ORIGINAL ARTICLE 10.1111/1469-0691.12582

Dynamics of methicillin-resistant *Staphylococcus aureus* and methicillin-susceptible *Staphylococcus aureus* carriage in pig farmers: a prospective cohort study

B. A. G. L. van Cleef[1,2,3], B. H. B. van Benthem[3], E. J. M. Verkade[1,2], M. van Rijen[1], M. F. Q. Kluytmans-van den Bergh[4], L. M. Schouls[3], B. Duim[5], J. A. Wagenaar[5], H. Graveland[3,6], M. E. H. Bos[6], D. Heederik[6] and J. A. J. W. Kluytmans[1,2,7]

1) *Laboratory for Microbiology and Infection Control, Amphia Hospital, Breda,* 2) *Laboratory for Medical Microbiology and Immunology, St Elisabeth Hospital, Tilburg,* 3) *Centre for Infectious Disease Control Netherlands, National Institute for Public Health and the Environment, Bilthoven,* 4) *Amphia Academy Infectious Disease Foundation, Amphia Hospital, Breda,* 5) *Department of Infectious Diseases and Immunology, Utrecht University, Utrecht,* 6) *Institute for Risk Assessment Sciences, Utrecht University, Utrecht and* 7) *Department of Medical Microbiology and Infection prevention, VU University Medical Centre, Amsterdam, the Netherlands*

Abstract

Our purpose was to determine the dynamics of livestock-associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA) carriage and its determinants in persons working at pig farms, in order to identify targets for interventions. This prospective cohort study surveyed 49 pig farms in the Netherlands on six sampling dates in 1 year (2010–11). Nasal and oropharyngeal swabs were collected, as well as environmental surface samples from stables and house. Of 110 pig farmers, 38% were persistent MRSA nasal carriers. The average cross-sectional MRSA prevalence was 63%. Methicillin-susceptible *S. aureus* (MSSA) nasal carriage was associated with fewer MRSA acquisitions (prevalence rate (PR) = 0.47, p 0.02). In multivariate analysis, an age of 40–49 years (PR = 2.13, p 0.01), a working week of ≥40 h (PR=1.89, p 0.01), giving birth assistance to sows (PR=2.26, p 0.03), removing manure of finisher pigs (PR=0.48, p 0.02), and wearing a facemask (PR = 0.13, p 0.02) were significantly related with persistent MRSA nasal carriage. A higher MRSA exposure in stables was associated with MRSA in pig farmers (p <0.0001). This study describes a very high prevalence of LA-MRSA carriage in pig farmers, reflecting extensive exposure during work. We identified the possible protective effects of MSSA carriage and of continuously wearing a facemask during work.

Keywords: Dynamics of carriage, epidemiology, livestock, methicillin-resistant *Staphylococcus aureus*, methicillin-susceptible *Staphylococcus aureus*, MRSA, MSSA, pigs, ST398, the Netherlands
Original Submission: 14 August 2013; **Revised Submission:** 27 December 2013; **Accepted:** 27 January 2014
Editor: G. Lina
Article published online: 4 February 2014
Clin Microbiol Infect

Corresponding author: B. A. G. L. van Cleef, Laboratory for Medical Microbiology and Immunology, St Elisabeth Hospital, P.O. Box 90151, 5000 LC Tilburg, the Netherlands
E-mail: brivancleef@gmail.com

Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a well-known pathogen inside and outside hospitals all around the world [1]. In the last years, the distinction between hospital-associated and community-associated MRSA has become less clear. In the Netherlands there still is a very low carriage rate of MRSA in the community (0.1%) [2]. Therefore, new strains, such as livestock-associated MRSA (LA-MRSA), are easily recognized.

The first LA-MRSA-positive pig farmer in the Netherlands was recognized in 2005 [3] and subsequent surveys showed a prevalence of LA-MRSA carriage ranging from 20–40% in people working with pigs [4–8]. Invasive infections were rarely described [9–11], and close contact with livestock was shown to be the major risk factor for LA-MRSA acquisition [6,7].

Currently there is a huge reservoir of MRSA in livestock with a relatively low impact on public health. This might change because *S. aureus* has proven to be very capable of exchanging genetic material, i.e. acquiring virulence factors [12]. To reduce the threat for public health the reservoir must be eliminated or reduced. Targets for intervention are needed, but at present unidentified. The purpose of this prospective cohort study was to determine the dynamics of LA-MRSA carriage and its determinants in persons working at pig farms.

