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Antibiotic susceptibility and resistance of *Staphylococcus aureus* isolated from fresh porcine skin xenografts: Risk to recipients with thermal injury

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ABSTRACT

The previous use of fresh porcine xenografts at the Prague Burn Centre had raised concerns over the transmission of zoonotic pathogens. This study examines the risk of zoonotic *Staphylococcus aureus* colonisation of burn patients from fresh porcine skin xenografts.

Samples were collected from the nares, skin and perineum of commercial pigs (n = 101) and were screened for methicillin sensitive *S. aureus* (MSSA) and resistant *S. aureus* (MRSA). The efficacy of the antibiotic wash used in decontamination of the pigskin was tested against planktonic- and biofilm-grown isolates. The *spa* type of each isolate was also confirmed.

All pig swabs were negative for MRSA but 86% positive for MSSA. All planktonic-grown isolates of MSSA were sensitive to chloramphenicol and nitrofurantoin and 44% of isolates were resistant to streptomycin. Isolates grown as biofilm exhibited higher rates of antimicrobial resistance. Sequence analysis revealed three distinct spa types of the MRSA ST398 clonal type.

This finding demonstrates the existence of a MSSA reservoir containing spa types resembling those of well-known MRSA strains. These MSSA exhibit resistance to antibiotics used for decontamination of the pigskin prior to xenograft. Amended use of procurement could allow the use of fresh pigskin xenografts to be reinstated.

1. Introduction

Due to the shortage of human skin for allo-transplantation, fresh pig skin was frequently used for xenografting at the Prague Burn Centre (PBC) [1]. However, the use of pigs as a skin source for xenotransplantation raises concerns over the potential for zoonotic infection [2,3].

In the treatment for severe burns patients, fresh or frozen porcine xenografts have proven to be an effective temporary

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Abbreviations: MRSA, methicillin resistant *Staphylococcus aureus*; MSSA, methicillin sensitive *Staphylococcus aureus*; CA, community acquired; HA, hospital acquired; LA, livestock associated; Spa gene, encoding pathogenic factor Protein A.

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biological skin substitute [1,4]. Xenografts adhere well to excised wounds and when removed a clean granulating wound remains. This promotes rapid epithelial in-growth in superficial burns and prepares the wound bed for autografting in deep dermal burns [1]. The PBC in the Czech Republic have successfully used commercial pigs for over 10,000 skin strip xenografts annually for severely burned patients and a skin tissue bank has been established [1,4,5]. The skin xenografts are in contact with the recipient for up to eight weeks during treatment. Fresh viable full thickness pig skin strips are treated with a saline/antibiotic solution prior to application [1]. Indeed, other centres currently use fresh porcine skin with ongoing success and procurement and preservation techniques are constantly being improved [1,6].

Previous studies on PBC patients receiving fresh porcine skin xenografts have reported that patients were colonised with a number of different pathogens with the majority of these being staphylococci [7]. In addition, methicillin sensitive *Staphylococcus aureus* (MSSA) has been shown to be the most frequently isolated organism from recipients followed by methicillin resistant *S. aureus* (MRSA) [5]. *S. aureus* is a common opportunistic pathogen associated with a wide range of diseases from superficial skin and soft tissue infections to severe life threatening infections including endocarditis and septicaemia [8]. MRSA is a documented problem in hospital settings [9] and since the late 1990s there has been a significant increase in the number of MRSA infections reported outside of the health care environment categorised as community acquired MRSA (CA-MRSA) [10]. In general CA-MRSA strains are more susceptible to antibiotics than hospital acquired MRSA (HA-MRSA) [11]. Over the past decade a third category of MRSA has emerged, the livestock associated MRSA (LA-MRSA [11]).

