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Occurrence of MRSA in air and housing environment of pig barns

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ABSTRACT

A high prevalence of MRSA among farm animals, especially pigs, has been observed for some time. However, knowledge on transmission routes of MRSA in livestock production is still scarce. Therefore, the aim of this study was to determine the occurrence of MRSA in pig house air as well as in samples from pigs and their housing environment in 27 MRSA positive pig barns of different sizes and production types.

In 85.2% of all barns MRSA was detected in the animal house air. Impingement turned out to be a more sensitive sampling technique than filtration. Other environmental samples such as boot swabs or faeces showed prevalences of MRSA from 55.6% to 85.2% at sample level. The level of MRSA was 88.3% for pooled and 82.1% for single nasal swabs, in skin swabs the one was 87.7%, the others was 78.7%. *Spa* typing of isolates from air and nasal swabs showed predominantly *spa* types t011 and t034. MRSA prevalences in pigs as well as in various environmental samples were significantly higher in fattening farms than in breeding farms. This study provides good reference that there could be an airborne transmission of MRSA within pig herds indicating a potential contamination of the environment of barns.

1. Introduction

Livestock associated (LA-) MRSA of the clonal complex 398 has been observed in pigs (Voss et al., 2005; Khanna et al., 2008), cattle (Lee, 2003; Spohr et al., 2011), poultry (Nemati et al., 2008; Pletinckx et al., 2011) and in humans being in contact with these animal species (Cuny et al., 2009a; Graveland et al., 2011; Spohr et al., 2011). It has also been identified in horses (Tokateloff et al., 2009), dogs (Floras et al., 2010) and in companion animals and rodents on farms (van de Giessen et al., 2009).

In pigs, LA-MRSA has been found in all levels of the production pyramid from nucleus herds (EFSA, 2009) to pigs at slaughter (de Neeling et al., 2007; Tenhagen et al., 2009). Herd size has been identified as a risk factor for herds to be positive and the likely role of trade in the transmission between herds has been pointed out (Battisti et al., 2010; EFSA, 2010).

Within herds, direct contact between animals and between animals and humans is a likely route of transmission. Furthermore, the intensity of contact and time spent in the barns have been identified as risk factors for humans (Cuny et al., 2009a; Graveland et al., 2011). However, more research on potential transmission pathways within and between herds is needed.

In animal husbandry it is known that, in addition to nasal colonisation, MRSA occurs in the barn environment. It could be regularly detected in dust (Springer et al., 2009; Van Den Broek et al., 2009), swabs from the animals' environment (Nathaus et al., 2010) or environmental wipes (Broens et al., 2011b). MRSA has also been detected

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in the air of pig barns (Schulz and Hartung, 2009; Harper et al., 2010) but the knowledge on the prevalence and load of MRSA in barn air is scarce. Contaminated barn air may lead to transmission from animals to humans without direct contact or between animal carriers and non-carriers. Exhaust air from barns may even be a source of MRSA for neighbouring animal houses as well as the entire environment (Gibbs et al., 2006). The objective of this study was to comprehensively investigate the occurrence of MRSA in barn air and housing environment of pig holdings of different production types to gain more information about the potentially airborne transmission of MRSA inside the animal house and within the whole farm.

2. Materials and methods

2.1. Selection of farms

Pig farms in Germany were preselected based on MRSA positive dust samples. These samples were taken within a maximum of 3 months before the investigation. 58.5% of all dust samples from pig farms ($n = 53$) were tested MRSA positive. In samples originating from fattening farms ($n = 26$) MRSA detection frequency was 76.9%, in those from breeding and weaner to grower farms ($n = 27$) 40.7%.

For this study a total of 27 pig farms was chosen. Fattening farms ($n = 15$) housing between 700 and 12 000 animals (median about 3025), ten breeding farms with 90 to 1600 sows (median about 450) and two weaner to grower farms with 960 and 2700 weaners were investigated. In each farm one representative animal house was chosen in which all samples were collected simultaneously. In breeding farms one of the farrowing houses was chosen, in fattening farms a house with pigs at the age of at least 90 days. If there were not enough animals for all samples animals of the neighbouring room were sampled. All farms were investigated once using the same sampling protocol.

