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Identification of *Staphylococcus* spp. Isolated in Different Production Stages of White Cheese and Detection of Enterotoxin

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SUMMARY

This study was performed to evaluate the *Staphylococcus* species and staphylococcal enterotoxin contamination in traditional production process of white cheese, a brined (or a pickled) cheese variety with a soft or semi-hard texture. Totally 640 samples including environmetal samples, raw milk samples, pasteurized milk samples and white cheese samples were taken from four traditional white cheese plants located in Konya region, Turkey. Total numbers of staphylococci isolates classified as coagulase-positive and coagulase-negative were 144 and 181, respectively. *S. aureus* and *S. intermedius* were identified as dominant species among isolates. *S. lentus* was most frequently identified coagulase-negative staphylococci species, followed by *S. xylosus* and *S. simulans*. Swabs taken from staff hands were determined more contaminated compared to other enviromental samples types. Level of staphylococcal enterotoxin was found under detectable level in all milk and cheese samples by ELFA technique. Nevertheless, the incidence of enterotoxin producer species indicates sanitation deficiency in traditional production of white cheese and potential public health problems due to consumption of white cheese produced under poor hygienic conditions. Additionally, number of isolate isolated from raw milk indicates that good sanitation practices must followed starting from farm level.

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Beyaz Peynir Üretiminin Farklı Aşamalarında *Staphylococcus* spp. İdentifikasyonu ve Enterotoksin Tespiti

ÖZET

Bu çalışma, beyaz peynir üretiminin farklı aşamalarında stafilokokların izolasyonu ve identifikasyonu ile staphylococcal enterotoksin düzeyinin tespiti amacıyla yapıldı. Konya'da beyaz peynir üretimi yapan dört firmadan çiğ süt, pastörize süt, beyaz peynir, personel ve farklı ekipmanlardan olmak üzere toplam 640 örnek alındı. Elde edilen stafilokok izolatların 144'ü koagülaz pozitif ve 181'i koagülaz negatif olarak tespit edildi. Koagülaz pozitif türler içinde *S. aureus* ve *S. intermedius* baskın tür olarak tespit edilmiştir. Koagülaz-negatif stafilokok türleri arasında en sık identifiye edilen *S. lentus* ve bunu *S. xylosus* ve *S. simulans* takip etmiştir. Personel elinden alınan swab örneklerindeki kontaminasyon düzeyi üretimde kullanılan ekipmanlardan daha yüksek bulunmuştur. Tüm süt ve peynir örneklerinde Stafilokok enterotoksin düzeyi tespit edilebilir düzeyin altında bulunmuştur. Bununla birlikte, beyaz peynir örneklerinde enterotoksin üretme yeteneğine sahip stafilokok türlerinin tespit edilmesi üretimde sanitasyon eksikliğini ve halk sağlığı içinde bir risk oluşturduğunu göstermektedir. Ayrıca, çiğ sütteki kontaminasyon düzeyi de iyi sanitasyon uygulamalarının çiftlik düzeyinden başlayarak takip etmesi gerekliliğini ortaya koymuştur.

INTRODUCTION

White cheese is a brined (or a pickled) cheese variety with a soft or semi-hard texture and a salty, acid taste (Hayaloglu et al 2002). *Staphylococcus* spp. are microorganisms that are naturally present in milk and dairy products and are often associated with food-borne diseases outbreaks due to the ability of some strains to produce thermostable enterotoxins (Viçosa et al 2010). Also Staphylococci are isolated from a wide range of foodstuff such as meat, cheese and milk, and from environmental sources such as soil, air and water (Heikens et al 2005). *S. aureus* is often found in milk, and has also been reported to be isolated from skin of udders and teats, milking equipment and shelves, floor, door (Jorgensen et al 2005b). *S. aureus* is the most important breed of enterotoxigenic staphylococcus. Besides, it is reported that certain coagulase-positive staphylococcus types, such as *S. intermedius* and *S. hyicus* and some coagulase-negative staphylococcus types, such as *S. epidermis* have the capacity to form enterotoxins (Küplülü et al 2002, Sutherland et al 2002). It is reported that the enterotoxins in foodstuff cannot be completely inactivated through heat treatments such as cooking and pasteurization, and at the same time they are resistant to proteolitic enzymes, drying and gamma rays (Normanno et al 2005).

Unless rules of hygiene are observed during the manucfacture stages of the milk products, contamination with microorganisms in different type and number may occur. Of these microorganisms, *Staphylococcus* spp. can lead to various infections and intoxications both in human beings and in animals. With all these points under consideration, in determining the microbiologic quality of foodstuff, to identify the number of some pathogen bacteria as well as of enterotoxins is of great importance to public health. From this point of view, research into staphylococcus and enterotoxins in foodstuff, particularly in milk and milk products has emerged as an important issue. The aim of the this study was the identification of the staphylococcal microflora isolated from different production stages of white cheese and detection of enterotoxin.

