Research Article

**Staphylococcus aureus** prevalence in the fresh salad and vegetables of the Quetta city

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**Abstract**

*Staphylococcal aureus* (*S. aureus*) has been reported as the most prevalent bacteria which can cause foodborne disease worldwide. Since fresh salads, vegetables are consumed uncooked and if contaminated with microbial pathogens may lead to severe food borne illness. This study aimed to evaluate the contamination rate of *Staphylococcal aureus* in fresh ready to eat salad, sold in local markets of Quetta city, Pakistan. Out of 100 samples tested, 54 were found contaminated with *S. aureus*. Out of 54 *S. aureus* isolates, 32 (59%) were coagulase positive *S. aureus*. However, 22 (40%) were coagulase negative. Enumeration of coagulase positive *S. aureus* samples revealed that only 10% (n=3) of the total collected samples were within the level of acceptance<20 cfu g⁻¹, while 40% (n=12) sample were almost on the threshold of Borderline (20-<10⁻⁴cfu g⁻¹). Whereas 50% (n=17) samples were at Unacceptable level (>10⁻⁴cfu g⁻¹). Furthermore, higher *S. aureus* contamination of fresh food samples was observed during early summer season.

**Keywords**: Bacterial count; Biochemical tests; *S. aureus*; Salad; Vegetables

**Introduction**

*Staphylococcus aureus* is a pathogenic bacterium which causes food borne illness along with several other diseases [1] and has been reported as most prevalent bacteria which cause intoxication and gastroenteritis throughout the world [2, 3]. *Staphylococcus aureus* is a gram positive, coccoid belonging to the family *Staphylococcaceae* [4]. The cells are spherical, often arranged like clusters of grapes [5, 6]. This organism produces coagulase and catalase. *Staphylococci* can survive in harsh condition of dryness and are even capable of high concentrate salt tolerance [7, 8].

Around 2.41 million foodborne illness per year is recoded in United States which is mainly caused by *S. aureus* and, yearly six
deaths out of 1000 of patients has been reported [9]. The vehicles which transmit S. aureus to food is during food processing by human source and by animals in dairy through mastitis. Human origin strains are main source of Staphylococcus food poisoning (SFPO). Early in 1936, Denison documented Staphylococcus food poisoning outbreaks occurred among the students of high school. Annually in Australia, almost 1300 cases have been reported of food poisoning by Staphylococcus aureus [10].

The severity of food born infection depends on the quantity of toxins consumed [11]. If the toxins in the body reach to the concentration of $10^5$ CFU/ml than it may lead to disease [12]. After the ingestion of food the common symptoms appear within 3-8 hour which lead to the abdominal cramps, nausea, vomiting and diarrhea. These symptoms are approximately for 24-48 hour [13, 14].

There are various virulence factors associated with the pathogenesis of S. aureus strains [15-17]. Some of them are hemolysins, hyaluronidase, lipases and thermo nuclease [18, 19], which cause invasion in tissues of the host cell. The main virulence factors are heat-stable enterotoxins (SEs), that cause the infrequent food-poisoning syndrome or foodborne related outbreaks, and also the Toxic shock syndrome toxin one (TSST-1), that weaken the immune system of a host [15, 17, 18, 20, 21].

In developing countries, pre- and post-harvest processes and provisions contribute highly to contamination of fresh products. For example, in Pakistan, a quarter of fresh products are irrigated with waste water [22]. The occurrence of foodborne related outbreaks by contaminated fresh vegetables has been increased during the last decade [23]. The consumption of green leafy vegetables provides numerous health benefits, and there is a direct relationship between consumption of these vegetables and the reduction of chronic diseases such as hypertension, diabetes, atherosclerosis, and cancer. The lack of thorough cooking in fresh cuisine can result in foodborne diseases if contaminated by pathogens [24].

The vaccine or immunotherapy designed to prevent S. aureus diseases have not been approved so far from US-Food and Drug administration (FDA). To avoid foodborne illness while consuming fresh vegetables and salad it’s pertinent to pay attention regarding the hygiene of consumed food [25].

