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Prevalence of *Staphylococcus aureus* in wild hedgehogs (*Erinaceus europaeus*) and first report of *mecC*-MRSA in Hungary

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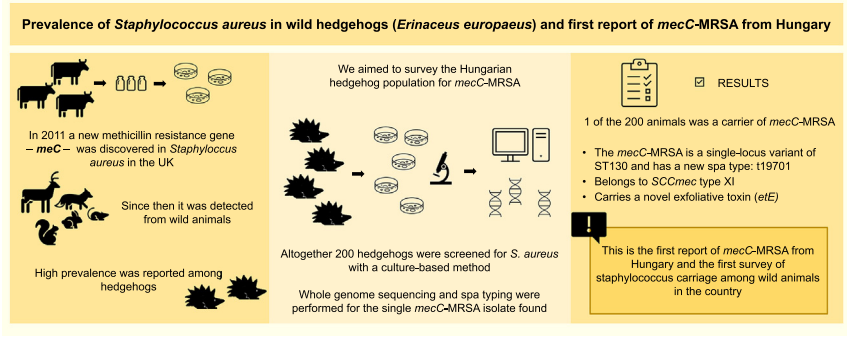
HIGHLIGHTS

- *MecC*-MRSA is an emerging zoonotic health risk.
- Here we report the WGS analysis of the first *mecC*-MRSA in Hungary.
- MRSA carriage among the Hungarian hedgehogs was 1%.
- Our isolate showed a novel sequence type (ST6736) and spa type (t19701).
- Our isolate carried a recently described exfoliative toxin (*etE*).

GRAPHICAL ABSTRACT



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ABSTRACT

In 2011 *mecC*, a new *mecA* gene homologue, was described in a bovine isolate in the UK. Since then, *mecC*-positive methicillin-resistant *Staphylococcus aureus* (*mecC*-MRSA) has also been found in wild animals. An especially high prevalence of *mecC*-MRSA has been reported among hedgehogs in Sweden (64%) and Denmark (61%). Based on these findings we aimed to survey the hedgehog population for *mecC*-MRSA in Hungary.

Altogether 200 hedgehogs were screened for *Staphylococcus aureus* using a culture-based method. The antibiotic susceptibility of the isolates to nine drugs was determined, their genetic relatedness was established by PFGE and *spa*-typing, and virulence genes were identified by PCR. Whole genome sequencing was performed for the single *mecC*-MRSA isolate found.

Of the 200 animals, 13 were carriers of *S. aureus* (6.5%). Among these, one isolate was *mecA* positive and one was *mecC* positive. The isolates were susceptible to non-beta-lactam antibiotics. Toxin genes were not found, but the majority carried genes responsible for adhesion and biofilm production. The *mecC*-MRSA isolate was a single-locus variant of ST130, had a new *spa* type (t19701) and belonged to *SCCmec* type XI. It carried a recently described, novel exfoliative toxin (*etE*).

This is the first report of *mecC*-MRSA in Hungary and the first survey of staphylococcus carriage among wild animals in the country. The *mecC* prevalence was much lower than in Northern European countries and rather similar to other countries in our region. *MecC*-MRSA could potentially emerge as a novel human pathogen, especially where close contact occurs between humans and animals.

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1. Introduction

Staphylococcus aureus is an important human pathogen that can cause several diseases ranging from minor skin infections to life-threatening conditions. Its significance has increased with its development of antibiotic resistance (Inagaki et al., 2019; Horváth et al., 2020; Zhen et al., 2020). MRSA was first reported in 1961 and it has become a serious global threat by now (WHO, 2017). Methicillin resistance was caused by the incorporation of the *mecA* gene into the bacterial chromosome. This gene codes an altered penicillin binding protein, PBP2a, which results in resistance to all β-lactam antibiotics except for the fifth generation cephalosporins (Lakhundi and Zhang, 2018). The *mecA* gene is located in the *SCCmec* mobile genetic element (Lakhundi and Zhang, 2018).

