Resistant Strains of Enterotoxigenic *Staphylococcus aureus*; Unknown Risk for Multiple Sclerosis Exacerbation

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Background: Despite all advances in neurological sciences, there are unknown aspects in the epidemiology of multiple sclerosis (MS). Based on this hypothesis, the enterotoxigenic strains of *Staphylococcus aureus* (*S. aureus*) are possible risk factors for exacerbations of MS.

Objectives: The present study was carried out to investigate the role of resistant strains of enterotoxigenic *S. aureus* in MS exacerbation.

Materials and Methods: Two-hundred nasal swab samples were collected from non-MS (n = 80), MS stable (n = 60) and MS exacerbation (n = 60) groups. Samples were cultured and those that were *S. aureus*-positive were analyzed for the presence of enterotoxins, using polymerase chain reaction (PCR). Antimicrobial susceptibility was performed using disk diffusion method.

Results: Ninety out of 200 nasal samples (45%) were positive for *S. aureus*. The highest levels of nasal colonization were seen in MS exacerbation group (68.33%). The most commonly detected enterotoxins were sea (30%), sec (15.55%) and seb (11.11%). There were significant differences between *S. aureus* colonization and type of samples (P = 0.026) and, also, between type of samples and prevalence of enterotoxins (P = 0.022). The highest levels of enterotoxigenic genes were seen in MS exacerbation group. The *S. aureus* strains had the highest levels of resistance against tetracycline (80%), ampicillin (72.22%), methicillin (66.66%), erythromycin (66.66%), oxacillin (63.33%), trimethoprim-sulfamethoxazole (61.11%) and cotrimoxazole (55.55%).

Conclusions: Our findings should raise awareness about the role of sea and sec enterotoxins, in resistant strains of *S. aureus*, as a risk factor for MS exacerbation. It is better to keep MS patients away from polluted environments of hospitals and health centers.

Keywords: Multiple Sclerosis; Antibiotic Resistance; Iran; Enterotoxins; *Staphylococcus aureus*

1. Background

*Staphylococcus aureus* (*S. aureus*) is a significant human pathogen, which colonizes the anterior nares of 20% - 60% of humans (1, 2). The *S. aureus* strains can cause a number of diseases, ranging from skin and soft tissue infections to urinary and respiratory infections, life-threatening endocarditis, pneumonia, endocarditis and osteomyelitis (1, 2).

Several of the *S. aureus* strains secrete a group of extracellular enzymes, which simplify tissue extinction and dispersal and membrane damaging toxins that cause catalytic effects on host cells and tissue damage (3, 4). The staphylococcal enterotoxins (SEs) are a group of low-molecular-mass and single-chain proteins that are similar in composition and biological activity, which differ in antigenicity (sea to sej) (5, 6).

Enterotoxigenic genes of the *S. aureus* can activate a high proportion of T cells, due to their ability to bind to both major histocompatibility complex (MHC) molecules in antigen presenting cells and specific V-β regions, in the T cell receptor (4, 7). This activation results in the polyclonal stimulation of T cells and an increased production of proinflammatory cytokines (4, 7). Based on the controversial theories and the results of previous studies, the enterotoxigenic genes of *S. aureus* may be involved in the fundamental etiology of multiple sclerosis (MS). The MS is a chronic disease of the central nervous system, with incompletely known etiology (4, 7-9). It is widely accepted as a complex autoimmune disease, generally targeting young adults (10). Although discovery of the activation of CD4+ Th immune cells represents a major step in disease pathology, the specific causes, responsible for activating this autoimmune disease, are unknown (10). Therefore, it is important to know the exact or potential mechanisms and also the risk factors of MS.

Although the possible role of *S. aureus*, in the occurrence of MS, is not entirely known, however, the presence of high levels of antibiotic resistance in the *S. aureus* strains increases the importance of this matter (11-17). According to the available data, almost 15% of *S. aureus* hospital infections were methicillin resistant (MRSA) (11-13) and between 20% - 70% of them were multi-drug resistant (14-17).

