Isolation of Staphylococcal Enterotoxins Causing Gastroenteritis

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Abstract: Staphylococcal enterotoxins (SE) A and B are two of the most important gastroenteritis causing agents. In some areas, more than 50% of food poisoning cases are caused by staphylococcal enterotoxin A (SEA). In USA and England, Staphylococcal enterotoxins A and B (SEA and SEB) are the most food poisoning causing agents (> 60%). Staphylococcal nasal carriage is constantly established in 20-40% of healthy human population and intermittently in 60% and only 10-20% of people are non-carriers. If food providers don't obey the rules of hygiene, they can transfer the contamination to food. A concentration of 10 bacteria per gram of food is sufficient for toxin production and induction of disease Staphylococcal enterotoxins are low molecular weight proteins (MW 26,900–29,600). They are encoded by genes embedded in mobile genetic elements such as phages and pathogenicity islands. There are several methods for detection of enterotoxigenic bacteria. The phenotypical methods are not reliable in specificity, because staphylococcal enterotoxins serotypes are antigenically similar. On the other hand, commercial serological kits can not detect all the serotypes and are limited in serotypes (A-B). Therefore, molecular techniques such as multiplex PCR and real-time PCR are recommended for detection of Staphylococcus aureus enterotoxins genes.

Keywords: Staphylococcal Enterotoxins (SE), Serological Kits, Molecular Techniques, Polymerase Chain Reaction (PCR)

1. Introduction

Several studies have shown that 15% to 80% of the Staphylococcus aureus isolated from various sources (dairy products, ice cream, meat products) is able to produce enterotoxin [1-3].

Staphylococcal enterotoxins (SE) A and B are two of the most important gastroenteritis causing agents. In some areas, more than 50% of food poisoning is caused by SEA. In USA and England Staphylococcal enterotoxins A & B are the most Food poisoning causing agents (> 60%) [4].

Staphylococcal enterotoxins are low molecular weight proteins MW 26,900-29,600 KD). These are encoded by genes embedded in mobile genetic elements such as phages and pathogenicity islands [5].

There are several methods for detection of enterotoxigenic bacteria. The phenotypical methods (agglutination, SRID) are not reliable in specificity, because SE serotypes are antigenically similar [6].

On the other hand, commercial serologic kits can not detect all the serotypes and is limited to serotypes (A-E) [7]. Therefore, molecular techniques such as PCR and real-time PCR are recommended for detection of Staphylococcus aureus enterotoxin genes [7].

In this study, genotypic method is utilized to detect Staphylococcal enterotoxins A and B genes. Furthermore, we used these methods to examine the contamination rate of traditional dairy products by Staphylococcus aureus.

2. Materials and Methods

2.1. Samples

One hundred samples of Milk and Milk products were
collected randomly from different areas in Prot Said governorate.

Table 1. Different samples of milk and milk products used in the study.

<table>
<thead>
<tr>
<th>Sample type</th>
<th>Number of samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raw Milk</td>
<td>20</td>
</tr>
<tr>
<td>Pasteurized Milk</td>
<td>6</td>
</tr>
<tr>
<td>Powdered Milk</td>
<td>4</td>
</tr>
<tr>
<td>Hand-made Yogurt</td>
<td>10</td>
</tr>
<tr>
<td>Canned Yogurt</td>
<td>10</td>
</tr>
<tr>
<td>Newziland Butter</td>
<td>10</td>
</tr>
<tr>
<td>Cream</td>
<td>10</td>
</tr>
<tr>
<td>Damitta Cheese</td>
<td>5</td>
</tr>
<tr>
<td>Karish Cheese</td>
<td>10</td>
</tr>
<tr>
<td>Rommy Cheese</td>
<td>5</td>
</tr>
<tr>
<td>Cheddar Cheese</td>
<td>5</td>
</tr>
<tr>
<td>Cooked Cheese</td>
<td>5</td>
</tr>
</tbody>
</table>

2.2. Processing of Samples:[8].

2.3. Bacteriological Identification

Media used in cultivation:
1- Cooked meat with 9% NaCl (Oxoid, UK)
2- Baird Parker agar with Egg Yolk Emulsion (Oxoid, UK)
a- Baird-Parker agar
b- Egg Yolk Tellurite Emulsion
3- Mannitol salt agar (Oxoid, UK)

Preparation:
Cultivation of samples: [9].
Colonial morphology

2.4. Identification of the Suspected Colonies

1- A smear from the suspected colonies was stained by Gram stain and examined under microscope.
2- Baird Parker agar with Egg Yolk Emulsion (Oxoid, UK)
a- Baird-Parker agar
b- Egg Yolk Tellurite Emulsion
3- Mannitol salt agar (Oxoid, UK)

Preparation:

2.5. Maintenance of the Selected Isolates

The selected isolates that fulfilled the criteria of being Staphylococcus aureus were inoculated on nutrient agar slopes. After an overnight incubation at 37°C, the slopes were kept at 4°C. Passage of the isolates was done every 2-3 weeks. Also, before starting any experiment, subculture was done twice to allow the cells to restore its viability. The isolate also inoculated on deep soft agar by direct stabbing and inoculated overnight at 37°C, then kept at freezer for one to three months.

2.6. Multiplex PCR for Detection of Sea & Seb Genes

2.6.1. DNA Extraction

1. DNA extraction kit: i-genomic BYF DNA Extraction Mini Kit, (Cat. No. 17361) (iNtRON Biotechnology, Korea).
2. Additional required equipment and reagent:

2.6.2. DNA Amplification

Material for DNA amplification: PCR Premix: 2x PCR Master mix Solution (i-Taq™) tubes (Cat. No. 25027 "1ml", 25028 "5 ml") (iNtRON Biotechnology, Korea).
2.6.3. DNA Detection by Gel Electrophoresis

All the following are used:
1. 0.5 M disodium ethylenediamine tetraacetate (EDTA).
2. Electrophoresis buffer: Tri acetate EDTA (TAE) (1X) buffer.
3. Agarose gel powder (Boehringer Mannheim, Germany): Molecular screening agarose for separation of small DNA fragments. It was stored at room temperature.
4. Ethidium bromide (Sigma, USA).
5. A DNA Molecular weight marker, SiZer™-100 DNA Marker (iNtRON Biotechnology, Korea).

