A New Specific DNA Target Sequence for Identification of *Staphylococcus epidermidis* using Modified Comparative Genomic Analysis

Reza Khoshbakht 1,2, Hosna Zare 1,2, Reza Kamali Kalhaki 1,2, Alireza Neshani 2,3, and Maryam Arfaatabar 4

1. Antimicrobial Resistance Research Center, Mashhad University of Medical Sciences, Mashhad, Iran
2. Student Research Committee, Mashhad University of Medical Sciences, Mashhad, Iran
3. Department of Laboratory Sciences, School of Paramedical Sciences, Mashhad University of Medical Sciences, Mashhad, Iran
4. Department of Medical Laboratory Sciences, Kashan Branch, Islamic Azad University, Kashan, Iran
† The first and the second authors have had equal contribution to this manuscript

**Abstract**

**Background:** *Staphylococcus epidermidis* (*S. epidermidis*) is the most frequently isolated pathogen from prostheses infections in the body. Therefore, improving its diagnostic methods, including rapid Nucleic Acid Amplification Tests (NAAT), seems necessary. Since the first step in designing a NAAT is to find a specific sequence and all DNA targets that have been introduced so far are not completely specific, we introduced a new 100% specific DNA target sequence to identify *S. epidermidis* in this study.

**Methods:** Modified comparative genomic analysis was used to find the best specific target sequence to detect *S. epidermidis*. A PCR method was designed for the evaluation of this target. To determine the detection limit and analytical specificity, pure genomic DNA of 18 bacteria include 12 standard strains (one *S. epidermidis* and 11 non-*S. epidermidis*) and six clinical isolates (five *S. epidermidis* and one non-*S. epidermidis*) were used.

**Results:** The 400 bp sequence of *S. epidermidis* ATCC 14990 was identified as the most specific sequence (Se400), having a 100% sequence similarity to *S. epidermidis* genomes but not with other bacteria. The detection limit of Se400-PCR was 10 fg, equal to about 4 copies of *S. epidermidis* genomic DNA/μl. All pure DNA templates from *S. epidermidis* generated a detectable amplicon by 264 bp length, but the PCR test was negative for the non-*S. epidermidis* group.

**Conclusion:** The Se400 sequence can be considered as a specific target for detecting *S. epidermidis*, based on our findings.

**Keywords:** Comparative genomic analysis, Detection, Pathogen, Polymerase chain reaction, Se400, *Staphylococcus epidermidis*

**Introduction**

One of the most isolated members of the coagulase-negative staphylococci (CoNS) group is *Staphylococcus epidermidis* (*S. epidermidis*). This bacterium colonizes mucous membranes and the skin, accounting for the majority of the bacterial flora in this environment. Genome study of *S. epidermidis* revealed that it is fully equipped with genes supposed to offer resistance from the severe circumstances faced in surrounding environment, allowing it to remain longer in dry conditions in hospitals. *S. epidermidis* is the most commonly implicated pathogen in infections related to any form of an indwelling medical device. This micro-organism has been detected with a relatively high prevalence from the Central Nervous System (CNS) shunts, joint prostheses, and prosthetic valves. Also, the mentioned bacterium has been repeatedly isolated from different specimens, such as blood, skin, wound, urinary tract, soft tissue infections, endocarditis, bactereemia, and pneumonia. According to scientific documents, CoNS cause half of all cases of Prosthetic Valve Endocarditis (PVE). More than 20% of people with implanted cardiac devices are infected by *S. epidermidis*, which in turn causes pain and purulence at the infection site and sepsis. The sepsis mortality rate resulting...
from *S. epidermidis* in infants could be as high as 4.8 and 9.4% \(^1\). The mortality rate due to endocarditis caused by CoNS is reported to be about 36% \(^6\), while it is estimated at 30% for septic shock \(^1\).

