Benha Veterinary Medical Journal 39 (2020) 117-121

Phenotypic detection of methicillin-resistant Staphylococcus aureus in some cheese varieties

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ABSTRACT

A Hundred-eighty random cheese samples includes varieties of cheese, soft (kareish and white), hard (Roomy), processed (Flamingo), beside machine and hand swabs were collected from different supermarkets (30 of each), at Qalubiya Governorate, Egypt to investigate the incidence of Staphylococci with especial reference to methicillin-resistant Staphylococcus aureus (MRSA) strains. The results revealed that the mean values of staphylococci and Staph. aureus counts (cfu/g) in kareish cheese samples were 3.16×10⁴ and 6.57×10⁷, respectively. For white cheese samples, they were 7.60×10⁴ and 5.12×10⁷, respectively. For Roomy cheese, samples were 5.79×10⁴ and 3.83×10⁷, respectively. For Flamingo cheese samples were 2.96×10⁴ and 1.05×10⁷, respectively. For machine swab samples were 1.02×10⁴ and 1.48×10⁴, respectively. For hand swab samples were 1.43×10⁴ and 3.71×10³ (cfu/ml), respectively. A total of 118/180 (65.5%) isolates of staphylococci species, includes 55 S. aureus (30.6%). All 55 isolated Staph aureus strains were coagulase positive while, other isolated staphylococcal strains (63) were coagulase negative. The isolated Staph. aureus was highly resistant for methicillin followed by oxacillin, nalidixic acid, ampicillin, oxetaxytracycline, cefotaxime and streptomycin. Meanwhile, it was highly sensitive to vancomycin followed by norfloxacine, gentamicin, meropenem and ciprofloxacine. A 49 out of 55 Staph. aureus strains grew well on ORSAB media (they were MRSA strains). Finally, Staph. aureus and MRSA strains were found in the examined cheese samples sold in markets at Qalubiya Governorate; beside that MRSA strains were detected that threats the consumer's health.

1. INTRODUCTION

Cheese considered as one of the most important dairy products in the diet for both young and old people as it is superior source of high-quality protein, bioactive peptides, fat, vitamins, minerals, and other essential elements (Walther et al., 2008). Poor hygienic practices in cheese processing plants may result in the contamination of cheeses with pathogens leading to prompting their decay, financial misfortunes, foodborne diseases in human and wellbeing hazard (Marjan et al., 2014). Staphylococcus aureus is very prominent in cheese as a contagious pathogen and it is considered the third-most important cause of food-borne disease in the world (Zinke et al., 2012). This was recovered from both hand and nose samples of persons (André et al., 2008).

Bacterial drug survival is a significant public health issue. The improvement of opposition both in human and bacterial microorganisms has been related with the extensive unwise administration of antimicrobials or due to extensive usage as growth enhancers in food animal production (Lowy, 2003). Meticillin-resistance Staph. aureus (MRSA) strains, mecA positive strains should be reported as oxacillin- resistant (CLSI, 2018). It is mainly referred to the activity of the specific mecA gene, present on Staphylococcal genome mec cassette (SCCmec), that translated to penicillin-binding protein 2a (PBP2a) leading to decrease its affinity essentially for all β-lactam antimicrobials (Thaker et al., 2013). MRSA is frequently associated with nosocomial outbreaks worldwide such as post-operative wound infections and pneumonia; in addition, it causes severe food poisoning infections (Khosravi et al., 2017). Nowadays, MRSA has been isolated from various food domestic animals all over the world (Antoci et al., 2013).

Staphylococcus aureus is viewed as one of the main causative infective specialist of mastitis in dairy production (Nam et al., 2011). Generally, methicillin and oxacillin are not utilized for mastitis treatment yet. Besides the presence of environmental MRSA may likewise be one of the sources of MRSA infection in animals and human as it can persist for long period in the environment (Lim et al., 2013).

