Distribution of Super Antigensgene of *Staphylococcus aureus* in Allergic Rhinitis Patients

Authors

Thanaa Shams Al-deen AL-Turaihi, Prof Dr Kareem Thamir Al-Kaabi

Corresponding Author

Thanaa Shams Al-deen AL-Turaihi Lecturer

Department of Medical Microbiology, Kufa College of Medicine

Ph.D Student in Kufa College of Medicine Department of Medical Microbiology

Email: thanaa.shams@yahoo.com

Summary

*Staphylococcus aureus* is a representative constituted pathogen related with the major community and hospital acquired sickness and has been taken into consideration for a long time as a major problem of Public Health, Several virulence factors engaged in the pathogenesis of *S. aureus* strains Perhaps the most notable virulence factors associated with this microorganism are the heat-stable enterotoxins that cause the sporadic food-poisoning syndrome or foodborne outbreaks (Martin et al., 2003).

**Hypothesis:** The pathogenesis of Allergic rhinitis (AR) has been a hot subject, recent studies had suggested that *Staphylococcus aureus* excretes exotoxins that may act as superantigens and can influence the activity of both immune modulatory and pro-inflammatory effector cell type and therefore, may have a potentially important role in the pathogenesis of chronic inflammatory disease or lead to exacerbation of upper airway disease.

**Methodology**

A case–control study has been conducted to determine the prevalence of enterotoxin producing *Staphylococcus aureus* in the nasal cavities of patients with allergic rhinitis during the period from March 2014 to November 2014. 100 patients with Allergic rhinitis and 100 control subjects, the patients attended Al-Sadder Medical City, outpatient clinic of ENT in Najaf city. For isolation of *S. aureus*, a nasal swab was taken from each of the 100 patients and 100 controls by using a sterile cotton swab. Isolates were identified by a conventional test and then confirmed by Biomerieux Vitek 2 Compact Automated Microbial Identification. The super antigen genes (SEA, SEB, SEC, SED, SEE, and TSST) were detected by using conventional PCR on the isolate then an enzyme immune assay RIDASCREEN ® SET A, B, C, D, E preformed on broth culture of these isolates in order to identify the enterotoxins production or libration.

**Result:**

Nasal *S. aureus* carriage was significantly more frequent in patients with allergic rhinitis than in non-allergic controls (*P* < 0.001), (Odds=3.4306 CI=1.8-6.26). The distribution of toxigenic *S. aureus* that recover from allergic rhinitis patients by PCR test was (63.16%) and (26.32%) from control groups and the difference was non-significant *P*= 0.434. The most frequently detected enterotoxin was staphylococcal enterotoxin B (SEB). ELISA test seem to be more sensitive for (SED, SEC, and SEE) and less sensitive for (SEA and SEB), however the specificity of ELISA is better in (SED, SEC, SED, SEE) than SEA.

**Conclusion:** We have demonstrated that the rate of nasal carriage of *Staphylococcus aureus* in allergic rhinitis patients was significantly higher than that of control subject, *S. aureus* isolated from allergic rhinitis patients have a probably a relationship with the disease and their superantigen have been a role in triggering and exacerbation of the disease.

**Keyword:** Allergic rhinitis, *Staphylococcus aureus*, Superantigens
Allergic Rhinitis

Allergic rhinitis (AR) is a disease that is a chronic airway inflammatory condition with the eosinophilic cell as a response to an inhalant allergic material in genetically liable patients (Liu et al., 2014). The following are the predominant symptoms of the AR patients: sneezing, nasal itching, congestion, of the nasal mucosa and running nose (Al-Abri et al., 2014) another organ of the body (nearby organs) are also involved which are the eyes, ears, sinuses, and throat, the smell sense can also be affected if the disease is for a prolong period (Guilemany, 2009).

Epidemiology

Allergic Rhinitis is a global health problem that affects 20%-40% of the population in developed countries and whose incidence is rising. It can be induced by different mechanisms and involves several etiological agents (Rondon et al., 2007).

