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Asymptomatic carriage of bacteria in food workers in Nilüfer district, Bursa, Turkey*

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Aim: This study was carried out in order to determine the nasal carriage of *Staphylococcus aureus* and the faecal carriage of *Salmonella* spp., *Shigella* spp., *Yersinia enterocolitica*, *Listeria monocytogenes*, and *Escherichia coli* O157:H7 in food industry workers.

Materials and methods: During the study, face to face interviews were conducted with the workers and 9-item questionnaires regarding demographic information, working time, and periodical medical examinations were administered. In the study nasal swab specimens of 1115 individuals and faecal specimens of 1061 individuals were bacteriologically investigated.

Results: The nasal carriage of methicillin-resistant *Staphylococcus aureus* (MRSA) was found to be significantly more common in the workers who have no health insurance and who never had a medical and bacteriological examination for carrier screening ($P < 0.05$).

Conclusion: This result supports that periodical health examination is a necessity for food industry workers.

Key words: Food workers, faecal carriage, nasal carriage, bacterial pathogens.

Bursa ili, Nilüfer ilçesinde, gıda işçilerinde asemptomatik bakteri taşıyıcılığı

Amaç: Bu çalışmada, gıda sanayii işçilerinde burunda *Staphylococcus aureus* ve dışkıda *Salmonella* spp., *Yersinia enterocolitica, Listeria monocytogenes* ve *Escherichia coli* O157:H7 taşıyıcılığını belirlemek amaçlanmıştır.

Yöntem ve gereç: Çalışma esnasında işçilerle yüz yüze görüşülerek demografik bilgiler, çalışma süresi ve periyodik tıbbi muayenelerle ilgili 9 sorudan oluşan anket formu doldurduldu. Çalışmada 1115 kişinin burun sürüntü örneklerinde ve 1061 kişinin dışkı örneklerinde bakteriyolojik araştırma yapıldı.

Bulgular: Metisiline Dirençli *Staphylococcus aureus* burun taşıyıcılığı sağlık karnesi olmayan, tıbbi muayene ve bakteriyolojik olarak taşıyıcılık taraması yapılmayan işçilerde daha sık bulundu ($P < 0,05$).

Sonuç: Bu sonuç gıda endüstrisinde çalışanlar için periyodik sağlık muayenesinin gerekliliğini desteklemektedir.

Anahtar sözcükler: Gıda çalışanları, dışkı taşıyıcılığı, burun taşıyıcılığı, bakteriyel patojenler.

Introduction

Foodborne diseases may be generally defined as the diseases caused by agents that enter the body through the consumption of food or drink (1). Food-borne diseases are a large group of diseases involving the diseases caused by biotoxins, chemical contaminants, parasites, and microbial pathogens (1). Foodborne diseases are a major public health problem (2). Although it is difficult to estimate the global incidence of these diseases, it is observed that 1.8 million individuals were died of diarrhoeal diseases only in 2005. The large numbers of these deaths are due to the diseases caused by consuming contaminated food and water. In the industrialized countries, it is reported

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that every year more than 30% of the population are exposed to foodborne diseases. For instance in the United States of America it is estimated that every year 76 million foodborne diseases occur and 325,000 of those are hospitalized and 5000 of them die (3).

New foodborne diseases have emerged due to several causes including demographic changes in recent years, changes in food production and distribution as well as in food choices, microbial adaptation, and inadequate infrastructure. Increase in the opportunities in commerce and travelling resulted in global spread of local regional foodborne diseases (2). Previous studies demonstrated that employees working in the food industry are the main source for spreading foodborne diseases or the epidemic ones (4,5).

In this study it was aimed to determine the main bacterial sources that take part in foodborne infection the carriage of nasal enterotoxygenic *Staphylococcus aureus* and faecal *Salmonella* spp., *Shigella* spp., *Yersinia enterocolitica, Listeria monocytogenes,* and *Escherichia coli O157:H7* in food industry workers. In addition, the rate of methicillin resistance in *S. aureus* was investigated.

Materials and methods

This study was carried out between January 1, 2005 and August 1, 2007 in Nilüfer District of Bursa Province. Bursa is the fourth largest province of Turkey. Nilüfer is 1 of the 7 central districts of the province with a population of 178,682. Nilüfer is an industrial district with organized industrial zones and many business centres. There are 1144 food manufacturers in the district, of which 174 were selected and all the employees working in those places were participated to the study. During the study, face to face interviews were conducted with the workers and a 9-item questionnaire regarding demographic information, working time, and periodical medical examinations were administered. Nasal swab specimen was taken from the workers by research assistants. The cups for faecal specimens were delivered to the study group and collected the next day.

