Staphylococcus aureus Contamination during Food Preparation, Processing and Handling

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Abstract—Throughout the world, food processing and handling is a major problem leading to food poisoning and infection. A total of 480 samples was analyzed for Staphylococcus aureus contamination which resulted from food processing. Most of the isolates were taken from food-handlers using nasal swabs. The most contaminated food was chicken pastries, followed by egg sandwiches and spring rolls. Isolates from all samples produced virulence factors hemolysin, coagulase, DNase and enterotoxins. Five different enterotoxins (SEs) were isolated and identified from different samples. The detected SEs are SEA to SEE. Most the isolates secreted SEA followed by SEB. The strains were multiple-resistant to several antibiotics. Ampicillin and penicillin were the most resisted followed by SEB. The strains were multiple-resistant to several antibiotics. The value of this investigation is to generate awareness about the dangers of food processing and handling leading to infections by foodborne microbes which constitute a potential health risk for the consumers.

Index Terms—Staphylococcus aureus, contamination, food products, food handlers.

I. INTRODUCTION

Food processing is an important industry worldwide. One of the major problems threatening food industry is the contamination with foodborne microbes of human origin resulting from improper handling and processing. Microbial contamination reduces shelf life and food quality leading to food infection and poisoning outbreaks, some of which are life threatening. Continuous monitoring of food processing is essential to avoid potential health problems.

Staphylococcus aureus is one of the major foodborne pathogens, frequently causing diseases globally as a result of food ingestion contaminated with staphylococcal toxin [1].

S. aureus is characterized by its ability to produce enterotoxins [2], which are considered to be the main causative agents of staphylococcal food poisoning [1]. S. aureus are commonly found on the skin of mammals, birds and fomites [2]. Humans are considered to be the major source of staphylococcal food poisoning [3]. S. aureus is found in nasal passages, throat, hair and skin of carriers [2]. Food is usually contaminated from nasal secretions, sneezing, coughing and direct hand contact of infected carriers [4], [5].

Three categories of S. aureus carriers have been recognized: persistent, intermittent (occasional) and never-carriers. Persistent carriers are infected with the same staphylococcal strain for months or even years. It was reported that 20% - 35% are persistent carriers. In addition, intermittent carriers represent 40% - 70%, while never-carriers are uninfected representing 10% - 40% of the population [6], [7].

S. aureus secretes several virulence factors and extracellular toxins of protein origin which contributes to the pathogenicity. It is the only species that produces beta hemolysin which lyse red blood cells at cold temperature [6].

Unlike other foodborne illnesses, staphylococcal food poisoning occurs shortly after, 30 min to 8 hrs, food ingestion contaminated with enterotoxin. Several symptoms result from SEs ingestion, which include abdominal cramping, vomiting, diarrhea, nausea, and chills. Infected individuals usually recover from the toxicity within 24 - 48 hrs [1].

SEs are pyrogenic toxins of the superantigen family due to their structural relatedness and their biological activities [8]. SEs are heat-stable at higher temperatures [2]. Serologically the toxins are classified into many types. The most common types are SEA, SEB, SEC,SED and SEE [9]. Other studies reported the existence of other types, such as SEG, SEI, SEH, SEK, SER and SET [1], [10]-[12].

Although the physiological stresses that stimulate secretion of SEs are not well understood, production of SEs occurs as a result of specific environmental stimulants present in food. Appropriate temperature, NaCl concentrations and pH are important factors affecting both growth and secretion of S. aureus enterotoxins [13], [14]. For example, growth of S. aureus declined with lower pH values [14]. Moreover, S. aureus can tolerate high osmotic sugar and salt concentrations.

SEs stability in high temperature has been well established. Temperature used in cooking is insufficient to destroy the SEs even after the microbe has been eradicated in food by cooking. The SEs in food are difficult to distinguish due to its lack of taste and food appearance [2].

Pathogenicity of S. aureus is related to its ability to produce several virulence factors. Most of staphylococcal virulence factors are exoproteins which include several types of superantigens and cytolysins as well as antibiotic resistant factors such as β-lactamases [15]. More than twenty staphylococcal exotoxins are classified as superantigens which cause a variety of human diseases including food poisoning SEs and enterotoxin-like proteins [16], [17]. The superantigens activate several immune cells including dendritic cells, T lymphocytes, antigen-presenting cells, and macrophages [16]. Hemolysin, DNase and coagulase are factors usually associated with SEs secretion [18], [19].

DOI: 10.7763/IJCEA.2014.V5.415

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Overuse of antibiotics has been reported to be the major factor for the emergence of antibiotic resistant bacteria affecting both human health and the environment [20]-[22]. Antibiotics have been used for treatment of human and animal diseases indiscriminately [23]-[25] and are also used as prophylactic in animal growth [23]. Several investigators reported the emergence of antibiotic-resistant *S. aureus* specifically carbapenems, cephalosporins, linoglycosides, macrolides, penicillin, and streptogramins. Resistant strains of *S. aureus* for penicillin, streptomycin, erythromycin and tetracyclin are increasing significantly in the last decades [26], [27].