Traditionally, *S. epidermidis* diagnosis has been performed according to the biochemical tests and morphological characteristics. Such methods are time-consuming (sometimes up to several days) and do not reliably distinguish *S. epidermidis* from other CoNS. Therefore, finding faster and more reliable methods has always been required. The development of Nucleic Acid Amplification Tests (NAATs) such as Polymerase Chain Reaction (PCR) in recent decades, has greatly increased the speed, sensitivity, and specificity of diagnostic tests \(^13-15\). One of the critical points for designing a NAAT is a completely specific DNA sequence for the desired micro-organism. The specific sequence should be present in all strains of such micro-organism but not found in any other micro-organism or has very low similarity \(^16\). In recent years, various PCR tests have been designed to detect *S. epidermidis* based on genes such as *serp0107*, *gseA*, *Staphostatin A*, and *sesC* \(^17-20\). Our bioinformatics evaluation showed that all genes introduced so far as diagnostic targets, are not 100% specific, and have many similarities with other species of *Staphylococcus*. Therefore, finding a specific target sequence that can be applied to design a completely specific PCR is still needed. Modified comparative genomic analysis or modified genome comparison is among the methods for finding a specific target sequence for organisms and was introduced by our team in 2018 \(^16,21\). So, this study aimed to introduce a novel target gene that is specific for the *S. epidermidis* complex, as well as to design highly specific and sensitive primers for the rapid detection of *S. epidermidis* using modified comparative genomic analysis.

**Materials and Methods**

*Staphylococcus epidermidis*-specific target mining

According to the described method in our previous studies \(^16,21\), the genomic sequence of *S. epidermidis* ATCC 14990 was compared with the available genomic sequences on the nucleotide collection database \(^22,23\), and the most specific sequence was selected (Figure 1). The steps are described below:

1. Genomic sequences of *S. epidermidis* on nucleotide collection database were determined. Then, one case which was preferably the NCBI reference sequence, was regarded as the reference.
2. The sequence of *S. epidermidis* ATCC 14990 (NZ_CP035288.1) was selected as the reference, and the sequence was obtained and cut to 5000 bp independent fragments, producing about 493 fragments.
3. Separately, each fragment was compared with other available sequences of nucleotide collection database by Basic Local Alignment Search Tool (BLAST). BLAST discovers similar regions between DNA sequences. The nucleotide sequences is compared with available sequences on database and the statistical significance is calculated by the program \(^22\).
4. After each analysis, results were screened, and the best fragments were selected. Evaluation of results was performed by two criteria:
   a. Presence of all *S. epidermidis* NCBI reference sequences in search results, having both identity and query cover 100%.
   b. No other microorganism except *S. epidermidis* would appear with the query cover >90%. The selection of these two criteria was based on our experiences and evaluation of the first 200 fragments.
5. Selected fragments of the previous step were compared separately with non-*S. epidermidis* sequences of the nucleotide collection database, and conserved parts of each fragment were determined.
6. Finally, the longer specific part was selected, and we named it Se400.

**Primer design and PCR**

To evaluate the specificity of Se400, an end-point PCR was designed with Oligo7 software \(^24\). The primers were then tested for secondary structure and anticipated melting temperature using Oligo Analyzer 3.1 (https://eu.idtdna.com/calc/analyzer) and were manufactured by DENAzist Asia Company. 264 bp amplicon and Primer sequences are provided in table 1. PCR reaction was prepared in 25 μl containing 2.5 μl of 10xPCR buffer (100 mM Tris-HCl [pH=8.3], 500 mM KCl), 1 μl of each 10 μM forward and reverse primers, 1 μl of DNA sample, 0.5 μl of 200 μM (each) of the four dNTPs, 1.5 μl of 25 mM MgCl₂, 0.625 U of Taq DNA polymerase, and PCR grade water. 10 ng of

---

**Figure 1. Method for mining *S. epidermidis*-specific nucleotide sequences.**
S. epidermidis ATCC 14990 pure DNA was used as the positive control, and water as the negative control.

For DNA amplification, 5 min of initial denaturation at 95°C was followed by 30 cycles of (i) 45 s of denaturation at 95°C, (ii) 45 s of annealing at 49°C for, (iii) 60 s of extension at 72°C for, and (iv) final 5 min of extension at 72°C. Finally, 3 μl of PCR product was visualized using 1.5% agarose gel electrophoresis and DNA green viewer. The presence of a 264 bp amplicon specifies the positive result.

**Bacterial isolates**

In this study, pure genomic DNA of 18 bacteria, including 12 standard strains (one S. epidermidis and 11 non-S. epidermidis) and six clinical isolates (S. epidermidis and one non-S. epidermidis) were used. All standard strains were acquired from the microbial bank of the Antimicrobial Resistance Research Center of Mashhad University of Medical Sciences, and all clinical isolates were acquired from Imam Reza Hospital of Mashhad (Table 2).

