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THE FREQUENCY OF *ebp*S AND *V*8 GENES IN *STAPHYLOCOCCUS AUREUS* ISOLATES FROM DIFFERENT SOURCES IN THIQAR PROVINCE-IRAQ

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Abstract

The current study was screened the profile of distribution of two virulence genes (*ebp*S and V8) in clinical isolates of *S. aureus* which isolated from three sources and comparison among them. Total isolates of *S. aureus* (*n* =63) recovered from 200 vaginal swabs from women with bacterial vaginal infections, 113 swabs from and tonsillitis infections and from 80 nasal swabs of healthy students in the college of Medicine, Thi-qar University through the period from February to November, 2017, the frequency of *ebpS* and *V8* genes was determine by PCR. The results showed that (100%) of *S. aureus* isolates from tonsillitis were carried *ebp*S gene with high significant differences (p< 0.001) when compared with those that isolated from nose and vagina with carriage percent 40% and 50%, respectively. While, there were no significant differences between isolates of *S. aureus* which isolated from vagina and nose at (P=0.061). A *S. aureus* isolates from vagina and nose were not harbored *V8* gene (0.0%), while this gene founded in 35.29% of tonsil isolates with significant differences under (P<0.001).The recent results recorded a high prevalence of *ebpS* gene among *S. aureus* isolates from different sources than *V8* gene and isolates differ in their harboring of the virulence genes according to the source of the infection which effect on the bacterial colonization and increased the pathogenicity.

***Keywords*:** *Staphylococcus aureus*, virulence factor

Introduction

A *Staphylococcus aureus* was an imperative human bacteria responsible for both nosocomial and community-acquired infections', like wise this bacterium found as commensal flora in healthy persons mostly colonized in nose (Peacock *et al*., 2001). The most transiently or persistently carriers with *S. aureus*, had a higher perils for developing clinical infections and those infections were more difficult to treatment (Safdar and Bradley, 2008). *S. aureus* strains caused severe skin and soft tissue infections, necrotizing pneumonia, and sepsis in else healthy kids, adolescences, and in neonates (Huang *et al*., 2009). Some studies documented that (5%) of women were harbor this bacteria in the vaginal tract and the postnatal women had high rates of colonization with *S. aureus*; moreover, genital-rectal carriage of *S. aureus* founded allied with development of postpartum fever (Chen *et al*., 2007).The frequency of community methicillin resistant infections diverged amongst unlike populations, publics and the pregnant women had threat factors and more susceptible to evolving those infections (Stumpf *et al*., 2008).

The pathogenicity of *S. aureus* was involved a miscellaneous array of virulence factors which expressed during various stages of infection by a net of virulence regulators (Cotar *et al*., 2010). So, the most persistent and chronic staphylococcal infections related to the expression of different virulence factors that mediate the bacterial adhesion and the formation of biofilms (Traber *et al*., 2008), and it contributed to a extensive variability of microbial infections like urinary tract infections, and infections of permanent indwelling devices' (Goerke *et al*., 2000).

Wide-ranging experimental evidences pointed on the standing of an entire group of secretory proteases in virulent *S. aureus* (Dubin, 2002). *S. aureus* isolates secreted two cysteine proteases, staphopain A and staphopain B', and numerous serine proteases, including V8 protease (SspA), and amount of serine protease-like proteins (Spl) (Sabat *et al*., 2008).

The serine protease of *S. aureus* isolates V8 was one of the important secreted enzymes by this microorganism (Drapeau *et al*., 1972), and also it contributed in the existence and growth of *S. aureus* (Houmard, and Drapeau, 1972).

The other virulence factors of cell wall-associated adhesion proteins was elastin-binding protein (*ebpS*) (Ghasemian *et al*., 2015) help the bacteria in colonization and increased the pathogenicity of this bacteria (Goudarzi *et al*., 2016). The objective of the present study was to detect the distribution of two virulence genes (*ebpS*, V*8*) in *S. aureus* isolates which isolated from different sources.

Material and Methods

Laboratory Methods

A total of 63 *S. aureus* isolates were collected from patients in two hospital wards Al-Habobi teaching hospital/Thi-Qar province-Iraq and from healthy students in the college of Medicine/Thi-qar University through the period from February to November, 2017.