Staphylococcus aureus

Staphylococci are spherical in shape and gram-positive bacteria which are non motile and do not form spores (Murray et al., 2003). Staphylococcus aureus is an important human pathogen especially in hospital-acquired infections (Guidey et al., 2014) and also in community-acquired infections, with methicillin-resistant S. aureus (MRSA) having a considerable public health threat. (Liu et al., 2009) S. aureus have the property to colonize asymptotically healthy individuals. The carriers are at higher risk of infection, and they are considered as an important origin of the S. aureus strains that spread among other individual (Chambers & DeLeo, 2009).

Some are considered to be part of the normal flora (normal inhabitant) lives within the body, colonizing the skin and mucous membranes in 10 to 20% of healthy adults of humans and also in the nose of a healthy human (Lowy, 1998). They can be a causative agent of different superficial and deep infections, where pus was formed in many times in human. Occasionally, S. aureus is regarded as an opportunistic pathogens as in case of infections of the urinary tract, respiratory tract, and gastrointestinal tract. The most commonly affected area of the body because of S. aureus infection is the skin (Daum, 2007). However, nasal colonization increases the risk of infection by four-fold (Safdar & Bradley, 2008). Superantigens: enterotoxins and toxic shock syndrome toxin

Two types of staphylococcal toxins are having superantigen activity. They are secreted by S. aureus enterotoxins, six antigenic types have been found in this category (named SE-A, B, C, D, E and G), and toxic shock syndrome toxin (TSST-1) (Schlievert et al., 2000). These enterotoxins after ingested with food induce diarrhea and vomiting and are responsible for staphylococcal food poisoning. TSST-1 has a systemic effect and is the main cause of toxic shock syndrome (TSS); in addition, enterotoxins also can induce toxic shock syndrome. However, nasal colonization increases the risk of infection by four-fold (Safdar & Bradley, 2008).

For routine detection of superantigens, commercially produced kits, such as reverse passive latex agglutination assays and enzyme-linked immunosorbent assays, were most commonly used. However, these methods were to date designed only to detect limited types of superantigens. As an alternative to these more traditional methods, the PCR approach can provide detection of toxin genes and is presently designed to detect the majority of (SAg) (McLauchlin et al., 2001). However, a gene’s presence does not establish its enterotoxigenic properties of a strain therefore, the expression of the gene should also be evaluated (Fooladi et al, 2010)

Material and Method

The study was included two groups: The first group (patients group): This group included 100 patients with Allergic rhinitis. These patients attended Al-Sadder Medical City, an outpatient clinic of ENT in Najaf city during the period from March 2014 to November 2014.

Exclusion criteria;
1. Patients who received antibiotics or oral corticosteroid therapy or had upper respiratory infections during the four weeks before enrollment.
2. Patients who already started or completed immunotherapy.

The control groups consist of 100 non allergic healthy volunteers not suffering from respiratory symptoms for isolation of S. aureus, a nasal swab was taken from each of the 100 patients

Isolation and Identification of S. aureus
Specimens were taken via the insertion of a sterile moistened swab in both nostrils to a depth of approximately 1 cm into the nostril and rotated five times. After collection, specimens were immediately transported to the lab for inoculation on the culture medium. Samples were directly inoculated onto mannitol salt agar, Chromoagar plates and incubated at 37°C for 24 hours. S. aureus isolate identification was based on morphology, Gram’s stain property, coagulase test, catalase test and mannitol salt agar fermentation.

Biomerieux Vitek 2 Compact Automated Microbial Identification
Automated VITEK® 2 ID cards this technique is used in order to provide reliable, accurate results for isolated bacteria.