In 2011, a new *mecA* gene homologue was found in bovine *S. aureus* isolates in the UK, and in human samples from Denmark, UK and Ireland. This new homologue, $mecA_{LGA251}$, was located in a novel *SCCmec* element, which was designated type XI (García-Álvarez et al., 2011; Shore et al., 2011). According to the recommendations of the International Working Group on the Classification of SCC Elements, the new homologue was named *mecC* (Ito et al., 2012).

The *SCCmec* structure has three basic structural elements. The first is the *mec* gene complex, which contains the *mec* gene itself accompanied by transducer and repressor protein coding genes. The second is the *ccr* gene complex, encoding cassette chromosome recombinase genes. And the third element is any other region of the cassette and is called a joining region (J1, J2, J3). The *mec* gene complex is divided into five classes, of which *mecC* belongs to the class E *mec* gene complex, which includes the *blaZ, mecC, mecR1* (transducer), *mecI* (repressor) genes (Shore et al., 2011; Lakhundi and Zhang, 2018). The various combinations of the different allotypes make up 9 different *ccr* gene complex types (Lakhundi and Zhang, 2018).

Since 2011, several *mecC*-positive isolates from humans and cattle have been found in diverse biological samples originating mainly from Western and Central European countries (García-Álvarez et al., 2011; Shore et al., 2011; Petersen et al., 2013; Kerschner et al., 2015; Monecke et al., 2016). In addition, in 2016 the first report of *mecC*-MRSA occurring outside Europe was published in Australia (Worthing et al., 2016) and the first report of *mecC*-MRSA from the American continent was published in 2021 (Silva et al., 2021).

Although the incidence of *mecC*-MRSA appears to be low in all sectors, including human healthcare settings, an increasing trend in human clinical cases has been observed in Denmark (Petersen et al., 2013; Dube et al., 2021). So far the human *mecC* isolates have either been detected during screening, or in clinical samples such as skin and soft tissue infections, surgical site infections, and bloodstream infections (Petersen et al., 2013).

The origin of the *mecC* gene is still debated. As a higher frequency of human cases has been detected in people in contact with confirmed *mecC*-MRSA-carrying farm animals and in rural areas, contact with livestock could be a possible risk factor for humans acquiring *mecC*-MRSA (Petersen et al., 2013).

In recent years *mecC* has been identified in other staphylococcal species from veterinary samples, such as *S. xylosus* (Harrison et al., 2013b; Desvars-Larrive et al., 2019) and *S. stepanovicii* (Loncaric et al., 2013).

Furthermore, *mecC*-MRSA has been recovered not only from domestic but also from wild animals (Loncaric et al., 2013; Monecke et al., 2013; Porrero et al., 2014a; Mrochen et al., 2018; L. Ruiz-Ripa et al., 2019a, 2019b; Heaton et al., 2020). The *mecC*-MRSA does not appear to have a particular host specificity; it has been isolated from several mammalian species including the European hedgehog, red fox, fallow deer, brown hare, wild rabbit, and wild boar (Monecke et al., 2016; L. Ruiz-Ripa et al., 2019a, 2019b; Heaton et al., 2020). Only a few studies have examined the possible role of the environment in the spread of this pathogen. One study found *mecC*-MRSA in river water in an area where *mecC*-MRSA had been found in wild boar and fallow deer, suggesting that water can be a shared site of exposure or transmission between the various animal species and the environment (Porrero et al., 2014b).

At first, domestic animals like cattle were linked with *mecC*-MRSA and they were thought to be the original source (Petersen et al., 2013). However, the wide distribution of *mecC*-MRSA in several wild animal species challenged this hypothesis. According to more recent results, wild rodents and insectivores or small carnivores could have served as the original reservoir for *mecC*-MRSA (Becker et al., 2014; Dube et al., 2021).

The European hedgehog is a small insectivore which is native throughout Europe, and prevalent both in rural and populated areas. In some countries garden owners often supply them with food and water. Therefore humans are prone to come into physical contact with these mammals (Rasmussen et al., 2019).

While the presence of *S. aureus* in humans, companion animals and livestock has been widely documented, there is only limited information on the prevalence of this pathogen in wildlife (Loncaric et al., 2013).

