Case Report

Molecular Detection of Methicillin-resistant *Staphylococcus aureus* (MRSA) from a Clinical Case of Myiasis Wound

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Abstract | The resistance of antibiotics to organisms is a matter of global concern. Methicillin-resistant *Staphylococcus aureus* (MRSA) infection is rapidly increasing in both humans and animals. A two and half years old indigenous calf brought with myiasis wound and bacteria associated with myiasis wound was studied and further molecular detection confirms the presence of methicillin-resistant *Staphylococcus aureus* from the outer part of the wound but not from the inner part. The study helps and aware the veterinarians, health-workers, and general people regarding the situation of antibiotic resistance. As maggot helps in the reduction of bacteria, this can be used as medical therapy in the case of a different wound.

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Introduction

Myiasis refers to the infestation of humans and/or animals by dipterous larvae (Maggot) (Juyena et al., 2013). Maggot is the larva of a dipterous insect with legless soft-body. Overall, more than 100 species of dipteran flies are present to cause Myiasis (Penner, 1958). Myiasis wound mostly occurs when fly larvae infest open wounds but not limited to larvae only, this can be infested by all dipteran life stages (Pezzi et al., 2019). Major predisposing factors of this sort of myiasis are poor socioeconomic conditions, extremes of age, and negligence (Huntington et al., 2008). Myiasis is a well-known condition to veterinarians or practitioner from underdeveloped regions and causes severe economic losses globally (Otranto, 2001). The prevalence of myiasis are reported both in human and animals mostly in rural, tropical and subtropical regions but now commonly seen in the temperate zone also (Noutsis and Millikan, 1994; Lwanga et al., 2018).

As with any sort of infestation of parasites, myiasis causes concerns for the possibility of secondary bacterial infection, since certain species of fly and their larvae harbor associated bacteria (Islam et al., 2015). Different gram-positive (*Corynebacterium* spp.) and gram-negative (*Proteus* spp., *Stenotrophomonas* spp., *Brevundimonas* spp.) bacteria are isolated in the case of maggot infestations (Toth et al., 2006). Of particular concern in our case, methicillin-resistant *Staphylococcus aureus* (MRSA) is a gram-positive round shaped, anaerobic bacteria which are genetically distinct from other strains of *Staphylococcus aureus*. MRSA is

usually a bacterium of both humans and animals and often described as community-associated, healthcare-associated, or livestock- associated. Around 10% of the sporadic infections are due to livestock-associated MRSA (Cuny et al., 2015). The MRSA is only susceptible to Vancomycin but it has a carcinogenic effect. Therefore, we report a case of methicillin-resistant *Staphylococcus aureus* isolated from a calf with myiasis wound in Bangladesh, as this is of global concern.

Case presentation

Two and the half-year-old indigenous male bovine calf was brought to Veterinary Teaching Hospital, Bangladesh Agricultural University, Mymensingh having body weight 158 kg with the complaint of a wound. Visual examination revealed the case of myiasis leading to wound.

Materials and Methods

The dressing was performed, swab sample from the wound before dressing was collected using sterile cotton bud both from the outer and inner part of the wound and transferred to the nutrient broth for molecular study (Figure 1). The collected broth samples were incubated at 37 ºC for 2 hours for the enrichment and inoculated into selective media i.e. Mannitol salt agar (MSA) and incubated at 37 ºC for overnight. The next day the growth of bacteria was observed and the pure culture of each bacteria was obtained by repeated culture of a single colony. The pure cultures of isolated bacteria were subjected to Gram´s staining for observation of bacterial morphology, arrangement, and staining characteristics under the light microscope at 10X magnification, as per the method described by Jaman et al. (2018). Bacteria from pure culture subjected to DNA extraction by boiling method, as per described by Hussain et al. (2016) and PCR was done using *S. aureus* specific *nuc* gene and MRSA specific *mec*A gene primers, with expected product size 279 bp and 533 bp respectively. PCR amplification was done by initial denaturation at 95 ˚C for 5 min, followed by 30 cycles of denaturation at 95 ˚C for 1 min, annealing temperature of primers was 55˚C for 45 sec and extension at 72 ˚C for 1 min. The final extension was conducted at 72 ˚C for 10 min. Forward and Reverse primers used to detect the *nuc* gene and *mec*A gene were mentioned in Table 1.

