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Journal of Consumer Protection and Food Safety
Journal für Verbraucherschutz und Lebensmittelsicherheit

ISSN 1661-5751
Volume 12
Number 4

J Consum Prot Food Saf (2017)
12:335-339
DOI 10.1007/s00003-017-1120-2

Springer

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RESEARCH ARTICLE

Enterotoxigenic *Staphylococcus aureus*: sources and strategy for control in food outlets

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Received: 12 April 2017 / Accepted: 31 July 2017 / Published online: 5 August 2017
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Abstract The current study was conducted to investigate the potential sources of enterotoxigenic *S. aureus* and the efficacy of ISO 22000:2005 with the technical specifications ISO/TS 22002-2:2013 for pathogen control in food outlets. A total of 724 samples (483 food, 120 equipment and 121 hand swabs from food handlers) were collected from 112 food outlets. All samples were processed for isolation and identification of *S. aureus* using cultural method. Furthermore, enterotoxigenic strains were identified among *S. aureus* isolates through detection of genes coding SEA-SEE, SEG and SHE by PCR. The isolation rates of *S. aureus* were 11.8, 3.3 and 10.7% from food, equipment and hand swabs, respectively. While the prevalence of enterotoxigenic *S. aureus* in the same samples was 3.5, 0.8 and 1.7%, respectively. Raw chicken meat showed highest isolation rate for enterotoxigenic strains (5.1%). Moreover, enterotoxigenic *S. aureus* possess SEC and/or SED were the most common strains whereas SEA, SEB, SEE were not detected. Phylogenetic analysis of SED sequences revealed that strains from cooked food and used knifes were grouped in the same cluster with the human sequence to highlight the human origin of the contamination. ISO 22000:2005 with technical specifications ISO/TS 22002-2:2013 was applied in all restaurants that yielded *S. aureus* for 45 days and samples were gathered again for retesting. None of the retested restaurants were positive. In conclusion, food handlers are the main source of cooked food contamination with enterotoxigenic *S. aureus* regarding ISO 22000:2005 with technical specifications ISO/TS 22002-2:2013 an efficient system for control.

Keywords Enterotoxigenic *S. aureus* · Food outlets · ISO 22000:2005 · Control system

1 Introduction

Food-borne illness is a major cause of morbidity and mortality throughout the world with increased burden in developing countries (WHO 2015). In US, food-borne diseases were responsible for 9.4 m cases, more than 55,000 hospitalizations and about 1300 deaths annually (Scallan et al. 2011). Staphylococcal food poisoning (SFP) is the most common food borne intoxication worldwide. The disease is caused by ingestion of food contained *S. aureus* enterotoxins (SEs), which were performed by enterotoxigenic strains of *S. aureus* in the food matrix. Already small quantities (100 ng) of thermostable SEs can be harmful to humans (Hennekinne et al. 2009). To date, SEs and their variants (SE*l*s) can be classified into several serotypes from SEA-SEE, which are called classical SEs and new types SEG-SE*l*U2. The classical SEs are the most common serotypes which incriminated in SFP outbreaks while SEH is the only new type which was reported in SFP (Argudín et al. 2010). Several types of foodstuffs are involved in SFP but milk, milk products, chicken and meats are most commonly implicated (Hennekinne et al. 2012). SFP in humans is characterized by a short incubation time

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(30 min to 6 h) followed by nausea, vomiting and abdominal pain but no fever. The symptoms are usually mild and self-limiting within 1–2 days, although some cases may require hospitalization (Fletcher et al. 2015). *S. aureus* is a ubiquitous pathogen that survives wide range of temperatures and fasts harsh environmental conditions including high salt concentration (15% NaCl) and dryness (Kadariya et al. 2014). Humans are the main source for *S. aureus*, present in nares and on the skin and even being considered healthy (Acton et al. 2009**)**. Accordingly, it is believed that food handlers are the main source of food contamination with enterotoxigenic *S. aureus*. Nonetheless, enterotoxigenic *S. aureus* has also been isolated from cows with subclinical mastitis and from the mouth and nose of pet dogs and cats, meaning that animals can be the cause for contamination of human foods, too (Fagundes et al. 2010; Abdel-Moein and Samir 2011). Unfortunately, the presence of enterotoxins from enterotoxigenic *S. aureus* in foodstuff does not change the appearance or taste of the food. Therefore, to combat the possible sources of enterotoxigenic *S. aureus* is an effective control strategy against such food borne intoxication. Many restaurants all over the world applied different hygienic measures in order to serve safe food for their clients. ISO 22000:2005 is a protocol that has been recently applied in different food outlets in Egypt. Therefore, the current study was carried out to determine the potential sources of enterotoxigenic *S. aureus* in food outlets and investigate the efficacy of ISO 22000:2005 with the technical specifications ISO/TS 22002-2:2013 in the control of such pathogens.