The first *mecC*-MRSA derived from hedgehogs was isolated in Sweden in 2013 from a sample which was taken in 2003 (Monecke et al., 2013). In an Austrian study, 40 different wild animal species were sampled, and they found three European brown hares, one European otter and one hedgehog carrying *mecC*-MRSA (Loncaric et al., 2013). In 2016, 2855 wild mammal samples, previously collected in Germany, Austria and Sweden, were analysed. Among these, 199 were hedgehog samples, but only two *mecC*-MRSAs were detected by Monecke et al. in Sweden in 2013 and two others in Germany (Monecke et al., 2013, 2016). However, in a screening in Sweden in 2017, 64% of the wild hedgehogs tested (35 out of 55) positive for *mecC*-MRSA (Bengtsson et al., 2017). A similarly high prevalence was reported in Denmark, where 114 out of 188 deceased hedgehogs (61%) carried *mecC*-MRSA in 2019 (Rasmussen et al., 2019).

In the middle of the 1960s a study conducted by Smith and Marples (Smith and Marples, 1964, 1965; Smith, 1965) reported a 86% prevalence of penicillin-resistant *S. aureus* in wild European hedgehogs in New Zealand. This study also noted that 45% of hedgehogs had chronic mycotic infections caused by a dermatophyte called *Trichophyton erinacei* (previously *T. mentagrophytes* var. *erinacei)*. Smith and Marples hypothesized that hedgehogs could potentially be natural reservoirs of penicillin-resistant *S. aureus* because an earlier study had demonstrated the production of penicillin-like compounds by dermatophytes (Peck and Hewitt, 1945), which could have provided a selective pressure to develop resistance. Subsequently, they were able to show that *Trichophyton* species present on hedgehogs' skin were able to produce a penicillin-like substance, with an anti-staphylococcal activity in vivo and in vitro, which could increase the penicillin resistance of the *S. aureus* strain residing in the ringworm infection site (Smith and Marples, 1965). In 2021, Dube et al. performed a similar screening study of the Swedish hedgehog population and they found that hedgehogs can carry benzylpenicillin-producing *T. erinacei*, and that this dermatophyte co-occurs with *mecC*-MRSA. Furthermore, this study confirmed the high (60%) prevalence and diversity of *mecC*-MRSA in Swedish hedgehogs (Dube et al., 2021).

Despite this relatively large amount of research on wildlife in some countries, there is no comprehensive work on the prevalence of MRSA strains in wildlife in Hungary. Taking into account the high prevalence of *mecC* strains in hedgehogs in Northern Europe, the main objective of this work was to explore Hungarian hedgehogs as possible reservoirs of this type of strain. This is the first study to examine the presence of staphylococci in the wildlife of our country and the first description of *mecC*-MRSA in Hungary.

2. Materials and methods

2.1. Sample collection

A total of 200 hedgehogs (125 alive and 75 dead) were screened for *S aureus* using a culture-based method between February and June 2020. Live animals were sampled in the Budapest region following admission to the Wildlife Rescue Center at the Budapest Zoo and Botanical Garden. Dead hedgehogs were collected as road kill from Budapest and its suburbs, an area approximately 7000 km^2 in size (Térport, 2013), or from wildlife

centers in Budapest where animals had died from complications after injury. Samples were taken from dead hedgehogs at the Department of Parasitology and Zoology, University of Veterinary Medicine, Budapest. Sampling for microbiological investigation was performed.

Two individual samples were collected from each animal: one from both nostrils, obtained with a mini-tip swab supplied with liquid Amies transport medium (Sigma Transwab ENT, Medical Wire & Equipment, Corsham, UK), and another from the skin between the spikes and fingers with traditional Stuart's charcoal transport swabs (Sigma Transwab Stuart's Charcoal, Medical Wire & Equipment, Corsham, UK). These were taken to the Institute of Medical Microbiology, Semmelweis University.

The study and its methodology were approved by the Semmelweis University Regional and Institutional Committee of Science and Research Ethics (reference number: SE RKEB 180/2020).

