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Authors: Kraemer JG, Pires J, Kueffer M, Semaani E, Endimiani A, Hilty M, Oppliger A

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Short communication

Prevalence of Extended-Spectrum β-Lactamase-Producing *Enterobacteriaceae* and Methicillin-Resistant *Staphylococcus aureus* in Pig Farms in Switzerland

JULIA G. KRAEMER[1,2], JOÃO PIRES[2], MARIANNE KUEFFER[2], EULALIA SEMAANI[3], ANDREA ENDIMIANI[2], MARKUS HILTY[2], ANNE OPPLIGER[1]*

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[1]*Institut universitaire romand de Santé au Travail (Institute for Work and Health), Universities of Lausanne and Geneva, CH-1066 Epalinges-Lausanne, Switzerland*

[2]*Institute for Infectious Diseases, University of Bern, CH-3001 Bern, Switzerland*

[3]*SUISAG, SGD - SSP Berne –Suisse Romande CH-1350 Orbe Switzerland*

*A. Oppliger

Corresponding author

Phone: +41 21 314 74 16

Fax: + 41 21 314 74 30

E-mail address: Anne.Oppliger@hospvd.ch

ABSTRACT

The presence of Methicillin-Resistant *Staphylococcus aureus* (MRSA) in pig farms has been widely reported, and the emergence of extended-spectrum β-lactamase-producing *Enterobacteriaceae* (ESBL-E) has been documented in several countries. However, data for Switzerland are very scarce. This study aimed to compare changes in the prevalence of MRSA in Swiss pig farms between 2008 and 2015 and make the first ever estimates of the presence of ESBL-E and carbapenemase producers in pigs and pig farm workers. Results showed that ESBL-E was present in both pigs and farm workers and that the proportion of farms with MRSA had increased fourfold in seven years (from 7% to 31%). Associations between antibiotic use and resistant bacteria carriage were shown.

1. Introduction

The worldwide increase in antibiotic resistant bacteria observed in animals, the natural environment and humans is a growing problem (WHO, 2015). In animal farming, antibiotics are used for therapy and/or prophylaxis, resulting in the potential selection of antimicrobial-resistant bacteria which can be transmitted to the surrounding environment and individuals working on the farm.

Among the widely-studied bacteria in livestock, the opportunistic pathogens Methicillin-Resistant *Staphylococcus aureus* (MRSA) and extended-spectrum β-lactamase-producing *Enterobacteriaceae* (ESBL-E) are of greatest concern (Schmithausen et al., 2015; Seiffert et al., 2013). Opportunistic pathogens can cause infections (e.g. skin infections, sepsis) in humans. In pigs, Escherichia coli can cause colibacillosis which is one of the most significant diseases in the swine industry (Xu et al., 2015). Even though there is no evidence that *S. aureus* causes diseases in pigs, MRSA carriage in pig farm workers has been linked to skin

and soft tissue infections (Nadimpalli et al., 2016). It is a great concern for both MRSA and ESBL-E that the antibiotic resistance genes can be transmitted from livestock-associated bacteria to human commensals and/or pathogens. In Switzerland, the prevalence of pig-associated MRSA is poorly documented compared to other countries (Garcia-Graells et al., 2013; van Cleef et al., 2014; van Cleef et al., 2015; Wardyn et al., 2015). In 2008 and 2009, it was shown that the prevalence of MRSA in Swiss pig farms was very low compared to other European countries (Oppliger et al., 2012; Overesch et al., 2011). The presence of ESBL-E has been widely observed in European pig farms (Ewers et al., 2012; Friese et al., 2013; Hansen et al., 2013; Hering et al., 2014; Jakobsen et al., 2015; Mesa et al., 2006; Randall et al., 2014; Rodrigues et al., 2013; Schmithausen et al., 2015; Von Salviati et al., 2014; von Salviati et al., 2015) and the transmission of ESBL genes from pigs to pig farm workers was suggested in Denmark, the Netherlands and Germany (Dahms et al., 2015; Dohmen et al., 2015; Hammerum et al., 2014; Moodley and Guardabassi, 2009). This study's objectives were to make the first ever investigation of the presence of ESBL-E in Swiss pig farms and to update previous data on the prevalence of MRSA.

