International Journal of Food Microbiology 211 (2015) 142–147

Contents lists available at ScienceDirect

International Journal of Food Microbiology

journal homepage: www.elsevier.com/locate/ijfoodmicro

Prevalence and diversity of enterotoxin genes with genetic background of *Staphylococcus aureus* isolates from different origins in China

Guoxiang Chao [a,b,*,1], Guangyu Bao [c,1], Yongzhong Cao [a], Wenguang Yan [a], Yan Wang [b], Xiaorong Zhang [a], Liping Zhou [b], Yantao Wu [a,**]

[a] Jiangsu Co-Innovation Center for Prevention of Animal Infectious Diseases and Zoonoses, College of Veterinary Medicine, Yangzhou University, Yangzhou 225009, China
[b] Yangzhou Center for Disease Control and Prevention, Yangzhou 225002, China
[c] Yangzhou No. 1 People's Hospital, Yangzhou 225001, China

ARTICLE INFO

Article history:
Received 5 March 2015
Received in revised form 16 June 2015
Accepted 12 July 2015
Available online 15 July 2015

Keywords:
Staphylococcus aureus
Staphylococcal enterotoxins (SE)
Gene cluster
spa types
Clonal complexes (CC)

ABSTRACT

Staphylococcal enterotoxins (SE) induce toxin-mediated diseases, such as food poisoning. In the present study, 568 isolates from different sources were tested for the prevalence of 18 SE genes and performed *spa* typing. In addition, we characterized the relationships between the distribution of SE genes and molecular clones based on multilocus sequence typing (MLST), *spa* and staphylococcal cassette chromosome mec (SCC*mec*) typing in selected 250 isolates. Approximately 54.40% of the isolates from different sources harbored one or more SE genes forming 120 distinct gene profiles. Seven genes, *sea*, *seb*, *seg*, *seo*, *sem*, *seq*, and *sel* were more frequently detected. The distributions of the SE genes among the isolates from human, animals, and foodborne origins were highly different with isolates from environments ($P < 0.01$). The classic SE genes in both foodborne and human origin isolates were significantly higher than that in animal origin isolates ($P < 0.01$), whereas the prevalence of genes of *egc* cluster and the other genes was similar in human, animal, and foodborne origin isolates ($P > 0.05$). We identified two important gene clusters, *sea–sek–seq*, which is closely related to hospital-acquired (HA) methicillin-resistant *Staphylococcus aureus* (MRSA)-III, and the *egc* cluster, which accounts for nearly half of all genes. Approximately 71% isolates could be typed by *spa*, yielding 103 *spa* types, of which 18 *spa* types were primary types. In clonal complex (CC) 239, an important Asian HA-MRSA-III clone from humans, nearly all isolates harbored complete or partial *sea-sek-seq* cluster; the main *spa* types were t030 and t037. In CC630, an important new community-associated (CA) MRSA-V CC in China, only sporadic SE genes, three main *spa* types, t4549, t2196, and t377 were observed. The *egc* cluster coexisting with other genes was present in isolates of CC5, CC9, CC1281, CC1301, CC30 and sequence type (ST) 25, but completely absent in isolates of CC239, CC59, CC7, and CC88. The results illustrate the genetic clonal diversity and the identity of *S. aureus* isolates from different sources with respect to SE genes and highlight a correlation between SE genes or gene clusters and CCs, *spa*, and MRSA clones. The foodborne and human origin isolates were the main potential causes of classic staphylococcal foodborne poisonings, whereas isolates harboring novel genes were new potential hazards to food safety.

1. Introduction

Staphylococcus aureus is one of the most frequently encountered zoonotic pathogens. This bacterium produced the notable virulence factors, staphylococcal enterotoxins (SE) encoded by SE genes, which can cause both isolated cases of staphylococcal food poisoning (SFP), as well as SFP outbreaks. Human, food animals, food contact surfaces and tools or environments can serve as vehicles for the transfer of *S. aureus* to foods (Argudín et al., 2010; Chao et al., 2014). Currently, 19 SE genes have been identified based on sequence homology. Certain SE genes have been grouped together basing on mobile elements such as prophages, transposons, plasmids, and pathogenicity islands (PIs). For example, *seg*, *sei*, *sem*, *sen*, and *seo* (*egc* cluster) are on *v*Saβ, *sed*, *sej*, and *ser* on pIB485 (Argudín et al., 2010; Varshney et al., 2009).

