blood	30	10(20)	20(40)	8(16)	22(44)
Catheter urine specimen	11	5(10)	6(12)	4(8)	7(14)
Wound and burn swab	6	5(10)	1(2)	5(10)	1(2)
Swab of Skin and nasal hospital staff	3	2(4)	1(2)	2(4)	1(2)
Total	50	22 (56)	28 (34)	19 (38)	31(62)

Table (3): Relationships between phenotype (biofilm production in three methods) and genotype (presence of Ica A, Ica D genes)

Presence/absence	CRA		Tube method		Microtiter plate	
genes	(+)	(-)	(+)	(-)	(+)	(-)
Ica A (+)	7	-	15	0	17	5
IcaA (-)	1	42	4	31	13	15
Total	8	42	19	31	30	20
Ica D(+)	7	1	12	7	15	4
IcaD(-)	1	41	7	24	15	26
Total	8	42	19	31	30	30

Results of colony phenotype on CRA plates showed that 7 isolates formed black colonies and were potential biofilm producers and positive for both ica genes (A and D) (Figure 1). In total, 15 and 12 strain of *Staph. epidermidis* were positive for both (Ica A and Ica D) and produced biofilm by tube method while 17 and 15 strain were positive to both IcaA and IcaD by Mtp methods. This study shown the presence of the Ica genes did not always correlate with biofilm production. Silva *et al.,* (2002) reported that shown only 59% of *S. epidermidis* strains positive for the Ica operon were biofilm producers by CRA method, so Cafiso *et al.,* (2004) demonstrated that 83.3% of the Ica-positive isolates produced biofilm by Mtp method , while Yazdani *etal.,* (2005) reported that only 54% and 52% of Ica positive were also positive by CRA and MTP methods respectively. Arciola *etal.,* (2005) reported that (IcaA/IcaD+)/MTP- strains represented 8%, however (IcaA/ IcaD)/MTP+ strains were 16%. Ica positive S. epidermidis in Chennai population which is an alarming finding since Ica operon is associated with strong biofilm formation. Several studies have reported a higher frequency of distribution of the ica locus in clinical isolates of S. epidermidis (Arciola *etal.,* 2002),While biofilm formation by strains that did not have the ica genes in this study could be explained by the presence of other genes, such as (bap) , which codes for the biofilm-associated protein (Bap), which can compensate for a deficiency of ica genes(Cucarella *etal.,* 2001). Muhammad (2013) study showen All S. epidermidis slime positive isolates were icaA positive, There was a greater correlation between the presence of both icaA and icaD and the slime production than the single expression of icaA or icaD and the presence of slime in all groups. Also present study showed five and four *S.epidermidis* strain were negative biofilm produced by MTP although possession Ica A and Ica D, that's mean Ica genes didn't always correlate with biofilm production. , but De Silva (2002) showed the relationships between presence of the Ica operon and phenotype. Of the 49 *S. epidermidis* strains found positive for the Ica operon, 29 (59%) were found to be biofilm producers by the CRA method , as well as all of the 73 *S. epidermidis* strains which lacked the Ica operon were found to be CRA negative (Gamal, 2009). Poliana (2013) was founded low correlation between the results of the PCR-based analysis and the CRA test, this finding indicates that the CRA test produces a high number of false negatives.

This is in contrast with other studies which, reported that the Dhanawade *et al.* (2010) showed results CRA and microtitre plate tests that were significantly correlated with the molecular analysis, While Jain & Agarwal (2009) showed the presence of the genes was best correlated with a positive CRA test. The hemagglutinin of *S. epidermidis* may play a major Role in the adherence of this organism to polymer containing biomaterials, thus hemagglutinin of *S. epidermidis* may be a virulence factor in pathogenesis of infection, hemagglutinin of *S. epidermidis* with the erythrocytes which detected macroscopically (Figure 2). The ability of *S. epidermidis* to mediate hemagglutination of erythrocytes was shown to be associated with the ability to adhere to plastic and to produce biofilm and therefore may be important for the pathogenesis of *S. epidermidis* infections (Rupp *et al.,* 1995). Cerca *et al.,* (2004) showed in his results *S. epidermidis* isolates were able to cause haemagglutination, as well as hemagglutination of erythrocytes is a common property of *Staphylococcus epidermidis* strains, which is related to adherence and biofilm formation and may be essential for the pathogenesis of biomaterial-associated infections caused by *S. epidermidis* (Dietrich *et al.,* 1999). PNAG/PIA also can agglutinate erythrocytes (Rupp and Archer, 1992) and a direct relation was found between the ability of a strain of *S. epidermidis* to agglutinate erythrocytes and to form a biofilm (Cerca *et al.,* 2005).

