Detection of methicillin resistance Staphylococcus aureus by Real-Time PCR technique

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Abstract

Real-Time PCR Assay is molecular methods for the rapid identification of methicillin-resistant Staphylococcus aureus (MRSA); it is generally dependent on the detection of an S. aureus-specific target methicillin (mecA gene). However, other methods cannot be applied for the direct detection of MRSA from direct specimens such as nasal samples without the previous isolation or enrichment of MRSA because these samples often contain S. aureus which cannot carry mecA gene. In this study, we used a real-time PCR assay that allows the detection of MRSA directly from clinical specimens (nasal samples) in horse with respiratory infection signs. The Real-Time PCR assay was conducted to amplified highly conserved region 94bp fragment of mecA gene Staphylococcus aureus. The results show specific detection of methicillin-resistant Staphylococcus aureus in nasal samples of horse. We concluded that Real-Time PCR assay provides a rapid and sensitive method which can be used for the detection of MRSA directly from specimens in horse.

تشخيص جرثومة المكورات العنقودية المقاومة للميثيسيلينات من الخيول باستخدام تقنية Real-Time PCR

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الخلاصة

Introduction

Staphylococcus aureus is a major pathogen that causes a wide spectrum of clinical manifestations, such as wound infections, pneumonia, septicemia, and endocarditis (1). Methicillin-resistant Staphylococcus aureus (MRSA) has been reported in a wide variety of animal species and has been referred to as livestock-associated MRSA as in pig, cattle, horses and poultry (2, 3, 4). Beta-lactam antimicrobial agents are the preferred drugs for serious S. aureus infections. However, since the introduction of methicillin into clinical use, methicillin-resistant S. aureus (MRSA) strains have emerged worldwide as important nosocomial pathogens, and the prevalence of these strains in the community is now increasing substantially (5). Methicillin resistance in S. aureus is caused by the acquisition of an exogenous gene, mecA, which encodes an additional ß-lactam-resistant penicillin-binding protein (PBP) (6). In recent years, methicillin-resistant S. aureus (MRSA) has become an infection problem worldwide in both human and animals. Even though the presence of MRSA in humans, companion animals and livestock have been widely documented (7). Another study recorded thr infections of horses

with MRSA of different clonal lineages in Animal hospital of the University of veterinary medicine Vienna (VTH) (8). Conventional screening for methicillin-resistant S. aureus generally relies on plate-based culture methods with or without prior broth enrichment, it is traditional methods are labour intensive and time-consuming and may necessitate a further 2 to 3days to confirm positive MRSA (9). For rapid confirmation of MRSA in pure cultures, several new methods have been developed in recent years, foremost the chromogenic media, the PBP-2a latex agglutination test and the mecA PCR using colonies from overnight cultures (10). Generally, these methods can speed up the identification of MRSA, but they cannot shorten the incubation steps (24–48 h) required after the sample reaches the laboratory. As a result, rapid detection methods and time consuming, Real-Time PCR assay can be used for the detection of MRSA directly from specimens in horse.

Materials and Methods

Samples collection:

A 50 nasal Samples were collected from nasal cavity of clinically infected horse with respiratory signs from different local field in Diwanyia city, by using sterile transport media swabs. Then the samples transport to laboratory for direct genomic DNA extraction.

Genomic DNA extraction:

Bacterial genomic DNA was extracted from 1ml transport media samples in 1.5ml microcentrifuge tubes by using (PrestoTM Mini gDNA Bacteria Kit, Geneaid. USA). Where, the extraction was done according to company instruction. After that, the extracted gDNA was checked by Nanodrop

spectrophotometer, then store in -20C at refrigerator until perform Real-Time PCR.

Real-Time PCR

Real-Time PCR assay was performed by using Syber green dye based amplification of mecA gene Staphylococcus aureus. The Primers were design in this study from Staphylococcus aureus strain 2793G MecA (mecA) gene, partial cds (GenBank: DQ320012.1) by using NCBI GenBank Database and the last version of Primer3 plus design online, then the primers were provided by (Bioneer company. Korea) as show in table (1). Real-Time PCR assay was carried out according to method described by Huletsky (11).

