Methicillin resistant *Staphylococcus aureus* (MRSA) in pastry cream products sold in Amol (Iran)

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Abstract:

BACKGROUND: Methicillin-resistant *Staphylococcus aureus* (MRSA) has been recognized as a matter of antibiotic resistance that is largely developed amongst common food-borne pathogens. MRSA is being considered as an important worldwide health threat and causes considerable concern to clinicians, food products manufacturers, governments and also consumers. **OBJECTIVES:** The objective of this study was to detect MRSA isolated from 360 samples of pastry cream products sold in the local markets in Amol, June 2016-May 2017, by plate count method and molecular technique. **METHODS:** The conventional plate counting method was conducted through inoculating appropriate dilutions of samples onto the Baired Parker Agar plates. MRSA isolates were detected by PCR method using mecA primers set. The resistance of isolated MRSA strains against some antibiotics was determined. **RESULTS:** Out of 360 pastry cream samples tested, 41.6% (150 samples) were contaminated by *S. aureus* with an average count of 4.94 log CFU/g in summer; 4.72 log CFU/g in autumn, 2.74 log CFU/g in winter and 3.62 log CFU/g in spring. Eleven samples out of 360 tested (3.05%) showed positive results for the mecA gene. No MRSA isolate was identified amongst winter samples. 56% of isolated strains showed sensitivity to oxacillin, 7% of isolates were sensitive to penicillin, 23 to ampicillin, 82% to gentamicin and 33% to tetracycline. **CONCLUSIONS:** According to the results, monitoring and improving the hygienic conditions of food production chain and educating food handlers and staff involved in food preparation is recommended in order to prevent MRSA prevalence.

Introduction

Staphylococcus aureus intoxication, caused through consuming different types of food including raw milk and dairy products (Jorgensen et al., 2005; Kamal et al., 2013), fast food sold in stands, retailed meat products (Lim et al., 2010; Lozano et al., 2009; Pu et al., 2009) and salad dishes, is considered as the third cause of food poisoning in the world (Asao et al., 2003). As the problem of antibiotic resistance of common pathogen bacteria is going to be worldwide, the serious health threat caused by meticillin-resistant *Staphylococcus aureus* (MRSA) is of increasing concern to clinicians, governments, public health care sectors and also the general public. During

the recent decade, MRSA has emerged as a significant life-threatening infective pathogen which does not respond to a wide range of antimicrobial agents (Kamal et al., 2013).

Regarding the high prevalence of *S. aureus* in food stuffs and food processing environments, several studies have investigated the presence of MRSA in several retail food products in different countries worldwide (Kamal et al., 2013; Lim et al., 2010; Lozano et al., 2009; Pu et al., 2009; Weese et al., 2010). In a study by Normanno et al. (2007) MRSA strains were detected in bovine milk and several cheese varieties in some parts of Italy. Also, Kamal et al. (2013) reported the prevalence of MRSA in raw milk and a number of dairy products (kariesh cheese and ice cream) distributed in the local markets and villages at Dakahlia province, Egypt. Caruso et al. (2016) indicated the prevalence of MRSA in sheep and goat bulk tank milk and also in the nasal part of people working on the positive farms in southern Italy. Although their results showed low prevalence of MRSA in sheep and goat milk, the detection of recognized zoonotic genotypes in milk of both farms and the isolation in people working in one of the positive farms of MRSA strains with a genetic profile identical to that of MRSA from milk, emphasizes the public health concern and highlights the need for additional surveillance including the detection of MRSA.

Furthermore, several food-borne acquired MRSA outbreaks have also been reported (Jones et al., 2002). Although the probability of transferring MRSA to human contaminated foodstuffs is considered low, contaminated food commodities can cause infection to community acquired MRSA (Herrera et al., 2016). The aim of this study was to evaluate the occurrence of MRSA isolated from pastry cream products sold in the local markets in Amol, collected June 2016- May 2017, via conventional methods and also molecular technique.