Staphylococcal food poisoning (SFP) occurs after food contamination with virulent enzymes producing Staph. aureus which have role in bacterial invasiveness and extracellular enterotoxins production (Prescott et al., 2005). Staphylococcal food poisoning is marked by gastro-intestinal disturbances including mild to severe abdominal pain, profuse watery diarrhea, and vomiting and usually the complete recovery occurs within 1-3 days (Shijia et al., 2016). As the level of contamination of cheese with Staphylococci constitute significant hazards to human consumers. Therefore, this study aimed to determine the
incidence of Staphylococci with special reference to Staph. aureus and MRSA in the examined different varieties of cheese, soft (Kareish and White); hard (Roomy); processed (Flamingo) purchased from different supermarkets, beside machine swabs and hand swabs collected from the same supermarkets, at Qalubia Governorate

2. MATERIAL AND METHODS

2.1. Collection of Samples

Hundred and eighty random samples include varieties of cheese, soft (Kareish and White); hard (Roomy); processed (Flamingo) purchased from different supermarkets, beside machine swabs and hand swabs (each swab was immersed in sterile tube with 10 ml peptone water 0.1%) collected from the same supermarkets (30 of each), at Kalobia governorate, Egypt, to throw light over the prevalence of staphylococci with special reference to Staph. aureus and MRSA beside the phenotypic characterization of the isolated Staph. aureus strains.

2.2. Bacteriological examination

2.2.1. Preparation of samples was performed according to APHA (2004).

2.2.2. Enumeration of Staphylococcus and Staph. aureus: after decimal serial dilution, the prepared samples were cultured on Baird-Parker agar, and incubated at 37°C for 24h, and then recorded according to FDA (2001).

2.2.3. Isolation and identification of suspected Staph. aureus. The isolated pure Staph. aureus colonies were identified morphologically by Gram's stain; biochemical reactions including coagulase activity was performed according to Quinn et al. (2002).

2.2.4. In-Vitro anti-microbial sensitivity test:
The isolated Staph. aureus strain was subjected to the sensitivity test against different antibiotics, using the antibiotic discs (Oxoid Ltd., England) and Mullar-Hinton agar diffusion method (Koneman et al., 1997).

2.2.5. Phenotypic detection of methicillin-resistant Staph. aureus (MRSA) according to Becker et al. (2002).

Typical 4-5 colonies of each isolated Staph. aureus strain was incubated in Brain Heart Infusion broth at 37°C/24 hrs.

By means of sterile loop, a loopful from the inoculated BHI broth was streaked over ORSAB agar base plates (Oxoid Ltd., England) supplemented with oxacillin (SR 195 E) and then incubated at 37°C/24 hours for detection of MRSA strains.

2.3. Statistical analysis

The obtained results were statistically analyzed following SnEdcor and Cochran (1969) using SPSS software program Differences in mean of analyzed data were considered significant at P≤0.05.

3. RESULTS

The results in table (1) declared that the examined Kareish cheese samples were the most contaminated with Staphylococci, followed by White cheese, Roomy cheese, and Flamingo cheese. While referring to machine and hand swabs, hands revealed higher contamination with Staphylococci, which also appeared as high incidence of machine contamination that indicated low hygienic practices. Moreover, significant differences (P≤0.5) were detected between kareish cheese and other examined samples.

The obtained results in table (2) revealed that Staph. aureus was the most frequently detected in Kareish cheese, followed by white cheese, Roomy cheese, and Flamingo cheese; while regarding to swab samples, hand swabs revealed higher contamination with Staph. aureus than machine swabs. Moreover, the statistical results revealed that, kareish cheese showed a significant increase of Staph. aureus counts when compared with Flamingo cheese, machine swabs and hand swabs samples. White cheese showed a significant increase of Staph. aureus counts when compared with hand samples. Meanwhile, there were no significant difference between kareish cheese, white cheese and Roomy cheese samples. There is no significant difference between white cheese, roomy cheese, flamingo cheese and hand swabs samples.