2. Material and methods

2.1. Pig farms and sampling design

Twenty-eight pig breeding units and one post-weaning farm, all located in western Switzerland, were visited between August and October 2015. Twelve farms had also been investigated in a 2008 study (Oppliger et al., 2012), and seven pig farmers were included in both studies. Sows were kept with their piglets (until 4 weeks old), in individual boxes (4–10 boxes per room) and directly on concrete; weaning piglets were kept in boxes (12–30 piglets per box; 2–12 boxes per room) and on wooden gratings, except in the post-weaning unit, where pigs were housed on straw.

Nasal swabs were taken from two to three immobilized piglets (n = 77) at each farm. Using a questionnaire, participating pig farm workers (n = 47) were asked about their personal antibiotic consumption over the last six months and that of their animals over the last four weeks. Farm workers collected their own anterior nasal sample using a sterile swab. All swabs were immediately transferred into 1 ml Amies transport medium (Copan, Brescia, Italy) and stored at 4 C for transportation. Pig fecal samples were collected by choosing a random pig box on each farm and collecting stool from the floor. Furthermore, all the farm workers were asked to send a stool sample, of which 49% (n = 25) from 19 farms, did so. All fecal samples were stored at -20°C until further processing. Airborne bacteria were sampled from the middle of each piggery (at about 1.5 m above the floor) over 5 minutes, using an impactor (MAS-100 Eco, MBV; Vevey, Switzerland) set to a flow rate of 100 l/min and onto MRSA chromogenic plates (Bio-Rad Laboratories, Cressier, Switzerland). After transport at 4°C, plates were incubated at 37°C for 24 hours and then screened for colonies. Results were expressed in Colony Forming Units (CFU) per m^3 of air. All statistical analyses were conducted using Stata statistical software (Version 11.2, StataCorp, College Station, Texas).

2.2. Animal and human fecal samples processing

Fecal samples (≈ 20 mg) were enriched overnight in 10 ml of LB broth. Enrichments were plated (50 µl) on ChromID BLSE, ChromID ESBL (bioMérieux, Geneva, Switzerland); and on ChromID Carba Smart plate (bioMérieux, Geneva, Switzerland) to detect carbapenemase producers.

Species were identified using MALDI-TOF MS (Bruker Daltonik, Bremen, Germany). At least three *Enterobacteriaceae* colonies per fecal sample were analyzed. Strains underwent the double-disk synergism test (DDST) on cation-adjusted Mueller-Hinton plates (Becton

Dickinson, Sparks, USA), with and without cloxacillin (250 mg/l; Sigma-Aldrich, St. Louis, USA).

β-lactamase genes (*bla*) were identified using a CT103XL microarray (Check-Points B.V., Wageningen, The Netherlands) and PCR/sequencing (Pires et al., 2016). Population structure was assessed using multilocus sequence typing (http://mlst.ucc.ie/mlst/dbs/Ecoli).

2.3. Animal and human nasal sample processing

Nasal swabs were vortexed and a 200 µl aliquot of the transport medium was transferred into 1 ml Luria-Bertani (LB) medium for putative ESBL producers or into 1 ml Staphylococcus broth for *S. aureus*. These enrichments were incubated overnight at 37°C. To screen for MRSA, 100 µl of Staphylococcus broth were plated on MRSASelect plates (BioRad, Reinach, Switzerland). After 24 hours of incubation, phenotypic screening for resistance to methicillin on the MRSASelect plates was followed by species identification using Matrix-assisted laser desorption/ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS; Bruker Daltonik, Bremen, Germany). PCR/sequencing of *S. aureus*-specific staphylococcal protein A was performed to identify the MRSA *spa* type (Shopsin et al., 1999).

To screen for ESBL-E, 100 µl of LB broth were plated on chromID BLSE agar plates (bioMérieux, France) and incubated for 24 hours at 37°C. Suspect colonies were isolated and processed in the same way as fecal samples.