Materials and Methods

Sample collection: A total of 360 samples of pastry cream products were collected from 15 confectionaries of the local markets in Amol (Mazandaran province, Iran) during the period of June, 2016 through May, 2017. All samples were kept at 4-6 ºC in ice box and transferred to the microbiology laboratory, Food Hygiene Department, Amol University of Special Modern Technologies and analyzed within 2 h of collection.

Sample preparation: Decimal dilutions were prepared through homogenizing 25 g of samples with 225 ml 0.1% sterile peptone water (Merck) for 2 min using Syclon-04C Stomacher blender (Ningbo Sklon, China) (Wehr and Frank, 2004).

***Staphylococcus aureus* count and isolation:** Briefly, 1 ml of appropriate dilution was inoculated onto the Baired Parker Agar (Merck Co., Darmstadt, Germany) plates and the inoculums were evenly distributed with a sterile glass spreader. The inoculated plates were incubated aerobically at 37 ºC for 24 h. Black and shiny colonies with a fine white rim, surrounded by a clear zone were counted as S. aureus. Then, *S. aureus* colonies were picked up and confirmed by coagulase, catalase and thermonulease tests (Wehr and Frank, 2004).

MRSA molecular identification DNA extraction: DNA was extracted using Roche Tripure reagent (Roche Co., USA) and according to manufacturer's manual guide. Twenty-five grams of samples were

vortexed with 225 ml of 0.1% sterile peptone water in the stomacher (Stomacher 450, Seward Co., London, England) at ambient temperature for 2 min. The contents of the stomacher were filtered into centrifuge tubes using sterile filter papers and glass funnels. Resulting filtrate was centrifuged (5,000 × g for 5 min at 2-8 ºC), and DNA was extracted from the pellets with the following order using Roche Tripure reagent. Pellets from pastry products juice were treated with 1 ml of Tripure reagent several times by pippeting and incubated at 25 ºC for 15 min to degrade nucleoproteins. Then 0.2 ml chloroform was added, the mixture was shaken for 15 sec, incubated at 25 ºC for 5 min and centrifuged at 12,000 × g for 15 min at 2-8 ºC in order to obtain three separate phases. All the colorless upper aqueous phase obtained above was removed carefully and discarded. In order to precipitate the DNA from the interphase and red organic phase, 0.3 ml 100% ethanol was added and the microtube was inverted several times to mix thoroughly. The samples were incubated for 2 to 3 min at 25 °C to allow the DNA precipitate to form and then the sample was centrifuged at 2,000 × g for 5 min at 2-8 ºC. The supernatant (containing phenol, ethanol, and protein) was removed. To remove any phenol present in the DNA from each sample, 0.1 M sodium citrate in 10% ethanol was added to the pellet remaining in the centrifuge tube, the samples were incubated, with occasional mixing, for 30 min at 25 °C and next centrifuged at 2,000 × g for 5 min at 2-8 ºC. The supernatant was discarded (the whole step of treating with sodium citrate was repeated 3 times). DNA pellets were washed in 2 ml of 75% ethanol, incubated for 15 to 20 min at 25 °C and centrifuged at 2,000 × g for 5 min at 2-8 ºC. The supernatant was discarded. The excess ethanol was removed from each DNA pellet by air-drying. DNA pellets were dissolved in 8 mM NaOH to approach a DNA concentration of 0.2 to 0.3 µg/µl.

PCR reaction: MRSA isolates were detected using the following primers set: mecA-Fw (TGGCAGACAAATTGGGTGGT) and mecA-Rev (TGAAGCAACCATCGTTACGGA) which were generated by Primer-BLAST software using mecA gene sequence obtained from Gene Bank (National Center for Biotechnology Information; NCBI). Amplification (ABI PRISM 7,500 Sequence Detection System, Applied Biosystems Co., Courtaboeuf, France) was done with one cycle at 55 ºC for 2 min, initial denaturation and enzyme activation (hot start), one cycle at 95 ºC for 2 min, 40 cycles as follow: denaturation at 95 ºC for 15 s, annealing and extension at 60 ºC for 1 min and melting curve analysis at 70-95 ºC (temperature gradient: 1 ºC/min). *S. aureus* ATCC 29213 was used as positive control and S. epidermidis (locally isolate) as negative control in PCR (Kamal et al., 2013).

