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RESEARCH ARTICLE

Prevalence of *Staphylococci* in Commercially Processed Food Products in Karachi-Pakistan

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ABSTRACT

Objectives: This study was designed to determine the prevalence of Staphylococci, Staphylococcus aureus (*S. aureus*), and Methicillin Resistant *S. aureus* (MRSA) in commercially prepared food items that involve human handling, in Karachi-Pakistan.

Methods: In a cross-sectional survey approach, in total 1012 food samples were analyzed. These samples were provided by different food processing industries during 2013 to 2016. Barid-Parker agar plates with egg yolk tellurite (Oxoid) along with Mannitol Salt Agar (BioM), Staph-chromo agar (Merck), Staphylococcus 110 Agar (BioM), and Blood Agar (Oxoid) were used for isolation and identification of Staphylococci. Polymerase chain reaction (PCR) was used for amplification of *mecA* gene and specie identification via 16s RNA.

Results: Total 723 samples (71.4%) showed the presence of Staphylococci. Out of 723 staphylococcal isolates, 367 (36.2%) were *S. aureus* and 85 (8.3%) isolates were confirmed as MRSA. Molecular studies for MRSA typing showed that most of the isolates (74.1%) belong to *SCCmecA* IV and 20% MRSA isolates were *SCCmecA* type II and 5.8% isolates carried *SCCmecA* type III. The MRSA isolates of *SCCmecA* type II belong to agr type I, while majority (67%) of the isolates carry *agr* type II.

Conclusion: This study suggested that majority of MRSA isolates recovered from commercial food items belongs to *SCCmecA* IV and human handling is a major factor for introduction of staphylococci in processed food items. *J Microbiol Infect Dis 2017; 7(2): 83-87*

Keywords: *Staphylococci, S. aureus*, Methicillin Resistant *S. aureus*

INTRODUCTION

Staphylococci are gram positive bacteria, known to survive under a wide range of environments e.g. on dry surfaces, high salt concentration and hospital set-ups [1,2]. *Staphylococci* exist in air, dust, sewage, water, milk, and food or on food equipment, environmental surfaces, humans, and animals. Humans and animals are the primary reservoirs [1]. Mostly, it colonizes persons in hospital as well as community set-ups and is transmitted via person to person contact as well as through inanimate objects [2]. The food borne cases of *Staphylococci* have been reported from all over the world [2]. Presence of pathogens in food products imposes potential hazard for consumers and causes grave economic loss via food-borne disease [3]. *Staphylococci* enter in a food set-up and contaminate the food products via human handlers or healthy nasal carriers. After entry, it multiplies in food and produce toxins that can cause food poisoning, a gastrointestinal illness [2,3]. Food items made by hands without cooking are at high risk to carry Staphylococcal toxins. However air, dust, and food contact surfaces may also serve as vehicles in the transfer of *Staphylococci* in foods. *Staphylococcus aureus* is one of the well-known pathogen of Staphylococci family [1-3]. It tolerates high salt concentration; desiccation and can grow in a wide range of temperatures (7 °C to 48.5 °C; optimum 30 to 37 °C), pH (4.2 to 9.3; optimum 7 to 7.5). These characteristics favor growth of the organism in many food products [3]. The highly resistant and pathogenic

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strain among Staphylococci is Methicillin Resistant *S. aureus* (MRSA). Originally, MRSA was found in hospital set-ups; in recent times it is frequently reported in the community as well, and considered as a source of food borne illness [4]. Food borne MRSA isolates are resistant to penicillin and oxacillin, only, whereas hospital isolates of MRSA are multi-drug resistant hence are difficult to control [4]. In *S. aureus* Methicillin resistance is mediated by *mecA* gene, which is located on mobile genetic element known as staphylococcal cassette chromosome *mec* (*SCCmec*). There are five major types of *SCCmec* elements (I-V). The majority of hospital-acquired MRSA strains carry *SCCmec* types I, II, or III, whereas community-acquired MRSA strains carry *SCCmec* types IV or V [8,9]. World Health Organization (WHO) defines food-borne disease as “disease of infectious or toxic nature caused by the consumption of food or water” [3]. *S. aureus* is one of the major pathogens responsible for food borne infections, world-wide. Present study describes the prevalence of Staphylococci, *S. aureus* and MRSA in different food items that involve human handling.

METHODS

In present study total 1012 food samples were analyzed. These samples were provided by different food processing industries during 2013 to 2016.

