Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 4, 2021, Pages. 8498 - 8508
Received 05 March 2021; Accepted 01 April 2021.

Molecular Detection of Eight Intsapi (S) In the Skin Pathogen *Staphylococcus Aureus* MRSA Clinical Strains in Iraq

Halah M. Mutar and Adnan Hamad Aubaid

Microbiology Department, College of Medicine, University of Al-Qadisiyah, diwaniya, Iraq.

Abstract

The present work highlights the tracing of eight intSapI(s) in thirty *Staphylococcus aureus* MRSA clinical strains isolated from skin lesions at Diwaniyah Teaching Hospital in Iraq in a previous study. A partial fragment of each intSaPI was amplified by conventional PCR using intSaPI specific primers. The amplified PCR products of the eight intSaPI(s) were 719, 539, 760, 673, 331, 472, 441, and 870 bp for intSaPI1, intSaPI2, intSaPI3, intSaPI4, intSaPI5, intSaPIn1/m1, intSaPIbov, and intSaPImw2, respectively. The frequency of occurrence of intSaPI1, intSaPI2, intSaPI3, intSaPI4, intSaPI5, intSaPIn1/m1, intSaPIbov1, and intSaPImw2 among the thirtyMRSA clinical strains were 6.66 (n=2/30), 16.66 (n=5/30), 13.33 (n=4/30), 20 (n=6/30), 10 (n=3/30), 23.33 (n=7/30), 3.33(n=1/30), and 6.66% (n=2/30), respectively. The present data would underpin the potential of intSapI(s) in *St.aureus* MRSA clinical strains in pathogenesis.

Keywords: *Staphylocccus aureus* MRSA; Skin infection; intSaPI1, intSaPI2, intSaPI3, intSaPI4, intSaPI5, intSaPIn1/m1, intSaPIbov1, and intSaPImw2

Introduction

Community acquired *Staphylococcus aureus* and hospital acquired *Staphylococcus aureus* are the most common etiological agents for various infections spanning from minor skin infections to serious infections like osteomyelitis or endocarditis (Eriksen et al. 1995; Wertheim et al.

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 4, 2021, Pages. 8498 - 8508
Received 05 March 2021; Accepted 01 April 2021.

2005). A set of virulence factors distributed in the genome play a crucial role in the pathogenicity of *S.aureus*. The mobile genetic elements (MGEs) are considered one of these virulence factors (Lindsay et al. 2004; Ruzin et al. 2001). *S. aureus* pathogenicity islands (SaPIs) are a type of MGEs. The SaPIs, 15 kb phage-inducible pathogenicity islands (Novick et al., 2010; Novick and Ram, 2015; Penadés and Christie, 2015), are derived from a heritable prophage or protophage, of which they could maintain a set of significant characteristics that promote their functionality. Generally, the horizontal transfer is responsible for the gaining of most MGEs. The mobilization mechanisms of SaPI(s) in *S. aureus* have been well studied (Ruzin et al. 2001; Malachowa et al. 2010, Cervera-Alamar eta l. 2018). The SaPIs constitute a big family with numerous natural *Staphylococcus aureus* strains, MW2, Mu 50, N315 and RF 122, E-MRSA16, MRSA252, COL, TW20, MN8, and USA300 (Cervera-Alamar eta l. 2018), comprising two or more. The consistency of the SaPI family is outlined by the truth that their open reading frames (ORFs) assign to big sets of orthologs, where the initial 8–10 are permanently SaPI genes, majority of them do not exist in other MGEs (Novick and Ram, 2015). The organization of SaPI(s) genome is prophage-comparable and include of a minor set of genes transcribed in one direction and a major set of genes transcribed in opposite direction, a beginning with an integrase (*int*) gene (Martínez-Rubio et al. 2017). The SaPIs are introduced into a single direction at specific positions on the bacterial chromosome. The packaging of SaPIs is performed through two mechanisms pac and cos; like bacteriophages (Feiss and Rao 2012). The SaPI(s), SaPI1, SaPI2, SaPI3, SaPI4, SaPI5, SaPIn1/m1 and SaPI1mw2, were previously detected in *S. aureus* clinical strains however, the SaPIbov1 and SaPIbov2 were identified in a *S. aureus* strain isolated from a bovine mastitis case (Novick et al., 2010).

