Commentary

Potential Public Health Consequences of Exposure Assessment for *Staphylococcus aureus*: Commentary on the Paper by Masclaux *et al.*

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The study of Masclaux *et al.*, in this issue of the *Annals of Occupational Hygiene*, describes measurement of *Staphylococcus* bacteria in pig farms using classical viable measurement techniques and molecular techniques. Molecular techniques find application in hygiene field studies more often and create new opportunities. Common dust sampling approaches can be used in combination with molecular techniques and high numbers of samples can be processed creating opportunities for hygiene control and for exposure assessment in the context of epidemiological surveys. Hygienists should be aware of the advantages and disadvantages of the use of these techniques. Aspects of microbial sampling are briefly reviewed.

Keywords: microbial exposure; microbiological assessment; microorganisms; MRSA

In this issue of the *Annals of Occupational Hygiene*, a paper has been published by Masclaux and others, which describes measurement of airborne *Staphylococcus aureus*, total bacteria, and endotoxins in pig farms (Masclaux *et al.*, 2013). The authors describe the results of measurement of *S. aureus* in the air as the most important result. *Staphylococcus aureus* is a Gram-positive, coagulase-positive, coccus of the family Staphylococcaceae. Staphylococcal species can be commensal colonizers of the skin of animals and humans (Vanderhaeghen *et al.*, 2010). Methicillin-resistant *S. aureus* (MRSA) strains have developed resistance to beta-lactam antibiotics through natural selection. Traditionally, MRSA has been considered a hospital-associated pathogen (Kluytmans and Struelens, 2009). Colonization with MRSA may result in difficult-to-treat infections in humans, especially after accidents (cuts, bites from

animals) or after operative treatment in hospital. Hospital infections with MRSA are associated with treatment failure and increased severity of disease.

THE PUBLIC HEALTH CONTEXT: MRSA ON LIVESTOCK FARMS

MRSA was rarely isolated from animals before 2000, and if isolated from animals, MRSA strains were generally assumed to be of human origin, especially when found in companion animals. The emergence of livestock-associated MRSA (LA-MRSA) changed this picture dramatically. The initial case was a 6-month-old girl. She was admitted in 2004 to a hospital in the Netherlands for major surgery. She appeared colonized with MRSA during preoperative screening (Voss *et al.*, 2005). The strain had been observed a few years earlier in France, in a study among pig farmers and their animals (Armand-Lefevre *et al.*, 2005). Further sampling and genotyping showed that her parents carried the same MRSA strain as did the pigs on the farm. Neighbouring pig farmers and their

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animals were also carriers. The strain is referred to as LA-MRSA, or MRSA ST398 (Sequence Type 398), and pigs or other animals are probably the natural host. MRSA ST398 probably originated in humans and jumped to animals where it acquired resistance genes possibly as a result of selective pressure due to antibiotics use in livestock production (Price *et al.*, 2012). Use of antimicrobials for treatment of animals, often at the herd level, probably contributed to the evolution of ST398 as well as presence of coagulase-negative staphylococci, which probably carried the resistance genes, which have been transferred to MRSA and led to the evolution of ST398. Nasal colonization with MRSA in the general population is low, in Northern Europe generally <1.5% (Tiemersma *et al.*, 2004). Studies among different occupational groups with intensive livestock contact, such as pig and veal farmers and veterinarians, show that their nasal carriage rate of MRSA ST398 can be as high as 70% (Voss *et al.*, 2005; Wulf *et al.*, 2008; Graveland *et al.*, 2010). Although transmission rates of LA-MRSA seem lower in hospital settings than observed for non-LA-MRSA (Wassenberg *et al.*, 2011), LA-MRSA might transmit to the general population through family members and other human–human contact (Porphyre *et al.*, 2012).

Assessing exposure to *S. aureus* including MRSA in the air contributes to understanding mechanisms of transmission from animals to humans and humans to humans. This makes the paper by Masclaux and others interesting and highly relevant. They were successful in measuring *S. aureus* using classical culturing techniques and molecular techniques. Their study shows the new possibilities of molecular techniques for measuring bioaerosols, as well as the dilemmas when designing an exposure assessment strategy, and the complexities in the interpretation of these data. It is of interest to discuss some elements of this study to make this type of work more accessible for occupational and environmental hygienists.