2.2. Cultivation, identification and antibiotic susceptibility testing

Samples were inoculated onto sheep blood agar and incubated at 37 °C overnight. The suspected *S. aureus* colonies, showing β-haemolysis and gold pigmentation, were further examined by catalase and Pastorex agglutination tests (Pastorex Staph-Plus Kit, Bio-Rad, Marnes-la-Coquette, France). The catalase- and coagulase-positive colonies were subcultured and an in-house duplex PCR was used to detect the *S. aureus* species-specific *nucA* gene, and the *mecA* gene which most commonly confers resistance to methicillin (Laub et al., 2011). For the detection of the novel *mecC* gene, we used the *mecC* gene primers of Paterson et al. (2012) with the following modified PCR conditions: 94 °C for 3 min, 30 cycles of 94 °C for 1 min, 57 °C for 1 min, 72 °C for 30 s and 72 °C for 10 min. ATCC 33591 (*mecA* +) and ATCC BAA-2312 (*mecC* +) strains were used as positive controls (Supplementary Table 1).

MIC test strips (Liofilchem®, Roseto degli Abruzzi, Teramo, Italy) were used to test antibiotic susceptibility to penicillin, erythromycin, clindamycin, gentamicin, ciprofloxacin, tetracycline and mupirocin. Susceptibility to cefoxitin and vancomycin were tested by disk diffusion and microdilution methods respectively. *S. aureus* strains were inoculated onto cation-adjusted Mueller-Hinton plates and the EUCAST breakpoints were used for interpretation (The European Committee on Antimicrobial Susceptibility Testing, 2020). *S. aureus* ATCC 29213 was included as a means of quality control of the MIC determinations.

2.3. Detection of virulence and toxin genes

The presence of the *icaD, cna, fnbA* and *fnbB* genes - responsible for adhesion and biofilm production - was examined in all *S. aureus* isolates by PCR (Campbell et al., 2008; Atshan et al., 2013). The presence of the toxin genes *sea, seb, sec, tsst, eta, etb* and *lukS-PV/lukF-PV* was also tested by PCR in each isolate (Becker et al., 1998; Monday and Bohach, 1999; Mehrotra et al., 2000; Al-Talib et al., 2009; Horváth et al., 2020) (Supplementary Table 2).

2.4. Genotyping

For genotyping, pulsed-field gel electrophoresis (PFGE) was performed after *SmaI* digestion for all *S. aureus* strains according to a previously published method (Szabó et al., 2009; Laub et al., 2011). Cluster analysis was done using Fingerprinting II software (Bio-Rad, Marnes-la-Coquette, France).

All isolated *S. aureus* strains were sent to the Biomi Ltd. laboratory (Gödöllő, Hungary) for *spa* genotyping by the Sanger sequencing method. Every new repeat combination was submitted to the Ridom Spa Server (https://spa.ridom.de/submission.shtml).

Multilocus sequence typing (MLST) of the *mecA*-MRSA was carried out according to the PubMLST protocol and the sequence type was assigned through the MLST database (https://pubmlst.org/organisms/staphylococcus-aureus). *SCCmec* typing of the *mecA*-MRSA was performed by PCR as described previously (Oliveira and de Lencastre, 2002; Milheiriço et al., 2007; Horváth et al., 2020) (Supplementary Table 3).

