Short communication

MRSA detection in raw milk, some dairy products and hands of dairy workers in Egypt, a mini-survey

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1. Introduction

Staphylococcus aureus (S. aureus) is a common member of the natural microflora of human skin and nasal passage (Hanson et al., 2011). In addition, as a potential pathogen, it may adversely affect human and animal health by causing severe necrotic lesions, abscesses (Lowy, 1998) and bacteremia (Reacher et al., 2000). Moreover, besides these pathogenic symptoms, toxicogenic foodborne strains of S. aureus, if they get multiplied in food to a certain level of about 10⁷–10⁹ CFU/g, may secret potent, heat stable enterotoxins responsible for food-borne intoxication (Tranter, 1990). S. aureus intoxication ranked third of food poisoning cases all over the world (Asao et al., 2003; Zhang, Landolo, & Stewart, 1998) and had been implicated with different categories of food including raw milk (Jorgensen, Mork, Hogasen, & Rovik, 2005), dairy products (Headrick et al., 1998), chicken, pork, beef and salad dishes (Bryan, 1998). Furthermore, S. aureus constitutes a primary cause of mastitis in dairy cattle (Virgin, Van Slyke, Lombard, & Zadoks, 2009).

Among S. aureus, Methicillin-resistant strains (MRSA), has recently emerged as a serious life-threaten infective agent which does not respond to a lot of antimicrobial treatments. Previous reports have shown an annual estimate of 94,000 MRSA infections in the United States, with nearly 20% mortality rate (Klevens et al., 2007).

MRSA synthesizes a penicillin binding protein (PBP2a), encoded by the mecA gene on a mobile genetic element (Staphylococcal cassette chromosome mec SCCmec), which has a role of counteracting the inhibitory effect of Beta-lactam (β-lactam) antibiotics by preventing them from effectively binding to cell wall proteins. Moreover, MRSA may also resist vancomycin (Cui, Murakami, Kuwahara-Arai, Hanaki, & Hiramatsu, 2000).

MRSA transmission has two main forms, hospital-acquired (HA) and community-acquired (CA). Although, HA MRSA infection is thoroughly investigated as the major form, CA MRSA presently represents an imminent hazard and may have severe consequences (Cui et al., 2004). Whilst, MRSA is strictly linked to hospitals and health workers, CA MRSA is more widespread and has no definite spreading vicinity (Wannet, Heck, Pluister, Spalburg, & De Neeling, 2004) and its risk against industrialized nations has been increased (Baggett et al., 2004; Doufour et al., 2002; Frank, Marcinak, Mangat, & Schreckenberg, 1999; Groom et al., 2001; O'Brien, Pearman, Gracey, Piley, & Grubb, 1999; Salmenlinna, Lyytikäinen, & Vuopio-Varkila, 2002; Witte, Cuny, Strommenger, Braulke, & Heuck, 2004).

Since S. aureus is highly prevalent in food and food environment, MRSA may have the same pattern of linkage. Many reports (De Boer...
et al., 2009; Kitai et al., 2005; Kwon et al., 2006; Lim et al., 2010; Lozano et al., 2009; Pu, Han, & Ge, 2009; Van Loo et al., 2007; Weese, Avery, & Reid-Smith, 2010; Weese, Reid-Smith, Rousseau, & Avery, 2010) have identified presence of MRSA in different retailed meat products from different regions worldwide with varied prevalence. Normanno et al. (2007) isolated MRSA strains from bovine milk and some cheese varieties in Italy. Moreover, several food-borne acquired MRSA outbreaks have been also reported (Jones, Kellum, Porter, Bell, & Schaffner, 2002; Klyutmans et al., 1995).

Recent reports revealed that MRSA was also associated with cases of bovine and caprine mastitis (Aras, Aydinb, & Kav, 2012; Vanderhaegen et al., 2010). MRSA strains have been found among the S. aureus strains isolated from bovine mastitis milk but they are not more prevalent (Hendriksen et al., 2008; Juhász-Kaszanyitzky et al., 2007; Kwon et al., 2005; Lee, 2003; 2006; Moon et al., 2007). In addition, specific MRSA strain CC398 has been linked with different food animals and people in contact, which arise a new MRSA form, livestock-associated (LA-MRSA). LA-MRSA has been isolated from both human and animal infections (Krziwanek, Metz-Gercek, & Mittermayer, 2009) and from bovine mastitis case (Monecke, S., Kuhnert, Hotzel, Slickers, & Ehrlich, 2007). Aforementioned reports may elucidate a possible way of transmitting either CA-MRSA or LA-MRSA between food/animal handlers and foods.

To the best of our knowledge, this is the first study to reveal the prevalence of MRSA strains in raw milk, some dairy products and on hands of dairy workers (Milkers and food handlers) in Egypt. Molecular MRSA identification was accomplished by PCR detection of mecA gene. Antibiotic susceptibility of isolated MRSA strains was also tested.

