

High risk for nasal carriage of methicillin-resistant *Staphylococcus aureus* among Danish veterinary practitioners

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Objectives Due to their multiple antibiotic resistance properties, methicillin-resistant *Staphylococcus aureus* (MRSA) are a major public health problem. After the recently described emergence of MRSA in animals, the authors investigated the risk of nasal MRSA carriage among people with professional contact with animals.

Methods Nasal swabs and information on animal exposure and known MRSA risk factors were obtained from participants (N=702) at five conferences organized by national veterinary and farmer associations and students at a business school in Denmark. All of the participants were screened by standard microbiological techniques for MRSA detection and characterization.

Results MRSA carriage was significantly ($P<0.02$) higher among the veterinary practitioners (3.9%) than among the participants not professionally exposed to animals (0.7%). Six of the nine MRSA strains isolated from veterinary practitioners belonged to clonal complexes (CC) previously associated with horses (CC8), small animals (CC22), and pigs (CC398). Although four of the nine positive veterinarians carried the CC associated with pigs, exposure to small animals, cattle, or horses, but not to pigs, was found to be a significant risk factor.

Conclusions The results indicate that veterinarians are at risk of MRSA carriage. Veterinary professionals should be informed about this emerging occupational health risk and educated about preventive measures. Collaboration between national medical and veterinary institutions is urgently needed to control the spread of these unwanted bacteria in the community.

Key terms antibiotic resistance; farmer; occupational health; veterinarian.

Staphylococcus aureus form one of the most important bacterial pathogens worldwide. Methicillin-resistant *S aureus* (MRSA) are a common cause of hospital-acquired infections that are associated with high morbidity and high mortality (1–3). MRSA are often resistant to most of the antibiotics available for treating staphylococcal infections, and they represent an economic burden on society due to increased therapeutic and diagnostics costs and the need for prolonged hospitalization. Recently, an increasing number of reports has documented cases of MRSA colonization or infection in animals, namely, dogs, cats, horses, and pigs (4–7). As

in humans, MRSA can colonize the skin and mucosa of healthy animals, especially nasal mucosa, which is used as the standard sampling site for surveys on human or animal carriage. MRSA were initially reported in pets owned by human patients infected with these bacteria (8) or by people having known risk factors for MRSA colonization, such as hospital employees (9). Thus it was hypothesized that MRSA in animals were mainly transmitted from humans. However, transmission can potentially occur in both directions, and direct exposure to MRSA-positive animals can lead to transmission to humans and potential infection. Various studies have

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recently demonstrated the transmission of MRSA between animals and people in daily contact with animals, such as veterinarians (10–12) and pig farmers (7).

To investigate possible occupational risks associated with the emergence of MRSA in animals, three independent studies were carried out to assess the prevalence of MRSA nasal carriage in veterinary personnel attending conferences [ie, the American College of Veterinary Internal Medicine (ACVIM) Forum in Louisville, Kentucky, USA (13), the International Pig Veterinary Society Congress (IPVS) in Copenhagen, Denmark (14), and a national livestock conference in the Netherlands (15)]. The proportion of nasal MRSA carriage observed in the three studies was high among the veterinary personnel (7.1%, 12.5%, and 3.9%, respectively) when compared with the estimated community prevalence (0.3% to 0.8%) (16, 17). At the ACVIM Forum, MRSA carriers were more frequently detected among technical staff (14.7%) and large animal personnel (15.6%) than among small animal personnel (4.4%) (13). Similarly, at the IPVS, 94% of the MRSA-positive persons had frequent contact with pigs, whereas only 47% of the MRSA-negative persons had frequent contact with pigs. This finding suggests that exposure to these animals is a risk factor for MRSA carriage (14). All strains isolated at the Dutch national livestock conference (15) and 31 out of 34 strains from the IPVS (14) belonged to clonal complex (CC) 398, the MRSA lineage associated with pigs.

The incidence of hospital-acquired MRSA has been maintained at low levels in Denmark since the mid-1970s, probably as a result of the national control program based on increased hospital hygiene awareness, the implementation of strict infection control practices, and prudent antibiotic consumption. Up until 2002, the annual incidence of MRSA infections was $\leq 1\%$ of total *S aureus* isolates from blood. Since then, an apparent increase has been observed, up to approximately 2% in 2005 (18). In view of the recent increase in the incidence of MRSA infections, since November 2006, MRSA infection and carriage have become notifiable (ie, all MRSA cases have to be reported to the national health authorities). However, Denmark remains a country with one of the lowest MRSA infection prevalences in Europe. Among animals, only one case of MRSA carriage has been reported to date (19).