3. Results

3.1 MRSA detection in farms

The prevalence of MRSA in the nasal swabs from pigs and workers in 2015 were 7.7% and 12%, respectively (Table 1 and Fig. 1). These values were about twice those of

2008 (Table 1). MRSA was also found in air samples of 20% of the farms investigated. The mean concentration of MRSA in these positive air samples was 408 CFU/m^3 (range: 10 CFU/m^3 to 1280 CFU/m^3). MRSA colonization in the farm environment (whether in pigs, workers or the air) increased in 2015 compared to 2008 (odds ratio: 5.7; 95%, confidence interval: 1.21–35.46) (Table 1). Twelve farms were visited both in 2008 and 2015; MRSA was detected in three of these farms in 2015, but in none of them in 2008 (Farms 1, 2 and 7; numbering according to Fig. 1). Seven pig farm workers were included in both studies and were negative for MRSA both times.

MRSA originating from human and pig nasal swabs were *spa* typed and no more than one individual *spa* type was found on any MRSA-positive farm. *Spa* types t034 and t1594 occurred on three farms each, and spa types t011 and t899 were only found on one farm each. The *spa* type of one MRSA isolate could not be identified (Fig. 1).

Table 1: MRSA prevalence in 2008 and 2015

	sampling 2008[a]		*sampling 2015*		
	number of farms/samples	MRSA prevalence	number of farms/samples	MRSA prevalence	odds ratio [95% Conf. Interval][b, c]
farmers	58	5 (8.6%)	47	6 (12.8%)	1.55 [0.36, 6.88]
pigs	343	11 (3.2%)	77	6 (7.7%)	2.55 [0.75, 7.79]
air	37	1 (2.7%)	29	6 (20.7%)	**9.39 [1.01, 443.60]**
farms	41	3 (7.3%)	29	9 (31%)	**5.7 [1.21, 35.46]**

[a] values from (Oppliger et al., 2012)
[b] values resulting from exact logistic regression
[c] values in bold are significant ($p \leq 0.05$)

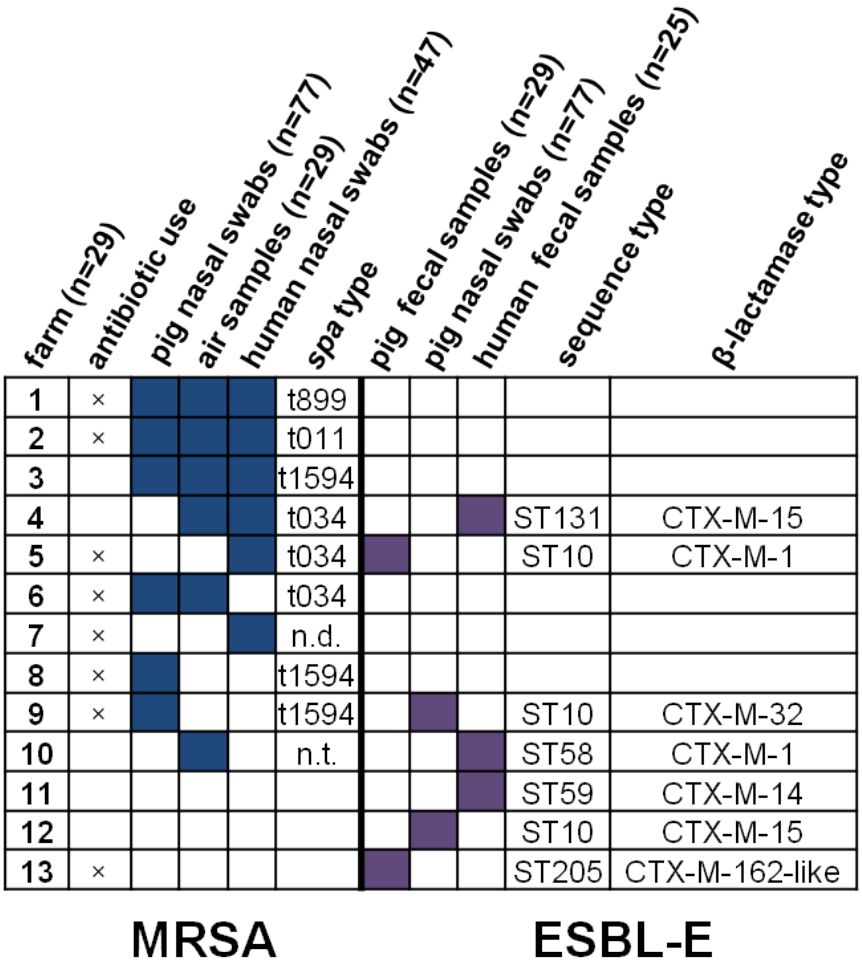

Fig. 1: MRSA and ESBL-E colonization in pigs, humans and air on farms.