2. Materials and methods

2.1. Samples collection

A total of 120 samples (Table 1) were collected from the rural areas (local markets and/or villages) located in Dakahlia province, Egypt during the period of December, 2011 through February, 2012. All samples were kept at 4 °C in insulated ice box and transferred to the dairy microbiology laboratory, Food control department, Zagazig University and analyzed within 4 h of collection.

2.2. Sample preparation

In order to prepare decimal dilutions, raw milk and swab soaking broth were used directly, whereas ice cream samples were initially left at room temperature to be melted, while kariesh cheese samples were homogenized (11 g of cheese sample with 99 ml 0.1% sterile peptone water (Oxoid) for 2 min) using Sycloon-04C Stomacher blender (Ningbo Sklon, China).

### Table 1

<table>
<thead>
<tr>
<th>Samples</th>
<th>Number of samples</th>
<th>Sample condition</th>
<th>Number of positive samples (%)</th>
<th>Mean count ± SD (CFU/ml or gm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raw milk</td>
<td>35</td>
<td>50 ml milk samples were collected from individual manually milked cow’s milk, farm tank milk, collector’s buckets milk and local market milk. Samples transferred immediately into sterile sampling tubes.</td>
<td>33 (94.3)</td>
<td>5.5 × 10^4 ± 3.2 × 10^4</td>
</tr>
<tr>
<td>Kariesh cheese</td>
<td>30</td>
<td>Acid coagulated skimmed soft cheese, locally manufactured. 250 g of freshly prepared cheese were collected.</td>
<td>28 (93.3)</td>
<td>6.2 × 10^3 ± 7.1 × 10^4</td>
</tr>
<tr>
<td>Ice cream</td>
<td>30</td>
<td>Produced at small scale; aseptically collected from markets and street vendors.</td>
<td>17 (56.7)</td>
<td>8.9 × 10^3 ± 4.8 × 10^2</td>
</tr>
<tr>
<td>Workers’/handlers’ hand swabs</td>
<td>25</td>
<td>Each swab represented a worker or a handler. Dorsal and palmar surfaces and fingertips were swabbed using moistened cotton sterile swab (using sterile ringers solution) which was rubbed gently against surfaces.</td>
<td>20 (80)</td>
<td>—</td>
</tr>
</tbody>
</table>

2.3. *Staphylococcus aureus* count and isolation

3M™ petrifilm™ Staph Express count plates (STX) (3M Corporate Headquarters; St. Paul, MN, USA) were used to enumerate and isolate *S. aureus* (AOAC, 2003). Briefly, 1 ml of appropriate dilution was inoculated onto petrifilm and the inoculum was evenly distributed with a sterile plastic spreader. After one minute at ambient temperature, the petrifilm was incubated aerobically at 35 °C for 24 h. According to the manufacturer’s instructions, red-violet colonies were counted as *S. aureus* and any plates with background colonies were subjected to, 3M™ Petrifilm™ Staph Express Disk testing to differentiate *S. aureus* according to the manufactures instructions. *S. aureus* colonies appear surrounded with halo zone, which were then counted, picked up and streak plated on Brain Heart infusion Agar (BHA, BD) to establish pure cultures. Pure cultures were maintained on Brain Heart Infusion broth (BHI) with glycerol (50% v/v) at −70 °C.

2.4. MRSA molecular identification

*S. aureus* isolates were grown in BHI prior to DNA extraction and purification according to the DNA Purification Kit (Qiagen) procedure. *S. aureus* (MRSA) isolates were detected using the following primers set: mecAFor (AAGCAATAGAATCATCAGAT) and mecAREv (AGTCTTCGACTCCGGATTTGC) which were generated by Primer-BLAST software using mecA gene sequence obtained from Gene Bank® (National Center for Biotechnology Information; NCBI). Amplification was done with the following profile: Initial denaturation at 94 °C for 5 min, 40 cycles as follow: denaturation at 94 °C for 30 s, annealing at 55 °C for 30 s and extension at 72 °C for 30 s, followed by final extension step at 72 °C for 5 min. The PCR product (451 bp) were electrophoresed on 0.5% agarose gel and visualized by ethidium bromide staining. GeneRuler® 100 bp DNA ladder (Fermentas) was used as a molecular weight standard. PCR was performed on *S. aureus* ATCC 33591 (positive control) and *Staphylococcus epidermidis* (locally isolate, negative control).

