

MRSA IN BREEDING PIGS IN GERMANY IN 2015

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Introduction

Methicillin resistant *Staphylococcus aureus* has been known to be prevalent in the pig production for nearly 15 years now (Meemken et al., 2010). In 2008 a survey carried out in the EU determined a high prevalence of MRSA in herds of breeding pigs also in Germany (EFSA, 2010). Likewise, MRSA were identified in Germany in herds of fattening pigs (Alt et al., 2011), pigs at slaughter (Tenhagen et al., 2009), on carcasses (Beneke et al., 2011) and in meat from pigs at retail (BVL, 2013). The current investigation was carried out to determine the current prevalence of MRSA in herds of breeding pigs, analyse patterns in the type of MRSA isolated from pigs and determine differences between the MRSA observed in units only housing sows and those housing weaned piglets. In previous studies it could be shown, that the prevalence of MRSA is higher in weaned piglets than in sows but little is known about differences in the types of MRSA that can be isolated in the different units.

Material and methods

Boot swab samples were collected in pig herds producing piglets for fattening purposes. Samples from herds were distributed across Germany based on the respective share of sows in the respective province (“Land”). A sample size of 384 samples was targeted. Within the herds two boot swab samples were collected: One sample was collected in the area where pregnant sows were housed, the other one was collected in the premises, where weaned piglets were housed. Samples were collected by the local veterinary authorities and submitted to the regional state laboratories for testing for MRSA.

Laboratory procedures

Boot swab samples were tested using a double selective enrichment method as previously described for environmental samples (Kraushaar et al., 2016). Briefly, samples were incubated with Mueller-Hinton broth containing 6% of NaCl and 1 ml of this broth after 18 to 24 hours was transferred to 9 ml of tryptic soy broth containing 50mg of aztreonam and 3.5mg of cefoxitin. 0.05ml of this broth were then plated on selective agar plates.

One colony from each positive sample was submitted to the National Reference Laboratory for coagulase positive staphylococci incl. *S. aureus* at the Federal Institute for Risk Assessment (BfR), where it was confirmed as MRSA using a multiplex PCR targeting the *nuc* gene for species identification, and the *mec* gene for methicillin-resistance (Kraushaar et al., 2016).

Data analysis: Only herds were included where samples were collected in both parts of the herd (i.e. sow and weaner premises) and where in case of positive results isolates were available for further characterization.

In pig herds agreement of samples and relative sensitivity of sow and weaner samples were calculated using the combined result of both samples as a reference. Results from the two samples were combined as a herd status. If any of the two samples was positive, the herd was considered positive, otherwise negative. Calculation of specificity was not done as this definition of a positive herd excluded false positive results.

Results

Data on 337 sow herds were finally included in the analysis. The overall prevalence of MRSA in the herds was 47.5 % (160/337). The observed MRSA prevalence was lower in sow stables (25.8 %, 87/337) than in the weaner premises (41.2 %, 139/337). MRSA were found in both, the sow stable and weaner premises in 66 herds (19.6 %). Agreement between the two samples was limited. Neither samples from sows nor those from weaners identified all positive herds. Relative sensitivity when compared to the combined results of the two samples was higher for the weaner samples than for the sow samples (86.9 vs 54.4 %).

From each positive sample, one isolate was further characterised. Overall 226 isolates were available for typing. Most of the MRSA- isolates (80.9 %) were from *spa*-types t011 and t034. This was similar in the sow and the weaner units (78.1 and 82.8 %, respectively). However, in the weaner units, slightly more MRSA of *spa*-type t011 were observed (50.4 % vs. 42.5 % in the sow herds). Eighteen other *spa*-types that could be assigned to the livestock associated clonal complex CC398 were identified and constituted 14.6 % of all MRSA-isolates were from. Eleven different additional CC398 associated types were seen in weaners, ten in sows. Seven isolates (4.5%) were from *spa*-types that could not be assigned to the CC398. However, all of those could be assigned to CC9: 6/7 t1430, 1/7 t15199. Type t1430 was seen in both, sows and weaners, with 3 isolates each, t15199 only in weaners.

With respect to *spa*-types of the isolates agreement between sow and piglet samples was limited. Only in 48 of the total of 160 positive farms (30.0 %), the same *spa*-type was identified in the sow stable and the weaner stable. In 39 of those 48 herds (81 %) both samples harboured either t011 or t034. In the remaining 18 herds with both samples positive the *spa*-type differed between the two parts of the herd.

Discussion

Overall, prevalence of MRSA in sow herds was similar to the prevalence determined during the EU-Baseline survey in 2008 (EFSA, 2009). However, the sampling methods differed and therefore comparison of the prevalences is not fully valid. The EU baseline survey included only sow premises and was based on dust samples. Purposeful inclusion of the weaner units in our study may have increased the prevalence as the proportion of positive samples was substantially higher in the weaner units than in the sow units. This agrees with previous studies on MRSA in pigs that found weaned piglets to have the highest prevalence of MRSA (Broens et al., 2010; Bangerter et al., 2016). The reason for this difference is not fully clear. However, frequently piglets from different sows are mixed in the weaner units and bacteria of

different farrowing crates may therefore contribute to the bacterial population in the weaner units. Additionally use of antimicrobials is frequent in weaners. Use of antimicrobials may foster detection of MRSA in the groups. As neither sampling weaners nor sows identified all positive herds, a combination of samples will be required for optimal sensitivity. Moreover, it is likely, that including more samples from other parts of the farm may further increase sensitivity.