2 Materials and methods

2.1 Samples

A total of 724 samples comprising 129 cooked pieces of chicken, 117 raw pieces of chicken, 123 cooked pieces of beef, 114 raw pieces of beef, 121 hand swabs and 120 equipment swabs (surface, cutting boards, knife, mincers) were collected from 112 food outlets. At least 6 samples were concurrently collected from each food outlet (one from each examined foodstuff as well as one hand and equipment swabs). All food samples were received in sterile bags while hand and equipment swabbing were done using liquid MRD swab sampling kit (TSC, UK). All samples were transferred in icebox with minimum delay to the laboratory for bacteriological examination.

2.2 Isolation and identification of *S. aureus*

Samples of 25 g raw beef, cooked beef, raw chicken and cooked chicken, respectively, were transferred to aseptic blender jar including 225 ml of 0.1% sterile peptone water. Each sample was then homogenized in the stomacher at 2000 rpm for 1–2 min to provide a homogenate (ISO 2003). 0.1 ml of the prepared samples as well as hand and equipment swabs were spread on Baird Parker agar plates (Oxoid, UK) and incubated at 37 °C for 24 h (ISO 2003). Suspected colonies were sub cultured and identified as *S. aureus* through Gram's stain films, biochemical tests, coagulase tests and serological confirmation using Staphytect Plus kit (Oxoid, UK) (Quinn et al. 2002).

2.3 Molecular detection of enterotoxigenic *S. aureus*

DNA was extracted from *S. aureus* isolates through rapid boiling procedure using a lysis buffer containing 1% Triton X-100, 0.5% Tween20, 10 mM Tris–HCL (pH 8.0) and 1 mM EDTA (Reischl et al. 1994). The amplification step was conducted to detect genes that are coding for SEs (SEA-SEE, SEG and SEH) using primer sets listed in Table 1. Conventional PCR was performed with 3 µl of bacterial DNA, 12.5 µl of 2X DreamTaq PCR master Mix (Thermo Scientific, Waltham, USA), 0.5 µl of each primer in concentration of 20 pmol and nuclease free water was added up to 25 µl. The reaction involved the following cycling conditions; 95 °C for 10 min, followed by 20 cycles of 95 °C for 1 min, 64 °C for 45 s, 72 °C for 1 min then final extension at 72 °C for 10 min (Kumar et al. 2011).

2.4 Sequencing and phylogenetic analysis

Selected PCR products of positive *S. aureus* enterotoxin type D from different isolation sources (raw chicken, cooked chicken, cooked beef, both equipment and hand swabs) were purified using a Qiaquick Kit (QIAgen, Hilden, Germany) according to manufacturer's instruction. The sequencing was conducted using Big Dye Terminator V3.1 sequencing kit (Applied Biosystems) with the forward primer SED. The obtained nucleotide sequences of enterotoxin type D of *S. aureus* isolates were compared with the sequences available in public domains using NCBI, BLAST server. Sequences were downloaded and imported into BIOEDIT version 7.0.1.4 for multiple alignments according to their deduced amino acid using the CLUSTAL W program of the BIOEDIT. The percent identity between sequences and

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Table 1 List of primer pairs used for detection of *S. aureus* enterotoxins

