

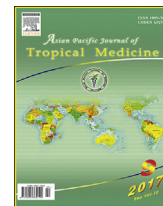
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journal homepage: <http://ees.elsevier.com/apjtm><http://dx.doi.org/10.1016/j.apjtm.2017.08.013>Diversity of livestock associated methicillin-resistant *Staphylococcus aureus*Henok A. Tegegne<sup>1,2</sup>, Ivana Koláčková<sup>1</sup>, Renata Karpíšková<sup>1</sup><sup>1</sup>Department of Bacteriology, Veterinary Research Institute, Brno, Czech Republic<sup>2</sup>Faculty of Veterinary Hygiene and Ecology, University of Veterinary and Pharmaceutical Sciences Brno, Czech Republic

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## ABSTRACT

**Objective:** To evaluate the diversity and molecular characteristics of livestock associated methicillin-resistant *Staphylococcus aureus* in livestock animals, food of animal origin and the environment in the Czech Republic.

**Methods:** After having been primarily enriched in buffered peptone water, the samples were cultured on Baird–Parker agar. Presumptive colonies were sub-cultured to blood agar and assessed morphologically. Furthermore, presumptive *Staphylococcus aureus* colonies were confirmed by MALDI-TOF. Multiplex PCR, *spa*-typing, and MLST have been used to characterize the strains. Each *mecA*-positive *Staphylococcus aureus* isolates were examined against 14 different antimicrobials by using disk diffusion method.

**Results:** In this study, 13 different *spa*-types belonging to five sequence types (ST) were detected. Ninety four percent of tested strains belonged to CC/ST398 for which t011, t034, t2123 and t2346 were the vast major *spa*-types. In addition, non-ST398 clones such as CC1(t127), ST5(t3598), ST8(t064) and ST361(t315) were detected, which are known as human associated clones.

**Conclusions:** The diversity of livestock associated methicillin-resistant *Staphylococcus aureus* has grown, and detecting lineages of human origin in animals and vice-versa becomes more common. Thus, livestock animal and its products will be a potential for the evolvement of methicillin-resistant *Staphylococcus aureus* in human population. Monitoring of pigs as well as other food-producing animal species and their products is therefore recommended.

## 1. Introduction

Livestock associated methicillin-resistant *Staphylococcus aureus* (*S. aureus*) (LA-MRSA) strains are mostly linked with clonal complex (CC) 398 [1]. Notably, sequence types (ST) 398, harbor the staphylococcal cassette chromosome *mec* (SCC*mec*VI, V) and *Staphylococcus aureus* protein A gene (*spa*) types t011, t034, t108, t567, t571, t899, t1254, t1451, t2011, t2510 and close relatives [1–3]. In most

European countries, CC398 is the most frequently identified type from livestock [4]. However, the epidemiology has been found to differ in different geographic areas across the globe [4]. Moreover, European baseline report mentioned clonal lineages of LA-MRSA other than CC398 such as CC9/ST9 (t1430), CC30/ST39 (t007) and CC97/ST97 (t3992, t5487) from pig production and CC9 (t1430) and CC5 (t002) from veal calf [1,5,6]. Several studies from Asia have demonstrated that CC9/ST9 (t1430) seems to be the prominent [3–7]. In the United States, the diversity of LA-MRSA is higher than that identified in Europe or Asia, with the reports of both CC398 as well as a variety of “human” associated strains [4]. Isolates belonging to MRSA ST5 and ST88 SCC *mec*IV, and not ST398, so far have been found in African livestock [8,9]. This study has been conducted to assess the diversity and molecular characteristics of LA-MRSA in livestock animals, food of animal origin and the environment in the Czech Republic.

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## 2. Materials and methods

Isolates used in this study originated from samples collected in the period 2012–2017 from livestock animals, food of animal origin and the environment and were taken on farm, slaughterhouse and retail levels in the Czech Republic. Livestock (pigs, calves, and cattle) and food of animal origin (pork, beef, veal, minced meat, milk, and cheese) samples have been collected. After having been primarily enriched in buffered peptone water for 24 h at 37 °C, the samples were cultured on Baird–Parker agar. Presumptive colonies were sub-cultured to blood agar and were assessed morphologically. Furthermore, presumptive *S. aureus* colonies were confirmed by MALDI-TOF.

Strains were obtained in accordance with the Commission Decision 2008/55/EC. Suspected *S. aureus* colonies were

(Ebersberg, Germany). *Spa*-types were assigned using the Bio Numerics Software.

