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ORIGINAL ARTICLE

Phylogenetic Relationships among *Staphylococcus epidermidis* based *on16S rRNA* Gene Sequence

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ABSTRACT

Background*: Diversity of clones is common in Staphylococci, but the focus on this diversity is less in coagulase negative group.* ***Objective****: We aimed to detect the biodiversity of Staphylococcus epidermidis isolated from skin lesions.* ***Methodology****: S. epidermidis were identified using Gram stain, catalase and coagulase tests, cultured on mannitol salt agar and tested for antibiotic susceptibility. Biodiversity was assessed using PCR of 16s rRNA genes. Phylogenetic analysis of isolate's sequences and sequences of S. epidermidis retrieved from Gene bank was done. The sequences were aligned to show their degree of similarity.* ***Results****: About 86% and 57% were resistant to penicillin and cefoxitin, fusidic acid, and erythromycin; respectively. Phylogenetic analysis revealed all S. epidermidis involved were belonged to the same cluster with S. epidermidis strain AFATF that was isolated previously from Egypt* ***Conclusion****: We confirmed the usefulness of 16s rRNA gene sequence in phylogenetic studies and the biodiversity of our isolates.*

INTRODUCTION

Genus staphylococcus is characterized by great level of diversity as it contains about 47 species and 23 subspecies[1]. It is classified on the basis of coagulase enzyme into two groups, a coagulase positive group which is characterized by the presence of coagulase enzyme. The most important and pathogenic species of this group is *Staphylococcus aureus* (*S. aureus*). While the second group is the coagulase negative one that is characterized by the absence of that enzyme and hence loses the ability to clot the plasma[2].

Coagulase negative group comprising many species, from which *Staphylococcus epidermidis* (*S. epidermidis*) and *Staphylococcus haemolyticus* are the most common ones[3]. Although *S. epidermidis* is classified as a commensal microorganism to the skin and mucous membranes of the human, now it is considered as an important microorganism in nosocomial infections especially when medical devices are used [4,5]. For *S. aureus*, there are many isolated strains from different sites of human body[6]. Sometimes many clones were isolated from the same site. Horizontal gene transfer can arise owing to different clones and subtypes of the microorganism. This transfer largely contributes to the pathogenicity and virulence of the microorganism [7].

Diversity of clones is a common feature in both coagulase positive and coagulase negative groups of Staphylococci, but the focus on this diversity is lower in coagulase negative group than coagulase positive one. For *S. epidermidis*, there are many published research methodologies for its identification. There is a great genome diversity among *S. epidermidis* which is higher than other species of the group. This fact was reported by pulsed field gel electrophoresis in many studies[8,9].

The genomic diversity of *S. epidermidis* is also analyzed by multi locus sequence typing in which a high level of genetic diversity and nine epidemic clonal lineages were observed to be disseminated worldwide with one single clonal lineage (clonal complex 2) comprised 74% of the isolates[10].

The assessment of genotypic diversity of *S. epidermidis* from expressed human breast milk is also done by other molecular methods as RAPD-PCR and REP-PCR in comparison to pulsed-field gel electrophoresis (PFGE). The study revealed a significant detection of the diversity by both RAPD-PCR and PFGE [11].

Multiplex PCR is another reported successful and effective method for detection of biodiversity in both coagulase positive and negative staphylococcal species isolated from patients with atopic dermatitis[12].

The genome of different phenotypes of closely related bacteria can be compared and analyzed phylogenetically. This phylogenetic analysis is a significant tool in detecting the pathogenicity of the microorganism [13,14]. For the phylogenetic relationship and identification of this microorganism, rRNA remains one of the most potent, objective, and accurate tools for identification [15].

The aim of the present study is to assess the genetic diversity among *S. epidermidis* and to estimate the

relationship among *S. epidermidis* with *S. aureus* using PCR assay of *16s rRNA* genes and phylogenetic analysis of their sequences.

METHODOLOGY

Bacterial strains, growth conditions, and susceptibility tests:

Seven *S. epidermidis* strains were isolated from patients with skin lesions. All patients age is ranging from 16 to 33 years. The isolates were subjected to Gram staining, catalase, and coagulase tests, and cultured on both nutrient agar (LAB M, England) and mannitol salt agar (LAB M, England) at 37°C for 18 to 24 h. The antibiotic susceptibility of these clinical isolates to penicillin, cefoxitin, tetracycline, doxycycline, gentamycin, fucidic acid, ofloxacin, chloramphnicol, erythromycin, and clindamycin was determined using disk diffusion method.