**Analytical sensitivity (limit of detection)**

The pure DNA concentration of S. epidermidis ATCC 14990 was calculated by a spectrophotometer (Thermo Scientific). A serial dilution of pure DNA was then prepared in water (10 ng, 1 ng, 100 pg, 10 pg, 1 pg, 100 fg, 10 fg, 1 fg) and the volume of 1 μl was applied as the template. The process was repeated three times to ensure the results.

**Analytical specificity**

The primer specificity was investigated by Blastn software to determine cross-reactivity with other human or bacterial genomes. To determine the analytical specificity for Se400-PCR, pure DNA of six S. epidermidis and 12 non-S. epidermidis were used (Table 2). 10 ng of pure DNA was applied in each reaction. Finally, sequencing was performed on all positive PCR products.

**Results**

**Target mining**

The 400 bp sequence containing nucleotides 242, 200 to 242,600 of S. epidermidis ATCC 14990 (NZ_CP035288.1) was recognized as the most specific sequence, having a 100% sequence similarity to S. epidermidis genomes but not with other bacteria. Se400 is a non-coding sequence located between EQW00_01195 and EQW00_01200 genes (Figure 2). The blastn search showed that Se400 could detect all strains of S. epidermidis among the registered complete genomes in the nucleotide collection database, and no similarity was observed with other microorganisms.
Analytical sensitivity (limit of detection)

The detection limit is defined as the lowest analyte concentration that can be reliably detected. Consequently, the Se400-PCR detection limit was 10 fg, equal to about 4 copies of S. epidermidis genomic DNA/μl (Figure 3).

The analytical specificity

PCR amplification using Se400-specific primers was performed with 18 bacteria using pure genomic DNA as the template to consider the analytical specificity of the Se400-PCR. As presented in figure 4, all genomic DNA templates from S. epidermidis generated a detectable amplicon by 264 bp length, but the PCR test was negative for the non-S. epidermidis group; subsequently, the Se400 primer was specific for detecting S. epidermidis. Finally, analysis of sequencing results for PCR products from 6 positive samples showed that the produced amplicon is related to the Se400 sequence of S. epidermidis.

Discussion

Before the discovery of molecular methods, phenotypic and biochemical tests were the only powerful methods for the detection and differentiation of various bacteria, including S. epidermidis. With the development of molecular methods, especially PCR in recent decades, the problems of traditional methods such as being slow and lacking reliability were solved. One of the challenges for designing a PCR test to detect S. epidermidis is the lack of a completely specific DNA target. According to the literature, most NAATs in the 1990s used genus-specific targets due to the unavailability of species-specific DNA targets. The amplified sequences based on such genes could only differentiate S. epidermidis from other close species when they were analyzed by confirmatory methods. The most famous method for analyzing these fragments was sequencing and then comparing the results with available sequences on GenBank. 16S rDNA, sodA, hsp60, and tuf were among the most used genes in such methods. Although the addition of the sequencing step reduced the speed and increased the costs, some cases were still seen in which the sequenced fragment was quite similar in several species. For example, although the 16S rDNA gene has been suggested as a target gene, it cannot be used as a distinct target to detect S. epidermidis in clinical specimens due to the significant similarity to Staphylococcus aureus (S. aureus). With an increased detection of S. epidermidis infections in the last two decades, the need to find species-specific targets for the detection of this bacterium has been increased significantly. Efforts eventually led to the introduction of serp0107, gseA, ecpB, and SesC genes as species-specific targets. However, our bioinformatics evaluation before starting this project showed that none of these diagnostic targets was 100% specific (Table 3).

In 2004, the gseA gene (GenBank acc. No. AB096695), responsible for the production of glutamic acid-specific 27-kDa serine protease (GluSE), was introduced by Ikeda et al as a species-specific target of S. epidermidis. This protease is involved in degrading human fibronectin, collagen, the complement protein C5, and slime formation. Thus, the protease may be associated with the pathogenesis of S. epidermidis. Despite an appropriate length of this gene (1214 bp), a comparison of its sequence with other available sequences at nucleotide collection database showed that this gene has similarity with some parts of the Staphylococcus saccharolyticus (S. saccharolyticus) genome with >68% query cover and >75% identity. This bacterium is a normal flora of the skin, and the pathogenesis is not apparent yet. Furthermore, very similar sequences to the gseA gene are present in other bacteria, reducing the specificity of this gene for the detection S. epidermidis. These bacteria include Staphylococcus capitis (S. capitis), Staphylococcus caprae (S. caprae), S.
A New Specific Target Gene for Identification of *Staphylococcus epidermidis*