Materials and Methods

Study design and selection of farms

This prospective cohort study surveyed persons working at 49 farrowing pig farms in the Netherlands for 1 year (2010–2011). Pig farms were randomly selected among participants from a previous study [13], which contained randomly selected farrowing pig farms from all Dutch pig farms.

Sampling occasions

During the 1-year study period, there were six sampling occasions: day 0, day 4, day 7, month 4, month 8, and month 12. Quantitative nasal and oropharyngeal swabs, extensive questionnaires, and wet wipe samples of four defined surfaces in house (backdoor handle, remote control of television, favourite chair of pig farmer, and dog neck/back) and four surfaces in the stables (farrowing and weaning stables, both sampled twice) were collected on day 0. Nasal swabs were introduced in the nostril and rotated once. Oropharyngeal swabs sampled the area of the inner cheek including the tonsils. Refrigerated swabs were transported to the laboratory, and cultured within 24 h. In addition, dry electrostatic dust collector cloths (EDCs) [14] were placed in the farrowing and weaning stables (two per stable) and on the highest cupboard in the living room of the house, and were left in place for 2 weeks before quantitative analysis.

On the remaining sampling occasions, subjects semi-quantitatively sampled their own nose and filled in a short questionnaire. Swab instructions were sent with the swabs. EDCs were placed on the same five locations in months 4, 8 and 12. An extra semi-quantitative sample of the throat was taken by the subjects themselves in month 12. Results of the individual cultures were disclosed at the end of the study.

Definitions

Persons working in pig farm stables for ≥20 h per week at the start of the study were defined as pig farmers, regardless of whether they lived on the farm premises or not. Persistent carriers were defined as persons with all nasal cultures positive for MRSA, non-carriers had no positive cultures, and intermittent carriers were the remaining persons.

Questionnaires

Extensive questionnaires were used to elucidate known and hypothetical determinants for (LA-)MRSA carriage. Data were collected on exact activities on the pig farm, contact with animals, hospital contact, personal use of antibiotics or immunosuppressive drugs, underlying disorders (e.g. eczema or other skin diseases) and presence of indwelling catheters and/or open wounds.

Laboratory analysis: cultures

Quantitative cultures were performed by diluting the elution buffer from ESwabs™ (swabs with 1 mL elution buffer; Copan, Brescia, Italy) up to 10^4 times in 0.9% NaCl, and incubating 100 μL of these dilutions on chromID *S. aureus* and chromID MRSA agar plates (BioMérieux, La Balme Les Grottes, France) overnight at 35°C. The number of CFU was counted on each agar plate, and plates with 10–100 CFU were used to calculate the original CFU number per swab. The remaining elution buffer and swabs were enriched overnight in Müller–Hinton broth with 6.5% NaCl, and subsequently cultured on *S. aureus* and MRSA selective agar plates.

Semi-quantitative cultures were performed by inoculating dry swabs (Copan) directly onto *S. aureus*, MRSA and Columbia agar plates with 5% sheep blood, and Müller–Hinton enrichment broth. Wet wipe samples (Sodibox, Nevez, France) were double-enriched in Müller–Hinton broth, followed by phenol mannitol broth with ceftizoxime (BioMérieux), which was subsequently cultured on blood and *Brilliance*™ MRSA agar plates (Oxoid, Basingstoke, UK), whether colour change occurred in the phenol mannitol broth or not.

All *S. aureus* strains were defined by green colonies in combination with a positive coagulase slide and DNase test. In case of discrepancies a tube coagulase test was performed. Methicillin susceptibility was tested for all *S. aureus* isolates, using the cefoxitin disk diffusion method according to EUCAST standards [15], followed by a duplex PCR for the *nuc* and *mec*A genes as described previously [16].

Laboratory analysis: EDC PCR

The EDC was suspended in FE-buffer (150 mM NaCl, 1 mM EDTA), mixed in a Stomacher blender (Seward Limited, London, UK), and stored at −20°C until further processing. DNA was isolated and purified with a Versant Molecular kPCR molecular system (Siemens Healthcare Diagnostics, The Hague, the Netherlands). For each sample, 5 μL was used to detect four targets with a LightCycler 480-II (Roche Diagnostics, Almere, the Netherlands): (i) *mec*A for methicillin-resis-