

## Detection of Mecithillin Resistant *S. aureus* Gene (*mec-A*) in Egyptian Equine Isolates Causing Toxic Shock Syndrome

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**Abstract:** The current study investigated an outbreak in equine farm (Arabian and foreign breeds) in which multiple drug resistant *S. aureus* was isolated from all excretions and tissues of infected and dead cases. The main objective of our study was to detect the *mec-A* gene using quantitative-PCR analysis to confirm that the multiple drug resistant *S. aureus* is MRSA as well to find a safe method for competing MRSA using probiotics. The results obtained from molecular analysis identified the *mec-A* gene in the tested samples with a great success. Moreover, the results revealed that *L. acidophilus* isolated from colostrums of mare showed great capability of hindrance of MRSA, followed by *L. acidophilus* isolated from goat colostrums. It can be concluded that MRSA strains caused a fatal toxic shock syndrome outbreak in the investigated equine stable.

**Key words:** MRSA · Quantitative-PCR · *Mec-A* gene · Toxic Shock Syndrome · Equine · Probiotic

### INTRODUCTION

*S. aureus* frequently living on the skin or in the nose of healthy animals can cause illnesses ranging from minor skin infections and abscesses, to life-threatening diseases such as pneumonia, meningitis, endocarditis, toxic shock syndrome (TSS) and septicemia which may be rapidly fatal [1, 2]. The importance of methicillin resistant *S. aureus* (MRSA) in veterinary medicine is not well documented [3]. However, MRSA outbreaks in horses suggested that this organism might be an emerging problem in the equine population [4, 5]. MRSA infection has been reported in hospitalized horses [6] and a suspected outbreak was first recorded in one Egyptian equine farm since 2009 [7]. Diagnosis of TSS depends upon; case history, pathological lesions, bacterial isolation, sensitivity assay and molecular techniques for detection of *mec-A* gen. In particular, PCR appears to be a rapid, sensitive and specific assay for *mec-A* gene compared with Southern blot hybridization, macrorestriction and fingerprinting [8, 9]. The aim of our study was further diagnosis of TSS in equine by molecular analysis for the detection of *mec-A* gene as well as to find a safe method for hindrance of isolated MRSA pathogen using probiotics.

### MATERIALS AND METHODS

In a previous study [7], an investigation was carried out on an outbreak in equine stable leading to high mortality rate reaching 18.82% with clinical and histopathological signs of toxic shock syndrome and with bacteriological isolation showing the presence of multiple drug resistant *S. aureus*. Pure *S. aureus* isolates from fecal samples of living equine, caecal and intestinal contents, lung, heart, liver of dead horses from the investigated farm were used for PCR assay.

#### Quantitative PCR

**DNA Extraction from Culture Samples:** According to Vannuffel *et al.* [10] DNA from cultured bacteria was extracted. Six multiple drug resistant *S. aureus* strains were selected for investigating the presence of *mec-A* gene.

**PCR Amplification:** On the basis of the DNA sequences of the *mec-A* gene, the following primers were used in PCR amplification (M1, 5'-TGG CTA TCG TGT CAC AAT CG-3' and M2, 5'-CTG GAA CTT GTT GAG CAG AG-3'), Ten micro liters of DNA samples was added to 90 ml of PCR mixture consisting of 10 mM Tris HCl (pH 8.8), 1.5

mM MgCl<sub>2</sub>, 50 mM KCl, 0.1% Triton X-100, 0.25 mM (each) deoxynucleoside triphosphates, 100 pmol of each primer and 1.25 U of DNA polymerase enzyme (Finnzymes, Inc., Espoo, Finland). After an initial denaturation step (3 min at 92°C), 30 cycles of amplification were performed as follows: denaturation at 92°C for 1 min, annealing at 56°C for 1 min and DNA extension at 72°C for 1 min with an increment of 2 s per cycle. The reaction was achieved with a final extension at 72°C for 3 min. After amplification, 15 µl of PCR samples was loaded on a 2% (wt/vol) agarose gel and horizontal electrophoresis was performed in 0.1 M Tris HCl (pH 8.6)-80 mM boric acid-1 mM EDTA containing 0.5 mg of ethidium bromide per ml. Amplified, ethidium bromide-stained DNA fragments were then visualized on a UV transilluminator at 300 nm.

**In vitro Antimicrobial Activity of Probiotic Bacteria Against MRSA (Well Diffusion Assay):** The *In vitro* antibacterial activity of the tested probiotic strains using agar well diffusion test [11] was carried out as follow;

Muller Hinton agar plates were prepared and inoculated with MRSA strains prepared in conc. Equivalent with 0.5 McFarland and streaked onto the agar plates using sterile swabs and then wells were drilled out using Pasture pipettes. Fifty µl aliquots of cell free culture supernatants in fresh M.R.S. broth of the probiotic strains (*Bifidobacterium*, *L. palantarum*, *L. acidophilus* (buffaloe-cow milk, cow milk, mare colostrum as well as goat colostrum) were suspended in the agar wells. Plates were incubated at 37°C/ 24 hrs under microaerophilic conditions and the diameters of inhibition zones around wells were measured.

## RESULTS

PCR results revealed that all tested strains carried *mec-A* gene (MRSA) (310 bp band) (Fig. 1). Well diffusion assay results displayed that *L. acidophilus* isolated from colostrums of mare showed great capability of hindrance of MRSA, followed by *L. acidophilus* isolated from goat colostrums (Table 1).