Isolation of genomic DNA:

Chromosomal DNA was isolated from overnight cultures grown on nutrient agar (LAB M, England) at 37°C. Genomic DNA was extracted by using the DNA extraction kit (Applied Biotechnology Co. Ltd, Egypt) according to manufacturer instructions. The concentration of the DNA was assessed spectrophotometrically.

PCR amplification of 16s rRNA gene:

PCR assay for *16s rRNA* gene was performed using a Gradient Thermacycler (MJ research thermal cycler, USA). The primers used for amplification were GAGTTTGATCCTGGCTCAG and GGTTACCTTGTTACGACTT (AlphaDNA Co., Canada). PCR reaction was performed in a 50 µl volume containing 100 ng of template DNA, 1 x of 2XRedmaster mix (Applied Biotechnology Co., Egypt), and 20 pmol of each primer. The volume for each PCR reaction was completed to 50 µl with nuclease free water (Promega, USA). The PCR products were electrophoresed on agarose gel and visualized under UV light.

DNA sequencing and alignment:

The amplified PCR products were submitted to Solgent Co Ltd (South Korea) for gel purification and sequencing. The obtained DNA sequences were edited and assembled using BioEdit (7.2) The sequences of our isolates were aligned using The Basic Local Alignment Search Tool (BLAST) algorithm in GenBank to find the regions of local similarity between our sequences and sequence databases (https://blast.ncbi.nlm.nih.gov/Blast.cgi). Name of the similar strains, the gene bank accession numbers, and the identity percentages were obtained.

The DNA sequences of the *16s rRNA* of 32 strains of *S. epidermidis* and 10 strains of *S. aureus* strains were downloaded from NCBI's GenBank. Different strains from different geographic regions including Egypt and some references strains were selected for retrieving their gene sequences for the subsequent analysis. *S. epidermidis* strain AFATF and *S. aureus* strain SVUB2, which isolated previously from Egypt, were included in our study (https://www.ncbi.nlm.nih.gov/nuccore/JX131632.1) and (https://www.ncbi.nlm.nih.gov/nuccore/AM982783.1); respectively.

Phylogenetic analysis:

Nucleotide sequences were aligned using MAFFT alignment (https://www.ebi.ac.uk/Tools/msa/mafft/)[16,17]. Phylogenetic tree was constructed along with determination of pairwise distances, sequence identity analysis and conservation analysis were performed using the Neighbor joining method[18] employing the Tamura-Nei Model[19].

RESULTS

The antibiotic susceptibility profiles of our isolates to penicillin, cefoxitin, tetracycline, doxycycline, gentamycin, fucidic acid, ofloxacin, chloramphnicol, erythromycin, and clindamycin were shown in table (1). About 86% were resistant to penicillin and 57% were resistant to cefoxitin, fusidic acid, and erythromycin. No resistance was detected against gentamycin, ofloxacin, chloramphnicol, and clindamycin.

Table 1: Susceptibility profiles of our clinical isolates of *Staphylococcus epidermidis* (OSE) strains.

Antimicrobial Agent	Concentration (µg)	OSE1	OSE2	OSE3	OSE4	OSE5	OSE6	OSE7
Penicillin	10	R	R	R	R	R	S	R
Cefoxitin	30	S	R	R	R	R	S	S
Tetracycline	30	S	I	S	I	R	S	S
Doxycycline	30	S	S	S	S	R	S	S
Gentamycin	10	S	S	S	S	S	S	S
Fucidic acid	10	S	R	R	R	R	S	S
Ofloxacin	5	S	I	I	S	S	S	S
Chloramphnicol	30	S	S	S	S	S	S	S
Erythromycin	15	S	R	R	R	R	S	S
Clindamycin	2	S	S	S	S	S	S	S

The seven sequences of our isolates were aligned with sequences from database. The accession numbers of the retrieved sequences were shown in table 2. Alignment of these sequences with the sequences of *S. epidermidis* strains already published in GenBank showed the regions of local similarity between our sequences and sequence databases. Table (3) showed the similarity percentage between our clinical isolates and the sequences in NCBI database. The highest identity percentage was obtained for sample OSE2 (99.13%) with strains *S. epidermidis*CDC121, MC10, and FDAARGOS_529 under the accession numbersCP034115.1, MK182856.1, and CP033782.1, respectively. While the lowest identity percentage was obtained for sample OSE6 (86.29%) with *S. epidermidis* CIFRI P-TSB7 under the accession number JF784023.1. Name of the similar strains, the gene bank accession numbers, and the identity percentages were shown in table (3) for all samples.

