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BRIEF REPORT

Evidence for Human Adaptation and Foodborne Transmission of Livestock-Associated Methicillin-Resistant *Staphylococcus aureus*

Jesper Larsen,[1] Marc Stegger,[1,5] Paal S. Andersen,[1,2] Andreas Petersen,[1] Anders R. Larsen,[1] Henrik Westh,[2,3] Yvonne Agersø,[4] Alexandra Fetsch,[7] Britta Kraushaar,[7] Annemarie Käsbohrer,[7] Andrea T. Feßler,[8] Stefan Schwarz,[8] Christiane Cuny,[9] Wolfgang Witte,[9] Patrick Butaye,[10,12] Olivier Denis,[11] Marisa Haenni,[13] Jean-Yves Madec,[13] Eric Jouy,[16] Frederic Laurent,[14,15] Antonio Battisti,[17] Alessia Franco,[17] Patricia Alba,[17] Caterina Mammina,[19] Annalisa Pantosti,[18] Monica Monaco,[18] Jaap A. Wagenaar,[20,22] Enne de Boer,[21] Engeline van Duijkeren,[23] Max Heck,[23] Lucas Domínguez,[24] Carmen Torres,[25] Myriam Zarazaga,[25] Lance B. Price,[5,6,a] and Robert L. Skov[1,a]

[1]Statens Serum Institut, Copenhagen, [2]University of Copenhagen, Frederiksberg, [3]Hvidovre Hospital, and [4]Technical University of Denmark, Lyngby; [5]Translational Genomics Research Institute, Flagstaff, Arizona; [6]George Washington University, Washington D.C.; [7]Federal Institute for Risk Assessment, Berlin, [8]Friedrich-Loeffler-Institut, Neustadt-Mariensee, and [9]Robert Koch Institut, Wernigerode, Germany; [10]Ghent University, and [11]Université Libre de Bruxelles, Brussels, Belgium; [12]Ross University School of Veterinary Medicine, Basseterre, Saint Kitts and Nevis; [13]French Agency for Food, Environmental and Occupational Health and Safety, Lyon, [14]Université Claude Bernard Lyon 1, [15]Hospices Civils de Lyon, and [16]French Agency for Food, Environmental and Occupational Health and Safety, Ploufragan, France; [17]Istituto Zooprofilattico Sperimentale delle Regioni Lazio e Toscana, [18]Istituto Superiore di Sanitá, Rome, and [19]University of Palermo, Italy; [20]Utrecht University, [21]Netherlands Food and Consumer Product Safety Authority, Utrecht, [22]Central Veterinary Institute, Lelystad, and [23]National Institute for Public Health and the Environment, Bilthoven, The Netherlands; [24]Complutense University of Madrid, and [25]University of La Rioja, Logroño, Spain

We investigated the evolution and epidemiology of a novel livestock-associated methicillin-resistant Staphylococcus aureus strain, which colonizes and infects urban-dwelling Danes even without a Danish animal reservoir. Genetic evidence suggests both poultry and human adaptation, with poultry meat implicated as a probable source.

Keywords. MRSA; host adaptation; foodborne transmission; poultry; livestock.

Food-producing animals constitute an expanding reservoir of so-called livestock-associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA) strains worldwide, of which clonal complex (CC) 398 predominates in Europe [1]. Whole-genome phylogenetic analysis showed that CC398 consists of 2 epidemiologically and evolutionarily distinct groups: a human-adapted progenitor subpopulation and a livestock-adapted subpopulation derived thereof [2]. The analysis also showed that the human-to-livestock host jump was accompanied by the loss of the ΦSa3 phage encoding proteins that protect *S. aureus* from the human innate immune response [2]. LA-MRSA CC398 is a frequent and increasing cause of infections among persons working in the livestock industry, and infection rates are also increasing among the general public [3, 4]. These patients usually live in rural areas where livestock is raised, suggesting that LA-MRSA CC398 in the general public is mainly due to spillover from nearby animal farms [3, 4].

