Molecular detection of methicillin resistant *staphylococcus aureus* (MRSA) from a clinical case of myiasis wound: a case report

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Abstract

Resistance of antibiotic to organisms is the matter of global concern. Methicillin resistant *Staphylococcus aureus* (MRSA) infection is rapidly increasing in both human and animals. A 2.5-year-old indigenous calf brought with myiasis wound and bacteria associated with myiasis wound was studied and further molecular detection confirms the presence MRSA.

Introduction

Myiasis refers to infestation of humans and/or animals by dipterous larvae [1]. Overall, more than 100 species of dipteran flies are present to cause Myiasis.[2] Myiasis wound mostly occurs when fly larvae infest open wounds. Major predisposing factors of this kind of myasis are poor socioeconomic conditions, extremes of age and negligence[3]. Myiasis is a well-known condition to veterinarians from underdeveloped regions and causes severe economic losses globally [4]. The prevalence of myiasis are reported both in human and animals mostly in rural, tropical and subtropical regions but now commonly seen in temperate zone also [5,6].

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 [7]. Of particular concern in our case, methicillin resistant*staphylococcus aureus* (MRSA) is a gram-positive round shaped, anaerobic bacteria that are genetically distinct from other strains of *Staphylococcus aureus.* MRSA is usually a bacterium of both human 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 [8]. 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 Report

Two and 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 wound. Visual examination revealed the case of myiasis leading to wound. Dressing was performed, swab sample from wound before dressing was collected using sterile cotton bud both from outer and inner part of wound and transferred to nutrient broth for molecular study (Figure 1). The collected broth samples were incubation at 37ºC for 2 hours for enrichment and inoculated into selective media i.e. Mannitol salt agar (MSA) and MacConkey agar (MAC) and incubated at 37º C for overnight. Next day the growth of bacteria was observed and pure culture of each bacteria were obtained by repeated culture of single colony. The pure cultures of isolated bacteria were subjected to Gram´s staining for observation of bacterial morphology, arrangement and staining characteristics under light microscope at 10x magnification, as per the method described by Jaman et al. [9] Bacteria from pure culture subjected to DNA extraction by boiling method, as per described by Khalid et al. [10] and PCR was done using *S. aureus* specific*nuc* gene and MRSA specific *mec* A gene primers, with an expected protocol size 279 bp and 533 bp respectively. Forward and Reverse primers used to detect *nuc* gene and *mec* A gene were mentioned in Table 1.

On the basis of cultural characteristics and staining properties*Staphylococcus aureus* was identified from outer part of wound as on Mannitol Salt Agar (MSA), *Staphylococcus aureus* produce golden yellow colony and on Gram´s staining it shows Gram positive, smooth, convex, grape-like clusters (Figure 2 and Figure 3). On the basis of PCR, it was further confirmed that the isolated bacteria are *Staphylococcus aureus* and methicillin resistant *staphylococcus aureus* by *nuc* gene and *mec* A gene respectively. (Figure 4 and Figure 5)

Discussion

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

In our study we report MRSA was detected from the outer part of the wound but not from 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. [7] The reason for no detection of bacteria from inner part is maggot itself, because maggot not allow to grow any bacteria and eat dead tissues. [12]

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

Authorship

PM, MMM: Performed clinical diagnosis and collected samples. PM, MMM, MAHA, AH and VKY: Performed the laboratory testing for the diagnosis. PM: Wrote and elaborate the manuscript. MH: Supervised the study. All authors: Contributed to drafting and revision of the manuscript and agree to be responsible for any aspect of the manuscript

Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this manuscript.