**Materials and methods**

**Sample collection**

A total 100 samples of fresh ready to eat salads vegetables were collected randomly from different street vendors, during the month of April to August 2017. The collected samples were transported in thermopol box filled with crushed ice to bacteriology laboratory, CASVAB, University of Balochistan for further processing. Samples were processed for bacterial analysis within 1-5 hour of collection.

**Isolation of Coagulase Positive S. aureus**

For isolation of S. aureus from food samples ISO 6888-1:1999 “Microbiology of food and animal feeding stuffs” method was adopted with some modifications. Briefly 10g sample was weighed aseptically into stomacher bags with 90 ml MRD (Maximum Recovery Dilution) and homogenized for 2 minutes. Homogenate was serially diluted $10^2$, 0.1 ml from each initial suspension and serial dilution was transferred onto Baird-Parker (BP) agar supplemented with egg yolk emulsion and incubated for 24-48h at 37 °C.

**Bacterial Quantification and enumeration**

To estimate the extent of contamination of fresh salad and vegetables with S. aureus was experiment carried out. Typical black-gray S. aureus colonies were with clear hallow zone appeared on BPA agar plates were enumerated and calculations were made to estimate the bacterial load on each sample. Further data was also analyzed according to
season of the year.

**Biochemical tests for identification of S. aureus**

The suspected typical colonies from BPA agar were further streaked on Nutrient Agar (NA) and incubated for 24 hrs at 37˚C. The colonies from NA surface were selected to perform Gram’s staining and microscopy. Identification of S. aureus isolates were made by series of biochemical tests such as thermonuclease (TNase), glucose, mannitol, salt tolerance, oxidase, MR-VP (Voges-Proskauer and Methyl Red) and indole test. Biochemically identified S. aureus isolates were further tested for coagulase production by performing coagulase tube test by adding colony in rabbit plasma for the observation of coagulation reaction.

**Results**

In this study, *Staphylococcus aureus* was isolated from 54 out of 100 samples. Growth of *staphylococcus aureus* on Baird-Parker agar are shown in (Figure 1). The suspected positive *S. aureus* samples were further confirmed by array of biochemical tests like Gram staining cocci positive organism were further confirmed as catalase positive, coagulase positive, negative for oxidase while positive for MR-VP and salt tolerance. While Oxidative fermentation of glucose and mannitol were positive without gas production as shown in (Table1) and (Figure 2).

![Figure 1. Coagulase positive S. aureus in Baird-Parker Agar isolated from salads and vegetables](image)

| Table 1. Preliminary biochemical identification of coagulase positive S. aureus |
|---------------------------------|-----------------|
| Coagulase                       | +               |
| Catalase                        | +               |
| Oxidase                         | -               |
| MR-VP                           | +               |
| Glucose                         | +               |
| Mannitol                        | +               |
| Salt tolerance                  | +               |
| Indole                          | -               |
Figure 2. Biochemical identification of coagulase positive *S. aureus*.

All 32 coagulase positive *S. aureus* were enumerated in cfu/g and converted the value into log$_{10}$ cfu/g. The results depicted that only 3 (10%) samples were within the range of <20 cfu g$^{-1}$ which were safe to be consumed, while 12 (40%) samples were almost on the threshold level (20 - $\leq 10^4$ cfu g$^{-1}$) and may not be consumed. Whereas 17 (50%) samples were very high in bacterial count (> $10^4$ cfu g$^{-1}$) and cannot be consumed (Table 2).

Tube coagulase test was performed on all biochemically identified *S. aureus*. Out of 54 tested samples 32 (59%) were found coagulase positive and 22 (40%) were found as coagulase negative (Figure 3).

Samples were collected and analyzed during the period of April to August 2017. The results depict that the peak season of *S. aureus* microbial contamination in fresh salad vegetables was May and June. It can be correlated with high yield of local ready to eat salad and vegetables and high temperature during these months of years. Bacterial contamination was decreased with decreasing temperature as shown in (Figure 4).

