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Concurrent Mithicillin Resistant *Staphylococcus aureus* (MRSA) and *Candida* ocular infection in a BALB/c mouse: A case report

Vinod Kumar Singh[1], Rahul Kumar[2], Amit Kumar[1]and Sharad KumarYadav[1]

[1.]Department of Veterinary Microbiology,
[2.]Department of Veterinary Pathology,
College of Veterinary Science and Animal Husbandry, DUVASU,
Mathura (UP)-281001, India

Corresponding author:
Vinod Kumar Singh,
Assistant Professor, Department of Veterinary Microbiology,
College of Veterinary Science & AH, DUVASU, Mathura
Email : vet.vinodsingh@gmail.com

Abstract

Polymicrobial infections tend to be more complex and results in aggressive forms of diseases that often exhibit multi drug resistance and impact the therapeutic measures. Despite the severity of such infections, polymicrobial etiology represents a neglected concept in making a clinical diagnosis. In present report, we describe a case of ocular infection associated with *Staphylococcus aureus* and *Candida albicans* in a female BALB/c mouse. This report not only underscores the importance of polymicrobial infection but also highlights the occupational risk of methicillin-resistant *Staphylococcus aureus* to research and animal care personnel's in animal research facility

Key words: Mouse; Ocular infection; Polymicrobial; MRSA; *Candida*

Introduction

The ocular mucosa lies at the interface of environment and the host immune system, is continually exposed to bacteria, viruses, and fungi. These pathogens can infect the eye either by direct introduction through trauma, by hematogenous dissemination or by extension from adjacent infected tissues (Shelite *et al*., 2014). Among the vast number of pathogens, *Staphylococcus aureus* is the second most commonly isolated bacteria from bloodstream infections (Goetghebeur *et al*., 2007). Many fungi are also often found associated with opportunistic eye infections in humans and among the yeast etiology; *Candida* remains the most common cause worldwide (Safneck, 2012). The present report describes a case of ocular candidiasis with concurrent methicillin resistant *Staphylococcus aureus* (MRSA) infection in a female BALB/c mouse.

Case Presentation

The Department of Veterinary Microbiology was consulted for culture and antimicrobial sensitivity test of ocular infection in a female BALB/c mouse for its rational treatment. The mice were being maintained in the laboratory animal house of the institute (Reg.No. 386/PO/ReBi/SL/01/CPCSEA) as per the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) for teaching and research. Anamnesis also revealed that the mouse was observed with epiphoric right eye during routine monitoring and was treated with gentamicin-dexamethasone eye-drop twice a day for 3 days as therapeutic measure, but no improvement was observed. The close clinical examination of mouse revealed progressively inflamed eyelids and adnexa with mucopurulent discharge from both the eyes (**Fig. 1a and 1b**). The samples were collected from both the eyes as described by Jarvis (1973) for microbiology. The samples were streaked over Blood-agar (BA) and Sabouraud dextrose agar (SDA) media and were incubated overnight aerobically at 37°C. The samples revealed growth on both BA and SDA media and the cultural, morphological and biochemical characters of the colonies were suggestive of *S. aureus* and *Candida albicans*. While the isolates were being characterized for species identity, the mouse was treated with insiltation of chloramphenicol (0.5%) and amphotericin B (0.15%) three to four times a day. The mice responded well to the treatment with reduced to no discharge from the eyes in 5 days to non inflamed and normal eyes within 10 days of treatment.

The *S. aureus* isolate was also confirmed by PCR amplification of 280 bp of *nuc* gene (**Fig. 2a**) specific for *S. aureus* as described by Jayshree *et al.* (2016). The germ tube test was

performed for confirmation of *Candida albicans* and was found positive (**Fig. 2b**). *In-vitro* antimicrobial sensitivity testing of *S. aureus* isolate by disc diffusion method as per the guidelines of Clinical and Laboratory Standards Institute (CLSI), reveal susceptibility to ciprofloxacin, gatifloxacin, moxifloxacin, norfloxacin, clindamycin, doxycycline, cefquinome, ceftriaxone, cefoperazone/sulbactam; intermediate sensitivity to cefotaxime, bacitracin, vancomycin and resistant to amikacin, gentamicin, colistin, amoxyclav, ampicillin/cloxacillin, ceftazidime/tazobactam, ceftizoxime, cefepime/tazobactam. The methicillin resistant was further confirmed by PCR amplification of *mec* A gene as described by Yadav *et al*. (2016) and was found positive (**Fig. 2c**). The *C. albicans* isolate was found sensitive to amphotericin B and resistant to ketoconazole, miconazole, clotrimazole and fluconazole.

