

First isolation of methicillin-resistant *Staphylococcus aureus* from pigs' clinical samples in Serbia

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Abstract

Methicillin-resistant *Staphylococcus aureus* is a highly important human pathogen that is also a significant concern in veterinary medicine. Despite the high prevalence of colonization, clinical infections with methicillin-resistant *Staphylococcus aureus* appear to be rare in pigs. Methicillin-resistant *Staphylococcus aureus* was isolated from a sow with endometritis and her five piglets with dermatitis originating from a Serbian farm. Identification of the strains was done by automated system and confirmed by polymerase chain reaction for *mecA* and *nuc* genes. Detection of Staphylococcal Cassette Chromosome *mec* type was performed by multiplex polymerase chain reaction. Antimicrobial susceptibility testing on erythromycin, clindamycin, gentamicin, kanamycin, tobramycin, ciprofloxacin, tetracycline, trimethoprim/sulfamethoxazole and vancomycin was done by disc diffusion method. Six isolated strains from the infected sow and her piglets showed resistance only to tetracycline beside resistance to all beta-lactam antibiotics. In the tested methicillin-resistant *Staphylococcus aureus* isolates, Staphylococcal Cassette Chromosome *mec* type V was present. To our knowledge, this finding is the first documented detection of methicillin-resistant *Staphylococcus aureus* from pigs' clinical samples in Serbia. The results of our study indicate the emergence of methicillin-resistant *Staphylococcus aureus* in a pig farm in Serbia highlighting the threat of this antibiotic-resistant microorganism as a pathogen causing both animal and human infections.

MRSA, sow, piglets, infection, metritis, dermatitis

Methicillin-resistant *Staphylococcus aureus* (MRSA) is an enormous problem in human medicine as both hospital- and community-associated pathogen. Although MRSA has emerged in animals at a slower rate, this pathogen is now a significant concern in veterinary medicine. In 2005, the presence of MRSA in pigs and the transfer to humans were reported for the first time (Voss et al. 2005). Soon thereafter, high prevalence of MRSA in pigs was detected in many countries (de Neeling et al. 2007; Witte et al. 2007; Dewaele et al. 2008). Despite the high prevalence of colonization, clinical infections with MRSA appear to be rare in pigs. Exudative epidermitis was reported in pigs on one farm in the Netherlands (van Duijkeren et al. 2007); another report described isolation of MRSA from pigs with skin infections, urinary tract infections and metritis, and mastitis agalactia (MMA) syndrome in Germany (Schwarz et al. 2008). The aim of this study was to report the first isolation of MRSA from pigs' clinical samples in Serbia.

Materials and Methods

In July 2009, a routine survey on a pig farm with 1600 sows located in the north-eastern part of Serbia was conducted. During observation of the sows' health status in the farrowing compartment of the farm, abundant purulent discharge from the vagina of one sow was noticed on the second day *post partum*. Further examination

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showed that the pus was discharged from uterus as a consequence of endometritis purulenta. Moreover, five piglets out of 12 that were farrowed by this sow, developed dermatitis resembling eczema. Samples from the cervix and skin lesions were collected for further testing. After sampling, the sow was treated with enrofloxacin and the diseased piglets locally with neomycin and AD3E vitamins. Control swabs from infected pigs were collected after 10 days.

Samples were plated on sheep blood agar and MacConkey agar (HiMedia, India), and incubated at 37 °C for 24 h. No growth was seen on MacConkey agar, but large numbers of beta-haemolytic white colonies were found on sheep blood agar. These colonies were identified as MRSA by Vitek2 System (bioMérieux, France) and confirmed by PCR for *mecA* and *nuc* genes. Antimicrobial susceptibility testing was performed by disk diffusion method with Neosensitab discs (Rosco, Denmark): erythromycin, clindamycin, gentamicin, kanamycin, tobramycin, ciprofloxacin, tetracycline, trimethoprim/sulphamethoxazole and vancomycin, in accordance to the Clinical and Laboratory Standard Institute recommendations (CLSI 2006). Determination of Staphylococcal Cassette Chromosome *mec* (SCC*mec*) type was done by multiplex PCR (Boye et al. 2007).

In order to investigate the possible source of MRSA, additional samples were taken from the nares of infected pigs, 19 healthy sows sharing the same accommodation with the MRSA-positive sow, as well as from the nares of three farm workers, veterinarian and veterinary technician who observed the sow during farrowing and *post partum*. Collected samples were investigated as previously described.

Results

According to both phenotypic and genotypic tests a total of six MRSA strains (one from the cervix and five from skin lesions) were isolated in this study. They showed resistance only to tetracycline beside resistance to all beta-lactam antibiotics. Staphylococcal Cassette Chromosome *mec* type V was present in tested MRSA isolates. No MRSA isolate was found from additional pig and human samples.

Discussion

MRSA strains are widely disseminated as nasal colonizer of pigs in countries with high-density pig farming investigated so far (Dewaele et al. 2008). In contrast, there are only a few reports of MRSA infections in pigs (van Duijkeren et al. 2007; Schwarz et al. 2008). This is the first report of MRSA infections in pigs in Serbia.

Metritis in the immediate post-farrowing period in sows and piglets' skin infections can be caused by MRSA (Papadopoulos et al. 2010). Metritis is more likely to occur when farrowing is prolonged or when there has been manual assistance. Piglets are infected during the birth process or soon after (van Duijkeren et al. 2007).

The recognition of MRSA in animals has raised concern over their role as potential reservoirs or vectors for human MRSA infections in the community. Considering the public health relevance of MRSA and association with pig farming, there is an urgent need to elucidate the transmission routes of MRSA on pig farms. In our study the origin of MRSA in the ill sow remains unclear. It is possible that the sow had already been colonized with MRSA before she became ill. The nare sample was negative, but it was taken after enrofloxacin administration. Alternatively, the MRSA strain may have been transmitted to pigs from another source. In a household contact, humans and companion animals may infect each other with MRSA. A similar event may have been the initial cause of the emergence of MRSA in pigs, although we did not find any MRSA isolate from the nares samples of healthy sows and farm workers. Methicillin-resistant *Staphylococcus aureus* may also be disseminated from contaminated feed. Tetracycline-resistant *S. aureus*, like isolates in our study, might survive or even thrive in feed medicated with tetracyclines.

The results of our study indicate the emergence of MRSA on a pig farm in Serbia highlighting the threat of this antibiotic-resistant microorganism as a pathogen causing both animal and human infections; therefore, continued surveillance is recommended. Close collaboration between human and veterinary medical practice is mandatory in order to identify human and animal carriers as well as to implement effective control measures in veterinary practice. Identification of colonized and infected animals is important for isolation and treatment purposes to prevent spreading of MRSA strains.

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