DNA Extraction
According to Mini gDNA Bacteria Kit protocol (Geneaid Biotech Ltd)

Polymerease Chain Reaction Protocols

<table>
<thead>
<tr>
<th>MONOPL EX GENE</th>
<th>TEMPERATURE/°C )/ TIME</th>
<th>Final extension</th>
<th>CYCLE NUMBER</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial denaturation</td>
<td>Cycling condition</td>
<td>Final extension</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Denaturation</td>
<td>Annealing</td>
</tr>
<tr>
<td>Sea</td>
<td>94°/5 min</td>
<td>94°/2 min</td>
<td>57°/2min</td>
</tr>
<tr>
<td>Seb</td>
<td>94°/5 min</td>
<td>94°/2 min</td>
<td>57°/2min</td>
</tr>
<tr>
<td>Sec</td>
<td>94°/5 min</td>
<td>94°/2 min</td>
<td>57°/2min</td>
</tr>
<tr>
<td>Sed</td>
<td>94°/5 min</td>
<td>94°/2 min</td>
<td>57°/2min</td>
</tr>
<tr>
<td>See</td>
<td>94°/5 min</td>
<td>94°/2 min</td>
<td>57°/2min</td>
</tr>
<tr>
<td>Tsst</td>
<td>94°/5 min</td>
<td>94°/2 min</td>
<td>57°/2min</td>
</tr>
</tbody>
</table>

Preparing the Primers Suspension
The DNA primers were suspended by addition the lyophilized product after spinning down briefly with TE buffer molecular grad depending on manufacturer instruction as stock suspension. Working primer tube was prepared by diluted with TE buffer molecular grad. The final picomoles depended on the procedure of each primer

Enzyme immunoassay for identifying of Staphylococcus enterotoxins A,B,C,D and E in cultures
Is a sandwich enzyme immunoassay for identification of Staphylococcus enterotoxins, all Staphylococcus aureus bacteria were examined in each allergic patient and each control subject. After preliminary growth on the agar plates, Staphylococcus aureus cultured were transferred to brain heart infusion BHI broth and cultured aerobically at 37°C, centrifuged supernatants of microbiological fluid cultures 5 min/at a minimum of 3500g/10°C sterile filtration of the supernatant is strongly advisable as any precipitated or re-suspended cells may influence the test reaction,
the ability of bacteria to produce toxin was measured by enzyme immune assay

**Primers for Monoplex PCR**

<table>
<thead>
<tr>
<th>TYPE</th>
<th>PRIMER NAME</th>
<th>OLI GO SEQUENCE (3'-5')</th>
<th>PRODUCT SIZE (BP)</th>
<th>REFERENCE</th>
<th>ORIGIN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Superantigens</td>
<td>sea</td>
<td>F: GGGTTATCAATGTGCGGGGTGG R: CCGCACCTTTTTTCTCTCGG</td>
<td>102</td>
<td>Mehrotra et al., 2000</td>
<td>Korea</td>
</tr>
<tr>
<td></td>
<td>seb</td>
<td>F: GTATGGGTGGTGAAGTGACG R: CCAAATAGTGAGAGTAGG</td>
<td>164</td>
<td>Mehrotra et al., 2000</td>
<td>Korea</td>
</tr>
<tr>
<td></td>
<td>sec</td>
<td>F: AGATGAAGTGTTATGTGATGTTAGG R: CACACCTTTTAGATCAACCG</td>
<td>451</td>
<td>Mehrotra et al., 2000</td>
<td>Korea</td>
</tr>
<tr>
<td></td>
<td>sed</td>
<td>F: CCAATATAGGAAGAAAATAAAAG R: ATTGGATATTTTTTCGTTC</td>
<td>278</td>
<td>Mehrotra et al., 2000</td>
<td>Korea</td>
</tr>
<tr>
<td></td>
<td>see</td>
<td>F: ACGTTTTTTTCAGGTCATCC R: CTTTTTTTTCTTCGGTCAATC</td>
<td>209</td>
<td>Mehrotra et al., 2000</td>
<td>Korea</td>
</tr>
<tr>
<td></td>
<td>tst</td>
<td>F: ACCCCTGTCCCCCTATTCATC R: TTCAGTATTTGTGAACGCC</td>
<td>326</td>
<td>Mehrotra et al., 2000</td>
<td>Korea</td>
</tr>
</tbody>
</table>