Table 1: *Primers used in PCR for nuc gene and mecA gene.*

Primers	Primer's sequence (5'-3')	Product size	Reference
nuc F	5'-GCG ATT GAT GGT GAT ACG GTD-3'	279bp	(Kalorey et al., 2007)
nuc R	5'-AGC CAA GCC TTG ACG AAC TAA AGC-3'		
*mec*A F	5'-AAA ATC GAT GGT AAA GGT TGGC-3'	533bp	
*mec*A R	5'-AGT TCT GGC ACT ACC GGA TTT TGC-3'		

Figure 1: *Collection of swab sample from myiasis wound.*

Results and Discussion

Based on cultural characteristics and staining properties *Staphylococcus aureus* was identified from the outer part of the wound as on Mannitol Salt Agar (MSA), *Staphylococcus aureus* produces smooth and convex golden yellow colony (Figure 2) and on Gram´s staining, it shows grape-like cluster which is the characteristics of Gram-positive bacteria (Figure 3).

Based on the PCR amplification result, it was further confirmed that the isolated bacteria are *Staphylococcus aureus* and methicillin-resistant *Staphylococcus aureus* by *nuc* gene and *mec*A gene respectively (Figures 4 and 5).

S. aureus is well known causing agents of skin and soft tissue infections as well as food poisoning (Hussain et al., 2016). Methicillin-resistant *Staphylococcus aureus* (MRSA) has emerged worldwide as a significant public health problem both in humans and animals and got zoonotic importance when scientists suggested the possibility of animals serving as reservoirs for human MRSA infection (Garoy et al., 2019).

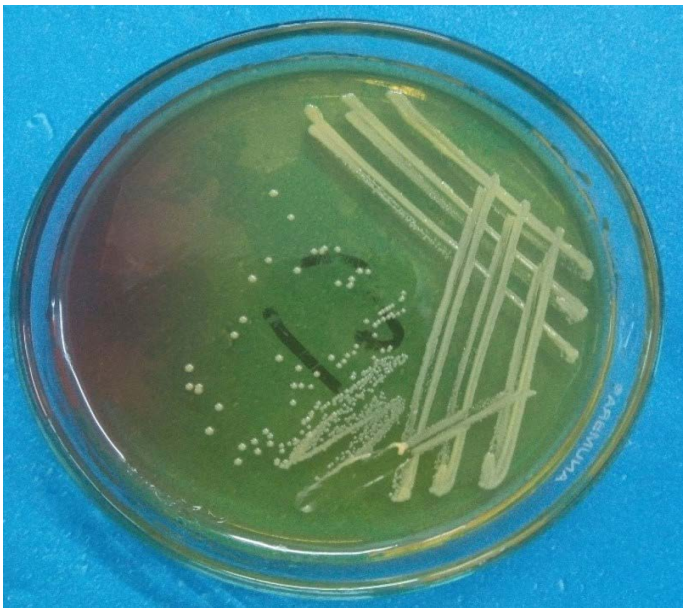

Figure 2: *Fermentation of MSA by Staphylococcus aureus indicated by Smooth and convex formation of a yellowish colony.*

