Journal of Applied Biological Sciences
Uygulamalı Biyolojik Bilimler Dergisi
E-ISSN: 2146-0108, 11 (3): 04-06, 2017

Şanlıurfa'da Yoğurtlarda Metisilin Dirençli *Staphylococcus aureus* (MRSA) Varlığının Real-Time PCR ile Belirlenmesi

Mehmet DEMIRCI[1], Akın YIĞIN[2], Serap KILIÇ ALTUN[3], Hikmet DINÇ[4]*, Serkan EROL[5]
[1]Beykent Üniversitesi tıp Fakültesi Tıbbi Mikrobiyoloji ABD, Istanbul
[2]Harran Üniversitesi Veteriner Fakültesi Genetik ABD, Şanlıurfa
[3]Harran Üniversitesi Veteriner Fakültesi Besin Hijyeni ABD
[4]Harran Üniversitesi Veteriner Fakültesi Farmakoloj ve Toksikoloji ABD
[5]Afyon Kocatepe Üniversitesi Veteriner Fakültesi Medikal Biyoloji ve Genetik ABD, Afyon

*Corresponding Author:
E-mail:hikmetdnc@gmail.com

Received: August 20, 2017
Accepted: November 8, 2017

Özet

Metisilin dirençli *Staphylococcus aureus* (MRSA), çoklu ilaç direncine sahip, insanlarda ve hayvanlarda şiddetli enfeksiyonlara neden olabilen en önemli patojenik mikroorganizmalardan birisidir. Yoğurt vb kontamine olmuş fazlaca tüketilen fermente süt ürünleri, tüketicilerde MRSA enfeksiyonlarının nedenlerinden biridir. Son yıllarda yapılan klonal incelemelerde, insanlardaki enfeksiyonlarda, hayvanlarda saptanan klonal kökenlerin bulunması nedeniyle, bu bakterilerin hayvanlarda ve hayvansal gıda ürünlerindeki epidemiyolojisi önem kazanmıştır. Bu bilgiler ışığında, Urfa bölgesinde üretilen yoğurtlarda *S. aureus* varlığı ve epidemiyolojik olarak MRSA'nın real-time PCR'la saptanması amaçlanmıştır. MRSA'nın real-time PCR ile tespiti için, yoğurtlardan elde edilen DNA'larda mecA geni real-time PCR sisteminde çalışılmıştır. Bu amaçla 9'u ambalajlı, 55 adeti ambalajsız olmak üzere incelenen 64 yoğurt örneğinde sadece ambalajsız olan yoğurt örneklerinin 2 adetinde (%3.13) real-time PCR ile MRSA mecA geni saptanmıştır. Bu çalışmada alınan sonuçlar, Şanlıurfa ilinde satışa sunulan yoğurtlardan direkt izolasyonla elde edilen DNA'larda, real-time PCR ile MRSA gibi insan ve hayvan sağlığı için önemli bir patojenin epidemiyolojik olarak araştırılabileceğini ve bölgede satışa sunulan ürünlerde varolduğunu göstermektedir. Ayrıca hijyen koşullarına dikkat edilmediğinde bu ürünler aracılığıyla ciddi hastalıklara neden olabileceğine de işaret etmektedir.

Anahtar Kelimeler: Kontaminasyon, MRSA, real-time PCR, yoğurt

Identification of Methicillin resistant *Staphylococcus aureus* in yoghurts from Şanlıurfa by real-time PCR

Abstract

Methicillin resistant *Staphylococcus aureus* (MRSA) is one of the most important pathogenic microorganisms that display multiple drug resistance, causing severe infections in animals and humans. Contaminated or raw milk products, such as yoghurt, are one of the root causes of such MRSA infections for the consumers. Recent studies on clonal investigation revealed the same clonal isolates of animals in human infections. Thus, the epidemiology of these bacteria in food products and animals has gained importance. Therefore, the aim of this study was to epidemiologically detect MRSA in yoghurt samples from Urfa via real-time PCR. To detect MRSA via real-time PCR, the mecA gene was investigated on the DNA obtained from the yoghurt samples. Among 64 yoghurt samples, of which 55 were unpackaged and 9 were packaged, the MRSA mecA gene was only detected in 2 (3.13 %) unpackaged samples via real-time PCR. These results showed that it was possible to detect and epidemiologically investigate MRSA, an important pathogen for human and animal health, via real-time PCR from yoghurt samples with direct DNA isolation. In addition, this pathogen can be found in the products sold in Şanlıurfa, which indicates that it may lead to severe diseases in case of consumption, if not conformed to hygienic conditions.