2.5. Whole genome sequencing and data analysis of the isolate containing the *mecC* gene

The *S. aureus* isolate carrying the *mecC* gene was sent for genome sequencing to Biomi Ltd., Gödöllő, Hungary. The genome coverage was 162×. The raw Illumina paired end reads were uploaded to the NCBI SRA database under the BioProject accession number PRJNA690008. The de-novo assembly was performed by Velvet Assembler (https://github.com/dzerbino/velvet/tree/master) and the annotation by RAST (https://rast.nmpdr.org/). The draft genome was submitted to the NCBI GenBank under the accession number JAEQMS000000000. Detection of resistance genes was carried out with ResFinder 2.3 (https://cge.cbs.dtu.dk/services/ResFinder/) (using the default settings) and CARD (https://card.mcmaster.ca/). For MLST typing, MLST 2.0 was used (https://cge.cbs.dtu.dk/services/MLST/). The allelic profile of the isolate was compared with allele sequences present in the PubMLST database (https://pubmlst.org/). The *SCCmec* type of the *mecC*-MRSA was determined by the SCCmecFinder 1.2 (https://cge.cbs.dtu.dk/services/SCCmecFinder/) Virulence and toxin genes were detected by VirulenceFinder 2.0 (https://cge.cbs.dtu.dk/services/VirulenceFinder/) (using the default settings) and by VFanalyzer (http://www.mgc.ac.cn/cgi-bin/VFs/v5/main.cgi). The identified genes and proteins were compared using Clustal Omega Multiple Sequence Alignment (https://www.ebi.ac.uk/Tools/msa/clustalo/). Sequences and annotations in search of genomic islands were compared using Geneious 11.1 (Biomatters Ltd., New Zealand).

3. Results

3.1. Carriage rate among hedgehogs

S. aureus strains were isolated in 13 of 200 animals (6.5%), 7/13 in nose samples and 4/13 in skin samples. Two animals had *S. aureus* at both locations (sharing identical PFGE and virulence gene patterns), from which only one isolate was selected, and hence 13 strains were included in this manuscript. Two isolates proved to be MRSAs, indicating a 1% MRSA prevalence (2/200).

3.2. Antibiotic susceptibility

The isolated *S. aureus* strains were susceptible to the majority of the examined antibiotics. The only exception was penicillin, to which 6/13 of the *S. aureus* samples showed resistance. Intermediate resistance, elevated MICs were detected to ciprofloxacin (13/13) and erythromycin (1/13). Phenotypically two isolates proved to be cefoxitin resistant (Tables 1 and 2). It was confirmed by further PCR analysis that these two strains were MRSAs: one carried the *mecA* gene, whereas the other sample (H68B1) carried the novel homologue *mecC* gene.

Table 1
Antibiotic susceptibility of 13 *S. aureus* isolates obtained from Hungarian hedgehogs (2020).

Antibiotics[a]	MIC range (mg/l)	Sensitive (%)	Intermediate (%)	Resistant (%)
PEN	0.032–32	54	NA[b]	46
MUP	0.094-0.5	100	0	0
CLI	0.032–0.38	100	0	0
ERY	0.064–1.5	92	8	0
TET	0.25–64	100	0	0
CIP	0.38–1	0	100	0
GEN	0.064–0.75	100	NA	0
FOX	20-33[c]	85	NA	15

[a] PEN = penicillin, MUP = mupirocin, CLI = clindamycin, ERY = erythromycin, TET = tetracycline, CIP = ciprofloxacin, GEN = gentamicin, FOX = cefoxitin.
[b] NA, not applicable.
[c] Inhibition zone diameter (mm).

Table 2
Antibiotic susceptibility results of the *mecC* carrying *S. aureus* isolate obtained from a Hungarian hedgehog (2020).

Sample	Antibiotics[a]								
	PEN	MUP	CLI	ERY	TET	CIP	GEN	VAN	FOX
	MIC (mg/l)								Zone diameter (mm)
H68B1	1.50	0.25	0.38	1.50	0.38	1	0.06	1	22
	R[b]	S	S	I	S	I	S	S	R

[a] PEN = penicillin, MUP = mupirocin, CLI = clindamycin, ERY = erythromycin, TET = tetracycline, CIP = ciprofloxacin, GEN = gentamicin, FOX = cefoxitin,
[b] R = resistant, I = intermediate, S = sensitive.

Table 3
Virulence genes detected with PCR in the 13 carried *S. aureus* isolates from Hungarian hedgehogs (2020).

Isolates	*icaD*[a]	*cna*[b]	*fnbA*[c]	*fnbB*[d]
SS2	+	+	+	+
SS13	+	−	+	+
SS70	+	−	+	+
18BO	+	−	+	+
H21B	+	−	+	+
H37O	+	−	+	+
H39O	+	−	+	+
H71B	+	−	+	+
H73B	+	−	+	−
H75O	+	−	+	+
H75B	+	−	+	+
H76O	+	−	+	+
H76B	+	−	+	+
H68B1 *mecC* +	+	−	+	−
H77O1 *mecA* +	+	−	+	+

+ = presence of the gene; − = absence of the gene.
[a] Intracellular adhesion B.
[b] Collagen binding adhesin.
[c] Fibronectin binding protein A.
[d] Fibronectin binding protein B.