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 4, 2021, Pages. 8498 - 8508
Received 05 March 2021; Accepted 01 April 2021.

The objectives of this study were to monitor the frequency of occurrence of eight foremost SaPI integrase genes among thirty*S. aureus* MRSA clinical strains isolated previously from patients suffered from skin infections at Diwaniyah Teaching Hospital in Iraq (unpublished data). These thirty*S.aureus* MRSA clinical strains were unraveled regarding the distribution of some virulent genes like *mecA*, *tst-1*, *Hla*, *Hlb*, *eta*, *etb*, *Erma*, and *Ermb* (unpublished data).

Materials and methods

Staphylococcus aureus MRSA strains

A total of thirty *St.aureus* MRSA clinical strains were employed in this study. These clinical strains were previously isolated form patients from Diwaniyah Teaching Hospital, Iraq suffering from skin lesions and identified with API STAPH (BIOMERIEUX, France) (unpublished data).

Cultivation conditions

Sheep blood agar [MacFaddin 1985] was utilized during the course of activation and culturing of *S.aureus* MRSA clinical strains. The medium was obtained from HiMedia Laboratories, India. All cultures were incubated at 37 ^{o}C.

Genomic DNA isolation

Genomic DNA was isolated from the thirty*S.aureus* MRSA clinical strains using Thermo Scientific GeneJET Genomic DNA purification kit according to the instructions of the manufacturer. The integrity of the genomic DNA was assessed by running the DNA on agarose gel electrophoresis followed by gel staining by ethidium bromide and visualization under ultraviolet illumination (Sambrook et al. 1989) through UV-transilluminator (Cleaver Scientific,

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 4, 2021, Pages. 8498 - 8508
Received 05 March 2021; Accepted 01 April 2021.

UK). However, the concentration of the genomic DNA in terms of ng/µl was determined by Nano-drop spectrophotometer (Thermo Scientific™ NanoDrop 2000, USA). All DNA preparations were stored at -20 ^{o}C until being processed further.

PCR detection of intSaPI (s)

The distribution of the eight intSaPI(s) among the thirty *S.aureus* MRSA clinical strains were investigated by conventional PCR. These eight inSaPI(s) were assigned to intSaPI1, intSaPI2, intSaPI3, intSaPI4, intSaPI5, intSaPIn1/m1, intSaPIbov, and intSaPImw2. Eight conventional PCR reactions were conducted by the aid of intSaPI (s) specific primers for each clinical strain. The nucleotide sequences of the primers used in this study were based on the previously designed primer sets (Alibayov et al. 2014). The list of the primer sets covering the PCR amplification of the eight intSaPI(s) were Fw-instSaPI1:5- ACAAAGACCCACTCACAAAC-3, Rv-instSaPI1:5-AAACCAATGGATTGTTCCGT-3, Fw-instSaPI2: 5-GCGTAGATGTTCATAGTGGTATT-3, Rv-instSaPI2: 5- TTCCTGGAGGTTGCTAAAA-3, Fw-instSaPI3:5- GTGATGAGTGGTTTCAGAGTTA-3, Rv-instSaPI3: 5-CTTTCAGTCTGCATTGGGT-3, Fw-instSaPI4:5- TTTGCCATCTTATCAGTAACATG-3, Rv-instSaPI4: 5- CTAAGAAAGCCCAAACCAGA-3, Fw-instSaPI5:5-GCAGTTTATAGATACGTCTAGTGT-3, Rv-instSaPI5: 5-CTCTTAAATGAGCGTATAGAAGAA-3, Fw- intSaPIn1/1m:5-TTAAACAGGTTGCTGACGAT-3, Rv- intSaPIn1/1m:5-GACCACTTCAATGCCAATAA-3, Fw-intSaPIbov1:5- AAGTATATGAGTTGTGGCTTGA-3, Rv-intSaPIbov1:5-CGTTCCATGTTAAAGCCATTAAT-3, Fw- intSaPImw2: 5-AGCATGTCCTAGCCTTTGA-3, and Rv- intSaPImw2: 5- ATGGACGAATTAGAGGGAGT-3. The lengths of the expected PCR products were 719, 539, 760, 673, 331, 472, 441, and 870 bp for intSaPI1, intSaPI2, intSaPI3,