Isolation and Enumeration of Staphylococci and *S. aureus*

Fifty grams of each sample were mixed with 450 ml of 0.1% peptone sterile physiological saline solution (0.85% NaCl) and homogenized. Three decimal dilutions of each sample homogenate were prepared for enumeration of *S. aureus*. From each dilution, 0.3, 0.3 and 0.4 ml was spread on Barid-Parker agar plates with egg yolk tellurite and were kept in an upright position until liquid was absorbed and then incubated at 35 °C for 24–48 h. Typical black colonies with white zone were considered as *S. aureus* [5]. Staph Latex Kit (Prolix Latex Agglutination System) and growth on Mannitol Salt Agar (BioM), Staph-chromo agar (Merck), Staphylococcus 110 Agar (BioM), and Blood Agar (Oxoid) was used for further confirmation.

Determination of plasma-coagulase and nuclease activity

Colonies of staphylococci were individually re-inoculated into test tubes containing 1.5 mL of Brain Heart Infusion Broth (BHI Broth, Oxoid). After 24h of incubation at 35°C, 0.5 mL of each sample was added to another test tube containing 1 mL rabbit plasma (Merck). Inoculated test tubes were incubated at 35°C. Formation of coagulum was considered as positive reaction. Results were evaluated after 1, 2, 3, 6 and 24 h. Each isolate was inoculated on the surface of DNase Agar (Oxoid) and was incubated at 35°C. After 24h of incubation, medium was flooded and acidified with 1 N hydrochloric acid, the DNA precipitated the turbidity of medium and clear zones around colonies indicated positive DNase reaction. The number of isolates that presented positive and negative results was recorded [10].

Determination of MIC for oxacillin

BHI Agar (Oxoid) was used to measure the oxacillin resistance level according to the guidelines of Clinical Laboratory Standard Institute (CLSI) [6]. Minimum inhibitory concentration (MIC) was re-confirmed by E-test using AB-Biodisk according to the manufacturer's instructions.

Polymerase chain reaction (PCR)

For molecular studies, genomic DNA was isolated by using the DNase Kit (Qiagen), following the manufacturer's instructions. PCR amplification of *mecA* genes was performed with an MWG Thermal Cycler in a volume of 50 µl of Promega Master Mix. Primers, described previously [6], were used for amplification of *mecA*, for *SCCmec* typing of MRSA isolates and for *agr* allele (I-IV) typing. 16S RNA was used as internal control for gene expression and specie identification.

Ethical approval

This study was approved by the institutional ethics committee.

RESULTS

Present study describes the presence of Staphylococci in different food products. Specially, foods that require considerable handling during preparation and that are kept at slightly elevated temperatures after preparation

are selected and included in this study (Table 1). Total 1012 samples were analyzed, 723 (71.4%) samples showed the presence of Staphylococci (Table 1, Figure 1).

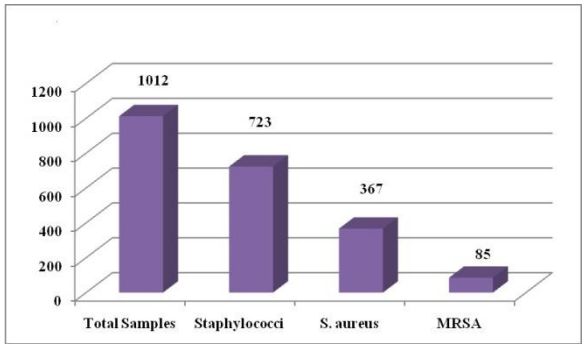

Figure 1. Isolation and identification of MRSA from food samples.

Out of 723 staphylococcal isolates, 367 (36.2%) were *S. aureus* and 85 (8.3%) isolates were confirmed as MRSA (Table 1, Figure 1). These isolates were identified on the basis of growth characters on selective and differential media. An isolate was considered within Staphylococcus genus when presented as gram-positive cocci in grape-like clusters, catalase, and mannitol fermentation positive. Coagulase test and growth on Baird-Parker agar and DNase agar confirmed the presence of *S. aureus* in subject food items. Positive reaction or amplification of 16s and *mecA* gene specific primers confirm the presence of MRSA isolates in 85 food items. Out of 1012 samples 338 were spices mix; 80.77% of these samples showed the presence of staphylococci. The pathogenic *S. aureus* were present in 43.79% of spices mix samples with 5.03% of MRSA isolates. Among candy samples, 80.95% were positive for Staphylococci; 23.81% of these were *S. aureus* and 9.52% were MRSA. In fish and meat products, 40% and 87% showed Staphylococcal growth, respectively. In fish products *S. aureus* were 28% with 16% MRSA and in meat products 39% *S. aureus* with 7% MRSA. Samosa and Paratha (Frozen products) showed *Staphylococci* in 55% and 64% of samples, respectively. In Parathas 30.67% isolates were identified as *S. aureus*, in Samosa the *S. aureus* were 45%, with 18.67% and 10%, respectively MRSA. Rice and lentils respectively, showed 40.37% and 27.5% *S. aureus* with 9.17% and 7.5% MRSA. About 20% samples of Formula Milk and 10% of Mayonnaise samples were positive for MRSA. Table 1 depicts the complete details of samples and isolates. Moreover, molecular studies for MRSA typing showed that most of the isolates (74.1%) belong to *SCCmecA* IV and 20% MRSA isolates were *SCCmecA* type II and 5.8% isolates carry *SCCmecA* type III. Interestingly, isolates recovered from formula milk and mayonnaises were *SCCmecA* type II. The isolates that carry *SCCmecA* type III were recovered from meat products and candy samples. The MRSA isolates of *SCCmecA* type II belong to agr type I, while majority (67%) of the isolates carry agr type II.