Table 2: Accession numbers of the *16SrRNA* gene sequences used in this study

Strain	Name of the strain	Accession number
SE1	*Staphylococcus epidermidis* strain AFATF	JX131632
SE2	*Staphylococcus epidermidis* JCM 5693 gene	LC462153
SE3	*Staphylococcus epidermidis*strain Fussel	NR_036904
SE4	*Staphylococcus epidermidis* ATCC 14990	D83363
SE5	*Staphylococcus epidermidis* strain NBRC 100911	NR_113957
SE6	Staphylococcus epidermidis *ATCC 146 (= MAFF 911486)*	D83362
SE7	Staphylococcus epidermidis *isolate H6-16S-LEGIO-BO*	FR775756
SE8	Staphylococcus epidermidis *isolate H5-16S-LEGIO-BO*	FR775755
SE9	Staphylococcus epidermidis *type strain DSM 20044T*	LN681574
SE10	Staphylococcus epidermidis*clone r16S_2*	HG326658
SE11	Staphylococcus epidermidis *clone f16S_1*	HG326657
SE12	Staphylococcus epidermidis*strain BGHMC11*	FR797807
SE13	Staphylococcus epidermidis *isolate 49/8*	HE962230
SE14	Staphylococcus epidermidis *strain CJBP1*	AM697667
SE15	Staphylococcus epidermidis *isolate AD10*	LT835144
SE16	Staphylococcus epidermidis *isolate AD9*	LT835133
SE17	Staphylococcus epidermidis *isolate DS8FE.134*	LN884112
SE18	Staphylococcus epidermidis *isolate D6N_3300*	LT677886
SE19	Staphylococcus epidermidis *isolate U4N_3099*	LT677685
SE20	Staphylococcus epidermidis *strain BGHMN1*	FR797794
SE21	Staphylococcus epidermidis *strain BGHMN2*	FR797795
SE22	Staphylococcus epidermidis *strain BGHMN3*	FR797796
SE23	Staphylococcus epidermidis *strain BGHMN4*	FR797797
SE24	Staphylococcus epidermidis*strain BGHMN5*	FR797798
SE25	Staphylococcus epidermidis*strain BGHMN10*	FR797802
SE26	Staphylococcus epidermidis*strain BGHMN9*	FR797801
SE27	Staphylococcus epidermidis*strain BGHMN8*	FR797800
SE28	Staphylococcus epidermidis*strain BGHMC1*	FR797803
SE29	Staphylococcus epidermidis*strain BGHMC5*	FR797804
SE30	Staphylococcus epidermidis*isolate W626T_307*	LT674893
SE31	Staphylococcus epidermidis*isolate 210N_1043*	LT675629
SE32	Staphylococcus epidermidis*isolate 471N_127*	LT674713
SA1	Staphylococcus aureus *strain ATCC 12600*	NR_118997
SA2	Staphylococcus aureus *strain S33 R*	NR_037007
SA3	Staphylococcus aureus *subsp. anaerobius strain MVF-7*	NR_036828
SA4	Staphylococcus aureus *strain NBRC 100910*	NR_113956
SA5	Staphylococcus aureus *strain ATCC 12600*	NR_115606
SA6	Staphylococcus aureus *ATCC 25923*	U02910
SA7	Staphylococcus aureus *strain CWS1*	FM207477
SA8	Staphylococcus aureus *JCM 5695*	LC462155
SA9	Staphylococcus aureus *strain: OA1*	D8356
SA10	*Staphylococcus aureus* strain SVUB2	AM982783

Table 3: Result of NCBI BLAST of *16S rRNA* genes of our isolates

Isolates	Maximum identity with BLAST Seq ID.		
	Identity percentage	**Strain**	**Gen Bank Accession no**
OSE1	96.69	PomC5.13	LM994806.1
OSE2	99.13	CDC121, MC10, and FDAARGOS_529	CP034115.1, MK182856.1, and CP033782.1
OSE3	90.27	CIFRI P-TSB7	JF784023.1
OSE4	98.37	OB027, OA162, 18CR, and isolate 211	KY622984.1, KY622801.1, KX214048.1, and LN623604.1
OSE5	91.88	4S01	MH392290.1
OSE6	86.29	CIFRI P-TSB7	JF784023.1
OSE7	98.53	Urmia-Culis-36	MK840753.1

Phylogenetic analysis of the *16Sr RNA* gene sequences for our clinical isolates and retrieved sequences of *S. aureus* and *S. epidermidis* strains revealed 3 distinct clusters with no out-group (Fig. 1). Interestingly, all isolated *S. epidermidis* strains involved in this study were belonged to the same cluster (cluster 1) together with the *S. epidermidis* strain AFATF that was isolated previously from Egypt (https://www.ncbi.nlm.nih.gov/nuccore/JX131632.1). The sequences of our *S. epidermidis* isolates were classified to subgroups that were closely related to each other as appeared in the phylogenetic tree (Fig. 1).

