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Short Communication

Detection and molecular characterisation of methicillin-resistant *Staphylococcus aureus* isolated from raw meat in the retail market

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ABSTRACT

Objectives: This study aimed to detect and characterise methicillin-resistant *Staphylococcus aureus* (MRSA) from retail meat in the Czech Republic.

Methods: Isolates were identified by PCR detection of the *S. aureus*-specific fragment Sa442 and *mecA* gene. *spa* typing, MLST, detection of genes encoding staphylococcal enterotoxins, Panton–Valentine leukocidin (*pvl*), exfoliative toxins A and B (*eta* and *etb*), toxic shock syndrome toxin (*tst*) and staphylokinase (*sak*), detection of φSa3 prophage and antimicrobial susceptibility testing were performed.

Results: Of 65 raw meat samples examined (poultry, beef, pork and rabbit), 23 (35.4%) were positive for MRSA. Twelve positive samples originated from poultry (12/33; 36.4%), while the remaining eleven came from pork (9/9; 100%) and pork/beef mixed minced meat (2/5; 40.0%). Eight *spa* types belonging to five different sequence types (STs) were identified. ST398 was the most frequent (28/36; 77.8%), presenting *spa* types t011, t034, t2576, t4132, t588 and t899. Other livestock-associated MRSA STs (ST9-t899, ST5-t002, ST692-t8646 or the newly described ST4034-t899) were also sporadically identified. In seven isolates (19.4%), one or more staphylococcal enterotoxin genes were detected, with *sea, seg* and *sei* prevailing. Three isolates from turkey [ST398-t899 ($n = 2$) and ST398-t011] harboured the *sak* gene, and the latter also harboured the *sea* gene. Seven isolates from poultry harboured the φSa3 prophage and were resistant to tetracycline.

Conclusion: Specific kinds of meat appear to be a possible source of MRSA, although the risk to humans is hard to define. Therefore, surveillance of MRSA in meat as well as hygienic practices should be improved.

1. Introduction

Staphylococcus aureus causes several diseases ranging from mild skin infections and food poisoning to more complicated cases of necrotizing pneumonia, endocarditis, osteomyelitis, staphylococcal scalded skin syndrome and toxic shock syndrome. *Staphylococcus aureus* can acquire resistance to many antibiotics, including methicillin and vancomycin [1], and has thus been listed by the World Health Organization (WHO) as one of the 'priority pathogens' threatening public health [2].

Since 2005, the livestock-associated methicillin-resistant *S. aureus* (LA-MRSA) clonal complex 398 (CC398) emerged as the major European clone, with food-producing animals as a reservoir [3]. Identification of LA-MRSA CC398 in food-producing animals is a matter of concern due to direct transmission to people in contact with infected animals and/or their products as well as possible contamination of food [4].

The presence of MRSA in the human food chain has been described [5,6]. MRSA prevalence differs depending on the type of food considered and its country of origin. MRSA from raw meat

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and meat products has been reported [1,5,7,8]. In the Czech Republic, only a few studies on MRSA in meat have been published and reported 1% of pork meat to be positive for MRSA [9].

The presence of *S. aureus* in food can trigger the production of enterotoxins that persist in foodstuffs even after the actual bacteria have been destroyed by heating, thus cause staphylococcal food poisoning (SFP) [6]. To date, 23 staphylococcal enterotoxins have been reported, of which the five classical enterotoxins (*sea, seb, sec, sed* and *see*) have often been associated with SFP [10]. Among the staphylococcal enterotoxins in MRSA, *sea* has a specific importance since it belongs, together with *scn, chp* and *sak* [11], to the immune evasion cluster (IEC) system allowing adaptation to the human host.

Understanding the genetic lineages of MRSA that are circulating in different environments is important for tracking its evolution in diverse niches. Thus, this study aimed to detect and characterise MRSA isolates from raw meat samples packed at the producer's level to determine the level of contamination of meat sold in the retail market of the Czech Republic to evaluate the possible risks for consumers.