Table 3. Comparison of Se400 sequence specificity with other introduced genes for detecting *S. epidermidis* in species level

| Target | Length (bp) | Similarity to non-*S. epidermidis* genomes with >35% query cover and >60% identity |
|--------|-------------|-----------------------------------------------------------------------------------|
| Se400  | 400         | No similarity to any non-*S. epidermidis* microbes                             |
| gseA (2004) | 1214       | - 3 strains of *S. saccharolyticus* with >68% query cover and >75% identity  
|          |             | - 5 strains of *S. caprae* with >58% query cover and >69% identity            |
|          |             | - 11 strains of *S. warneri* with 55% query cover and 67% identity            |
|          |             | - > 1300 strain of *S. aureus* with 50% query cover and 67-69% identity      |
|          |             | - 6 strains of *S. pasteuri* with 46-51% query cover and 67-69% identity     |
| serp0107 (2006) | 882     | - 3 strains of *S. saccharolyticus* with 98% query cover and 69% identity  
|          |             | - 7 strains of *S. capitis* with 95% query cover and 68% identity            |
|          |             | - 5 strains of *S. caprae* with 66% query cover and 70% identity            |
|          |             | - 3 strains of *S. haemolyticus* with 42% query cover and >68% identity     |
|          |             | - Other microorganisms: *S. warneri* *S. hominis* *S. argenteus*            |
| sesC (2016) | 2031       | - 3 strains of *S. saccharolyticus* with 83-100% query cover and >69% identity  
|          |             | - 7 strains of *S. capitis* with 42% query cover and >67% identity          |
|          |             | - 5 strains of *S. caprae* with 52% query cover and 66% identity            |
| ecpB (2019) | 318        | - 3 strains of *S. saccharolyticus* with 100% query cover and >77% identity  
|          |             | - 19 strain of *S. aureus* with 65% query cover and 67% identity            |

The specificity of the genes was tested bioinformatically using Blastn software.

*aureus*, *Staphylococcus haemolyticus* (*S. haemolyticus*), *Staphylococcus saprophyticus* (*S. saprophyticus*), *Staphylococcus hominis* (*S. hominis*), *Staphylococcus equorum* (*S. equorum*), *Staphylococcus simulans* (*S. simulans*), *Staphylococcus lugdunensis* (*S. lugdunensis*), and *Staphylococcus schleiferi* (*S. schleiferi*).

serp0107, which is a putative transcriptional regulator gene, was introduced by Liu et al in 2006 as the species-specific diagnostic target to detect *S. epidermidis*. The sequence of this gene with 882 bp length was obtained from the (nt 91276–92157) of *S. epidermidis* RP62a (GenBank Accession No. CP000029). Our assessment of this sequence using the BLASTN search tool showed that a highly similar sequence is also present in *S. saccharolyticus* and *S. capitis*. Also, similar points to the serp0107 gene are found in other Staphylococcus species, including *S. caprae*, *S. haemolyticus*, *Staphylococcus warneri* (*S. warneri*), *S. hominis*, and *Staphylococcus argenteus* (*S. argenteus*) lead to a lack of complete specificity of this gene for the detection of *S. epidermidis*.

*S. epidermidis* surface protein C (*sesC*) gene was introduced by Khodaparast et al in 2016 as a specific diagnostic target of *S. epidermidis*. *sesC* protein is expressed more in *S. epidermidis* biofilm-associated cells than planktonic ones. Also, this target is appropriate to design various NAATs due to the suitable length (2031 bp). Nevertheless, our evaluation of this gene showed that similar sequences are found in three other species of the *Staphylococcus* genus, which reduces its specificity. The highest similarity was seen for the *S. saccharolyticus* with 83-100% query cover and >69% identity, followed by *S. capitis* and *S. caprae*.

Finally, the last introduced species-specific gene for this bacterium was the *ecpB* gene, encoding the Staphostatin A protein. This 318 bp gene was initially introduced to differentiate *S. aureus* and *S. epidermidis*. However, the high similarity with a sequence in *S. aureus* with 65% query cover and 67% identity makes it unspecific. The assessment of available sequences in NCBI also showed that this gene is also found in *S. saccharolyticus* with 100% query cover and >77% identity.

According to the results obtained using the BLASTN search tool, the Se400 sequence is completely specific to the *S. epidermidis* and is not found in any other micro-organisms, unlike the other sequences having nonspecific regions in several points. Therefore, using this sequence seems to solve the unavailability of a completely specific target for *S. epidermidis*.