All isolates were collected from different sources, including: 20 *S. aureus* isolates from 80 nasal swabs of students, 26 isolates from 200 vaginal swabs from women suffering of bacterial vaginal infections, and 17 isolates from 113 pharyngitis and tonsillitis patients. The isolates were identified depending on cultural properties including colony morphology and hemolysis (LAB/ United Kingdom), followed by biochemical tests including the growth on mannitol salt agar, coagulase test and DNase test (Baird, 1996; Brooks *et al*., 2007). The diagnosis was confirmed by using API Staph. system (BioMerieux/France).

Preparation of bacterial DNA

The Bacterial DNA was extracted using Genomic DNA Extraction kit (Geneaid/Korea) from entirely *S. aureus* isolates.

PCR diagnosis of *ebpS* and *V*8 genes

The specific primer pairs of *ebpS* with the following sequences were used: forward: '5-CAATCGATAGACACAAATTC-3 and reverse: 5-CAGTTACATCATCATGTTTA-3'. While, for *V*8 gene: forward: 5-TTGTTCTTCGAAACTT-3 and reverse: 5-GGCTTTGGCTTTATTG-3' (Bodenand Flock, 1992).

Amplification of the *ebpS* and *V*8genes was done using primers described at above. The final volume of reaction tubes was 20µl, consist of 5µl Master Mix (Bioneer-Korea), 1µl from each forward and reverse primers, 5µl of DNA template and the volume was completed by adding nuclease free water. The PCR cycling conditions of *ebpS* gene involved: initial denaturation at 94°C for 5 min, followed by 30 cycles of denaturation at 94°C for 1min, annealing at 50°C for 1min, extension at 72°C for 1 min and final extension for 2 min (Boden and Flock, 1994). Whereas for *V8* gene: initial denaturation at 94°C for 10 min, followed by 30 cycles of denaturation at 94°C for 1min, annealing at 45°C for 1 min, extension at 72°C for 2 min and final extension for 10 min after the last cycle (Boden and Flock, 1992). Electrophoresis of PCR product was carried out in 1.4% agarose gel and the presence of a 526 bp and1550 bp band indicate a positive result for *ebpS* and *V*8 genes, respectively.

Statistical Analysis

The results of the present study were statistically analyzed by using SPSS program Version 22. *P* values below to 0.05 were considered statically significant by Chi square.

Results and Discussion

A *S. aureus* isolates from vagina and nose were not harbored *V8* gene (0.0%), while this gene founded in 35.29% of tonsil isolates with significant differences under (P<0.01) as shown in table 2; and the size of the V8 gene showed in figure (2).

The protease enzymes had importance in virulence of *S. aureus* (Dubin, 2002). The staphylococcal proteases had different functions such as virulence factors by a diversity mechanisms, including inactivation of antimicrobial peptides, cleavage of human immunoglobin (Ig) molecules and permitting dissemination of tissue destruction (Selsted *et al*., 1996). V8 protease might cleave and inactivate the heavy chains of Ig classes (Arvidson *et al*., 2000) and alter the fibronectin-binding phenotype of *S. aureus* isolates; this enzyme played a vital role for modifying the host proteins that assistance of the microbes and might play chief role in the posttranslational regulation the activity *S. aureus* exo-protein (McGavin *et al*., 1997). There were different influence of SspA concluded on the advance of tissue abscess infectivity model, it essential for maturation of SspB, controlled of autolytic activities (Rice *et al*., 2001).

The geographic areas may be effects the distributions of different virulence genes of *S. aureus* isolates (Campbell *et al*., 2008) and also whom recorded the prevalence of *V8* and *ebpS* genes in isolates of *S. aureus* was 51/56 (91%) and 56/56 (100%), respectively.

The importance of presence V8 proteases might be modulates the virulent and pathogenicity of *S. aureus*, also Dubin, (2002) suggested that proteases enzyme contributed in high pathogenicity of *S. aureus*.

The V8 gene was one of genes present in virulent *S. aureus* strains. So, this gene used as a marker to identify a *S. aureus* that founded in most strains of *S. aureus* (Campbell *et al*., 2008).

The current results showed lower percentage of V8 gene; these results were disagreed with results of Zdzalik *et al*. (2012) which revealed a high abundance of the gene encodingV8 protease.

The results of current study showed that *S. aureus* isolates recovered from tonsil carried *ebp*S gene in complete percent (100%), when compared with those that recovered from nose and vagina with percent 40% and 50%, respectively. The results significantly was high under (P<0.01) as shown in table 1. While there was no significant differences between isolates of *S. aureus* which isolated from vagina and nose at (P=0.061). There was a significant correlation shown between *S. aureus* isolates from tonsil and nose (P<0.05), with no correlation present with vaginal isolates, and the PCR products of the goal gene showed in figure (2).