Samples from pigs, humans and the air were collected on 29 farms. Only farms with at least one positive sample (whether MRSA or ESBL-E) are shown, with the specific samples that tested positive indicated by blue (MRSA) or purple (ESBL-E) squares. Molecular features are also shown (spa type for MRSA; sequence type and β-lactamase type for ESBL-E), as is antibiotic use during the sampling period.

n.d. – not determined

n.t. – not tested

3.2. Nasal and fecal prevalence of ESBL-E in animals and workers

ESBL-E screening showed that 12% (3/25) of the workers who provided fecal samples were ESBL-E carriers and none of the 47 participants showed nasal colonization. Two piglet fecal samples (6.9%) and two nasal samples from pigs (2.6%) were positive for ESBL-E (Fig. 1). Only one positive ESBL-E sample was found per farm. All ESBL-positive bacteria were identified as *E. coli*, and all β-lactamase genes belonged to CTX-M group 1, except for one, which belonged to CTX-M group 9. Within CTX-M group 1, the following β-lactamase types were found: 1 (n = 2), 15 (n = 2), 32 (n = 1) and 162-like (n = 1) (See Fig. 1). Population structure revealed that isolates belonged mostly to sequence type (ST) 10 (n = 3), and the remaining isolates belonged to ST131, ST58, ST59 and ST205. On four farms, there was a co-occurrence of MRSA and ESBL-E. All the isolates were susceptible to carbapenems, and no plasmid-mediated AmpC producers were detected.

3.3. Use of antibiotics and presence of resistant bacteria

Among the 13 farms showing resistant bacteria, eight had administered antibiotics to their piglets before or during the sampling period, either for prophylactic treatment or a specific health problem. Three other farms that had used antibiotics showed no presence of resistant strains. The association between antibiotic use and the presence of resistant bacteria (whether MRSA or ESBL-E) on the farms (pig nasal swab, human nasal swabs, pig fecal samples, human fecal samples or air) was statistically significant (Yates corrected Chi squared: 5.227, $P = 0.022$).

During the six months prior to the study, the eight farm workers colonized with resistant bacteria (MRSA or ESBL-E) had not undergone any antibiotic treatment, whereas the six that had, were not found to be colonized.

4. DISCUSSION

This study showed that the prevalence of MRSA on Swiss pig farms increased significantly between 2008 and 2015 (from 7.3% to 31%), with a very high increase in the prevalence in pig farm workers (from 6.6% to 12%). Nasal MRSA carriage among Swiss pig farm workers was, therefore, similar to the mean worldwide and European prevalences observed in people in contact with livestock (14% and 15.9%, respectively) (Liu et al., 2015). This prevalence was considerably lower than in Germany, where a maximum prevalence of 84% has been detected (Fischer et al., 2016), and it was higher than the prevalence observed in the general population (1%–2%) (DeLeo et al., 2010). Our observed MRSA prevalence in pigs was lower than the prevalence found in Swiss pigs at the slaughterhouse (25.7%) in 2015 (Anresis, 2016). We assume that this is because our samples were taken from piglets, not from finishing pigs, and it has previously been shown that MRSA prevalence is higher in these ready-to-be-slaughtered pigs than in suckling or weaning piglets (Friese et al., 2012).