2. Materials and methods

2.1. Meat sample collection

A total of 65 different meat samples from poultry [$n = 33$, mainly from turkey ($n = 21$)], beef ($n = 12$), pork ($n = 9$), rabbit ($n = 6$) and mixed minced meat (beef and pork) ($n = 5$) were randomly collected from supermarkets between 2017 and 2018 in the Czech Republic. The retail meat samples originated from different countries including the Czech Republic ($n = 25$), Belgium ($n = 1$), Germany ($n = 5$), France ($n = 4$), Poland ($n = 15$), Hungary ($n = 7$), Brazil ($n = 6$) and China ($n = 2$). Meat samples were originally packed by the producer, and 28 were minced meat (8 poultry, 9 pork, 6 beef and 5 mixed). Samples were transported to the laboratory and were kept at 4°C until the time of processing. Each meat sample (25 g) was homogenised aseptically using a Stomacher® 400 (Seward, Norfolk, UK) for 2 min in 225 mL of buffered peptone water (BPW) (Oxoid Ltd., Basingstoke, UK) and incubated overnight at 37°C. Then, 10 μL of the enriched broth was streaked on MRSA Agar (Oxoid Ltd.) and incubated overnight at 37°C.

2.2. Bacterial isolates and determination of methicillin resistance

One to three presumptive MRSA colonies per sample were transferred onto blood agar and identified using matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF/MS) (autoflex™; Bruker Daltonik GmbH, Bremen, Germany). MRSA isolates were confirmed by PCR using the *mecA* gene encoding resistance to methicillin [12], and *S. aureus*-specific fragment Sa442 [13] was used as a positive control. Simultaneously, cefoxitin resistance in *S. aureus*-positive isolates was tested by the disk diffusion method using a 30 μg cefoxitin disk (Oxoid Ltd.) to avoid exclusion of *mecA*-negative MRSA isolates [14]. Confirmed MRSA isolates were stored at −80°C for further characterisation.

2.3. spa typing and multilocus sequence typing (MLST)

Typing of the *S. aureus* protein A locus (*spa* typing) was performed according to the methodology of Friedrich et al. [15]. Primers were used according to Stegger et al. [16]. Specific *spa* sequences were analysed using the Ridom StaphType software v.2.2.1 (Ridom, Münster, Germany).

MLST was performed based on seven housekeeping genes according to Enright et al. [17]. Sequence types (STs) were assigned according to the MLST website (http://saureus.mlst.net). Sequencing for *spa* and MLST determination was performed by Eurofins MWG Operon (Ebersberg, Germany).

2.4. Detection of staphylococcal enterotoxins and virulence-encoding genes

Detection of the epidemiologically most important staphylococcal enterotoxin genes (*sea, seb, sec, sed, see, seg, seh, sei* and *sej*) and genes encoding virulence factors [Panton–Valentine leukocidin (*pvl*), exfoliative toxins A and B (*eta* and *etb*) and toxic shock syndrome toxin (*tst*)] was performed by PCR [18,19]. All MRSA isolates were analysed for acquisition of markers of human host adaptation identified by staphylokinase (*sak* gene) and *Sa*3int integrase gene according to Sung et al. [20] and Goerke et al. [21], respectively. Reference stains were used as a positive control.

2.5. Antimicrobial susceptibility testing

Susceptibility to a panel of 10 antimicrobial agents was assessed by the disk diffusion method following the guidelines of the Clinical and Laboratory Standards Institute (CLSI) [14]. The following antibiotic disks (Oxoid Ltd.) were used: clindamycin (2 μg); erythromycin (15 μg); trimethoprim/sulfamethoxazole (25 μg); gentamicin (10 μg); tetracycline (30 μg); chloramphenicol (30 μg); ciprofloxacin (5 μg); rifampicin (5 μg); and teicoplanin (30 μg). A cefoxitin disk (30 μg) was added for phenotypic confirmation of resistance to methicillin.

3. Results

Of the 65 meat samples tested, 23 (35.4%) were positive for MRSA, of which 12 came from poultry meat [12/33 (36.4%), including 1 chicken and 11 turkey meat samples], while the remaining 11 came from pork minced meat (9/9; 100%) and beef and pork mixed minced meat (2/5; 40.0%). Samples from beef ($n = 12$) and rabbit meat ($n = 6$) did not contain any MRSA isolates. The country of origin for positive samples are given in Table 1 and Fig. 1.

