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Microbiological Risks and Portion Analyse of Stuffed Mussels Sold in Istanbul

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ABSTRACT In this study, stuffed mussels were collected from 50 selling points in Istanbul, consist of street vendors (n=38) and retail shops (n=12), analyzed microbiologically and weighted per portion. Mean Total Aerobic Count result was 7.38±1.01 log10 cfu/g among the samples. *Coliform* was isolated from 44% of samples (2.85±0.57 log cfu/g), *Escherichia coli* from 12% of samples (3.76±0.71 log cfu/g), *Staphylococcus aureus* from 4% (4.15±0.30 log cfu/g), *Bacillus cereus* in 2% (2.78 log cfu/g) and *Bacillus licheniformis* in 4% (3.18±0.08 log cfu/g) of samples. *Listeria ivanovii* was isolated from 2% of samples (in 25 g). No *Clostridiu*m spp., *Campylobacter* spp., *Salmonella* spp. and *Listeria monocytogenes* were detected. Level of *Staphylococcus* spp. (*S. aureus* + *S. epidermidis*) contamination was significantly higher in the retail shop (P <0.05). The mean portion per stuffed mussel was 16.78±5.62 g. Except for the result of *Staphylococcus* spp., microbiological analyses and a gram of portions did not show a significant difference between retail shops and street vendors (P >0.05). However, an inappropriate level of *S. aureus* contamination in retail shops remain a food safety gap. Furthermore, the mean results of portions are assumed to be used in predictive microbiology studies.

Keywords: *Food Safety, Food Microbiology, Mussels*

ÖZ

İstanbul'da Satılan Midye Dolmalardaki Mikrobiyolojik Riskler ve Porsiyon Analizi

Bu çalışmada İstanbul'da sokakta (n=38) ve kapalı restoranda (n=12) satışa sunulan toplam 50 farklı noktadan midye dolma örneği toplanarak mikrobiyolojik inceleme ve porsiyon analizi yapılmıştır. Örneklerin Toplam Aerobik Bakteri sayısı ortalama 7.38±1.01 $\log_{10}$ kob/g olarak bulunmuştur. *Coliform* grubu %44 (2.85±0.57 log kob/g), *Escherichia coli* %12 (3.76±0.71 log kob/g), *Staphylococcus aureus* %4 (4.15±0.30 log kob/g), *Bacillus cereus* %2 (2.78 log cfu/g) ve *Bacillus licheniformis* %4 (3.18±0.08 log cfu/g) oranında izole edilmiştir. *Listeria ivanovii* %2 oranında tespit edilmiştir. *Clostridium* spp., *Campylobacter* spp., *Salmonella* spp. ve *Listeria monocytogenes* tespit edilmemiştir. *Staphylococcus* spp. (*S. aureus* + *S. epidermidis*) kontaminasyon düzeyi restoranlarda anlamlı olarak daha yüksek bulunmuştur (*P* <0.05). Midye dolmaların adet porsiyonu ortalama 16.78±5.62 g olarak tartılmıştır. *Staphylococcus* spp. sonuçları dışında mikrobiyolojik analizler ve porsiyon gramajları restoranlar ile sokak satış noktaları arasında anlamlı bir fark göstermemiştir (*P* >0.05). Ancak *S.aureus*'un kapalı restoranlarda tüketim için uygun düzeyin üzerinde tespit edilmesi bir gıda güvenliği açığının bulunduğuna işaret etmektedir. Porsiyon ortalamalarının ileride prediktif mikrobiyoloji çalışmalarında kullanılabileceği öngörülmektedir.

Anahtar Kelimeler: *Gıda Güvenliği, Gıda Mikrobiyolojisi, Midye*

INTRODUCTION

Stuffed mussel is one of the most popular street food consumed in Istanbul, Turkey (Güzeler and Özbek 2017). It is mostly sold by street vendors and available in retail shops (Kisla and Uzgun 2008). In most case, unemployed immigrants collectively help for the preparation of stuffed mussel and sell on the streets (Eranıl Demirli et al. 2015). Thus, in retail shops is the expected health risk is low, however unregulated street vendors bear the risk of illness.

