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Pathogenicity of *Staphylococcus sciuri* in murine *in vivo* model

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This study was conducted to assess the pathogenic potential of exfoliative toxin gene-positive *Staphylococcus sciuri*. Twenty eight eight weeks-old mice were used for the study. The animals were randomly assigned into four groups of 7 mice per group. Groups 1 and 2 were infected with 1 × 10^8 colony-forming unit (cfu)/ml of the organism intraperitoneally (i.p) and subcutaneously (s.c) respectively, while groups 3 and 4 were inoculated with sterile phosphate buffered saline (PBS) i.p and s.c, respectively. Groups 3 and 4 served as the controls. The mice were monitored daily for 15 days for skin lesions, morbidity and mortality, and rectal temperatures. The body weights of the individual mouse were also taken at two days interval. The mean values of rectal temperatures and body weights were subjected to one-way analysis of variance (ANOVA) and a value of $p<0.05$ was considered significant. The morbidity rates for groups 1 and 2 were 57 and 71%, respectively, while mortality rate for both groups was 11%. No mortality was recorded in groups 3 and 4. Skin lesions were recorded in all the mice in *S. sciuri*-infected groups, while no skin lesions were recorded in the control groups. Significant ($p<0.05$) increase in rectal temperatures were recorded in the infected groups compared to the control groups. The body weight significantly ($p<0.05$) reduced in infected groups against the controls. This study has shown that exfoliative toxin gene-containing *S. sciuri* is potentially pathogenic.

Key words: *Staphylococcus sciuri*, skin lesion, mortality.

INTRODUCTION

Coagulase-negative staphylococci (CoNS), with the exception of *Staphylococcus saprophyticus*, are generally considered to be bacteria of doubtful pathogenic potential in humans and animals (Stepanovic et al., 2003). They have long been regarded as harmless skin commensals and dismissed as culture contaminants (Shittu et al., 2004). But with the substantial rise in the incidence of CoNS reported as causative agents of nosocomial infections following the use of prosthetic valves and other invasive technologies (Peters, 1988; Huebner and Goldmann, 1999; Petinaki et al., 2001; Shittu et al., 2004), a lot of interests in the pathogenic potential of these microorganisms have been generated.

Amongst the CoNS, *Staphylococcus sciuri* (*S. sciuri*) is

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widely distributed in nature and found principally as skin commensal in animal species (Kloos, 1980; Devriese et al., 1985; Stepanovic et al., 2001a; Nagase et al., 2002), but they may colonize humans, and their isolation from various human clinical specimens has been reported (Kloos, 1980; Kloos et al., 1997; Masou et al., 1999; Couto et al., 2000; Nagase et al., 2002). However, from the clinical standpoint, most *S. sciuri* isolates recovered from humans have not been considered important (Shittu et al., 2004) and at present, only seven cases of *S. sciuri* infection have been established in humans. These include serious infections such as endocarditis (Hedin and Winderstrom, 1998), peritonitis (Wallet et al., 2000), septic shock (Horii et al., 2001), urinary tract infections (Stepanovic et al., 2003), wound infections (Stepanovic et al., 2002; Shittu et al., 2004, Coimbra et al., 2011), pelvic inflammatory disease (Stepanovic et al., 2005) and bacteraemia (Ahoyo et al., 2013).

In animals, *S. sciuri* has been reported to be an invasive pathogen where it caused wound infections (Adegoke, 1986; Devriese, 1990), mastitis in goat (Poutrel, 1984) and cow (Rahman et al., 2005), and a highly fatal exudative epidermitis in piglets (Chen et al., 2007). The isolation of *S. sciuri* from these clinical specimens has generated a lot of interest in the potential of this microorganism to cause infections in humans and animals.

The ability of *S. sciuri* to cause these wide array of diseases, has been attributed to its capacity to produce virulent factors such as deoxyribonuclease (DNase), biofilm, clumping factor, proteinase, lipase, delta toxin, capacity to stimulate nitric oxide production (Stepanovic et al., 2001b) and ability to harbour exfoliative toxin gene (Chen et al., 2007).

Although there are reports on infections caused by *S. sciuri* and possible virulent factors involved, its pathogenic potentials have not been fully investigated. Moreover, its effects on body organs have not been assessed and the exact contribution of its virulent factors in the course of infection is still controversial. The aim of the present study was to investigate the effects of exfoliative gene-positive *S. sciuri* on the skin and some internal organs of mice.