Based on MLST, *spa* typing and antimicrobial resistance profile, in nine samples up to three different MRSA strains were detected, so that a total of 36 MRSA isolates were collected (Table 1). Presence of the *mecA* gene was confirmed in all of them. The obtained MRSA isolates belonged to five different sequence types (ST5, ST9, ST692, ST4034 and ST398), with ST398 being the most frequent (28/36; 77.8%) (Fig. 2B). Of the 28 ST398 isolates, 15 originated from poultry meat (15/20; 75.0%), while 11 were from pork (11/13; 84.6%) and two were from pork/beef minced meat (2/3; 66.7%) samples. Six different *spa* types (t011, t034, t588, t899, t4132 and t2576) were detected among ST398 isolates, of which t034 was the most frequent ($n = 16$). Eight isolates were non-ST398, comprising five ST9-t899 (from poultry, beef and pork meat), one ST5-t002 (poultry meat), one ST692-t8646 (poultry meat) and the newly described ST4034-t899 (pork meat). *spa* type t899 was identified in ST398 ($n = 2$), ST4034 ($n = 1$) and in all ST9 ($n = 5$) isolates (Table 1).

In addition to β-lactams, resistance to six different antimicrobial agents was detected. Resistance to tetracycline was the most frequently identified (28/36; 77.8%). The macrolide–lincosamide–streptogramin B (MLS_B) phenotype was identified in 17 isolates presenting resistance to both erythromycin and clindamycin (17/36; 47.2%), while resistance to clindamycin was detected in 26 isolates (26/36; 72.2%). Gentamicin resistance was found only in three isolates, including ST398 and the ST4034. This latter isolate is also one of the most resistant isolates of the present collection. Most of the isolates (20/36; 55.6%) were concurrently resistant to

Table 1
Characteristics of the 36 methicillin-resistant *Staphylococcus aureus* isolated from retail meat

Strain no.	Sample no.	Animal origin	Country of origin [a]	MLST	*spa* type	Enterotoxin genes	*sak* gene	*Sa*3int	Resistance [b]
SAV0154	2180/13	Pork	CZ	ST4034	t899	–	–	–	FOX, TET, ERY, CLI, GEN, CHL, CIP
SAV0990	170/17	Turkey	PL	ST398	t034	–	–	–	FOX, TET, ERY, CLI, CIP
SAV0995	170/17	Turkey	PL	ST398	t011	–	–	–	FOX, TET
SAV0999	171/17	Pork	PL	ST398	t034	–	–	–	FOX, TET, CLI
SAV1034	344/17	Pork/beef	PL	ST398	t034	–	–	–	FOX, TET, CLI, CIP
SAV1035	344/17	Pork/beef	PL	ST9	t899	*seg, sei*	–	–	FOX, CIP
SAV1103	718/17	Turkey	PL	ST692	t8646	–	–	+	FOX, TET, ERY, CLI, CIP
SAV1104	720/17	Pork/beef	PL	ST398	t034	–	–	–	FOX, TET, ERY, CLI, SXT
SAV1109	915/17	Turkey	PL	ST398	t899	–	+	–	FOX, TET, ERY, CLI, SXT, CHL, CIP
SAV1110	916/17	Pork	PL	ST398	t034	–		–	FOX, TET, ERY, CLI, SXT, GEN
SAV1146	1138/17	Turkey	DE	ST398	t899	–	+	–	FOX, TET, CIP
SAV1147	1139/17	Turkey	PL	ST398	t011	*sea*	+	+	FOX, TET, ERY, CLI, CIP
SAV1148	1253/17	Turkey	DE	ST398	t034	–	–	–	FOX, TET, ERY, CLI
SAV1149	1253/17	Turkey	DE	ST9	t899	*seg, sei*	–	–	FOX, CIP
SAV1150	1253/17	Turkey	DE	ST9	t899	*seg, sei*	–	–	FOX, ERY, CLI, CIP
SAV1157	1345/17	Turkey	DE	ST398	t034	–	–	–	FOX, ERY, CLI
SAV1158	1345/17	Turkey	DE	ST9	t899	*seg, sei*	–	–	FOX, CIP
SAV1159	1345/17	Turkey	DE	ST398	t034	–	–	–	FOX, TET, ERY, CLI
SAV1169	1529/17	Turkey	CZ	ST5	t002	*seg, sei*	–	+	FOX, TET, ERY, CLI, CIP
SAV1187	1660/17	Pork	CZ	ST398	t034	–	–	–	FOX, TET, CLI
SAV1217	1965/17	Pork	CZ	ST398	t034	–	–	–	FOX, TET, CLI
SAV1218	1966/17	Pork	CZ	ST398	t034	–	–	–	FOX, TET, ERY, CLI
SAV1219	1966/17	Pork	CZ	ST398	t034	–	–	–	FOX, TET, CLI
SAV1228	1985/17	Pork	CZ	ST9	t899	*seg, sei*	–	–	FOX, CIP
SAV1229	1985/17	Pork	CZ	ST398	t011	–	–	–	FOX, TET, CLI
SAV1230	1985/17	Pork	CZ	ST398	t2576	–	–	–	FOX, TET, ERY, CLI
SAV1231	1986/17	Pork	CZ	ST398	t034	–	–	–	FOX, TET, CLI
SAV1232	1986/17	Pork	CZ	ST398	t011	–	–	–	FOX, TET
SAV1233	2024/17	Turkey	PL	ST398	t4132	–	–	+	FOX, TET, CLI, CIP
SAV1235	2091/17	Chicken	FR	ST398	t588	–	–	+	FOX, TET, ERY, CLI, CIP
SAV1244	2096/17	Turkey	CZ	ST398	t011	–	–	+	FOX, TET, ERY, CLI, GEN, CIP
SAV1245	2096/17	Turkey	CZ	ST398	t034	–	–	–	FOX, TET, CLI, CIP
SAV1246	2096/17	Turkey	CZ	ST398	t011	–	–	+	FOX, TET, CIP
SAV1256	227/18	Turkey	DE	ST398	t034	–	–	–	FOX, CIP
SAV1257	227/18	Turkey	DE	ST398	t034	–	–	–	FOX, ERY, CLI, CIP
SAV1260	310/18	Pork	BE	ST398	t011	–	–	–	FOX, TET, SXT, CIP

