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EVALUATION OF GENOTYPE AND PHENOTYPE METHODS FOR DETECTION OF BIOFILM FORMED BY *STAPHYLOCOCCUS EPIDERMIDIS ISOLATED FROM PATIENTS IN SOME HOSPITALS OF BAGHDAD CITY*

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ABSTRACT

Coagulase-negative staphylococci have important role as pathogen and most common cause of bacteremia related to indwelling device, S. epidermidis was the most frequently isolated species in blood cultures and most important of the skin flora, it can cause severe infections after penetrates anatomic barriers, because it produces various proteases, peptidases, surface lipoproteins that promote host tissue adherence and biofilms. 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 480 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. Slime producing which detected by three phenotyping methods 60% of bacterial isolate were positive biofilm produced by microtiter plate, while (38, 16)% respectively were positive for both tube and CRA methods. While (34,20,10,2)% 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.The results indicated that the IcaA gene had the highest rate in blood culture (34 %) and Catheter urine specimen (20%) followed by IcaD gene which was detected in 38 % of blood culture and 20% of Catheter urine specimen. The prevalence of IcaA gene (66%) was low versus that of IcaD gene (72%). 27, 28 strain of staph.epidermidis were biofilm-positive for Ica A and Ica D respectively by Mtp while 15 strain were positive to both IcaA and IcaD by tube methods. Most positive both

genotype (Ica A, Ica D) and biofilm produced Were 28(56%) while negative for both of them and negative biofilm produced were 13(26%), While 5(10%) were positive for Ica A / D and negative for phenotype (biofilm produce). In relation of S.epidermidis, 66% (29/50) of clinical strain present Ica genes and slime producing. While four only out of 50 clinical strain are producing slime but losing the Ica genes also 26% (13/50) of clinical strain were non-slime producing and Ica genes were negative.

KEYWORDS: *Staph. Epidermidis*, biofilms, Ica(Intercellular adhesion).

INTRODUCTION

Staphylococcus epidermidis is part of the normal microbiota of the human skin, but it is leading cause device-associated infections in critically ill patients, Commensal and clinical *S. epidermidis* isolates differ in their ability to form biofilms on medical devices. [Kozitskaya,2005; Samah,2013]**.** It is recognized as an important nosocomial pathogen, especially associated with individuals a compromised immune system as cancer patients and with implanted foreign body materials as heart valves [Orebro, 2011],The pathogenesis of S. epidermidis in device associated infections mostly relies on the potential of the bacterium to adhere to the device surface[Sujata, 2012].

Increasing frequency of *Staph. epidermidis* as pathogen of nosocomial sepsis, and accounts for approximately 30% of all nosocomial blood stream infections [knobloch, 2001] and catheter, more than 60% of all infections are caused by biofilm [Kim *et al*., 2001].

Therefore in recent years, a lot of work has been focused on this bacteria which a leading cause of biofilm-related infections, particularly, in patients with indwelling medical devices [Ziebuhr, 2001].

Biofilms are heterogeneous mixtures of bacteria that are held together by a secreted matrix called extracellular polymeric substances (EPS)[Zegan , 2002]. biofilm an important stage in the pathogenesis of numerous bacterial species[Donlan2001] by helps the bacteria to form stable communities of protection rather than live as freeplanktonic cells [Yarwood ,2003], as well as is the most important factor for the establishment of S. epidermidis as a nosocomial pathogen[Hansan et al., 2007], Also biofilm form represents an important virulence factor of Staphylococcus epidermidis , which ability these bacteria to aggregate.

The biofilm made of carbohydrate and protein molecules, major part of the biofilm is called polysaccharide intercellular adhesion (PIA) encoded on the *Ica* gene operon [Wojtyczka 2014].

The intracellular adhesion (*Ica*) locus consists of the four genes *IcaABCD*, these genes encode proteins mediating the synthesis of the PIA, and the *IcaA* gene is encoding the N-acetylglucosamyltransferase. This enzyme is not very active *in vitro*, but co-expression of the *IcaD* gene due to increases the activity[Mirzaee.etal.2014].

In the case of staphylococci, formation of the biofilm requires polysaccharide intercellular adhesion (PIA), which is synthesized by enzymes encoded by the intercellular adhesion cluster (*Ica*) [Gotz, 2002].