It has been previously described that MRSA strains evolve from MSSA strains by acquisition of the mecA gene, which is carried in a mobile genetic element called the staphylococcal cassette chromosome mec (SCCmec). This gene confers resistance to virtually all β-lactam antimicrobials by encoding an alternative penicillin binding protein (PBP2a), which has a lowered affinity for β-lactam antibiotics [12]. Epidemiological information on *S. aureus* can be obtained through various methods such as multilocus sequence typing (MLST) and spa typing, which is based on the sequencing of the polymorphic X region of the *S. aureus* protein A gene (*spa* gene). [11]. It is suggested that LA-MRSA has been evolved by multiple introductions of the SCC*mec* element into MSSA and studies have shown that strains with identical spa-types can carry different SCC*mec* elements [13,14].

To date, two major lineages of LA-MRSA have been described. MRSA multilocus sequence type (ST) 398 of swine origin is predominant in Europe and North America and ST9 in Asia [13,15]. As with humans, colonisation occurs in the nasal passage, perineum and skin and it has now become an important global zoonotic pathogen documented in horses, cats, dogs and cattle. European surveillance studies show that LA-MRSA ST398 isolates typically belong to 4 major *spa* types (t011, t108, t034, and t899) and several minor *spa* types (e.g., t1197, t1451, t1456) [16].

Many studies have also reported the prevalence of MSSA ST398 in swine [14] and porcine MSSA is known to be present on pigskin and has been described in China, Japan and Denmark [15–18].

S. aureus, including LA-MRSA, is a significant cause of skin infections in pigs. Transmission of LA-MRSA between pigs and pig farmers, pig farmers and their families, veterinarians and companion animals in small-animal healthcare, and in equine hospitals has been reported [19,20]. While it has been shown that LA-MRSA can cause disease in humans, there is currently no evidence to date, that LA-MRSA has been transmitted between humans [21].

Finally, it is known that bacteria can exist in two growth states, planktonic and sessile. Most studies are carried out using planktonic bacteria, however, in nature, bacterial populations exist as biofilms. Biofilms exhibit altered phenotypes with respect to growth, antibiotic resistance and gene expression and are thought to be the predominant bacterial phenotype on both healthy and diseased skin [22–24]. Therefore, the carriage of MRSA and/or MSSA on pigskin used for xenografts could potentially be a risk factor for colonisation and subsequent infection in burn patients [25].

As a moratorium had been introduced in the Czech Republic preventing the continuation of this technique, the purpose of the study was to demonstrate if there was any risk from the use of fresh viable commercial pig skin. Based on previous studies demonstrating *S. aureus* as a common commensal on pigs skin [7] and the reports on zoonotic *S. aureus* infections in individuals [11] the aim of this study was to; (i) screen a commercial pig herd used for skin xenografts for the presence of MSSA and MRSA, (ii) assess the clonal structure of *S. aureus* isolated from these pigs, (iii) and determine the antimicrobial susceptibility of planktonic- and biofilm-associated strains to antimicrobials used to decontaminate porcine skin prior to xenografting at the PBC.

2. Materials and methods

2.1. Animal sampling

A total of 101 pigs from a commercial herd in Prague used for skin xenografts were swabbed for the presence of *S. aureus*. Three swabs were taken from each pig, one from the perineum, one from the nasal cavity and an additional swab from the skin. Each swab was placed into Amies charcoal medium (Sterilin, UK) for transport and shipped at 4 °C within 24 h of collection.

2.2. Isolation of S. aureus

Each swab was initially cultured on a blood agar plate (BAP) and in Contrast™ MRSA broth (Oxoid Ltd., Hampshire, UK) and incubated for 24 h at 37 °C. Positive Contrast™ MRSA broth cultures were inoculated onto Brilliance™ chromogenic agar (Oxoid, Hampshire, UK), non-selective 5% BAP (Oxoid Ltd., Hampshire, UK) [26] and incubated overnight at 37 °C in air. After incubation, BAP and Brilliance chromogenic agar were examinded and potential *S. aureus* were submitted to a catalase, DNase and Staphaureux™ latex agglutination test (Oxoid Ltd., Hampshire, UK) as per manufacturer's instruction [27]. The positive control used was the *S. aureus* NCTC 6571