2. Objectives

As far as we know, there were scarce available data on the prevalence of *S. aureus* and its enterotoxins, in the cases of MS. Therefore, the present study was carried out to...
investigate the prevalence of *S. aureus* in the nasal swabs of MS patients and, also, investigate the role of enterotoxigenic genes of this bacterium in the exacerbation of MS.

### 3. Materials and Methods

#### 3.1. Samples and Staphylococcus aureus Identification

From September 2013 to August 2014, a total of 200 nasal swab samples were collected from non-MS persons (n = 80), patients who had not experienced a relapse of MS in the past 6 months (MS stable group) (n = 60) and those who had suffered a relapse of MS within 30 days of study recruitment (MS exacerbation group) (n = 60). All samples were collected from the educational hospitals and private health centers of the Tehran province, Iran. The swab samples were rapidly transferred to the laboratory in cooler, with ice-packs.

Samples were directly cultured onto 7% sheep blood agar (Merck, Darmstadt, Germany) and incubated aerobically at 37°C, for 48 hours. After incubation, suspicious colonies were examined by the use of morphologies compatible with *Staphylococcus* spp. (microscopical morphology, catalase and coagulase production). Studied colonies were cultured on tryptic soy broth (TSB) (Merck, Darmstadt, Germany) and tryptic soy agar (TSA) (Merck, Darmstadt, Germany). After growth, staphylococci were identified on the basis of colony characteristics, Gram staining, pigment production, hemolytic and the following biochemical reactions: catalytic activity, coagulated test (rabbit plasma), oxidase test, glucose O/F test, resistance to bacitracin (0.04 U), mannitol fermentation on mannitol salt agar (MSA) (Merck, Darmstadt, Germany), urease activity, nitrate reduction, novobiocin resistance, phosphatase, deoxyribonuclease (DNase) test and carbohydrate (xylose, sucrose, trehalose and maltose, fructose, lactose, mannose) fermentation test (18).

#### 3.2. Antimicrobial Susceptibility Test

The pattern of antimicrobial resistance was studied using the simple disk diffusion technique. The Mueller-Hinton agar (Merck, Darmstadt, Germany) medium was used for this purpose. Antibiotic resistance of *S. aureus* strains against 16 commonly used antibiotics in the cases of urinary tract infections was determined using the instruction of clinical and laboratory standards institute (CLSI) guidelines (19). Susceptibilities of *S. aureus* isolates were tested against ampicillin (10 μg/disk), gentamycin (10 μg/disk), amikacin (30 μg/disk), imipenem (30 μg/disk), methicillin (30 μg/disk), tetracycline (30 μg/disk), vancomycin (5 μg/disk), ciprofloxacin (5 μg/disk), norfloxacin (30 μg/disk), cotrimoxazole (30 μg/disk), clindamycin (2 μg/disk), trimethoprim-sulfamethoxazole (25 μg/disk), penicillin G (10 μg/disk), oxacillin (1 μg/disk), erythromycin (15 μg/disk) and azithromycin (15 μg/disk) antibiotic agents (Oxoid, Basingstoke, UK). The plates containing the discs were allowed to stand for at least 30 minutes before incubation at 35°C, for 24 hours. The diameter of the zone of inhibition produced by each antibiotic disc was measured and interpreted using the CLSI zone diameter interpretative standards (19). *S. aureus* ATCC 25923 and *Escherichia coli* ATCC 25922 were used as quality control organisms in antimicrobial susceptibility determination.