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 4, 2021, Pages. 8498 - 8508
Received 05 March 2021; Accepted 01 April 2021.

intSaPI4, intSaPI5, intSaPIn1/m1, intSaPIbov1, and intSaPImw2, respectively. The PCR conditions were set as follow: 95 ^{o}C , 5 min for initial denaturation, 30cycles each ; 94 ^{o}C , 1 min for denaturation, 55 ^{o}C (for intSaPI1, intSaPI3, intSaPI4, intSaPI5), 56 ^{o}C (intSaPI2), 51^{o}C (for intSaPIn1/1m), 53 ^{o}C (for intSaPIbov1), 54 ^{o}C (for intSaPImw2), 30 sec for annealing, 42 sec (for intSaPI1, intSaPI3, intSaPI4), 30 sec (for intSaPI2), 20 sec (intSaPI5, intSaPIn1/m1, and intSaPIbov1), 72^{o}C (for extension), and 10 min for 72 ^{o}C. The PCR products were analyzed by 1% agarose gel electrophoresis followed by visualization under UV-transilluminator. All agarose gels were run in the presence of 100 bp DNA ladder (abm, Canada). Each PCR reaction (50 µl), regardless of the name of the partial amplified fragment, consisted of 30 ng genomic DNA, 0.5µM of each forward and reverse primer, and 25µL of PCR Master mix (2X) (iNTRON, Korea).

Results

DNA fingerprint of intSaPI(s) among *St.aueus* MRSA clinical strains

The molecular profile of thirty*S.aureus* MRSA clinical strains, previously isolated and identified (unpublished data), was determined by conventional PCR regarding eight intSaPI(s). The eight traced intSaPI(s) were intSaPI1, intSaPI2, intSaPI3, intSaPI4, intSaPI5, intSaPIn1/m1, intSaPIbov, and intSaPImw2. The resultant PCR products derived from PCR amplification of the eight intSaPI(s) were 719, 539, 760, 673, 331, 472, 441, and 870 bp for intSaPI1, intSaPI2, intSaPI3, intSaPI4, intSaPI5, intSaPIn1/m1, intSaPIbov1, and intSaPImw2, respectively as shown in Figures 1-5. Generally, there existed a discrepancy in the distribution of the eight intSaPI(s) among the thirty*S.aureus* MRSA clinical strains under study. The frequency of occurrence of intSaPI1, intSaPI2, intSaPI3, intSaPI4, intSaPI5, intSaPIn1/m1, intSaPIbov1, and intSaPImw2 among the thirtyMRSA clinical strains were 6.66 (n=2/30), 16.66 (n=5/30), 13.33

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 4, 2021, Pages. 8498 - 8508
Received 05 March 2021; Accepted 01 April 2021.

(n=4/30), 20 (n=6/30), 10 (n=3/30), 23.33 (n=7/30), 3.33(n=1/30), and 6.66% (n=2/30), respectively. The intSaPI(s) intSaPIn1/m1 and intSaPIbov1 did exhibit the highest and lowest frequencies 23.33 and 3.33% of occurrence among the thirty*S.aureus* MRSA clinical strains, respectively. Generally speaking, the frequency of occurrence of the eight traced intSaPI(s) was low among the thirty*S.aureus* MRSA clinical strains under study.