Although it was reported that the elimination of nasal *S. aureus* by 63% with mupirocin, the emergence of resistant strains to this antibiotic from infected individuals is possible [28]. The increase of mupirocin resistant *S. aureus* as an attempt to eliminate mexitillin resistant strains [29].

The aim of this study is to analyze the frequency of infection among food handlers and to relate *S. aureus* virulence factors, such as SEs, blood hemolysis, DNase and antibiotic resistance to food industries and food handlers.

II. MATERIALS AND METHODS

A. Selection of Samples

The selected samples were taken at random from food handlers. A total of 120 samples were collected from food handlers nasal swabs. The swab samples were inoculated on mannitol salt agar MSA and Baird-Parker agar (BP) [30]. The plates were incubated at 37°C for 24 hours. Colonies with typical *S. aureus* morphology were selected and biochemically identified by Analytical Profile Index (API) strips, (USA) for *Staphylococcus* species.

B. Selection of Food Samples and Detection of SEs

Three hundred and sixty samples from three types of food: 120 spring rolls (Sr), 120 sambosa or chicken pie (Chkp) and 120 egg sandwiches (Egs) were analyzed for the presence of *S. aureus*. Ten grams of sample foods were transferred to a sterile stomacher bag and 90 ml of ringer solution was added [2], [31]. The sample was homogenized in a stomacher (Lab. Lemco 400) for 1 min. 1.0 ml from the supernatant was inoculated into salt-meat broth (enrichment media) and incubated at 37°C for 24 hrs. A loopful from the enrichment media was streaked onto MSA and BP. The plates were incubated at 37°C for 24 hrs. Colonies with typical *S. aureus* morphology were identified biochemically using API strips.

*S. aureus* isolates were further analyzed for their virulence factors in relation to their SE production following the standard bacteriological methods [30]. For hemolysis reaction, the isolates were grown on blood agar and incubated for 24 hr at 37°C. Their ability to hemolyze blood cells was determined. For DNase reaction, bacteria were grown on DNase agar (Oxoid, UK) and incubated for 24 hr at 37°C. A concentration of 1 N HCl was added to the plates. A clear zone around the colonies was considered a positive reaction. The coagulase reaction was done by using the Stphytect Plus test (Oxoid DR 595) [2].

The isolates were screened for *S. aureus* SEs by using an ELISA kit according to the manufacturer recommendations (TECRA International Pty Ltd, Australia).

C. Resistance of *S. aureus* to Antibiotics

All nasal swab and food sample isolates were screened for thirteen antibiotics using antibiotic susceptibility disk diffusion assay [32]. DST agar (Oxoid, UK) was used. The following antibiotics disks were used: ampicillin 10μg (Amp), chloramphenicol 30μg (C), clindamycin 2μg (DA), erythromycin 15μg (E), gentamycin 10μg (CN), methicillin 5μg (Meth), mupirocin 20μg (Mup) neomycin 30μg (N), penicillin 10μg (P), streptomycin 10μg (S), tetracycline 30μg (TE), tobramycin 10μg (Tob) and vancomycin 30μg (VA).

*Staphylococcus aureus* NCTC 6571 was used as a control. The samples were incubated at 37°C for 24 h and the inhibition zones were measured.

III. RESULTS

A total of 480 samples was used to isolate *S. aureus* from Ns, Sr, Chkp and Egs. Most of the isolates (66.2 %) were from Ns, Sr (35.7%), Chkp (39.3%) and Eggs (28.7%) (Fig. 1).

The enterotoxin producers from Ns samples (30.3%), Sr (38.3%), Chkp (56.1%) and Egs (49.5%) (Fig. 2).

Five types of enterotoxins were analyzed. All the 5 types were secreted by the isolates. SEA showed the highest production (48.9%) followed by SEB (43.9%), SEE (18.7%) SED (10.5%) and SEC (6.9%) (Fig. 3).

The SE types in Sr were as follows: A (48.4%), B (36.1%), C (3.3%), D (1.3%) and E (10.9%). Chkp samples were less contaminated with A (51.3%), B (30%), C (1.2%), D (11.2%) and E (6.3%). On the other hand, the Egs samples were contaminated with A (41.8%), B (40.6%), C (3.5%), D (2.2%) and E (6.5%) (Fig. 4).
The relation of enterotoxin secretion to other virulence factors was analyzed. All of the enterotoxin secreting strains were also positive for hemolysin and DNase enzymes. However, not all strains secreted coagulase enzymes. 92.1% were coagulase positive (Fig. 6).

The highest resistant was Amp (86.3%) followed by P (85.93%) and Te (61%). Only 15.5% were resistant to Meth. resistance to Tob was the lowest (4.93%) (Fig. 7).