Our strains sequences and the sequence of *S. aureus* isolated earlier from Egypt (https://www.ncbi.nlm.nih.gov/nuccore/AM982783.1) were seen in phylogenetically distinct two branches (cluster 1 and 2), cluster 2 contained the majority of *S. aureus16S rRNA* gene sequences.

Cluster 3 was distinct for strain SE9; *S. epidermidis*, type strain DSM 20044T which was isolated from USA.

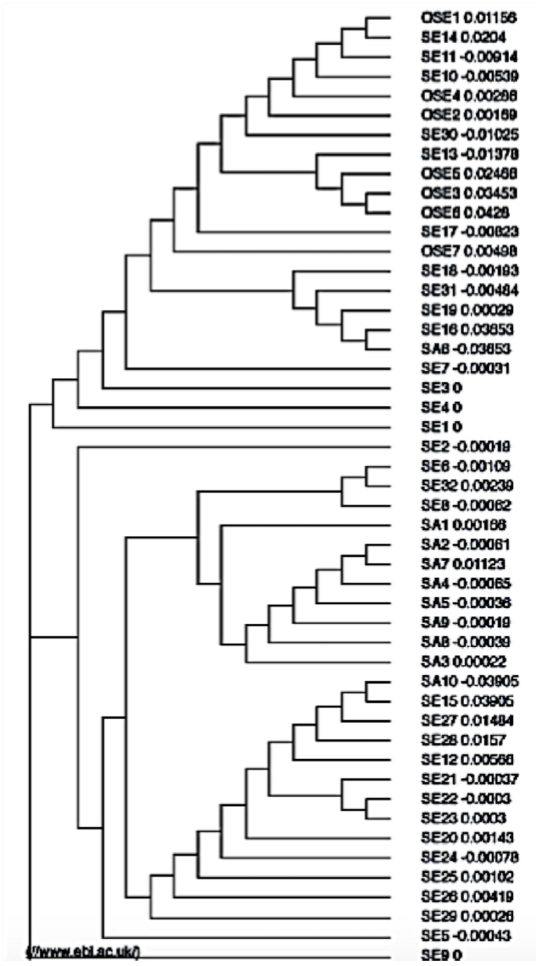

Fig. 1: Phylogenetic tree created using the neighbour-joining method based on the partial nucleotide sequences of *16S rRNA* gene of *S. epidermidis* and *S. aureus*. The tree generated from the alignment of the reference sequences with samples from this study using MAFFT alignment (https://www.ebi.ac.uk/Tools/msa/mafft/).

DISCUSSION

Suitable antimicrobial drug therapy is an important issue for the treatment of infectious diseases caused by coagulase negative staphylococci today; this is due to great diversity of the species and the emergence of antimicrobial resistance. Antimicrobial susceptibility test was performed to our *S. epidermidis* strains and high resistance pattern to penicillin was observed. The elevated rate of resistance to this antimicrobial agent was detected in other studies regardless the site of isolation. High resistance pattern of multidrug resistant *S. epidermidis* to penicillin was reported early [20] and also high resistance rate to penicillin G was predicted[21].Of notice, we reported no resistance to neither gentamycin, ofloxacin, chloramphnicol, nor clindamycin. This is in accordance with a study performed in a near area; Saudi Arabia, who detected that all their *S. epidermidis* isolates were susceptible to gentamycin, levofloxacin, and moxifloxacin[22]. In contrast, Hellmark group reported 79% of *S. epidermidis* isolates from Sweden were resistant to gentamycin [23]. Resistance detected to fusidic acid in our study was 57% and this is closely similar to what reported before in Europe 46% and 60%[24, 25].