## 3. Results

In total, 757 strains were analyzed which were collected from livestock animals (221), food of animal origin (414) and environmental samples (122). A majority (38%) of strains were from pigs, and 18.0%, 9.6%, 7.0% and 9.5% were obtained from cattle, goat, sheep and poultry respectively. From a total of 159 MRSA positive strains, 13 different *spa*-types were identified. Detected *spa*-types with their sequence types are summarized in Table 1. Out of 105 *spa*-typed strains 9 (8.5%) isolates did not belong to the *spa*-types related to CC398. These were attributed to four *spa*-types that clustered in four different STs such as ST361, CC/ST1 (t127), CC/ST8 (t064) and CC/ST5 (t3598).

**Table 1**

Number of strains with MLST and *spa* types from different sample origins.

Sample origin	Strains identified (n = 757)	<i>mecA</i> positive strains (n = 159)	MLST (no. of isolates) (n = 156)	<i>Spa</i> -types (no. of isolates) (n = 105)
Livestock animal				
Pig	109	59	ST398 (59)	t011(24), t034(21), t2123(7), t2346(2), t4659(1)
Cattle	34	18	ST398 (11) ST361(6) ST1(1)	t011(3), t034(1), t2346(1), t1255(1) t315(1) t127(1)
Goat	38	12	ST398 (12)	t011(2)
Sheep	25	1	ST398(1)	t011(1)
Poultry	10	–	–	–
Other food animals	5	–	–	–
Food of animal origin				
Pork, ham, and bacon	179	19	ST398(18)	t011(6), t034(8), t2346(1), t4652(2), t899(1)
Cattle meat and milk	102	25	ST398(24) ST5(1)	t011(5), t034(1) t3598(1)
Goat milk	35	9	ST398(6) ST8(1)	t011(2) t064(1)
Sheep milk	29	1	ST398(1)	t011(1)
Poultry meat and liver	62	1	ST398(1)	t011(1)
Other meat	7	–	–	–
Environment				
Milking equipment, feeder, handrail, cart, fence, etc.	122	14	ST398(14)	t011(5), t1773(1), t2123(3)

confirmed using the polymerase chain reaction method detecting species specific fragment SA442 [10] and *mecA* gene [11]. Strains were stored in deep freezer at –80 °C.

Each *mecA*-positive *S. aureus* isolates was examined against 14 different antimicrobials that belong to different antibiotic classes by using disk diffusion method. Antimicrobial susceptibility testing and breakpoints have been established according to the Clinical and Laboratory Standards Institute guideline.

Selected strains methicillin-resistant *Staphylococcus aureus* (MRSA) were analyzed by *spa*-typing and MLST. *Spa*-typing was performed according the methodology published on the website SeqNet.org maintained by the European Network of Laboratories [12,13]. Primers according to Stegger *et al.* [14] were used for *spa*-typing and 7 housekeeping genes for MLST were used according to Enright *et al.* [15]. Sequencing was performed in a sequencing facility of Eurofins MWG Operon

## 4. Discussion

Most of the strains were *spa*-types associated with ST/CC398 (94%) such as t011, t034, t2123, t2346, t4652, t899, t1255, and t4659. The *spa*-types t011, t034, t1255, t899, and t3495 are widely distributed in most of Europe countries [1,3,5,16,17]. However, *spa*-type t2346 principally is prevalent in central Europe such as Germany, Czech Republic and Austria [1–5]. *Spa*-type t4652 and t2123 which are reported in this study are rarely identified and only reported from Germany and Netherlands [18]. However, *spa*-type t4659 has been reported only from the Czech Republic [5].

*Spa*-types belonging to lineages ST361, CC/ST1(t127), and CC/ST8(t064) are commonly identified in Europe and are known as human strains [1,19,20]. A study from Sweden reported CC/ST5 (t3598) as MSSA human strain (available from: <http://spa.ridom.de/spatypes.shtml>), but in our study we identified it from

livestock product (milk) and it harbored *mecA* gene. Detecting human strains from food of animal origin may be a sign of cross-contamination during processing [21]. However, colonization of livestock animal with human strains and jumping of animal strain to human host are increasing time to time [22]. In addition to this, host specific strains are widening their host range. Thus, colonization of pigs and other livestock animals with MRSA no matter which strain belongs to, will be potential for the evolution of strains [23]. Therefore, it is paramount important to assess the existing situation, and close monitoring of animal husbandry and food of animal origin processing line is to safeguard the public and minimize the risk of development of wide host range antibiotic resistant strains.

Though alike other European countries, vast majority of strains detected in this study belong to CC/ST 398, with the most common *spa*-type of t011, clonal diversity of LA-MRSA in livestock animals growing. Lineages other than ST398 which are commonly known as human strains are also detected in this study. In addition to the impact of LA-MRSA in livestock production, our finding illustrates that livestock animals may have a role in perpetuation and widespread distribution of human strains in the community. Therefore, it is vital to take breakthrough measure before LA-MRSA becomes a major health system challenge of the country.

### Conflict of interest statement

The authors declare no conflict of interest.

### Acknowledgments

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