Diversity of *spa*-types was substantial. However, most isolates were from *spa*-types that had already been identified in previous studies on pigs and other livestock in Germany. As only one isolate per positive sample and unit was typed, variability is probably even larger than observed here. In line with that, *spa*-types did not always agree between sows and weaners, indicating the presence of several *spa*-types in the same herd. However, most isolates could still be assigned to the clonal complex CC 398, the so-called livestock associated MRSA (LA-MRSA) and within that complex to the *spa*-types t011 and t034. This indicates persistence of this type of MRSA in the population over time. Other CC398 *spa*-types may indicate further evolution of MRSA in the pig population which is underlined by the frequent identification of new *spa*-types that can still be assigned to CC398 the MLST-based clonal complex that is most widely spread in the German farm animal population.

The proportion of isolates not assignable to CC398 was low and all of these isolates belonged to a *spa*-type related to the clonal complex CC9, that has frequently observed in isolates from poultry in Germany (Vossenkuhl et al., 2014; Kraushaar et al., 2016) . This type differs from most CC398 isolates by frequent resistance to ciprofloxacin, a feature that is less frequently seen in CC398 isolates. Whether these isolates originate from poultry farms or are also fully established in the pig population is not known.

In terms of food safety, MRSA positive herds constitute a permanent source of these bacteria in the food chain and although appropriate slaughter hygiene can limit the spread to carcasses, it is well established that MRSA can also be found on pork and be introduced in the households of consumers. Moreover, people working on pig farms are frequently carriers of the bacteria and may introduce them into the healthcare system.

Currently there is no specific strategy in place to contain MRSA in the pig population in central Europe. Therefore all efforts should be made to control the introduction of livestock associated MRSA into the healthcare system.

References

- Alt, K., A. Fetsch, A. Schroeter, B. Guerra, J.A. Hammerl, S. Hertwig, N. Senkov, A. Geinets, C. Mueller-Graf, J. Braeunig, A. Kaesbohrer, B. Appel, A. Hensel, and B.A. Tenhagen. 2011. Factors associated with the occurrence of MRSA CC398 in herds of fattening pigs in Germany. *BMC Vet Res* 7:69. doi:10.1186/1746-6148-7-69.
- Bangerter, P.D., X. Sidler, V. Perreten, and G. Overesch. 2016. Longitudinal study on the colonisation and transmission of methicillin-resistant *Staphylococcus aureus* in pig farms. *Vet. Microbiol.* 183:125–134. doi:10.1016/j.vetmic.2015.12.007.
- Beneke, B., S. Klees, B. Stührenberg, A. Fetsch, B. Kraushaar, and B.-A. Tenhagen. 2011. Prevalence of methicillin resistant *Staphylococcus aureus* (MRSA) in a fresh meat pork production chain. *J.Food Prot.* 74:126-129.

Broens, E.M., E.A. Graat, P.J. van der Wolf, A.W. van de Giessen, D.E. Van, J.A. Wagenaar, N.A. Van, D.J. Mevius, and M.C. De Jong. 2010. MRSA CC398 in the pig production chain. *Prev Vet Med* 98:182-189.

BVL. 2013. Berichte zur Lebensmittelsicherheit 2009 - Zoonosen-Monitoring. BVL Rep. 6 (4).

EFSA. 2009. Analysis of the baseline survey on the prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) in holdings with breeding pigs, in the EU, 2008, Part A: MRSA prevalence estimates; on request from the European Commission. *EFSA J.* 2009:82pp.

EFSA. 2010. Analysis of the baseline survey on the prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) in holdings with breeding pigs, in the EU, 2008, Part B: factors associated with MRSA contamination of holdings. *EFSA J.* 2010:1597.

Kraushaar, B., B. Ballhausen, D. Leeser, B.-A. Tenhagen, A. Käsbohrer, and A. Fetsch. 2016. Antimicrobial resistances and virulence markers in Methicillin-resistant *Staphylococcus aureus* from broiler and turkey: A molecular view from farm to fork. *Vet. Microbiol.* doi:10.1016/j.vetmic.2016.05.022.

Meemken, D., T. Blaha, R. Tegeler, B.A. Tenhagen, B. Guerra, J.A. Hammerl, S. Hertwig, A. Kasbohrer, B. Appel, and A. Fetsch. 2010. Livestock associated methicillin-resistant *Staphylococcus aureus* (LaMRSA) isolated from lesions of pigs at necropsy in northwest Germany between 2004 and 2007. *Zoonoses.Public Heal.* 57:e143–e148.

Tenhagen, B.-A., A. Fetsch, B. Stührenberg, G. Schleuter, B. Guerra, J.A. Hammerl, S. Hertwig, J. Kowall, U. Kämpe, J. Bräunig, A. Schroeter, A. Käsbohrer, and B. Appel. 2009. Prevalence of MRSA types in slaughter pigs in different German abattoirs. *Vet.Rec.* 165:589-593.

Vossenkuhl, B., J. Brandt, A. Fetsch, A. Käsbohrer, B. Kraushaar, K. Alt, and B.A. Tenhagen. 2014. Comparison of spa Types, SCCmec types and antimicrobial resistance profiles of MRSA isolated from Turkeys at farm, slaughter and from retail meat indicates transmission along the production chain. *PLoS One* 9. doi:10.1371/journal.pone.0096308.