Resistance of MRSA against antibiotics: In this experiment, the resistance of isolated MRSA strains against penicillin (20 µg), ampicillin (20 µg), gentamicin (10 µg), tetracycline (30 µg) and oxacillin (1 µg) was determined. Gradient concentrations of antibiotics on discs were placed on Muller-Hinton agar plates inoculated with S. aureus. Plates were incubated at 37 ºC for 24 h (Kamal et al., 2013).

Statistical analysis: Data were analyzed based on descriptive statistical analysis applying the Statistical Package for Social Science Software 22 (SPSS version 22.0). Results were presented as the mean and the standard error of the mean (±SE).

Table 1. Count of *S. aureus* and MRSA positive samples in pastry cream products in Amol.

Sampling time	Number of samples	Number of positive samples (%)	Mean count ± SD (log CFU/g)	Number of MRSA positive samples (%)	MRSA count ± SD (log CFU/g)
Jun2016	30	16 (53.3%)	4.92 ± 3.51	2 (6%)	3.23± 2.00
Jul 2016	30	16 (53.3%)	4.94 ± 3.39	1 (3%)	3.07 ± 1.00
Aug 2016	30	18 (60%)	4.96± 3.70	3 (10%)	3.17 ± 2.20
Summer	90	50 (55.5%)	4.94 ± 3.55	6 (6.6%)	3.14 ± 2.14
Sep 2016	30	17 (56.6%)	4.90 ± 3.74	2 (6%)	3.07 ± 2.20
Oct 2016	30	13 (43.3%)	4.85 ± 3.65	0	-
Nov 2016	30	11 (36.6%)	3.80± 2.25	0	-
Autumn	90	41 (45.5%)	4.72± 3.54	2 (2.2%)	2.60 ± 1.30
Dec 2016	30	10 (3.33%)	2.77± 1.54	0	-
Jan 2017	30	7 (23.3%)	2.71 ± 1.44	0	-
Feb 2017	30	8 (26.6%)	2.74 ± 1.61	0	-
Winter	90	25 (27.7%)	2.74 ± 1.53	0	-
Mar 2017	30	9 (30%)	2.69 ± 1.59	0	-
Apr 2017	30	11 (36.6%)	3.82± 2.64	1	2.30± 0.69
May 2017	30	14 (46.6%)	3.74± 2.43	2	2.20 ± 0.00
Spring	90	34 (37.7%)	3.62 ± 2.38	3 (3.3%)	2.07 ± 0.30
Total	360	150 (41.6%)	-	11 (3.05%)	-

Results

Results of *S. aureus* counts in tested products are shown in Table 1. Out of 360 pastry cream samples tested during one year in this study, nearly 41.6% (150 samples) were contaminated by *S. aureus* with an average count of 4.94 log CFU/g in summer; 4.72 log CFU/g in autumn, 2.74 log CFU/g in winter and 3.62 log CFU/g in spring. The highest and lowest number of contaminated samples and also bacteria count was observed in summer and winter, respectively.

Counting MRSA positive samples using mecA gene, PCR detection showed that 3.05% of the whole samples contained MRSA. According to the PCR amplification results, only 11 samples out of 360 tested showed positive results for the mecA gene. No MRSA isolate was identified amongst winter samples.