**Result**

**Table (1)** Total studied patients with allergic rhinitis and control included in the study

<table>
<thead>
<tr>
<th>Study groups</th>
<th>NO</th>
<th>Positive for <em>S.aureus</em></th>
<th>%</th>
<th>Negative for <em>S.aureus</em></th>
<th>%</th>
<th>P value</th>
<th>Odds=3.4306 CI=1.8-6.26</th>
</tr>
</thead>
<tbody>
<tr>
<td>Allergic rhinitis patients</td>
<td>100</td>
<td>52</td>
<td>52%</td>
<td>48</td>
<td>48.0%</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>control</td>
<td>100</td>
<td>24</td>
<td>24.0%</td>
<td>76</td>
<td>76%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>200</td>
<td>76</td>
<td>38%</td>
<td>124</td>
<td>62%</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Table (2)** show the distribution of toxigenic and non-toxigenic of *S. aureus* isolates recovered from allergic rhinitis patients and control by PCR

<table>
<thead>
<tr>
<th></th>
<th><em>SAUREUS ISOLATED FROM PATIENTS GROUP</em></th>
<th>%</th>
<th><em>SAUREUS ISOLATED FROM CONTROL GROUP</em></th>
<th>%</th>
<th>TOTAL</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>toxigenic</td>
<td>48</td>
<td>63.16%</td>
<td>20</td>
<td>26.32%</td>
<td>68</td>
<td>89.48%</td>
</tr>
<tr>
<td>Non toxigenic</td>
<td>4</td>
<td>5.26%</td>
<td>4</td>
<td>5.26%</td>
<td>8</td>
<td>10.52%</td>
</tr>
<tr>
<td>Total</td>
<td>52</td>
<td>68.42%</td>
<td>24</td>
<td>31.58%</td>
<td>76</td>
<td>100%</td>
</tr>
</tbody>
</table>

P-value=0.434
Table (3) show the distribution of superantigens among (76 S. aureus) isolates recovered from allergic rhinitis patients and control by both PCR and Elisa test

<table>
<thead>
<tr>
<th>ENTEROTOXIN</th>
<th>PCR</th>
<th>100%</th>
<th>ELISA</th>
<th>100%</th>
<th>SENSITIVITY</th>
<th>SPECIFICITY</th>
</tr>
</thead>
<tbody>
<tr>
<td>SEA</td>
<td>52</td>
<td>68.42</td>
<td>16</td>
<td>21.052</td>
<td>23.1%</td>
<td>83.3%</td>
</tr>
<tr>
<td>SEB</td>
<td>64</td>
<td>84.21</td>
<td>8</td>
<td>10.53</td>
<td>12.5%</td>
<td>100%</td>
</tr>
<tr>
<td>SEE</td>
<td>16</td>
<td>21.05</td>
<td>12</td>
<td>15.79</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>SEC</td>
<td>8</td>
<td>10.53</td>
<td>8</td>
<td>10.53</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>SED</td>
<td>8</td>
<td>10.53</td>
<td>8</td>
<td>10.53</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>TSST</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Table (4) distribution of superantigen genes among toxigenic S aureus in AR patients and control groups

<table>
<thead>
<tr>
<th>S AUREUS</th>
<th>ONE TYPE OF TOXIN</th>
<th>100%</th>
<th>TWOTYPE OF TOXIN</th>
<th>100%</th>
<th>THREE TYPE OF TOXIN</th>
<th>100%</th>
<th>FOUR TYPE OF TOXIN</th>
<th>100%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients</td>
<td>48</td>
<td>8</td>
<td>16.67%</td>
<td>32</td>
<td>66.66%</td>
<td>8</td>
<td>16.67%</td>
<td>4</td>
</tr>
<tr>
<td>Control</td>
<td>20</td>
<td>8</td>
<td>40%</td>
<td>8</td>
<td>40%</td>
<td>4</td>
<td>20%</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>68</td>
<td>16</td>
<td>56.67%</td>
<td>40</td>
<td>106.66%</td>
<td>12</td>
<td>36.67%</td>
<td>4</td>
</tr>
</tbody>
</table>