Among Ica genes, *IcaA* and *IcaD* have been reported to play a significant role in biofilm formation in *S. epidermidis and S. aureus* [yazdani,2006].The main components of EPS in *Staphylococcus epidermidis are* Polysaccharide intercellular adhesion (PIA) and capsular polysaccharide/adhesion (PS/A).

The bacteria communicate with each other by production of chemotactic particles or quorum sensing (pheromones), influence biofilm formation,[Thomas *et al*., 2007].

The other very important factor in establishment of S. epidermidis as nosocomial pathogen is methicillin resistant, is known to be associated with the presence of the mecA gene, which encodes a penicillin-binding protein with low affinity for b-lactam antibiotics (PBP2A), The mecA gene is carried by a genetic mobile element called the staphylococcal chromosomal cassette mec (SCCmec) [Ziebuhr et al., 2001], five major SCCmec types have been identified, and SCCmec shown to be transferable among staphylococcal species.

Aim of the study

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

2-In vitro study of 3 phenotypes of biofilm which includes:-Formation of biofilm on microtiter plates, Tube method and Congo red agar.

3- Determine the presence of the IcaA and IcaD genes in S. epidermidis from catheter urine , blood, burn and wound infections was detected by PCR and specific primers.

4- Study the correlation between the presence of IcaA, IcaD with biofilm production.

MATERIAL AND METHODS

1- Congo red agar (CRA): This media used to detection and identification slime producing by staphylococcus epidermidis. It is composed of: 37 g/L of brain–heart infusion broth,36 g/L of sucrose,15 g/L of agar agar, 0.8 g/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 55C and poured in sterile petri dishes, plates with Congo red medium were incubated aerobically for 24 hours 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.

2-Primers

10µl (100µM) of each primer was added to a 0.2 ml tube then 90µl of nuclease free D.W. was added to get 100µl of 10µM primer solutions.

3- Patients and controls

3.1. Patients

Four hundred and eighty (480) patients were included in this study which was carried out in the (Kindy, Imam Ali, Al- Wasete, Ibn-Albalady, Baghdad) Hospitals in Baghdad from February -2014 to july-2014.

3.2. Controls

Fifty (50) samples were collected from of skin and nasal hospital staff.

3.3. Grouping of patients

Patients were divided into three groups as the following: urine catheter septicemia, wound and burn infections, blood septicemia.

3.4. Sampling

3.4.1. 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.

3.4.2. Culturing of the samples.

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

3.4.2.2. 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 24 hours before reported negative cultures.

3.4.2.3 blood culture For blood samples, 2 ml 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 subcultured onto blood and MacConkey agar plates and incubated at 37 °C for 24–48 h.

3.5 Biofilm study

3.5.1.Phenotypic characterization of slime-producing bacteria

Qualitative detection of the phenotypic production of biofilm formation by all strains was studied by.

3.5.1.1 Detection of Biofilm by Culturing on Congo red agar plates (CRA) [*chaieb2005*]. The morphology of the colonies and their phenotypic changes were studied using CRA cultures as previously described. Briefly, plates with Congo red medium were incubated aerobically for 24 hours 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.

3.5.1.2 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 hours. The cultures were aspirated and the 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.

3.5.1.3 Detection of biofilm by microtiter plate method (Mtp)

Quantitation of biofilm was performed by a spectrophotometric method, which measures the total biofilm biomass(bacterial cells and extracellular matrix), Biofilm formation assays and the characterization of the isolates were performed according to[Eftekhar and Speert,2009] The isolates were cultured in Muller-Hinton agar (Oxoid, Basingstoke, UK) for 24 h at 35ºC and 20 µL inocula of bacterial suspensions were prepared (0.5 McFarland). These inocula were added to each well of a sterile 96-well polystyrene flat-bottom microtitre plate (Costar 3599, Corning, NY, USA). 3- the wells were filled with 180 µL of tryptIcase soy broth (TSB) medium (Oxoid; Basingstoke, UK) supplemented with 0.25% glucose for *Staphylococcus epidermidis*, The plates were incubated for 18 h at 35ºC. 4- remove non-adherent cells, the wells were rinsed three times with sterile saline. 5- The attached bacteria were fixed with methanol for 20 min and dried for 30 min at room temperature.6- Crystal violet (0.5%) was used to stain the bacteria for 15 min. 7-The biofilm was eluted with ethanol for 30 min without shaking. 8-The absorbance at 450 nm was measured in a microtitre plate reader (Behring EL 311, Hoechst, Akasaka, Japan).

