Pharmacophore

ISSN-2229-5402

Journal home page: http://www.pharmacophorejournal.com

Pharmacophore
an International Journal
ISSN 2229-5402

EVALUATION OF ICAABCD OPERON EXPRESSION IN STAPHYLOCOCCUS AUREUS STRAINS BIOFILM PRODUCERS

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ARTICLE INFO

Received:
03th Jun 2017
Accepted:
29th Nov 2017
Available online:
14th Dec 2017

Keywords: *Staphylococcus aureus, biofilms, icaADBC operon, pathogenesis*

ABSTRACT

Background and aim: Staphylococcus aureus biofilm formation is key role in resistance to antibiotic. The possibility of biofilm formation on the surface and implicated devices such as catheters is one of the most important virulence factors in S .aureus. The aim of this study was assessment of biofilm formation and determination of the frequency of ica ADBC genes among these isolates.
Materials and methods: In this study, 350 samples of blood, sputum, skin lesions, sneezing and urine were collected from hospitalized patients. A total 280 clinical isolates of S. aureus were obtained and identified from various infectious origins. Phenotypic biofilm formation was performed by using Congo red agar method. Prevalence of each of icaA, icaB, icaC and icaD genes were conducted by RT- PCR method. Results: Biofilm formation on CRA showed that 61 strains were biofilm producers, which 4 (6.6%), 43 (70.5%) and 14 (23%) of them were strong, mediate and weak biofilm producers, respectively. All strains has a strong biofilm formation that has icaD gene expression and 75% of these isolates were positive for icaA , icaC and icaB genes. The frequency of icaA/icaD genes in strains with mediate biofilm formation was 76.7%, however, these value for strains with weak biofilm formation was 28.6 %.
Conclusion: in this study, more than half of S. aureus isolates showed biofilm formation among which the majority amplified icaA, icaC, icaD genes, and therefore it was shown a significant relationship between biofilm formation and icaADBC genes.

To Cite This Article: Pedram Ehterami*, Mahboobeh Rajabpour, Sahar Honarmand Jahromy, Fatemeh Noorbakhsh, vahid hasani, (2017), "Evaluation of icaabcd operon expression in staphylococcus aureus strains biofilm producers", ***Pharmacophore, 8(6S),*** *e-1173108.*

Introduction

Staphylococcus aureus is one of the most significant infectious factors in hospitals and society. The bacteria are able to produce several toxins including Enterotoxin, Pantone valentine, and exofoliative toxin [1]. Biofilm formation is one of pathogenic factors of staphylococcus a. providing connectivity to different surfaces and also an increase in antibiotic resistance pattern. Biofilms are a group of growing bacteria's on living and not living surfaces causing connection of bacteria to the surfaces and the connection is a key phase in biofilm formation [2, 3]. Biofilm is a structure composed of a bacteria population, produced through an exopdyroeric matrix provided by enclosed bacteria. Researchers showed that the first phase in staphylococcus a. infectivity is its connectivity to surfaces like medical devices, host tissues and so on relating to a combination of external factors regarding cells such as connectivity and biofilm formation [4, 5]. Intercellular cohesive

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polysaccharide is supposed to be main factor in biofilm formation in staphylococcus aureus strains. Infections resulting from S. aureus relating to biofilm are still a serious clinical concern in patients using medical tools. Biofilm formation causes a decrease in sensitivity to antibacterial treatments, leading high treatment expenses for patients [6, 7]. Biofilm formation is a result of operon activity called icaABCD, as the most important factor in creation of ISO polysaccharide matrix and it is polysaccharide intracellular adhesion (PIA). The genes icaC, icaB, icaA and icaD are controlled by various regulatory systems. The mentioned systems include sar A and sigma (B). In addition to ica ABCD operon system, agr system plays a role in biofilm formation, too. Molecular studies have shown that in the last phase of adhesion in which the structures adore to each other for the first time and they develop after that and then create a biofilm, all of them occur using polysaccharide intercellular adhesion (PIA), that is synthetic product of the operon icaABCD. The synthetic ability of polysaccharide intercellular adhesion (PIA) is the most important factor in adhesion and development of staphylococcus aureus biofilm. Intercellular adhesion (ICA) is composed of four genes of icaABCD, coding the mediated protein PIA. Operon icaABCD expression in internal environment of anaerobic makes an increase in biofilm development. Other influential conditions in gene expression includes: the amount of sugar, ethanol, an increase in osmolality, temperature, and antibiotics like tetracycline. The necessity for search on the changes and expression and the rate of icaABCD expression is obvious more than ever, according to their direct impact on development of biofilm and pathogenicity of staphylococcus aureus, aiming to attain a strategy to struggle this phenomenon. The real- time PCR approach can be used to study the effect of contributing factors in pathogenicity including biofilm. The present study is carried out in order to discuss the frequency of genes ica ABCd among staphylococcus aureus strains isolated from clinical samples.