Among the resistant strain, about 40.2% had multiple resistance to two antibiotics. Resistance to one antibiotic was 10.3%. Multiple resistance to 3 antibiotics (13.1%), 4 (8.4%) and 5 (1.9) (Fig. 8).

IV. DISCUSSION

In this study most of the isolates were from nasal swabs. During the last decades several studies were conducted on *S. aureus* in which nasal carriage was the major cause of wound and surgical infections accounting for 40-100% of staphylococcal infections [33]. In the present investigation it was focused on processed food contamination.

Food handlers were considered to be the main sources of contamination and the cause of staphylococcal food poisoning outbreaks. Food processing cookware was also the source of staphylococcal contamination [2].

In this study, among the tested food handlers, 34% were asymptomatic *S. aureus* carriers. The percentage of *S. aureus* carriers among the populations differs from one to another. It is well established that nasal carriers of *S. aureus* probably originated from staphylococcal infections [6]. The nasal carriage rate of *S. aureus* in adult populations has been
reported to be between 30 to 50%, some of which are SE producers [7]. It was reported that S. aureus isolated from animals are different from humans biotypes [34]. The human biotypes were isolates from nasal passages with the highest proportion of SE secretion. In addition it was reported that the source of SEA producing S. aureus was mainly from humans and the source for the isolates producing SEC were from animals [35]. This suggests a possible transmission of S. aureus biotypes from animals to humans and vice versa.

In this study, most of food handlers were carriers of S. aureus. The most contaminated food was chicken pastries and egg sandwiches, thus, these processed foods were considered to be the most common vehicle for S. aureus due to substantial human handling during processing and preparation responsible for its contamination. Another study showed that S. aureus was present on skin of animals such as chickens contaminating about 24% of chicken samples examined [36]. Rapid food cooling, proper storage conditions and hygienic care reduce S. aureus contaminations [37].

Most of the enterotoxin producers were isolated mainly from chicken pastries, followed by egg sandwiches. Although most of the isolates were from nasal swabs, the isolates from this source were the least SE producers.

S. aureus secretes several types of enterotoxins (SEs) which are responsible for causing food poisoning. Different SE serological types have been recognized and designated as SEA, SEB, SEC, SED, SEE, SEG, SHE, SEI and SEJ [1], [6], [7], [10]-[12], [15]. The toxins are superantigens which have the ability to induce diarrhea when ingested [16], [17]. In the present investigation, only SEA, SEB, SEC, SED and SEE were screened. These are the most common S. aureus enterotoxins [9].

In the present study, strains producing SEA were the most common, mostly from chicken pastries. Strains producing enterotoxin A are known to be the most frequent in foodborne outbreaks. SEA is a leading cause of staphylococcal food poisoning. SEA is an extremely potent gastrointestinal toxin, with a low as 100 ng sufficient to cause toxicity. SEA has two known biological effects. SEA is a super antigen that acts on gastrointestinal cells, stimulating non-antigen-specific T cells proliferation [9]. Since SEA was detected from nasal swabs, contamination of SEA in chicken pastries was probably from a human source. All the food samples from this study involved extensive preparation and handling, which is a clear indication that the main source of contamination was from nasal samples.

SEB was the second most detected enterotoxin from all samples. Our results are in agreement with Carmo et al, (2002) [38] who reported SEA and SEB are the most frequently detected in both human and food samples. However, in our study, SEC, SED and SEE were the least common enterotoxin detected in both nasal swabs and food samples.

In reference to virulence factors, results indicated that all producing strains were positive for hemolysis and DNase tests, but lesser for coagulase. The enterotoxigenicity of S. aureus was found to be highly correlated with their potential production of DNase [39]. Therefore, DNase test was implemented as an index for staphylococcal enterotoxigenicity. Furthermore, hemolysis was used in this study, as an indicator for the presence of staphylococci and their enterotoxins. Almost all of S. aureus produced coagulase, and were correlated with the production of SEs staphylococcal enterotoxins [18].

Presence of MRSA strains is a serious problem since it can cause outbreak when transmitted from one person to another. In Spain a large outbreak of MRSA resulted from new patients admitted to hospitals with 39% infected from surgical and skin infections. Most of the strains were resistant to MRSA. MRSA are the common cause of hospital-acquired infections. They are treated with vancomycin, which is often the only drug of choice for severe MRSA infections [32]. In this study S. aureus resistance was detected at low frequency in both methicillin and vancomycin.

V. CONCLUSION

Staphylococcus aureus strains isolated from nasal swabs secreted a wide range of enterotoxins. The strains from food contaminated with S. aureus, were most likely related to human infection. Most of the strains were resistant to the tested antibiotics. The lowest frequency of resistance was exhibited to Tob and CN. There were few strains found to be resistant to vancomycin which is the first drug of choice for treatment of MRSA infections.

This study may enhance public awareness of the importance and appropriate procedures for food processing and care in handling. Improper handling may inflict serious health problems.

REFERENCES


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