#### 3.3. DNA Extraction and Polymerase Chain Reaction Confirmation

Total genomic DNA was extracted from the bacterial colonies. A single colony was inoculated on 5 mL of brain heart infusion broth and incubated overnight, at 37°C. Then, 1.5 mL of a saturated culture were harvested with centrifugation, for 5 minutes, at 14000 rpm. The cell pellet was resuspended and lysed in 200 μL of lysis buffer (40 mM Tris-acetate, pH 7.8, 20 mM sodium-acetate, 1 mM ethylenediaminetetraacetic acid, 1% sodium dodecyl sulphate) by vigorous pipetting. To remove most proteins and cell debris, 66 μL of 5M NaCl solution were added and mixed well, and then, the viscous mixture was centrifuged at 12000 rpm for 10 minutes, at 4°C. After transferring the clear supernatant into a new eppendorf tube, an equal volume of chloroform was added, and the tube was gently inverted at least 50 times when a milky solution was completely formed. Following centrifugation at 14000 rpm for 5 minutes, the supernatant was then removed to another eppendorf tube and double volume of 100% ethanol was added. The tubes were inverted five to six times, gently, and then centrifuged at 10000 rpm, for 5 minutes. The supernatant was discarded and 1 mL of ethanol (70%) was added to the pellet, and tubes were centrifuged at 10000 rpm, for 5 minutes. Finally, the supernatant was discarded and the pellet was dried for 10 minutes at room temperature, after which it was resuspended by 100 μL H2O. The stock was kept at −20°C until use. The DNA concentration has been determined by measuring absorbance of the sample at 260 nm using spectrophotometer (20).

Presence of *S. aureus* in each DNA sample was confirmed using the Banada et al. method (21). The polymerase chain reaction (PCR) reaction mix consisted of 1X PCR buffer (10 mM Tris-HCl, pH 8.3, 50 mM KCl and 0.01% (w/v) gelatin) with 4 mM MgCl2, 250 mM of each nucleotide (denuxynucleoside triphosphate), 0.5 mM of each primer (forward and reverse), 4 ng of the molecular beacon and 4 U of Jumpstart *Taq* DNA polymerase (Fermentas, Vilnius, Latvia).

#### 3.4. Polymerase Chain Reaction Amplification for Enterotoxigenic Genes

The PCR method was used to study the distribution of
sea, seb, sec, sed, see, seg, seh, sei, and sej enterotoxins of the S. aureus (3, 22-24). Oligonucleotide primers, annealing temperature, PCR programs and size of products are shown in Table 1. A programmable thermal cycler (Eppendorf, Mastercycler® 5330, Eppendorf-Netheler-Hinz GmbH, Hamburg, Germany) PCR device was used in all PCR reactions. All runs included a negative DNA control, consisting of PCR grade water and strains of S. aureus ATCC 13565 (sea), ATCC 14458 (seb), ATCC 19095 (sec), FRI 361 (sed, seg, sei and sej), ATCC 27664 (see) and FRI 137 (seh) that were used as positive controls.

3.5. Statistical Analysis

The results were transferred to a Microsoft Excel spreadsheet (Microsoft Corp., Redmond, WA, USA) for analysis. Statistical analysis was performed using SPSS 16.0 software (SPSS Inc., Chicago, IL, USA) for significant relationship between incidences of enterotoxigenic genes of S. aureus isolated from the nasal swab samples of non-MS, MS stable and MS exacerbation groups. The chi-square test and Fisher’s exact two-tailed test analysis were performed in this study. Statistical significance was considered at a P < 0.05.

3.6. Ethical Considerations

The present study was accepted by the ethical committees of the educational Hospitals. Written informed consent was obtained from all of the study patients or their parents.

| Target Gene | Primer Sequence (5’ – 3’) | PCR Product, bp | Annealing Temperature, °C |
|-------------|-----------------------------|-----------------|----------------------------|
| sea         | F: TTGGAAACGGTTAAAAGCAA    | 120             | 50                         |
|             | R: GAACCTTCCCATCAAAAAACA   |                 |                            |
| seb         | F: TCGCATCAAACGCAAAACG     | 478             | 50                         |
|             | R: GCAGGTACCTCTAAGTGCC     |                 |                            |
| sec         | F: GACATAAAAGCTAGGAATTT    | 257             | 50                         |
|             | R: AAATCGGATACATATATCC     |                 |                            |
| sed         | F: CTAGTTTTGGTAATATCTCCT   | 317             | 50                         |
|             | R: TAATGCTATATCTTATAGGG    |                 |                            |
| see         | F: AGGTTTTTTTACAGGTATCC    | 209             | 50                         |
|             | R: CTTTTTTTTTCGGTCAATCC    |                 |                            |
| seg         | F: AGTAGACATTITTTGGCCITT   | 287             | 55                         |
|             | R: AGAACCATAAACTCGTATAGCC |                 |                            |
| seh         | F: GTCTATATGGAGTACACACT    | 213             | 46.4                       |
|             | R: GACCTTACTATTTTCGTTGC    |                 |                            |
| sei         | F: GGTGATATTGGTGAGGTGAAC   | 454             | 50                         |
|             | R: ATCCATATTITTCGCTTTTACCAG|                 |                            |
| sej         | F: CATCAGAAGCTTGTCCGCTAG   | 142             | 50                         |
|             | R: CTAATTTTACATCAAGGTAC    |                 |                            |