Primer	Primer sequence	Size (bp)
SEA F.	GCAGGGAACAGCTTTAGGC	521
SEA R.	GTTCTGTAGAAGTATGAAACACG	
SEB F	ACATGTAATTTTGATATTCGCACTG	667
SEB R.	TGCAGGCATCATGTCATACCA	
SEC F.	CTTGTATGTATGGAGGAATAACAA	284
SEC R.	TGCAGGCATCATATCATACCA	
SED F.	GTGGTGAAATAGATAGGACTGC	385
SED R.	ATATGAAGGTGCTCTGTGG	
SEE F.	TACCAATTAACTTGTGGATAGAC	171
SEE R	CTCTTTGCACCTTACCGC	
SEG F.	CGTCTCCACCTGTTGAAGG	328
SEG R.	CCAAGTGATTGTCTATTGTCG	
SEH F.	CAACTGCTGATTTAGCTCAG	359
SEH R	GTCGAATGAGTAATCTCTAGG	

phylogenetic analysis with the neighbor joining approach was performed using MEGA version 6 (Fig. 1).

2.5 GenBank accession numbers

The sequences have been deposited in the GenBank data base under accession numbers KY348536–KY348540.

2.6 Control system for *S. aureus* in food outlets

ISO 22000:2005 food safety management system (ISO 22000 2005) with the details mentioned in ISO/TS 22002-2:2013 (ISO/TS 22002-2 2013) was applied for 45 days with regular inspection every 2 weeks to ensure perfect application in all food outlets that showed positive results to *S. aureus* (n = 56). After the application period (45 days), 362 samples were collected from food outlets which included 56 cooked pieces of chicken, 70 raw pieces of chicken, 61 cooked pieces of beef meat, 52 raw pieces of beef meat, 64 hand swabs, and 59 equipment swabs. Samples were tested for isolation and identification of *S. aureus* as previously described.

3 Results

Out of 724 samples (483 food, 120 equipment, 121 hand swabs from food handlers), the isolation rate of *S. aureus* was 11.8, 3.3 and 10.7% from food, equipment and hand swabs, respectively. The prevalence of enterotoxigenic *S. aureus* in the same samples was 3.5, 0.8 and 1.7%, respectively. Among the examined foodstuff, the highest isolation rate for enterotoxigenic *S. aureus* was in raw chicken meat samples (5.1%), followed by cooked chicken samples (3.9%) (Table 2). The following enterotoxins coding genes

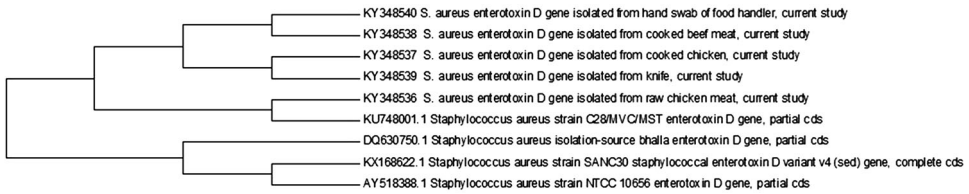

Fig. 1 Phylogenetic analysis of the obtained sequences was carried out based on partial sequence of *S. aureus* enterotoxin D coding gene using neighbor joining approach by MEGA 6 software. *Bootstrap consensus tree* revealed the evolutionary history of these sequences

Table 2 Prevalence of *S. aureus* and enterotoxogenic strains among the examined samples

Samples	No. of examined samples	No. positive to *S. aureus*	%	Numbers positive to enterotoxigenic *S. aureus*	%
Cooked chicken	129	18	14	5	3.9
Raw chicken	117	16	13.7	6	5.1
Cooked meat	123	10	8.1	3	2.4
Raw beef	114	13	11.4	3	2.6
Total food samples	483	57	11.8	17	3.5
Hand swab	121	13	10.7	2	1.7
Equipment swab	120	4	3.3	1	0.8
Total	724	74	10.2	20	2.8

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Table 3 Distribution of *S. aureus* enterotoxin coding genes among enterotoxigenic isolates

Staphylococcal enterotoxins coding gene	No.	%	Sources
SEC	6	30	Raw chicken, raw beef, cooked beef, hand swabs
SED	4	20	Raw chicken, raw beef, cooked beef, hand swabs
SEG	1	5	Raw beef
SEC + SED (together in one isolate)	7	35	Raw chicken, cooked chicken, equipment (knife)
SED + SHE	1	5	Raw chicken
SEC + SED + SEG (together in one isolate)	1	5	Cooked chicken
Total	20	100	

were detected in 20 enterotoxigenic strains SEC, SED, SEG, SEH; some isolates possess more than one SEs genes while none of them carried SEA, SEB or SEE (Table 3). Positive results were recorded in 56 food outlets whereas none of them (56) yielded *S. aureus* when retested after 45 days from application of ISO 22000:2005 with ISO/TS 22002-2:2013 specifications.