S. aureus has a highly clonal population structure identified through multilocus sequence typing (MLST) and *spa* genotyping. *spa* genotyping is based on variations of the polymorphic region within the protein A gene (*spa*), while MLST is based on chosen housekeeping genes to determine genetic diversity. *spa* typing and MLST are highly consistent, thus the results of MLST and *spa* can be compared between laboratories (Argudín et al., 2010; Varshney et al., 2009).

In the present study, 568 isolates from different sources were tested for the prevalence of 18 SE genes (*sea* to *see*, *seg* to *sgr*, and *seu*) and *spa*

* Correspondence to: G. Chao, Yangzhou center for Disease Control and Prevention, Yanfu East Road, No. 36, Yangzhou 225002, China.
** Correspondence to: Y. Wu, Yangzhou University, Wenhui East Road, No. 48, Yangzhou 225009, China.
E-mail addresses: cgx1816@sina.com (G. Chao), ytwu@yzu.edu.cn (Y. Wu).
[1] These two authors contributed equally to this work.

http://dx.doi.org/10.1016/j.ijfoodmicro.2015.07.018
0168-1605/

typing was performed. In addition, 250 isolates examined through MLST and staphylococcal cassette chromosome mec (SCC*mec*) typing in a previous study (Chao et al., 2014) were analyzed in the present study to determine SE gene profiling, identify gene clusters, and detect the diversity of SE genes in correlation with the genetic background (MLST, *spa*, and MRSA clones). The aim of the present study was to investigate the distribution of SE genes and gene clusters and identify differences within defined molecular clones from different sources.

2. Materials and methods

2.1. Bacterial isolates

Among the 568 isolates used in the present study, 304 isolates were obtained from human (primarily patients from three tertiary hospitals); 89 isolates were obtained from animals (56 isolates from pigs, and the remaining isolates from animals, such as chickens, dogs, sheep, and pigeons, from animal hospitals); 109 isolates were foodborne (74 isolates from retail food sources, principally from farmers' markets or supermarkets; 35 isolates from raw cow's milk of distinct farms); and 66 isolates were obtained from environments or tools such as hair-cutting equipment, pedicure tools, bedclothes and towels in hotels or barbershops (designed as environments in the present study) (Table 1). All isolates were collected in Jiangsu Province from 2006 to 2013.

2.2. PCR assays for 18 SE genes

A total of 18 toxin genes (*sea*, *seb*, *sec*, *sed*, *see*, *seg*, *seh*, *sei*, *sej*, *sek*, *sel*, *sem*, *sen*, *seo*, *sep*, *seq*, *ser*, and *seu*) were examined through PCR assays as previously described (Varshney et al., 2009). The amplicon sequences were analyzed using DNASTAR software for verification against *S. aureus* database sequences to ensure specificity.

2.3. spa typing

The *S. aureus* protein A (*spa*) repeat region was amplified according to a published protocol (Shopsin et al., 1999). DNA sequencing was performed commercially using GenScript (http://genscript.bioon.com.cn/, Nanjing, China). The *spa* types were randomly assigned using the SpaServer website (http://spaserver2.ridom.de).

3. Results

3.1. Prevalence and distribution of enterotoxin genes

Among the 568 isolates analyzed, 309 isolates (54.40%) harbored 845 SE genes forming 120 distinct SE gene combinations groups. The enterotoxin genes *sea*, *seb*, *sec*, *sed*, *see*, *seg*, *sei*, *sem*, *sen*, *seo*, *seu*, *seh*, *sej*, *sek*, *sel*, *sep*, *seq*, and *ser* were identified in 18.13%, 10.40%, 3.70%, 2.29%, 4.05%, 16.90%, 8.45%, 12.85%, 6.16%, 17.25%, 6.16%, 4.05%, 1.54%, 9.33%, 10.39%, 3.87%, 11.44% and 2.11% of all isolates, respectively. The genes of the *egc* cluster (*seg*, *sei*, *sem*, *sen*, *seo*, and *seu*) accounted for 45.56% (385/845), and classic SE genes (sea, *seb*, *sec*, *sed*, and *see*) accounted for 25.68% (217/845) of the 845 detected genes.