Table (1):

Primer	Sequence		Amplicon
mecA	F	**TCAGGTTACGGACAAGGTGA**	94bp
	R	**GAGGTGCGTTAATATTGCCATT**	

The Real-Time PCR amplification reaction was done by using (AccuPower^{TM} 2X Green star qPCR master mix kit, Bioneer. Korea) and the qPCR master mix were prepared for each sample according to company instruction as following table:

qPCR master mix	Volume
Genomic DNA template	2.5µL
2X Green star master mix	25µL
mecA Forward primer (10pmol)	1µL
mecA Reverse primer (10pmol)	1µL

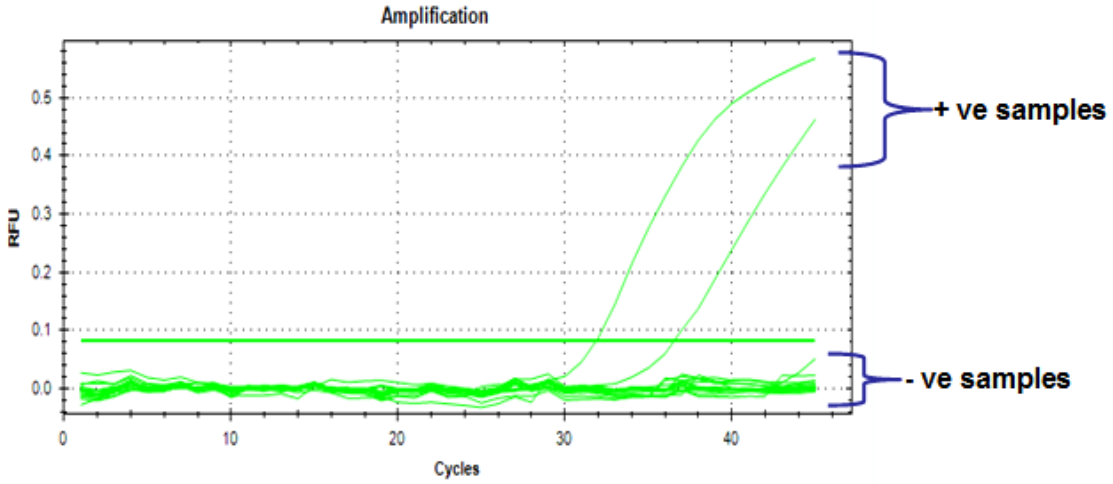
DEPC water	20.5µL
Total volume	50µL

These qPCR master mix reaction components that mentioned in table was placed in sterile white qPCR strip tubes and transferred into Exispin vortex centrifuge for 3minutes, the place in MiniOpticon Real-Time PCR system and applied the following thermocycler conditions as the following table:

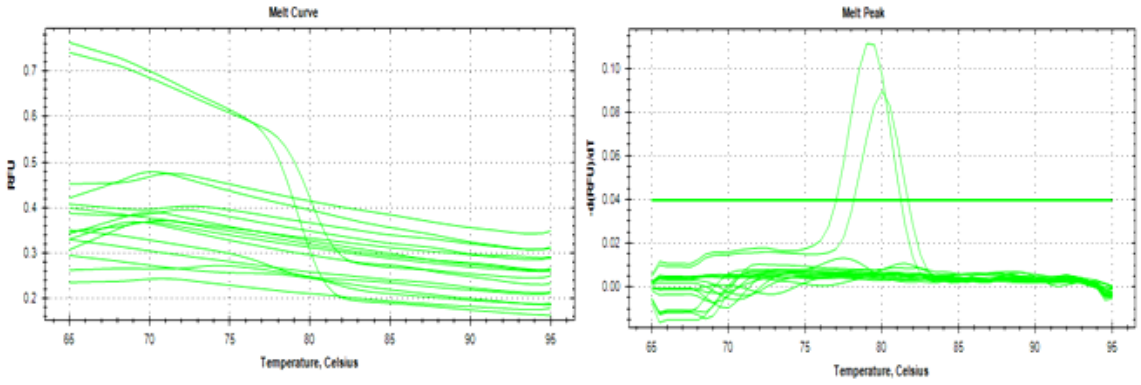
qPCR step	**Temperature**	**Time**	**Repeat cycle**
Initial Denaturation	95 °C	3 minute	1
Denaturation	95 °C	10 sec	45
Annealing\ Extension	55 °C	30 sec	
Detection(scan)			
Melting	60-95°C	0.5 sec	1