MEASURING (MR)SA IN THE AIR

Masclaux and others have sampled (MR)SA in two different ways. First, by viable sampling, on nutrient media with an impactor for bioaerosols at an airflow of 100 l min^{-1}. A total volume of only 10 l was sampled for *S. aureus* and 250 l for MRSA. Sampling duration for viable sampling was short, only 6 s for *S. aureus* and 2.5 min for MRSA. Sampling was followed by species identification of cultured colonies by use of selective media and subsequent typing. A distinction between *S. aureus* and MRSA and different MRSA strains can be made by molecular

typing techniques after DNA extraction from the colonies. For MRSA, usually multi-locus sequence typing on a series of household genes is used. This approach is highly specific because molecular techniques are able to identify species or strains on a very detailed level. Culturing colonies for analysis is laborious and time consuming, especially in case of high numbers of environmental samples, because of the long incubation step.

Staphylococcus aureus levels were also assessed using an alternative approach by taking dust samples, DNA extraction of dust samples and measurement of the presence of *S. aureus* DNA in the dust, using a 370-bp fragment of the tuf-gene, which is specific for *Staphylococcus*. An advantage of these measurements was that they could be taken over a longer period of 4 h. This makes application in larger scale exposure control studies or epidemiological studies possible. This has been a major limitation of viable sampling so far. The difference in sampling strategies for viable and non-viable (MR)SA describes issues related to all bioaerosol sampling in a nutshell:

- Viable sampling is limited to a maximum sampling time to avoid overgrowth of media. Pig farms usually have high microbial levels and therefore a short sampling duration had to be chosen. As a result, the variation in measured concentrations will be large. When *S. aureus* was present at low levels in the air in some stables, it may have been missed because of this short sampling duration.

- The analysis is performed on DNA extracted directly from the dust but cannot distinguish viable and non-viable *S. aureus*. These polymerase chain reaction (PCR)-based methods usually overestimate viable levels because DNA from dead *S. aureus* can also be detected. This is a general problem with measuring microorganisms in air. On the other hand, these methods are extremely useful when microorganisms are viable but difficult or impossible to culture. Microorganisms are exposed to osmotic shock and high force upon impact, when they are carried at high velocity through an air sampler. The trauma incurred may lead to loss of viability and the rates at which these events occur are species dependent. True levels can differ substantially from measured levels, depending on the microorganism. Levels obtained in this study, with direct analysis of *S. aureus* DNA in dust, yielded considerably higher levels than the viable samples suggesting that the viability of the *S. aureus* species is low and that viable levels might give a poor reflection of true viable levels.

Aggregation of colonies might lead to underestimation of viable levels. These issues are illustrated by the paper of Masclaux *et al.* (2013), and others who also attempted to measure *S. aureus* in the air, by viable and non-viable sampling (Gibbs *et al.*, 2004, 2006; Green *et al.*, 2006; Schulz *et al.*, 2011; Friese *et al.*, 2012, 2013a,b; Gilbert *et al.*, 2012). Marginally better results have been obtained for *S. aureus* with impingement, using the all glass impinger with phosphate-buffered saline, relative to a filtration technique (IOM sampler with polycarbonate filter) with sampling times of 30 and 150 min, respectively (Friese *et al.*, 2012). The geometric mean count of MRSA was 257 CFU m^{-3} for impingement and 802 CFU m^{-3} for filtration, but the number of MRSA positive samples was higher for impingement complicating an accurate comparison. Gilbert *et al.* (2012) observed higher levels at lower flow rates with an inhalable sampler and suggested that sampling trauma at higher flow rates might explain these differences.

- Viable MRSA ST398 was detected in only one air sample, with a level of 300 CFU m^{-3}. The interpretation of this finding is complicated by the fact that the MRSA ST398 status of the farms in the study has not been assessed. It would have been useful to take a series of swab samples of the animals present to assess what the MRSA ST398 farm prevalence was as done by others to verify what *S. aureus* strains circulate on a farm. Earlier studies in Switzerland showed that 75% of pig farms had MRSA positive animals (Oppliger *et al.*, 2012). Thus, it seems that MRSA ST398 could not be detected with the strategy chosen in most of the farms, probably because of low levels and high variability related to the short sampling time.