In order to evaluate the antibiotic resistance of isolated MRSA strains, several antibiotics (penicillin, ampicillin, gentamicin, tetracycline and oxacillin) were used. These antibiotics are extensively used to treat most MRSA infections (Labrou et al., 2012). 56% of MRSA isolated strains in the present study showed sensitivity to oxacillin. 7% of MRSA isolates were sensitive to penicillin, 23% to ampicillin, 82% to gentamicin and 33% to tetracycline.

Discussion

Among several factors contributing to *S. aureus* contamination of food stuffs, poor hygienic practices are considered as the main factor. According to Petinaki and Spiliopoulou (2012), MRSA is considered as an important public health concern regarding its potential to contaminate foodstuffs of animal origin and to infect humans and animals. MRSA is known as a common cause of bovine mastitis and thus contami-

nated dairy products which can be considered vehicles of transmission of MRSA to humans (Mancini et al., 2015).

The present study reports the occurrence of MRSA in pastry cream products collected from confectionaries in local markets in Amol. As the results show, *S. aureus* was isolated from 41.6% of the samples and 3.05% of the whole samples were contaminated by MRSA. It reveals considerable prevalence of *S. aureus* in pastry cream products and seems aserious food safety hazard. In comparison to other studies, prevalence of MRSA in the present work is low. For instance, Basanici et al. (2017) evaluated the occurrence and the characteristics of MRSA isolated from 3760 samples of milk and dairy products in a previous survey conducted in southern Italy during 2008-2014; out of 484 *S. aureus* strains isolated, 40 (8.3%) were MRSA. Hererra et al. (2016) assessed a collection of 8 MRSA, isolated from samples of fresh cheese (Doble Crema) elaborated from raw cow milk in small dairies in Colombia. All the isolates harbored the mecA. The isolates belonged to the community-acquired MRSA group, suggesting a human source of contamination. Caruso et al (2016) investigated the prevalence of MRSA in sheep and goat bulk tank milk from southern Italy. MRSA was detected in 2 of the 162 (1.23%) bulk tank samples analyzed, the first from a sheep and the second from a goat. Although their results showed low prevalence of MRSA in sheep and goat milk, the detection of recognized zoonotic genotypes in milk emphasizes the public health concern and highlights the need for additional surveillance including the detection of MRSA.

In a study conducted by Riva et al. (2015), samples were collected from various dairy herds in the province of Milan (northern Italy). They found that the prevalence of *S. aureus* was 9.1% in raw milk. About half (45.7%) of the strains were enterotoxigenic, and 37.1% were resistant to at least one of the antimicrobial drugs tested. Seven (20%) of 35 isolates were identified as MRSA.

Kamal et al. (2013) reported the prevalence of *S. aureus* in raw milk, kariesh cheese, ice cream sold in the local markets and villages and also hand swabs of dairy workers at Dakahlia province, Egypt. The high prevalence and counts of detected coagulase positive *S. aureus* throughout all of the examined samples and swabs, reflected the neglected hygienic practices either in the production of raw milk and dairy products or in the personal hygiene. In this work, the isolates showed resistance to tetracycline, penicillin, and ampicillin. The whole MRSA isolated in a study by Hererra et al. (2016) were resistant to penicillin, ampicillin, cefoxitin and oxacillin and susceptible to the non-β-lactams antibiotics tested. Also, 20% of MRSA isolated by Kamal et al. (2013) showed resistance against both oxacillin and vancomycin, while other isolates showed resistance against oxacillin alone. Hosseini Jazani and Babazadeh (2012) evaluated the prevalence of enterotoxigenic and methicillin resistant *S. aureus* in cream filled pastries in Urmia (Azarbayjan, Iran). They isolated *S. aureus* from 15% of the samples and all the isolates were susceptible to oxacillin. 40% of the isolates were enterotoxigenic. The resistance to oxacillin in our work was 56% that is a very critical hazard in public health. The highest antibiotic resistance amongst the isolates was observed against penicillin, rifampin and teicoplanin. In this study, the same result was found about resistance to penicillin.