The importance of *ebpS* gene in activity of virulent *S. aureus* isolates was documented in several drudies, the *ebpS* gene had related with biofilm formation which mentioned by Ghasemian *et al*. (2015) that determined a relationship between *S. aureus* isolates and formation of biofilm and whom recorded the prevalence of *ebpS* gene was 7% which disagree with our results about the frequency of the gene.

The frequency of *ebpS* gene as one of Microbial Surface Components Recognizing Adhesive Matrix Molecules (MSCRAMM) genes was 82 (65%) in *S. aureus* isolates (Kuźma *et al*., 2006).

The current data revealed a complete isolates of *S. aureus* that recovered from tonsils had *ebpS* gene, Likewise, Nemati *et al*. (2009) showed all MRSA isolates had *ebpS* gene and other genes like *icaD*, can and *clfB* that related with biofilm formation and all genes identified as MSCRAMM gene, there were good genetic capacity for adherence. Furthermore, Voss *et al*. (2005) described that the MRSA isolates which carry these genes were easily transferred from animals such as pigs to humans. The differences in the harboring of virulence genes under this study among the *S. aureus* isolate from different sources indicate the fact that the site of infection affects the selection of bacterial isolate that cause the infection, where the site with more harsh environment colonize and infected by more virulent isolates.

In the study performed by Paniagua and coworkers, (2014) documented that *ebpS* gene was one of the most prevalent virulence genes (65.6 %) in *S. aureus* isolates related catheter.

Table 1: The frequency of *ebp*S gene among *S. aureus* isolated from different sources.

Frequency / Sources	Positive		Negative		Total	
	No.	%	No.	%	No.	%
Tonsil	17	100	0	0.0	17	100
Nose	8	40	12	60	20	100
Vagina	13	50	13	50	26	100

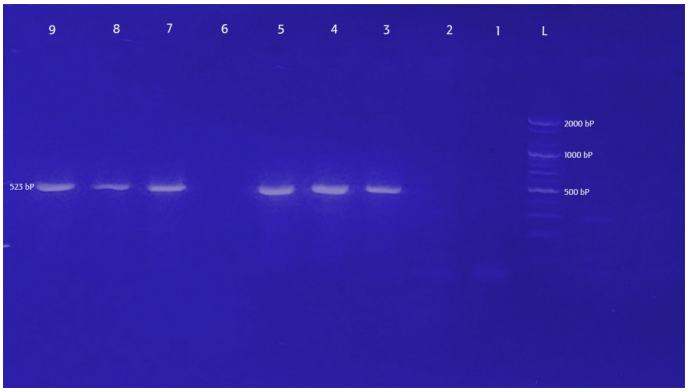

Fig. 1 : Agarose gel electrophoresis of *ebpS* gene that give a PCR products of (523) bp . L: ladder; Lanes 3,4,5,7,8,9 gives PCR products of gene, lane 1,2,6 not shown PCR products.

Table 2 : The frequency of *V8* gene among *S. aureus* isolated from different sources.

Frequency / Sources	Positive		Negative		Total	
	No.	%	No.	%	No.	%
Tonsil	6	35.29	11	64.71	17	100
Nose	0	0.0	20	100	20	100
Vagina	0	0.0	26	100	26	100

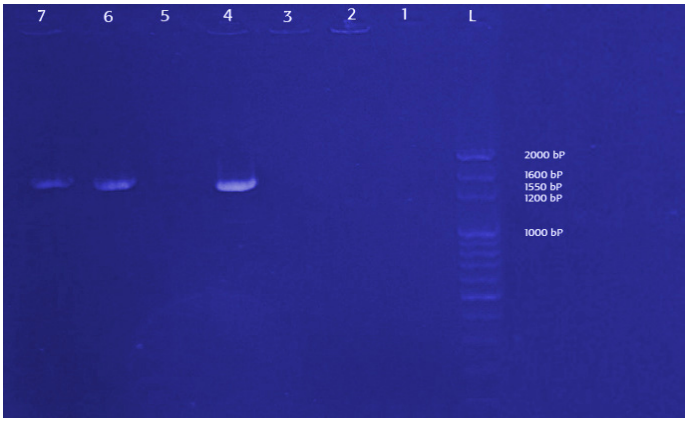

Fig. 2 : Gel electrophoresis of *V8* gene show a PCR products of (1550) bp. L: ladder; Lanes 4,6,7 gives the positive results of gene, lane 1,2,3,5 not shown the PCR products.