3.6 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 as previously described.

3.6.1 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 20 ml 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.

3.6.2 Detection of IcaA and IcaD loci

The presence of *IcaA, IcaD* DNA were detected by polymerase chain reaction using forward and reverse primers for *IcaA, IcaD,* as described previously [Arciola,2001]. From this suspension, a 5 µl aliquot was directly used as a template for PCR amplification, The sequences of *icaA* and *icaD* were taken from the GenBank sequence database of the National Center for Biotechnology Information. Primers specific for *icaA* and *icaD* were picked on the gene sequences by the Primer3 program. The 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, and visualized under ultraviolet transillumination. The reaction volume was 50 µL containing PCR buffer 5 µL (10 mM), the forward and reverse primers (1 µL each), together with 1 µL of the extracted DNA, 1 µL of dNTP, 1 µL of *Taq* DNA polymerase, and 40 µL of double distilled H2O (ddH2O). A thermal step program was used, including the following parameters: incubation at 94°C for 10 minutes, followed by 30 cycles at 94°C for 1 minute (denaturation), 55°C for 30 seconds (annealing), and 72°C for 10 minutes after conclusion of the 30 cycles. Amplification products were analyzed using 2% agarose gel during 50 min at 80V. The bands were stained with ethidium bromide (0.5 µg/ml), after electrophoresis, gels were seen under UV light.

2.7 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. The Pearson correlation coefficient (r) and its significance (P) were calculated between groups; probability values less than 0.05 were considered statistically significant.

RESULTS

Biofilm assay

Detection of slime-production by *S. epidermidis* isolates

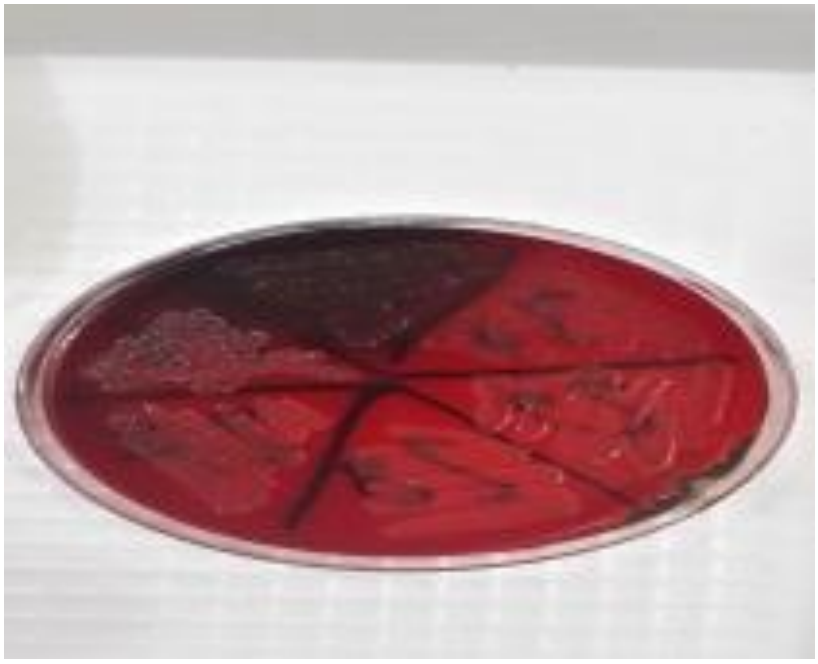
Ability of *S. epidermidis* to form Slime can be inferred by phenotypic characteristic when grown on Congo red agar. Colonies of strains that produce Slime strains form rough, black colonies, while the colonies that do not produce slime are red in color, as those seen in Figure 1 which showed Phenotypic 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) 8(16%) S. epidermidis were slime producers and the remaining 42(84%) strains were non-slime producers on Congo red agar (in figure 1 and table 2).