2.5. Antibiotic resistance of MRSA

This experiment was designed to determine the minimal inhibitory concentration (MIC) of oxacillin and vancomycin against isolated MRSA strains. Graded-concentration antibiotic strips (M.I.C.E. strips; Oxoid) were used. The strips were used according to the manufacturer’s protocol. Simply, Muller-Hinton agar plates (MH) was used and 0.5 McFarland inoculum of the *S. aureus* isolated strains was swab-spread over the plate, then, M.I.C.E. strips were aseptically placed on the dried surface within 15 min. Plates were incubated at 37 °C for 24 h.
2.6. Statistical analysis

Data were analyzed based on descriptive statistical analysis using SAS software (SAS, 2006), and results were reported as mean value ± standard error of mean (SE).

3. Results and discussion

Counting and isolating S. aureus in food depends usually on culturing viable cells on Baird Parker medium (Wehr & Frank, 2004); although, many constraints have been associated with this medium, such as low selectivity and laborious preparation (Schoeller & Ingham, 2001). Another important limitation of Baird parker method, its inability to distinguish between thermonuclease positive and negative S. aureus strains, Thus, STX plates were used in this study to overcome all these shortages with Baird Parker medium, since STX plates offer the selective differentiation between the DNase producer and non-producer S. aureus strains, in addition to a more convenient applicability. While, regarding the performance more than standard Baird Parker medium (Viçosa, Moraes, Yamazi, & Nerso, 2010).

Results of S. aureus counts in tested products varied between each other (Table 1). Certainly, many factors are responsible for variations in prevalence and counting of food-borne pathogens, and mostly, the neglected hygienic practices were the main factors in case of S. aureus.

Out of 35 raw milk samples tested, nearly 94% (33 samples) were contaminated by CP S. aureus with an average count of 5.5 × 10^3 CFU/ml; for the karish cheese, 28 out of 30 (93%) samples tested were positive for CP S. aureus, with an average count of 6.2 × 10^5 CFU/g. On the other hand, CP S. aureus contamination for ice cream was 17 out of 30, (57%) with an average count of 8.9 × 10^3 CFU/ml. Many surveys and reports have been done concerning the prevalence of CP S. aureus in raw milk and dairy products. Makita, Desisa, Teklu, Zewe, and Grace (2012) remarked a high prevalence of S. aureus in raw bulk milk (43.5% of examined samples), and this value increased significantly at the raw milk collection centers (72.0% of examined samples), which was attributed to faulty handling. André et al. (2008) tested 24 samples of raw milk and 24 samples of minas frescal cheese (soft cheese) for the presence of CP S. aureus, and they reported that these samples were contaminated with CP S. aureus with a percentage of 66.7 and 70.8% respectively, which were slightly less than our results. Although, a higher prevalence in raw milk was reported by other researchers (Adesiyun, Webb, & Romain, 1998). However, several reports revealed higher values than ours in case of contaminated soft cheese (Araújo, Pagliares, Queiroz, & Freitas-Almeida, 2002; Rosengren, Fabricius, Guss, Sylven, & Lindqvist, 2010). In a recent report of incidence of CP S. aureus in Turkish cheese, 9.5% were contaminated with a mean log of 4.79 CFU/g (Yesim Can and Haluk Çelik, 2012); although, higher incidences were reported in other studies (Kuplülu, Sammelmetoglu, & Celik, 2004; Normanno et al., 2005). In another survey, 100% of examined soft raw milk cheese samples were contaminated by S. aureus strains which were confirmed by PCR for the presence of enterotoxin genes (sea, sed and sej), which were detected in many S. aureus isolates (Cremonesi et al., 2007).

On the other hand, reports concerning ice cream contamination with CP S. aureus have reported different percentages. Normanno et al. (2005) found that out of 350 examined ice cream samples, only 23 were contaminated by CP S. aureus. Warke, Kamat, Kamat, and Thomas (2000) studied the surveillance of CP S. aureus in packed and open retailed ice cream samples in India, and they found that 100% of both brands were contaminated with CP S. aureus with an average count of 2–3 log CFU/ml.

As a leading causative agent of bovine mastitis all over the world (Buzzola et al., 2001), S. aureus usually finds its way to bulk milk and in turn contaminates other raw milk products (André et al., 2008).

Hand Swabs of dairy workers and food handlers revealed high frequency of CP S. aureus colonizing their skins. 80% of hand swab sample were positive for CP S. aureus; consequently, they may constitute another sustainable source of CP S. aureus contamination of dairy products. As an evidence of the seriousness of hand contamination with CP S. aureus, toxic shock syndrome toxin 1 (TSST-1) gene was detected in S. aureus isolated from food handlers (Rapini, Cerqueira, Carmo, Veras, & Souza, 2005) and from milk of clinical and subclinical mastitis cases and from bulk tank milk (Takeuchi, Ishiguro, Ikegami, Kaidoh, & Hayakawa, 1998). On the controversy of our findings, a lower incidence of S. aureus isolated from dairy workers’ hands has been mentioned (Sospenda, Maehs, & Soriano, 2012).