**Notes:**

- PCR programs: one cycle at 94°C for 5 minutes; 30 cycles at 94°C for 2 minutes, 72°C for 1 minute and one cycle at 72°C, for 5 minutes.
- PCR volume, 50 μL: 5 μL PCR buffer, 10X 1.5 mM MgCl₂, 200 μM dNTP (Fermentas), 0.5 μM of each primers F (forward) and R (reverse), 1.25 U Taq DNA polymerase (Fermentas), 2.5 μL DNA template.
4. Results

The study enrolled 200 patients, 80 in the non-MS, 60 in the MS stable groups and 60 patients in the MS exacerbation group. Of 200 nasal swabs collected for this study, 90 (45%) were positive for *S. aureus* (Table 2), with significant differences identified between MS exacerbation group and non-MS group (*P* = 0.019) and also, between MS stable groups and non-MS group (*P* = 0.032). On the other hand, the most commonly infected group was MS exacerbation group (68.33%).

Distribution of enterotoxigenic genes of the *S. aureus* strains of various studied groups is shown in Table 3. Results of the gel electrophoresis for enterotoxigenic genes of the *S. aureus* are shown in Figures 1 - 4. The most commonly detected enterotoxins were sea (30%), sec (15.55%), and seb (11.11%). There were no positive results for see and seh enterotoxins. Significant differences were seen between the prevalence of sea and seg (*P* = 0.015), sea and sei (*P* = 0.018), sea and sed (*P* = 0.033), sec and seg (*P* = 0.035) and seb and sei (*P* = 0.041) genes. Significant differences were also seen for the prevalence of sea gene, between MS exacerbation and MS stable groups (*P* = 0.020) and also between MS exacerbation and non-MS groups (*P* = 0.023). In addition to sea, there were significant differences for the prevalence of sec gene between MS exacerbation and MS stable groups (*P* = 0.039).

Antibiotic resistance pattern of *S. aureus* isolated from various studied groups is shown in Table 4. We found that the *S. aureus* strains of MS exacerbation group had the highest levels of resistance to various types of antibiotics (*P* = 0.028). The *S. aureus* isolates of our investigation had the highest levels of resistance against tetracycline (80%), ampicillin (72.22%), methicillin (66.66%), erythromycin

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**Table 2. Distribution of *Staphylococcus aureus* in Various Studied Groups**

| Studied Groups of Patients | No. Samples Collected | No. of Positive Samples a |
|---------------------------|-----------------------|---------------------------|
| Non-MS group              | 80                    | 19 (23.75)                |
| MS stable group           | 60                    | 30 (50)                   |
| MS exacerbation group     | 60                    | 41 (68.33)                |
| Total                     | 200                   | 90 (45)                   |

a Values are presented as No. (%).

**Table 3. Distribution of Enterotoxigenic Genes of *Staphylococcus aureus* in Various Studied Groups a**

| Studied Groups of Patients | No. of Positive Samples | sea | seb | sec | sed | see | seg | seh | sei | sej |
|----------------------------|-------------------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Non-MS group               | 19                      | 1   | 1   | 1   | -   | -   | -   | -   | -   | -   |
| MS stable group            | 30                      | 4   | 3   | 3   | 1   | -   | -   | -   | -   | -   |
| MS exacerbation group      | 41                      | 22  | 6   | 10  | 3   | 1   | 1   | 1   | 1   | -   |
| Total                      | 90                      | 27  | 10  | 14  | 4   | 1   | 1   | 1   | 1   | -   |

a Values are presented as No. (%).