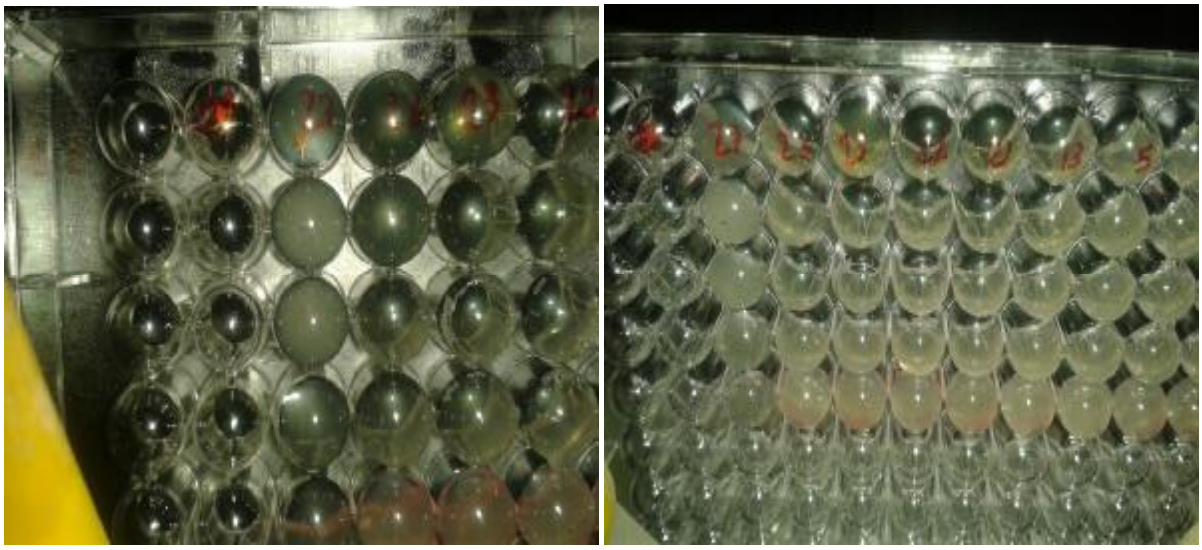


Figure (2): hemagglutination among staph.epidermidis isolates responsible for infection.

Table (4): Relationship between biofilm formation by Mtp method, hemagglutination assay and Ica A gene.

Source of *S. epidermidis*	Mtp Method (+)	Ica A (+)	Hemagglutination assay			
			Macroscopically		Microscopically	
			+	-	+	-
Blood	18	10	6	12	8	10
Catheter urine	6	5	5	1	6	0
Wound and burn swab	5	5	2	3	3	2
Swab of skin and nasal hospital staff	1	2	0	1	1	0
Total No. (%)	30	22	13	17	18	12

Hemagglutination is the aggregation of erythrocytes caused by bacteria adhering to Erythrocytes, which commonly used assay to demonstrate bacterial adherence Also, hemagglutinin may play a role in the pathogenesis of infections or may serve as Alternative marker for adherence isolates.

Table results showed 13 and 18 of the 30 strains, were the relationship between positive both (Macroscopically and microscopically) hemagglutination assay respectively and biofilm formation (30)strain, This is entirely consistent with Cerca *et al.,* (2009) in his studies have shown For 9 of the 11 strains, was derived describing the relationship between biofilm formation and hemagglutination. Hemagglutinating isolates were significantly more likely to be recovered in high number from blood culture (6 and 8) respectively strain comparing to catheters urine (5 and 6) strains respectively. Also this study appearance a strong correlation between hemagglutination (18 strain) and adherence to plastic (30 strain) and Ica A genes (22 strain). This is entirely consistent with the results of Paul *et al.,* (1999) who showed that relationship between biofilm formation and hemagglutination in *Staphylococcus epidermidis*, and the presence of icaA, a strong association existed between biofilm formation, and hemagglutination. PIA and Ica-containing plasmid, is not only able to mediate biofilm formation on glass but is also able to mediate hemagglutinationas , Therefore, PIA is involved in both intercellular adhesion between individual *S. epidermidis* cells and adherence to erythrocytes, causing hemagglutination, this may allow *S. epidermidis* to colonize biomaterials that are associated with host cellular milieu, including erythrocytes, so Fey, (1999) showing the strong between biofilm formation, which has been linked to strains that produce polysaccharide intercellular adhesion (PIA), and hemagglutination.

PIA as the hemagglutinin of *S. epidermidis* or at least as a major functional component (Dietrich *et al.,* 1999), the mechanism of hemagglutination mediated by PIA could be related to co- interactions of the differentially charged polysaccharide species with the negatively charged surface of the erythrocyte, but purified PIA inhibits hemagglutination, PIA might interact with a specific

receptor for the hemagglutinin on the erythrocyte surface (Gerke *etal.,* 1998).

Cerca *et al.* (2005), Showed all strains forming biofilms were able to agglutinate erythrocytes , and the ability of *S. epidermidis* to produce biofilm on plastic surfaces has capacity to mediate hemagglutination of erythrocytes (16 biofilm-positive/hemagglutination-positive strains and 19 biofilm-negative/hemagglutination-negative strains) within the 39 clinical strains tested (Fey *et al.,* 1999). Adherence to biomaterials and form biofilm is thought to be pivotal in the pathogenesis of prosthetic device infection by *Staphylococcus epidermidis* and strong association of hemagglutination with adherence and biofilm production (Rupp *et al.,* 1995), therefore may be important for the pathogenesis of *S. epidermidis* infections (cerca, 2005).

Conclusions

Staph. epidermidis isolated from blood culture, urine catheters specimen, wound and burn infection as well as swab of skin and nasal hospital staff were capable to forming biofilm. The ability of *S. epidermidis* to hemagglutination of erythrocytes is associated with the ability to produce biofilm and therefore may be important for the pathogenesis of *S. epidermidis* infections

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