OCCURRENCE OF ENTEROTOXIGENIC STAPHYLOCOCCUS AUREUS IN SOME CHEESE VARIETIES IN ASWAN CITY – UPPER EGYPT

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ABSTRACT

A total of 120 random samples of some cheeses were collected from various dairy shops, street vendors and supermarkets located in Aswan city (upper Egypt) and The samples were examined microbiologically for enumeration, isolation and identification of Staph. aureus strains. These samples included fresh Kareish, pickled Kareish, Domiati and Processed cheese (30 samples each). Of the examined cheese samples 28(93%), 25(83%), 21(70%) and 6(20%), respectively were contaminated with Staph. aureus. With mean counts of 3.08 X 10⁶ ± 1.79 x10⁶, 3.40 x 10⁵ ± 1.59x10⁵, 7.24 x10⁵ ± 4.10 x10⁵ and 1.15 x10²±8.98, respectively. Incidence of coagulase positive and coagulase negative strains of S. aureus in the examined cheese samples were (15 (54%) and (13 (46 %), (7 (28%) and (18(72%), (13(62%) and (8 (38%) and (0% and 6(100%), respectively. Methicillin resistant S. aureus (MRSA) was isolated in an incidences of 8(26%), 7(23%), 9(30%), and 0(0%) from examined cheese samples respectively while vancomycin resistant Staph aureus (VRSA) was isolated in an incidences of 3(10%), 5(17%), 8(27%) and 0(0%). Three out of twelve strains of MRSA and VRSA were isolated from fresh Kareish cheese 4 strains from Pickled Kareish cheese and 5 strains of Domiati cheese. Two strains produced enterotoxins A were isolated from fresh Kareish cheese which can also synthesis enterotoxins B and another 2 strains isolated from Domiati cheese produce enterotoxins A and also one strain synthesis enterotoxins B, enterotoxins C, and enterotoxins D. The selected 12 strains were strongly produce coagulase only 4 strains were enterotoxigenic.

Key words: Staph.aureus, Enterotoxigenic, cheese.

INTRODUCTION

Cheeses are ready to eat food products that do not undergo any further treatment to ensure their safety before consumption. Although cheeses have been characterized as one of the safest food products by some authors (Little et al., 2008). In 2006 the consumption of contaminated cheese accounted for the 0.4% of the total food borne outbreaks in Europe (European Food Safety Authority "EFSA", 2008), furthermore, the scientific literature has reported severe food poisoning out breaks associated with various types of cheese. (Kongo et al., 2008).

Staphylococcus aureus is a leading cause of gastroenteritis resulting from the consumption of contaminated food. Staphylococcal food poisoning is due to the absorption of Staphylococcal enterotoxins preformed in the food (Loir et al., 2003)

Many contaminants find their way to raw milk, from which they gain access to dairy products (Al–khatab and Al-Mitwalli, 2009). Chapaval et al., (2010) found staphylococcal enterotoxins in milk when milk was stored at temperatures of 37 ºC to 42 ºC or when exposed to variations in temperature.

On heating at normal cooking temperature, the bacteria may be killed but the toxins remain active (Presscott et al., 2002). Staphylococcal enterotoxins are highly heat resistant and are thought to be more heat resistant in foodstuffs than in a laboratory culture medium (Bergdoll 1983).

Besides enterotoxins producing S. aureus are most dangerous and harmful for the human health about 50% of this organism are able to produce enterotoxins associated with food poisoning (Payne, 1974). Illness through S. aureus ranges from minor skin infection such as pimples, boils, cellulites, toxic shock syndrome impetigo, and abscesses to life threatening
The present study was planned to deal with the following:

1- Enumeration, isolation and identification of staph. aureus in some cheese varieties sold in Aswan city.

2- Identification of antibiotics resistant strains of staph. aureus.