LA-MRSA CC9/CC398 displaying *spa* type t899 represents a unique genotype that consists of a CC398 chromosomal backbone and a smaller CC9 region, which contains the staphylococcal protein A (*spa*) gene [2]. By analyzing national surveillance data, we observed that LA-MRSA CC9/CC398 has caused sporadic illness in persons living in urban areas of Denmark despite an apparent lack of a Danish livestock reservoir for this genotype (Supplementary Appendix 1 and Supplementary Table 1), raising questions about possible sources and modes of transmission. In the present study, we investigated the epidemiology of all LA-MRSA CC9/CC398 cases in Denmark and used whole-genome phylogenetic analysis to compare the Danish isolates with a European collection of *S. aureus* CC9/CC398 isolates from humans, animals, and retail foods.

METHODS

We reviewed the national MRSA database at Statens Serum Institut (Supplementary Appendix 2) for laboratory findings of all MRSA isolates collected from 1 January 1999 to 31 December 2015. MRSA isolates with *spa* type t899 (or derivatives thereof) were further investigated with a polymerase chain reaction assay that detects the *sau1-hsdS1* variant present in CC398 [5]. The staphylococcal cassette chromosome *mec* (SCC*mec*) types and subtypes were evaluated for all CC9/CC398 isolates [6]. A case was defined as a person with a CC9/CC398-positive screening culture from the nares, throat, or perineum or with a CC9/CC398-positive clinical culture from a site of infection.

For comparative purposes, we obtained 110 CC9/CC398 isolates from different laboratories across Europe. The CC9/CC398 isolates, including 101 methicillin-resistant and 9 methicillin-susceptible isolates, were collected from humans, animals, and retail foods during 2006–2012 (Supplementary Table 2). We used whole-genome phylogenetic analysis to study the relationships among isolates from Danish CC9/CC398 cases, the European collection of CC9/CC398 isolates, and a worldwide collection of 85 archetypal CC398 isolates [2]. Genomes were

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[a]L. B. P. and R. L. S. are joint senior authors.

Correspondence: J. Larsen, Microbiology and Infection Control, Statens Serum Institut, Artillerivej 5, DK-2300 Copenhagen S, Denmark (jrl@ssi.dk).

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screened for specific genes by mapping sequence reads against reference sequences (Supplementary Table 3). Methodological details are provided in Supplementary Appendix 2.

RESULTS

We identified 12 persons meeting the case definition between 2009 and 2015 (cases A–L), including 10 urban cases and 2 mink farmers (Table 1). Epidemiologic details of each case are provided in Supplementary Appendix 3. Whole-genome phylogenetic analysis showed that the CC9/CC398 isolates from the 12 Danish cases formed a separate group within the livestock-associated CC398 subpopulation, together with the 110 CC9/CC398 isolates from other European countries (100% bootstrap support) (Supplementary Figure 1). The isolates from the 10 urban cases (A–C and F–L) belonged to a clade of 49 CC9/CC398 isolates, which harbored a ΦSa3 phage integrated into the *hlb* β-hemolysin gene (68% bootstrap support) (Supplementary Figure 2) (hereafter referred to as the *ΦSa3 clade*). The ΦSa3 phage contained *scn*, *chp*, and *sak*, which encode the staphylococcal complement inhibitor protein (SCIN), the chemotaxis inhibitor protein (CHIPS), and staphylokinase (SAK), respectively (Supplementary Table 4). All the ΦSa3-positive CC9/CC398 isolates furthermore carried the same type IV(2B)a SCC*mec* element (Supplementary Table 4). Isolates from the epidemiologically linked urban cases (B and C, G and H, and J–L, respectively) differed by only a few single-nucleotide polymorphisms (Supplementary Figure 2).

Isolates from 7 of the urban cases (A–C and F–I) belonged to a distinct subclade within the ΦSa3 clade (100% bootstrap support) (Supplementary Figure 2). The subclade contained isolates from humans (n = 15), animals (n = 5), and retail foods (n = 17) (Supplementary Table 4). Notably, it contained 95% (18 of 19) of all CC9/CC398 isolates from poultry and poultry meat, compared with only 7% (4 of 56) of the CC9/CC398 isolates from other animal species and retail foods (Supplementary Figure 2) (hereafter referred to as the *poultry-associated subclade*). Three isolates belonging to the poultry-associated subclade were recovered from distinct chicken meat products sold in Denmark. European Union labeling showed that these 3 products originated from French production facilities, but there was no information on the origins of the animals before slaughter. Five of the isolates, including those from cases B and C and 3 from turkey meat, harbored 2 putative genetic markers of poultry adaptation, SAAV_2008 and SAAV_2009 [7].