MATERIALS AND METHODS

The experimental protocol used in this study was approved by the Ethics Committee of the University of Nigeria, Nsukka, and conforms with the guide to the care and use of animals in research and teaching of University of Nigeria Nsukka, Enugu State, Nigeria.

Animals

Eight-week-old mice of both sexes, weighing between 19 and 22 g were obtained from the laboratory animal unit, Faculty of Veterinary Medicine, University of Nigeria, Nsukka. They were fed on commercial growers mash (Guinea feeds®) and water was provided *ad libitum*. These mice were acclimatized for 2 weeks in the animal house at the Department of Veterinary Pathology and Microbiology, University of Nigeria, Nsukka.

Pathogen and preparation of inoculum

A strain of *S. sciuri* isolated from the skin of an apparently healthy dog in Nsukka, Nigeria and fully identified and characterized at the Molecular Biology and Biochemistry laboratory, University of Logrono, Spain (Courtesy of Prof. Carmen Torres) was used in the study. The *S. sciuri* strain used is positive for exfoliative toxin A (*eta*) gene. The isolate was maintained on nutrient agar slant at 4°C in the Microbiology unit of the Department of Veterinary Pathology and Microbiology, University of Nigeria, Nsukka. Prior to use, the strain was sub-cultured on mannitol salt agar, incubated aerobically at 37°C for 24 h. Colonies were homogenized in sterile PBS and the turbidity adjusted to correspond to 0.5 McFarland's turbidity standard (equivalent to 1×10^8 cfu/ml).

Animal groups and infection

Twenty-eight adult eight weeks-old mice were randomly assigned into four groups of seven mice per group. Groups 1 and 2 were infected with 1×10^8 cfu/ml exfoliative toxin-positive *S. sciuri* intraperitoneally (i.p.) and subcutaneously (s.c.) respectively, while groups 3 and 4 were similarly inoculated with sterile PBS and served as the controls.

General observation, sample collection and bacterial culture

The animals were observed daily for fifteen days post-infection (dpi) and their physical condition, rectal temperature, morbidity and mortality were recorded. The weights of the individual animals were taken at two days interval. Skin lesions and internal organs (spleen, kidney and liver) of mice that developed skin lesions and/or died were processed for bacteria isolation and histopathology.

Positive culture was confirmed if the morphology and biochemical characteristics of the isolated strain were identical with that of the inoculum. No colony growth in 48 hours was regarded as a negative culture. Systemic infection was defined by positive culture of *S. sciuri* from one or more internal organs.

Histopathology

Formalin-fixed and paraffin wax-embedded sections of skin lesions, liver, spleen and kidney were stained with haematoxylin and eosin (H and E).

Statistical analysis

The data collected for morbidity and mortality were subjected to descriptive statistics and expressed in percentages, while the mean values of temperature and body weight parameters were subjected to one-way analysis of variance (ANOVA). Statistical analysis was performed at 5% probability level.

RESULTS

Effect of *S. sciuri* infection on clinical signs and skin lesions

Mice inoculated i.p became lethargic by 2 dpi and they

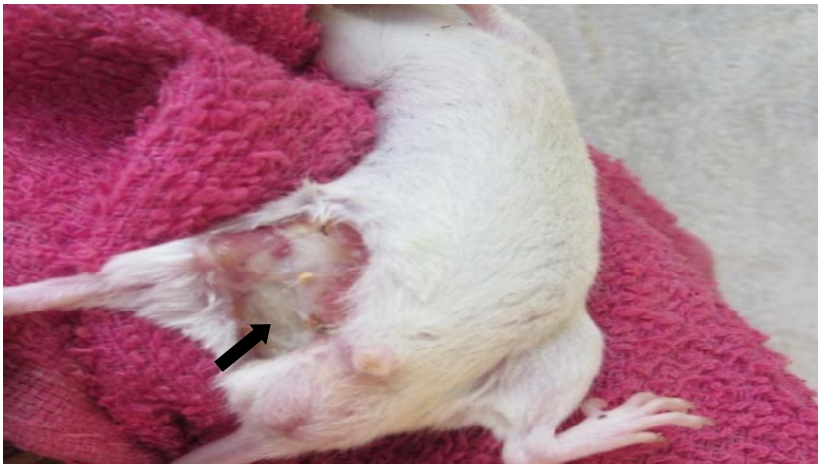

Figure 1. Sloughed skin in mouse infected intraperitoneally (i.p) with *S. sciuri* at 8 dpi (arrow).