The three previous studies on MRSA carriage among veterinary staff lacked control groups of people without professional contact with animals and had relatively low sample sizes (179 to 272 persons tested), and two of the studies included heterogeneous study populations consisting of veterinarians from many different countries. The objective of our cross-sectional study was to determine whether people with different forms of professional contact with animals are at risk of MRSA

carriage in Denmark, a country where MRSA are uncommon in both the human and animal populations. For this purpose, standard microbiological techniques were used to screen approximately one-tenth (N=231) of the reference population of veterinary practitioners in Denmark for MRSA carriage. The survey also included cattle farmers, other professionals exposed to animals, and a control group of people without professional contact with animals, for a total of 725 tested persons.

Study population and methods

Study population

The sampling was performed between August 2006 and February 2007 at the following five national conferences attended mostly by veterinary staff or farmers: (i) the Annual Veterinary Professional Week, Nyborg; (ii) a meeting of the Danish Pig Veterinary Society, Kolding; (iii) a general meeting of the Danish Equine Veterinary Association, Ebeltoft; (iv) a general meeting of the Danish Small Animal Veterinary Association, Århus; and (v) the Danish cattle conference, Herning. The study population comprised the following four categories: (i) veterinary practitioners in contact with production (eg, pigs and cattle) or companion animals (eg, horses, dogs, and cats), (ii) cattle farmers, (iii) other nonveterinary professions with daily contact with animals (eg, veterinary or agricultural students or veterinary nurses), and (iv) unexposed persons (ie, persons without professional exposure to animals). Students from the Copenhagen Business School were also recruited for their lack of professional contact with animals.

Sampling and questionnaire information

The participants were provided with a single cotton swab and instructed to insert the cotton swab ~1 cm into each nostril and rotate along the mucosal membrane for 5 seconds. Sampling was performed under the supervision of one member of the scientific staff to ensure that the swabs were taken correctly. Information about the amount of weekly professional contact with specific animal species and information on recognized risk factors for MRSA carriage, such as previous hospitalization (within the last 6 months), antimicrobial therapy (within the last 6 months), and residence with a human health care worker, was obtained using a standardized questionnaire filled out prior to the sampling. Participation was on a strict voluntary basis, and signed informed consent was obtained from all of the participants. Personal information was kept anonymous except for the volunteers from the Danish cattle conference, who were sampled after 1 November 2006 when MRSA became

mandatorily reportable in Denmark. The protocols used were approved by the Danish National Committee on Biomedical Research Ethics (H-KF-2007-0007).

Bacterial identification and characterization

MRSA were detected by the following two methods: (i) direct streaking onto oxacillin resistance screening agar (ORSAB, Oxoid, Hampshire, United Kingdom) and (ii) after streaking onto ORSAB swabs were placed into a MRSA-selective enrichment broth (Statens Serum Institut, Copenhagen, Denmark), followed by streaking on MRSA-ID agar plates (BioMérieux, Marcy l'Etoile, France). After 24 and 48 hours of incubation at 37°C, presumptive MRSA colonies on either of the chromogenic agar plates were subcultured onto 5% blood agar plates and a 1-µg oxacillin disc (Oxoid). MRSA isolates were confirmed through the detection of the gene responsible for methicillin resistance (*mecA*) by polymerase chain reaction (PCR) (20) and by PCR detection and sequencing of the staphylococcal protein A (*spa*) gene (21). The sequences were analyzed using Ridom StaphType 1.5.2 software and submitted to the Ridom *spa* server database (<http://spa.ridom.de/spatypes.shtml>, last accessed on 11 May 2007) to determine possible associations with CC identified by multi-locus sequence typing. SCC*mec* types were determined with a multiplex PCR as described by Oliveira et al (22). The presence of the Panton-Valentine leukocidin (PVL) toxin gene was determined by PCR as described by Lina et al (23).

Antimicrobial susceptibility

Antimicrobial susceptibility testing was performed by the disk diffusion method of the Clinical and Laboratory Standards Institute (24), unless stated otherwise. The following antimicrobial disks (Oxoid) were used: chloramphenicol (30 mg), ciprofloxacin (5 mg), clindamycin (2 mg), erythromycin (15 mg), fusidic acid (10 mg), kanamycin (30 mg), mupirocin (5 mg), quinupristin or dalfopristin (15 mg), rifampicin (5 mg), tetracycline (30 mg), and trimethoprim and sulfamethoxazole (1.25 + 23.75 mg). Disc diffusion susceptibility was tested for fusidic acid and mupirocin as previously described (25, 26). The reference strain *S aureus* ATCC 25923 was used for quality control.