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**Figure 1. Results of the Gel Electrophoresis for Identification of Enterotoxigenic Genes in *Staphylococcus aureus* Strains**

M: 100 bp DNA ladder (Fermentas, Vilnius, Latvia), lines 1 - 4: positive samples for sed (37 bp), seb (478 bp), sea (120 bp) and sec (257 bp), lines 5 - 8: positive controls and line 9: negative control.

**Figure 2. Results of the Gel Electrophoresis for Identification of Enterotoxigenic Genes in *Staphylococcus aureus* Strains**

M: 100 bp DNA ladder (Fermentas, Vilnius, Latvia), lines 1 - 3: positive samples for see (209 bp), sei (454 bp) and sej (142 bp), lines 4 - 6: positive controls and line 7: negative control.
5. Discussion

The results of our investigation showed that resistant strains of enterotoxigenic S. aureus may be the risk factors for MS exacerbation. We found that the total prevalence of S. aureus in the nasal swab samples of non-MS, MS stable and MS exacerbation groups were 23.75%, 50% and 68.33%, respectively. The high prevalence of S. aureus in the MS exacerbation group may be due to the fact that these patients are more frequently under treatment with immunosuppressing drugs. Therefore, the levels of immunity in this group of patients are reduced and several infections, like S. aureus, will occur. Higher prevalence of enterotoxigenic genes and antibiotic resistance were

Table 4. Antibiotic Resistance Pattern of Staphylococcus aureus Isolated From Various Studied Groups

| Type of Samples | AM10 | Gent10 | Amk30 | Imp30 | Met30 | Tet30 | VAN | Cip5 | Nor | Cotr | Clin | TM/Sul | Pen10 | Ox | Em15 | Az15 |
|----------------|------|--------|-------|-------|-------|-------|-----|------|-----|------|-----|--------|-------|----|------|------|
| Non-MS group   | 19 (52.63) | 10 (31.57) | 6 (20.69) | 5 (26.32) | 1 (5.26) | 8 (42.10) | 12 (61.54) | 2 (10.52) | 5 (26.32) | 3 (15.78) | 8 (42.10) | 4 (21.05) | 9 (47.37) | 4 (21.05) | 8 (42.10) | 3 (15.78) |
| MS stable group | 30 (66.66) | 20 (40) | 12 (24) | 10 (20) | 3 (6) | 17 (34) | 22 (44) | 11 (22) | 15 (30) | 9 (18) | 15 (30) | 16 (32) | 9 (18) | 15 (30) | 3 (6) |
| MS exacerbation group | 41 (85.36) | 35 (70.73) | 29 (58.33) | 24 (47.22) | 9 (18.6) | 35 (70.73) | 38 (76.47) | 8 (16) | 19 (38) | 29 (58.33) | 29 (58.33) | 15 (30) | 25 (50) | 16 (32) | 18 (36) |
| Total          | 90 (65) | 47 (31.57) | 39 (26.32) | 13 (8.69) | 60 (40) | 72 (48) | 15 (10) | 25 (16.67) | 22 (14.81) | 50 (33.33) | 27 (18) | 20 (13.33) | 55 (36.67) | 38 (26.67) | 57 (38.67) | 60 (40) | 30 (20) |

a In this table AM10 = ampicillin (10 μg/disk), Gent10 = gentamycin (10 μg/disk), Amk30 = amikacin (30 μg/disk), Imp30 = imipenem (30 μg/disk), Met30 = methicillin (30 μg/disk), Tet30 = tetracycline (30 μg/disk), VAN = vancomycin (5 μg/disk), Cip5 = ciprofloxacin (5 μg/disk), Nor = norfloxacin (30 μg/disk), Cotr = cotrimoxazole (30 μg/disk), Clin = clindamycin (2 μg/disk), TM/Sul = trimethoprim-sulfamethoxazole (25 μg/disk), Pen10 = penicillin G (10 μg/disk), Ox = oxacillin (1 μg/disk), Em15 = erythromycin (15 μg/disk) and Az15 = azithromycin (15 μg/disk).

b Values are presented as No. (%).
also seen in the *S. aureus* strains of the MS exacerbation group. Mulvey et al. (4), in a similar study, which was conducted in Canada, showed that the total prevalence of *S. aureus* in non-MS, MS stable and MS exacerbation groups were 30%, 21.2% and 27.3%, respectively, which was entirely different from our results. Probably the *S. aureus* isolates of their investigation were related to the host. In fact, they showed that there is no difference in the host-pathogen interaction, making MS patients more susceptible to colonization with *S. aureus*, and this finding is related to those of Hu et al. (25).

