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Prevalence of methicillin-resistant *Staphylococcus aureus* among veterinarians: an international study

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ABSTRACT

Pig farmers and veterinarians in contact with livestock in The Netherlands have a higher risk of methicillin-resistant *Staphylococcus aureus* (MRSA) carriage than the general population. The objective of this study was to investigate whether this is also true for other professionals in contact with pigs in an international setting. A convenience sample of 272 participants at an international conference on pig health in Denmark was screened for MRSA carriage using combined nose/throat swabs and were asked to complete a questionnaire concerning animal contacts, exposure to known MRSA risk-factors, and the protective measures taken when entering pig farms. In total, 34 (12.5%) participants from nine countries carried MRSA. Thirty-one of these isolates were non-typeable by pulsed-field gel electrophoresis following *Sma*I digestion of chromosomal DNA. All of the non-typeable isolates belonged to *spa* types (t011, t034, t108, t571, t567 and t899) that correspond to multilocus sequence type 398. All of the above-mentioned *spa* types, with the exception of t899, have been isolated previously from either Dutch pigs, pig farmers and/or veterinarians. Protective measures, e.g., masks, gowns and gloves, did not protect against MRSA acquisition. Transmission of MRSA from pigs to staff tending to these animals appears to be an international problem, creating a new reservoir for community-acquired MRSA (CA-MRSA) in humans in Europe, and possibly worldwide. The rise of a new zoonotic source of MRSA could have a severe impact on the epidemiology of CA-MRSA, and may have consequences for the control of MRSA, especially in those countries that maintain a low prevalence by means of search-and-destroy policies.

Keywords Community-acquired, methicillin-resistant *Staphylococcus aureus*, pigs, *Staphylococcus aureus*, surveillance, veterinarians

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INTRODUCTION

Methicillin-resistant *Staphylococcus aureus* (MRSA) is an important cause of nosocomial disease worldwide. Recent reports indicate that the epidemiology of MRSA is undergoing a major change following the emergence of community-acquired MRSA (CA-MRSA) [1–3]. CA-MRSA can cause serious infections in otherwise healthy individuals and has, in some instances, even surpassed methicillin-susceptible *S. aureus* as a pathogen [4].

In 2004, contact with livestock, especially pigs, was identified as a risk-factor for MRSA carriage in The Netherlands [5]. Surveys of Dutch pig farmers [5] and veterinarians [6] showed a significantly higher MRSA carriage rate in these groups (26% and 4.6%, respectively) than in the general Dutch population (0.03%) [7]. A survey of slaughterhouse pigs showed that 39% of pigs were MRSA-positive [8]. Isolates from pigs, pig farmers and veterinarians were non-typeable by standard typing using pulsed-field gel electrophoresis

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(PFGE), following digestion of chromosomal DNA with *Sma*I, because of a novel DNA methylation enzyme present in these isolates [9]. Typing of these isolates showed that they belonged to a number of closely related *spa* types (t011, t034, t108, t567 and t571), all of which corresponded to multilocus sequence type (ST) 398. Strains that were non-typeable by *Sma*I PFGE were first observed in The Netherlands in 2003 and are increasing in frequency (12th International Symposium on Staphylococci and Staphylococcal Infections, Maastricht, The Netherlands, 2006; abstract 0.26).

A strict 'search-and-destroy' policy in The Netherlands has kept the prevalence of MRSA in hospitals at 1% [10–12]. In order to preserve the effectiveness of this policy, the national guidelines were recently changed so that all individuals in professional contact with pigs are now isolated and screened for MRSA upon admission to a hospital. It is currently unknown whether this new source of CA-MRSA is limited to The Netherlands, or whether it is an international problem. However, the latter is probable, since the meat and livestock market is international. In order to investigate whether contact with pigs might be a risk-factor for MRSA carriage in countries other than The Netherlands, the present study screened a random selection of participants at an international conference on pig health in Denmark.

MATERIALS AND METHODS

A convenience sample of 272 individuals from among *c.* 2500 participants at a conference in Copenhagen, Denmark, concerning pig health was screened. One swab per individual was taken from both anterior nares and throat by either a qualified physician or by the participants themselves under the direct supervision of this physician. Each of the individuals sampled was asked to complete a questionnaire seeking information concerning profession and the type and intensity of contact with pigs, protective measures taken in pig farms, recent hospital admissions, and contact with known MRSA-positive family members.

All swabs were incubated in a semi-selective Tryptone Soy broth containing NaCl 2.5% w/v, cefoxitin 3 mg/L and aztreonam 10 mg/L (SSI Diagnostika, Hillerød, Denmark). After 24 h, the broths were subcultured on sheep blood 5% v/v agar plates and MRSA-ID agar plates (bioMérieux, La Balme Les Grottes, France). Staphylococci were initially identified on the basis of colony morphology and tube coagulase tests. Methicillin resistance was determined by disk-diffusion using cefoxitin disks according to CLSI recommendations [13].