Conclusion

The recent study showed a high prevalence of *ebpS* gene among *S. aureus* isolates from different sources than *V8* gene and the isolates differs in their harboring of the virulence genes according to the source of the infection which effect on the bacterial colonization and increased the pathogenicity.

References

Arvidson, A. (2000). Extracellular proteases, p. 379–385. *In* Fischetti, V.A.; Novick, R P.; Ferretti, J J.; Portnoy, D.A. and Rood, J.I. (ed.), Gram positive pathogens. American Society for Microbiology, Washington, D.C.

Baird, D.S. (1996). *Staphylococcus*: cluster forming Gram positive cocci. In: Collee JG, Fraser AG, Marmion BP, Simmons A, editors. Mackie and McCartney practical medical microbiology. 14th ed. New York: Churchhill Livingstone., 247.

Boden, M.K. and Flock, J.I. (1992). Evidence for three different fibrinogen binding proteins with unique properties from *Staphylococcus aureus* strain Newman. J. Microb. Pathog., 12: 289–298.

Boden, M.K. and Flock, J.I. (1994). Cloning and characterization of a gene for a 19 kDa fibrinogen-binding protein from *Staphylococcus aureus*. J. Mol. Microbiol. 12: 599–606.

Campbell, S.J.; Deshmukh, H.S.; Nelson, C.L.; Bae, I.G.; Stryjewski, M.E.; Federspiel, J.J.; Tonthat, G T.; Rude, T.H.; Barriere, S.L.; Ralph, C.R. and Vance, G.F.V. (2008). Genotypic Characteristics of *Staphylococcus aureus* isolates from a multinational trial of complicated skin and skin structure infections. J. Clin. Microbiol., 46(2): 678–684.

Chen, K.T.; Campbell, H.; Borrell, L.N.; Huard, R.C.; Saiman, L. and Della-Latta, P. (2007). Predictors and outcomes for pregnant women with vaginal-rectal carriage of community associated methicillin resistant *Staphylococcus aureus.* Am. J. Perinatol., 24: 235-240.

Cotar, A.I.; Chifiriuc, M.C.; Dinu, S.; Bucur, M.; Iordache, C.; Banu, O. *et al*. (2010). Screening of molecular virulence markers in *S. aureus* and *Pseudomonas aeruginosa* strains isolated from clinical infections. Int. J. Mol. Sci., 11: 5273-5291.

Drapeau, G.R.; Boily, Y. and Houmard, J. (1972). Purification and properties of an extracellular protease of *Staphylococcus aureus*. J. Biol. Chem. 247: 6720–6726.

Dubin, G. (2002). Extracellular proteases of *Staphylococcus* spp. J. Biol. Chem. 383: 1075–1086.

Ghasemian, A.; Peerayeh, S.N.; Bakhshi, B. and Mirzaee, M. (2015). Several Virulence Factors of Multidrug-Resistant *Staphylococcus aureus* isolates from hospitalized patients in Tehran. Int. J. Enteric. Pathog., 3(2): e25196.

Goerke, C.; Campana, S.; Bayer, M.G.; Doring, G. and Botzenhart, K. (2000). Direct quantitative transcript analysis of the *agr* regulon of *Staphylococcus aureus* during human infection in comparison to the expression profile *in vitro*. J. Infect. Immun., 68: 1304-1311.

Goudarzi, M.; Goudarzi, H.; Sa Figueiredo, A.M.; Udo, EE.; Fazeli, M.; Asadzadeh, M. (2016). Molecular Characterization of Methicillin Resistant *Staphylococcus aureus* strains isolated from intensive care units in Iran: ST22-SCCmec IV/t790 emerges as the Major Clone. J. PLoS One., 11(5):0155529.

Houmard, J. and Drapeau, G.R. (1972). Staphylococcal protease: a proteolytic enzyme specific for glutamoyl bonds. Proc. Natl. Acad. Sci. USA 69: 3506–3509.

Huang, Y.C.; Chao, A.S.; Chang, S.D. (2009). Association of *Staphylococcus aureus* colonization in parturient mothers and their babies. Pediat. Infect. Dis. J., 28:742-744.