4 Discussion

SFP is a common food borne disease and the actual number of cases are beyond the ones reported. Also, it has an economic burden due to hospitalization costs and financial losses for food industries. Therefore, the control of SFP has a great social and economic impact (Argudín et al. 2010**)**. Our results revealed that the prevalence of *S. aureus* and enterotoxigenic strains among examined raw chicken meat samples was 13.7 and 5.1%, respectively. Our results are comparable with those by Madahi et al. (2014), who isolated *S. aureus* in 6.42% of the examined chicken nuggets, where the vast majority of isolates were enterotoxigenic. Much higher results were obtained by Kitai et al. (2005) who recovered *S. aureus* from 65.8% of the examined raw chicken samples while the prevalence of enterotoxigenic strains was 17.6%. On contrary, Bystron et al. (2005) and Pepe et al. (2006) failed to isolate enterotoxigenic *S. aureus* from chicken samples. Furthermore, the recovery rates for *S. aureus* and its enterotoxigenic strains from raw beef meat samples were 11.4 and 2.6%, respectively. Those results were lower than those recently published by Shawish and Al-Humam (2016), that were 26.8% for *S. aureus* and 8% for enterotoxigenic *S. aureus*. It is noteworthy that raw chicken meat showed the highest isolation rates for both *S. aureus* and its enterotoxigenic strains, which underlines that raw chicken meat may be an important source for contamination of kitchen environment with such enterotoxigenic strains.

On the other hand, the high prevalence of *S. aureus* and enterotoxin producing *S. aureus* among cooked chicken and beef meat, which are ready to eat, highlights the potential contamination of these foodstuffs after cooking and during final preparation before serving, which may be owed to improper handling from infected food handlers through direct contact and/or respiratory secretions (Fletcher et al. 2015). Strikingly, SED coding gene sequences from the selected isolates which have been recovered in this study and those retrieved from GenBank showed 100% identity. Whereas the results of phylogenetic analysis revealed that sequences of both cooked chicken and beef as well as sequences from the knife were grouped in the same cluster as the human sequence, and not in the raw chicken sequence. This suggests the incrimination of food handlers in the contamination of not only cooked food but also of kitchen tools such as knife mentioning that this knife isolate was obtained from a knife used only for cutting cooked food. Unexpectedly, none of the isolated *S. aureus* harbors SEA gene a result which was conflicted with other studies that found SEA as a common staphylococcal enterotoxin (Di Giannatale et al. 2011; Madahi et al. 2014). However, some recent studies showed that SEA begins to lose ground against other *S. aureus* enterotoxins (Fagundes et al. 2010; Mashouf et al. 2015). Importantly, this study highlights the efficacy of ISO 22000:2005 for control SFP as all positive food outlets for *S. aureus* and/or enterotoxigenic *S. aureus* yielded negative results when retested after 45 days of application of ISO 22000:2005 with technical specifications ISO/TS 22002-2:2013. The efficacy of ISO 22000:2005 to control SFP may be owed to strict measures which were implemented in this standard such as:

- purchasing food from reputable supplier,
- checking delivery conditions,

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- inspecting food on arrival,
- instant storage,
- adequate personal hygiene,
- applying exclusion policy and visitors' policy,
- reducing handling,
- segregation of high-risk and raw food (color coding),
- effective cooling and defrosting systems, training of food handlers.

Finally, the results of the current study prompt us to conclude that ISO 22000:2005 with technical specifications ISO/TS 22002-2:2013 is an efficient system to control SFP in food outlets regarding food handlers stand behind contamination of cooked food with enterotoxigenic *S. aureus*, whereas raw chicken meat should be considered as a potential source of contamination of the kitchen environment.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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