MLST, multilocus sequence typing.
[a] BE, Belgium; CZ, Czech Republic; DE, Germany; FR, France; PL, Poland.
[b] FOX, cefoxitin; TET, tetracycline; ERY, erythromycin; CLI, clindamycin; GEN, gentamicin; CHL, chloramphenicol; CIP, ciprofloxacin; SXT, trimethoprim/sulfamethoxazole.

Table 2
Multidrug resistance profiles of methicillin-resistant *Staphylococcus aureus* strains to the ten tested antibiotics

Resistance profile	Frequency	MAR index [a]
R7=FOX, TET, ERY, CLI, SXT, CHL, CIP	1	0.7
R7=FOX, TET, ERY, CLI, GEN, CHL, CIP	1	0.7
R6=FOX, TET, ERY, CLI, SXT, GEN	1	0.6
R6=FOX, TET, ERY, CLI, GEN, CIP	1	0.6
R5=FOX, TET, ERY, CLI, SXT	1	0.5
R5=FOX, TET, ERY, CLI, CIP	5	0.5
R4=FOX, TET, ERY, CLI	4	0.4
R4=FOX, TET, SXT, CIP	1	0.4
R4=FOX, TET, CLI, CIP	3	0.4
R4=FOX, CLI, ERY, CIP	2	0.4
R3=FOX, TET, CLI	6	0.3
R3=FOX, TET, CIP	2	0.3
R3=FOX, ERY, CLI	1	0.3
R2=FOX, CIP	5	0.2
R2=FOX, TET	2	0.2

FOX, cefoxitin; TET, tetracycline; ERY, erythromycin; CLI, clindamycin; SXT, trimethoprim/sulfamethoxazole; GEN, gentamicin; CHL, chloramphenicol; CIP, ciprofloxacin.
[a] MAR index = multiple antibiotic resistance index (no. of antimicrobials to which the isolate is resistant/no. of antibiotics to which the isolate is subjected).

three or more different antimicrobial groups in addition to cefoxitin (Table 2). All five isolates belonging to ST9-t899 were susceptible to tetracycline and trimethoprim/sulfamethoxazole and resistant to ciprofloxacin. No resistance to teicoplanin was recorded.

The presence of enterotoxin genes was low (7/36; 19.4%). One turkey isolate (ST389-t011) harboured the immune evasion cluster (IEC) with the *sea* and *sak* genes. Two isolates (ST389-t899) from turkey also carried the *sak* gene but not the *sea* gene. The *seg–sei* genes were detected in six isolates belonging to ST9 and ST5. Finally, genes encoding virulence factors (*pvl, eta, etb* and *tst*) were not detected in the obtained isolates.