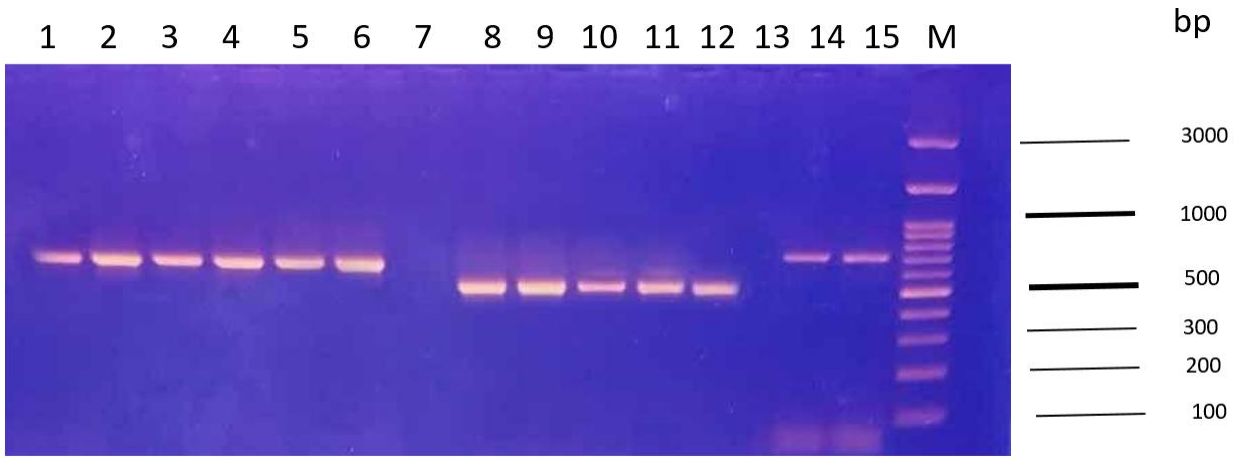

Fig 1: Agarose gel electrophoresis (1.5%) showing the PCR products of amplification of SapI1, SaPI2, and SaPI4 from *St.aureus* MRSA clinical strains. Lanes (1-6): represent PCR products (673 bp) of SaPI4 from six positive out of thirty *S.aureus* MRSA clinical strains. Lanes (8-12): represent PCR products (539 bp) of SaPI2 from five positive out of thirty*S.aureus* MRSA clinical strains. Lanes (14-15): represent PCR products of SaPI1 (719 bp) from two positive out of thirty*S.aureus* MRSA clinical strains. M: 100 bp DNA. Lanes 7 and 13: empty lanes.

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 4, 2021, Pages. 8498 - 8508
Received 05 March 2021; Accepted 01 April 2021.

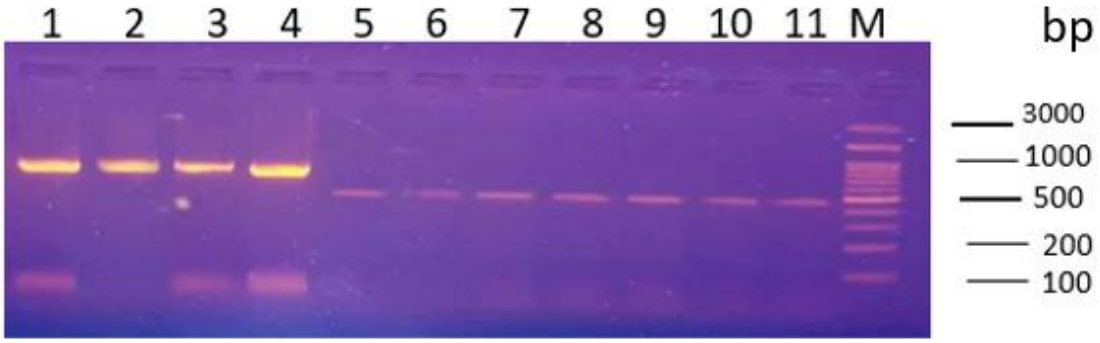

Fig 2: Agarose gel electrophoresis (1.5%) showing the PCR products of amplification of SaPI3 and intSaPIn1/m1 from *S.aureus* MRSA clinical strains. Lanes (1-4): represent PCR products (760 bp) of SaPI3 from four positive out of thirty*S.aureus* MRSA clinical strains. Lanes (5-11): represent PCR products (472 bp) of intSaPIn1/m1 from seven positive out of thirty*S.aureus* MRSA clinical strains. M: 100 bp DNA