2.2. Samplings at pig farms

2.2.1. Air samples

Air samples were collected using impingement and filtration methods in parallel. For impingement ($n = 81$) All-Glas-Impingers (AGI-30, Ace Glass Inc., Vineland, USA) filled with 30 ml phosphate buffered saline (PBS) were used. The collection time was about 30 min and the air flow (about 11.5 l/min) was controlled by using a rotameter (Analyt-MTC GmbH, Müllheim, Germany). The second technique was air filtration ($n = 80$) using personal air sampler pumps (GilAir-5, Sensidyne, USA and SKC Gulf Coast Inc., USA) in combination with an I.O.M. dust sampler (Institute of occupational medicine, Edinburgh, UK and SKC Gulf Coast Inc., USA). The filter with a diameter of 25 mm consisted of polycarbonate with an 8 μm pore size (Whatman, USA). The collection time was 150 min with an air flow of 2.5 l/min. Air samples were collected 1.50 m above ground floor at three different locations inside the animals house distributed symmetrically alongside the central alley in the animal house.

2.2.2. Environmental samples

In each barn pooled samples of about 2.5 g dust ($n = 27$) were collected. Dust was sampled from at least five different locations without contact to animals (excluding window sills) by using a sterile brush. About 250 g faeces ($n = 27$) and a boot swab sample ($n = 27$) of the whole length of the service alley in the investigated barn were collected. Additionally, pooled feed samples (about 250 g) were collected directly from the feeder in 17 farms. In ten farms there was no feed from the feeder available in this certain time. All pooled samples were taken from at least five different locations within the barn.

2.2.3. Animal samples

Sixty pigs were sampled by nasal and skin swabs in every herd to detect 5% prevalence in an infinite population with 95% confidence. Nasal swabs were taken from one anterior nare using dry cotton swabs (Sarstedt AG & Co. KG, Nümbrecht, Germany). Skin swab samples were taken by swabbing three to four times the skin surface behind one ear using cotton swabs humidified by PBS.

2.3. Laboratory analysis

All samples were stored at 4 °C until its analysis on the day after sampling (within 24 h).

2.3.1. Animal samples and environmental samples

All nose and dust samples were investigated with and without enrichment. Samples were enriched by using Mueller Hinton broth (868517, Oxoid Lt., Hampshire, UK) with 6.5% NaCl (MHB+) and tryptone soy broth (583439, Oxoid Lt., Hampshire, UK) including 75 mg/l aztreonam and 3.5 mg/l cefoxitin (TSB+) subsequently. Nasal and skin swab samples were investigated individually and pooled. One pool was built out of 4 randomly chosen swabs resulting in 12 pools per farm for nasal and skin swab samples. The remaining swabs of 12 animals were analysed individually. For the determination of bacterial counts individual nasal swabs were extracted in 1.5 ml PBS and 100 μl of the original fluid as well as the first 1:10 dilution was streaked onto a chromogenic MRSA screen agar (CHROMagarMRSA™, MAST Diagnostica GmbH, Reinfeld, Germany). Individually analysed skin swab samples were streaked directly onto the selective agar. The plates were aerobically cultured at 37 °C for 24 h. Nasal and skin swabs were placed into MHB+ simultaneously, single samples into 10 ml and pooled samples into 20 ml of MHB+. After 24 h of incubation at 37 °C 1 ml of MHB+ was transferred into 9 ml TSB+. This selective broth was incubated for 17 h at 37 °C. A loop-full of TSB+ was streaked onto chromogenic MRSA screen agar and then incubated at 37 °C for 24 h.

For quantitative analysis of dust 0.1 g of dust was dissolved in 10 ml PBS + 0.01% TWEEN20. 100 μl of the original fluid and the first 10-fold dilution were streaked onto chromogenic MRSA screen agar. For enrichment 1 ml of the original fluid was transferred into 9 ml MHB+. Boot swab samples and 25 g of pooled faeces and feed samples respectively were inoculated in 225 ml MHB+ and