Table 1 Total Staphylococci counts/gm. in the examined samples of cheese and swab samples (n=30 for each)

| Samples            | No. | % a  | Min. | Max. | Mean ±SEM** |
|--------------------|-----|------|------|------|-------------|
| Kareish cheese     | 25  | 83.3 | 2.3×10⁵ | 4.5×10⁶ | 6.5×10⁶ ±0.18×10⁶ |
| White cheese       | 23  | 76.7 | 1.5×10⁵ | 3.4×10⁶ | 7.6×10⁶ ±0.19×10⁶ |
| Roomy cheese       | 17  | 56.7 | 1.3×10⁵ | 3.9×10⁵ | 5.79×10⁵ ±0.23×10⁵ |
| Flamingo cheese    | 16  | 53.3 | 1.2×10⁵ | 1.2×10⁵ | 2.96×10⁵ ±0.07×10⁵ |
| Machine swabs      | 17  | 56.7 | 4.5×10⁴ | 2.3×10⁵ | 1.02×10⁵ ±0.08×10⁵ |
| Hand swabs         | 20  | 66.7 | 5.8×10⁴ | 2.0×10⁵ | 1.43×10⁵ ±0.02×10⁵ |
| Total              | 118 | 65.6 | 4.5×10⁴ | 4.5×10⁶ | 9.79×10⁵ ±0.39×10⁵ |

* Percentage in relation to total number of each sample in each row. ** Minimum count for positive samples. ***Standard error of mean.

Table 2 Total Staph. aureus counts/g (for cheese samples) or ml (for swab samples) in the examined cheese and swab samples (n=30 for each)

| Samples            | No. | % a  | Min. ** | Max. ** | Mean ±SEM*** |
|--------------------|-----|------|---------|---------|-------------|
| Kareish cheese     | 12  | 40.0 | 9.4×10² | 2.6×10³ | 6.57×10³ ±0.20×10³ |
| White cheese       | 11  | 36.7 | 8.0×10² | 1.9×10³ | 5.12×10³ ±0.18×10³ |
| Roomy cheese       | 8   | 26.7 | 4.3×10² | 8.8×10³ | 3.38×10⁴ ±0.12×10⁴ |
| Flamingo cheese    | 7   | 23.3 | 1.5×10² | 5.1×10³ | 1.05×10⁴ ±0.07×10⁴ |
| Machine swabs      | 8   | 26.7 | 1.5×10² | 4.5×10³ | 1.48×10⁴ ±0.06×10⁴ |
| Hand swabs         | 9   | 30.0 | 3.7×10² | 6.3×10³ | 3.71×10³ ±0.07×10³ |
| Total              | 55  | 30.6 | 1.5×10² | 2.6×10³ | 3.17×10³ ±0.07×10³ |

* Percentage in relation to total number of each sample in each row. ** Minimum count for positive samples. ***Standard error of mean. 
In addition, the results of coagulase activities of isolated staphylococcus species strains (Table, 3) declared that, out of 118 isolated staphylococcus species strains, 55 were coagulase positive and all of them were *Staph. aureus* strains, with the incidence of 65.6%, where kareish sample was the mostly contaminated, followed by white cheese, Flamingo cheese, and Roomy cheese respectively. Moreover, table (4) revealed that 125 samples out of 180 ones were accepted as they were free form Coagulase Positive *Staph. aureus*.

The in-vitro sensitivity tests for the isolated *Staph. aureus* (Table 5) revealed that, the isolated *Staph. aureus* strain was resistant to ampicillin, cefotaxime, methicillin, nalidixic acid, oxacillin, oxytetracycline, and streptomycin; while showed intermediate sensitivity to erythromycin, and sensitive to ciprofloxacin, gentamicin, meroenen, norfloxacin, and vancomycin. The methicillin-resistance *Staph. aureus* (MRSA) strains, mecA positive strains should be reported as oxacillin- resistant. The recorded results appeared that, 49 *Staph. aureus* strains out of 55 ones were grown well on ORSAB media with blue colored colonies due to an acid-dependent chromogenic component (aniline blue) (Table, 6).

| Table 3 Coagulase activities of isolated Staphylococcus species strains |
|-------------------------------------------------|
| Samples          | No. | Staphlococci positive Coagulase positive Staph. aureus strains | coagulase negative strains |
|                  | No. | %*                  | No. | %*                  | No. | %*                  |
| Kareish cheese   | 30  | 25 83.3%            | 12  | 40.0%               | 13  | 45.3%               |
| White cheese     | 30  | 23 76.7%            | 11  | 36.7%               | 12  | 40.0%               |
| Roomy cheese     | 30  | 17 56.7%            | 8   | 26.7%               | 9   | 30.0%               |
| Flamingo cheese  | 30  | 16 53.3%            | 7   | 23.3%               | 9   | 30.0%               |
| Machine swabs    | 30  | 17 56.7%            | 8   | 26.7%               | 9   | 30.0%               |
| Hand swabs       | 30  | 20 66.7%            | 9   | 30.0%               | 11  | 36.7%               |
| Total            | 180 | 118 65.6%**         | 55  | 30.6%**             | 63  | 35%**               |