Materials And Methods

Sample collection:

In this cross-sectional study of 12-month (2016), a total of 350 samples of blood, phlegm, dermal wounds, sneezes and urine were collected from patients in hospitals located in Mazandaran province. To identify S. aureus isolates, biochemical tests including catalase, coagulase, mannitol salt agar, desoxyribonuclease enzyme, bacitracin test and Novobiocin test were used to identify S.aureus strain [11]. Demographic data regarding collected clinical samples such as collecting place, patient's gender, age and collecting date were study on biofilm formation in Congo red agar (CRA) method:

In the present study, staphylococcus aureus isolates were (Sigma, USA) for 24 hours at 37°C, to form biofilm of strong, weak and medium.

RNA extraction and CDNA synthesis:

To extract RNA, RN easy kit (Qiagen Company) was used. Thereby, logarithmic phase of bacteria's growth was applied to extract RNA. Treatment and omission of DNA were done using DNase kit (Qiagen) and then c DNA synthesis was carried out using reverse transcriptase and primer Oligo-d T, [11].

RT-PCR reaction:

The polymerase chain reaction was done in 25 µL and included mastermix composed of PCR primer buffer 1x for each primer, 0/3 µM 0/2 µM each dNTP, µM MgCl and 1/5 UTaq polymerase. The used polymerase to amplify gene areas of ica ABCD is shown in table 1. The polymerase chain reaction was done in BioRAD device as follows.

The initial denaturation step was done at 94°C for 33 cycles, including secondary denaturation at 94°C for 60″seconds, then annealing step or connection temperature at 55°C for 60 seconds, for ica A gone (56°C for 1 minute for ica D gene) , (59°C for 1 minute for genes ica B and ica C), and extension step at 72°C for 1 minute and the final extension phase at 72°C for 1 minute.

After PCR, to identify ica ABCD genes, electrophoresis was done on agarose gel 1/5% and after staining the gel, in order to observe band on agarose gel using Etihidium bromide, the gel Doc device Biorad was used.

Statistical analysis:

Results from statistical software of SPSS 720 were used to specify expressed gene's frequency among biofilm forming strains and frequency syrxhronization. The chi-square test was used to examine the relation among each gene using bacteria biofilm formation.

Results

A number of 350 clinical samples of blood, phlegm, dermal wounds, sneezes and urine of patieats in hospitals were collected. A total of 280 S.aureus isolates were confirmed by phenotypic and biochemical tests. A total of 61 samples of S-aureus included 15 wounds, 21 urin samples, 10 chips, 7 throats, 5 sneezings and 3 blood ones in which biofilm formation on Congo red agar showed that 61 isolates were able to form biofilm of strong, average and weak, 4 (6/6%), 43 (70/5%) and 14 (23%), respectively. The gene ica D was observed by a band of 198 bp, ica C by 192 bp, ica B by 190 bp, and ica A by 188 bp (figure 1). According to table 2, a total of 4 samples formed strong biofilms in which the frequency of gene ica D was 3 samples, the relation in genes ica B and ica C was the same but in ica D, 4 positive samples were observed. In addition, in average biofilm production a number of 23 samples out of 43 ones included ica A and a number of 11 ones included ica B and 10 samples ica C, the 33 samples included ica D.

But in weak samples in this relation a number of 4 samples out of 14 ones had ica A, 2 samples had ica B, 3 ones had ica C and the other 4 ones had ica According to table 3, the most frequent percentage of ica D in strong biofilm samples was 100%. Also, in samples of weak biofilm by a total of 43 samples, the most gene frequency related to ica A and ica D (by 76/7%). And in weak biofilm samples, the most frequency belonged to ica D and ica A (by 28/6%), that is significant (P < 0/001). Based on table 4, the relation between expression of each gene in biofilm formation strains (strong, average) and those lacking biofilm (weak) is significant.