The CC9/CC398 isolates from the 2 mink farmers (cases D and E) were nearly identical but only distantly related to the isolates from the 10 urban cases (Supplementary Figure 2). They carried a distinct type V(5C2&5)c SCC*mec* element, lacked the ΦSa3 phage, and were negative for SAAV_2008/2009 (Supplementary Table 4). Cases D and E and their isolates were not epidemiologically linked to the urban cases and will not be discussed further.

DISCUSSION

The present study describes the epidemiologic and genomic details of a novel hybrid LA-MRSA CC9/CC398 genotype, which has been observed among persons living in urban areas of Denmark. These cases were intriguing, because this genotype had never been detected in Danish livestock and epidemiologic investigations showed that none of the 10 urban cases had direct livestock exposure.

In contrast to Denmark, CC9/CC398 has been isolated from pigs, cattle, poultry, and retail foods in other European countries, including France, Germany, Italy, the Netherlands, and Spain (Supplementary Appendix 1 and Supplementary Table 1). Seven of the 10 urban isolates were closely related to poultry and poultry meat isolates from these countries, and 2 of the urban isolates even shared putative poultry-adaptive genes with 3 turkey meat isolates, providing strong support for a poultry origin.

It is unclear how these urban-dwelling Danes became colonized or infected with CC9/CC398. Two scenarios seem plausible: foodborne and human-to-human transmission. The presence of CC9/CC398 in poultry meat produced in other European Union countries but sold in Denmark, as documented here, suggests that Danes have been exposed to this pathogen with some frequency through consumption or handling of contaminated food products. Persons who handle raw meat professionally, such as case A, are probably at higher risk for *S. aureus* exposure than the average consumer. Alternatively, CC9/CC398 may have arrived in urban Denmark through chains of person-to-person transmission where the first link in each chain was a colonized or infected livestock worker. For example, it may be that case F acquired CC9/CC398 from his brother, who owns an animal farm in Egypt. Previous reports have shown that transmission of CC398 indeed occurs from livestock workers to their family members [8–10]. In contrast, sustained chains of person-to-person transmission appear to be rare, both in the community and hospital settings [3, 11]. Thus, we believe that human-to-human transmission is a less likely source of CC9/CC398 in the remaining urban cases, because none of them reported contact with livestock workers or had a history of travel to areas where CC9/CC398 is endemic in livestock.

Isolates from the epidemiologically linked urban cases were nearly identical to each other, suggesting either transmission between these persons or acquisition from a common source, for example, a contaminated food product. No attempts were made to identify possible food sources for the urban cases at the time of diagnosis, as *S. aureus* is not considered to cause foodborne disease, except from enterotoxin-produced gastroenteritis. Such investigations would also be difficult to perform under any circumstance because of the indefinite colonization period between exposure and disease.

The ΦSa3 phage encoding SCIN, CHIPS, and SAK was present in all 10 urban isolates and in several CC9/CC398 isolates

Table 1. Description of Danish Livestock-Associated Methicillin-Resistant *Staphylococcus aureus* CC9/CC398 Cases and Isolates