In order to investigate the carriage of nasal *S. aureus*, swab specimens taken from both nostrils and transferred to Stuart-transport media were sent to the laboratory immediately. In the laboratory, the specimens were cultured at 35 °C and for 24 h after inoculating to 2 different Oxacillin Resistant Screening Agars (ORSA), with and without oxacillin supplement (Oxoid, Basingstoke, England). After the evaluation, *S aureus* colonies grown on both types of media were stored in microbank tubes (Cryobank, Mast Diag.) at -20 °C for the process, which will be carried out later on.

In order to investigate *Salmonella* spp. and *Shigella* spp. from the faecal specimens, Selenit F broth, Eozilen Metilen Blue (EMB) Agar and *Salmonella Shigella* (SS) Agar (BD, BBL Heidelberg Germany) in order to investigate *Y. enterocolitica* Cefsurodin Irgasan Novobiosin (CIN) Agar (BD, BBL Heidelberg Germany) and 5 mL Phosphate Buffer; for *E. coli O157:H7* Mac Conkey Sorbitol Agar (BD, BBL Heidelberg Germany) and in order to investigate *L. monocytogenes* Listeria Enrichment Broth (BD, BBL Heidelberg Germany) and Oxford Agar (BD, BBL Heidelberg Germany) medias were used. Inoculations were incubated at 35 °C and evaluated 24 and 48 h later. Cultures were followed by transferring from growth media to selective media after a reasonable time with classical methods.

To identify the colonies that were considered as a possible factor, Crystal Diagnosis System (BD, Sparks MD USA) was used. After the biochemical identification typing was carried out with type serums (BD, Sparks MD, USA). For the molecular investigation of the capability of enterotoxin secretion in *S aureus* strains, after the microbank storing systems fixed at room temperature; each beads taken from inside of it was swabbed to 5% Columbia sheep blood agar (BD, BBL Heidelberg Germany) media and incubated at 35 °C for 24 h. After the formed colonies were verified as *S aureus* with the catalase and coagulase tests, several colonies taken from the fresh passages performed to 5% Columbia sheep blood agar were suspended in sterile physiological saline in a 0.5 ml reaction tube and boiled for 10 min at 99 °C to isolate DNA.

Primers belonging to *S aureus* enterotoxin Mehrotra et al. (6) were selected. Synthesis of oligonucleotides was carried out at the Iontek Ltd Şti. Primers and anticipated DNA sizes after amplification are presented in Table 1.

Table 1. Nucleotide sequences and anticipated sizes of PCR products for the *S. aureus* gene-specific oligonucleotide primers.

Gene	Primer	Oligonucleotide sequence (5'-3')	Size of the amplified product (bp)
S aureus enterotoxin A (sea)	GSEAR GSEAR	1 GGTTATCAATGTGCGGGTGG 2 CGGCACTTTTTTCTCTTCGG	102
S aureus enterotoxin B (seb)	GSEBR GSEBR	1 GTATGGTGGTGTAACTGAGC 2 CCAAATAGTGACGAGTTAGG	164
S aureus enterotoxin C (sec)	GSECR GSECR	1 AGATGAAGTAGTTGATGTGTATGG 2 CACACTTTTAGAATCAACCG	451
S aureus enterotoxin D (sed)	GSEDR GSEDR	1 CCAATAATAGGAGAAAATAAAAG 2 ATTGGTATTTTTTTTCGTTC	278
S aureus enterotoxin E (see)	GSEER GSEER	1 AGGTTTTTTCACAGGTCATCC 2 CTTTTTTTTCTTCGGTCAATC	209

Multiplex primer set contained 200 μM deoxynucleoside triphosphates; 5 μL reaction buffer (100 mM Tris-HCl (pH 8.3), 500 mM KCl); 1.5 mM $MgCl_2$; 20 pmol (each) of sea, seb, sec, see, and 40 pmol of sed primer; 2.5 U of Taq-polymerase (Fermentas), and 10 μL of template DNA. The volume of this mix was adjusted to 50 μL with sterile distilled water. Evaporation of the reaction mixture was prevented by addition of 100 mL of sterile mineral oil. DNA amplification was carried out in a Biometra T3000 Thermal Cycler (Biometra, Göttingen, Germany) with the following thermal cycling profile: an initial denaturation at 94 °C for 5 min was followed by 35 cycles of amplification (denaturation at 94 °C for 2 min, annealing at 57 °C for 2 min, and extension at 72 °C for 1 min), ending with a final extension at 72 °C for 7 min.