Several foodborne bacterial pathogens, such as *E. coli*, *B. cereus*, *S. aureus*, *Salmonella* spp. and *C. perfringens* and

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also viruses were isolated from stuffed mussels in former studies (Bingöl et al. 2008; Ateş et al. 2011). High frequency of *Coliform* and different amount of *B. cereus*, *S. aureus* and Vibrio spp. were detected in stuffed mussels collected from street vendors of Turkey (Ergönül et al. 2014). According to the microbiological results of Bingöl et al. (2008), 18.4% of stuffed mussels sold in Istanbul were contaminated in an unacceptable limit of consumption. Additionally, related to the consumption of stuffed mussels a life-threatening case has been also reported in Turkey (Urazel et al. 2014).

Stuffed mussel is particularly consumed in high demand areas of Istanbul. Most of the sellers prefer to calculate the portion according to the number of consumed stuffed mussels. However, consumed portion, i.e. the amount of rice and mussel, varies by the consumer's preference. However, there is lack of information in the literature on the average grammage of the edible portion of stuffed mussel, which can be useful for studies on the risk/benefit assessment not only microbial but also chemical contaminants and dietary value of this food item.

Hence, in this study stuffed mussels sold in street vendors and retail shops (restaurants) in major places in Istanbul were selected for investigation. Ready-to-eat stuffed mussels were collected from 50 different selling points and analyzed microbiologically. Regard to the amount of edible portion per stuffed mussel, the results of this study is assumed to be used for future predictive microbiology and dietary studies.

MATERIALS and METHODS

Sampling plan

Stuffed mussels were collected from 50 different locations (10 samples from each) including 38 street vendors and 12 retail shops in Istanbul. Sampling was performed with the assumption of increased temperature and decreased hygienic quality (related to the high temperature) in the summer month (June 2016). All the samples were transported to the laboratory in a refrigerated box and immediately analyzed.

Microbiological analyses

Edible components were weighed and 25 g of each sample was transferred to Stomacher filter bags and 225 ml of enrichment solutions were added (1:10 ratio). Afterward, samples were homogenized in Stomacher bags and tenfold serial dilution was prepared with Maximum Recovery Diluent (Oxoid CM0733). Appropriate dilutions were spread onto Total Aerobic Count (TAC) and selective agar plates for enumeration. The name of the bacteria investigated and media used in the isolations are shown in Table 1.

Edible portion weights

Three samples from each selling point were weighted without shell (only mussel meat and rice components). Mean results of grammage were calculated and compared between street vendors and restaurants.

Data analyses

Results of microbiological analyses transformed to log10 unit by using MS Excel and all counts were analyzed statistically with SPSS (version 11.5). A possible relationship between the microflora and weight results was observed for street vendors and retail shops according to t-test and one-way Anova test. P <0.05 was considered statistically significant.

Table 1. Isolation and identification procedures of the investigated bacteria

Investig. bacteria	Media	Incuba.	Reference
TAC	Plate Count Agar (Oxoid CM0325)	30°C, 48 h	(ISO 2003)
E.coli	Coli ID agar (BioMeriéux 42017)	42°C, 24 h	(Omurtag et al. 2012)
Coliform	Coli ID agar (BioMeriéux 42017)	42°C, 24 h	(Omurtag et al. 2012)
Staphylococcus spp.	Baird-Parker Agar (Merck Nr.1.05406) with egg yolk (Merck Nr.1.03784) ID 32 STAPH (BioMeriéux)	37°C, 48 h	(Omurtag et al. 2012)
Bacillus cereus	BACARA agar (BioMeriéux) API 50 CHB/E (Biomerieux)	37°C, 24 h	(Thepaut and Soriano 2012, Tallent et al. 2012)
Salmonella spp.	Buffered Peptone Water (Oxoid CM0509) MSRV motility agar (Oxoid CM0910) XLD-agar (Merck Nr.1.05287) API 20 E (BioMeriéux)	37°C, 24 h 42°C, 24 h 37°C, 24 h	(Omurtag et al. 2012)
Clostridium spp.	TSC agar (Merck Nr. 111972) API 20 A, rapid ID 32 A	37°C, 24 h anaerobically	(Rhodehamel and Harmon 2001)
Listeria spp.	Fraser Broth [Fraser Broth Base (Oxoid CM0895) Fraser Listeria selective supplement (Merck Nr.1.00093), Ammonium-ferric(III)-citrate (Merck 3762) ALOA Agar (BioMeriéux) API Listeria (BioMeriéux)	37°C, 37 h	(Omurtag et al. 2012)
Campylobacter spp.	Bolton Broth (Oxoid CM0983, with supplement Oxoid SR0208E) m-CCDA (Oxoid CM0739, with supplement Oxoid SR0155E) API CAMPY (Biomerieux)	42°C, 48 h, microaerobically	(Omurtag et al. 2012)