*Percentage in relation to total number of each sample in each row (30 for each).

| Table 4 Acceptance of the examined samples in relation to *Staph. aureus* (n=30 of each). |
|-------------------------------------------------|
| Samples          | No. of accepted samples** | No. of non- accepted samples** |
|                  | No. | %        | No. | %        |
| Kareish cheese   | 18  | 60%      | 12  | 40%      |
| White cheese     | 19  | 63.3%    | 11  | 36.6%    |
| Roomy cheese     | 22  | 73.3%    | 8   | 26.6%    |
| Flamingo cheese  | 23  | 76.6%    | 7   | 23.3%    |
| Machine swabs    | 22  | 73.3%    | 8   | 26.6%    |
| Hand swabs       | 21  | 70%      | 9   | 30%      |
| Total            | 125 | 69.4%    | 55  | 30.5%    |

**Accepted and non- accepted samples according to EOS (2005) for cheese samples and to EC (2001) for hand and machine swabs in relation to the isolation of Coagulase Positive *Staph. aureus* (free). EOS 1007:2005-par4 for Roomy cheese. EOS 1007:2005-par2 for Flamingo cheese. EOS 1008:2005-par4 for Kareish cheese. EOS 1008:2005-par5 for white cheese.

| Table 5 In-Vitro anti-macrobial Sensitivity test for isolated *Staph. aureus* strains |
|-------------------------------------------------|
| Antimicrobial agents  | Disk Concentrations | Sensitive | Intermediate | Resistant | AA |
|                      | No. | %            | No. | %            | No. | %            |
| Ampicillin            | 20 µg | 10 18.2% | 1 1.8% | 44 80.0% | R |
| Cefotaxime            | 30 µg | 4 7.3% | 8 14.5% | 43 78.2% | R |
| Ciprofloxacin         | 5 µg  | 44 80.0% | 8 14.5% | 3 5.5% | S |
| Erythromycin          | 15 µg | 7 12.7% | 29 52.7% | 19 34.6% | IS |
| Gentamicin            | 10 µg | 46 83.6% | 5 9.1% | 4 7.3% | S |
| Meropenem             | 10 µg | 46 83.6% | 6 10.9% | 3 5.5% | S |
| Methicillin           | 5 µg  | 4 7.3% | 0 0.0% | 51 92.7% | R |
| Nalidixic acid        | 30 µg | 3 5.5% | 5 9.1% | 47 85.4% | R |
| Norfloxacin           | 10 µg | 47 85.4% | 4 7.3% | 4 7.3% | S |
| Oxacillin             | 1 µg  | 6 10.9% | 0 0.0% | 49 89.1% | R |
| Oxytetracycline       | 30 µg | 2 3.6% | 9 16.4% | 44 80.0% | R |
| Streptomycin          | S/10 | 5 9.1% | 22 40.0% | 28 50.9% | R |
| Vancomycin            | 30 µg | 49 89.1% | 1 1.8% | 5 9.1% | S |

No: Number of isolates. %: Percentage in relation to total number of isolates (55). AA: Antibiomarial activity. R: Resistant. S: Sensitive. IS: Intermediate.

| Table 6 Incidence of methicillin resistant *Staph. aureus* strains on ORSAB agar |
|-------------------------------------------------|
| Samples          | Coagulase positive *Staph. aureus* strains | *Staph. aureus* on ORSAB media |
|                  | No. | %*                  | No. | %*                  |
| Kareish cheese   | 30  | 12 40.0%            | 12  | 40.0%               |
| White cheese     | 30  | 11 36.7%            | 11  | 36.7%               |
| Roomy cheese     | 30  | 8 26.7%             | 7   | 23.3%               |
| Flamingo cheese  | 30  | 7 23.3%             | 7   | 23.3%               |
| Machine swabs    | 30  | 8 26.7%             | 7   | 23.3%               |
| Hand swabs       | 30  | 9 30.0%             | 5   | 16.7%               |
| Total            | 180 | 55 30.6%            | 49  | 27.2%               |

%:* Percentage in relation to total number of samples in each row.