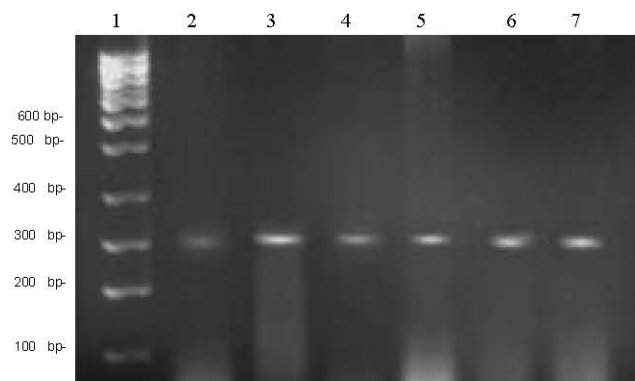


Fig. 1: Gel electrophoresis of DNA fragments isolated from the six multiple antibiotic resistant *S. aureus* strains showing a band at 310 bp representing the *mec A* gene

Table 1: Antimicrobial activity of the probiotic strains against the tested MRSA (reconstruct this table showing the relation between zone of inhibition and % of sensitivity and not the average of the total inhibition zones)

Probiotic strains	Zone of Inhibition in mm of Tested MRSA Strains						Average
	A	B	C	D	E	F	
<i>Bifidobacterium</i>	8.00	0.00	8.00	0.00	0.00	0.00	2.67
<i>L. palantarum</i>	0.00	0.00	0.00	0.00	0.00	0.00	0.00
<i>L. acidophilus</i>	8.00	0.00	9.00	0.00	0.00	0.00	2.83
Buffaloe-cow milk							
<i>L. acidophilus</i>	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Cow milk							
<i>L. acidophilus</i>	10.00	9.00	11.00	10.00	9.00	9.00	9.67
Mare colostrum							
<i>L. acidophilus</i>	8.00	9.00	9.00	9.00	8.00	8.00	8.50
Goat colostrum							
Total	5.67	3.00	6.17	3.17	2.83	2.83	3.945



Fig. 2: MRSA strain (1); Faecal sample of living horse, show sensitivity toward Bifidobacterium, *L. acidophilus* (Buffaloe-cow milk), *L. acidophilus* (Mare colostrums) and *L. acidophilus* (Goat colostrums)



Fig. 3: MRSA strain (3); Lung of dead horse1, show sensitivity toward Bifidobacterium, *L. acidophilus* (Buffaloe-cow milk), *L. acidophilus* (Mare colostrums) and *L. acidophilus* (Goat colostrums)

## DISCUSSION

In the present study, PCR results verified that all the multiple antibiotic resistant *S. aureus* strains isolated from living and dead horses in the investigated farm carried *mec-A* gene and the toxic shock syndrome (TSS) and septicemia affecting horses in the Egyptian equine stable might be caused by these MRSA. On the basis of these results, the quantitative PCR strategy could give rapid and reliable information to clinicians not only for the identification of pathogenic bacteria but also for therapeutic management [10].

These findings agree with Anderson and Weese [12] who found that clinical MRSA infection in horses' ranges from simple skin and soft tissue infections to bacteremia/septicemia, pneumonia, septic arthritis, endocarditis and osteomyelitis. Also, our results agree with the Public Health Agency of Canada [13] which reported that some

strains of *S. aureus* carry toxic shock syndrome toxin 1 (TSST-1) which is superantigen that cause toxic shock syndrome if it is released systemically.

Results of the present work agree with Quinn *et al.* [14] who abused MRSA of being a critical pathogen responsible for a great morbidity and mortality especially among immune suppressed cases. Also, our results agree with Anderson and Weese [12] who mentioned that animals at high-risk of MRSA infection are the immune suppressed, antimicrobially-treated and surgically incised. Our study revealed that MRSA infection may be an emerging disease in the investigated horses stable which in contact with dog farms and subjected to over stress and excessive non effective antibiotic treatments. As horse stable where the outbreak occurred was closely situated near a large dog farm. Dogs are asymptomatic carriers [15] for MRSA therefore they might be accused of being the source of infection. These findings agree with Weese *et al.* [5, 16, 17] who proved that MRSA infection may be an emerging disease in horses, this infection become endemic in horse farms because of the extensive movement of horses, especially thoroughbreds and standard breeds. The probiotic activity of different probiotic strains isolated from different sources was carried out to study the capability of different probiotic strain to hinder the growth of MRSA, results revealed that *L. acidophilus* isolated from colostrums of mare showed great capability of hindrance of MRSA, followed by *L. acidophilus* isolated from goat colostrum. Results agree with Karska-Wysocki *et al.* [18] who studied the antibacterial activity of lactic acid bacteria against MRSA including ten human clinical isolates and MRSA standard strain ATCC 43300 which were tested *in vitro* and reported that lactic acid bacteria give elimination of 99% of the MRSA cells after 24 h of their incubation at 37°C.

In our study, we may suggest that MRSA could be transmitted from animals' fecal contaminant as well as animals' by-products which will act as zoonotically transmitted highly pathogenic bacteria to human. Therefore, our findings imply a modification in the hygienic strategies for handling, decontamination and therapy of MRSA in animal population. Our study investigated an outbreak of MRSA infection in horses suggesting that this organism could cause an emerging problem in equine population. The most effective probiotic strain against MRSA proved to be *L. acidophilus* isolated from Mare colostrum. Researchers recommended that veterinary hospitals initiate surveillance programs for MRSA infections to clarify the role of MRSA in animal (equine) outbreaks.

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