RESULTS

Among 50 selling points, TAC levels of samples ranged from 4.31 to 8.60 log10 cfu/g, with a mean of 7.38±1.01 log cfu/g. TAC levels did not show a significant difference between street vendors and retail shops. Almost half of the samples were contaminated with *Coliform* (44%), whereas *Escherichia coli* (12%) was most frequently isolated pathogen, which from this follows *Staphylococcus aureus* (4%) and *Bacillus cereus* (2%). Except for one stuffed mussel, most of the samples contaminated with *E.coli* did not contain *Coliform*, which is usually associated with the poor hygienic condition. One sample was contaminated with *Listeria ivanovii* (2%), and two samples with *Bacillus licheniformis* (4%). All of the results of microbiological analyses were presented in Table 2.

Table 2. Comparison of microbiological analyses (mean log10 cfu/g) and portion results of stuffed mussels according to the selling point (n=50)

	TAC (n=50)		*E.coli* (n=6)		*Coliform* (n=22)		*S. aureus* (n=2)		*S. epidermidis* (n=1)		*Bacillus cereus* (n=1)		*Bacillus licheniformis* (n=2)		*Listeria ivanovii** (n=1)		Weight of edible component
Selling point	mean	%	mean	%	mean	%	mean	%	mean	%	mean	%	mean	%	+/-	%	g / stuffed mussel
Street vendor (n=38)	7.37 ± 0.98	100	4.09 ± 0.58	8	2.83 ± 0.62	14	2.30	2	-	-	-	-	3.09 ± 0.12	4	+	2	15.63 ± 5.58
Retail shop (n=12)	7.39 ± 1.17	100	3.11 ± 0.47	4	2.88 ± 0.47	30	6	2	5.57	2	2.78	2	-	-	-	-	20.40 ± 5.97
Overall	7.38 ± 1.01	100	3.76 ± 0.71	12	2.85 ± 0.57	44	4.15 ± 0.30	4	5.57	2	2.78	2	3.09 ± 0.12	4	+	2	16.78 ± 5.62
p-value	0.96		0.83		0.25		0.019**				0.78				-		0.336

**Listeria* spp. was not enumerated, ** *Staphylococcus* spp. level of contamination was significantly higher in retail shops (P <0.05)

Escherichia coli was twice more frequently isolated in street vendors when compared to retail shops, whereas *S. aureus* contamination in the retail shop was higher than the appropriate level of consumption (6 log kob/g) and this result showed a significant difference from street vendors (P <0.05). Another toxin-producing bacteria, B.cereus, was obtained only from a retail shop. In this study, *Clostridium* sp., *Salmonella* sp., *Listeria monocytogenes* and *Campylobacter* spp. were not detected.

The weight of edible portion (rice and mussel meat) of stuffed mussels ranged from 7.68 g to 32.20 g, with a mean of 16.78±5.62 g. No significant difference was found in weight of samples between the selling points (Table 2).

DISCUSSION

As a result of being street food, in this study samples were mostly available in street vendors (38 of 50 selling points). TAC results varied between 4.31 log and 8.60 log cfu/g and was certainly higher than the recent studies conducted in Turkey, which varies from <1 log to 6.44 log cfu/g (Ergönül et al. 2014; Kök et al. 2015). Low microbiological quality of water, that mussels were collected from and additionally unhygienic condition during food preparation were suggested as some of the reasons of an unacceptable load of bacterial and viral contamination in stuffed mussel (Yılmaz et al. 2010; Ateş et al. 2011).

