Typing of *coa* gene polymorphism coding Coagulase in *Staphylococcus aureus* isolated from foodstuffs by PCR and correlation to antibiotic resistance

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Abstract

Background: *Staphylococcus aureus* could be considered a major cause of community acquired infections. Food borne diseases are important problem around the world. Coagulase is expressed in all strains of this bacterium. The main objective of this study was to determine PCR-RFLP *coa* patterns and relationship between *coa* gene and antibiotic resistances in food samples.

Materials and Methods: 1050 food samples were collected during 8 months in Hamadan, Iran. Food samples were studied for *S.aureus*. The antibiotic susceptibility testing was performed by disk diffusion agar. After extraction of genomic DNA, *nuc* and *coa* genes were detected. Finally, with PCR-RFLP method *coa* typing was performed. All statistical analyses were performed using SPSS, version 22

Results: Ninety eight cases (9.33%) of *S. aureus* were isolated. The most frequent resistance was observed to tetracycline (41.8%), erythromycin (38.8%) and gentamicin (36.7%), respectively. *Coa* gene was present in all isolates and had four different patterns. Digestion of the PCR products generated four different restriction patterns for all isolates. The most frequent sizes were 700 bp and 600 bp, respectively

Conclusion: In according to the increased resistance to antibiotics in strains isolated from food samples, rapid and accurate typing of *S. aureus* to identify the transmission of infectious agents is very important. This data can be used to better develop control measures for foods contaminated with *S. aureus* origin.

KEY WORDS: *Staphylococcus aureus*, PCR-RFLP, *coa* gene.

1. INTRODUCTION

Staphylococcus aureus is a leading cause of community acquired infections in the world and has become an important public health problem due to the high resistance to antibiotics and undesirable condition (Mashouf, 2015). Food-borne diseases are considered as a major public health problem, and annually millions of people in the world infected and a part of them may be hospitalized or expired (Chen, 2014; Eshraghi, 2009). *S. aureus* have more than 20 different species which scattered in different habitats and some of them there are in the skin, glands, mucous membranes of animals and humans and transport to the animal products such as; milk, meats and environmental resources such as; soil, sand, dust, air and natural waters (Hosseini, 2015; Hosseini, 2016).

Pathogenicity in *S. aureus* depends on the expression of a wide variety of secreted molecules associated with cell wall of bacteria and escape from host immune system and responses of host tissue (Frenay, 1994). Coagulase, a product of the coagulase gene (*coa*) is expressed in all strains of *S. aureus*. This enzyme, is the basic criterion for the identification of *S. aureus* isolates. The *coa* gene has polymorphic repeat sequences in the 3 ′ coding regions that can be used to differentiate *S. aureus* isolates (Tang, 2000; Mousavi, 2016).

The PCR-RLFP typing technique is a simple and rapid method for monitoring of variations in *S. aureus* isolate. In recent years this technique frequently used for the typing of *S. aureus* isolated from food samples (Zhang, 2013, Afrough, 2012) With molecular typing of this enzyme can prevent of epidemics and reduces the number of infections and costs result from nosocomial infections (Afrough, 2012; Mousavi, 2016; Ahmadi, 2013). Present study aimed to evaluate the relationship between *coa* gene polymorphism and antibiotic resistance with using PCR-RFLP technique in *S. aureus* isolated from food samples.

2. MATERIALS & METHODS

Sample collection: This cross-sectional study was conducted during a period of 8 months from October 2013 to June 2014. 1050 food samples were randomly collected from different parts of the Hamadan province. Each of the samples in sterile containers with special bolts were dumped in the minimum time while maintaining cold conditions was transferred to the microbiology laboratory University of Medical Sciences.

Isolation and identification of *S. aureus:* Samples were subjected to homogenization with the help of vortex after addition of 0.5 to 1 ml of sterile saline at room temperature. The enrichment process perform with help of enrichment broth (QUELAB) containing 3.5% Potassium Tellurite) for *S.aureus* incubated for 24 hours at 37 °C. Then samples cultured in Bird Parker agar medium (Merk) containing 5% egg yolk and tellurite and incubated at 37 ° C for 48 hours. Colonies with black appearance with a bright halo were considered as positive, and cultured on blood agar medium again, and for all of isolates in addition to the gram staining, catalase, coagulase, DNase tests, mannitol

fermentation test using mannitol salt agar medium (QUELAB) and VP test were performed (Mashouf, 2015, Ghannad, 2016). *S. aureus* with ATCC 25423 in all steps was used as a positive control.

