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SHORT NOTE

MICROBIOLOGY AND HISTO-PATHOLOGY OF MANDIBULOFACIAL ABSCESS IN A BALB/c MOUSE

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Among the various laboratory animals, mice and rats with their extensive use in bioscience research, have achieved an unparalleled level. These animals are no different from others with respect to the possibilities of adventitious and opportunistic infections. These organisms usually do not cause any clinical disease but instead causes physiological alterations rendering the animals unsuitable for experimental studies. Although the number and prevalence of these pathogens have declined considerably, but many are still around and may infect the colony. Facial abscess is the one among such infections and the present report describes the microbiology and histopathology of a mandibulofacial abscess. Earlier, a few cases of natural occurrence of maxillofacial abscesses in mice has been reported (Lawson, 2010); however, the present case is first of its kind with dual infection of methicillin resistant *Staphylococcus aureus* (MRSA) and *Pseudomonas aeruginosa* resistant against multiple antimicrobial drugs.

During routine monitoring of the mice kept for teaching and research, a swelling was noticed on the right side of lower jaw of a female 6 months old female BALB/c mouse, which progressed into a mass of about 1 cm diameter within 5-6 days (Fig. 1a). On examination, a well circumscribed abscess sac with caseous material was observed. The material was aspirated aseptically for cytological and microbiological examinations. The mouse was treated with systemic ciprofloxacin (Cipla Ltd., Mumbai, India). Although, the abscess wound started healing, the mouse was found dead after four days. The carcass was thoroughly necropsied but no appreciable gross lesions in any visceral organs except mild congestion was found. The abscess cavity wall containing cutaneous tissue was collected along with the visceral organs in 10% neutral buffered formalin till fixation. The tissues were then cut and washed overnight in running tap water, dehydrated in ascending grades of alcohol, cleared in xylene and embedded in paraffin for routine histopathology (Luna, 1968). Thin sections of 4-5 µm were cut and stained with H&E stain for microscopic examination (Luna, 1968).

Microscopic examination of aspirated material smear stained with Gram's stain showed Gram-positive cocci and Gram-negative rod-shaped bacteria. The smear stained with Leishman stain revealed aggregates of bacterial cells surrounded by inflammatory cells. The infiltrating cells were predominantly polymorphonuclear cells with transition from polymorphs to mononuclear cells. The aspirated material was inoculated on blood agar medium which revealed the growth of *S. aureus* and *P. aeruginosa*, confirmed on the basis of morpho-cultural and biochemical properties (Holt *et al*., 1994). The identity of isolates was confirmed by polymerase chain reaction (PCR) amplification of 280 bp of *nuc* gene, specific for *S. aureus* (Fig. 1b) and 618 bp of 16S rRNA specific for *Pseudomonas* (Fig. 1c), as described by Jayshree *et al*. (2016) and Singh *et al.* (2017), respectively. *S. aureus* isolate DUVASU/MRSA-1 (MH092064) and *P. aeruginosa* isolate DUVASU/4580 (KY930657) were used as positive control and nuclease-free water as negative control in PCR amplification. Further, the PCR product of *Pseudomonas* isolate was sequenced and identified as *P. aeruginosa* on the basis of sequence homology in BLAST analysis (submitted to Genbank; accession No. KY930661). *In vitro* anti-microbial susceptibility test by disc diffusion method revealed *S. aureus* isolate to be MRSA with resistance against methicillin, ceftriaxone/sulbactam, ceftriaxone/tazobactam, cefepime/tazobactam,