Results

A total of 1144 participants were interviewed in this study. Faecal specimens were taken from 1061 (92.8%) of them, nasal specimens were collected from all participants, 1115 of which (97.4%) were found suitable for evaluation. The mean age of the participants was 32.8 ± 10.4 years, and 59.8 % of them were male, whereas 40.2% were female.

The rate of production workers was 55.3%. The rest (44.7%) include technical staff, service staff, and managers. The average working time is 82.3 ± 101.7 months (1 month to 600 months). The rate of the participants who completed the primary school was 43.2%, whilst 3.6% of the participants was illiterate. Considerable amount of the participants (15.8%) did not have any periodical medical examination.

Staphylacoccus aureus was grown in the nasal cultures from 169 samples. Twenty-nine (17.2%) of them were MRSA. This data demonstrates that the Nasal *S. aureus* carriage was 15.2% , whereas nasal MRSA carriage was 2.6%. When the 21of 29 isolates were further investigated for their enterotoxin production capacity, *S. aureus* enterotoxin A production was detected in 3 specimens, whilst *S. aureus* enterotoxin E was produced in one specimen.

According to the faecal analyses *E. coli 0157* strain was grown in 22 specimens. Further evaluation of these isolates by using type serum for the pathogenic *E. coli O157:H7* strain demonstrated that none of them was *E. coli O157:H7. Listeria monocytogenes* was isolated from one of the faecal samples. No *Salmonella spp., Shigella spp., and Y. enterocolitica* growth was isolated from any faecal specimen.

The features of the participants who had nasal carriage according to the risk factors are presented in Table 2. Having no health insurance, having no periodical medical examination for nasal or faecal carriage, working in production, and working less than 1 year in a workplace were taken as risk factors.

Table 2. The distribution of participants who have nasal MRSA carriage or not, according to the risk factors.

Risk factors	Nasal MRSA carriage n = 29		Normal group n = 1086		Total n = 1115		Fisher- P
	n	%	n	%	n	%	
Health Insurance							
Available	21	2.1	961	97.9	982	100.0	**0.016**
Not available	8	6.0	125	94.0	133	100.0	
Periodical Medical Examination							
Available	20	2.1	943	97.9	963	100.0	**0.032**
Not available	9	4.9	172	95.1	181	100.0	
Branch of work							
Production worker	18	2.9	608	97.1	626	100.0	0.57
Technical staff	11	2.1	507	97.9	518	100.0	
Working time							
Less than 1 year	9	3.4	256	96.6	265	100.0	0.37
More than 1 year	20	2.3	859	97.7	879	100.0	

Discussion

In Turkey, employees who work in food production companies and health organizations are obligated to take a periodical medical examination every 3 months and obtain a health report documenting the employee's carriage of contagious diseases. It is necessary to give faecal culture for *Salmonella* and *Shigella* at least once a year; for microscopic examination of stool for *Entamoeba histolytica* cysts, *Giardia lamblia* cysts, and helminth eggs at least every 6 months; and throat and nasal culture for *S. aureus* and lung X-ray for tuberculosis screeining at least once a year.

Although it is a legal obligation, in our study the rate of the employees without health insurance was 12.3% and the rate of employees not taking periodical medical examinations was 15.8%. Previous studies involving the food sector employees in our country demonstrated that the rate of employees with no health insurance ranges from 58.8% to 93.5% (7,8). The reason for the lower rate observed in our study may be due to the fact that it was carried out in a developed region where regular inspections are carried out by the municipality.

Escherichia coli O157:H7 has been recognised as a major pathogen for food industry since 1982 when it was first identified as a human pathogen. It causes severe diseases, such as hemorrhagic colitis, haemolytic uremic syndrome, and thrombotic thrombolytic purpura, and affects all age groups (9). Epidemic diseases developing in a wide spectrum from asymptomatic carriage caused by *E. coli 0157* to serious diseases, such as hemorrhagic colitis and haemolytic uremic syndrome, are reported from every part of world (10).