MATERIAL AND METHODS

Samples were collected from different manufacture white cheese, obtained from four dairy plants located in the city of Konya, Turkey. The samples were collected at different stages of white cheese manufacture, considering sampling from different sources, e.g. raw milk, pasteurized milk, white cheese, the swap specimens taken from the hands of the workers and from the equipment (milk tank, cheese cans, cutting wire and extractor cloth). Samples were transported to the laboratory under refrigeration for microbiological analyses.

Surfaces and equipments were sampled during processing and after the cleaning and disinfection procedure following the International Organization for Standardization (PN-EN ISO 18593-2004). *Staphylococcus* spp. was counted on Baird-Parker agar (BPA, Oxoid, CM0275) with added egg yolk tellurite, incubated at 37ºC for 48 h. After growth, *Staphylococcus* colonies were counted and classified as typical for *S. aureus* (jet black to dark gray, smooth, convex, entire margins with an opaque zone, clear halo beyond the opaque zone) and atypical (jet black to dark gray colonies, entire margin without a halo). For each sampling point, 5 to 10 colonies on plates of BPA were randomly selected and plated on Nutrient agar (Oxoid, CM0309). The strains were preliminary screened by Gram staining and coagulase test (FDA BAM 2001). API Staph biochemical test kit (bioMeriéux REF 20 500) and VITEK 2 GP (bioMeriéux, 21 342) identification system were used for identification.

VIDAS® Staph enterotoxin II (SET2; bioMeriéux, REF 30 705, 2004) Enzym Linked Floresance Analysis (ELFA) technique was used to identify enterotoxins in raw milk, pasteurized milk and white cheese. 500 µL was taken from the infiltrate that was obtained at the end of the extraction process and added to the sample unit of a VIDAS SET2 reactive stripe. The results were automatically read by the device. For instance, the detected value of the toxin concentration is given as 0.5 ng/g in the method.

RESULTS AND DISCUSSION

In this study, isolation and identification of *Staphylococcus* spp. carried out in the different production stages of white cheese (raw milk, pasteurized milk, white cheese and the swab specimens taken from the hands of the workers and from the equipment) of manufacture white cheese in dairy plant in Konya, Turkey. Besides, Staphylococcus enterotoxin analysis was conducted on the samples of raw milk, pasteurized milk and white cheese. No staphylococcus enterotoxins (SE) were encountered in raw milk, pasteurized milk and white cheese. The distribution of *Staphylococcus* isolates based on the result of the coagulase test given in Table 1.

The distribution of coagulase-positive staphylococcus identified in the samples taken from raw milk, white cheese, equipment and the hands of the staff is shown in Table 2.

The distribution of coagulase-negative staphylococcus identified in the samples taken from raw milk, white cheese, equipment and the hands of the staff is shown in Table 3.

Table 1. The Distribution of Staphylococcus Isolates Based on The Result of The Coagulase Test

Sample Type	No. Samples	No. Staphylococcus Isolates	No. Coagulase-Positive Staphylococcus Isolates	No. Coagulase-Negative Staphylococcus Isolates
raw milk	80	145	93	52
white cheese	80	69	23	46
pasteurized milk	80	-	-	-
milk tank	80	41	9	32
extractor cloth	80	10	-	10
cheese can	80	14	-	14
cutting wire	80	-	-	-
the hands of the staff	80	46	19	27
Total	**640**	**325**	**144**	**181**

Table 2. The Distribution of Coagulase-Positive Staphylococcus Identified in The Samples Taken From raw Milk, White Cheese, Equipment and The Hands of The Staff

Coagulase-Positive Staphylococcus Types	Sample Type					
	Raw Milk	White Cheese	Milk Tank	Extractor Cloth	Cheese Can	Staff
S aureus	49	3	1	-	-	8
S intermedius	35	18	7	-	-	11
S hyicus	9	2	1	-	-	-
Total	**93**	**23**	**9**	**0**	**0**	**19**

Table 3 The Distribution of Coagulase Negative Staphylococcus Identified In The Samples Taken From Raw Milk, White Cheese, Equipment and The Hands of The Staff

Coagulase Negative Staphylococcus Types	Sample Types					
	Raw Milk	White Cheese	Milk Tank	Extractor Cloth	Cheese Can	Staff
S epidermis	3	-	-	-	-	-
S xylosus	7	24	-	-	-	-
S carnosus	-	13	-	-	-	-
S saprophyticus	5	7	4	2	-	-
S simulans	17	-	-	8	-	-
S lentus	13	-	27	-	2	4
S auricularis	3	2	-	-	3	-
S haemolyticus	4	-	-	-	-	-
S chromogenes	-	-	1	-	-	-
S hominis	-	-	-	-	9	23
Total	**52**	**46**	**32**	**10**	**14**	**27**

It is seen that the number of *Staphylococcus* isolates isolated from raw milk samples is higher than those isolated from the other samples (Table 1). The number of coagulase-positive *Staphylococcus* isolates is higher than that of coagulase-negative staphylococcus isolates (Table 2-3).