4. Discussion

MRSA is a significant public-health concern with the potential to contaminate food and to infect consumers [5]. In this study, 65 samples of retail meat collected in the Czech Republic but originating from different European and non-European countries were analysed, of which 23 (35.4%) contained MRSA. This proportion is higher than previous studies conducted in England (7.3%; 9/124) [5], Belgium (8.0%; 11/137) [4], the Netherlands (11.9%; 264/2217) [7] and Spain (21.8%; (2/101) [22] on different types of retail meat.

In this study, CC398 was by far the most frequently recovered lineage. This result is in accordance with other studies conducted in England, Belgium, the Netherlands and Spain on different types of retail meat where 100% [5], 97% [4], 85% [7] and 64.1% [22] of MRSA isolates, respectively, belonged to CC398. In this study, one isolate detected in a pork meat sample belonged to ST4034-t899, a single-locus variant (*arcC* gene) of ST398 [23]. The diversity of CC398 is clearly on the rise, although ST398 belonging to a few *spa* types (t011, t034, t899) remains predominant [24].

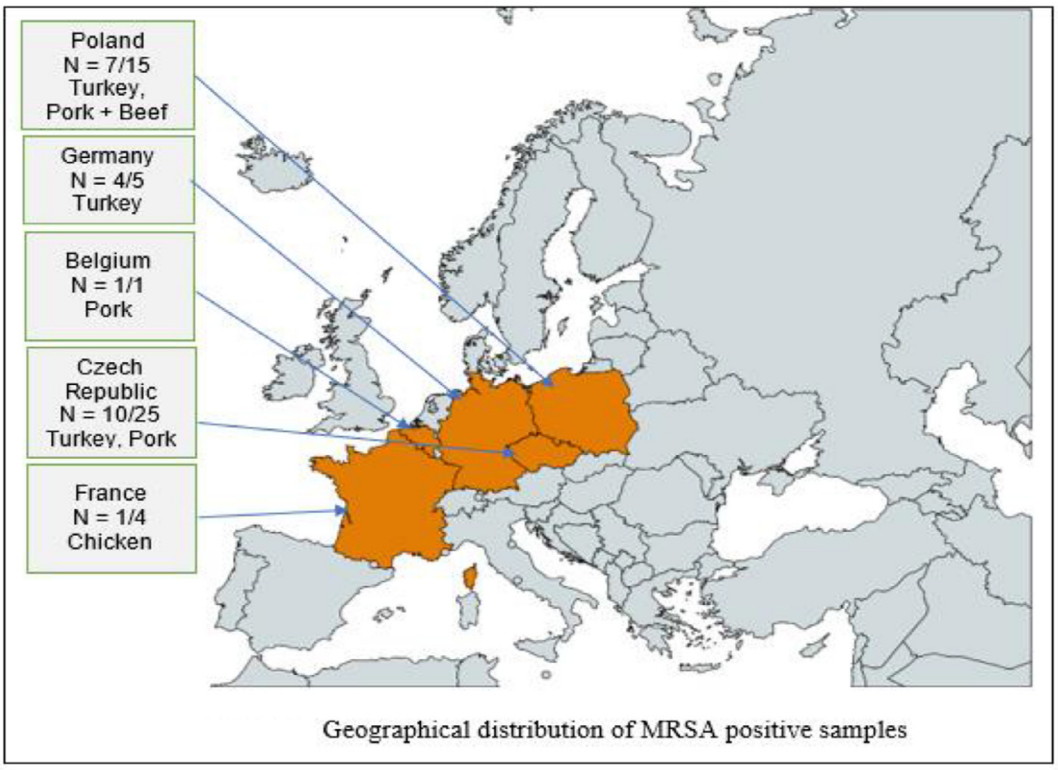

Fig. 1. Geographical distribution of methicillin-resistant *Staphylococcus aureus* (MRSA)-positive sample.