Listeriosis generally occurs by consuming vegetable or meat contaminated with *L. monocytogenes*. Because of its high fatality rate, listeriosis is one of the foodborne diseases among major public health problems (11). *Listeria* causes more death than other bacteria causing food poisoning. In high risk individuals, 20%-30% of foodborne listeriosis results in death (12). There is little information about the prevalence of *L. monocytogenes* in human faeces. In our study *L. monocytogenes* was detected only in one faecal specimen (0.9%). Similarly, the prevalence was found to be varied between 0.12% and 0.8% in the studies performed in healthy individuals (13-15). Although the prevalence is low as observed here and by others, when the high fatality rate is considered, it will be appropriate to investigate the carriage of *Listeria* in faeces of employees working in food preparation.

In present study *Salmonella spp., Shigella spp.,* and *Y. enterocolitica* were not detected in any specimens. In the study carried out in Jakarta, Salmonella was isolated from 7% of the faecal specimens of the street vendors and restaurant employees (16). In another study performed in Ghana, typhoidal *Salmonella* was detected in 2.3% of the faecal specimens of the food vendors in Ghana (17). In a salmonella epidemic that occurred in a hospital, Salmonella was detected in 12.3% of food handlers and it was indicated that asymptomatic food handlers can be the source of nosocomial salmonella epidemic (18). In the study carried out in 151 food workers working in a medical college in North India, *Salmonella* and *Shigella* were not detected in any of the faecal specimens (19). To our knowledge there is no study about prevalence of *Y. enterocolitica* in food company workers. However, no Salmonella, Shigella, or *Y. enterocolitica* was detected in the faecal specimens of 2000 healthy individuals in a previous study from Germany (13). The reason for not detecting any types of Salmonella, Shigella and any *Y. enterocolitica* in our study could be that those pathogens are rarely seen in asymptomatic individuals.

Staphylococcus aureus may be responsible for staphylococcal food-poisoning outbreaks by producing enterotoxins. Staphylococcal food-poisoning is the second common cause of food-borne diseases after Salmonella in France (20). The cause of the acute gastroenteritis outbreak at a school in Austria was determined as *S. aureus* that transmitted from a kitchen staff (21). The source of the *S. aureus* outbreak that affected 180 individuals in Brazil was also the food handlers carrying *S. aureus* in their throats (22). In our study nasal carriage rate of *S. aureus* was found to be 15.2%, whereas this rate was 2.6% for MRSA. Among the 21 specimens where MRSA was detected, 4 were able to produce enterotoxin. *S. aureus* enterotoxin A and *S. aureus* enterotoxin E were determined in 3 and 1 specimens, respectively. It may be claimed that the rate of MRSA colonisation changes due to both the existence of risk factors and geographic region (23). The carriage rate of nasal MRSA in the community was found between 0.2% and 3% in the studies carried out in various countries (24). Information concerning the carriage of MRSA in this society is inadequate (24). In previous studies from Turkey, nasal carriage rate of MRSA was reported as 2.6% and 5%, respectively (25,26).

In the studies carried out on food employees, nasal carriage rate of *S. aureus* varies between 24% and 41%. Oteri et al. determined a nasal carriage rate of 24% in their study involving 161 employees working in the food sector within hospitals (27). It was 26.6% in another study involving 500 restaurant employees in Kuwait City. Most of the *Staphylococcus aureus* strains produced enterotoxin (86.6%) and 28% of them were enterotoxin A producer (28). In a study carried out by Figueroa et al., *S. aureus* carriage was found to be 19.0% on specimens taken from retro-pharynx of restaurant employees and the most of them were enterotoxin A producer (29). In Chile the frequency of enterotoxigenic *S. aureus* was 41% on nose, throat, hand, and nail samples of the staff working in a cafeteria of a university. The most frequent enterotoxin type was type B (30). There are a limited number of studies concerning nasal carriage of *S. aureus* in Turkey.

Although nasal carriage rate of *S. aureus* is lower in our study in comparison to other studies, the rate of nasal MRSA carriage was similar to the studies developed in Turkey and in other countries.

Participants who have no health insurance and no periodical medical examination for nasal or faecal carriage were significantly more common in the nasal MRSA carriers compared with the remaining participants.

Our data demonstrated that nasal MRSA carriage was found significantly high in individuals with no health insurance and/or had no periodical medical examination, which suggests that the periodical medical controls are not enough and not appropriately managed in the food industry. Increasing the frequency of controls, providing health education programmes for food industry workers, and regular medical examinations of food employees may be beneficial to prevent foodborne diseases.

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