Keywords: MRSA, contamination, real-time PCR, yoghurt

INTRODUCTION

Methicillin resistant S. aureus (MRSA) is one of the most potent pathogenic microorganisms that can cause severe infections in humans, which may also have multidrug resistance [1]. Moreover, MRSAs may also lead to critical infections in animals for instance mastitis in cows and lameness in chickens [2,3,4]. Since the clonal origins found in animals were detected in the infections of humans in the recent clonal evaluations, the significance of their epidemiology in animals and animal products have escalated [5]. These MRSA strains detected in animals carry potential risk to colonize in or infect the consumers [6]. Contaminated or unprocessed milk or dairy products are one of the sources of infection. The MRSA strains found in contaminated dairy products is thought to emerge from the mammary glands of the cows [7]. In Turkey, the number of either family businesses or dairy farm type establishments is quite abundant, which affects the microbiological quality of the milk and dairy products. In the Şanlıurfa province, many medium or small sized enterprises produce milk and dairy products that are available in district bazaars and convenience stores daily. There is no epidemiological information on the MRSA condition in dairy products accessible in this region or Turkey. Therefore, in this study, it was aimed to detect methicillin-resistant S. aureus (MRSA), which is perilous for human health, by the real-time PCR method from the DNA obtained by direct isolation from the yoghurts available in the Şanlıurfa province and to evaluate its epidemiological condition in the yoghurts on the market.

MATERIAL and METHODS

Material

Within the scope of this study, 64 yoghurt samples, 9 packaged weighing 1000gr and 55 unpackaged weighing 500gr, that were produced and put on the market in the Şanlıurfa province in March 2016 were used. Sterile sample

dishes were used for the samples taken from the unpackaged yoghurts, all were brought to the laboratory under cold chain and preserved in -20°C until DNA isolation.

Method

***Staphylococcus aureus* isolation**

From the yoghurt samples, 10 g were transferred to sterile pouches, 90 mL sterile buffered peptone containing water was added and the samples were homogenized in the homogenizer for 3 minutes.Then, dilutions up to 108 decimals were prepared from the homogenized samples. Samples from each dilution were planted onto Baird-Parker Agar (Merck 1.0546) supplemented with Egg-yolk Tellurite Emulsion (Merck 1.03785) and incubated at 37 ° C for 24-48 hours under aerobic conditions [8]. Gram staining, catalase, oxidase, urease and coagulase tests were applied to the black, bright convex colonies in the petri dishes.

DNA extraction from the yoghurt samples

From each sample, 10g yoghurt was mixed with 90ml sterile ¼ Ringer solution (Sigma, St. Louis, USA) and 200µl of each mixture was used for DNA isolation [9].DNA extraction was performed using DNeasy Tissue Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Concentrations of the obtained DNA were measured with NanoDrop ND-1000 (Thermo Fisher Scientific, Wilmington, USA) spectrophotometer. Extracts were preserved at -20°C until the real-time PCR application.

Primer selection specific for the mecA gene for Methicillin resistant *S. aureus* (MRSA) detection

The primer and probe set, specific for the mecA gene that was used, are given in Table 1 [10], which were obtained from TIB MOLBIOL (Berlin, Germany). The final concentrations of the primers were set as 0.5 µM and the probes as 0.2 µM.

Table 1. Oligonucleotide primers and probe sequences used for the MRSA mecA gene detection by real-time PCR.

Primer	Oligonucleotide sequences	Ref.
mecA-F	5'-CAAGATATGAAGTGGTAAATGGT-3'	Sherastha et al. (2002)
mecA-R	5'-TTTACGACTTGTTGCATACCATC-3'	
mecA-HP-1	5'-CAGGTTACGGACAAGGTGAAATACTGATT-[FAM] 3'	
mecA-HP-2	5'[Red 640]-ACCCAGTACAGATCCTTTCAATCTATAGCG-p3	

The amplification and detection of the MRSA mecA gene by Real-time PCR

Real-time PCR applications were performed on the LightCycler 480 II real-time PCR system usingthe Light Cycler Fast Start DNA Master Hybridization Probes Kit (Roche Diagnostics, Mannheim, Germany) according to the manufacturer's instructions. The total volume was set as 20 µl for the PCR application. To each reaction,5 µl (50 ng) Template DNA were added and the PCR protocol given in Table 2 was followed. The ATCC 33592 MRSA strain was used as positive control, and molecular biology grade water (ddH2O) as negative control.