Statistical analysis

Because of the low number of MRSA-positive isolates in most of the groups, the association between the occurrences of MRSA between each hypothesized risk factor was assessed with the use of the exact logistic analysis with the statistical package LogExact 4 (27). Odds ratios were estimated from the following general formula on

the basis of the estimates of the logistic analysis as follows: $\ln(P_i/(1-P_i)) = B_i$, where $P_i = \text{Pr}(\text{MRSA} + | \text{risk factor } i)$ and B_i is the effect of the i th risk factor group. The difference in the prevalence of MRSA between the risk factor groups was tested using the exact test. Conditional maximum likelihood estimates were calculated. If the observed value was at the extreme (eg, zero positive persons in a group), the conditional maximum likelihood estimate did not exist and was replaced by the median unbiased estimate.

The numbers of hours spent with animals were grouped as follows: (i) 0 hours/week, (ii) >0 and ≤5 hours/week, (iii) >5 and ≤20 hours/week, and (iv) >21 hours/week. These time groups were stratified by animal species (small animals, pigs, cattle, horses, and other) and also considered as a combined group.

Results

Questionnaires and samples were obtained from 725 persons. Altogether 23 observations were excluded from the data analyses for the following reasons: 20 samples were from foreigners (three from Finland, one from France, one from Germany, five from Norway, seven from Sweden, one from Switzerland, one from the Netherlands, and one from the United Kingdom) and therefore did not represent the target population; one was from a human health care nurse and therefore constituted a separate risk group; two persons did not fill in relevant information in the questionnaire. All the samples from these 23 persons were MRSA-negative. The remaining 702 persons comprised veterinary practitioners (N=231), nonveterinarians (N=72), farmers (N=98), and unexposed persons (N=301).

The distribution of MRSA carriers from each of the sampling events is given in table 1.

Table 1. Distribution of methicillin-resistant *Staphylococcus aureus* (MRSA) obtained during six distinct sampling events.

	MRSA		Total
	Positive	Negative	
Danish Veterinary Association—horse section, Ebeltoft	1	48	49
Danish cattle conference, Herning	–	169	169
Danish Pig Veterinary Society, Kolding	2	65	67
Annual Veterinary Professional Week, Nyborg	5	185	190
Danish Small Animal Veterinary Association, Århus	3	96	99
Copenhagen Business School	–	128	128
Total	11	691	702

MRSA was isolated from 11 persons: 3.9% (N=9) of veterinary practitioners and 0.7% (N=2) of the unexposed group. The veterinary practitioners had a significantly ($P=0.02$) higher odds of carrying MRSA than did the practitioners in the unexposed group (table 2). The chance of carrying MRSA was not higher among the farmers and other persons with professional contact with animals than among the unexposed group.

Time spent with animals was a significant risk factor only for people spending 5 to 20 hours/week with small animals ($P=0.02$), cattle ($P<0.001$), or horses ($P<0.001$), but not pigs ($P=0.86$). This time frame was typical for veterinary practitioners, but, because of the sparse data material, it could not be determined whether this association was due to the number of hours spent with the animals or to other factors associated with their profession. Not all of the veterinarians stated whether they used gloves while handling antibiotics. Therefore, it was not possible to determine if the handling of antibiotics without gloves was a risk factor for MRSA carriage. Of the veterinary practitioners that answered this question

(149 of 231), 135 stated that they did handle antibiotics without using protective gear. Four of the five MRSA-positive veterinarians stated that they handled antibiotics without gloves. There was no apparent risks associated with visits to the hospital, the occupation of the spouse, the personal consumption of antibiotics within the past 6 months, and the presence of pets in the household (data not shown).

All of the MRSA isolates were negative for the *pvl* toxin gene. Of the 11 strains, 5 carried SCCmec type IV cassettes, and the remaining isolates were nontypeable by the Oliveira method, including all of the CC398 isolates. Nine MRSA strains were resistant to erythromycin, and all of the strains belonging to CC398 were resistant to tetracycline (table 3). Sequencing of the protein A gene (*spa*) revealed high genetic diversity (table 3), including *spa* types associated with CC8 (t008), CC22 (t020 and t022), and CC398 (t011 and t034).

