

Investigation of MRSA transmission between pigs and the environment following intra-nasal inoculation

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Abstract

Meticillin-resistant *Staphylococcus aureus* (MRSA) ST398 has not been detected in pigs in Ireland. However, other strains of MRSA, including MRSA t002, have been isolated from animals and humans in Ireland. The aim of this study was to determine if nasal colonization of pigs with a non-ST398 strain of MRSA could be reproduced using intra-nasal inoculation and to investigate subsequent transmission of this strain. Six pigs were inoculated intra-nasally with 2×10^9 cfu MRSA t002. Six days post-inoculation these pigs were washed and moved to a clean house with 15 unexposed pigs (In-contact group). Another 15 unexposed pigs were added to the vacated house (Environment group). The inoculated pigs and In-contact group were moved to a clean house every 2 days to minimise exposure to MRSA in the environment. Nasal swabs were taken for MRSA culture from all pigs and counts were undertaken from samples from inoculated pigs. Inoculated pigs were euthanized 5 weeks post-inoculation and exposed pigs were euthanized 4 weeks post-exposure to MRSA. Upper respiratory tract samples were taken at postmortem for MRSA culture. All inoculated pigs were MRSA-positive for 5 days post-inoculation. Thereafter the number of positive pigs decreased with no inoculated pig positive after 17 days post-inoculation. However, MRSA was isolated from three inoculated pigs following postmortem culture. Ten of the 15 pigs from the Environment group were MRSA-positive during the first 3 days post-exposure but all 15 were negative on subsequent samplings. Only 3 of 15 pigs in the In-contact group were MRSA positive during the first 3 days post-exposure; 4 other pigs in this group were MRSA-positive on subsequent samplings. This study demonstrates that, experimentally, pigs can become colonised with a non-ST398 strain of MRSA which can be transmitted by both direct contact with colonised pigs and by environmental exposure alone. However, transmission by both routes appeared to be inefficient and risk of persistence was low under these conditions.

Introduction

While meticillin-resistant *Staphylococcus aureus* (MRSA) has been isolated from pigs and their environment in several European countries, MRSA has not been detected in pigs in Ireland thus far (1, 2). MRSA has been isolated from companion animals and horses in Ireland and a high proportion of staphylococcal bloodstream infections in humans in Ireland are caused by MRSA (3, 4). In humans the primary site of carriage of *S. aureus* and MRSA is the anterior nares (5). In pigs, MRSA carriage is usually determined by nasal swab culture. However, the importance of staphylococcal carriage in other sites has not been assessed. Pharyngeal carriage of *S. aureus* has been described in humans and the tonsillar tissue of the pharyngeal area is an important site of entry and replication for several porcine pathogens (6, 7). Transmission of MRSA between humans usually occurs as a result of direct contact. While those carrying or infected with MRSA can shed the bacterium into their environment, the importance of the environment as a source of MRSA for the colonisation of humans is debated (8). In pig production settings, where large numbers of animals are kept in relatively unclean environments, the environment may play an important role in the transmission of MRSA. The aim of this study was to determine if a strain of MRSA, present in animals and humans in Ireland, could be used to experimentally reproduce nasal colonisation in pigs. An associated aim of this study was to determine the relative importance of animal- and environmental-sources in the transmission of MRSA. We also sought to determine if the pharyngeal tissues of the pigs were a significant site of colonisation for *S. aureus* and MRSA.

Materials and Methods

Forty pigs from a commercial pig-herd were selected for inclusion in this study based on the results of nasal swab culture for *S. aureus*. All 40 pigs were *S. aureus*-positive on at least 2 sampling occasions prior to the start of the trial.

The inoculating strain, MRSA t002, was grown on blood agar overnight then suspended in sterile phosphate-buffered saline.

Six pigs were inoculated intra-nasally with 2×10^9 cfu MRSA by syringe application to the nasal cavity. These 6 pigs were allowed to contaminate their environment for a period of 6 days. On day 6 post-inoculation (PI), the 6 pigs were removed from their house, washed in a disinfectant solution to remove MRSA from the body surface and moved to a new house.

Fifteen pigs (In-contact group) were added to the house now containing the 6 inoculated and washed pigs. All 21 pigs in this house were moved to a clean house every 2 days and the vacated house was disinfected. This group of pigs was primarily exposed to MRSA via nasal shedding from the inoculated pigs. Fifteen pigs were also added to the contaminated house vacated by the 6 inoculated pigs on day 6 PI (Environment group). These pigs were exposed to MRSA from the environment only. Four negative-control pigs were inoculated intra-nasally with sterile saline and housed separately from the other groups.

Nasal swabs were taken from inoculated pigs for MRSA enrichment culture and MRSA direct counts on Brilliance MRSA agar. Nasal swabs were taken from both groups of exposed pigs and negative-control pigs for MRSA enrichment culture only. Nasal swabs were taken daily for the first 3 days PI and post-exposure (PE) and then every 2-3 days. Environmental samples were taken from a 0.372 m² portion of a shelf in each pig-house for MRSA enrichment culture and direct counts. This shelf was not in direct-contact with the animals in the house.