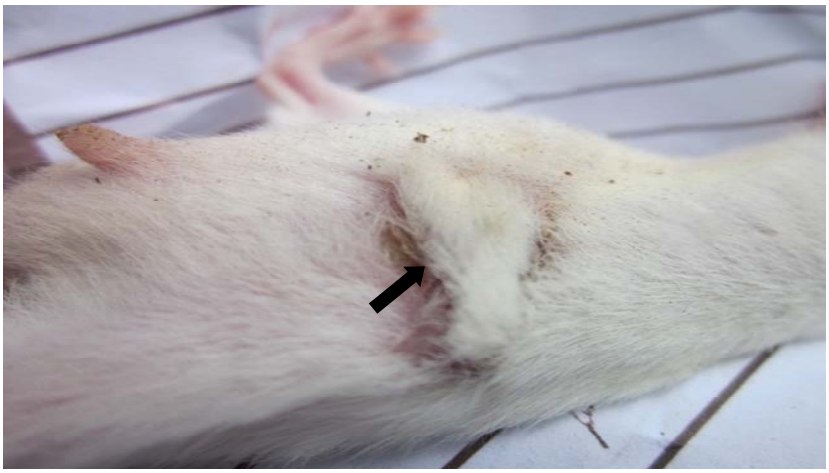

Figure 2. Sloughed skin in mouse infected subcutaneously (s.c) with *S. sciuri* at 7 dpi (arrow).

also had ruffled hair coat, reduced feed and water intake. Some of the mice developed swollen limbs. These signs lasted till end of the experiment by which the morbidity was 57%. In s.c inoculated mice, similar clinical signs were observed, however morbidity by 15 dpi was 71%. These signs were not observed in any of the mice in the control groups.

Skin lesions which started as erosions and then progressed to ulcerations developed in all the mice in the *S. sciuri*-infected groups by 6 to 8 dpi (Figures 1 and 2). Healing of the skin lesions started by 9 dpi in both groups and by 15 dpi, healing was complete (Figure 3). No skin lesions were observed in mice in the control groups.

Death of 3 mice occurred by 5 and 6 dpi in groups 1 and 2 respectively, which gave 11% mortality rate for both, while no mortality was recorded in the control groups. Positive bacterial cultures were obtained from skin lesions and internal organs of all the mice in *S. sciuri*-inoculated groups, while negative cultures were observed in the control groups.

Grossly, the liver of all the mice in the infected groups appeared pale and contained multiple abscesses, visible on both the parietal and visceral surfaces. Microscopically, the important changes observed in the skin of all the mice in the *S. sciuri*-infected groups involved mainly the dermis, characterized by the appearance of dermal abscesses which appeared as necrotic areas admixed with numerous dead and viable polymorphonuclear leucocytes, cellular debris, bounded peripherally by granulation tissue (Figure 4).

Microscopically, their livers showed a mild to moderate periportal leucocytic infiltration comprising of predominantly polymorphonuclear leucocytes, macrophages and a few lymphocytes (Figure 5). Occasionally, multifocal

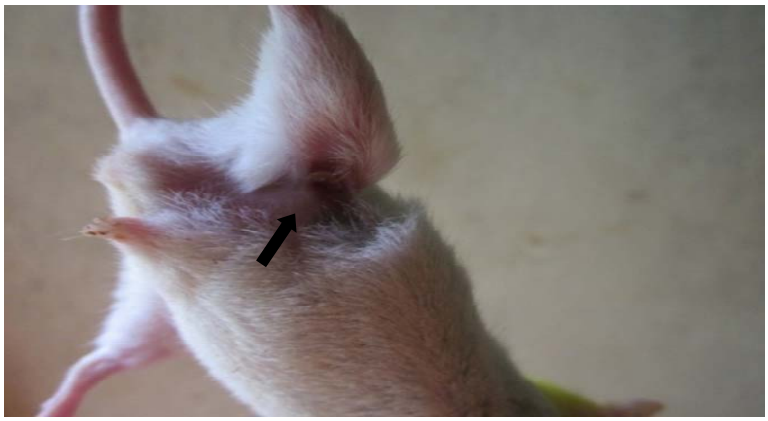

Figure 3. Healed skin in mouse inoculated i.p with *S. sciuri* by 14 dpi (arrow).