Table 1. MICs and typing of *SCCmecA* genes and agr in MRSA isolated from different food products.

Source	No. of Samples	Staphylococci, n (%)	*S. aureus*, n (%)	MRSA, n (%)	MICs (µ/ml)			AGR Typing		*SCCmecA* Typing		
					32	64	128	I	II	II	III	IV
Candies	105	85 (80.95)	25 (23.81)	10 (9.52)	2	4	4	2	8	2	2	6
Candy mix	50	40 (80.00)	19 (38.00)	4 (8.00)	0	4	0	0	4	4	0	0
Dates	85	40 (56.25)	17 (20.00)	6 (7.06)	2	4	0	0	6	0	0	6
Fish Products	25	10 (40.00)	07 (28.00)	4 (16.00)	0	0	4	0	4	0	0	4
Formula Milk	15	10 (66.67)	08 (53.33)	3 (20.00)	0	0	3	3	0	3	0	0
Lentils	40	19 (47.50)	11 (27.50)	3 (7.50)	2	1	0	1	2	0	0	3
Mayonnaise	30	12 (40.00)	08 (26.67)	3 (10.00)	0	3	0	3	0	3	0	0
Meat Products	100	87 (87.00)	39 (44.83)	7 (7.00)	2	5	0	3	4	0	3	4
Paratha	75	48 (64.00)	23 (30.67)	14 (18.67)	4	7	3	6	8	3	0	11
Rice	109	77 (70.64)	44 (40.37)	10 (9.17)	0	5	5	2	8	0	0	10
Samosa	40	22 (55.00)	18 (45.00)	4 (10.00)	4	0	0	4	0	2	0	2
Spices Mix	338	273 (80.77)	148 (43.79)	17 (5.03)	17	0	0	0	17	0	0	17

DISCUSSION

In the present study, commercial food items, those involve human handling, during processing and packaging, showed high rate of staphylococcal contamination, including *S. aureus* and MRSA. In present study it has been observed that food handlers are the major

source of staphylococcal contamination. Although, the original source for staphylococcal introduction to food items was not traced out, even then this study creates doubt about food safety and highlights influence of food handling and processing on consumer health. Of more concern is the presence of MRSA; out of 1012 samples 367, (36.2%) were contaminated with *S. aureus* and 85 (8.3%) with MRSA. This is a very serious situation. Due to multi-drug resistance and enterotoxins production, MRSA could be more fatal as compared to methicillin sensitive *S. aureus*. According to Jones at al [7] MRSA are as likely to produce enterotoxins as are methicillin-sensitive strains. Normally, it is considered that MRSA can survive in limited food items e.g. dairy products, meat and through cross contamination during kitchen processing of the recipe. However, during present study, recovery of MRSA from different variety of foods e.g. Lentils, Rice, Spices mix, Sweet mix and Dates, suggest the versatility of this pathogen. Moreover, majority of these isolates exhibited low-level oxacillin resistance (MIC ranges 32 to 128µg/ml), a character of the community of MRSA. According to Boyle-Vavra et al. [8], community-acquired MRSA (CAMRSA) isolates usually carry *SCCmec* type IV. Out of 87 subject isolates of MRSA, 63 (72.4%) belong to *SCCmec* type IV. According to Song et al. [9] *SCCmec* type IV is the most predominant community type clone in the Asian countries. Interestingly, MRSA that belongs to *SCCmec* type II was recovered from sweet products only e.g. candies, mayonnaise, and formula Milk; these products involve more human handling as compared to the other products tested. The *SCCmec* type IV normally belongs to community acquired isolates of MRSA and *SCCmec* type II is associated with hospital acquired isolates. It is reported that *SCCmec* type II isolates are highly resistant type of MRSA, whereas *SCCmec* type IV exhibited low-level of resistance and are only resistant to ß-lactam antibiotics; as noticed in the present study. Although, this study is based on food items that involve human handling, but the true source of MRSA remains to be elucidated. Of more concern is to determine how these isolates of *S. aureus* develop oxacillin resistance. According to best of our knowledge, in Pakistan the use of antibiotics in agriculture is not common. However, use of antibiotics as a growth promoter in poultry industry is a common practice but good data are not available. So, this study which is based on wide range of samples provides us intimation about the prevalence rate of MRSA is our community.

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Competing interests:

None to declare.

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