Table- 1 Prevalence of Staph.epidermidis from study groups.

Study groups	No. of bacterial isolated	%
Blood	30	60
Catheter urine specimen	11	22
Wound and burn	6	12
Skin and nasal hospital staff	3	6
Total	50	100

The staph.epidermidis were isolated mostly from blood (60%) followed by Catheter urine specimen (22%), Wound and burn swab (12%) as well as (6%) from swab of Skin and nasal hospital staff.

A1-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

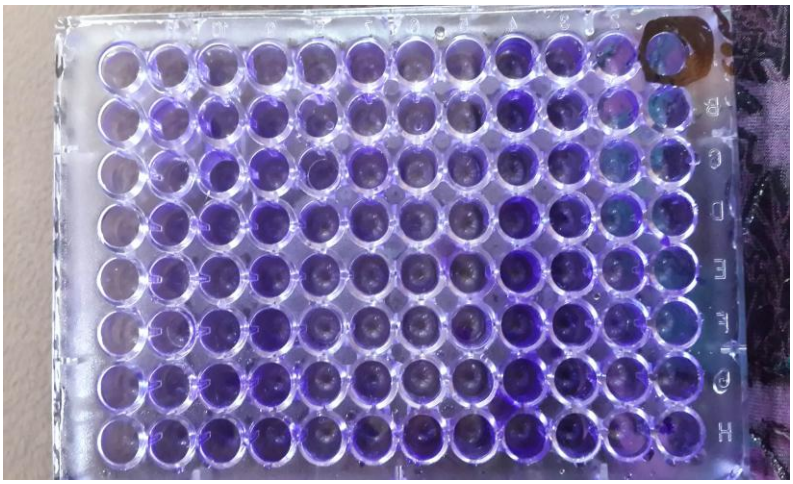


Figure 1 (a) Biofilm producer and no producer on Congo-red agar (b) Biofilm production by tube method with positive and negative control and (c) microtiter plate method for detection of biofilm production.

Table -2 Detection biofilm of staph.epidermidis by phenotyping methods.

Phenotyping methods	Positive number (%)	Negative number (%)	Total
CRA	8 (16)	42 (84)	50 (100)
Tube method	19 (38)	31 (62)	50 (100)
Microtiter plate	30 (60)	20 (40)	50 (100)

Slime producing which detected by three phenotyping methods 60% of bacterial isolate were positive biofilm produced by microtiter plate , while (38, 16%) respectively were positive for both tube and CRA methods .in same time table 2 showed negative result as (84, 62, 40%) respectively for CRA, Tube and microtiter plate method.

Table -3 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	17(34)	5	6	17	13(26)
Catheter urine	11	10(20)	1	10	7	1 (2)
Wound and burn swab	6	5(10)	1	3	5	1 (2)
Skin and nosal swab	3	1(2)	1	0	1	2(4)
Total	50	33(66)	8(16)	19(38)	30(60)	17(34)

Also founded (16%) of the *S. epidermidis* isolates produced black colonies on the Congo red agar (CRA) and were therefore classified as biofilm producers. *S. epidermidis* isolates, which were gained from blood by (34 %), (20 %) of the isolates from urine catheters as well as

wound and burn swab produced biofilm phenotypically by 10 % so (26, 2, 2)% respectively of the *S. epidermidis* isolates from blood culture, urine catheters, wound and burn swab as well as (4%) from swab of skin and nasal hospital staff produced no biofilm phenotypically.

PCR detection of *IcaA, IcaD* All biofilm and non-biofilm producing *S. epidermidis* (50 isolate) were performed by PCR detection genes which included the IcaA, *IcaD* genes for identify and confirm biofilm producing strains. It was found that some biofilm producing staphylococci strains were positive for *IcaA also positive for IcaD* genes, *giving 188 bp and 198 bp band for the IcaA and IcaD* (66,72)% respectively slime producing *S. epidermidis* isolates was positive for both IcaA and IcaD (Table-4). 17, 19 of 30 *S. epidermidis* isolated from blood culture were found to be positive for both *Ica (A &D*).