The *S. aureus* may carry toxigenic genes, which can act as superantigens that trigger large numbers of CD4+ cells and have been involved in various autoimmune diseases, including MS, rheumatoid arthritis and Wegener’s granulomatosis (26). As far as we know, the present study is the first prevalence report of enterotoxigenic *S. aureus* in the anterior nares of MS patients, in Iran. We found that the total prevalence of enterotoxigenic genes in the *S. aureus* strains of MS exacerbation patients were higher than those of MS stable and non-MS patients. The total prevalence of sea, seb, sec, sed, seg and sei genes in the *S. aureus* isolates of MS exacerbation patients were 30%, 11.11%, 15.55%, 4.44%, 1.11% and 1.11%, respectively. Higher prevalence of superantigens and enterotoxigenic genes, in the patients who suffered from MS, were reported previously by Mulvey et al. (4), Franca et al. (7) and Kumar et al. (27).

The identification of *S. aureus* harbored enterotoxins and especially sea and sec, as a possible risk factor in MS exacerbations, increases the possibility of novel treatment choices for managing this disease. Potentially, antimicrobial decolonization regimes, which have been used successfully to decolonize individuals with MRSA in the hospital and community scenery, could be used to MS patients, colonized with *S. aureus* (28). We found that all the *S. aureus* strains of our study were resistant to more than three antibiotics and the prevalence of resistance against methicillin was 66.66%, which was high, especially for patients who suffered from the MS. Our results also indicate that the total prevalence of antibiotic resistance in the *S. aureus* strains of MS exacerbation group, against ampicillin, gentamicin, methicillin, tetracycline, cotrimoxazole, trimethoprim-sulamethoxazole, oxacillin and erythromycin were 85.36%, 70.73%, 85.36%, 92.68%, 70.73%, 75.60%, 73.17% and 87.80%, respectively, which was compared with those of MS stable and non-MS patients. Of the studies that have been conducted in this field, in Iran, all have shown a high levels of antibiotic resistance of *S. aureus* against the studied antimicrobial agents of our investigation (14, 29-33).

Although *S. aureus* enterotoxins and superantigens have been recognized as risk factors in other immunological diseases, like rheumatoid arthritis and Wegener’s granulomatosis, this is one of the first studies examining the potential association of enterotoxigenic genes in the *S. aureus* isolates of nasal swabs and the potential association with MS exacerbations. The data presented in this study seem to highlight the need for a more extensive trial, to better define the role of the colonization of *S. aureus* containing sea, seb, sec, sed, seg and sei enterotoxigenic genes, and their potential role in the etiology of MS. In the current setting of Iran, prescription of antibiotics, especially in the cases of MS, should be done based on the results of disk diffusion method. The results of our investigation showed that prescription of imipenem and vancomycin, due to their low levels of antibiotic resistance, is effective.

**Authors’ Contributions**

Farzad Mehrabi: writing and editing, the main concept. Ali Asgari: submission and revision.