Studies conducted on the microbiological safety of this food item sold in Turkey found contamination with several foodborne pathogens, such as; *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli*, *Salmonella* sp., *Clostridium perfringens*, and *Vibrio* spp. (Bingöl et al. 2008; Yılmaz et al. 2010; Ateş et al. 2011; Ergönül et al. 2014). Food inspectors evaluate stuffed mussel in the Food Security Criteria of Turkish Food Codex (TFC) under the ready-to-eat cold snack category (TFC 2011). Therefore, it is limited for *E. coli* as 101 cfu/g, and required no staphylococcal enterotoxins, Salmonella and Listeria monocytogenes contamination in 25 g of sample. According to the Production Hygiene Criteria of TFC cooked crustaceans with/without shell (which is a component of stuffed mussel) have limits for *E. coli* and coagulase-positive staphylococcus as 101 (1 log) cfu/g and 103 (3 log) cfu/g, respectively (TFC, 2011). In the present study, average *E. coli* (3.76 log cfu/g) and *S. aureus* (4.15 log cfu/g) contamination were above these values, which indicates a requirement of production hygiene improvement. Although there is no limit set for *B. cereus* within these criteria, its contamination bear risk for food safety, due to its toxin production potential.

Listeria ivanovii is rarely causing illness in human, but still one of the important foodborne pathogens. It is isolated in several studies from stuffed mussels (Kök et al. 2015; Guillet et al. 2010). However, *L. monocytogenes* was not isolated in most of the studies conducted on stuffed mussels (Terzi et al. 2015; Kök et al. 2015). In this study, *L. monocytogenes* is not isolated either, but *L. ivanovii* was determined in one sample from a street vendor. As well as other important foodborne pathogens such as Salmonella spp., *Campylobacter* spp. and *Clostridium* spp. were not found. Thus, these results are promising when compared with the former studies.

On the other hand, retail shops in this study were more frequently contaminated with coliforms, *Staphylococcus* spp. and *B. cereus*. Regard to the significantly higher contamination rate in retail shops (particularly for

S. aureus) it is assumed that chance of cross-contamination, i.e. contact with other foodstuffs, is more common in retail shops than street vendors. Studies on technological applications in stuffed mussels showed that modified atmosphere packaging extended the shelf-life of stuffed mussels (Ulusoy and Ozden 2011) and lemon juice dressing decreased the level of *Salmonella* Typhimurium in the stuffed mussel (Kişla 2007). However, bacterial toxins resistant to food processing treatments; e.g. *S. aureus* enterotoxins and emetic toxin of *B. cereus* (EFSA 2012) should also be considered as an important hazard for retail shops.

In this study grammages of edible portions had a wide range (from 7.68 g to 32.20 g). This difference might be the result of the type of mussels used for preparation, e.g. some mussel species are smaller and take less amount of rice. Nevertheless, the mean amount of edible portions did not show statistical significance between street vendors (15.63±5.58 g) and retail shops (20.40±5.97 g). Following this result, as a standardized portion grammage by weighing the food item before selling, can be suggested rather than count per mussel.

In most of the studies, stuffed mussels have a high-level TAC and *S. aureus*, *E. coli*, *B. cereus*, *L. ivanovii* were most isolated bacteria with different level of contamination. In this study, *S. aureus* contamination in a retail shop exceeded twice more (6 log cfu/g) the accepted limit in TFC (<3 log cfu/g). Thus, the high contamination count of TAC and Coliform, and *E. coli*, when compared with other studies, indicates a low hygienic status in both of the street vendors and retail shops. These results give an overview that *L. ivanovii* might be evaluated as a normal flora agent or a hygienic indicator of this food item. On the other hand, edible portion size affects the risk of exposure to several contaminants, as well as carry nutritional importance. Therefore it is assumed that the data of edible portion may be useful with additional information like pH and aw for future predictive microbiology studies.

CONCLUSION

As a remarkable result of this study, both of *S. aureus* and *B. cereus* were found in samples. Therefore, a possible exposure to these bacteria via consumption of contaminated stuffed mussels can be realized for a further risk assessment research.

CONFLICT of INTEREST

The authors declare that they have no conflict of interest.

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