Table -4 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	17(34)	13(26)	19(38)	11(22)
Catheter urine specimen	11	10(20)	1(2)	10(20)	1(2)
Wound and burn swab	6	5(10)	1(2)	5(10)	1(2)
Swab of Skin and nasal hospital staff	3	1(2)	2(4)	2(4)	1(2)
Total	50	33(66)	17(34)	36(72)	14(28)

In table 4 showed prevalence of IcaA, IcaD genes were (66, 72)% respectively , The results indicated that the IcaA gene had the highest rate in blood culture (34 %) and Catheter urine specimen (20%) followed by IcaD gene which was detected in (38 %) of blood culture and (20%)of Catheter urine specimen. The prevalence of IcaA gene (66%) was low versus that of IcaD gene (72%).

Table -5 Relationships between phenotype (biofilm production in three methods) and genotype (presence of Ica A, Ica D genes)

Presence / absence genes	CRA		Tube method		Microtiter plate	
	(+)	(-)	(+)	(-)	(+)	(-)
Ica A (+)	7	-	15	0	27	5
IcaA (-)	1	42	4	31	3	25
Total	8	42	19	31	30	20
Ica D(+)	7	1	15	2	28	4
IcaD(-)	1	41	4	17	2	26
Total	8	42	19	19	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 2). Of the organisms which formed pink colonies on CRA plates.

In total, 15 strain of staph.epidermidis were positive for both (Ica A and Ica D) and produced biofilm by tube method while 27,28 strain were positive to both *Ica*A and *Ica*D by Mtp methods.

Table -6 Relationship of phenotype and genotypic biofilm production

Genotype ***Ica A / D***	**Biofilm**		
	Positive (%)	**Negative (%)**	**Total**
Positive 33(66)	28(56)	5(10)	33(66)
Negative17(34)	4(8)	13(26)	17(34)
Total 50(100)	33(66)	17(34)	50

In table 6 showed most positive both genotype (Ica A, Ica D) and biofilm produced Were 28(56%) while negative for both of them and negative biofilm were 13(26%),While 5(10%) were positive for Ica A / D and negative for phenotype (biofilm produce). In relation of S.epidermidis, 66% (29/50) of clinical strain present Ica genes and slime producing. While four only out of 50 clinical strain are producing slime but losing the Ica genes also 26% (13/ 50) of clinical strain were non slime producing and Ica genes were negative, as well as table showed all biofilm negative strain were negative for both genes .

DISSCUSION

Coagulase-negative staphylococci (CoNS) are a major component of the normal microbial flora of humans and are often found as contaminants in clinical specimens [Rupp, 1994]. The problem has been intensified with surgical wound infections, increased use of prosthetic and indwelling devices and the growing numbers of immunocompromised patients in hospitals [Andremont, 2003]. *S epidermidis* is a ubiquitous member of the normal flora and the species most commonly encountered in clinical specimens, usually as a contaminant [NCCLS,.2002]. Within 24 h, burned patients can start suffering from opportunistic bacterial attacks that can vary from simple infections, to more complicated bacteria, which may resistance to drugs. Infection by multiple drug resistant bacteria could create additional complexity to the problem [Ahmad, 2002].

In studies carried out by Arciola *et al.* Sixty strains of *S. epidermidis* were isolated from infections associated with the implementation of vascular catheter and ten strains were isolated from the skin and mucous membranes of healthy subjects.

The result in table 2 compatible with Wojtyczka study who showed that 97.1% of strains were assessed as biofilm producers with the use of TCP method, 73.6% by TM, and only 6.8% in the case of CRA method [Wojtyczka 2014] as well as de Silva *et al*. explained the low effectiveness of the CRA method in evaluation of biofilm production [De Silva *et al*.,2002].

In table 3 we found difference in the capacity to form biofilms, by S. epidermis isolates from symptomatic patients compared to swab of skin and nasal hospital staff using three phenotypic methods, Staphylococci isolated from blood samples showed a higher extent of biofilm production than that isolated from catheter urine these result comparable with results of Mertens who shown 75% of the *S. epidermidis* isolates from blood culture and central venous catheters tip produced biofilm phenotypIcally [Merten 2013].