Discussion And Conclusion

Biofilm is a population of growing bacteria's on living or not-living surfaces, locating in an extracellular matrix produced by itself (self-product) from Exopolysaccharide (EPS), proteins, and a number of Nano molecules like DNA. The most crucial microorganisms playing a role in multi microbial biofilm formation, include staphylococcus aureus, pseudomonas aeruginosa, staphylococcus epidermis, some of the family of Entrobacteriaceae and the yeast candida albicans [13]. The first phase in infecting of S.aureus is its adhesion to surfaces like medical tools, host tissues and so on, contributing to a mixture of extracellular factors like the ability of adhesion and biofilm formation [14]. In the present study, S.aureus isolates from hospital were cultured in CRA for 24 hours at 37°C in order to examine biofilm formation, and were studied regarding weak, average and strong and disability in biofilm formation. Synthesis of this polysaccharide happens following the related enzyme by ica A. Association of this loci with ica B causes an increase in polysaccharide synthesis and capsule phenotype formation. The role of ica B is to acetylate the polysaccharide before adhesion to cell membrane and ica C encodes a membranous protein, helping elongation and secretion of this polysaccharide from the cell. The expression and operon ica ABCD increase using regulatory systems such as Sar A and Sigma B [14]. In the present study, biofilm formation on Congo red Agar showed that 61 isolates were able to form biofilm of strong, average, and weak by 4 (6/6%), 43 (70/5%) and 14 (23%), respectively, also, ica ADBC expression was higher in biofilm forming isolates than biofilm-lacking isolates. In this study, strong biofilm isolates had ica ADBC, meaningfully. For instance, 100% of strong biofilm forming isolates had ica D and 75% of them were ica A and ica C and ica B. While prevalence of these genes in weak and average biofilm isolates or biofilm-lacking isolates was much less. In a study by Mirzaee et.al, similar results were observed [15]. Regarding synchronization of biofilm forming genes, a total of 75% isolates were strong, 23/3% were average and 14/3% were weak holding all genes, and synchronization of ica A and ica D was more rather than other genes. In a study by Ghasemian and Mirzaee, it was observed that adhesion genes of CIFA/B, Fnb A/B, Can, Fib and also eno, along with ica genes showed a higher frequency in biofilm-forming strains [16, 17].

Gad G et.al studied S.aureus regarding biofilm production using molecular method of PCR an Tissue culture plate (TCP), technique and found out that 83% of isolates were positive regarding having genes related to biofilm ica D and ica A. From among 302 strains resistant to Methicillin, 257 isolates (97%) had ica D and 27 strains (9%) didn't show the gene. In addition, ica A was reported positive in all negative ica D strains [19]. In a study by Martin, the frequency of ica B was reported as 89% [20]. Furthermore, in a study by Atshan, all 4 genes were found in all sensitive and resistant isolates to Methicillin [21]. The reasons of variance in the results of present study and the others lie on the number of isolates under study, bacteria isolation source, biofilm production condition in under study strains and the molecular method that is used. It can be stated from the results of studies, that genes ica A and D play an important role in polysaccharide bio-synthesis, if there exists biofilm production relating to operon ica; and the gene ica B plays a minor role in synthesized polysaccharide process.

Conclusion

As observed in the present study, more than half of staphylococcus aureus clinical isolates were able to produce biofilm and the expression of all 4 genes of ica in these isolates existed to a high extent, than there is a significant relation between biofilm formation and presence of ica ADBC.