Most of the MRSA *spa* types identified in this study are known to be associated with carriage on pig farms, e.g. t034 and t011 (Anresis, 2016; Friese et al., 2012), and these *spa* types have also been identified in humans, particularly people working with livestock (Fischer et al., 2016). However, we also found a *spa* type less frequently isolated on pig farms (t1594), and further investigation is needed to identify its origin. Interestingly, this *spa* type has been found in a hospital-acquired infection in Switzerland (Fenner et al., 2008). Moreover, our results showed that airborne MRSA was present in one fifth of farms, sometimes in very high concentrations (> 1000 CFU/m^3), confirming that air exposure can indeed be a route of MRSA transmission to pig farm workers (Bos et al., 2016; Friese et al., 2012; Masclaux et al., 2013; Schmithausen et al., 2015; van Cleef et al., 2015).

We found ESBL-E in 12% of pig farm workers – a result in agreement with those obtained in Denmark (13%, 18/136) (Hammerum et al., 2014) and the Netherlands (11%, 7/64) (Dohmen et al., 2015). The prevalence in pig farm workers was higher than the observed prevalence for the general population (Central Europe: 3%) (Karanika et al., 2016). Among the STs identified, ST58, ST10 and ST131 have been previously associated with livestock and humans (Ewers et al., 2012; Leverstein-van Hall et al., 2011). ST131 and ST10 are particularly high-risk clones and are highly associated with both human and animal-associated infections. Animals colonized with these clones could, therefore, be at a greater risk of developing infections (Ewers et al., 2012).

Previous studies have also shown that human and pig isolates within the same farm harboured similar ESBL genes and plasmid types, suggesting clonal transmission between animals and humans (Dohmen et al., 2015). The present study cannot derive any conclusions concerning the transmission of ESBL-E, as we found only one positive sample per farm. However, ESBL-encoding genes are most often located on mobile genetic elements which ultimately favours their dissemination (Madec et al., 2017). In Germany, a lower prevalence (6.3%) was observed by using inguinal swab samples from 32 pig farmers (Dahms et al., 2015). Our results confirmed that ESBL-E could be found in pigs' noses, as previously observed in Swiss pigs at slaughterhouses (Endimiani et al., 2012). This could be explained by their natural affinity to rooting in the soil for food, putting their snouts in contact with their fecal matter. In Germany, ESBL-E were not only isolated from the air on farms housing pigs with ESBL-E positive fecal carriage (Schmithausen et al., 2015; Von Salviati et al., 2014) but also in the ambient air surrounding those farms (Von Salviati et al., 2014). Therefore, the airborne spread of MRSA and ESBL-E within pig farms and across surrounding areas is possible and could lead to environmental contamination and public health consequences. Given the cross-sectional nature of our study, transmission of resistant bacteria and the transmission routes to

other hosts within farms was not possible to assess. Furthermore, our study included a limited number of samples and only one time point for ESBL-E (2015) sampling or two time points for MRSA sampling (2008 and 2015), respectively. Nevertheless, since increasing trends in MRSA and ESBL-E carriage are observed in other countries, we assume that the prevalence of antibiotic resistant bacteria is likely to increase in the future (Burow et al., 2014; Cuny et al., 2015; Van Boeckel et al., 2015).

We also observed a significant association between resistant bacteria (whether MRSA or ESBL-E) and the use of antibiotics. However, we had no information about whether farmers regularly used antibiotics as prophylactics or only in the case of disease.

This study is the first to have found ESBL-E in Swiss pig farm workers, and it confirms that these resistant bacteria are emerging in Swiss livestock, as has already been shown in Denmark, the Netherlands and Germany. In addition, we observed that the prevalence of pig-associated MRSA in Swiss pig farm workers had increased over the last eight years and was now at the same level as in other European countries. Measures therefore need to be implemented in order to prevent potential MRSA and ESBL-E transmission from animals to humans. For instance, very strict hygiene measures should be recommended (hand washing, using piggery-specific work clothing, taking a shower at the end of work shifts, disinfection and protection of any small cuts or grazes on the skin), as well as wearing a respirator mask when carrying out activities that generate large amounts of dust.

Further studies, using a One Health approach, are needed to better identify any reservoirs of these resistant zoonotic strains and their routes of dissemination.

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Ethical clearance for this study was sought and obtained from Human Research Ethics Committee of the Canton Vaud (243/14 and P_2017-00265) and the Veterinary Ethics Committee of the Canton Vaud (VD2903).

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