Reference

1. Zumpt F. Myiasis in man and animals in the Old World. A textbook for physicians, veterinarians and zoologists. Myiasis in Man and Animals in the Old World. A Textbook for Physicians, Veterinarians and Zoologists. 1965.
2. John D, Petri W, Detro K. Concerning a rabbit cuterebrid, the larva of which may penetrate the human skin (Diptera: Cuterebridae). *J Kansan Entomol Soc* . 2006; **8** :328-334.
3. Huntington TE, Voigt DW, Higley LG. Not the usual suspects: human wound myiasis by phorids. *J. Med. Entomol* . 2008;**45** (1):157-159.
4. Otranto D. Myiasis: devils in the flesh of humans and animals.*Hosp Prac* . 2001; **53** :98-102.
5. Noutsis C, Millikan LE. Myiasis. *Dermatol Clin* . 1994;**12(4)** :729-736
6. Lwanga A, Anis M, Ayoubi M, Sharma J, Khosla P. Two cases of myiasis associated with malignancies in patients living in the continental United States. *Cureus* . 2018;**10(1)** .
7. Islam MT, Al-Maruf A, Mannan MA, Rahman HM, Tarafder MM, Samad MA, Al Noman A, Hossain MB, Rahman MM. Isolation and identification of associated bacteria and maggots from myiasis affected wounds of cattle and goats in Bangladesh. *J Adv Vet Anim Res* . 2015;**2(2)** :95-100.
8. Cuny C, Wieler LH, Witte W. Livestock-associated MRSA: the impact on humans. *Antibiotics* . 2015; **4(4)** :521-543.
9. Jaman MM, Mishra P, Rahman M, Alam MM. Clinical and laboratory investigation on the recurrence of the umbilical hernia after herniorrhaphy in bovine calves. *J Bangladesh Agril Univ* . 2018;**16(3)** :464-470.
10. Hussain K, Rahman M, Nazir KHMNH, Rahman H, Khair A. Methicillin resistant Staphylococcus aureus (MRSA) in patients of community based medical College Hospital, Mymensingh, Bangladesh. *Am J Biomed Life Sci* . 2016; **4(3)** :26-29.
11. Kalorey DR, Shanmugam Y, Kurkure NV, Chousalkar KK, Barbuddhe SB. PCR-based detection of genes encoding virulence determinants in Staphylococcus aureus from bovine subclinical mastitis cases. J Vet Sci. 2007; **8(2)** :151-154.
12. Arnold C. New Science Shows How Maggots Heal Wounds. *Scientific American.* 2013.

List of tables:

Table 1. Primers used in PCR for *nuc* gene and *mec* A gene.

Primers	Primer's sequence (5'-3')	Product size	Reference
nuc F	5'-GCG ATT GAT GGT GAT ACG GTD-3'	279bp	[11]
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	[11]
*mec*A R	5'-AGT TCT GGC ACT ACC GGA TTT TGC-3'		

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

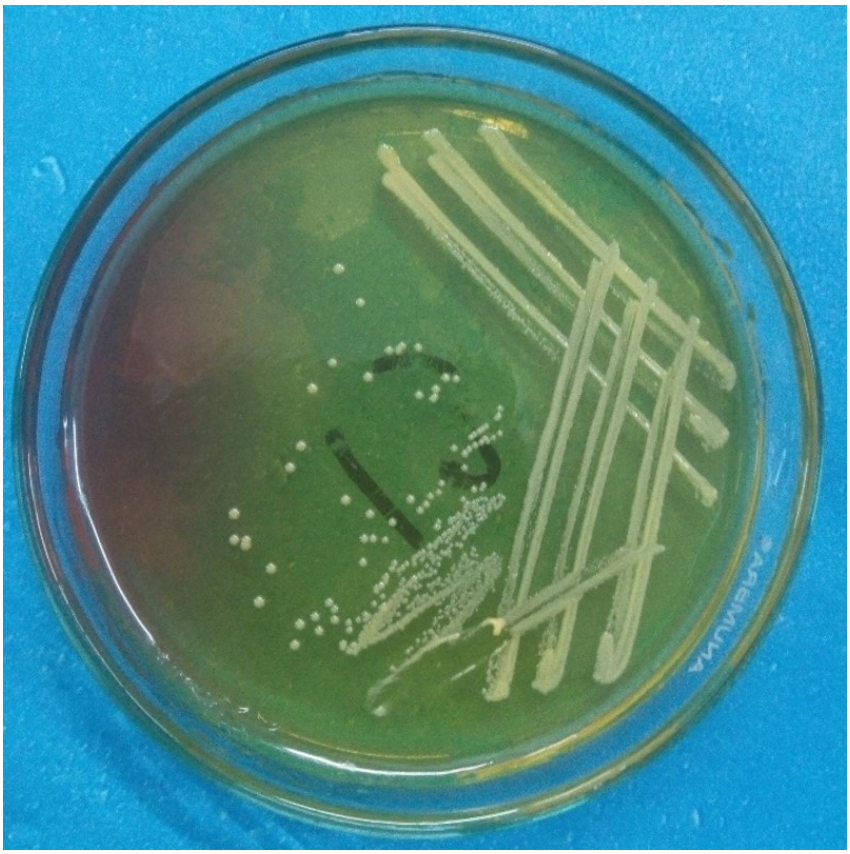

Figure 2: Fermentation of MSA by *Staphylococcus aureus* indicated by formation of yellowish colony