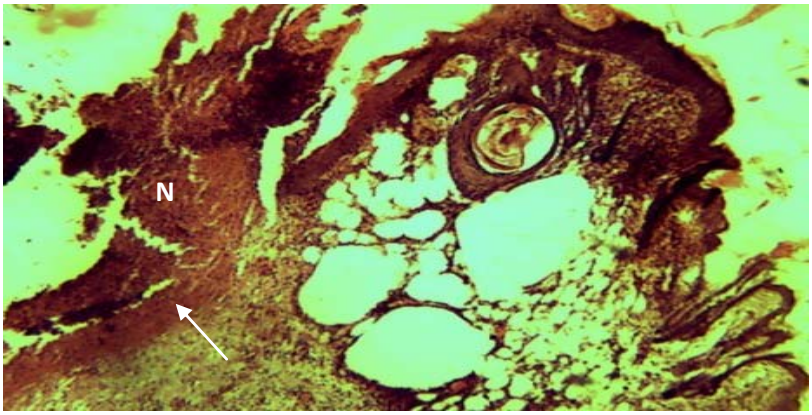

Figure 4. Skin showing necrotic area (N) admixed with polymorphonuclear leucocytes and cellular debris, bounded peripherally by granulation tissue (arrow).

microabscesses were found, which appeared as multiple aggregates of neutrophils (Figure 6).

Effect on body weight

Changes in the mean body weight of *S. sciuri*-infected and uninfected mice are presented in Figure 7. Significant ($p<0.05$) variations were observed in the mean body weights of mice in the four groups. The mean body weights of mice in the infected groups were lower than those of the uninfected groups.

Effect on rectal temperature

Figure 8 shows the effects of *S. sciuri* on the rectal temperature. The mean rectal temperatures of mice in the infected groups did not vary significantly ($p>0.05$) from each other (groups 1 and 2). They varied significantly from those of the uninfected groups (group 3 and 4).

DISCUSSION

The morbidity rates of 57 and 71% recorded for the intraperitoneally and subcutaneously-infected mice respectively, suggest that the *S. sciuri* isolate used has some pathogenic potential. The pathogenic capability of the inoculum is further supported by the mortalities (11%) as well as the skin lesions recorded in the infected groups.

The mortalities by 5 to 6 dpi which coincided with development of skin lesions by 6 dpi in the infected groups may have resulted due to septicaemia and/or toxaemia. *S. sciuri* isolates have been reported to be invasive pathogens capable of producing variety of toxins (Stepanovic et al., 2001b).

The *S. sciuri* strain used in this study is positive for

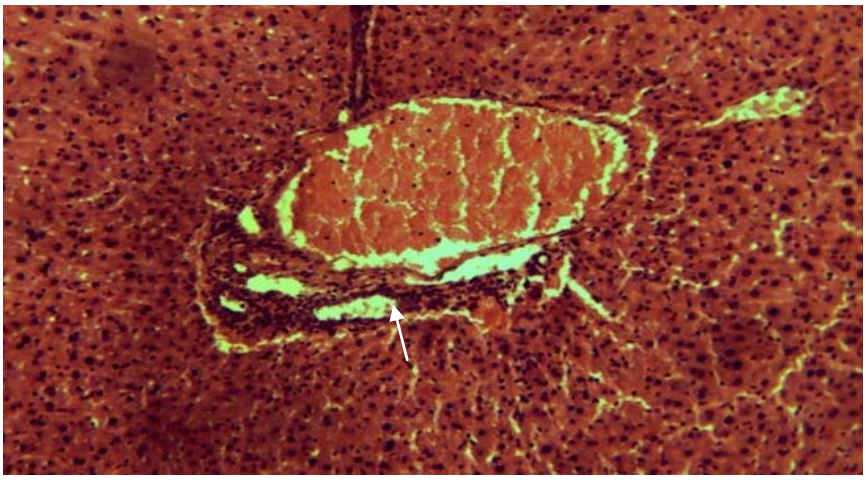

Figure 5. Section of the liver of i.p inoculated mouse showing a mild periportal leukocytic infiltration (arrow) Mag x100 (H and E).