**References**

1. Tokajian S, Haddad D, Andraos R, Hashwa F, Araji G. Toxins and Antibiotic Resistance in Staphylococcus aureus Isolated from a Major Hospital in Lebanon. *ISRN Microbiol.* 2011:2011:412049.
2. Mehndiratta PL, Bhalla P, Ahmed A, Sharma YD. Molecular typing of methicillin-resistant Staphylococcus aureus strains by PCR-RFLP of SPA gene: a reference laboratory perspective. *Indian J Med Microbiol.* 2009;27(2):16-22.
3. Mehrotra M, Wang G, Johnson WM. Multipart PCR for detection of genes for Staphylococcus aureus enterotoxins, exfoliative toxins, toxic shock syndrome toxin 1, and methicillin resistance. *J Clin Microbiol.* 2000;38(1):1032-5.
4. Mulvey MR, Doupe M, Prout M, Leong C, Hizon R, Grossberndt A, et al. Staphylococcus aureus harbouring Enterotoxin A as a possible risk factor for multiple sclerosis exacerbations. *Mult Scler.* 2013;19(5):597-603.
5. Yahagi E, Imani Fooladi AA, Amin M, Mirnejad R, Nezamzade R, Amani J. Detection of Class 1 Integrons in Staphylococcus aureus Isolated From Clinical Samples. *Iran Red Crescent Med J.* 2014;16(10):e16234.
6. Fleming K, Ackermann G. Prevalence of enterotoxin producing Staphylococcus aureus in stools of patients with nosocomial diarrhoea. *Infection.* 2007;35(5):356-8.
7. Franca TG, Chiuso-Minicucci F, Zorzella-Pezavento SF, Ishikawa LL, da Rosa LC, Colavite PM, et al. Previous infection with Staphylococcus aureus strains attenuated experimental encephalomyelitis. *BMC Neurosci.* 2014:15:8.
8. Brocke S, Gaur A, Piercy C, Gatum A, Gibbels K, Fathman CG, et al. Induction of relapsing paralysis in experimental autoimmune encephalomyelitis by bacterial superantigen. *Nature.* 1993;365(6447):642-4.
9. Schifffenbauer J, Johnson HM, Burkofskii EI, Wegryn L, Soos JM. Staphylococcal enterotoxins can reactivate experimental allergic encephalomyelitis. *Proc Natl Acad Sci U S A.* 1993;90(18):8543-6.
10. Ozenci V, Kouwenhoven M,-link H. Cytokines in multiple sclerosis: methodological aspects and pathogenic implications. *Mult Scler.* 2002;8(5):396-404.
11. Frazee BW, Lynn J, Charlebois ED, Lambert L, Lowery D, Perdreaume R. High prevalence of methicillin-resistant Staphylococcus aureus in emergency department skin and soft tissue infections. *Ann Emerg Med.* 2005;45(3):311-20.
12. Manzur A, Vidal M, Pujol M, Cisnal M, Hornero A, Masuet C, et al. Predictive factors of meticillin resistance among patients with Staphylococcus aureus bloodstream infections at hospital admission. *J Hosp Infect.* 2007;66(2):235-41.
13. Shen H, Akoda E, Zhang K. Methicillin-Resistant Staphylococcus aureus Carriage among Students at a Historically Black University: A Case Study. *Int J Microbiol.* 2013:2013:979734.
14. Dibah S, Arzanlou M, Jannati E, Shapouri R. Prevalence and antimicrobial resistance pattern of methicillin resistant Staphylococcus aureus (MRSA) strains isolated from clinical specimens in Ardabil, Iran. *Iran J Microbiol.* 2014;6(3):163-8.
15. Onanuga A, Temedie TC. Nasal carriage of multidrug-resistant Staphylococcus aureus in healthy inhabitants of Amassoma in Niger delta region of Nigeria. *Afr Health Sci.* 2011;11(2):176–81.

16. Tiwari HK, Sapkota D, Sen MR. High prevalence of multidrug-resistant MRSA in a tertiary care hospital of northern India. *Infect Drug Resist.* 2010;3:57–61.

17. Mishra SK, Rijal BP, Pokhrel BM. Emerging threat of multidrug-resistant bugs—Acinetobacter calcoaceticus baumannii complex and methicillin-resistant Staphylococcus aureus. *BMC Res Notes.* 2013;6:98.

18. Znaantar T, Chaieb K, Ben Abdallah F, Ben Kahla-Nakbi A, Ben Has sen A, Mahdouani K, et al. Multiplex PCR detection of the antibiotic resistance genes in Staphylococcus aureus strains isolated from auricular infections. *Folia Microbiol (Praha).* 2008;53(4):357–62.

19. Clinical and Laboratory Standards Institute (CLSI). *Performance standards for antimicrobial susceptibility testing twenty-second informational supplement.* Wayne Pa: CLSI; 2012.