In this study, the specificity of the Se400 target sequence was confirmed by PCR. The specificity of Se400 in all *S. epidermidis* strains highlighted both the efficacy of the comparative genomic analysis for finding possible targets and the significance of experimental research. Also, the Se400-PCR test was able to detect very low levels of genomic DNA template. One of our limitations was the small number of bacterial strains tested, and it is recommended that further research be conducted with a large sample size. Future studies might include comparing the previously known target gene with Se400 sequence in clinical samples.

**Conclusion**

In conclusion, *S. epidermidis*-specific target sequences were identified using a new comparative genomics method for finding species-specific nucleotide sequences. Many nucleotide targets were assessed, and a target sequence was applied to design a PCR test to detect *S. epidermidis* in clinical samples. Further surveys are being planned to include more bacterial strains for the evaluation of the particular targets. Unique tar-
gets may be found using this method for the detection of any micro-organism, for which a genome sequence is available.

Conflict of Interest
None declared.

References
1. Morse SA, Mietzner TA, Miller S, Riedel S, Jawetz Melnick & Adelbergs Medical Microbiology 28 E: McGraw-Hill Education; 2019.
2. Stacy A, Belkaif Y. Microbial guardians of skin health. Science 2019;363(6424):227-8.
3. Rendboe AK, Johannesen TB, Ingham AC, Månsson E, Iversen S, Baig S, et al. The Epidome - a species-specific approach to assess the population structure and heterogeneity of Staphylococcus epidermidis colonization and infection. BMC Microbiol 2020;20(1):362.
4. Chu V, Miro JM, Hoen B, Cabell CH, Pappas PA, Jones P, et al. Coagulase-negative staphylococcal prosthetic valve endocarditis—a contemporary update based on the International Collaboration on Endocarditis: prospective cohort study. Heart 2009;95(7):570-6.
5. Sabaté Brescó M, Harris LG, Thompson K, Stanic B, Morgenstern M, O'Mahony L, et al. Pathogenic mechanisms and host interactions in Staphylococcus epidermidis device-related infection. Front Microbiol 2017;8(1401).
6. Rogers KL, Fey PD, Rupp ME. Coagulase-negative staphylococcal infections. Infect Dis Clin North Am 2009;23(1):73-98.
7. McCann MT, Gilmore BF, Gorman SP. Staphylococcus epidermidis device-related infections: pathogenesis and clinical management. J Pharm Pharmacol 2008;60(12):1551-71.
8. Bennett JE, Dolin R, Blaser MJ, Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases E-Book: Elsevier Health Sciences; 2019.
9. Namvar AE, Bastararah S, Abbasi N, Ghebi GS, Farhadbaktiariat S, Arezi P, et al. Clinical characteristics of Staphylococcus epidermidis: a systematic review. GMS Hyg Infect Control 2014;9(3):Doc23.
10. Lalani T, Kanafani ZA, Chu VH, Moore L, Corey GR, Pappas P, et al. Prosthetic valve endocarditis due to coagulase-negative staphylococci: findings from the International Collaboration on Endocarditis Merged Database. Eur J Clin Microbial Infect Dis 2006;25(6):365-8.
11. Dong Y, Speer CP, Glaser K. Beyond sepsis: Staphylococcus epidermidis is an underestimated but significant contributor to neonatal morbidity. Virulence 2018;9(1):621-33.
12. Kumar G, Kumar N, Toneja A, Kaleekal T, Tarima S, McGinley E, et al. Nationwide trends of severe sepsis in the 21st century (2000-2007). Chest 2011;140(5):1223-31.
13. Sah S, Bordlopi P, Vijaya D, Amarnath SK, Devi CS, Indumathi V, et al. Simple and economical method for identification and speciation of Staphylococcus epidermidis and other coagulase-negative Staphylococci and its validation by molecular methods. J Microbiol Methods 2018;149:106-19.
14. Roberts AL. Identification of Staphylococcus epidermidis in the clinical microbiology laboratory by molecular methods. Methods Mol Biol 2014;1106:33-53.
15. Safdari H, Neshani A, Sadeghian A, Ebrahimi M, Iranshahi M, Sadeghian H. Potent and selective inhibitors of class A ß-lactamase: ß-prenylxyco coumarins. J Antibiot (Tokyo) 2014;67(5):373-7.
16. Neshani A, Kakhki RK, Sankian M, Zare H, Chichaklu AH, Sayyadi M, et al. Modified genome comparison method: a new approach for identification of specific targets in molecular diagnostic tests using Mycobacterium tuberculosis complex as an example. BMC Infect Dis 2018;18(1):517.
17. Ikeda Y, Ohara-Nemoto Y, Kimura S, Ishibashi K, Kikuchi K. PCR-based identification of Staphylococcus epidermidis targeting gseA encoding the glutamic-acid-specific protease. Can J Microbiol 2004;50(7):493-8.
18. Liu D, Swiatlo E, Austin F, Lawrence M. Use of a putative transcriptional regulator gene as target for specific identification of Staphylococcus epidermidis. Lett Appl Microbiol 2006;43(3):325-30.
19. Khodaparast L, Khodaparast L, Van Mellaert L, Shahrrooei M, Van Ranst M, Van Eldere J. sscC as a genetic marker for easy identification of Staphylococcus epidermidis from other isolates. Infect Genet Evol 2016;43:222-4.
20. Ghattas MZ, ElRakayby MT, Aziz RK, Zedan HH. A novel PCR method targeting staphostatin genes differentiates Staphylococcus aureus from Staphylo-coccus epidermidis in clinical isolates and nasal microbiome samples. Research square 2019. Unpublished.
21. Kakhki RK, Neshani A, Sankian M, Ghazvini K, Hooshyar A, Sayyadi M. The short-chain dehydro-genases/ reductases (SDR) gene: a new specific target for rapid detection of Mycobacterium tuberculosis complex by modified comparative genomic analysis. Infect Genet Evol 2019;70:158-64.
22. Sayers EW, Beck J, Bolton EE, Bourexis D, Brister JR, Canese K, et al. Database resources of the national center for biotechnology information. Nucleic Acids Res 2021;49(D1):D10.
23. Sharma S, Ciufo S, Starchenko E, Darji D, Chlumsky L, Karsch-Mizrachi I, et al. The NCBI biocollections database. Database (Oxford) 2018;2018:bay006.
24. Rychlik W. OILG 7 primer analysis software. Methods Mol Biol 2007;402:35-60.
25. Stackebrandt E, Goebelel BM. Taxonomic note: a place for DNA-DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. Int J Systematic Evolutionary Microbiology 1994;44(4):846-9.
26. Geh SH, Potter S, Wood JO, Hemmingsen SM, Reynolds RP, Chow AW. HSP60 gene sequences as universal targets for microbial species identification: studies with coagulase-negative staphylococci. J Clin Microbiol 1996;34(4):818-23.
A New Specific Target Gene for Identification of *Staphylococcus epidermidis*