Figure (1.): Ethidium bromide-stained agarose gel of PCR, amplified products from extracted DNA of S. aureus isolates and amplified with forward and reverse primer sea. The electrophoresis was performed at 70 volt for 1.5hr. Lane (L) DNA molecular size marker (1000 bp ladder), Lanes (1, 2, 3, 4, 5, 6 and 7) shows positive results with sea gene (102 bp).

Figure (2.): Ethidium bromide-stained agarose gel of PCR, amplified products from extracted DNA of S. aureus isolates and amplified with forward and reverse primer sea. The electrophoresis was performed at 70 volt for 1.5hr. Lane (L) DNA molecular size marker (1000 bp ladder), Lanes (8, 9, 12 and 14) shows positive results with sea gene (102 bp) Lanes (S10, 11, and 13) show negative results with sea gene.

Figure (3): Ethidium bromide-stained agarose gel of PCR, amplified products from extracted DNA of S. aureus isolates and amplified with forward and reverse primer seb. The electrophoresis was performed at 70 volt for 1.5hr. Lane (L) DNA molecular size marker (1000 bp ladder), Lanes (S2, 3, 4, 5, 6 and 7) shows positive results with seb gene (164 bp), Lanes (1) show negative result with seb gene.
Figure (4): Ethidium bromide-stained agarose gel of PCR, amplified products from extracted DNA of *S. aureus* isolates and amplified with forward and reverse primer *seb*. The electrophoresis was performed at 70 volt for 1.5hr. Lane (L) DNA molecular size marker (1000 bp ladder), Lanes (S9,11,12,14, and 16) shows positive results with *seb* gene (164 bp), Lanes (10,13 and 17) show negative result with *seb* gene.

Figure (5): Ethidium bromide-stained agarose gel of PCR, amplified products from extracted DNA of *S. aureus* isolates and amplified with forward and reverse primer *sec*. The electrophoresis was performed at 70 volt for 1.5hr. Lane (L) DNA molecular size marker (1000 bp ladder), Lanes (S3 and 4) shows positive results with *sec* gene (451 bp), Lanes (1,2,5,6,7 and 8) show negative results with *sec* gene.

Figure (6): Ethidium bromide-stained agarose gel of PCR, amplified products from extracted DNA of *S. aureus* isolates and amplified with forward and reverse primer *sec*. The electrophoresis was performed at 70 volt for 1.5hr. Lane (L) DNA molecular size marker (1000 bp ladder), Lanes (S10 and 11) shows positive results with *sec* gene (451 bp), Lanes (9,13,14,15,16, and 17) show negative results with *sec* gene.

Figure (7): Ethidium bromide-stained agarose gel of PCR, amplified products from extracted DNA of *S. aureus* isolates and amplified with forward and reverse primer *sed*. The electrophoresis was performed at 70 volt for 1.5hr. Lane (L) DNA molecular size marker (1000 bp ladder), Lanes (S11 and 13) shows positive results with *sed* gene (278 bp), Lanes (S10,12,14,15,16 and 17) show negative results with *sed* gene.

Figure (8): Ethidium bromide-stained agarose gel of PCR, amplified products from extracted DNA of *S. aureus* isolates and amplified with forward and reverse primer *sed*. The electrophoresis was performed at 70 volt for 1.5hr. Lane (L) DNA molecular size marker (1000 bp ladder), Lanes (S10,12,14,15,16 and 17) show negative results with *sed* gene.
18) shows positive results with sed gene (278 bp), Lanes (16,17,19,20,21,22, and 23 ) show negative results with sed gene