3- Detection of enterotoxigencity of the isolated staph. aureus organisms

**MATERIALS and METHODS**

### A) Collection of samples:
A total of 120 random samples of some cheese varieties were collected from various dairy shops, street vendors and supermarkets located at Aswan city. These samples included Fresh Kareish, pickled Kareish, Domiati and Processed cheese (30 samples each). Collected samples were transferred in an ice box directly to the laboratory with a minimum of delay to be examined.

### B) Preparation of serial dilutions (ISO 8261: 2001):

1- Enumeration and isolation of Staph. aureus (A.O.A.C., 2000): Over duplicated plates of a dry surface of Baird Parker (B-P) agar, 0.1 ml from each prepared dilutions of examined samples were transferred and evenly spread using surface plating technique (Thatcher and Clark, 1988).

- The inoculated B-P agar plates were incubated at 37°C for 24-48 hrs. Suspected colonies are circular, smooth, convex, moist, 2-3 mm in diameter, gray to jet black, shiny, with light color (off-white) narrow margin, surrounded by hallow zones and had buttery to gummy consistency when touched with inoculating needle were counted. The plates were then reincubated for additional 18-24 hrs before being counted for further growth, Staph. aureus count/ g were recorded.

- Furthermore, an appropriate amount from each prepared sample was inoculated into sterile Nacl 10% broth. Inoculated Nacl 10% broths were incubated at 37 °C for 24 hrs. A loopful of the incubated broth was streaked onto sterile plates of Mannitol Salt agar. (A.O.A.C., 2000)

Identification of Staph. aureus recovered from the examined samples:

A- Morphological characters for all isolates:

1- Staining reaction (A.P.H.A., 2004):- B- Biochemical reactions: catalase activity (Koneman et al., 2005), anaerobic mannitol fermentation (Baird-Parker, 1962), coagulase test. according to (Cruickshank et al., 1973).

2- Isolation of antibiotics resistant strain of staph. aureus.

a- Identification of methicillin resistant Staph aureus (MRSA): (Simor et al., 2001).

The cultures of Staph aureus were subcultured on Oxacillin Resistance Screen Agar Base (ORSAB) (Oxoid Limited, Basingstoke, England) containing ORSAB Selective Supplement contained two antibiotics-oxacillin at 2mg/L and polymyxin B
50,000 IU/l. The plate was incubated at 37°C for 24-48 h and examined for the presence of MRSA colonies, which were blue on ORSAB.

b- Identification of vancomycin resistant Staph. aureus (VRSA) ©Tiwari and Sen, 2006).

3- Detection of the enterotoxigenicity of Staph. aureus (Park et al., 1994)

RESULTS

Table 1: Statistical analytical results of Staph. aureus count in the examined cheese samples.

<table>
<thead>
<tr>
<th>Examined samples</th>
<th>No. of examined samples</th>
<th>Positive samples</th>
<th>Count /g</th>
<th>No. of samples above E.S.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh Kareish cheese</td>
<td>30</td>
<td>28</td>
<td>93</td>
<td>1x10^7</td>
</tr>
<tr>
<td>Pickled Kareish cheese</td>
<td>30</td>
<td>25</td>
<td>83</td>
<td>4.17x10^2</td>
</tr>
<tr>
<td>Domiati cheese</td>
<td>30</td>
<td>21</td>
<td>70</td>
<td>1x10</td>
</tr>
<tr>
<td>Processed cheese</td>
<td>30</td>
<td>6</td>
<td>20</td>
<td>1x10</td>
</tr>
</tbody>
</table>


Table 2: Frequency distribution of positive cheese samples based on their S. aureus count.

<table>
<thead>
<tr>
<th>Count /g</th>
<th>Fresh Kareish cheese</th>
<th>Pickled Kareish cheese</th>
<th>Domiati cheese</th>
<th>Processed cheese</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No./28</td>
<td>%</td>
<td>No./25</td>
<td>%</td>
</tr>
<tr>
<td>10^4&gt;</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>10^5&gt;</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>10^6&gt;</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>10^7&gt;</td>
<td>8</td>
<td>28.5</td>
<td>11</td>
<td>44</td>
</tr>
<tr>
<td>10^8&gt;</td>
<td>6</td>
<td>21</td>
<td>2</td>
<td>8</td>
</tr>
<tr>
<td>10^9&gt;</td>
<td>5</td>
<td>18</td>
<td>9</td>
<td>36</td>
</tr>
<tr>
<td>10^10&gt;</td>
<td>8</td>
<td>28.5</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>10^12&gt;</td>
<td>1</td>
<td>4</td>
<td>-</td>
<td>4</td>
</tr>
</tbody>
</table>

Table 3: Incidence of coagulase positive and coagulase negative strains of S. aureus in the examined cheese.