Species identifications and susceptibility testing results were confirmed using the Vitek II Automated Microbiology System with ID card GP and AST card AST-P554 (bioMérieux), which includes susceptibility tests for ciprofloxacin, clindamycin, erythromycin, fusidic acid, gentamicin, linezolid, quinupristin–dalfopristin, rifampicin, vancomycin, teicoplanin, trimethoprim–sulphamethoxazole and tetracycline. All cefoxitin-resistant isolates were also investigated by PCR for carriage of the *mecA* gene [14], and their staphylococcal cassette chromosome (SCC)*mec* type was determined using the primers described by Zhang *et al.* [15]; the isolates were also typed by PFGE following *Sma*I digestion of chromosomal DNA [16] and their *spa* type was determined [17].

Data were analysed by univariate logistic regression analysis, with carriage of MRSA as a dependent variable, and contact hours with pigs, country of origin, protective measures and contact with cows as independent variables. The best model was selected with the backward likelihood ratio method. If p was >0.05, the coefficient was discarded. All analyses were performed using SPSS v.12 (SPSS Inc., Chicago, IL, USA).

RESULTS

Of the 272 participants who were screened, 34 (12.5%) carried a *mecA*-positive *S. aureus* strain. Table 1 summarises the main characteristics of the participants, together with data concerning animal contact and the use of protective clothing.

Table 1. Main characteristics of 272 conference participants screened for carriage of methicillin-resistant *Staphylococcus aureus* (MRSA)

	Non-carriers (n = 238)	MRSA carriers (n = 34)	p[a]
Mean age (years)	42 (range 22–69)	42 (range 28–57)	
Gender			
Male	166 (70%)	27 (80%)	
Female	65 (28%)	4 (12%)	
Unknown	7 (3%)	3 (9%)	
Type of activity			
Veterinarian	202 (84%)	33 (97%)	
Commercial	9 (4%)	0	
University	9 (4%)	1 (3%)	
Research	8 (3%)	0	
Student	5 (2%)	0	
Other	8 (3%)	0	
Frequency of pig contact[b]			
Frequent	113 (47%)	32 (94%)	0.0001
Sometimes	83 (35%)	2 (6%)	0.0003
Seldom	42 (18%)	0	0.0001
Use of protective equipment			
Gown	150 (63%)	17 (50%)	0.02
Gloves	64 (27%)	25 (74%)	
Mask	74 (31%)	19 (56%)	
Type of animal contact			
Pigs	238 (100%)	34 (100%)	0.002
Dairy cows	62 (26%)	18 (53%)	
Meat cows	36 (155)	7 (20%)	
Poultry	24 (10%)	1 (3%)	
Sheep	30 (12%)	5 (15%)	
Goats	11 (5%)	0	
Horses	39 (16%)	4 (12%)	
Companion animals	99 (42%)	10 (30%)	
Pets at home	160 (67%)	21 (62%)	
Recent hospital stay	5 (2%)	1 (3%)	
MRSA-positive family member	5 (2%)	1 (3%)	

[a]Values <0.05 are shown.
[b]Frequent, i.e., daily and/or more than 5 h/week; sometimes, <5 h/week, but with a minimum of once per month; seldom, less than once per month.

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Table 2. Number of participants per country and the distribution of methicillin-resistant *Staphylococcus aureus* (MRSA) carriers and *spa* types among countries

Country	Participants/ country	No. (%) of MRSA carriers/ country	*spa* types (*n*)[a]
Australia	6	0	–
Austria	8	0	–
Belgium	6	1 (16)	t011 (1)
Brazil	8	0	–
Bulgaria	1	0	–
Canada	16	1 (6)	t034 (1)
Cyprus	1	0	–
Czech Republic	5	0	–
Denmark	29	1 (3)	t022 (1)
Finland	4	0	–
France	6	1 (16)	t111 (1)
Germany	39	13 (33)	t011 (8), t034 (4) t108 (1)
Ireland	5	0	–
Italy	13	8 (61)	t108 (1), t899 (5) t1730 (1)
Japan	1	0	–
Korea	1	0	–
Lithuania	2	0	–
Malaysia	1	0	–
Malta	1	0	–
Mexico	3	0	–
The Netherlands	26	6 (23)	t011 (3), t108 (1), t567 (1), t571 (1)
New Zealand	2	0	–
Norway	5	0	–
Philippines	1	0	–
Poland	3	0	–
Portugal	3	0	–
Serbia	2	0	–
Slovakia	1	0	–
South Africa	3	0	–
South Korea	1	0	–
Spain	11	2 (18)	t011 (2)
Sweden	12	0	–
Switzerland	12	0	–
Taiwan	1	0	–
Thailand	9	1 (11)	t034 (1)
UK	8	0	–
USA	14	0	–
Vietnam	2	0	–

[a]No. of isolates with the indicated *spa* type.