Table1. primer sequence used for genes icaA/B/C/D for RT-PCR

srequency of using primers	**Gene**	**The size of amplification band (bp)**
5-GAGGTAAAGCCAACGCACTC-3 **5-CCTGTAACCGCACCAAGTTT-3**	**icaA**	**188**
5-ATACCGGCGACTGGGTTTAT-3 **5-TTGCAAATCGTGGGTATGTGT-3**	**icaB**	**190**
5-CTTGGGTATTTGCACGCATT-3 **5-GCAATATCATGCCGACACCT-3**	**icaC**	**192**
5-ACCCAACGCTAAAATCATCG-3 **5-GCGAAAATGCCCATAGTTTC-3**	**icaD**	**198**

Table2. number and frequency of genes icaABCD

P value	Weak (n=14)	Medium (n=43)	Strong (n=4)	Biofilm / genes formation
P<0.001	14.4	33.43	3.4	*icaA*
P<0.001	2.14	11.43	3.4	*icaB*
P<0.001	9.14	10.43	3.4	*icaC*
P<0.001	4.14	33.43	4.4	*icaD*

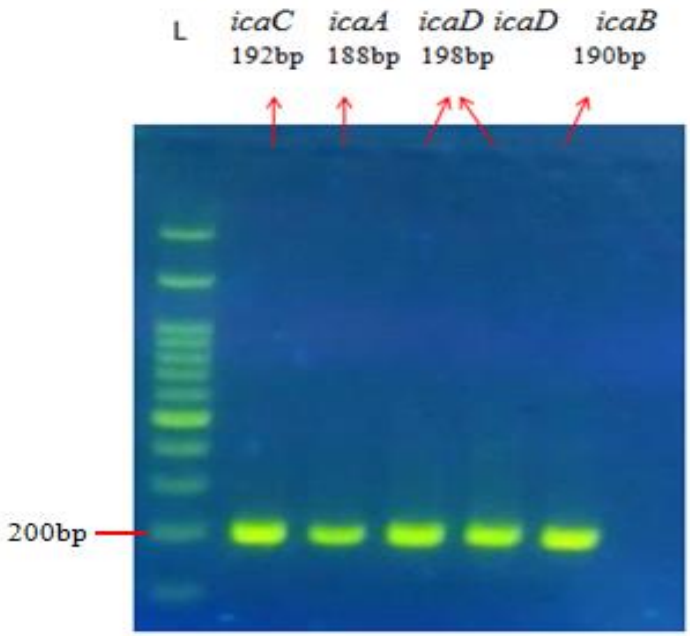
Table3. percent of frequency of genes icaABCD

P value	**Weak (n=14)**	**medium (n=43)**	**Strong (n=4)**	**Biofilm / genes formation**
P<0.001	**%28.6**	**%76.7**	**%75**	**icaA**
P<0.001	**%14.3**	**%25.6**	**%75**	**icaB**
P<0.001	**%64.3**	**%23.3**	**%75**	**icaC**
P<0.001	**%28.6**	**%76.7**	**%100**	**icaD**

Table4. result of chi-square test

(sig) Significant level	**Value**	**test**
*0.019	15.231	**chi-squar**

Represent significant at probability level 5% and nonsignificant ns*



Figures 1. Frequency electrophoresis of icaA, icaB, icaC and icaD genes among clinical isolates of staphylococcus aureus