<table>
<thead>
<tr>
<th>Examined samples</th>
<th>No. of examined samples</th>
<th>No. of isolated S. aureus</th>
<th>coagulase positive</th>
<th>coagulase negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh Kareish cheese</td>
<td>30</td>
<td>28</td>
<td>15</td>
<td>54</td>
</tr>
<tr>
<td>Pickled Kareish cheese</td>
<td>30</td>
<td>25</td>
<td>7</td>
<td>28</td>
</tr>
<tr>
<td>Domiati cheese</td>
<td>30</td>
<td>21</td>
<td>13</td>
<td>62</td>
</tr>
<tr>
<td>Processed cheese</td>
<td>30</td>
<td>6</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
### Table 4: Frequency distribution of MRSA and VRSA and recovered from the examined cheese samples.

<table>
<thead>
<tr>
<th>Examined samples</th>
<th>No. of isolated S.aureus</th>
<th>MRSA</th>
<th>VRSA</th>
<th>Both</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
</tr>
<tr>
<td>Fresh Kareish cheese</td>
<td>28</td>
<td>8</td>
<td>3</td>
<td>11</td>
</tr>
<tr>
<td>Pickled Kareish cheese</td>
<td>25</td>
<td>7</td>
<td>5</td>
<td>20</td>
</tr>
<tr>
<td>Domiati cheese</td>
<td>21</td>
<td>9</td>
<td>8</td>
<td>38</td>
</tr>
<tr>
<td>Processed cheese</td>
<td>6</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

### Table 5: Incidence of MRSA and VRSA strains isolated from the examined cheese

<table>
<thead>
<tr>
<th>Examined samples</th>
<th>No. of examined samples</th>
<th>MRSA</th>
<th>VRSA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
</tr>
<tr>
<td>Fresh Kareish cheese</td>
<td>8</td>
<td>26</td>
<td>3</td>
</tr>
<tr>
<td>Pickled Kareish cheese</td>
<td>7</td>
<td>23</td>
<td>5</td>
</tr>
<tr>
<td>Domiati cheese</td>
<td>9</td>
<td>30</td>
<td>8</td>
</tr>
</tbody>
</table>

### Table 6: Enterotoxins produced by some strains of *Staph. aureus* isolated from the examined cheese samples.

<table>
<thead>
<tr>
<th>Examined samples</th>
<th>No. of strains tested</th>
<th>No. of strains producing enterotoxins</th>
<th>Types of produced enterotoxins</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>A</td>
</tr>
<tr>
<td>Fresh Kareish cheese</td>
<td>3</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Pickled Kareish cheese</td>
<td>4</td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>Domiati cheese</td>
<td>5</td>
<td>2</td>
<td>2</td>
</tr>
</tbody>
</table>

### Table 7: Enterotoxins production in relation to coagulase producing *Staph. aureus* strains.

<table>
<thead>
<tr>
<th>No. of strains tested</th>
<th>Coagulase producing strains</th>
<th>Enterotoxigenic Staph. aureus strains</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>12</td>
<td>12</td>
<td>100</td>
</tr>
</tbody>
</table>
DISCUSSION

The results recorded in Table 1 showed that 28(93%), 25(83%), 21(70%) and 6(20%) of the examined fresh Kareish, Pickled Kareish, Domiati and processed cheese samples were contaminated with Staph. aureus, respectively.