In this study, a higher variability of ST398 *spa* types (t011, t034, t588, t899, t2576 and t4132) was observed compared with that commonly reported in the literature. In a study conducted on 124 raw meat samples including pork ($n = 63$), chicken ($n = 50$) and turkey ($n = 11$) from retail outlets in North-West England, CC398 belonged to only three different *spa* types (t011, t034 and t899) [5]. Another study conducted to assess the prevalence and genetic diversity of LA-MRSA in Belgian pork reported a similar result [4]. The higher diversity of *spa* types observed in our study may be related to the multiple European and non-European origins of the meat samples. Indeed, countries with high import of food of animal origin from different sources may have a higher diversity of strains [25].

Non-ST398 isolates, including ST9 ($n = 5$), were more sporadically reported in this study. ST9 is the major LA-MRSA clone in Asia with a marked variation in its prevalence [26]. This clone has also been reported in Europe since 2008 when its occurrence was described in pigs from Italy [27]. Since then, LA-MRSA ST9 has been found in different food animals in Germany [8] and in retail meat from the UK [28]. Although this lineage is typically swine-associated, human infections have also been reported [29]. Several studies found that most of LA-MRSA ST9 strains carried at least one enterotoxin gene [26], which is in agreement with our findings since all our of ST9-t899 isolates harboured the *sei–seg* genes. This lineage also shows a wide variety of *spa* types with a certain geographic distribution and are mainly originated from poultry. All strains were susceptible to many antibiotics including tetracycline, but resistant to ciprofloxacin.

Only one ST5-t002 harbouring the enterotoxin genes *seg and sei* was described in this study. CC5 is a common and widespread clonal complex that comprises both community-associated and healthcare-associated MRSA [30]. This lineage can be also considered as an animal-adapted clone since it has been recovered from livestock (poultry, pigs and cattle) and companion animals [31]. Most studies on poultry have shown that MRSA isolates belonged to CC5 besides CC398 [32]. This lineage has been identified in France, Portugal, Colombia, Argentina and the USA [33].

Finally, one LA-MRSA ST692-t8646 was identified from turkey minced meat originated from Poland. ST692 is a host-specific lineage predominantly found in domestic and wild birds from Turkey, Northern Ireland, Sweden, Germany and Korea [34,35], although it was also observed recently in a grey seal [36] and wallabies [37]. Interestingly, all of the isolates reported in the previous studies were susceptible to methicillin. Only one study from South Korea reported the presence of ST692-t2247 MRSA in the food chain, where it was isolated from chicken carcasses and from personnel at a chicken slaughterhouse [35,38].

In addition to colonisation/infection, MRSA strains have been involved in SFP outbreaks [6]. In a single isolate (ST398-t011), we detected *sea*, one of the five classical enterotoxins (*sea, seb, sec, sed* and *see*) most frequently associated with SFP [10]. *sei* and *seg* genes were the most prevalent enterotoxin genes detected in this study. Six isolates coming from five different animal-origin meat samples carried these genes. A similar combination of the *seg–sei* genes among *S. aureus* isolates was previously observed from different food matrixes [39]. The staphylococcal enterotoxin genotypes *seg–sei* or *sea–seg–sei* are known to be associated with food-poisoning outbreaks [40].

Presence of the IEC genes, which is a marker of human adaptation, was found only in one *Sa*3int-positive poultry isolate (ST389-t011) that carried the *sea* and *sak* genes. Presence of the IEC cluster in a CC398 isolate within the livestock clade shows the circulation of this lineage in the human population as well [41]. Six ST398 and one ST5 isolates from poultry meat (six from turkey and one from chicken) most likely carried the φAvβ prophage described by Price et al. [42].

The small number of samples described here is a limitation of this study, and further studies are needed to strengthen our