The alignment results showed high degree of similarity (99.13%) between the sequence of OSE2 with the sequence of *S. epidermidis* CDC121, MC10, and FDAARGOS_529 which are isolated from South Korea, Spain, and USA; respectively. This is coincident with the concept that the genetic diversity between strains is widely distributed all over the world. On the other side, low degree of similarity was detected between OSE6 and *S. epidermidis* CIFRI P-TSB7, this may be attributed to the difference in environmental conditions between the two strains as the later one was identified in east coast of India.

The comparison of the *16S rRNA* gene sequences has been useful in many phylogenetic studies of Staphylococcus[26, 27]. The similarity of the *16S rRNA* sequence has been shown to be very high; 90% to 99% in 29 Staphylococcus species[28].

Phylogenetic analysis of the *16Sr RNA* gene sequences for our isolates showed that all the strains were located on the same cluster. This cluster group also contains sequences retrieved of other *S. epidermidis* from different countries as Egypt, Japan, and USA which indicates the high level of biodiversity of our isolates. Interestingly, these results were similar to what reported before about the great genome diversity among *S. epidermidis* which is higher than other species of the group[8, 9].

Our strains sequences and the sequence of *S. aureus* isolated earlier from Egypt were seen in phylogenetically distinct two branches (cluster 1 and 2), cluster 2 contained the majority of *S. aureus16S rRNA* gene sequences. These results were in accordance with a previous study performed in Iraq who also supported the biodiversity of their *S. aureus* isolates [29].

CONCLUSION

Our study indicated that *S. epidermidis* strains isolated from Egypt were highly diverse and the phylogenetic analysis using *16s rRNA* gene sequence is a helpful step in this research area.

Author contribution

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Shymaa Enany and Samira Zakeer. The first draft of the manuscript was written by Samira zakeer and Shymaa Enany and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Compliance with Ethical Standards

This study was approved by Suez Canal University, Egypt ethical board. Permission and informed consent to collect samples were obtained from all patients attending the skin Hospital at Cairo Governorate, Egypt.

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- Each author listed in the manuscript had seen and approved the submission of this version of the manuscript and takes full responsibility for it.
- This article had not been published anywhere and is not currently under consideration by another journal or a publisher.

REFERENCES

1. Lamers RP, Muthukrishnan G, Castoe TA, Tafur S, Cole AM, Parkinson CL. Phylogenetic relationships among Staphylococcus species and refinement of cluster groups based on multilocus data. BMC evolutionary biology. 2012;12:171.
2. Baron S. Medical Microbiology. 4th edition. Chapter 12 Staphylococcus. Galveston (TX): University of Texas Medical Branch at Galveston; 1996.