The S. aureus count ranged from 1 x10^7 to 5.25 x 10^7 with a mean count of 3.08 x 10^6 ± 1.79 x 10^6 in fresh Kareish cheese samples, from 4.17 x10^2 to 4.70 x10^6 with a mean Count of 3.40 x 10^5 ± 1.59x10^5 in Pickled Kareish cheese samples, from 1 x10 to 1.27 x10^7 with a mean count of 7.24 x10^5 ± 4.10 x10^5 and for Processed cheese the counts ranged from 1 x10 to 1.90 x10^2 with a mean count of 1.15 x10^2±8.98 cfu/g.

Egyptian standards (2005) pointed out that the cheese must be free from Staph. aureus and its toxins. It was evident that 28(93%), 25(83%), 21(70%) and 6(20%) of the examined fresh Kareish, Pickled Kareish Domiati and processed cheese samples failed to comply with the standard, respectively.

The results in Table 2, revealed that the highest frequency distribution of fresh Kareish cheese samples was 8(28.5%) lied in the range of 10^7 <10^8 and 10^6 <10^7 cfu/g, while the rest of the positive samples were distributed as 21,18 and 4% lied in between 10^4 <10^5, 10^5 <10^6 and 10^7 <10^8 cfu/g, respectively. In case of Pickled Kareish cheese samples the highest frequency distribution was (44%) lied in the range of 10^3 <10^4, 10^2 <10^3, 10^1 - <10^2 and 10^0 <10^1 cfu/g, respectively.

For Domiati cheese samples the highest frequency distribution was (43%) lied in the range of 10^2 -10^3 cfu/g, while the rest of the positive samples were distributed as 33, 14 and 10 % lied between 10^1 <10^2, 10^2 <10^3 and 10^3 <10^4, respectively. While the Processed cheese positive samples all of them lie in range10^2 <10^3 fresh Kareish cheese samples ,comparatively higher counts were obtained by Tawfek et al. (1988) Relatively lower counts were detected by Shelailah (1979), Ahmed (1980). Ewida (2009), De Reu et al. (2002) and In case of the examined Pickled Kareish cheese samples comparatively lower counts were obtained by Kaldes (1997). While for Domiati cheese samples results, higher counts obtained by Sallam et al. (1985). Relatively lower counts obtained by El-Malt (1993) and Hassan (2009). Lower incidence of S. aureus in curd (6.66 %) was reported by Kumar and Prasad (2010) and (3.33 %) Thaker et al. (2013). The difference in the prevalence of S. aureus between different types of cheese may originate from the method of manufacture, storage and handling.

Fresh Kareish cheese is a popular Egyptian food. It is made at farmers houses from raw skim milk and the fresh product is either consumed fresh or consumed after pickling in its salted fresh whey with added spices and medical plants (mish cheese) so the source of Staph. aureus in Kareish cheese are the raw milk, during processing and distribution in village markets these sources lead to the expected high count of Staph. aureus. Domiati cheese is considered to be the main national popular pickled soft cheese produced in Egypt. It is traditionally prepared from raw milk without addition of lactic acid culture starters but by addition of appreciable amount of salt to milk before renneting. It can be consumed fresh, but usually it is consumed after pickling in its salted fresh whey for a period of not less than 8 weeks, the product may be soiled with pathogens which enter through different environmental sources that render it a source of infection by many diseases. Genigeorgis (1989) demonstrated that the higher concentration of competing microorganisms in milk, the lower the rate of S. aureus growth and SE production. Competition with lactic acid bacteria has been reported in other research on cheese (Otero et al., 1988).

For Domiati cheese samples the highest frequency distribution was (43%) lied in the range of 10^2 -10^3 cfu/g, while the rest of the positive samples were distributed as 33, 14 and 10 % lied between 10^1 <10^2, 10^2 <10^3 and 10^3 <10^4, respectively. While the Processed cheese positive samples all of them lie in range10^2 <10^3 fresh Kareish cheese samples ,comparatively higher counts were obtained by Tawfek et al. (1988) Relatively lower counts were detected by Shelailah (1979), Ahmed (1980). Ewida (2009), De Reu et al. (2002) and In case of the examined Pickled Kareish cheese samples comparatively lower counts were obtained by Kaldes (1997). While for Domiati cheese samples results, higher counts obtained by Sallam et al. (1985). Relatively lower counts obtained by El-Malt (1993) and Hassan (2009). Lower incidence of S. aureus in curd (6.66 %) was reported by Kumar and Prasad (2010) and (3.33 %) Thaker et al. (2013). The difference in the prevalence of S. aureus between different types of cheese may originate from the method of manufacture, storage and handling.