Kuźma, K.; Malinowski, E.; Kłossowska, A.; Kaczmarowski, M. and Smulski, S. (2006). Detection of adhesin genes by PCR and analysis of their distribution in *Staphylococcus aureus* strains isolated from bovine mastitis. J. Bull. Vet. Inst. Pulawy. 50: 319-323.

McGavin, M.J.; Zahradka, C.; Rice, K. and Scott, J.E. (1997). Modification of the *Staphylococcus aureus* fibronectin binding phenotype by V8 protease. J. Infect. Immun. 65: 2621–2628.

Moreillon, P.; Entenza, J.M.; Francioli, P.; McDevitt, D.; Foster, T.J.; Francois, P. and Vaudaux, P. (1995). Role of *Staphylococcus aureus* coagulase and clumping factor in pathogenesis of experimental endocarditis. J. Infect. Immun., 63:4738–4743.

Nemati, M.; Hermans, K.; Devriese, L.A.; Maes, D. and Haesebrouck, F. (2009). Screening of genes encoding adhesion factors and biofilm formation in *Staphylococcus aureus* isolates from poultry. J. Avian. Pathology. 38(6): 513-517.

Paniagua-Contreras, G.; Monroy-Pérez, E.; Gutiérrez-Lucas, R.; Sainz-Espuñes, T.; Bustos-Martínez, J. and Vaca, S. (2014). Genotypic characterization of methicillin-resistant *Staphylococcus aureus* strains isolated from the anterior nares and catheter of ambulatory hemodialysis patients in Mexico. J. Folia. Microbiol. (Praha). 59(4): 295-302.

Peacock, S.J.; de Silva, I. and Lowy, F.D. (2001). what determines nasal carriage of *Staphylococcus aureus*? Trends Microbiol. 9: 605-610.

Rice, K.; Peralta, R.; Bast, D. Azavedo, J. and Mcgavin, M.J. (2001). Description of *Staphylococcus* Serine Protease (*ssp*) Operon in *Staphylococcus aureus* and Non polar inactivation of *sspA*-Encoded Serine Protease. J. Infect. Immun., 69(1): 159–169.

Sabat, A.J.; Wladyka, B.; Kosowska-Shick, K.; Grundmann, H.; van Dijl, J.M.; Kowal, J.; Appelbaum, P.C.; Dubin, A. and Hryniewicz, W. (2008). Polymorphism, genetic exchange and intragenic recombination of the aureolysin gene among *Staphylococcus aureus* strains. J. BMC. Microbiol., 8: 129.

Safdar, N. and Bradley, EA. (2008). The risk of infection after nasal colonization with *Staphylococcus aureus*. Am. J. Med., 121: 310-315.

Selsted, M.E.; Tang, Y.Q.; Morris, W.L.; McGuire, P.A.; Novotny, M.J.; Smith, W.; Henschen, A.H. and Cullor, J.S. (1996). Purification, primary structures, and antibacterial activities of beta-defensins, a new family of antimicrobial peptides from bovine neutrophils. J. Biol. Chem., 271:16430.

Stevens, M.P. and Edmond, M.B. (2009). The rising tide: skin and soft tissue infections due to community associated methicillin resistant *Staphylococcus aureus*. South. Med. J., 102: 121–122.

Stumpf, P.G.; Flores, M. and Murillo, J. (2008). Serious postpartum infection due to MRSA in an asymptomatic carrier: case report and review. Am. J. Perinatol., 25*:* 413-415.

Traber, K.E.; Lee, E.; Benson, S.; Corrigan, R. and Cantera M. (2008). *Agr* function in clinical *Staphylococcus aureus* isolates. J. Microbio., 154(Pt 8): 2265-2274.

Voss, A.; Loeffen, F.; Bakker, J.; Klaassen, C. and Wulf, M. (2005). Methicillin-resistant *Staphylococcus aureus* in pig farming. J. Emerg. Infect. Dis., 11: 1965-1966.

Zdzalik, M.; Karim, A.Y.; Wolski, K.; Buda, P.; Wojcik, K.; Brueggemann, S.; Wojciechowski, P.; Eick, S.; Calander, A.; Jonsson, I.; Kubica, M.; Polakowska, K.; Miedzobrodzki, J.; Wladyka, B.; Potempa, J. and Dubin, G. (2012). Prevalence of genes encoding extracellular proteases in *Staphylococcus aureus* important targets triggering immune response in vivo. J. FEMS. Immun. Med. Microbiol. 66: 220–229.