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The prevalence of serine-aspartate dipeptide-repeat region (sdr C and E) putative virulence gene among local isolates of *Staphylococcus aureus* from different sources

Ahmed N. Fayad

Collage of Veterinary Medicine, University of Thi-Qar

ahmed.fayad@sci.utq.edu.iq

Abstract

Staphylococcus aureus is an important bacterial pathogen associated with a wide range of infections due to its ability to produced several types of virulence factors. The study aimed to determine the prevalence of serine-aspartate dipeptide-repeat region (sdr C and E) genes among *S. aureus* isolates from different sources. A total of 37 isolates was conducted in this study divided into 17 isolates from tonsils infections and a 20 isolates from nasal carriage, the prevalence of *sdrC* and *sdrE* genes was detected by polymerase chain reaction technique. The results showed high prevalence of *sdrC* and *sdrE* genes among bacteria from tonsils infections in compared with isolates of nasal carriage with high significant differences. Also, *sdrC* gene was high prevalent than *sdrE* in tonsils isolates than in nasal carriage. In brief, the *sdr* genes highly prevalent in *S. aureus* isolates from invasive infections when compared with nasal carriage isolates with predominant of *sdrC* gene.

Keywords: *Stapylococcus aureus*, *sdr C*, *sdr E*.

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Introduction

Staphylococcus aureus is an opportunistic pathogenic gram positive microorganism causing various infections in humans and animals, with the ability to invade and persist in non-professional phagocytes: fibroblasts, osteoblasts and different types of epithelial cells (1,2). Both localized and systemic infections can occurs, such as abscesses, impetigo, cellulitis, sepsis, endocarditis, bone infections, and meningitis (3). *S. aureus* is also responsible for diseases caused by secreted toxins such as exfoliatins (scalded skin syndrome), enterotoxins, or toxic shock syndrome toxin (4,1,5). Most *S. aureus* infections start as minor colonization of skin or soft tissue, then the organism can spread to the bloodstream and disseminate into various tissues (6,7). At present, *S. aureus* causes a large number of dangerous community acquired infections that have a significant impact on public health.

S. aureus is also regarded as one of the most important causes of nosocomial infections, especially infections of surgical sites, catheters, and implants (4,8). The fate of the infectious process is determined by the host's immunity, strain virulence and resistance to antibiotics (9,10). It seems that *S. aureus* strains associated with human infection have variable combinations of pathogenic determinants and that either the presence or the

expression of those combinations varies according to the type of infection and genetic susceptibility of the infected host (11).

A group of *S. aureus* virulence factors responsible for the initial attachment with host cells is mediated mainly through a family of surface proteins, referred to as microbial surface components recognizing adhesive matrix molecules (MSCRAMMs), such as clumping factor A (ClfA), and B (ClfB), elastin binding protein (EbpS), collagen adhesin (Cna), serine-aspartate repeat protein C (SdrC), SdrD, SdrE, bone sialoprotein binding protein (Bbp, isoform of SdrE), fibronectin binding proteins A (FnBPA) and B (FnBPB), (4,12,13) and that attachment regarding as important step in biofilm formation (5). The characteristic feature of Sdr proteins is the presence of R region containing multiple serine-aspartate repeats (14).

The *sdr* locus encodes three proteins, SdrC, D, and E; though, not all three genes are present in all *S. aureus* strains (15). It was noticed that the carriage of invasive strains is always correlated with the presence of *sdrE* genes (16). SdrC binds β-neurexin 1 exodomain and expression of the protein raises adherence to cultured mammalian cells that expressing β-neurexin on their surface (17). A *sdrD* is important in abscess formation (18). Molecular typing studies play an important role in epidemiological studies, particularly in tracing the passage of pathogens from the food chain to humans and vice versa, such information is necessary to assess the efficacy of an outbreak detection and prevention; and to help in studying evolutionary relationship of MRSA and other pathogens (8).

The successful eradication of *S. aureus* infection in patients become difficult when biofilm mentioned above was formed, it can protects *S. aureus* from the damage of antibiotics and host immune system etc. (5,6). In addition, *S. aureus* biofilms could promote horizontal spread of antibiotic resistance determinants, which were mainly through increasing the prevalence of plasmid transfer events by both conjugation and mobilization (5).

The objective of this study to determine the prevalence of serine-aspartate dipeptide-repeat region(sdr) C and E genes among *S. aureus* isolates from different sources.