Table 2 shows the number of participants and MRSA carriers according to country. Thirty-one of the 34 isolates were non-typeable by PFGE following *Sma*I digestion of chromosomal DNA. However, s*pa* typing of these 31 isolates revealed that 26 belonged to closely related *spa* types (i.e., t011, t034, t108, t571, t567), all of which were shown in previous studies to correspond to ST398 [8] (Table 2). The remaining isolates belonged to *spa* type 899, which was also shown by multilocus sequence typing (MLST) to belong to ST398. Isolates of *spa* type t011 were isolated from participants from four different European countries; *spa* type t034 was isolated from the two delegates from outside Europe.

The three isolates that were typeable by PFGE belonged to *spa* types t022, t111 and t1730, and were recovered from Danish, French and Italian delegates, respectively; *spa* type t022 corresponds to ST22, and *spa* type t111 corresponds to ST5.

All 34 isolates were susceptible to vancomycin, rifampicin, quinupristin–dalfopristin, fusidic acid and linezolid. Further resistance phenotypes of the 31 ST398 MRSA isolates are shown in Table 3. Nine (29%) isolates were resistant to four antibiotic classes, and 18 (58%) to five or more antibiotic classes. All isolates were tetracycline-resistant, 22 (70%) isolates had an MLS_B phenotype, and 15 (48%) were resistant to trimethoprim–sulphamethoxazole. The four isolates that were resistant to ciprofloxacin were from Italy (2) and Spain (2). There was no clear association between the *spa* type and the resistance pattern.

The most frequent SCC*mec* type was SCC*mec*V (n = 24, 70.6%), followed by type IVa (n = 3) and type III (n = 2). No SCC*mec* type could be assigned for five isolates when the primers described by Zhang *et al.* [15] were used.

Univariate analysis, with MRSA carriage as the endpoint, showed a significantly increased risk of MRSA carriage for individuals having frequent (daily or a minimum of 5 h/week) pig contact, as compared with those seldom having contact (less than once per month), with an OR of 16.3 (CI 3.75–70.6). Individuals with infrequent contact (<5 h/week, but a minimum of once per month) had a non-significant trend towards a higher risk, with an OR of 2.4 (CI 0.58–9.8, p not significant) as compared with those seldom having contact. Contact with cows, country of origin, and use of protective measures, especially wearing of a mask, had no influence on the rate of MRSA colonisation. Indeed, statistically, not wearing a mask was protective, with an OR of 0.38 (CI 0.12–0.99).

DISCUSSION

Community-acquired MRSA is rapidly becoming a widespread pathogen worldwide, primarily as a cause of skin and soft-tissue disease, but sometimes of invasive infection, e.g., necrotising pneumonia, in otherwise healthy individuals [1–4]. The source of CA-MRSA is unknown, but clinical and molecular epidemiological studies have indicated two separate evolutionary pathways for CA-MRSA and hospital-acquired MRSA. MRSA strains belonging to several different multilocus sequence types have been associated with infection and colonisation in both humans and animals, suggesting bidirectional transmission [18–26]. However, most reports are

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Table 3. Resistance phenotypes of 31 methicillin-resistant *Staphylococcus aureus* isolates that were non-typeable by pulsed-field gel electrophoresis with *Sma*I and that belonged to sequence type 398

Isolate[a]	Country of residence	*spa* type	Ciprofloxacin	Clindamycin	Erythromycin	Gentamicin	Tetracycline	Trimethoprim–sulphamethoxazole
13	Germany	t011	S	S	S	S	R	S
28	The Netherlands	t567	S	S	S	S	R	S
29	The Netherlands	t108	S	S	S	S	R	S
1	Thailand	t034	S	S	S	S	R	R
6	Canada	t034	S	S	S	R	R	S
14	Italy	t899	I	R	S	S	R	S
27	The Netherlands	t571	S	S	S	S	R	R
2	Italy	t899	S	R	R	S	R	S
4	Spain	t011	R	R	S	S	R	S
5	Germany	t011	S	R	R	S	R	S
8	Belgium	t011	S	S	S	R	R	R
9	Italy	t108	S	R	R	I	R	S
15	Germany	t011	S	R	R	S	R	I
18	Germany	t011	S	R	R	S	R	S
26	Germany	t011	S	R	R	S	R	S
30	Germany	t011	S	R	R	S	R	S
3	Spain	t011	R	R	R	S	R	S
7	Germany	t034	S	R	R	S	R	R
10	Germany	t034	S	R	R	S	R	R
11	Germany	t034	S	R	R	S	R	R
12	Germany	t108	S	R	R	S	R	R
17	Italy	t899	S	R	R	S	R	R
19	Germany	t011	S	R	R	R	R	S
20	Italy	t899	S	R	R	S	R	R
23	Germany	t011	S	R	R	S	R	R
24	Germany	t034	S	R	R	S	R	R
25	The Netherlands	t011	S	R	R	S	R	R
31	Italy	t899	R	R	R	S	R	S
16	The Netherlands	t011	S	R	R	R	R	R
21	Italy	t899	R	R	R	S	R	R
22	The Netherlands	t011	S	R	R	R	R	R

[a]Isolates are listed in order of resistance, from least to most resistant.

anecdotal or describe outbreaks in a single institution or country.