Discussion and conclusion

Authors corroborate the isolation of multiple drug resistant *C. albicans* and *S. aureus* from ocular infection in a female BALB/c mouse. Eye infections are common in rodents and among the various infectious causes; fungal infections continue to be a challenge for clinicians, both in terms of diagnosis and treatment. In the present case, the mouse was observed for the ocular affection. The microbiological observations of the samples from eyes of the affected mouse revealed presence of *S. aureus* and *C. albicans*. Earlier, Saunders (1967) reported *S. aureus* isolation from eyes of rats. Young and Hill (1974) reported the isolation of *Pasteurella pneumotropica* while Needham and Cooper (1975) reported the isolation of *Pasteurella pneumotropica* and *S. aureus* from infected eyes of rats and mice respectively. *Candida* has been reported to be commonly involved in ocular infections in humans (Motukupally *et al*., 2015) but no clinical case has been reported in mice as per the available literature. However, the animal model of ocular infection by *Candida* and various other pathogens of human are well established in mice (Arendrup *et al*., 2002) indicating the susceptibility of mice to these pathogens. The involvement of the *Candida* concomitantly with multi-drug resistant *S. aureus* in the present case is one probable reason for the failure of initial treatment with eye drop containing steroid. The immunosuppressive effect of steroid in the eye drop explains the progression of infection in the eye as reported by Mitisui and Hanabusa (1955). Ley and Sanders (1956) inferred that cortisone facilitates the existing fungal infection. The therapeutic failures are high in ocular fungal infections due to delayed or misdiagnosis, poor drug penetration in eye, resistance to antifungal drugs, host factors like compromised immune system (Kunimoto *et al*., 1999) and polymicrobial etiology, similar to the case in the present study. It was also reported that *C. albicans* stimulate infection in mice by a number of bacteria when both organisms are inoculated intra peritoneally (Carlson, 1983). *In-vitro*, *C. albicans* has been found to enhance the growth of a number of bacteria, including *S. aureus* (Staib and Geier, 1971). Moreover, a synergistic effect of *C. albicans* and *S. aureus* on mouse mortality has also been reported (Carlson, 1983). In clinical candidiasis, *C. albicans* is frequently reported associated with *S. aureus* and other *Streptococcus species* (Palestine *et al*., 1983). In addition, *S. aureus* has been found to be a frequent opportunist in experimentally induced candidiasis (Staib *et al*., 1976). As per the available literature, a few clinical case of staphylococcus ocular infection have been reported in mice (Needham and Cooper, 1975) and among the different farm animals the bacteria are reported to be a major bacterial cause in eye infection (Udegbunam *et al*., 2014). *Candida* infections are generally treated with amphotericin B, although flucytosine and fluconazole have also been recommended for the treatment of *Candida* infections of the eye in humans (Hamada *et al*., 2013). The *C. albicans* and *S. aureus* isolates from eye in the present study responded well to insiltation of chloramphenicol (0.5%) and amphotericin B (0.15%) which were also found effective in *in-vitro* testing. The improvement observed in the condition of eyes with provided treatment further supported the findings. Although several drugs are being used for treatment, very limited literature is available regarding the antifungal susceptibility of *Candida* isolated from ocular infections (Therese *et al*., 2006) and there is a need of regular screening of ocular infections for the *Candida* etiology followed by antifungal drug susceptibility to formulate guidelines for the treatment of ocular candidiasis.

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Figure 1a and b: Mouse having inflamed eyes with mucopurulent discharge

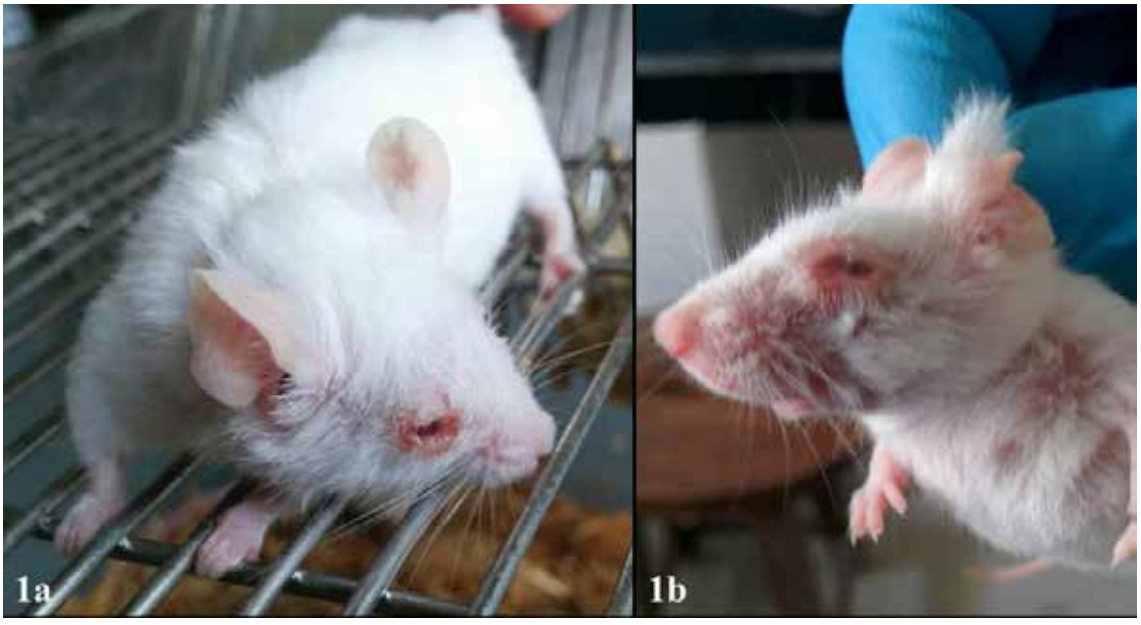

Figure 2: a. PCR amplification product of 280 bp *nuc* gene specific for *S. aureus* **b.** Germ tube formation (400x) **c.** PCR amplification product of 533 bp *mec* A gene responsible for methicillin resistance. Ladder used in AGE is100 bp.