Table 2. MRSA mecA gene detection real-time PCR protocol

Step	Cycle	Temperture, Duration and reading
Denaturation	1 cycle	95°C – 600s - none
Amplification	45 cycles	95°C – 10s – none 50°C – 15s – single reading 72°C – 20s – none
Melting	1 cycle	95°C - 60 s – none 45°C – 60s – none 75°C – continuous reading
Cooling	1 cycle	40°C –30s – none

RESULTS

Among the 64 yoghurt samples evaluated in this study, *S. aureus* was isolated from 3 and MRSA was detected in 2 of them. Results are given in Table 3. The *S. aureus* counts of the samples were noted between 4.60-6.10 log10 kob/g. There was no mecA positivity detected in the packaged products with real-time PCR, yet 2 (3.63%) of the unpackaged products showed mecA positivity. Thus, 62 (96.87%) of the samples were found mecA negative.

The mecA gene amplification curve is shown in Figure I, where the red curves indicate the mecA positive samples and green the mecA negative.

Table 3. The genetic distribution of MRSA-positivity detected by real-time PCR (n (%))

	Culture(+)	Culture(-)	mecA (+)	mecA (-)
Packaged (9)	0 (0)	9 (100)	0 (0)	9 (100)
Unpackaged (55)	3 (5.4)	52 (94.5)	2 (3.6)	53 (96.3)
Total (64)	3 (4.6)	61 (95.3)	2 (3.1)	62 (96.8)

DISCUSSION

Many recent studies indicate that real-time PCR method can be used to detect methicillin-resistant *S. aureus* (MRSA), an important pathogen for humans and animals, in a more rapid and accurate manner, compared to the classical culture and latex agglutination methods currently in use [11,12,13,14,15]. Al-Ashmawy et al.[16], reported MRSA in 35% of yoghurt samples in their mecA gene study in Egypt. Moreover, in their study in Egypt, Fadel and İsmail [17], found that 80% of their yoghurt samples were *S. aureus*

positive, and 50% were MRSA positive. In another study from Egypt, Kamal et al.[18], detected *S. aureus* with a very high rate as 94.3% (33 samples) inraw daily dairy products and in 5 the mecA gene was found using molecular methods. Rahimi[19] reported *S. aureus* in 20 (5.8%) of 347 packaged and unpackaged dairy products from Iran and underlined that there is a significant biological threat for public health in traditional (unpackaged) dairy products. Furthermore, in Colombia, Herrera et al.,[20] isolated MRSA from 8 cheese samples produced from raw cow milks and demonstrated the importance of this pathogen in dairy products. In a study in China, MRSA was found in 11 of 200 raw milk samples that were investigated and its implication for both humans and animals was pointed out by the researchers[21]. In France,Laurent et al.[22]compared the mecA positive S.aureus isolates detected in infected people and animals, and reported that they were similar. In Turkey, Gezgen and Şeker[23] in their study conducted on raw cow milk samples from Izmir, found S.aureus in 32.97% of 972 samples that 4 (2.2%) were mecA positive. Moreover, in this presented study 64 yoghurt samples were evaluated and among unpackaged products, MRSA strains were found in 2 (3.63%). Unpackaged yoghurt samples were collected from the local bazaars and markets in the Şanlıurfa province. The situation can be better examined and the impact of MRSA on public health can be assessed with more extensive further studies covering various regions of Turkey.

CONCLUSION

Finally, the obtained data revealed the presence of MRSA in unpackaged yoghurts available in local bazaars and markets and showed the necessity of hygienic regulations and control for human health in food of animal origin. It also suggested that more extensive studies on the epidemiology of MRSA should be undertaken to determine its prevalence.

REFERENCES

[1] Köck R, Becker K, Cookson B, van Gemert-Pijnen JE, Harbarth S, Kluytmans J, Mielke M, Peters G, Skov RL, Struelens MJ, Tacconelli E, Navarro Torné A, Witte W, Friedrich AW.2010. Methicillin-resistant Staphylococcus aureus (MRSA): burden of disease and control challenges in Europe. Euro Surveill, 15(41).

[2] Graveland H1, Duim B, van Duijkeren E, Heederik D, Wagenaar JA. 2011. Livestock-associated methicillin-resistant Staphylococcus aureus in animals and humans. Int J Med Microbiol, 301(8),630-4.

[3] Petinaki E, Spiliopoulou I. 2012. Methicillin-resistant Staphylococcus aureus among companion and food-chain animals: impact of human contacts. ClinMicrobiol Infect, 18(7),626-34.

[4] Iqbal Z, Seleem MN, Hussain HI, Huang L, Hao H, Yuan Z. 2016. Comparative virulence studies and transcriptome analysis of Staphylococcus aureus strains isolated from animals. Scientific Reports, 6,35442. J Dairy Sci, 99(10), 7872-7876.

[5] Lozano C, Gharsa H, BenSlama K, Zarazaga M, Torres C . 2016. Staphylococcus aureus in Animals and Food: Methicillin Resistance, Prevalence and Population Structure. A Review in the African Continent. Poirel L, ed. Microorganisms, 4(1),12.