**Figure (9):** Ethidium bromide-stained agarose gel of PCR, amplified products from extracted DNA of *S. aureus* isolates and amplified with forward and reverse primer see. The electrophoresis was performed at 70 volt for 1.5hr. Lane (L) DNA molecular size marker (1000 bp ladder), Lanes (S 3) shows positive results with see gene (209 bp), Lanes(1,2,4,5,6,7,8 and 9 ) show negative results with see gene

**Figure (10):** Ethidium bromide-stained agarose gel of PCR, amplified products from extracted DNA of *S. aureus* isolates and amplified with forward and reverse primer see. The electrophoresis was performed at 70 volt for 1.5hr. Lane (L) DNA molecular size marker (1000 bp ladder), Lanes (S 18) shows positive results with see gene (209 bp), Lanes (19,20,22,23 and 24 ) show negative results with see gene

**Discussion**

**The carriage rate of *S. aureus* in nasal cavity:**

It was focused in table (1) regarding the rate of isolation of *S. aureus* from the nasal cavity of patients with allergic rhinitis (AR) in comparison to that of nasal cavity of control subjects (not-allergic subject) it was seen from the table that 52(52%) of the nasal swab from patients with allergic rhinitis (AR) showed growth of *S. aureus* in comparison to 24(24%) of the swab that was taken from the nasal cavity of control subject, the difference between the two results was shown to be significant P<0.001,Odds 3.43306. The patients with allergic rhinitis showed to be higher carriers of *S. aureus* in their noses. The result of this table is in agreement with (Shiomori et al.,2000)

**Superantigenic exotoxins produced by *S. aureus***

*Staphylococcus aureus* (SA) is one of the most common human bacterial pathogens and produces enterotoxins that act as toxins and superantigens. Staphylococcal enterotoxins are a family of structurally related proteins comprised of different serological types: *Staphylococcus* enterotoxins A (SEA), B (SEB), C, D, E (up to U), and toxic shock syndrome toxin-1 (TSST-1). (Bachert et al.,2007) the stimulatory role of superantigens in the development of inflammation in chronic rhinosinusitis and nasal polyposis has been documented. It has been postulated that SA and its products are related to the pathogenesis of allergic inflammatory diseases, including rhinitis and atopic dermatitis (Bachert et al., 2010; Ikezawa et al., 2010; Liu et al., 2014). There has recently been much interest in the role of bacterial superantigens in allergic inflammatory reactions. Numerous studies have demonstrated that bacterial superantigens regulate the activity of immunomodulatory (T lymphocytes) and pro-inflammatory cell types (dendritic cells, eosinophils and epithelial cells etc), and play an important role on allergic disease. Mechanistic studies have shown that superantigens stimulate the T cells by cross-linking the variable part on the beta chain of the T-cell receptor (TCR) with MHC class II
molecules outside the peptide-binding groove area. This leads to stimulation of up to 30% of the naive T-cell population in a nonspecific way, compared with stimulation of only about 0.1% of the T cell population via the conventional allergen-specific MHC-restricted route utilizing both TCR-Vα and b chains. Thus, direct binding of SEB to an MHC class II molecule loaded with antigen-derived peptides might enhance the antigenicity of the allergen and the development of allergic disease. In addition, SEB may act as allergens. SEB can induce antigen-specific T cells that are able to promote the generation of antigen-specific IgE antibodies, which subsequently play a role in conventional allergen-mediated reactions. Humans are natural carriers for staphylococcus aureus, the nasal passage and skin being the most common site for staphylococcus aureus colonization. (Jusufagic et al., 2006; Tang., 2012)

The destination of this study was to evaluate the frequency of genes that code for superantigens including enterotoxins through A, B, CD, E and toxic shock syndrome toxin (TSST) in S. aureus isolates recovered from allergic rhinitis patients, all (76) S. aureus isolates diagnostic positive were applied for toxin gene distribution analysis. Sequences specific for staphylococcal toxin genes were detected by PCR.