Table 3 showed that 15(54%), 7(28%) and 13 (62 %) of the examined staphylococci strains recovered from fresh Kareish cheese, Pickled Kareish cheese and Domiati cheese, respectively, were coagulases positive. While, 13(46%), 18(72%), 8(38%) and 6(100%) of the isolated strains of staphylococci of the examined cheese, respectively, were coagulase negative. Concerning the result of coagulase positive S. aureus in the examined fresh Kareish cheese samples, higher results were obtained by Ahmed (1980), Al-Hawary et al. (2009) and Helmy et al. (2009). Lower results were detected by Aman (1994); Kaldes (1997) and Kolluman et al. (2011).

In case of coagulase positive S. aureus in the examined Domiati cheese samples, lower results were detected by Coveney et al. (1994), Kaldes (1997); Normanno et al. (2005) and Kolluman et al. (2011).

Various examples of staphylococcal food poisoning are described in the literature. In one case, cheese was involved in an outbreak because it had been made from unheated treated milk, milk contaminated after pasteurization and before inoculation with lactic starter culture or did not use culture starter. In this particular case, the starter culture did not grow properly, resulting in a fermentation accident that allowed the S. aureus strain to develop and produce SE (Vasavada, 1988). Although milk and milk products are frequently contaminated with S. aureus, dairy products are rarely involved in staphylococcal
food poisoning because the critical cell density of >105 cfu/g-1 is usually not reached (Altekruse et al., 1994).

The result in Table 4 showed that 8(29%), 3(11%) and 3(11%) showed staph aureus strains isolated from fresh Kareish cheese, 4 strains of Pickled Kareish cheese and 5 strains of Domiati cheese. The frequency distribution of MRSA and VRSA in the Domiati cheese samples were 9(43%) and 8(38%) while 3(14%) can be resistant for both drugs. In Processed cheese there is no of isolated strains of MARSA, VRSA or resistant for both drugs.

In Table 5 the incidences of MRSA and VRSA from the examined samples were 26%, 3(10%), 7(23%), 5(17%), 9(30%) and 8(27%) of fresh Kareish cheese, Pickled Kareish cheese and Domiati cheese respectively.

Inspection of Table 6 revealed that out of 12 methicillin and vancomycin resistance Staph aureus strains tested for enterotoxins production 3 strains of fresh Kareish cheese, 4 strains of Pickled Kareish cheese and 5 strains of Domiati cheese. From fresh Kareish cheese 2 strains were synthesized enterotoxins A and SEB (one strains) in Pickled Kareish cheese there is no strains producing enterotoxins. Domiati cheese 2 strains were isolated and produce enterotoxins A(2 strains), SEB (one strain), SEC (one strain) and SED (one strain).

This finding was in contrast to other studies from Spain, Kenya, Switzerland, Brazil, South Korea, the USA, Slovakia and Palestine, where most of the enterotoxigenic Staph aureus isolated usually synthesized the toxins SEA, SEC, or SED (Scherrer et al., 2004).

The higher percentages of SEA among Staph aureus strains isolated from milk and milk products may be due to the fact that enterotoxins A are less common among the strains of animals origin than from human origin (Hajek and Marsalek, 1973). These strains of human origin contaminate milk and milk products during different stages of production or at consumer outlet. On the other side the presence of SEC and SED can be attributed to the increased incidence of Staphylococcal mastitis as enterotoxins C and D were found to be produced by Staph aureus strains isolated from bovine mastitis and were designated as ‘animal strains’ (Olson et al., 1970).