[6] Marshall BM, Levy SB .2011. Food animals and antimicrobials: impacts on human health. Clin. Microbiol. Rev, 24,718–733.

[7] Sasidharan S, Prema B, Yoga LL. 2011. Antimicrobial drug resistance of Staphylococcus aureus in dairy products. Asian Pac J Trop Biomed, 1(2):130-132.

[8] Ertaş, N, Serhat, AL, Karadal, F,Gönülalan, Z .2014. Kayseri ilindesatışasunulanmandayoğurtlarınınmikrobiyolojikkalitesi. İstanbul ÜniversitesiVeterinerFakültesi-Dergisi, 40(1), 83-89.

[9] Galanis A, Kourkoutas Y, Tassou CC, Chorianopoulos N. 2015. Detection and Identification of probiotic Lactobacillus plantarum strains by multiplex PCR using RAPD-Derived primers. Int J MolSci, 16(10), 25141-53.

[10] Shrestha NK, Tuohy MJ, Hall GS, Isada CM, Procop GW. 2002. Rapid Identification of Staphylococcus aureus and the mecA Gene from BacT/ALERT blood culture bottles by using the light cycler system. J. Clin. Microbiol , 40(7), 2659-2661.

[11] Rohrer S, Tschierske M, Zbinden R, Berger-Bächi B. 2001. Improved methods for detection of Methicillin-Resistant Staphylococcus aureus. Eur J ClinMicrobiol Infect Dis, 20 , 267–270.

[12] Grisold AJ, Kessler HH. 2016. Use of Hybridization Probes in a Real-Time PCR Assay on the LightCycler® for the Detection of Methicillin-Resistant Staphylococcus aureus Diagnostic Bacteriology Protocols in Volume 345 of the series Methods in Molecular Biology™ , 79-89.Humana Press.

[13] Wada M, Lkhagvadorj E, Bian L, Wang C, Chiba Y, Nagata S, Shimizu T, Yamashiro Y, Asahara T, Nomoto K. 2010. Quantitative reverse transcription-PCR assay for the rapid detection of methicillin-resistant Staphylococcus aureus. J ApplMicrobiol, 108 ,779–788.

[14] Zhang Z, Liu W, Xu H, Aguilar ZP, Shah NP, Wei H .2015. Propidiummonoazide combined with real-time PCR for selective detection of viable Staphylococcus aureus in milk powder and meat products. J Dairy Res, 1625–1633

[15] Onen SP, Elmalı M. 2016. Determination of L.monocytogenes, and its Antibiotic Resistance of Local Produced Cheese Consuming in Hatay. Van Vet J, 27(1), 25-29.

[16] Al-Ashmawy MA, Sallam KI, Abd-Elghany SM, Elhadidy M, Tamura T. 2016. Prevalence, Molecular Characterization, and Antimicrobial Susceptibility of Methicillin-Resistant Staphylococcus aureus Isolated from Milk and Dairy Products.Foodborne Pathog Dis, 13(3), 156-162.

[17] Fadel HM, Ismail J. 2015. Occurrence and zoonotic importance of methicillin-resistant Staphylococcus aureus in raw milk and some dairy products at Ismailia City, Egypt,Hanaa,Zagazig Veterinary Journal (ZVT), 43(3), 95-104.

[18] Kamal RM, Bayoumi MA, Abd El Aal SFA. 2013. MRSA detection in raw milk, some dairy products and hands of dairy workers in Egypt, a mini-survey.Food Control, 33(1), 49–53.

[19] Rahimi E. 2013. Enterotoxigenicity of Staphylococcus aureus isolated from traditional and commercial dairy products marketed in Iran. Braz J Microbiol , 44 (2), 393-399.

[20] Herrera FC, García-López ML, Santos JA. 2016. Characterization of methicillin-resistant Staphylococcus aureus isolated from raw milk fresh cheese in Colombia.

[21] Zhang L, Li Y, Bao H, Wei R, Zhou Y, Zhang H, Wang R. 2016. Population structure and antimicrobial profile of Staphylococcus aureus strains associated with bovine mastitis in China. Microb Pathog,103-9.

[22] Laurent F, Chardon H, Haenni M, Bes M, Reverdy ME, Madec JY, Lagier E, Vandenesch F, Tristan A. 2012. MRSA harboring mecA variant gene mecC, France.Emerg Infect Dis, 8 (9), 1465.

[23] Gezgen C, Seker E. 2016. Investigation of methicillin resistance and Panton-Valentine leukocidin in Staphylococci isolated from bovine mastitis.ActaSci Vet, 44, 01-09.