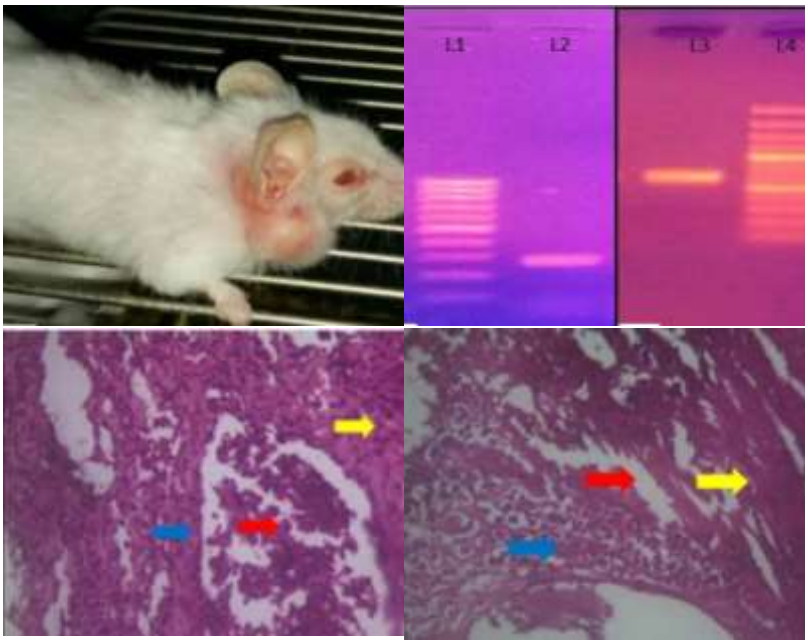

Fig. 1: a) Affected mice with development of abscess of about 1 cm dia; b) PCR amplification product of 280 bp *nuc* gene specific for *S. aureus* (Lane 1: 100 bp DNA ladder; Lane 2: test *S. aureus* isolate); c) PCR amplification product of 618 bp 16S rRNA gene specific for *Pseudomonas* spp. (Lane 3: test *Pseudomonas aeruginosa* isolate; Lane 4: 100 bp plus DNA ladder); d) A section of abscess cavity showing micro-abscess (red arrow) surrounded by fibrous connective tissue (yellow arrow) and infiltration of inflammatory cells (blue arrow) in the surrounding subcutaneous tissue. 400x, H&E; e) A section of cutaneous lesion showing fragmentation (red arrow) and hyalinization of muscle bundles (yellow arrow) with myofilbrils infiltrated with mononuclear and poly-morphonuclear inflammatory cells (blue arrow). 400x, H&E

cefoperazone/ tazobactam, cefuroxime, ceftazidime/ tazobactam, cefotaxime, gentamicin and amoxycillin/ clavulinic acid while susceptible to doxycycline, clindamycin, gatifloxacin, ciprofloxacin, ofloxacin and trimethoprim/sulphamethoxaz ole. *P. aeruginosa* isolate was found susceptible to amikacin, gentamicin, tobramycin, ciprofloxacin, ofloxacin, gatifloxacin, gemifloxacin, cefquinome, imipenem and moxifloxacin and resistant to cefepime/ tazobactam, cefoperazone/ sulbactam, ceftazidime/ tazobactam, ceftazidime, ceftriaxone, cefotaxime, peperacillin and polymixin-B (HiMedia, Mumbai, India). Histo-pathology revealed severe infiltration of inflammatory cell in the wall of abscess with few developing and well-formed micro-abscesses containing pus. The abscess wall was lined by fibrous connective tissue (Fig. 1d) and the surrounding tissue was severely infiltrated with mono-nuclear inflammatory cells, suggestive of aged lesion. The subcutaneous muscular tissue showed fragmentation of muscle bundles with hyalinization of muscle fibers and infiltration of mononuclear and polymorphs in-between the myofibril at few places (Fig. 1e). However, the histology of visceral organs revealed no significant changes.

Although the occurrence of abscesses in mice has earlier been reported (Hong and Ediger, 1978), the overall reports of facial abscesses are only a few (Clarke *et al*., 1978; Lawson, 2010). Blackmore and Francis (1970) isolated *Staphylococci* of several phage types from a variety of lesions, including abscesses at various sites and suggested that the most probable route of infection was oral mucosa. Wilson (1976) reported the occurrence of abscesses in masseter muscle of a HT strain mice associated with *Pasteurella pneumotropica*. Needham and Cooper (1976) isolated a variety of phage types of *S. aureus* from abscesses of bulbourethral gland and observed indifferent strains of barrier-maintained mice. The mandibulofacial abscess in present case was found associated with *P. aeruginosa* and MRSA. *P. aeruginosa* is as an opportunistic pathogen with ability to develop multi-drug resistance. At present there is no report describing the isolation of *P. aeruginosa* alone or associated with other pathogens from mandibulofacial abscess in mice. However, the association of *S. aureus* in abscesses affecting the face and jaws of mice and mandibular abscess has been reported (Clarke *et al*., 1978), suggesting oral mucosa as the probable route of infection. Recently, a study on 139 facial abscess cases with 30 maxillofacial abscesses and 9 mandibulofacial abscesses has

proposed the etiopathogenesis of mandibulofacial and maxillofacial abscesses as *S. aureus* inoculation in gingival sulcus of molar teeth from masticated hair fragments due to barbering or grooming activities (Lawson, 2010). This proposed etiopathogenesis could be a probable explanation for the isolation of *S. aureus* and *P. aeruginosa* from mandibulofacial abscess as barbering has also been observed in the present case. Histopathological findings are in coherence with Clarke *et al*. (1978) and Joiner *et al.* (1980) for abscess associated with *S. aureus* and *B. fragilis* which further support the isolation of *S. aureus* and *P. aeruginosa* as etiology behind the abscess formation. The isolation of multiple antimicrobial resistant MRSA and *P. aeruginosa* from laboratory mice depicts the importance of occupational risk to research and animal care personnel in animal research facility. Similarly, a clinical case of MRSA in pigs used for experimental purposes in a research animal setting was found associated with spread of infection to research staff (Sergio *et al*., 2007). In recent years, MRSA infection has occurred in various animals, with increasing incidence of transmission between animals and human (Walther *et al*., 2006). Recognizing the possibility of early diagnosis of multiple antibiotic resistant bacterial infections is important to prevent the spread to other animals, including human, in research facilities.

Ethical Statement: The clinical case described was part of an IAEC approved project with approval No. 12/02/2016 vide letter No. 110/IAEC/16 dated 16.09.2016. Also, the authors declare that they have no conflict of interests.

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