As seen in Table 1, *S. aureus* count was higher in warm-weather months of the year; it may be due to the providing optimum temperature for *S. aureus* growth in warm months. There are some studies about the prevalence of *S. aureus* infection in relation to its seasonality, with higher incidence of infection in the summer, end of the spring and beginning of the autumn or in warmer months reported in all studies. Early studies of seasonality of S. aureus originated from tropical countries where conditions such as impetigo are seen more frequently (Kakar et al., 1999; Leekha et al., 2012). Two studies of acute diarrheal illness (performed in the same population in South Korea during different time periods) reported more cases in the summer (Cho et al., 2006; Cho et al., 2008). The prevalence of MRSA strains (3.05%) among the strains isolated from pastry cream products shows the pathogen has entered into the food chain and should be considered as a potential consumer health risk. Therefore, it is recommended to monitor and improve the hygienic conditions of food production chain and educate food handlers and staff involved in food preparation.

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مجله طب دامی ایران، ۱۳۹۷، دوره ۱۲، شماره ۲، ۱۷۳-۱۶۷

شیوع استافیلو کوکوس اورئوس مقاوم به متیسیلین در محصولات قنادی خامه‌ای عرضه شده در شهرستان آمل (ایران)

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چکیده

زمینه مطالعه: استافیلو کوکوس اورئوس مقاوم به متیسیلین (MRSA) یکی از موارد حائز اهمیت در بحث مقاومت آنتی بیوتیکی است که به میزان قابل ملاحظه‌ای در بین باکتری‌های بیماریزای غذایی شایع در حال گسترش می‌باشد. MRSA تهدیدی برای بهداشت جهانی به شمار می‌رود و موجب بروز نگرانی در جامعه پزشکان، تولید کنندگان مواد غذایی، دولت‌ها و نیز مصرف کنندگان گردیده است. **هدف:** هدف از این مطالعه بررسی شیوع استافیلو کوکوس اورئوس و MRSA جدا شده از ۳۶۰ نمونه محصولات قنادی خامه‌ای عرضه شده در شهرستان آمل، از خرداد ۱۳۹۵ الی تیر ۱۳۹۶، به روش کشت معمولی و روش مولکولی بود. **روش کار:** روش متداول کشت در پلیت از طریق تلقیح مقدار مناسب از رقت‌های نمونه‌ها روی محیط برد پارکر آگار انجام شد. جدایه‌های MRSA به روش PCR با استفاده از پرایمرهای اختصاصی mecA شناسایی شدند. مقاومت جدایه‌های MRSA در برابر چند آنتی بیوتیک متداول نیز تعیین گردید. **نتایج:** از ۳۶۰ نمونه محصولات قنادی خامه‌ای مورد بررسی ۴۱/۶٪ (۱۵۰ نمونه) آلوده به استافیلو کوکوس اورئوس بودند، میانگین شمارش در تابستان ۴/۹۴ log CFU/g، در پائیز ۴/۷۲ log CFU/g، در زمستان ۲/۷۴ log CFU/g و در بهار ۳/۶۲ log CFU/g بود. یازده نمونه از ۳۶۰ نمونه مورد بررسی (۳/۰۵٪) حامل ژن mecA بودند. ۵۶٪ از جدایه‌ها به اگزاسیلین، ۷٪ به پنیسیلین، ۲۳٪ به آمپی سیلین، ۸۲٪ به جنتامایسین و ۳۳٪ به تتراسایکلین حساس بودند. **نتیجه‌گیری نهایی:** مطابق یافته‌ها، اعمال کنترل و ارتقای بهداشت در زنجیره تولید مواد غذایی و آموزش بهداشت به کارکنان و افراد در تماس با مواد غذایی جهت جلوگیری از شیوع MRSA توصیه می‌گردد.

واژه‌های کلیدی: آنتی بیوتیک، مقاوم به متی سیلین، محصولات قنادی، واکنش زنجیره‌ای پلیمراز، استافیلو کوکوس اورئوس

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