Materials and Methods

The experimental study was included 37 isolates of *S. aureus* isolated from different sources in AL-Habobi teaching hospital in Thi-Qar province in the period from January to April-2017. The isolates were divided into 17 isolates from tonsils infections and 20 isolates of nasal carriage.

DNA extraction

The DNA was extracted according to the kit index that provided by manufacturer (Favorgen-Taiwan).

PCR analysis

Genomic DNA from *S. aureus* was conducted to amplification by PCR in the thermal cycler (Eppendorf-Germany), the final volume of multiplex PCR mixture was 20 µ involved 5 µ of master-mix (Bioneer-Korea), 2 µ from each *sdrC* primer (forword & reverse) and *sdrE* primer (forword & reverse) (11), 4 µ of target DNA and 3 µ of DNase free water. The sequences of primers used and amplification conditions was illustrated in table (1). After 30 cycles, the PCR reaction mixtures were investigated by 1% agarose gel electrophoresis.

Table (1): The primers sequences, PCR conditions and products length of PCR analysis.

Genes		Sequences	PCR conditions	Product size Bp
sdrC	Forward	5′-ACGACTATTAAACCAAGAAC-3′	1 min, 94°C; 1 min, 45°C; 1.5 min, 72°C	560
	Reverse	5′-GTACTTGAAATAAGCGGTTG-3′		
sdrE	Forward	5′-CAGTAAATGTGTCAAAAGA-3′	1 min, 94°C; 1 min, 45°C; 1.5 min, 72°C	767
	Reverse	5′-TTGACTACCAGCTATATC-3′		

Statistical analysis

T-test was used for statistical analysis of the data. Also, standard deviation was calculated for PCR test (SPSS Version 22).

Results

The *sdrC* gene presents in a high prevalence in tonsils infection causing *S. aureus* where 14 (82.35%) isolates carried this gene with significant differences under (P< 0.05). While, no significant differences seen in the prevalence of *sdrE* gene were 9 (52.94%) of isolates gave a positive PCR results for *sdrE* as illustrated in table (1), (figure 1).

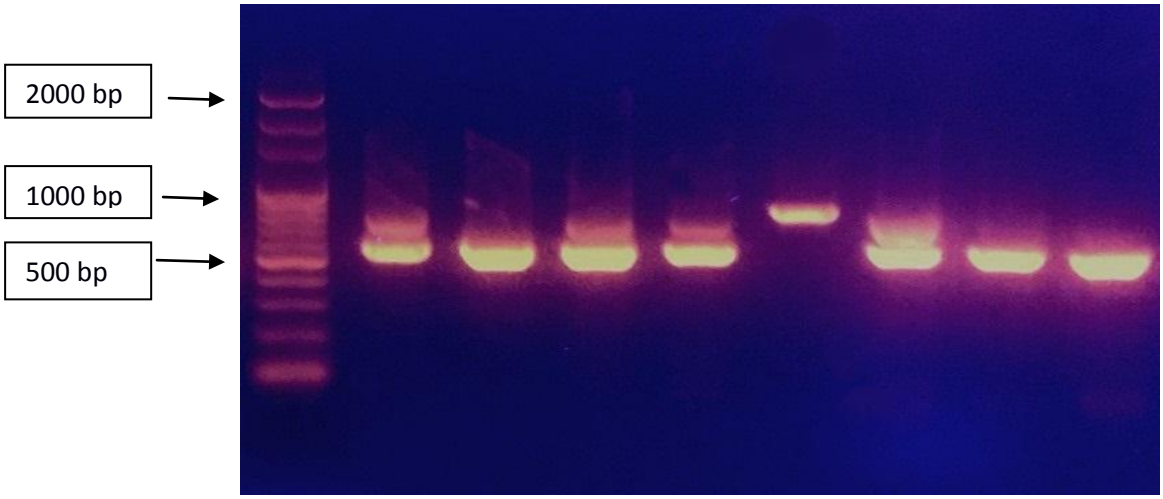
Table 1: The prevalence of *sdrC* and *sdrE* genes among S. aureus isolates from tonsils infections

Genes / Prevalence	*sdrC*		*sdrE*		Proportion values
	No.	%	No.	%	
Positive	14	82.35	9	52.94	< 0.01
Negative	3	17.65	8	47.06	
Total	17	100	17	100	

The prevalence of *sdrC* and *sdrE* genes among nasal carriage *S. aureus* isolates was 3,6 represents (15, 30)%, respectively. With high significant differences under ($P < 0.001$) in case of *sdrC* and significant ($P < 0.05$) for *sdrE* gene (table 2).