Antimicrobial susceptibility testing: Antimicrobial susceptibility testing was performed for all isolates using Kirby Bauer disk diffusion method. Antimicrobial susceptibility of *S. aureus* isolates was determined using gentamicin (10μg), tetracycline (30 μg), trimethoprim / sulfamethoxazole(25 μg), ciprofloxacin (5 μg), erythromycin (15 μg), clindamycin (2 μg), rifampin (5 μg) and cefoxitin (15μg) antibiotics (MAST Laboratories Ltd., Bootle, Merseyside, UK) according to the guidelines of Clinical and laboratory standards institute (CLSI), (Jorgensen and Turnidge, 2015).

DNA extraction and PCR amplification: DNA of *S.aureus* isolates were extracted using commercial DNA extraction kits (Bioflux, Japan) and stored in the freezer -20 °C. For identification of *nuc* and *coa* genes, 2μl of extracted DNA was added to 18μl of PCR reaction mixture (final volume 20μl). The primers sequences for *nuc* and *coa* genes were as follows; F- *nuc:5*- GCGATTGATGGTGATACGGTT-3; R- *nuc:5* AGCCAAGCCTTGACGAACTAAAGC-3,F-coa:5- ATAGAGATGCTGGTACAGG -3; R- coa:5- GCTTCCGATTGTTCGATG -3. PCR cycle program for *nuc* gene was as follows: one cycle for initial denaturation at 94 ºC for 5 minutes, 37 cycles with denaturation at 94 ºC for 1 min, annealing stage in 55 ºC at 30 seconds, elongation step at 72 ºC for 1.5 minute andfinal elongation stage in 72 ºC for 10 minutes; also for *coa* gene, the program was as follows: Initial denaturation: 94°C for 5 min, denaturation at 95°C for 30 seconds, annealing temperature; 55°C for 45 seconds, extension step:72°C for 2 min, 30 cycles and final extension:72 °C in 7 min(Bartels, 2014; Dallal, 2010; Kazemian, 2016; Aydin, 2011).

RFLP of the coagulase (*coa*) gene: PCR products were digested in one sterile micro-tube, 1μl of *Alu*I restriction enzyme was added to 1μl of PCR products. Then 2μl of enzyme buffer 10x added and finally, 16μl of distilled water was added to the final volume (20μl), and then micro-tubes containing the mixture were incubated in 37 °C overnight. After this time, micro-tubes were incubated for 20 min at 65°C for deactivation of the enzyme. In the next step, enzymatic digestion products were electrophoresed for two hours on agar gel 2% in voltage of 90 volts (Rezaei-Soufi, 2016; Zhao, 2012).

3. RESULTS

Frequency of *S. aureus* isolates: In this study, 1050 food samples were studied for *S.aureus* contamination. A total of 98 strains (9.33%) were isolated and confirmed by a nuc-based PCR test.

Result of Antimicrobial susceptibility test: Of 98 *S.aureus* isolates, the most antibiotic resistance was observed to tetracycline (41.8%), erythromycin (38.8%) and gentamicin (36.7%), respectively. also our results showed that *S. aureus* isolates from food samples had very little resistance to cefoxitin (6.1%) which representing the resistance to methicillin antibiotic. The resistance to other antibiotics was as follows; clindamycin with (26.5%), ciprofloxacin (32.7%), and rifampin (25.5%), finally, trimethoprim-sulfamethoxazole (13.2%).

PCR of the *coa* gene: In this study, were detected several different gene amplicons in the different samples, which data are shown in Table 1. Also, the results of electrophoresis of PCR products are presented in Figure 1.

Table.1. Coagulase gene RFLP patterns

Total N (%)	Bucher N (%)	Cheese N (%)	Cream milk N (%)	Chicken meat N (%)	Red meat N (%)	Milk N (%)	Yogurt N (%)	Cream N (%)	PCR product N (%)
7(7.14)	-	3(3.06)	-	-	2(1.96)	2(1.96)	-	-	Bp500
31(31.63)	1(1.02)	8(8.16)	-	4(4.08)	9(9.18)	7(7.14)	1(1.02)	1(1.02)	Bp600
44(44.89)	2(1.96)	5(5.10)	-	5(5.10)	11(11.22)	18(18.36)	1(1.02)	2(1.96)	Bp700
11(11.22)	3(3.06)	2(1.96)	-	1(1.02)	2(1.96)	2(1.96)	-	1(1.02)	Bp800
5(5.10)	-	1(1.02)	2(2.04)	1(1.02)	1(1.02)	-	-	-	Bp850
98(100)	6(6.12)	19(19.38)	2(2.04)	11(11.22)	25(25.52)	29(29.06)	2(2.04)	4(4.08)	Total