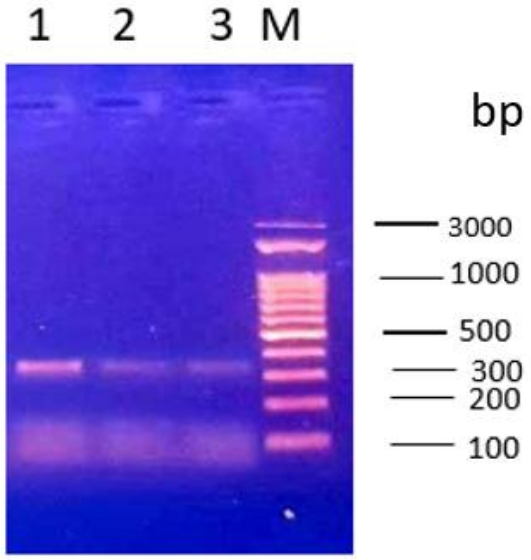

Fig 3: Agarose gel electrophoresis (1.5%) showing the PCR products of amplification of SaPI5 from *S.aureus* MRSA clinical strains. Lanes (1-3): represent PCR products (331bp) of SaPI5 from three positive out of thirty*S.aureus* MRSA clinical strains. M: 100 bp DNA.

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 4, 2021, Pages. 8498 - 8508
Received 05 March 2021; Accepted 01 April 2021.

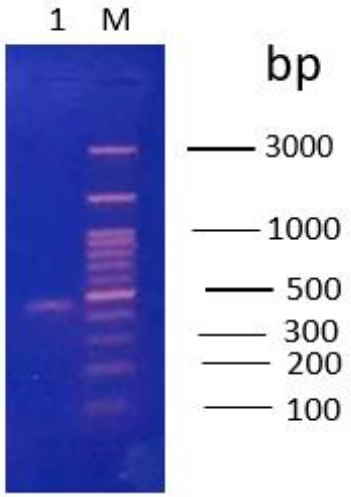

Fig 4: Agarose gel electrophoresis (1.5%) showing the PCR products of amplification of intSaPIbov1 from *S.aureus* MRSA clinical strains. Lane 1: represents PCR products (441) of intSaPIbov1 from one positive out of thirty*S.aureus* MRSA clinical strain. M: 100 bp DNA.

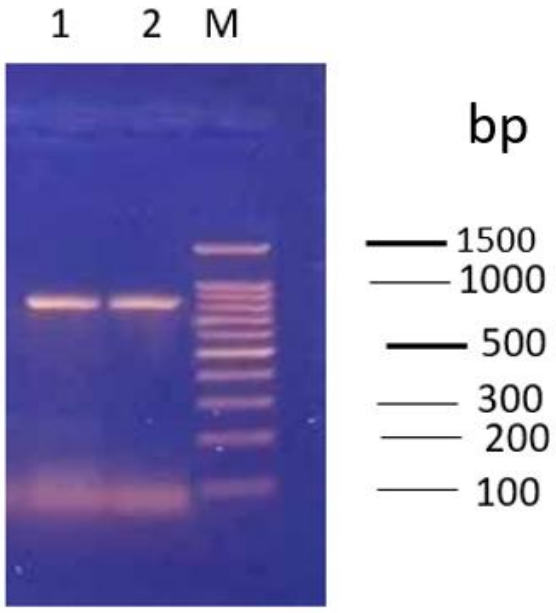

Fig 5: Agarose gel electrophoresis (1.5%) showing the PCR products of amplification of intSaPImw2 from *S.aureus* MRSA clinical strains. Lanes 1 &2: represent PCR products (870) of intSaPImw2 from two *S.aureus* MRSA clinical strains. M: 100 bp DNA.