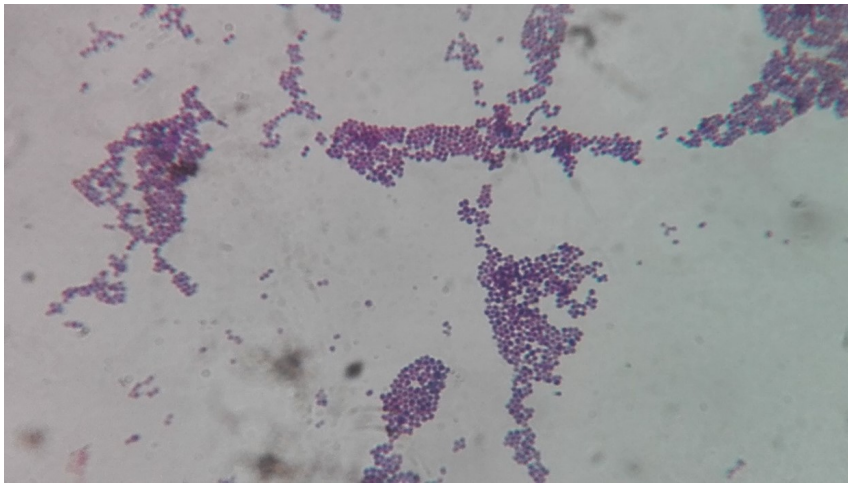

Figure 3: Smooth, convex, grape-like clusters of *Staphylococcus aureus* on Gram's staining

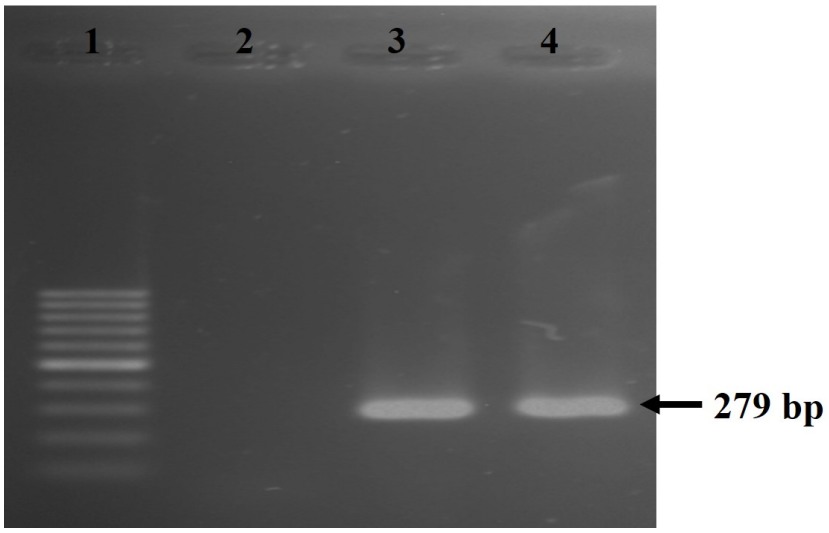

Figure 4: Results of PCR of *S. aureus* specific nuc gene (size=279 bp). Here Lane 1- 100 bp ladder, Lane 2- negative control, Lane 3- positive control, Lane 4- amplified nuc gene of *S. aureus*

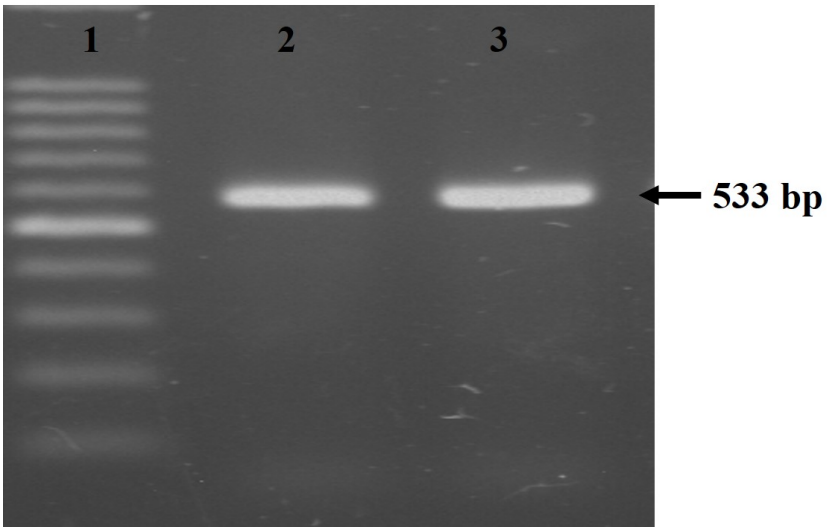

Figure 5: Amplification of *mec*A gene (size=533 bp). Here Lane 1- 100 bp ladder, Lane 2- positive control, Lane 3- amplified *mec*A gene of *S. aureus*