Table 2: Table 1: The prevalence of *sdrC* and *sdrE* genes in S. aureus isolated from nose

Genes / **Prevalence**	***sdrC***		***sdrE***		**Proportion values**
	No.	%	No.	%	
Positive	3	15	6	30	< 0.05
Negative	17	85	14	70	
Total	20	100	20	100	



Fig(1):- Agarose gel electrophoresis of *sdrC and sdrE* primers that give a PCR products of (560) and (767) bp, respectively. L: ladder; other lanes represents a positive amplification products for the useful primers.

Discussion

S. aureus is a gram positive cocci present as normal flora in skin and mucus membrane especially in nose of many peoples. At the same time this bacteria responsible for a different types of an important infections in health care sitting and communities, the infections ranging

from mild skin and wound infections to life-threatening diseases such as toxic shock syndrome, the bacteria transmitted easy by direct contact contaminated objects like: hands or body secretions and droplet transmission, or its transmitted indirectly through the breathing of contaminated air the environments (19,20,21). The ability of bacteria to survive in the different milieu play an important role in transmission which assists by bacterial production of many significant factors that help in successful and distribution of *S. aureus*, these factors including development of resistance to many antimicrobial agents and its ability to express a numerous virulence factors including toxins such as enterotoxins and adhesive factors like clumping factor and sdr proteins (22,23).

There is a wide range of adhesive factors whose correlated to the pathogenesis of *S. aureus* infections and colonization of bacteria on the body surfaces. The ability of bacteria to produce these factors is detected by PCR to determine the genes that responsible for production including genes of cell wall-associated adhesion proteins such as *fnb*, *can* and *sdr* (11).

Sdr proteins (from SD Repeat) are members of a structural related family of cell wall surface proteins which characterized by the presence of R region containing multiple serine-aspartate repeats. The sdr locus codes three proteins, SdrC, SdrD, and SdrE; but, not all these genes found in all *S. aureus* strains (23,24). The current study showed high prevalence of *sdrC* gene among *S. aureus* isolates from tonsils infections which was noted in several studies (23,24,25) in *S. aureus* isolates from patients this may indicate the importance of the protein in bacterial colonization and invasiveness of host tissues. While, sdrE present in moderate percent. But, the distribution of *sdr* genes was low *S. aureus* isolated from nasal carriage which is an indicated to high diversity of bacteria from different sources and the colonization of nose may not requires to express high number of adhesive molecules.

Conclusion

In brief, the *sdr* genes have high prevalence among *S. aureus* from invasive isolates when compared with nasal carriage isolates with predominant of *sdrC* gene that present in high percent in compared with *sdrE* gene.

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أنتشار جينات ثنائي ببتيد اسبارتيت السيرين المتعدد (النوعين سي و أي) المسببة لضراوة جرثومة المكورات العنقودية الذهبية في عزلات محلية من مصادر مختلفة

احمد ناصر فياض

كلية الطب البيطري، جامعة ذي قار

ahmed.fayad@sci.utq.edu.iq

المستخلص

تعد المكورات العنقودية الذهبية جرثومة ممرضة مهمة ترتبط بمجموعة واسعة من الاصابات نظرا لقدرتها على إنتاج عدة أنواع من عوامل الضراوة . تهدف هذه الدراسة لتحديد انتشار ثنائي ببتيد اسبارتيت السيرين المتعدد (النوعين سي و اي) لعزلات المكورات العنقودية الذهبية المأخوذة من مصادر مختلفة.

جرت هذه الدراسة على ٣٧ عزلة مقسمة إلى ١٧ عزلة أخذت من اللوزتين و ٢٠ عزلة أخذت من افرازات الأنف، تم الكشف عن انتشار الجينات *sdr C* و *sdr E* بواسطة تقنية تفاعل سلسلة البوليمريز.

أظهرت النتائج لانتشار جينات *sdr E* و*sdr C* نسبة عالية للبكتريا المعزولة من اللوزتين بالمقارنة مع افرازات الانف مع وجود فرق محسوس جدا. ايضا كانت النسبة عالية لجينات *sdr C* بالمقارنة مع جينات *sdr E* للعزلات المأخوذة من اللوزتين .

باختصار، فإن جينات *sdr* منتشرة بشدة في عزلات المكورات العنقودية الذهبية في الاصابات الشديدة مع عزلات اللوزتين و جينات *sdr C* . .

الكلمات المفتاحية: *Stapylococcus aureus, sdr C, sdr E.*