References

1.Freeman-Cook L, Freeman-Cook KD, Alcamo IE, Heymann DL. Staphylococcus aureus infections: Infobase Publishing; 2006.
2.El-Feky MA, El-Rehewy MS, Hassan MA, Abolella HA, Abd El-Baky RM, Gad GF. Effect of ciprofloxacin and N-acetylcysteine on bacterial adherence and biofilm formation on ureteral stent surfaces. Pol J Microbiol. 2009;58(3):261-7.
3.Fox L, Zadoks R, Gaskins C. Biofilm production by Staphylococcus aureus associated with intramammary infection. Veterinary microbiology. 2005;107(3):295-9.
4.Singhal D, Foreman A, Bardy JJ, Wormald PJ. Staphylococcus aureus biofilms. The Laryngoscope. 2011;121(7):1578-83.
5.Zhu Y, Weiss EC, Otto M, Fey PD, Smeltzer MS, Somerville GA. Staphylococcus aureus biofilm metabolism and the influence of arginine on polysaccharide intercellular adhesin synthesis, biofilm formation, and pathogenesis. Infection and immunity. 2007;75(9):4219-26.
6.Boles BR, Thoendel M, Roth AJ, Horswill AR. Identification of genes involved in polysaccharide-independent Staphylococcus aureus biofilm formation. PloS one. 2010;5(4):e10146.
7.Periasamy S, Joo H-S, Duong AC, Bach T-HL, Tan VY, Chatterjee SS, et al. How Staphylococcus aureus biofilms develop their characteristic structure. Proceedings of the National Academy of Sciences. 2012;109(4):1281-6.
8.Beenken KE, Dunman PM, McAleese F, Macapagal D, Murphy E, Projan SJ, et al. Global gene expression in Staphylococcus aureus biofilms. Journal of bacteriology. 2004;186(14):4665-84.
9.Lauderdale KJ, Boles BR, Cheung AL, Horswill AR. Interconnections between Sigma B, agr, and proteolytic activity in Staphylococcus aureus biofilm maturation. Infection and immunity. 2009;77(4):1623-35.
10. Satpathy S, Sen SK, Pattanaik S, Raut S. Review on bacterial biofilm: An universal cause of contamination. Biocatalysis and Agricultural Biotechnology. 2016;7:56-66.
11. Hamdan-Partida A, Sainz-Espuñes T, Bustos-Martínez J. Characterization and persistence of Staphylococcus aureus strains isolated from the anterior nares and throats of healthy carriers in a Mexican community. Journal of clinical microbiology. 2010;48(5):1701-5.
12. Knobloch JK-M, Horstkotte MA, Rohde H, Mack D. Evaluation of different detection methods of biofilm formation in Staphylococcus aureus. Medical microbiology and immunology. 2002;191(2):101-6.
13. Donlan RM. Biofilm formation: a clinically relevant microbiological process. Clinical Infectious Diseases. 2001;33(8):1387-92.
14. Iwase T, Uehara Y, Shinji H, Tajima A, Seo H, Takada K, et al. Staphylococcus epidermidis Esp inhibits Staphylococcus aureus biofilm formation and nasal colonization. Nature. 2010;465(7296):346-9.
15. Mirzaee M, Najar Peerayeh S, Ghasemian A-M. Detection of icaABCD genes and biofilm formation in clinical isolates of methicillin resistant Staphylococcus aureus. Iranian Journal of Pathology. 2014;9(4):257-62.
16. Ghasemian A, Najar Peerayeh S, Bakhshi B, Mirzaee M. Comparison of Biofilm Formation between Methicillin-Resistant and Methicillin-Susceptible Isolates of Staphylococcus aureus. Iranian biomedical journal. 2016;20(3):175-81.
17. Mirzaee M, Najar-Peerayeh S, Behmanesh M, Moghadam MF. Relationship between adhesin genes and biofilm formation in vancomycin-intermediate Staphylococcus aureus clinical isolates. Current microbiology. 2015;70(5):665-70.
18. Gad GFM, El-Feky MA, El-Rehewy MS, Hassan MA, Abolella H, El-Baky RMA. Detection of icaA, icaD genes and biofilm production by Staphylococcus aureus and Staphylococcus epidermidis isolated from urinary tract catheterized patients. The Journal of Infection in Developing Countries. 2009;3(05):342-51.
19. Grinholc M, Wegrzyn G, Kurlenda J. Evaluation of biofilm production and prevalence of the icaD gene in methicillin-resistant and methicillin-susceptible Staphylococcus aureus strains isolated from patients with nosocomial infections and carriers. FEMS Immunology & Medical Microbiology. 2007;50(3):375-9.
20. Martín-López JV, Pérez-Roth E, Claverie-Martín F, Gil OD, Batista N, Morales M, et al. Detection of Staphylococcus aureus clinical isolates harboring the ica gene cluster needed for biofilm establishment. Journal of clinical microbiology. 2002;40(4):1569-70.
21. Atshan SS, Nor Shamsudin M, Sekawi Z, Lung LTT, Hamat RA, Karunanidhi A, et al. Prevalence of adhesion and regulation of biofilm-related genes in different clones of Staphylococcus aureus. BioMed Research International. 2012;2012.
22. Szweda P, SCHIELMAnn M, Milewski S, FRAnKOWSKA A, JAKuBCZAK A. Biofilm production and presence of ica and bap genes in Staphylococcus aureus strains isolated from cows with mastitis in the eastern Poland. Pol J Microbiol. 2012;61(1):65-9.
23. Vancraeynest D, Hermans K, Haesebrouck F. Genotypic and phenotypic screening of high and low virulence Staphylococcus aureus isolates from rabbits for biofilm formation and MSCRAMMs. Veterinary Microbiology. 2004;103(3):241-7.