At postmortem examination, samples of the nasal skin, nasal mucosa, palatine tissue and para-epiglottic tissue were taken from each pig. MRSA direct counts and enrichment culture were carried out on all samples. In addition, *S. aureus* enrichment culture and direct counts were carried out on all samples.

Results (Table 1)

All 6 inoculated pigs were MRSA-positive on enrichment culture for 5 days PI with detectable counts of MRSA in each pig on at least one occasion. No further counts of MRSA were detected in the inoculated pigs after day 6 PI and the number of pigs MRSA-positive on enrichment culture decreased thereafter. All 6 inoculated pig were MRSA-negative on enrichment culture after day 17 PI. MRSA was detected in the palatine tissue of 2 pigs at postmortem examination. MRSA was detected in both the nasal mucosa and para-epiglottic tissue sample in one inoculated pig.

Seven of the 15 pigs (In-contact group) exposed to MRSA by contact with the inoculated pigs were MRSA-positive on enrichment culture between days 1 and 30 post-exposure (PE). Six of these pigs were MRSA-positive on only one occasion while the seventh pig was MRSA-positive on 2 sampling occasions. One pig was MRSA-positive on culture of the para-epiglottic tissue sample at postmortem.

Ten of the 15 pigs (Environment group) exposed to MRSA in the environment were MRSA-positive on enrichment culture between days 1 and 3 PE. Four of these pigs were MRSA-positive on more than one occasion during that period. None of the 15 pigs were MRSA-positive after day 3 PE. One of the 15 pigs was MRSA-positive on culture of the nasal skin sample at postmortem examination.

The environment of the inoculated pigs was MRSA-positive on days 1-3 PI with detectable counts on each occasion. MRSA were not detected in the environment of the inoculated pigs immediately prior to their removal from this house and their replacement with un-inoculated pigs. MRSA were not detected in the environment of any of the houses for the remainder of the study.

Direct counts of *S. aureus* were recorded on all 40 palatine tissue samples at post mortem with a median count of 4×10^5 cfu/gram of tissue. *S. aureus* was less frequently detected on enrichment culture of the other 3 tissue samples taken at postmortem and the median *S. aureus* count for each of these samples was zero.

Table 1: Number of inoculated and exposed pigs MRSA-positive on enrichment culture of nasal swabs and post-mortem tissue samples

Days post-inoculation or post-exposure	Inoculated pigs	Pigs exposed to MRSA-inoculated pigs	Pigs exposed to MRSA-contaminated environment
Day 1	6/6	2/15	6/15
Day 2	6/6	0/15	3/15
Day 3	6/6	1/15	6/15
Day 5-10	6/6	2/15	0/15
Day 11-28	2/6	2/15	0/15
Total no. pigs positive	6/6	7/15	10/15
Post-mortem samples			
Nasal skin	0/6	0/15	1/15
Nasal mucosa	1/6	0/15	0/15
Palatine tissue	2/6	0/15	0/15
Para-epiglottic tissue	1/6	1/15	0/15
Total no. of pigs positive at postmortem examination	3/6	1/15	1/15

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Discussion

The inoculated pigs in this study appeared to be successfully colonised with MRSA for a period of at least 5 days. Since these pigs were kept in a clean environment and MRSA was not detectable in the environment after day 3 PI, these results are unlikely to reflect contamination from the environment. One pig was MRSA-positive on 9 separate sampling occasions and was also MRSA-positive on culture of 2 tissue samples at postmortem examination; it is likely that this animal was persistently colonised with MRSA. A recent study suggested that intra-nasal inoculation may not be an efficient method of reproducing nasal colonisation in pigs (9); therefore, the results of this study may not reflect the true colonising capacity of this strain of MRSA in pigs. Successful colonisation with *S. aureus* is dependent on several host and microbial factors. In this study, individual variation between pigs in their susceptibility to colonisation may have influenced the results observed. In the pigs exposed to MRSA by exposure to inoculated pigs, transmission of MRSA occurred but only to a limited extent. The number of inoculated pigs shedding MRSA and the numbers of bacteria shed may not have been sufficient to establish colonisation in the in-contact animals.

The number of pigs (10 of 15) testing positive for MRSA from the group exposed to MRSA in the environment alone suggests that transmission of MRSA from the environment to pigs occurs. It has been suggested that environmental survival of MRSA may be strain-specific (10). It is possible that the inoculating strain of MRSA t002 was not adapted to survival in the environment of the pig-house and so transmission of MRSA did not occur after day 3 PE.

A previous study found greater numbers of *S. aureus* in the tonsillar tissue of the pharynx of pigs compared with the nasal cavity (11) and is in agreement with our findings. It is possible that the pharyngeal tissues represent a significant site for the colonisation of pigs with *S. aureus* and MRSA. Since most studies rely on demonstrating MRSA on nasal swab culture, this finding has implications for sampling strategies and interpretation of the results of such studies.

Conclusions

Intra-nasal inoculation can be used to reproduce colonisation of pigs with MRSA t002; however, the efficiency of this method is questionable and is likely to be influenced by individual variation between animals. Transmission of MRSA t002 can occur as a result of direct-contact between pigs and contact with a contaminated environment. In this study, transmission by either route did not appear to result in persistent colonisation. The tissues of the pharynx may be a significant site of carriage of *S. aureus* and MRSA in pigs.

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