Table 2. Distribution of methicillin-resistant *Staphylococcus aureus* (MRSA) in different occupational groups. (OR = odds ratio, 95% CI = 95% confidence interval)

Occupation group	MRSA		Total	OR	95% CI	P-value
	Positive	Negative				
Veterinary practitioners	9	222	231	6.0	1.23–58	0.02
Nonveterinarians ^a	–	72	72	1.7 ^b	–∞–22	1.0
Farmers	–	98	98	1.3 ^b	–∞–16	1.0
Unexposed	2	299	301	1.0	.	.
Total	11	691	702	.	.	.

^a The persons associated with veterinary tasks included veterinary students, veterinary nurses, and animal health advisors in contact with animals.

^b The conditional maximum likelihood estimate was not available and was replaced by the median unbiased estimate.

Discussion

This study confirmed that veterinary staff form a professional category at risk of MRSA carriage in that the veterinarians in our study had a higher MRSA prevalence (3.9%) than the members of the unexposed group (0.7%). The MRSA carriage rate observed for the veterinary staff was also high when compared with the expected prevalence among healthy persons in the Danish community, which is well below 1%. In particular, practicing veterinarians with 5 to 20 hours of professional contact with small animals, cattle, and horses were shown to have a higher chance of being MRSA carriers than the other groups. In contrast with the findings of previous studies, no statistically significant association could be shown between MRSA carriage and contact with pigs.

Table 3. Description of the methicillin-resistant *Staphylococcus aureus* carriers and strains. (CHL = chloramphenicol, CIP = ciprofloxacin, CLI = clindamycin, ERY = erythromycin, FD = fusidic acid, KAN = kanamycin, MUP = mupirocin, Q-D = quinupristin/dalfopristin, TET = tetracycline, SXT = trimethoprim/sulfamethoxazole, NA = not applicable)

Risk group	Type of practice	<i>spa</i> type	Clonal complex	SCCmec type	Antibiotic resistance profile
Veterinarian	Horse	t166	CC1	NT	ERY, KAN, SXT, TET
Veterinarian	Cattle, horse, small animal	t008	CC8	IV	CIP, CLI, ERY, KAN
Veterinarian	Cattle, horse, small animal	t020	CC22	IV	CIP, CLI, ERY
Veterinarian	Cattle, horse, small animal	t022	CC22	IV	CIP
Veterinarian	Cattle	t216	CC59	IV	CIP, CLI, ERY
Veterinarian	Cattle, small animal	t186	CC88	NT	CIP, ERY, TET
Veterinarian	Small animal	t034	CC398	NT	CHL, CLI, ERY, FD, MUP, SXT, TET
Veterinarian	Pig	t034	CC398	NT	TET
Veterinarian	Small animal	t034	CC398	NT	ERY, TET
Unexposed	NA	t011	CC398	NT	CHL, CLI, ERY, FD, SXT, TET
Unexposed	NA	t002	CC5	IV	CLI, ERY, KAN, Q-D

This discrepancy could reflect geographic differences in the prevalence of MRSA in pigs. A recent survey in the Netherlands (28) showed a high prevalence (39%) among pigs in that country. However, the prevalence of MRSA in Danish pigs is likely to be substantially lower (19, 29), and this lower prevalence may explain the rare occurrence of these bacteria in Danish pig practitioners. Although one of the previous surveys of MRSA carriage in veterinary staff took place at a veterinary conference (IPVS) in Denmark (14), MRSA were detected in only one Danish pig practitioner despite the high prevalence of MRSA observed among pig practitioners from other countries attending the conference (14).

Interestingly, cattle farmers were not found to be a risk group, and none of the cattle farmers tested were found to carry MRSA. This result suggests that the risk of MRSA carriage may differ between distinct professional groups working in contact with the same animal species. It should be noted that veterinary practitioners generally work with a variety of different animal species and are exposed to many animal populations, whereas farmers are in contact with one or a few animal species and populations. Therefore, the risk of MRSA carriage may be related to the type of professional contact.

Although most of the recorded factors were considered risk factors, the observed low prevalence of MRSA made inference with respect to these factors difficult. Exact logistic analyses were used to derive odds ratios and 95% confidence intervals. However, conditional maximum likelihood estimates were not always available and were therefore replaced by median unbiased estimates. The point estimates of the latter should be interpreted with care, and more weight should be put on the confidence intervals. In general, a low prevalence in the various risk groups constitutes a special problem that renders studies of risk factors difficult. By the use of exact methods, it is possible to some extent to circumvent some limitations concerning sample size, but a larger sample size would still be required for appropriate inferences with respect to some risk factors. It should be noted that the sample size of this study was approximately 2.5 times larger than those used in the three previous surveys. Furthermore, this survey was conducted at the national level and therefore targeted a smaller reference population than those of two previous studies performed at international veterinary conferences. Denmark is a small country with approximately 747 practicing veterinarians registered with the national veterinary association, including 439 small animal, 265 cattle, 149 pig, and 192 horse practitioners (it should be noted that a veterinary practitioner may be a member of more than one section at a time) (Charlotte Borelli, Danish Veterinary Association, personal communication, 2007). Thus the sample size of our study was relatively high (approximately one-third of the total target popula-

tion) when the low number of practicing veterinarians in this country is taken into consideration.

