

# An *Ex Vivo* Porcine Nasal Mucosa Explants Model to Study MRSA Colonization

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## Abstract

*Staphylococcus aureus* is an opportunistic pathogen able to colonize the upper respiratory tract and skin surfaces in mammals. Methicillin-resistant *S. aureus* ST398 is prevalent in pigs in Europe and North America. However, the mechanism of successful pig colonization by MRSA ST398 is poorly understood. To study MRSA colonization in pigs, an *ex vivo* model consisting of porcine nasal mucosa explants cultured at an air-liquid interface was evaluated. In cultured mucosa explants from the surfaces of the ventral turbinates and septum of the pig nose no changes in cell morphology and viability were observed up to 72 h. MRSA colonization on the explants was evaluated followed for three MRSA ST398 isolates for 180 minutes. The explants were incubated with  $3 \times 10^8$  CFU/ml in PBS for 2 h to allow bacteria to adhere to the explants surface. Next the explants were washed and in the first 30 minutes post adhering time, a decline in the number of CFU was observed for all MRSA. Subsequently, the isolates showed either: bacterial growth, no growth, or a further reduction in bacterial numbers. The MRSA were either localized as clusters between the cilia or as single bacteria on the cilia surface. No morphological changes in the epithelium layer were observed during the incubation with MRSA. We conclude that porcine nasal mucosa explants are a valuable *ex vivo* model to unravel the interaction of MRSA with nasal tissue.

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## Introduction

*Staphylococcus aureus* is an opportunistic pathogen colonizing the upper respiratory tract and skin surfaces of humans as well other mammalian species. The nose is considered to be the primary ecological niche of *S. aureus* colonization in humans [1]. Nasal carriage of *S. aureus* has been identified as a risk factor for the development of various infections in humans [1].

In 2004 a new distinct clone of methicillin-resistant *S. aureus* (MRSA) ST398 has been found in pigs in the Netherlands [2]. Since then, MRSA ST398 has been detected in pigs, veal calves and poultry around the world [3,4]. The transmission of MRSA ST398 from livestock to humans has been reported in many countries [5,6] and contact with livestock is recognized as a risk factor for human colonization [4,7]. Additionally, ST398 isolates may cause infections in humans [8]. However, the mechanisms underlying successful colonization of pigs are poorly understood. Determination of the essential bacterial colonization factors is crucial to develop new treatment strategies to prevent colonization and consequently reduce MRSA ST398 interspecies transmission. Animal models are useful to study MRSA colonization. Murine [9] and rat models [10] have been developed specifically for studying *S. aureus* colonization in humans. However, the study of Gonzalez-Zorn showed that the murine nasal cavity is not a

natural habitat of *S. aureus* and that this model may not be optimal to study *S. aureus* colonization [11]. Recently, *in vivo* pig colonization models have been applied [12–14]. Inoculation of pigs however, yielded variable results possibly due to unstable colonization [13,14] or too low numbers of bacteria to detect with the sampling and/or isolation method used [13]. *In vivo* *S. aureus* colonization may be further difficult to control due to the presence of undefined local microbial and environmental factors. A suitable alternative system to gain better understanding of nasal colonization may be the use of porcine nasal mucosa explants in which bacterial and host factors can be evaluated under controlled conditions. At present there is no *ex vivo* model to study pig nasal colonization although some models based on the nasal primary tissue culture are used in virological studies [15–17]. In the present study we for the first time established porcine nasal mucosa explants as a novel tool to study MRSA ST398 colonization in pigs using bacterial observation of CFU changes in time or maintenance on the explants as indicators of colonization.

## Materials and Methods

### Isolation and Cultivation of the Nasal Mucosa Explants

Animals (Landrace, 6 months old sows, 70–75 kg) were MRSA negative and came from van Beek SPF pig farm B.V. (Lelystad, the