3. Becker K, Heilmann C, Peters G. Coagulase-negative staphylococci. Clinical microbiology reviews. 2014;27(4):870-926.
4. Otto M. Staphylococcus epidermidis--the 'accidental' pathogen. Nature reviews Microbiology. 2009;7(8):555-67.
5. Widerstrom M, Wistrom J, Sjostedt A, Monsen T. Coagulase-negative staphylococci: update on the molecular epidemiology and clinical presentation, with a focus on Staphylococcus epidermidis and Staphylococcus saprophyticus. European journal of clinical microbiology & infectious diseases : official publication of the European Society of Clinical Microbiology. 2012;31(1):7-20.
6. Rodriguez M, Hogan PG, Burnham CA, Fritz SA. Molecular epidemiology of Staphylococcus aureus in households of children with community-associated S aureus skin and soft tissue infections. The Journal of pediatrics. 2014;164(1):105-11.
7. Cespedes C, Said-Salim B, Miller M, Lo SH, Kreiswirth BN, Gordon RJ, et al. The clonality of Staphylococcus aureus nasal carriage. The Journal of infectious diseases. 2005;191(3):444-52.
8. Lina B, Vandenesch F, Etienne J, Kreiswirth B, Fleurette J. Comparison of coagulase-negative staphylococci by pulsed-field electrophoresis. FEMS microbiology letters. 1992;71(2):133-38.
9. Lang S, Livesley MA, Lambert PA, Elliott J, Elliott TS. The genomic diversity of coagulase-negative staphylococci associated with nosocomial infections. The Journal of hospital infection. 1999;43(3):187-93.
10. Miragaia M, Thomas JC, Couto I, Enright MC, de Lencastre H. Inferring a population structure for Staphylococcus epidermidis from multilocus sequence typing data. Journal of bacteriology. 2007;189(6):2540-52.
11. Begovic J, Jovcic B, Papic-Obradovic M, Veljovic K, Lukic J, Kojic M, et al. Genotypic diversity and virulent factors of Staphylococcus epidermidis isolated from human breast milk. Microbiological research. 2013;168(2):77-83.
12. Soares J, Lopes C, Tavaria F, Delgado L, Pintado M. A diversity profile from the staphylococcal community on atopic dermatitis skin: a molecular approach. Journal of applied microbiology. 2013;115(6):1411-9.
13. Lefebure T, Stanhope MJ. Evolution of the core and pan-genome of Streptococcus: positive selection, recombination, and genome composition. Genome biology. 2007;8(5):R71.
14. Soyer Y, Orsi RH, Rodriguez-Rivera LD, Sun Q, Wiedmann M. Genome wide evolutionary analyses reveal serotype specific patterns of positive selection in selected Salmonella serotypes. BMC evolutionary biology. 2009;9:264.
15. Zakrzewska-Czerwinska J, Gaszewska-Mastalarz A, Pulverer G, Mordarski M. Identification of Staphylococcus epidermidis using a 16S rRNA-directed oligonucleotide probe. FEMS microbiology letters. 1992;100(1-3):51-8.
16. Madeira F, Park YM, Lee J, Buso N, Gur T, Madhusoodanan N, et al. The EMBL-EBI search and sequence analysis tools APIs in 2019. Nucleic acids research. 2019;47(W1):W636-W41.
17. Katoh K, Standley DM. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Molecular biology and evolution. 2013;30(4):772-80.
18. Saitou N, Nei M. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Molecular biology and evolution. 1987;4(4):406-25.
19. Tamura K, Nei M. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Molecular biology and evolution. 1993;10(3):512-26.
20. Haque N, Hossain MA, Bilkis L, Musa AK, Mahamud C, Bari MS, et al. Antibiotic susceptibility pattern of Staphylococcus epidermidis. Mymensingh medical journal : MMJ. 2009;18(2):142-7.
21. Ma XX, Wang EH, Liu Y, Luo EJ. Antibiotic susceptibility of coagulase-negative staphylococci (CoNS): emergence of teicoplanin-non-susceptible CoNS strains with inducible resistance to vancomycin. Journal of medical microbiology. 2011;60(Pt 11):1661-8.
22. Eladli MG, Alharbi NS, Khaled JM, Kadaikunnan S, Alobaidi AS, Alyahya SA. Antibiotic-resistantStaphylococcus epidermidisisolated from patientsand healthy students comparing with antibiotic-resistant bacteriaisolated from pasteurized milk. Saudi Journal of Biological Sciences. 2018.
23. Hellmark B, Unemo M, Nilsdotter-Augustinsson A, Soderquist B. Antibiotic susceptibility among Staphylococcus epidermidis isolated from prosthetic joint infections with special focus on rifampicin and variability of the rpoB gene. Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases. 2009;15(3):238-44.
24. McLaws F, Chopra I, O'Neill AJ. High prevalence of resistance to fusidic acid in clinical isolates of

Staphylococcus epidermidis. The Journal of antimicrobial chemotherapy. 2008;61(5):1040-3.

25. Hamad T, Hellmark B, Nilsdotter-Augustinsson A, Soderquist B. Antibiotic susceptibility among Staphylococcus epidermidis isolated from prosthetic joint infections, with focus on doxycycline. APMIS : acta pathologica, microbiologica, et immunologica Scandinavica. 2015;123(12):1055-60.
26. Ghebremedhin B, Layer F, Konig W, Konig B. Genetic classification and distinguishing of Staphylococcus species based on different partial gap, 16S rRNA, hsp60, rpoB, sodA, and tuf gene sequences. Journal of clinical microbiology. 2008;46(3):1019-25.
27. Taponen S, Simojoki H, Haveri M, Larsen HD, Pyorala S. Clinical characteristics and persistence of bovine mastitis caused by different species of coagulase-negative staphylococci identified with API or AFLP. Veterinary microbiology. 2006;115(1-3):199-207.
28. Kwok AY, Su SC, Reynolds RP, Bay SJ, Av-Gay Y, Dovichi NJ, et al. Species identification and phylogenetic relationships based on partial HSP60 gene sequences within the genus Staphylococcus. International journal of systematic bacteriology. 1999;49 Pt 3:1181-92.
29. Saleh RO, Raheema RH, Jameel ZJ. Phylogenetic tree and Submission of Staphylococcus aureus Isolate from Skin Infection. Journal of Pure and Applied Microbiology. 2018;12(4):2199-204.