Six of the nine MRSA isolated from veterinary practitioners belonged to CC groups (CC8, CC22, and CC398) that have previously been associated with horses, small animals, and pigs, respectively (10, 30–32). In a previous study (12), it was shown that MRSA isolated from small animal practitioners in the United Kingdom and Ireland belonged to the same genetic lineage as MRSA occurring in small animals, whereas MRSA isolated from horse practitioners were genetically related to strains occurring in horses. These associations were considered to be indirect evidence for the zoonotic transmission of MRSA from animals to veterinary staff. The CC occurring in small animals and horses in Europe (CC22 and CC8, respectively) are established human pathogens, and they probably originated from humans. The genetic lineage that recently emerged in pigs (CC398) is likely to be of animal origin and may be regarded as true zoonosis. Several cases of human infections associated with this zoonotic MRSA lineage (including severe infections) have been reported in the Netherlands (32), Germany and Austria (33), and, more recently, in Denmark (Robert Skov, unpublished results).

Four veterinarians carried the pig-associated clone CC398. In concordance with the results of previous studies in the Netherlands (7, 15), in our study, CC398 isolates were resistant to tetracycline, the antibiotic most frequently used in pig farming. However, all four CC398 isolates had very heterogeneous resistance patterns, even within the same *spa* type (table 3). Furthermore, only one of the four people harboring MRSA CC398 (three veterinary practitioners and one unexposed person) noted contact with pigs (25 hours/week). The remaining two veterinary practitioners had 37 hours/week with small animals, a finding suggesting that CC398 may be linked to animals other than pigs. A previous study by Witte et al (33) provided a similar indication, as MRSA strains belonging to CC398 were isolated from cases of sinusitis and wound infections in dogs and horses. The unexposed CC398 carrier was a sales representative at a pharmaceutical company, who was likely to have had frequent contact with veterinary practitioners. The remaining MRSA isolates belonged to CC groups previously identified in Denmark and known to cause community-acquired infections. The occurrences of MRSA belonging to these CC groups have not been reported in animals to date.

Factors other than exposure to animals should be considered as possible risk factors. For example, handling antimicrobials without gloves might be an alternative risk factor. *S aureus* is part of the normal skin flora, and repeated exposure to antimicrobials could lead to selection for MRSA. In this study, it was not possible to determine whether the use of protective gear during

the handling of antibiotics was associated with MRSA carriage. However, handling antimicrobials without gloves seemed to be a common practice among Danish veterinarians and, more importantly, among most of those who were found to carry MRSA. Veterinary professionals should be informed about this emerging occupational health risk and educated about preventive measures. For example, they should wear gloves when handling antimicrobial agents and take adequate protective measures when attending animals at risk of MRSA infection (eg, wound and postoperative infections) and working in farm environments, which can be contaminated with MRSA (eg, pig farms). Skin lesions should always be protected from exposure to domestic animals, as indicated by recent documentation on skin MRSA infections transmitted from horse to veterinary personnel (34). The risk of zoonotic MRSA infection can be minimized by the early recognition of infected animals, proper animal handling, biosecurity precautions, and, most importantly, personal hygiene.

This study indicates that, even in a country such as Denmark, where MRSA are rare in both human and animal populations, the prevalence of MRSA carriage among veterinary practitioners is significantly higher than in the rest of the community. This finding raises important questions related to both occupational and public health concerns. Animals can act as reservoirs of novel MRSA lineages, and veterinarians should be aware of the importance of MRSA in animals and should be able to detect and manage animal patients, as well as to prevent the spread of MRSA within the animal population. Comprehensive guidelines on how to manage MRSA in veterinary practices have recently been developed by the British Small Animal Veterinary Association (BSAVA) and are available on the Internet (<http://www.bsava.com/resources/mrsa/mrsaguidelines/mrsaguidelines.htm>).

In summary, veterinary practitioners spending between 5 to 20 hours a week had a higher chance of being MRSA-positive than an unexposed group. In view of this finding, increased awareness, appropriate intervention, and control measures are required to control the spread of MRSA among veterinarians.

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