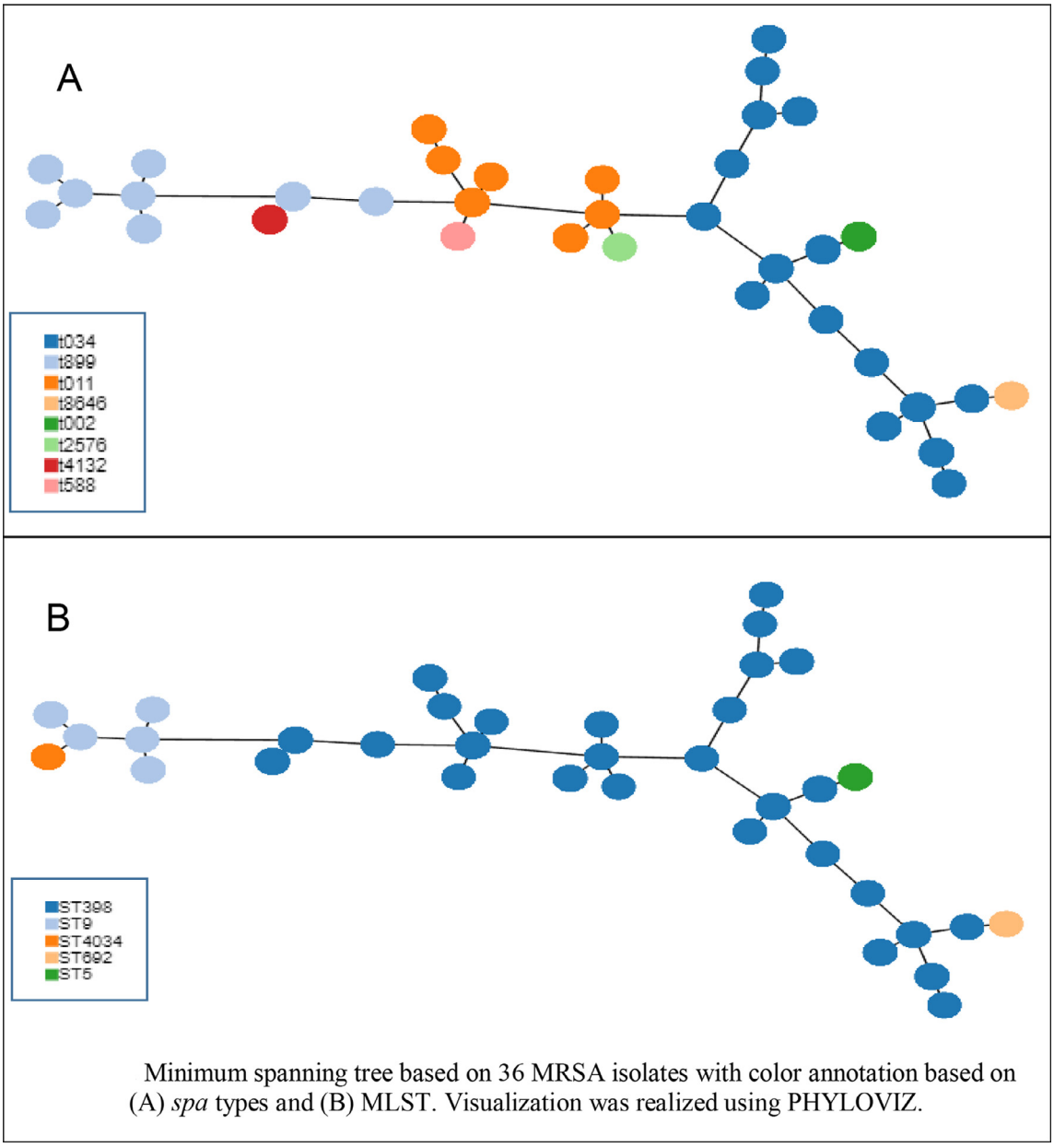

Fig. 2. Minimum spanning tree based on 36 methicillin-resistant *Staphylococcus aureus* (MRSA) isolates with colour annotation based on (A) staphylococcal protein A (*spa*) typing and (B) multilocus sequence typing (MLST). Visualisation was realised using PHYLOViZ.

results. Likewise, the fact that pork meat is more frequently associated with CC389 *spa* types t011 and t034 than poultry meat (10/13 versus 11/20), while poultry meat is the only meat that is likely to carry the *Sa*3int (7/20), remains to be confirmed.

5. Conclusions

In this study, we demonstrated that 35.4% (23/65) of the retail meat sold in the Czech Republic but originating from European countries was contaminated by MRSA isolates. ST398 was by far the most frequent clone, indicating the presence of animal-associated genotypes in the food chain and the potential public-health hazard related to colonisation/infection. Besides, detection of enterotoxins genes (*sea, seg* and *sei*) commonly associated with food poisoning is also a public-health concern. Therefore, harmonised monitoring systems and hygiene measures would be needed along the food chain to lower the risk of MRSA transmission to consumers.

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Competing interests

None declared.

Ethical approval

Not required.

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