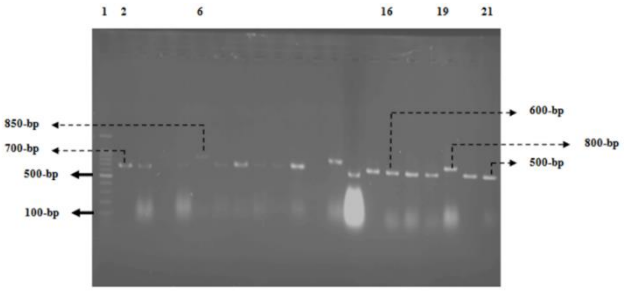

Figure.1. Electrophoresis of *coa* gene products

RFLP PCR of the *coa* gene: After PCR, products were cut using restriction enzymes *Alu*I. The PCR- RFLP patterns of *coa* gene products were different. The frequencies are shown in Table 2.

Table.2. The results of PCR-RFLP for *coa* gene products in *S. aureus* isolates

N (%)	PCR-RFLP pattern	PCR products
7(7/1)	Uncutted	500 bp
30(30/6)	150-350 bp	600 bp
41(41/8)	260-450 bp	700 bp
14(14/3)	150-350-400 bp	800-850 bp
1(1)	Uncutted	850 bp

DISCUSSION

S. aureus is worldwide the most important gram-positive bacteria which have numerous role in producing of various infection. This pathogen is also one of the most common and important cause of food poisoning (Mashouf, 2015). Prevalence of *S. aureus* in this study was 9.3%, which was similar to the study of Soltan Dallal, (9.5%) in Tehran (Dallal, 2010). In contrast, Saadat, reported higher incidence (27%) of *S. aureus* in Tabriz (Saadat, 2014). In the study of Ali aydin, in Turkey, 2011, the prevalence of *S. aureus* in food samples was 13.8% (Aydin, 2011). As well as, in similar study by B. Crago, in Canada during 2007 to 2010, the prevalence rate of *S. aureus* 10.53% was reported (Crago, 2012).

In our study, contamination between dairy products and meat with prevalence rate 62 (9.24%) and 36 (9.49%), respectively, had no significant difference in bacterial abundance ($p > 0.05$). But, in the study of Soltan Dallal, there was significantly difference between the frequency of bacteria isolated from dairy products (17%) and meat products (5/3%), ($p<0.05$), (Dallal, 2010). Another research in Italy in 2014 has presented the prevalence of *S. aureus* in milk and dairy products 39% (Wu, 2010), which is higher than results of present study. Ertas, in Turkey in 2010 have reported the prevalence of 57% (Ertas, 2010). The reason for this high rate of infection, likely is due to the receiving of raw milk from cows or sheep with mastitis disease. Other factors are contaminated equipment, as well as personnel. In addition, production of raw milk and unpasteurized cheese (cottage cheese) is an important concerning factor for development of food poisoning.

In present study, the high resistance was observed to tetracycline, erythromycin and gentamicin antibiotics with frequency 41 (41.8%), 38 (38.8%) and 36 (36.7%), respectively. The results from this study were similar to other studies which conducted on strains isolated from food samples in Iran (Mashouf, 2015). Overall, in comparison of antibiotic resistance in strains that isolated from food samples and hospital isolated, it determined that nosocomial isolates have a much higher proportion of resistance than isolates obtained from community (Normanno, 2007) .So, it can be concluded that the isolates exist in community if entered to the hospital, they can acquire resistance to different antibiotics through genetic elements.

As mentioned previously, after the PCR, four patterns were observed which among them; 700 bp and 600 bp were the most frequent sizes, respectively. In a previous study carried out by Sahebekhtiari, in Tehran and Mashhad, on 111 *S. aureus* isolated from raw milk samples, three different *coa* types were observed after enzyme digestion (Sahebekhtiari, 2011), which were in agreement with results of this study. As well as, our findings are in accordance with a study by Dehkordi, (2015) and Momtaz, (2011) in ChaharMahal VA Bakhtiyari province in west of iran wich reported two different *coa* types (Dehkordi, 2015). This could indicate that the strains isolated from one geographic region have almost identical patterns.

Coelho, (2009) tested 65 *S. aureus* isolates from milk samples of cows with subclinical mastitis in Rio de Janeiro and found four PCR products of 900, 700, 600 and 400 bp, with a 600 bp product being the predominant product (Coelho, 2009). These results are similar to findings of present study.

4. CONCLUSION

In according to the increased resistance to antibiotics in strains isolated from food samples, rapid and accurate typing of *S. aureus* to identify the transmission of infectious agents is very important. With molecular typing of *coa* gene can prevent of epidemics and reduces the infections and costs of nosocomial infections. However, further studies using a large collection of strains are needed to determine the common characteristics of the predominant strains.

5. ACKNOWLEDGEMENT

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Conflict of interest: None declared

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