Results

Real-Time PCR assay based SYBR Green dye for detection of mecA gene that responsible for methicillin-resistant in Staphylococcus aureus (MRSA) were the results show specific direct detection of mecA gene out from (2/50) from nasal samples of horse with respiratory infection signs. The specificity of mecA gene primers that amplification by SYBR Green dye based Real-Time PCR was determined by dissociation curve (Melt Curve). Where the positive amplification product samples show specific amplification at melt peak mainly at (Tm: 80C°) without primer diamer or nonspecific products. (Figure-1,2,3)



(Fig. 1): Display Real-Time PCR amplification plots that appeared from the (31 to 35 cycles). The samples with amplification appeared at 31 cycles contained large amount of DNA while the samples with the amplification appeared at 35 cycles contained lower quantity of DNA for S. aureus, so the amplification appeared later, samples were negative which appeared under threshold line.



(Fig. 2): Display Real-Time PCR Melt curve that shows the melting point for S. aureus mecA gene ranged from 79.5°C to 80.5°C for positive samples, whereas no melting point for S. aureus mecA gene negative samples.

Well	Fluor	Content	Sample	End RFU	Call
E02	SYBR	Unkn	Staph.aureus	0.378	(+) Positive
G02	SYBR	Unkn	Staph.aureus	0.540	(+) Positive
A01	SYBR	Unkn	Staph.aureus	0.0108	
A02	SYBR	Unkn	Staph.aureus	-0.000279	
B01	SYBR	Unkn	Staph.aureus	0.00442	
B02	SYBR	Unkn	Staph.aureus	-0.00284	
C01	SYBR	Unkn	Staph.aureus	0.00863	
C02	SYBR	Pos Ctrl	Staph.aureus	0.0173	
D01	SYBR	Unkn	Staph.aureus	-0.00602	
D02	SYBR	Unkn	Staph.aureus	-0.000713	
E01	SYBR	Unkn	Staph.aureus	-0.00240	
F01	SYBR	Unkn	Staph.aureus	0.0125	
F02	SYBR	Unkn	Staph.aureus	0.00161	
G01	SYBR	Unkn	Staph.aureus	0.00428	
H01	SYBR	Unkn	Staph.aureus	-0.00196	
H02	SYBR	Neg Ctrl	Staph.aureus	0.00371	

(Fig. 2): Display Real-Time PCR end point amplification positive results of mecA gene in methicillin-resistant in Staphylococcus aureus (MRSA) in horse.

Discussion

Methicillin-resistant Staphylococcus aureus (MRSA) has become very important infection problem worldwide in both human and animals (8). In recent years the transfer of MRSA isolates between animals and humans gained specific attention, especially in the case of MRSA which has been the most commonly reported MRSA strain found in association with livestock (Livestock-associated (LA)-MRSA) (12). MRSA has been isolated from most domestic animal species. MRSA strains from horses, as well as from dogs and cats, typically belong to human S. aureus lineages (13,14). In this study we, focused on molecular detection method for Methicillin-resistant

Staphylococcus aureus (MRSA) in nasal samples of horses, where, the results show that Real-Time PCR assay rapid, specific and sensitive technique in direct detection of mecA gene. These results were agreed with (11) who used New Real-Time PCR Assay as Molecular methods for the rapid identification of methicillin-resistant Staphylococcus aureus (MRSA) are generally based on the detection of an S. aureus-specific gene target and the mecA gene. However, standard culture methods for the identification of S. aureus and the determination of Methicillin susceptibility are time-consuming, usually requiring 2 to 4 days. For these reasons, it has become important to develop rapid diagnostic tests for the detection of MRSA.

The molecular detection of MRSA directly from clinical specimens containing a mixture of staphylococci represents an important challenge for the rapid detection of MRSA carriers (15). To overcome this challenge, we have used a Real-Time PCR Assay which provides a rapid, specific detection mecA in Staphylococcus aureus.

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