In the present study, monoplex PCR assay was designed to detect toxin genes employed here. Table (2) shows that, (63.16%) of S. aureus isolated strains from AR patients were producing one or more enterotoxins, whereas (26.32%) of S. aureus from the control subjects produced detectable enterotoxins the difference non-significant (p=0.434), and S. aureus non toxigenic isolated in equal number in AR patients and control group (5.26%), Our finding were in agreement with those of (Azzazy et al., 2015) who reported that the frequency of nasal carriage of S. aureus in the patients group was 25/45 (55.56%), while in control group was 6/45 (13.33%) with highly significant difference (χ² = 17.8 and P_0.001).

The results of molecular investigations for the finding of genes encoding the toxins; SEA, SEB, SEC, SED, SEE and TSST.

In table no (3) show the distribution of superantigens among (76 S. aureus) isolates recovered from allergic rhinitis patients and control by both PCR and Elisa test S. Aureus containing enterotoxins were (84.21%) for SEB, (68.42%) for SEA, (21.05%) for SEE, (10.53%) for SED, (10.53%) for SEC and (0%) for TSST in patients by PCR method. As noticed, the most frequently detected enterotoxin was staphylococcal enterotoxin B (SEB).

While by ELISA method we found that the main type of toxin was SEA (21.052%), SEB (10.526%), SEC (10.526%), SED (10.526%), TSST (0) and SEE (15.789%).

While in case of PCR results for gene detection the present results was also not much different from that seen by (Shiomori et al., 2000) who found that the rates of culture supernatants containing the individual superantigenic exotoxins were 13% for SEA, 54% for SEB, and the rate of SEB was the highest among the enterotoxins, the result was similar with little difference especially in TSST, which was 20% in case of Shiomori study and 0% in the present study, but it was the same as in case of (Azzazy et al., 2015) who found that TSST toxin gene was 0% both in case of PCR, and also when ELISA detection was performed, he also found that SEB toxin was higher than other enterotoxin a result which was slightly different from the present study in which SEA was found to be the highest. The most frequent enterotoxin type was type B (PALA et al., 2010).

ELISA test seem to be more sensitive for (SED, SEC, and SEE) and less sensitive for (SEA and SEB), however the specificity of ELISA is better in (SED, SEC, SED, SEE) than SEA.

However, a possible limitation of this procedure is that the molecular methods are only able to demonstrate the existence of the genes encoding for SEs in bacteria but cannot prove that production of SEs protein occurs unless RT-PCR is carried out (Morandi et al., 2007). High
percentages of *S. aureus* with enterotoxin genes, especially SEA, were confirmed phenotypically and the results of PCR showed a clear relationship with immune assay results, this was in agreement with other previous study as (Anvari *et al.*, 2008). Nevertheless, with regard to SEB, more differences are observed between genotypical and phenotypical methods (Fooladi *et al.*, 2010).

**Distribution of enterotoxin genes in toxigenic *S. aureus* in patients and control groups**

Table (4) show the distribution of enterotoxin genes in toxigenic *S. aureus* in patients and control groups toxigenic *S. aureus* that produced one type of toxin isolated from patients and control group from 16 strains (56.67%), *S. aureus* that produced two type of toxin isolated from 40 isolated strain (106.66%) *S. aureus* that produced three type of toxin isolated from 12 isolated strain (36.67%) and *S. aureus* that produced four type of toxin isolated from 4 isolated strain (8.33%). In fact when a revision of the results of this table was done, we could found that the bacterial isolates which excreted 2 type of enterotoxin was higher than that which excreted either one type or 3,4 type of the enterotoxins, at the same time there were a scanty of studies which explained or demonstrated the combined existence of enterotoxigenic both in case of molecular studies or in ELISA studies.

Conclusion we have demonstrated that the rate of nasal carriage of *Staphylococcus aureus* in allergic rhinitis patients was significantly higher than that of control subject. *Staphylococcus aureus* isolated from allergic rhinitis patients have a probably a relationship with the disease and their superantigen have been areol in triggering and exacerbation of the disease

**References**