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 4, 2021, Pages. 8498 - 8508
Received 05 March 2021; Accepted 01 April 2021.

Discussion

S.aureus MRSA clinical strains, most causative agent of skin lesions in human, pose a major public health threat worldwide. Methicillin-resistant *S. aureus* (MRSA) is a pathogen of growing magnitude in the hospitals and the community. *S.aureus* MRSA displays antibiotic resistance to against methicillin, penicillin, and vancomycin due to the presence of antibiotic resistance genes (Pal et al. 2020). Actually, the potential reorganization of virulence factors imposed by SaPI is of great significance from the standpoint of epidemiology. As it will in turn help in the regulation and emergence of novel resistant strains (Perillo et al., 2012). In the present study, the frequency of occurrence of SapI(s) among the thirty *S.aureus* MRSA clinical strains is in a good accordance with the findings of previous reported studies where each strain does harbor an average of one SaPI per strain and other strains carry two or more SaPI(s) (Cervera-Alamar et al. 2018). The frequency of occurrence of intSaPI1, intSaPI2, intSaPI3, intSaPI4, intSaPI5, intSaPIn1/m1, intSaPIbov1, and intSaPImw2 among the thirtyMRSA clinical strains were 6.66 (n=2/30), 16.66 (n=5/30), 13.33 (n=4/30), 20 (n=6/30), 10 (n=3/30), 23.33 (n=7/30), 3.33(n=1/30), and 6.66% (n=2/30), respectively. SaPI(s) harbor a distinguishing genes required for their unique life style and carry auxiliary genes, comprising toxic shock syndrome toxin (TSST-1) and other super-antigens (Schlievert et al. 2010). The frequency of occurrence of TSS-1 among the thirty*S.aureus* MRSA clinical strains, present strains enrolled in this study, was 100% (unpublished data). Reportedly, SaPIs are the only genetic carriers of TSST-1 (Schlievert et al. 2010).

On the other hand, SaPI(s) present a more significant role for the staphylococci than mere high-speed carriers of virulence genes. Despite their derivatization from prophages, they diverged, forming a highly distinctive and separate lineage. They control the transfer of non-linked

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 4, 2021, Pages. 8498 - 8508
Received 05 March 2021; Accepted 01 April 2021.

chromosomal genes (Chen et al. 2015). SapI(s) interfere with the reproduction of phage reproduction (Ram et al. 2014) and determine animal host specificity (Viana et al. 2010).

Hence, the SaPIs seem to be crucial for the evolution and adaptation of the organism.