Case/ Isolate	Sex	Age, y	Isolation Date	Specimen	Residence	Travel	Epidemiologic Findings	SCC*mec*	Resistance Profile	ΦSa3 Clade[a]	Poultry-Associated Subclade[a]	SAAV_2008/ 2009[a]
A	M	54	29 April 2009	Wound swab	Urban	No	Professional exposure to poultry meat	IV(2B)a	Clindamycin-erythromycin-norfloxacin-tetracycline	+	+	–
B	F	57	7 April 2012	Blood culture	Urban	No	No exposure to livestock; stayed in hospital A during 4–26 April 2012	IV(2B)a	Norfloxacin-tetracycline	+	+	+
C	M	72	30 April 2012	Wound swab	Urban	No	No exposure to livestock; stayed in hospital A during 3–10 and 23–30 April 2012	IV(2B)a	Norfloxacin-tetracycline	+	+	+
D	F	70	10 June 2014	Wound swab	Rural	No	Professional exposure to mink; family A	V(5C2&5)c	Clindamycin-tetracycline	–	–	–
E	M	71	19 June 2014	Screening (throat)	Rural	No	Professional exposure to mink; family A	V(5C2&5)c	Clindamycin-tetracycline	–	–	–
F	M	65	22 July 2014	Wound swab	Urban	Egypt	No exposure to livestock; contact to chicken farmer (Egypt)	IV(2B)a	Norfloxacin-tetracycline	+	+	–
G	F	27	30 January 2015	Wound swab	Urban	No	No exposure to livestock; stayed in hospital B during 13–17 January 2015	IV(2B)a	Norfloxacin-tetracycline	+	+	–
H	F	34	1 February 2015	Screening (nares)	Urban	Kosovo	No exposure to livestock, stayed in hospital B from 16 December 2014 to 6 January 2015	IV(2B)a	Clindamycin-erythromycin-norfloxacin-tetracycline	+	+	–
I	M	52	21 September 2015	Wound swab	Urban	Egypt	No exposure to livestock	IV(2B)a	Norfloxacin-tetracycline	+	+	–
J	F	0	14 October 2015	Eye swab	Urban	No	No exposure to livestock; family B	IV(2B)a	Norfloxacin-tetracycline	+	–	–
K	F	36	13 November 2015	Screening (nares)	Urban	No	No exposure to livestock; family B	IV(2B)a	Norfloxacin-tetracycline	+	–	–
L	M	36	20 November 2015	Screening (nares)	Urban	No	No exposure to livestock; family B	IV(2B)a	Norfloxacin-tetracycline	+	–	–

spa type = t899.

Abbreviation: SCC*mec*, staphylococcal cassette chromosome *mec*.

[a] Plus signs denote positive findings; minus signs, negative findings.

from animals and food products. The phylogenetic distribution of the ΦSa3 phage suggests that it was reintroduced into CC9/CC398 in a single horizontal gene transfer event, after which it has been stably maintained as a prophage. SCIN, CHIPS, and SAK are antihost defense proteins that enable *S. aureus* to evade the human innate immune response and thus may contribute to colonization and virulence [12]. This raises the possibility that ΦSa3-positive CC9/CC398 isolates are more likely to be disseminated both by the foodborne route and via human-to-human transmission chains than their ΦSa3-negative CC9/CC398 and CC398 relatives.

Taken together, these findings suggest that a subpopulation of CC9/CC398 has become adapted to humans and that poultry meat may serve as a vehicle for the transmission of such isolates. However, the cases described here are not sufficient to change the generally accepted view that foodborne transmission plays only a minor role in the epidemiology of LA-MRSA in humans. Nonetheless, we and others have shown that LA-MRSA strains are versatile organisms that have proven readily adaptable to a wide range of animal species, including humans, which clearly demonstrates that the future of *S. aureus* in livestock is unpredictable. Thus, from a public health perspective, it is essential to continue *S. aureus* surveillance at the human-animal interface to quickly detect evolutionary and epidemiologic changes and intervene to protect human health.

Supplementary Data

Supplementary materials are available at http://cid.oxfordjournals.org. Consisting of data provided by the author to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the author, so questions or comments should be addressed to the author.

Notes

Author contributions. J. L., L. B. P., and R. L. S. designed the study and wrote the article. M. S. and P. S. A. performed whole-genome sequencing and phylogenetic analysis of the isolates. A. P., A. R. L., H. W., and Y. A. collected and analyzed all isolates and data from Denmark. A. F., B. K., A. K., A. T. F., S. S., C. C., W. W., P. B., O. D., M. H., J.-Y. M., E. J., F. L., A. B., A. F., P. A., C. M., A. P., M. M., J. A. W., E. d. B., E. v. D., M. H., L. D., C. T., and M. Z. gathered isolates and data from other European countries. J. L. coordinated the network of participating laboratories and managed the database. All authors proofread the article.

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