In general, the rate of colonisation with MRSA among non-hospitalised individuals is very low [27]. In The Netherlands, the prevalence of MRSA upon admission to a hospital was 0.03% [7]. Even in countries with a high prevalence of MRSA, e.g., the USA and Portugal, carriage rates in the general population are only 0.2–3% [27–30]. For this reason, the high prevalence of MRSA carriage (12.5%) among attendees at an international conference on pig health is of great concern and, combined with the significant association between the time spent on pig farms and the risk of colonisation, indicates that contact with pigs could be an important source of MRSA carriage.

Of the 34 MRSA carriers in the present study, 31 veterinarians from seven countries carried a strain that was non-typeable by PFGE. The non-typeable isolates belonged to *spa* types (t011, t034, t108, t571, t567, t899) that correspond to ST398. All of the above-mentioned *spa* types, with the exception of t899, have also been found either in Dutch pigs, pig farmers and/or veterinarians. Carriage of a methicillin-susceptible ST398 strain by pigs and pig farmers has been described previously [31], suggesting that this clone is capable of colonising both pigs and humans. The source of MRSA in pigs is presently unknown, but dissemination of MRSA among pigs could be facilitated by the trade of live animals among different countries and by the use of antibiotics for mass treatment of livestock. All of the isolates in the present study were resistant to tetracycline, which is one of the main antibiotics used in pig farming in The Netherlands (http://www.cidc-lelystad.wur.nl/NL/publicaties/rapporten/maran/). Of further concern is the fact that 58% of the ST398 isolates were truly multiresistant, in the sense that they were resistant to five or more classes of antibiotic (Table 3).

Selection of multidrug-resistant microorganisms of clinical relevance in humans has been associated previously with antibiotic consumption by livestock. A reservoir of vancomycin-resistant enterococci was discovered among pigs and poultry, and led to a ban on the use of the glycopeptide avoparcin as a growth promoter in animals [32]. Later, a high proportion of poultry farmers were found to be carrying vancomycin-resistant enterococci [33]. A more severe challenge is presented by MRSA, since it is a much

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more virulent microorganism than vancomycin-resistant enterococci.

The protective measures taken by veterinarians did not prevent them from becoming colonised with MRSA. This could be a result of breaches in adherence to these measures, e.g., poor hand hygiene after removal of gloves or the reuse of contaminated dust masks, or because of contamination outside pig farms. Gibbs *et al.* [34] showed that antibiotic-resistant bacteria from the environment of pigs, including ampicillin- and tetracycline-resistant *S. aureus*, could be recovered up to 150 m downwind of an (open) pig-breeding facility. The possibility that airborne MRSA can colonise veterinarians or other individuals in the direct vicinity of a pig farm can therefore not be excluded.

When the allelic profile of ST398 is compared with predominant clones in Europe by means of the MLST database, there is no relationship with epidemic healthcare-associated MRSA or common CA-MRSA at the present time. The situation in The Netherlands shows an increasing prevalence of ST398 among MRSA isolates from all sources. This clone has also been reported in Germany from cases of ventilator-associated pneumonia [35] and in infections in Denmark (R. Skov, unpublished data). In the present study, participants from The Netherlands, Germany, Spain, Belgium, Canada, Thailand and Italy carried 'pig-related' MRSA strains, thereby indicating that these strains are far more widespread than reported previously. If these strains are allowed to spread freely among pigs, and from pigs to humans, they could constitute an important new source of CA-MRSA. Apart from the fact that individuals in contact with pigs have a higher risk of developing MRSA infection, the high rate of carriage also has an economic effect on search-and-destroy policies for MRSA because of the extra screening and isolation measures required.

The high carriage rate of 'pig-related' MRSA among professionals in contact with pigs indicates that livestock may serve as an important source of CA-MRSA in Europe, and possibly worldwide. The rise of a new 'zoonotic' source of MRSA could have a severe impact on the epidemiology and control of CA-MRSA, especially in countries currently using a search-and-destroy policy. In order to preserve the low prevalence of MRSA in such countries, and to prevent a further increase of CA-MRSA in others, it is important to know the extent to which these strains may have spread in livestock and in the community, and whether screening for MRSA in individuals in contact with pigs is necessary and cost-effective.

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