119
4. DISCUSSION

Dairy products are common vehicle of foodborne illness, Staphylococci mainly *Staphylococcus aureus* and MRSA are the most important causes of outbreaks and the presence of them in cheese has relevant public health implications (Verkade and Kluytmans, 2014). Staphylococcal contaminated milk and dairy products have been reported as one of the most frequent elements in Staphylococcal food poisoning due to food intoxication with Staphylococcal heat resistant enterotoxins secreted during cheese production in case of low sanitary conditions (Can and Celik, 2012).

Regarding to the obtained results in Table (1), they came in accordance with those reported by Al-Hawary et al. (2009). Meanwhile, the results were disagreed with El-baradei et al. (2007), who recorded lower counts. The obtained results may be due to using raw milk with high initial flora, poor processing conditions; inadequate sanitation and disinfection of food contact surfaces and utensils sanitation and disinfection, besides of inadequate refrigerating and surfaces or and/or lack personal cleanliness.

The presence of *Staph. aureus* in cheese commonly indicates direct contamination from worker’s hands with abrasion and wounds or inadequately cleaned equipment resulting in *Staph. aureus* intoxication. Accordingly, the total *Staph. aureus* count can be taken as an index of sanitary conditions under which the varieties of cheese are manufactured and handled (Gonzalez et al., 2017).

Referring to the obtained results in Table (2), nearly similar counts were recorded by Hassan and Gomaa (2016). Meanwhile, the results were disagreed with those of Zinke et al. (2012) and Serrano et al. (2018), who reported lower *Staph. aureus* counts and with Ibrahim et al. (2015) who recorded higher *Staph. aureus* counts in their examined samples.

In addition, the obtained results of Coagulase activity of isolated staphylococcus species in table (3) came in agree with those obtained by Zinke et al. (2012) and Serrano et al. (2018).

The presence of *Staph. aureus* in cheese indicates personal cleanliness as well as deficient equipment sanitation and disinfection. Lack of sanitary practices with *S. aureus* contamination leads to its growth and proliferation producing staphylococcal enterotoxins which are incriminated in many food poisoning outbreaks; also, several workers have reported the occurrence of multidrug resistant *Staph. aureus* in milk and dairy products (Omoshaba et al., 2017).

The obtained in-vitro sensitivity tests for the isolated *Staph. aureus* in Table (5) came in line with those recorded by Gonzalez et al. (2017) and Omoshaba et al. (2017). The resistance to oxacillin, methicillin occurred mainly due to the presence of mecA gene on *Staphylococcus aureus* chromosome that responsible for the production of Penicillin binding protein PBP2a (Ito et al., 2004). In addition, the results proved that multiple antibiotic resistances are widely spread among isolated strains of *Staph. aureus* as proved the fact of Shalini and Rameshwar (2005) that the zoonotic transmission of multi-antibiotic unsusceptible microorganisms between the food producing animals and human is mainly come through contaminated food intake. Moreover, a significant relation between Methicillin insusceptibility and resistance to other non-ß-lactam antibacterials has been reported (Otalu et al., 2011).

The methicillin-resistance *Staph. aureus* (MRSA) strains, mecA positive strains should be reported as oxacillin-resistant (CLSI, 2018). The recorded results of suspected MRSA isolates (Table 5) appeared grown well on ORSAB media with blue colored colonies due to containing chromogenic aniline blue. The obtained results came in line with those recorded by Becker et al. (2002); and Serrano et al. (2018) who reported that all the isolates that were classified as MRSA on CHROM agar (ORSAB) contained mecA gene.

5. CONCLUSIONS

The recorded results concluded that *Staph. aureus* and MRSA strains were detected in the examined cheese samples that in cheese sold in markets in Kalabria governorate. Detection of MRSA in examined cheese, is considered a serious health problem may causing severe food poisoning. In addition, many efforts and practices have to be applied to improve cheese safety, including raise the awareness about good hygienic personnel practices during production and storage, collection of raw milk, as well as ensuring proper sanitary procedures of production related equipment.

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