27. Martineau F, Picard FJ, Ke D, Paradis S, Roy PH, Ouellette M, et al. Development of a PCR assay for identification of staphylococci at genus and species levels. J Clin Microbiol 2001;39(7):2541-7.

28. Poyart C, Quesne G, Boumaila C, Trieu-Cuot P. Rapid and accurate species-level identification of coagulase-negative staphylococci by using the sodA gene as a target. J Clin Microbiol 2001;39(12):4296-301.

29. Sivadon V, Rottman M, Quincampoix JC, Avettand V, Chaverot S, de Mazancourt P, et al. Use of sodA sequencing for the identification of clinical isolates of coagulase-negative staphylococci. Clin Microbiol Infect 2004;10(10):393-42.

30. Zakrzewska-Czerwińska J, Gaszewska-Mastalarz A, Pulverer G, Mordarski M. Identification of *Staphylococcus epidermidis* using a 16S rRNA-directed oligo-nucleotide probe. FEMS Microbiol Lett 1992;100(1-3):51-8.

31. Bahador A, Esmaeili D, Khaledi A, Ghorbanzadeh R. An in vitro assessment of the antibacterial properties of nanosilver Iranian MTA against Porphyromonas gingivalis. J Chem Pharmaceut Res 2013;5(10):65-71.

32. Khaledi A, Khademi F, Esmaeili D, Esmaeili SA, Rostami H. The role of HPaA protein as candidate vaccine against Helicobacter pylori. Der Pharma Chemica 2016;8(3):235-7.

33. Hosseini SMJ, Nacini NS, Khaledi A, Daymad SF, Esmaeili D. Evaluate the relationship between class 1 integrons and drug resistance genes in clinical isolates of *Pseudomonas aeruginosa*. Open Microbiol J 2016;10:188-96.