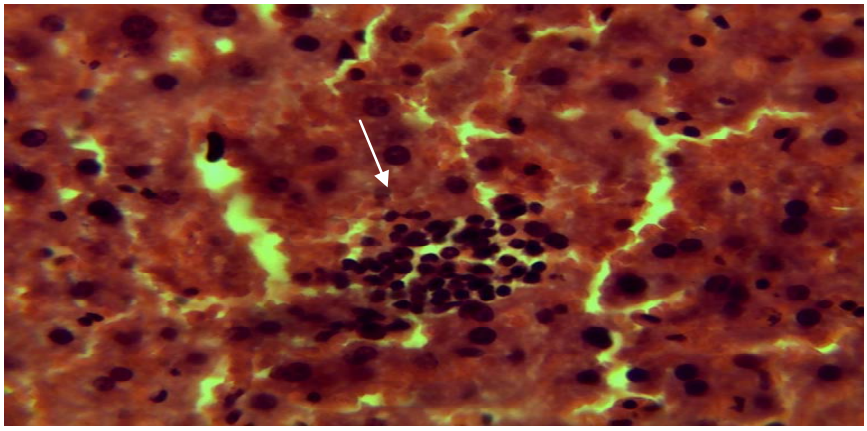

Figure 6. Section of the liver of s. c inoculated mouse showing an aggregation of leukocytes within the liver parenchyma (arrow) Mag x400 (H and E).

exfoliative toxin A gene (*eta*), which encodes exfoliative toxin A production (Sheehan et al., 1992). Exfoliative toxin A has been reported as one of the virulent factors associated with skin lesions produced by coagulase-positive staphylococci such as *S. hyicus*, *S. pseudintermedius* (*intermedius*) and *S. aureus* in pigs, dogs and man respectively (Quinn and Markey, 2003; Bukowski et al., 2010). Therefore, sloughing of the skin of animals in the infected groups observed at 8 dpi may be due to production of exfoliative toxin by the *S. sciuri* isolate inoculated into the animals. This toxin has also been reported to be a possible virulent factor in *S. sciuri* infection (Chen et al., 2007).

Progression of the skin lesions from erosions to ulceration may be due to the ability of the inoculated *S. sciuri* strain to stimulate nitric oxide (NO) production. This capacity has been reported as a possible mechanism of pathogenicity in the course of *S. sciuri* infection (Stepanovic et al., 2001b). Nevertheless, healing of the skin lesions without treatment by 10 dpi may suggest a kind of self-limiting infection. It may also be that the animals had tremendous ability for skin regeneration. However, in natural infections, secondary infection of the skin lesions by pyogenic bacteria such as *S. aureus* and *Pseudomonas aeruginosa* may delay their healing (Qiunn and Markey, 2003).

Significant ($p<0.05$) variations in the mean body temperatures (with temperature of infected groups higher than that of control groups at each observation period) of mice between 1 and 13 dpi suggests that the *S. sciuri* was able to invade the animals resulting to bacteraemia and fever. The increased body temperature may also be due to production of toxins by the *S. sciuri* strain. But the lack of statistical difference observed by 14 and 15 dpi coincided with the healing period; this suggests that bacteraemia may have receded by that time. Although

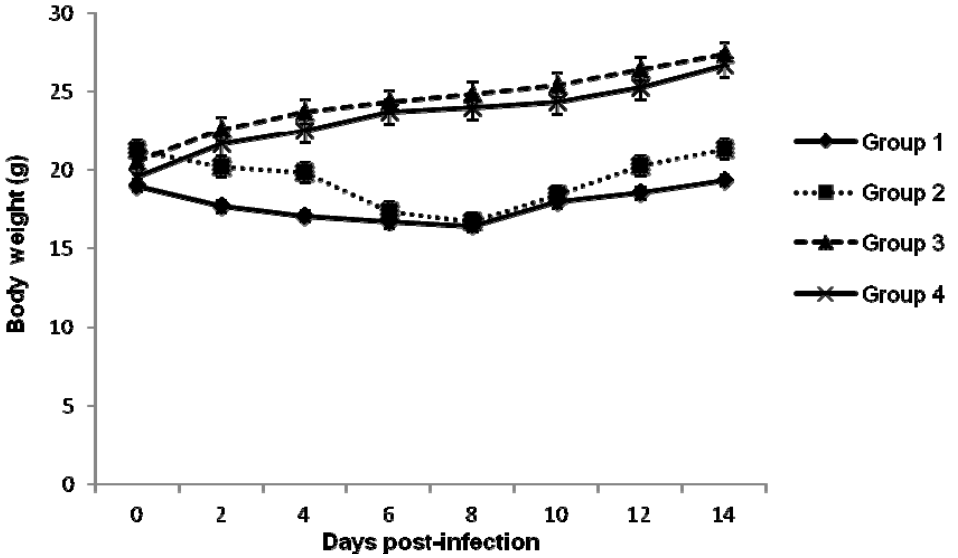

Figure 7. Effect of *S.sciuri* on body weight.