These results bear similarity to the results of the studies conducted by some researchers (Adwan et al 2005, Al-Tahiri 2005, Ekici et al 2004). These results are an indication of the fact that the hygienic quality of raw milk in the conditions of Turkey is low because raw milk is obtained, especially in family corporations, not with modern milking machines, cooling process isn't applied following the milking, and proper attention isn't paid to the hygiene of milking (Saltan et al 2003). Another source of contamination can be shown as the fact that the milk from animals with mastitis problem is mixed with that from healthy animals.

Growth of *Staphylococcus* spp. isn't seen in pasteurized milk samples. Also no staphylococcus enterotoxins were encountered. The results obtained show that raw milk was pasteurized at proper temperature. However, as opposed to the results obtained, Omurtag and Akın (1981) detected *enterotoxigenic Staphylococcus* in 23%, of the 80 pasteurized milk sample. The researchers reported that the pasteurized milks were not pasteurized in proper conditions and that these products posed risk to the health of consumers. Again Küplülü et al (2002) established SEA to be >0.1 ng/ml in two samples out of 250 pasteurized milk samples. This is explained as resulting from keeping raw milk into which milk with mastitis was added in ambient temperature for too long in hot seasons and from not reducing the heat of the milks to below 10°C following pasteurization. Besides, it is claimed that enterotoxins that are characteristically thermo-stable may pose a danger because they cannot be broken down even if the milk has been pasteurized.

It is seen that the number of coagulase-negative *Staphylococcus* isolates in *Staphylococcus* isolates obtained from white cheese samples is higher than that of coagulase-positive isolates (Table 2-3). However, it is reported that, as well as coagulase-positive staphylococcus, some coagulase-negative staphylococcus spp. (*S. epidermidis, S. saprophyticus, S. sciuri, S. warneri*) have the capacity to generate enterotoxins (Carmo et al 2003, Cunha and Calsolari 2007, Irlinger 2008). Günşen and Yörük (2003) reported that 64% of the vacuum packed fresh cheddar samples they analyzed were incompatible with Turkish Food Codex in terms of *S. aureus* (80/125). Normanno et al (2005) established that 20.7 % of the 3097 milk and milk product samples they examined (631/3097) were contaminated with coagulase-positive staphylococcus, that 362 of the 364 suspected strains chosen for identification were *S. aureus* and 59.9% of them were characteristically enterotoxigenic. They noted that, of these isolates, 4 (10.8%) had SEA, 20 (54.1%) had SEB, 4 (10.8%) had SEC, 6 had SED and 3 (8.1%) had SEE enterotoxin gene. Al-Tahiri (2005) argued that the results he obtained in the corporations that conducted conventional production were alarming, that these values might reach the capacity to generate enterotoxins that cause food-poisoning when the appropriate conditions were available for bacterial multiplication, and that this posed a serious risk to human health. Lamprell et al (2004) identified *S. aureus* in 852 of 1036 cheese samples (82.2%), *S. intermedius* in 138 (13.3%) and coagulase-negative staphylococcus in 46 (4.5%). 62.1% of the *S. aureus* strains were determined to form SEC bovine; 4.1 % to form SEC ovine; 26.6 % to form unidentified toxin; 2.9 % to form SEA, 1.3 % to form SEB and 2,9 % to form SED type enterotoxins. They also established that, 6 of the *S. intermedius* strains formed SEC, and 2 formed SED and SEA+SED type enterotoxins. Bone et al (1989) didn't encounter any live patogens in the cheese made from sheep milk, whereas they detected the presence of SEA in cheese samples caused by *S. aureus*. Küplülü et al (2002) detected SEA in 2 samples at >0.1 ng/ml level out of 250 pasteurized milk samples.

In the equipment used in production, as well as coagulase-negative *Staphylococcus* spp., coagulase-positive *Staphylococcus* spp., though few, were isolated. This detection shows that proper attention isn't given to cleaning and disinfecting the equipment. Also reproduction of staphylococcus types in the samples taken from the hands of the workers employed in production is an indicator of lack of hygiene.

CONCLUSION

Those who milk the animals must be trained regarding milking and the hygiene of the milking place; tanks which have the suitable requirements for cooling and storing the milk must be set up. The staffs who work in dairy plant must be trained concerning hygienic applications. The equipment used in production must be cleaned and disinfected properly after the production. Also the program for cleaning and disinfecting must be supervised by those involved. Food analyses must look not only for *S. aureus* but also for coagulase-positive staphylococcus in food. Also, it is agreed that, especially in the case of a food poisoning complaint resulting from heat treated foodstuff, to demand a staphylococcus enterotoxin analysis instead of

looking for staphylococcus type bacteria would be more useful in that the period of analysis is shorter and the reason for the case could be thus better understood. The studies conducted regarding milk and milk products in accordance with the results obtained underline the food safety risks in milk industry resulting from staphylococcus and thus from enterotoxins.

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