References

1. Riewerts Eriksen, N. H., Espersen, F., Thamdrup Rosdahl, V. & Jensen, K. Carriage of *Staphylococcus aureus* among 104 healthy persons during a 19-month period. *Epidemiol. Infect.* **115**, 51–60 (1995).
2. Wertheim, H. F. *et al*. The role of nasal carriage in *Staphylococcus aureus* infections. *Lancet Infect. Dis.* **5**, 751–762 (2005).
3. Lindsay, J. A. & Holden, M. T. G. *Staphylococcus aureus*: Superbug, super genome? Trends in Microbiology **12**, 378–385 (2004).
4. Ruzin, A., Lindsay, J. & Novick, R. P. Molecular genetics of SaPI1–a mobile pathogenicity island in *Staphylococcus aureus*. Mol. Microbiol. **41**, 365–77 (2001).
5. MacFaddin J. F., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. I, Williams and Wilkins, Baltimore.
6. Green MR, Sambrook J (1989) Molecular cloning: A Laboratory Manual. Cold Spring Herber Laboratory Press.
7. Alibayov B, Zdenkova K, Sykorova H, Demnerova K. Molecular analysis of *Staphylococcus aureus* pathogenicity islands (SaPI) and their superantigens combination of food samples. J Microbiol Methods. 2014: 107; 197–204
8. Novick RP, Ram G. (2015). The floating (pathogenicity) island: a genomic dessert. Trends Genet 32: 114–126.
9. Novick RP, Christie GE, Penadés JR. (2010). The phage related chromosomal islands of Gram-positive bacteria. Nat Rev Microbiol 8: 541–551
10. Penadés JR, Christie GE. (2015).The phage-inducible chromosomal islands: a family of highly evolved molecular parasites. Annu Rev Virol 2: 181–201.
11. Martínez-Rubio R, Quiles-Puchalt N, Martí M, Humphrey S, Ram G, Smyth D, Chen J, Novick RP, Penadés JR. Phage-inducible islands in the Gram-positive cocci. The ISME Journal (2017) 11, 1029–1042.
12. Ruzin, A., Lindsay, J. & Novick, R. P. Molecular genetics of SaPI1–a mobile pathogenicity island in Staphylococcus aureus. Mol. Microbiol. 41, 365–77 (2001).

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 4, 2021, Pages. 8498 - 8508
Received 05 March 2021; Accepted 01 April 2021.

13. Malachowa, N. & Deleo, F. R. Mobile genetic elements of *Staphylococcus aureus*. Cellular and Molecular Life Sciences 67, 3057–3071 (2010).

14. Feiss, M. & Rao, V. B. Te bacteriophage DNA packaging machine. Adv. Exp. Med. Biol. 726, 489–509 (2012).

15. Cervera-Alamar M, Guzmán-Markevitch K, Žiemytė M, Ortí L, Bernabé-Quispe P, Pineda-Lucena A, Pemán J, Tormo-Mas MA. Mobilisation Mechanism of Pathogenicity Islands by Endogenous Phages in *Staphylococcus aureus* clinical strains. Scientific Reports. (2018) 8:16742 | DOI: 10.1038/s41598-018-34918-2.

16. Mahendra Pal, Kerorsa GB, Marami LM, Kandi V, "Epidemiology, Pathogenicity, Animal Infections, Antibiotic Resistance, Public Health Significance, and Economic Impact of *Staphylococcus aureus*: A Comprehensive Review." American Journal of Public Health Research, vol. 8, no. 1 (2020): 14-21. doi: 10.12691/ajphr-8-1-3.

17. Perillo, J., Ceccarelli, D., Spagnoletti, M., Lollai, S., Cappuccinelli, P., Colombo, M.M., (2012). Molecular characterization of enterotoxigenic and borderline oxacillin resistant Staphylococcus strains from ovine milk. Food Microbiol. 32, 265–273.

18. Schlievert PM, Strandberg KL, Lin YC, Peterson ML, Leung DY. Secreted virulence factor comparison between methicillin-resistant and methicillin-sensitive *Staphylococcus aureus*, and its relevance to atopic dermatitis. J Allergy Clin Immunol. 2010 Jan; 125(1):39-49.

19. Chen J, Ram G, Penadés JR, Brown S, Novick RP. Pathogenicity island-directed transfer of unlinked chromosomal virulence genes. Mol Cell. 2015 Jan 8; 57(1):138-49.

20. Ram G, Chen J, Ross HF, Novick RP. Precisely modulated pathogenicity island interference with late phage gene transcription. Proc Natl Acad Sci U S A. 2014. 7; 111(40):14536-41.

21. Viana D, Blanco J, Tormo-Más MA, Selva L, Guinane CM, Baselga R, Corpa J, Lasa I, Novick RP, Fitzgerald JR, Penadés JR. Adaptation of *Staphylococcus aureus* to ruminant and equine hosts involves SaPI-carried variants of von Willebrand factor-binding protein.

22. Mol Microbiol. 2010; 77(6):1583-94.