From the aforementioned results, it can be clearly concluded that the hygienic qualities of examined raw milk and dairy products in this study were poor, in addition to poor personal hygiene, which might play an important role in toxin production (Rapo, 2002). MRSA were isolated from dairy products (Normanno et al., 2007). Even though, in this study, the detection of preformed enterotoxin in foods was not addressed, the numbers of recovered S. aureus in either of raw milk and dairy products were quite enough for toxin production (>10^6 CFU/ml or g.m, Tranter, 1990).

Regarding to MRSA identification, two identical CP S. aureus colonies from each positive sample were selected for MRSA identification, which was accomplished using mecA gene PCR detection. Routine epidemiological surveys usually choose only one colony for molecular identification of MRSA (Gouloumès et al., 1996); however, the selection of two colonies was adopted here to confer more credibility to the experiment. MRSA identification based on mecA gene detection is considered the best method for this purpose (Al-Ruais & Khiali, 2011; Aras et al., 2012; Normanno et al., 2007; Vanderhaegen et al., 2010) rather than phenotypic methods which have usually many errors (Oliveira & Lancaster, 2002).

Based on PCR amplification results, only 5 samples out of 95 tested yielded positive results for the mecA gene. Of the 5 isolates, 3 were from raw milk samples and 1 each from Karish cheese and ice cream samples. While, none of the CP S. aureus isolates from swabs of workers’ hands was positive for the mecA gene.

Reports regarding prevalence of MRSA in food and in particularly in milk and dairy products are generally scarce. Eleven MRSA strains were identified among 118 S. aureus previously identified as a causative agent of mastitis (Vanderhaegen et al., 2010). Another report revealed the detection of 2 MRSA strains among 42 S. aureus strains isolated from caprine clinical mastitis cases (Aras et al., 2012). Another study was able to isolate MRSA strains from raw goat’s milk and associated workers in Czech Republic (Stastkova, Karpiska, & Karpskova, 2009).

Considering MRSA identification in other food stuffs, many reports have identified its prevalence (retail chicken meats, Kwon et al., 2006; cage-cultured Tilapia, Atyah, Zamri-Saad, & Siti-Zahrah, 2010; retail meats and meat products, Hanson et al., 2011; Pu et al., 2009; Weese, Avery, et al., 2010).

Bulk tank milk analyzes concerning MRSA detection were yet very few; based mainly on either phenotypic or antibiotic susceptibility differentiation. While, Erskine, Walker, Bolin, Bartlett, and White (2002) and Makovec and Ruegg (2003) were able to detect MRSA in Bulk tank milk (with a limited frequencies), Anderson, Lyman, Bodeis-Jones, and White (2006) reported that out of 357 S. aureus strains isolated from raw milk at North Carolina, no strain has any resistance against oxacillin.
To check out the antibiotic resistance of isolated MRSA strains, two antibiotics (vancomycin and oxacillin) were chosen. Both antibiotics have been widely used as the drug of choice for most MRSA infections (Domaracki, Evans, & Venezia, 2000; Labrou et al., 2012). Graded antibiotic strips were used to determine the MIC of each antibiotic, which can be defined as “the lowest antibiotic concentration that will inhibit the visible growth of a microorganism after overnight incubation” (Andrews, 2001). Generally, no statistical differences for the minimum inhibitory concentration of oxacillin were found between the tested 5 isolates and it was clearly demonstrated that all detected MRSA strains in this study showed resistance to oxacillin (MIC were >256 µg/ml). 4 MRSA isolates were susceptible to vancomycin (MIC ranged from 0.06 to 0.12 µg/ml), however, only one MRSA isolate (isolated from raw milk) showed resistance against both oxacillin and vancomycin (MIC was >256 and >2 µg/ml, respectively).

Multidrug resistance of MRSA strains is not uncommon. MRSA strains isolated from Turkish Tulum cheese were found to have resistance to multiple antibiotics (Yesin Can and Haluk Celik, 2012; Sawant, Sordillo, and Jayarao, 2005) reported LA MRSA resistance against β-lactam, aminoglycosides and macrolides. Multidrug resistance by MRSA strains isolated from raw meat was also reported by Hanson et al. (2011). Moreover, different antibiotic resistances by S. aureus strains isolated from milk and dairy products were reported repeatedly (Arestrup, Wegener, & Rosdahl, 1995; Giannesschi, Concha, & Franklin, 2002; Lange, Cardoso, Senczek, & Schwarz, 1999; Tondo, Guimaraes, Henriques, & Ayub, 2002).

4. Conclusion

CP S. aureus high prevalence among tested raw milk, milk products and hand swabs highlighted the necessity of enforcement of hygienic implementations and practices within dairy facilities. Although, the experimental results showed a relatively low MRSA detection in raw milk and dairy products samples, they possibly represent major threats for transmission of this multidrug resistant pathogen. As a future work, subsequent molecular and ecological characterization of isolated MRSA strains should follow.

References