Approximately 54.60% (166/303) isolates from human harbored 492 genes: the classic genes accounted for 26.62% (131/492), and the *egc* genes accounted for 42.68% (210/492). Approximately 61.80% (55/89) isolates from animals harbored 128 genes; the classic genes accounted for 10.94% (14/128), and *egc* genes covered 73.44% (94/128). About 16.67% (11/66) isolates from environments harbored 15 SE genes. Approximately 70.64% (77/109) foodborne strains from raw milk and food harbored 210 genes: the classic genes accounted for 33.81% (71/210), and the *egc* genes accounted for 34.76% (73/210) (Table 1).

Approximately 20.25% (115/568) of the isolates harbored one SE gene forming 14 different profiles, of which the most frequent profiles were *seb*, *sea*, *sel*, *seq*; 10.21% possessed two genes forming 29 different

Table 1
Distributions of the 18 types of SE genes in 568 isolates obtained from different sources.

Sources of strains		No. of strains	Classic SE genes						Non-classical SE genes: *egc* cluster					Non-classical genes: other SE genes							Total	Mean
			sea	seb	sec	sed	see		***seg***[b]	***sei***	***sem***	***sen***	***seo***	***seu***	seh	sej	sek	sel	sep	seq	ser		
Humans (%)		304	67 (22.04)	32 (10.53)	18 (5.92)	6 (1.97)	8 (2.63)		54 (17.76)	34 (11.18)	36 (11.84)	21 (6.91)	47 (15.46)	18 (5.92)	4 (1.32)	4 (1.32)	42 (13.82)	34 (11.18)	13 (4.28)	49 (16.12)	5 (1.64)	492	1.62
Environments[a]		66	1						1		1	3	3					6				15	0.23
Animals	Pigs	56	6						19	5	20		19	10				1		9		89	
	Chickens	13	2						1				2	1	1							7	
	Ducks	2								1	1		1	1			1					5	
	Goose	9	3				1								1			1				6	
	Dogs	2	1															1				2	
	Sheep	6	1						3	1	2		4	3			3	2				19	
	Peacocks	1																					
Total in Animals (%)		89	13 (14.61)				1 (1.12)		23 (25.84)	7 (7.87)	23 (25.84)		26 (29.21)	15 (16.85)	2 (2.25)		4 (4.49)	5 (5.62)		9 (10.11)		128	1.44
Foodborne	Raw milk	74	13	11	3	6	8		14	5	10	9	14	2	9	3	4	14	8	5	6	144	1.95
	Food	35	9	14		1	6		4	2	3	2	8		8	2	3		1	2	1	66	1.89
Total in foodborne (%)		109	22 (20.18)	25 (25.94)	3 (2.75)	7 (6.42)	14 (12.84)		18 (16.51)	7 (6.42)	13 (11.93)	11 (10.09)	22 (20.18)	2 (1.83)	17 (15.60)	5 (4.59)	7 (6.42)	14 (12.84)	9 (8.26)	7 (6.42)	7 (6.42)	210	1.93
Total in all (%)		568	103 (18.13)	57 (10.04)	21 (3.70)	13 (2.29)	23 (4.05)		96 (16.90)	48 (8.45)	73 (12.85)	35 (6.16)	98 (17.25)	35 (6.16)	23 (4.05)	9 (1.54)	53 (9.33)	59 (10.39)	22 (3.87)	65 (11.44)	12 (2.11)	845	1.49

[a] Isolates from hair-cutting equipment, pedicure tools, bedclothes and towels in hotels or barbershops.
[b] The genes of the *egc* cluster are shown in bold italic font.