- More specific direct measurement of MRSA ST398 in dust with quantitative PCR was attempted by amplification of multiple genetic targets in another study (Gilbert *et al.*, 2012; Bos *et al.*, 2013). A major challenge is to find DNA targets that are uniquely specific for the organism or strain, which one wants to measure. This was not easily possible for MRSA ST398 with the implication that the targets measured may not be present on one and the same DNA molecule and origin from different DNA molecules from different strains or even microorganisms. The *mecA* gene was targeted, which codes for a variant of the penicillin-binding protein (PBP2a). The *mecA* gene resides on a mobile genetic element, the staphylococcal Cassette Chromosome *mec* (SCCmec), but can also be present in coagulase-negative staphylococcal species present in a dust sample. An ST398 PCR was used to specifically target *S. aureus* isolates belonging to ST398. However, this target is also present in methicillin-sensitive *S. aureus* (MSSA) ST398, thus it measures MRSA and MSSA ST398. The SCCmec element was also targeted, but this element is highly heterogeneous, therefore requires multiple primers and some elements could have been missed making SCCmec potentially a poor proxy for MRSA. Although none of the targets was specific for MRSA ST398 only, correlation analysis showed relatively high correlations between the different targets suggesting that they probably shared a common source. This makes it likely that in many samples MRSA ST398 in the air was sampled. This issue, that DNA targets measured in dust can come from different DNA molecules and thus different organisms, is one of the potential culprits of environmental sampling of microorganisms in dust using molecular techniques. This seems paradoxal because genetic targets have been used to identify ST398 and distinguish ST398 from other strains (van Meurs *et al.*, 2013). However, these targets have been used at the end of a diagnostic process, when an individual MRSA strain has to be tested. Here, the test is applied on one strain with unique DNA, which has already been identified as MRSA, and the issue, which occurs with dust samples that contain multiple microorganisms, does not occur.

Thus, the major advantage of direct analysis of dust samples is that more samples can be processed over a shorter time interval without laborious microbiological work. A potential disadvantage is the loss of specificity and thus information about the target organism and the lack of information about viability. Whether these two aspects are truly disadvantages depends on the specific context and research questions. Alternatively, meta-genomic approaches can be applied that amplify and identify each individual strain of DNA in a sample to analyse the whole spectre of species present. However, this approach is still relatively expensive and requires a sufficient amount of DNA in a sample (Tringe and Rubin, 2005; Tringe *et al.*, 2008).

CONSEQUENCES FOR PUBLIC HEALTH

Many farmers are nasal carriers of MRSA ST398. Epidemiological studies among pig and veal farmers have shown high carriage rates. A considerably lower number of farmers are persistent carriers. Intermittent carriage most likely reflects recent exposure or contamination of the nasal nares.

An association has been observed between environmental MRSA levels and nasal carriage (Bos *et al.*, 2013). Whether transmission through air is a relevant route in addition to direct contact (hand–mouth, hand–nose contact, and smoking) will require further study. One can hypothesize that it should be possible to establish exposure response relations on the basis of air measurements especially because there is a clear exposure response relation with duration of animal contact (Graveland *et al.*, 2010). On the other hand, it seems unlikely that low levels so far measured in outdoor air, near pig and poultry stables at distances between 50 and 150 m, create a major risk for residents who live near livestock farms (Gibbs *et al.*, 2004, 2006; Green *et al.*, 2006). Studies among residents did not show more nasal carriage among residents that could not be explained by direct contact to farm animals (van Cleef *et al.*, 2010; Bisdorff *et al.*, 2012). Meta-population models exist, which facilitate risk assessment for MRSA ST398 and describe transmission from different occupationally exposed (sub-)populations to the general population (Porphyre *et al.*, 2012). Modelling is strongly based on relatively crude assumptions regarding the likelihood that animal–human contact and human–human contact lead to transmission. Exposure studies will help to specify the role of different transmission routes (air, direct contact, and hand–mouth/nose behaviour). Exposure response models, possibly for different transmission routes, in combination with information about exposure distributions can improve risk assessments for microbial agents and contribute to effective preventive strategies.

In conclusion, exposure assessment of microbial agents is rapidly developing as a result of access to new molecular approaches. Occupational health specialists will benefit from these developments as new possibilities emerge for application of occupational hygiene expertise in transmission studies and risk assessments for microbial agents.

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