While Gamal et al., 2009 show 88.6%of S. epidermidis were biofilm producers also Arslan and Ozkarde showen CRA method demonstrated positive results in 38.5% of staphylococci isolated from clinical specimens [Arslan and Ozkarde, 2007] ,on other hand Iveta etal., study founded (38.8%) biofilm-positive *S. epidermidis* isolates from blood cultures and (55.2%) biofilm-positive isolates from catheters [Iveta etal.,2012].

Result in table 4 are consistent with the findings of Eftekhar study which showed 30% of the samples collected from patients were *Ica* positive compared to 8% of the samples collected from healthy individuals [Eftekhar F, 2009] In another study, 53% of the samples isolated from patients were *Ica* positive compared to 0% of samples collected from healthy volunteers [El-DinSS, 2011]. While iveca founded presence of *IcaA* genes and biofilm formation was slightly more often observed in catheter related isolates than blood culture isolates[Iveta, 2012].

the Ica A and Ica D genes in (34%) clinical S.epidermidis isolated from blood is highest percentage when compare with catheter urine, wound and burn swab in (20, 10) % respectively, while 2% in swab of skin and nasal hospital staff.

Arciola *et al.* reported that all *S. epidermdis* biofilm positive strains isolated from intravenous catheters were positive for *IcaA* and *IcaD* genes, both these genes are required for full slime synthesis, and present in all biofilm producing strains[Arciola et al., 2005]. Important role of *Ica* genes as virulence markers in staphylococcal infections, also Gad et al. founded all staphylococcal biofilm producing strains were positive for IcaA and

IcaD[Gad,2009], while Silva et al. was founded no association between the presence of the Ica operon and biofilm formation by clinical isolates of S. epidermidis [De Silva et al., 2002].

These result are Consistent with the Cafiso results which suggested that Ica D and mec A is important virulence markers for clinical staphylococcal isolated [Cafiso, 2004] and also can be considered as useful virulence for rapid determination of severity of S.epidermidis infection while Samah showed the IcaA and IcaD was not well correlated with biofilm production on MTP methods [Samah,2013].

Therefore S.epidermidis as a potential pathogen because of its ability to produce polysaccharide slime, which accumulation is mediated by the chromosomal Ica genes; Our study results are in agreement with those of Mack et al. [Mack et al, 2000] but Arciola et al. reported that all S. epidermdis biofilm positive strains isolated from intravenous catheters were positive for IcaA and IcaD genes, Ica genes as virulence markers in staphylococcal infections because both these genes are required for slime synthesis[Arciola et al., 2005].

The Ica A gene encodes the enzyme is N- acetylglucosaminyl-transferase, which involved in PIA synthesis. Also Ica D has been reported to a play a critical role in the maximal expression of N- acetylglucosaminyl-transferase, leading to the full phenotypic expression of the capsular polysaccharide [Arciola, 2005]. In S. epidermidis detection of the Ica locus is a good predictor of biofilm formation for distinguishing blood and catheter- related infecting organisms from contaminating bacteria (Aricola et al., 2005].
Results in table 4 are consistent with the findings of the Arciola and Vogel study that shown the biofilm form in staphylococcus associated with the presence of both IcaA and I caD genes [Arciola, 2001; vogel, 2006].

Satorres and Alcara also reported that 42.2% of staph.epidermidis isolated from blood and intravascular catheter were positive for IcaA and Ica D genes.Several studies have shown that formation of biofilm in staphylococci causing catheter associated and nosocomial infections is associated with the presence of both IcaA and IcaD genes [Satorres , 2004 ; Vogel,2006]. Coexpression of these genes is necessary for the full phenotypic expression of biofilm in clinical staphylococcal isolates [Cafiso,2004].

Results in Table 5 demonstrated the presence of the Ica genes did not always correlate with biofilm production. Silva et al. reported that only 59% of S. epidermidis strains positive for the Ica operon were biofilm producers by CRA method[Silva et al., 2002]. In correlating also to MTP method, Cafiso et al. demonstrated that 83.3% of the Ica-positive isolates produced biofilm by both methods[Cafiso et al,2004], while Yazdani et al. reported that only 54% and 52% of Ica positive were also positive by CRA and MTP methods respectively[Yazdani, 2006] while mertens show on Congo agar, approximately 96% of the *icaA/D*-negative isolates did not produce biofilm in vitro[Mertens ,A.2013].