Table 2. Microbial quantity of coagulase positive *S. aureus* in salads vegetables according to HPA (Health Protection Agency)

<table>
<thead>
<tr>
<th>Classification</th>
<th>CFU Range</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acceptable</td>
<td>&lt;20 cfu g$^{-1}$</td>
<td>3 (10%)</td>
</tr>
<tr>
<td>Borderline</td>
<td>20 - $\leq 10^4$ cfu g$^{-1}$</td>
<td>12 (40%)</td>
</tr>
<tr>
<td>Unacceptable</td>
<td>&gt; $10^4$ cfu g$^{-1}$</td>
<td>17 (50%)</td>
</tr>
</tbody>
</table>

Figure 3. Prevalence of coagulase positive *S. aureus* in salad and vegetables
Discussion
In recent past the salad and vegetables have become an integral part of daily food. These fresh and uncooked foods may also lead to several foodborne infections if contaminated. Foodborne illnesses pose a great health risk worldwide which can lead to personal distress, economic burden and in certain cases may lead to death [26]. In this study, out of 100 fresh salads/vegetables collected samples tested, 57 samples were found positive for *S. aureus*, importantly 32 of these positive samples were identified as coagulase positive. Several studies in past have reported the fresh food contamination with *S. aureus* and its adverse effect on human health [27, 28]. Ideally the food should be free from any pathogen, however, the addible food contaminated with bacterial pathogens under 4 log_{10}CFU/g is still considered as safe however, any food exceeding this threshold should be discarded straightaway [29]. Our study not only detected the *S. aureus* in fresh food but also estimated the load of contamination to get a fair idea about the healthiness of food used by inhabitants of the city. Astonishingly, the 56% of positive samples harbor more than 4 log_{10}CFU/g bacterial count. The food contaminated with such high number of *S. aureus* is unhealthy and may pose serious health risk to consumers. Even some samples of fresh vegetables and salad were contaminated with *S. aureus* as high as 5 log_{10}CFU/g, which is nowhere suitable for human consumption. The coagulase *S. aureus* proliferates in several foods and in suitable condition provide the organism to expend the growth at certain concentration to make food toxic by producing enterotoxins that causes gastroenteritis [30]. The results in this study revealed that the minimum bacterial count was 1.0 x 10^{-3} cfu/g and maximum bacterial count was 2.1 x 10^{-5} cfu/g. The maximum level of bacterial count in fresh food products may be because of irrigation with waste water [31]. We have also observed the same factor as salad and vegetables harvested in Quetta city are irrigated mainly with untreated waste/sewage water and this water serves as a main source of contamination. Similar findings of utilizing waste water for irrigating salads and vegetables is reported in urban region of Quetta city [32]. However, there are also
several other factors which may contribute to contamination of food items such as poor hygienic practices during collection, storage and transportation. The bacterial contamination of fresh food items which are rich in carbohydrates provide an ideal proliferation condition for microbes [31]. Higher microbial counts have been reported in leafy salads and vegetables (51.5%) [33]. Our results are also in agreement with the study recently conducted in Indonesia, which reported is 4 to 6 log_{10}cfu/g S. aureus contamination [34]. Although, in this study we have only analyzed the occurrence of S. aureus in fresh vegetables and salads samples but still there may be certain other pathogens which may pose serious health risk to human if consumed. Even this study may not be conclusive in term of all bacterial contamination of collected food samples but these results indicate the high S. aureus contamination in analyzed samples.

**Conclusion**

In developing countries especially those having a very low rain fall, irrigation of salads and vegetables is carried with waste water and this may lead to several foodborne diseases. In Quetta region, due to scarcity of water, farmers cultivate leafy green vegetables and salads in untreated waste water which has significantly high microbial burden and such practices make the food unhealthy.

**Authors’ contributions**

Conceived and designed the experiments: F Abbas, A Samad & S Saifullah, Performed the experiments: S Saifullah, Analyzed the data: Saima, Roomeela & M Yousaf, Contributed materials/ analysis/ tools: M Rizwan, FS Bugti, T Mykhaylo & A Razig, Wrote the paper: S Saifullah, Saima & M Yousaf.

**References**