Although the selected 12 isolates were strongly producing coagulase, only 4 were enterotoxigenic which confirm what was stated by A.P.H.A. (1992) and Ryser (2001) Table (7) that attempts to associate enterotoxin production by Staph aureus with specific biochemical properties were generally failed. Consequently, confirmation of the toxin by serological or other means provide the only proof that the particular strain is enterotoxigenic.

Park et al. (1994) evaluated the RIDASCREEN SET kit for its efficiency. They concluded the major advantages of the kits were a high degree of specify (neither false –positive results due to the growth of nonstaphyloccal microorganisms nor cross –reaction among reagents of the kits was reported), excellent sensitivity, simplicty rapidity (results can obtained in 3h) and semi quantitative results.

CONCLUSION

The obtained results allow concluding that fresh Kareish cheese, Pickled Kareish cheese, Domiati cheese and Processed cheese samples sold in Aswan city markets were produced, handled, packed and distributed under neglected hygienic measures. The information given by the achieved results proved that most of the examined fresh Kareish cheese, Pickled Kareish cheese, Domiati cheese and Processed cheese samples sold in Aswan city market were highly contaminated with high number of Staph aureus which may lead to undesirable changes of these products that render them unfit for consumption and indicate unpersonal hygiene and un- sanitary conditions during processing and handling of such product in compared with examined Processed cheese which show lower incidences or counts.

In the resent years, the extensive therapeutic use of antimicrobials or with their administration as growth promoters in food animal production lead to development of resistance both in human and animal's pathogens as occurred with Staph aureus. Nowdays, Staph aureus can resist many antibiotics as methicillin and vancomycin which lead to difficulty in their susceptibility of Staph aureus to these antibiotics and other group of antibiotics. In this study, methicillin resistance Staph aureus (MRSA) and vancomycin resistance Staph aureus (VRSA) could be isolated from examined samples in different percentages.

REFERENCES


American Public Health Association New York.


توجد المكور العنقودي الذهبي المفرز للسموم المعوية في بعض الجبنة المباعة بمحافظة أسوان مصر العليا

Email: lailael.malt@ymail.com

يعد الميكروب المكور العنقودي الذهبي من أهم الميكروبات التي تساهم في حدوث حالات التسمم الغذائي التي إما إعراضها الإسهال، وما زال يسبب الكثير من المشاكل الصحية الأخرى على مستوى العالم مثل تسمم الدم البكتيري والتهاب عضلة القلب والتهاب الأمعاء. هذا وتعتبر الآلام ومنお得اتها من أهم الأعراض التي يمكن أن يصل إليها الميكروب حيث أنه يعيش في الأمعاء والجهارات التنفسية، وعلى جلود الإنسان والمثلثة وكذلك أحياناً يوجد في ضرع الماشية، ويسبب الالتهاب المخاطي، وذلك يكون من السهولة تثبيت البكتيريا.

جرى الدراسة على عدد من في جبة مصنع محلية ثلاث عينات عينة من كل من (الجبن الفريش الطازج، الجبن الفريش المملح، الجبن المطبخ المصنوع في شركات الألبان) وقد جمّعت العينات من النواحى الجليدية وأسواق مدينة أسوان بطريقة عشوائية لفحصها. وقد وجد بفحص البكتيريولا أن توجد الميكروب المكور العنقودي الذهبي (28%) في أسواق مدينة أسوان وجبة مصنع محلية ثلاث عينات عينة من كل من (الجبن الفريش الطازج، الجبن الفريش المملح، الجبن المطبق المبذول بالصورية)، وجد وجد أن أربعة عينات من (عينات عينات اثنين من جبن الفريش، وعينات من جبن الفريش المملح، وعينات عينات من جبن المطبخ المصنوع في شركات الألبان) وجد وجد أن أربعة عينات من (عينات اثنين من جبن الفريش، وعينات من جبن المطبخ المصنوع في شركات الألبان) لم توجد علامة على

A: إفراغ سم من الجبن

B, C&D: من السموم

70