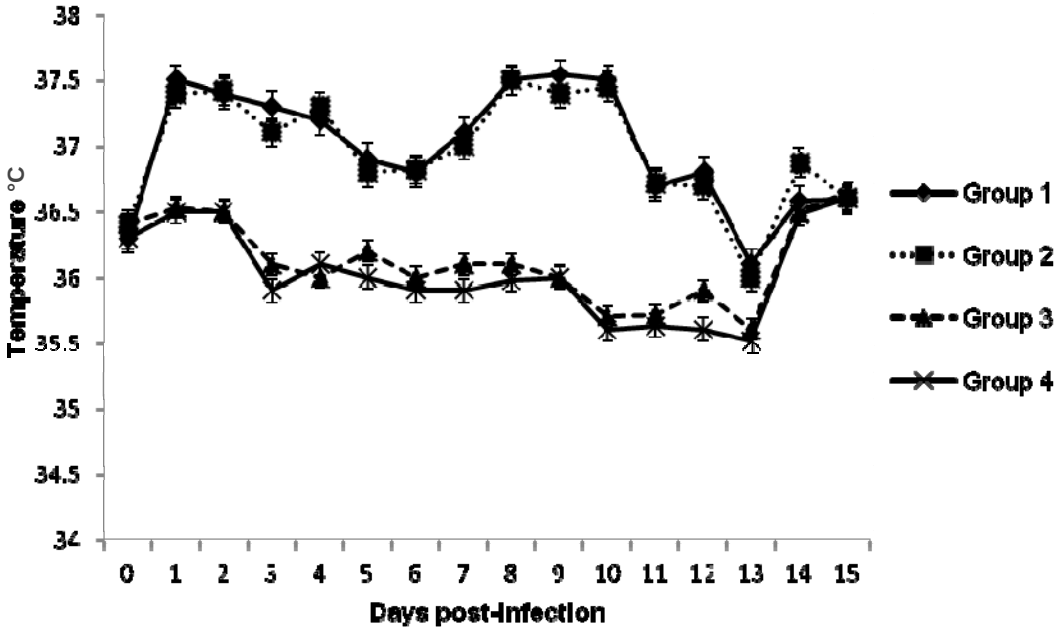

Figure 8. Effects of *S. sciuri* on rectal temperatures.

there was increased body temperature of the *S. sciuri* infected mice, this increase did not exceed the normal body temperature (35 to 38°C) of mice.

The significant ($p<0.05$) decrease in body weight of the infected groups by 2 to 14 dpi, may be as a result of their reduced feed intake observed in the course of the experiment. Significantly ($p<0.05$) decreased body weight observed in group 1 compared to 2 at 2 to 4 dpi, may suggest that intraperitoneal inoculation resulted to faster establishment of infection than the subcutaneous route. Isolation of pure cultures of *S. sciuri* from skin lesions and internal organs and the histopathological findings, further suggests that systemic infection occurred in *S. sciuri*-infected groups. The histopathological changes in form of dermal abscess in the skin and microabscess in liver, may suggest that the organism invaded the organs and caused necrosis which triggered formation of the abscesses. Dermal abscesses are not uncommon findings in *Staphylococcus aureus* infection, and exfoliative toxin C (*Ex*hC) of *S. sciuri* has been suggested

to be a mammalian necrosis inducer (Li et al., 2011). This might be the first report of dermal abscess associated with *S. sciuri* containing exfoliative toxin A gene.

In conclusion, this study has shown that exfoliative gene-positive *S. sciuri* is potentially pathogenic. The pathogenic effect as evidenced by sloughing of the skin in infected mice is probably due to elaboration of exfoliative toxin. There were significant alterations in the temperature, body weight and liver histology of mice infected with the organism. These findings indicate that the significance of *S. sciuri* as a potential pathogen may have been somewhat underestimated. It is therefore suggested that further studies using larger experimental animal models be carried out.

Conflict of interests

The author(s) have not declared any conflict of interests.

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