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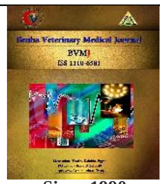

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Original Paper

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 3.16×10^5 and 6.57×10^4, respectively. For white cheese samples, they were 7.60×10^4 and 5.12×10^4, respectively. For Roomy cheese, samples were 5.79×10^4 and 3.38×10^4, respectively. For Flamingo cheese samples were 2.96×10^4 and 1.05×10^4, respectively. For machine swab samples were 1.02×10^4 and 1.48×10^3, respectively. For hand swab samples were 1.43×10^4 and 3.71×10^3 (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, oxytetracycline, cefotaxime and streptomycin. Meanwhile, it was highly sensitive to vancomycin followed by norfloxacin, gentamicin, meropenem and ciprofloxacin. 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 Qalubuiya 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-utmost 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). Methicillin-resistance *Staph. aureus* (MRSA) strains, *mec*A positive strains should be reported as oxacillin- resistant (CLSI, 2018). It is mainly referred to the activity of the specific *mec*A gene, present on Staphylococcal genome *mec* cassette (SCCmec), that translated to penicillin-binding protein 2a (PBP2a) leading to decrease its affinity essentially for all ß-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

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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 Qalubiya Governorate

2. MATERIAL AND METHODS

2.1. Collection of Samples

Hundred and eighty random samples include verities 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 Kaliobia 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 37ºC 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 Snedecor and Cochran (1969) using SPSS software program Differences in mean of analyzed data were considered significant at $P\leq0.05$.

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 ($P\leq0.5$) 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 swabs 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	Positive		Min.	Max.	Mean ±SEM**
	No.	%*			
Kareish cheese	25	83.3	2.3×10^4	4.5×10^6	$3.16\times10^5 \pm0.18 \times10^{5a}$
White cheese	23	76.7	1.5×10^4	3.4×10^5	$7.60\times10^4 \pm0.19 \times10^{4ab}$
Roomy cheese	17	56.7	1.3×10^4	3.9×10^5	$5.79\times10^4 \pm0.23\times10^{4ab}$
Flamingo cheese	16	53.3	1.2×10^4	1.2×10^5	$2.96\times10^4 \pm0.07 \times10^{4ab}$
Machine swabs	17	56.7	4.5×10^3	2.3×10^4	$1.02\times10^4 \pm0.08 \times10^{4b}$
Hand swabs	20	66.7	5.8×10^3	2.0×10^4	$1.43\times10^4 \pm0.02 \times10^{4b}$
Total	118	65.6	4.5×10^3	4.5×10^6	$9.79\times10^4 \pm0.39 \times10^4$

***** 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	Positive		Min. **	Max. **	Mean ±SEM***
	No.	%*			
Kareish cheese	12	40.0	9.4×10^3	2.6×10^5	$6.57\times10^4 \pm0.20 \times10^{4a}$
White cheese	11	36.7	8.0×10^3	1.9×10^5	$5.12\times10^4 \pm0.18 \times10^{4ab}$
Roomy cheese	8	26.7	4.3×10^3	8.8×10^4	$3.38\times10^4 \pm0.12\times10^{4abc}$
Flamingo cheese	7	23.3	1.5×10^3	5.1×10^4	$1.05\times10^4 \pm0.07 \times10^{4bc}$
Machine swabs	8	26.7	1.5×10^2	4.5×10^3	$1.48\times10^3 \pm0.06 \times10^{3c}$
Hand swabs	9	30.0	3.7×10^2	6.3×10^3	$3.71\times10^3 \pm0.07 \times10^{3bc}$
Total	55	30.6	1.5×10^2	2.6×10^5	$3.17\times10^4 \pm0.07 \times10^4$

***** 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, meropenem, norfloxacin, and vancomycin. The methicillin-resistance *Staph. aureus* (MRSA) strains, *mec*A 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.	Staphylococcus positive		Coagulase positive Staph. aureus strains		coagulase negative strains	
		No.	%*	No.	%*	No.	%*
Kareish cheese	30	25	83.3	12	40.0	13	43.3
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). **Percentage in relation to total number of the examined samples (180).

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-part4 for Roomy cheese. EOS 1007:2005-part2 for Flamingo cheese. EOS 1008:2005-part4 for Kareish cheese. EOS 1008:2005-part5 for white cheese

Table 5 In-Vitro anti-microbial 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: Antibiogram activity. R: Resistant. S: Sensitive. IS: Intermediate

Table 6 Incidence of methicillin resistant *Staph. aureus* strains on ORSAB agar

Samples	No.	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.

4. DISUCSSION

Dairy products are common vehicle of foodborne illness, Staphylococci mainly *Staph. 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 enerotoxins 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 recorded 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 *mec*A gene on *Staph. 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 micro-organisms 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, *mec*A 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 *mec*A gene.

5. CONCULOSIONS

The recorded results concluded that *Staph. aureus* and MRSA strains were detected in the examined cheese samples that in cheese sold in markets in Kaliobia 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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