2.7. Statistical Analysis of the Results

The results were calculated, tabulated and statistically analyzed. The collected data were entered, checked and analyzed using chi square ($\chi^2$) according to knapp and Odds ratio Using statistical computer program SPSS version II under windows 7 as follow:

**Chi square $\chi^2$:**
Used to compare between more than 2 percentages and determine whether there is a significant difference between the expected frequencies and the observed frequencies in one or more categories.

$$\chi^2 = \sum \frac{(O - E)^2}{E}$$

3. Results

The present work was carried out on 100 samples of milk and milk products collected randomly from different areas in Prot Said Governorate.

- Milk samples were 30; they were 20 samples of raw milk, 6 samples of pasteurized milk and 4 samples of powdered milk.
- Cheese samples were 30; they were 10 samples of karish cheese, 5 samples of damitta cheese, 5 samples of rommy cheese, 5 samples of cheddar cheese and 5 samples of cooked cheese.
- Yoghurt samples were 20; they were 10 samples of canned yoghurt and 10 samples of hand-made yoghurt.
- Also 10 samples of cream and 10 samples of butter were examined.

![Figure 5. Staphylococcal enterotoxin genes isolated by PCR in milk and milk products.](image)

**Table 2. Type of Staphylococcal enterotoxin genes isolated by PCR in milk and milk products.**

<table>
<thead>
<tr>
<th>Sample type %</th>
<th>Positive samples for S. aureus</th>
<th>Positive samples for enterotoxin by PCR No. (%)</th>
<th>Negative samples No. (%)</th>
<th>Odds ratio 95% CI</th>
<th>$X^2$</th>
<th>P. value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cheese</td>
<td>15</td>
<td>4 (33.3)</td>
<td>11 (66.7)</td>
<td>0.25 (0.04-1.43)</td>
<td>3.35</td>
<td>0.078</td>
</tr>
<tr>
<td>Milk</td>
<td>16</td>
<td>8 (50.0)</td>
<td>8 (50.0)</td>
<td>1 (0.2-5.01)</td>
<td>0.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Cream</td>
<td>4</td>
<td>2 (50.0)</td>
<td>2 (50.0)</td>
<td>1 (0.0-39.77)</td>
<td>0.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Butter</td>
<td>4</td>
<td>1 (25.0)</td>
<td>3 (75.0)</td>
<td>0.11 (0.0-5.15)</td>
<td>2.1</td>
<td>0.457</td>
</tr>
<tr>
<td>Yogurt</td>
<td>7</td>
<td>3 (42.8)</td>
<td>4 (57.2)</td>
<td>0.56 (0.04-7.46)</td>
<td>0.29</td>
<td>0.593</td>
</tr>
<tr>
<td>Total</td>
<td>46</td>
<td>18 (41.3)</td>
<td>28 (58.7)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$X^2$</td>
<td>1.54</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P. value</td>
<td>0.843</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Figure 6. Showing agarose gel electrophoresis for PCR of 270 bp sea gene and 165 bp seb gene. (Lane M) is the (100 bp ladder marker), (Lane 2) is for sea, (Lane 3) is for seb, (Lane 4) is for both genes (sea& seb).

4. Discussion

Among food stuffs implicated in food poisoning; milk, dairy products, meat, poultry and eggs, specially handled food, play an important role since enterotoxigenic strains have been frequently isolated from food handlers [12, 13]. Milk products can cause sever health hazards to people as they are highly susceptible to variety of microorganisms because of their high nutritive value [14]. They are responsible of many outbreaks of food poisoning [15]. The importance of the enterotoxins comes from their heat stability and their resistance to inactivation by gastrointestinal proteases like pepsin. Although Staphylococcus can be killed at normal cooking temperature, the toxins remain active [16]. Also they are potent even in very small amount ranging from 20 ng to < 1µg can produce symptoms to human beings [17].

These results agreed with Niskanen and Aalto [18] who detected Staphylococcus growth on Baird-Parker agar in 83% of samples versus 59.4% by Mannitol Salt agar, on the other hand AlKhafaji [19] detected equal growth of S. aureus on both media.

All enterotoxins are superantigens which are encoded by mobile genetic elements including phages, plasmids and pathogenicity islands [20-22]. Detection of SE gene does not indicate presence of biologically active molecules or production of enough amount of toxin to cause disease. But it allows the determination of potentially enterotoxigenic strains of S. aureus [23]. In the present study enterotoxin genes were detected in 19 isolated strains of S. aureus out of 46 strains positive for S. aureus in a percent of (41.3%). This result is higher than that detected by Imanifooladi et al [9] who detected enterotoxin gene in (31.1%) in isolated S. aureus colonies from milk and milk products While S. aureus...
organism is heat labile, its produced enterotoxin is heat stable. Hence the importance of Multiplex PCR technique in detecting genes encoding enterotoxigenic strains especially in food poisoning outbreak.

5. Conclusion

The phenotypical methods are not reliable in specificity, because staphylococcal enterotoxins serotypes are antigenically similar. On the other hand, commercial serological kits can not detect all the serotypes (A-B). Therefore, molecular techniques such as multiplex PCR and real-time PCR are recommended for detection of \textit{Staphylococcus aureus} enterotoxins genes.

References