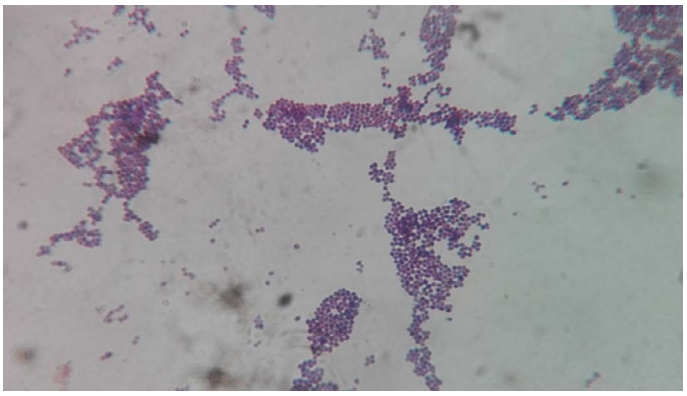

Figure 3: *Staining characteristics of bacteria: Grape like clusters were found which are the characteristics of Staphylococcus aureus.*

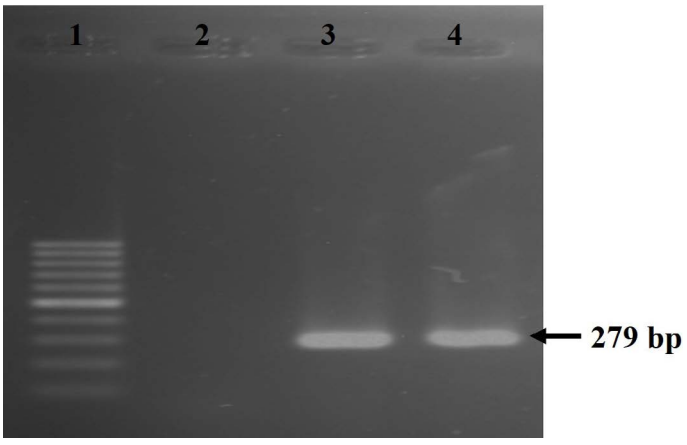

Figure 4: *Results of PCR of Staphylococcus aureus specific nuc gene (size=279 bp). Here, Lane 1: 100 bp ladder, Lane 2: negative control, Lane 3: positive control, Lane 4- amplified nuc gene of S. aureus.*

In our study we reported that MRSA was detected from the outer part of the wound but not from the inner part, this might be because of the unhygienic environmental conditions and untreated condition of myiasis wound. This result is also supported by Islam et al. (2015). The reason for no detection of bacteria from the inner part is maggot itself because maggot does not allow to grow any bacteria and eat dead tissues (Huntington et al., 2008; Arnold, 2013).

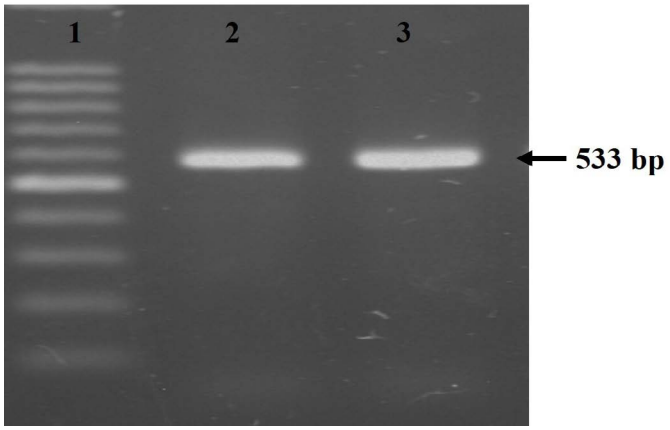

Figure 5: *Amplification of the mecA gene (size=533 bp). Here, Lane 1: 100 bp ladder, Lane 2: positive control, Lane 3: amplified mecA gene of Staphylococcus aureus.*

In conclusion, maggot inhibits the presence of MRSA. So, maggot can be used as medical therapy to make the wound clean. As MRSA is a global concern, the veterinary practitioner should be careful during antibiotic treatment.

Authors Contribution

PM and MMM performed clinical diagnosis and collected samples. PM, MMM, MAHA, AH and VKY performed the laboratory testing for the diagnosis. PM wrote and elaborates the manuscript. MH supervised the study. All authors read the manuscript and agree to be responsible for any aspect of the manuscript.

Conflict of interest

The authors have declared no conflict of interest.

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