20. Sambrok JA. *Molecular Cloning: A Laboratory Manual.* 3 ed. New York: Cold Spring Harbor Laboratory Press; 2001.

21. Banada PP, Chakravorty S, Shah D, Burday M, Mazzella FM, Alland D. Highly sensitive detection of Staphylococcus aureus directly from patient blood. *PLoS One.* 2012;7(2):e31126.

22. Johnson WM, Tyler SD, Ewan EP, Ashton FE, Pollard DR, Rozee KR. Detection of genes for enterotoxins, exfoliative toxins, and toxic shock syndrome toxin 1 in Staphylococcus aureus by the polymerase chain reaction. *J Clin Microbiol.* 1991;29(3):426–30.

23. Nashef D, Toshkova K, Salasia S, Hassan AA, Lammler C, Ziehock M. Distribution of virulence genes of Staphylococcus aureus isolated from stable nasal carriers. *FEMS Microbiol Lett.* 2004;233(1):45–52.

24. Omoe K, Ishikawa M, Shimoda Y, Hu DT, Ueda S, Shinagawa K. Detection of seg, seh, and sei genes in Staphylococcus aureus isolates and determination of the enterotoxin productivities of S. aureus isolates Harboring seg, seh, or sei genes. *J Clin Microbiol.* 2002;40(3):857–62.

25. Hu L, Umeda A, Kondo S, Amako K. Typing of Staphylococcus aureus colonising human nasal carriers by pulsed-field gel electrophoresis. *J Med Microbiol.* 1995;42(2):227–32.

26. Torres BA, Kominsky S, Perrin GQ, Hobeika AC, Johnson HM. Superantigens: the good, the bad, and the ugly. *Exp Biol Med (Maywood).* 2000;226(3):364–76.

27. Kumar P, Kretzschmar B, Herold S, Nau R, Kreutzfeldt M, Schutze S, et al. Beneficial effect of chronic Staphylococcus aureus infection in a model of multiple sclerosis is mediated through the secretion of extracellular adherence protein. *J Neuroinflammation.* 2015;12:22.

28. Ellis MW, Griffith ME, Dooley DP, McLean JC, Jorgensen HJ, Patterson JE, et al. Targeted intranasal mupirocin to prevent colonization and infection by community-associated methicillin-resistant Staphylococcus aureus strains in soldiers: a cluster randomized controlled trial. *Antimicrob Agents Chemother.* 2007;51(10):3591–8.

29. Tashakori M, Mohseni Moghadam F, Ziasheikholeslami N, Jafarpour P, Behisoun M, Hadavi M, et al. Staphylococcus aureus nasal carriage and patterns of antibiotic resistance in bacterial isolates from patients and staff in a dialysis center of southeast Iran. *Iran J Microbiol.* 2014;6(2):79–81.

30. Japoni A, Ziyaeyan M, Jmalidoust M, Farshad S, Alborzi A, Rafaatpour N, et al. Antibacterial susceptibility patterns and cross-resistance of methicillin resistant and sensitive Staphylococcus aureus isolated from the hospitalized patients in Shiraz, Iran. *Braz J of Microbiol.* 2010;41(3):567–73.

31. Ghaseemian R, Najafi N, Makhloough A, Khademlooo M. Frequency of nasal carriage of Staphylococcus aureus and its antimicrobial resistance pattern in patients on hemodialysis. *Iran J Kidney Dis.* 2010;4(1):218–22.

32. Rahimi F, Bouzari M, Katolui M, Pourshafee MR. Antibiotic Resistance Pattern of Methicillin Resistant and Methicillin Sensitive Staphylococcus aureus Isolates in Tehran, Iran. *Jundishapur J of Microbiol.* 2011;6(2):144–9.

33. Zarei M, Erami M, Kosha H, Mohammadmi A. Survey of Antibiotic Resistance Pattern of Isolated Staphylococcus Coagulase Negative Species from Patients with Bacteremia in Shahid Beheshti Hospital of Kashan. *Iran J Med Sci.* 2012;37(3):251–414.