In some isolates presence of the Ica operon but non biofilm production may be has the insertion of a sequence element, known as IS256 [Cho, 2002 ; Kiem ,2001]. In the study of Satorres and Alcaraz, only one out of 65 staphylococci was found to be biofilm negative by CRA while possessing the IcaA and IcaD genes[Satorres and Alcaraz, 2004].

Dobinsky et al. showen the expression of the Ica m-RNA has been shown to occur in biofilm negative S. epidermidis or biofilm producing strains under experimental conditions which did not promote biofilm formation, that biofilm accumulation is controlled by regulatory mechanisms other than the Ica operon[Dobinsky et al., 2003].

While Gad etal. Reported Showed all Staphylococcal biofilm producing strain were positive for IcaA and Ica D [Gad etal, 2009].

De saliva etal. Reported that only 59% of S.epidermidis strain positive for Ica operon were biofilm produced by CRA method[De saliva etal., 2002].

Arciola et al. reported that (IcaA/IcaD+)/MTP- strains represented 8%, however (IcaA/ IcaD)/MTP+ strains were 16%[Arciola, 2005]. In the study of Oliveira and Cunha Mde one CoNS was classified as strongly adherent by MTP assay but did not carry any of the Ica genes[Oliveira and Cunha, 2010].

The presence of *Ica* operon is always associated with biofilm formation but the absence of *Ica* operon is not always associated with absence of biofilm formation. In fact, one sample in which *Ica* operon was not detected showed a strong biofilm forming capability. This confirms the fact that multiple genes are involved in biofilm production and *Ica* operon alone is not a crucial cluster for causing an implant associated infection.

Ica genes didn't always correlate with biofilm production. While de Silva 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[De Silva, 2002]. All of the 73 *S. epidermidis* strains which lacked the *Ica* operon were found to be CRA negative before they can form a biofilm [Gamal, 2009].

Poliana was founded low correlation was found 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 [Poliana2013].

This is in contrast with other studies which, reported that the Dhanawade *et al.*, showed results CRA and microtitre plate tests that were significantly correlated with the molecular analysis [Dhanawade *et al.*, 2010].

While Jain & Agarwal showed the presence of the genes was best correlated with a positive CRA test [Jain & Agarwal, 2009].

Ica positive *S. epidermidis* in Chennai population which is an alarming finding since *Ica* operon is associated with strong biofilm formation. The study in a relatively large population may be useful to ascertain the incidence and prevalence of biofilm producing *S. epidermidis* also the screening for the presence of aap gene in healthy skin isolates will also be useful for such assertion.

Several studies have reported a higher frequency of distribution of the *Ica* locus in clinical isolates of *S. epidermidis*, emphasising its utility as a virulence marker [Arciola *et al.*, 2002].

The 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 can compensate for a deficiency of *Ica* genes. According to other studies, the *bap* gene in strains isolated facilitated biofilm formation [Cucarella *et al.*, 2001]. As well as Tormo reported explained the presence of certain genes as accumulation- associated protein (aap) and Bap homolog protein (bhp) genes were induce an alternative PIA-independent mechanism of biofilm formation[Tormo, 2005].

Chaieb et al. determined higher proportion of *IcaA/D*-positive isolates (72.7%), which phenotypically produced biofilm [Chaieb et al., 2005].

In some *Ica*-positive strains, biofilm production was not observed; this can be explained by an extreme sensitivity of this feature to different environmental factors like antibiotic concentration in a patient's body, high temperature , stress , glucose level, etc. [Frebourg et al.2000 And Rachid, 2000].

CONCLUSIONS

1-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 of forming biofilm.

2- CRA, although easier and faster to perform, remains TCP a better tool for screening method for detection of bacterial biofilm in laboratories.

3-Both *IcaA* and *IcaD* can support the adhesion mechanisms of *S. epidermidis* in the infections associated with medical devices.

4- The presence of both Ica A and D gene was not always associated with in vitro formation of biofilm. 5- In S. epidermidis detection of the Ica locus is a good predictor of biofilm formation for distinguishing blood and catheter- related infecting organisms from contaminating bacteria.

RECOMMENDATION

Study effect of virulence factor of *S. epidermidis like AtlE* gene, *sarA* family and the *agr* quorum sensing system.

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