3.3. Virulence factors and toxin genes

By PCR, the examined toxin genes were not found, whereas the majority of the isolated *S. aureus* strains carried genes responsible for adhesion and biofilm production (*icaD* 13/13, *fnbA* 13/13, *fnbB* 11/13, *cna* 1/13). Only one isolate, the SS2 strain, carried the collagen collagen-binding adhesin gene. The only difference of note between the two MRSAs was that the *fnbB* gene was absent in the *mecC*-MRSA (Table 3).

3.4. Genotyping

3.4.1. PFGE, MLST, spa and SCCmec results

The PFGE dendrogram of the 13 *S. aureus* isolates shows the presence of two major clusters at 93% similarity level, and only the *mecA*-MRSA has a notably divergent banding pattern (Fig. 1).

The *spa*-typing of the *mecC*-positive MRSA strain showed a new repeat combination (04-82-17-25-17-25-25-110) and a new *spa* type (t19701). The *mecA*-MRSA isolate belonged to the t330 *spa* type, CC45-ST3060 MLST type and was *SCCmec* type IV. According to the results of the *spa* typing of all of the *S. aureus* isolates, the strains belonged to seven different *spa* types: t091 ($n = 3$), t843 (n = 3), t18328 ($n = 2$), t008 (n = 2), t449 ($n = 1$), t330 (n = 1), and t19701 (n = 1) (Fig. 1).

3.4.2. WGS of the mecC positive MRSA isolate

The single *mecC* gene gene-containing isolate was examined further with molecular methods. The MLST type of the isolate was a single-locus variant of ST130 with one nucleotide difference in the *arcC* gene. A new allele number (*arcC*-793) and a new sequence type (ST6736) were assigned to it by the pubMLST curators. During the analysis of the WGS data, the presence of *blaZ*, *mecC*, and *lmrS* resistance genes was detected. The latter codes for an antibiotic efflux pump capable of extruding several antibiotics, including linezolid, trimethoprim, chloramphenicol, erythromycin, streptomycin, kanamycin, and fusidic acid (https://card.mcmaster.ca/).

The only toxin genes in the bacterial genome were the gamma hemolysin genes (*hlgA, hlgB, hlgC*), also called the *hlg*-locus (Dalla Serra et al., 2005), and the bicomponent leukocidins, *lukD-lukE, lukH-lukG* (Seilie and Bubeck Wardenburg, 2017). The Panton-Valentine leukocidin and the animal-associated leukocidin homologue lukM/lukF-P83 (Monecke et al., 2013) were absent. The epidermal cell differentiation inhibitor gene *edinB*, as well as a relatively new exfoliative toxin gene homologue named *etE* (Imanishi et al., 2019) (previously called *etD2* (Monecke et al., 2013)), were detected in close proximity to each other, separated only by a 227 bp noncoding region. The genes associated with beta haemolysin-converting phages, also called the immune evasion cluster (IEC) (*sea, sep, chp, sak and scn)*, were absent (Monecke et al., 2016; Ahmadrajabi et al., 2017). During our analysis, the genes coding for aureolysin (*aur*), thermonuclease (*nuc*), coagulase (*coa*), lipase (*geh, lip*), and protease (*sspA, sspB, sspC, splA, splB, splC, splD, splE splF*) enzymes were found. Furthermore, the *mecC*-MRSA isolate contained the ica operon, which is responsible for the poly-n-succinyl-β-1,6-glucosamine (PNSG) polysaccharide production during infection. This saccharide allows the bacteria to adhere to each other, thereby promoting biofilm formation (http://www.mgc.ac.cn/VFs/). In addition, several other genes responsible for the production of microbial surface component-recognizing adhesive matrix molecules (MSCRAMMs) were found, such as clumping factors (*clfA, clfB*), fibrinogen

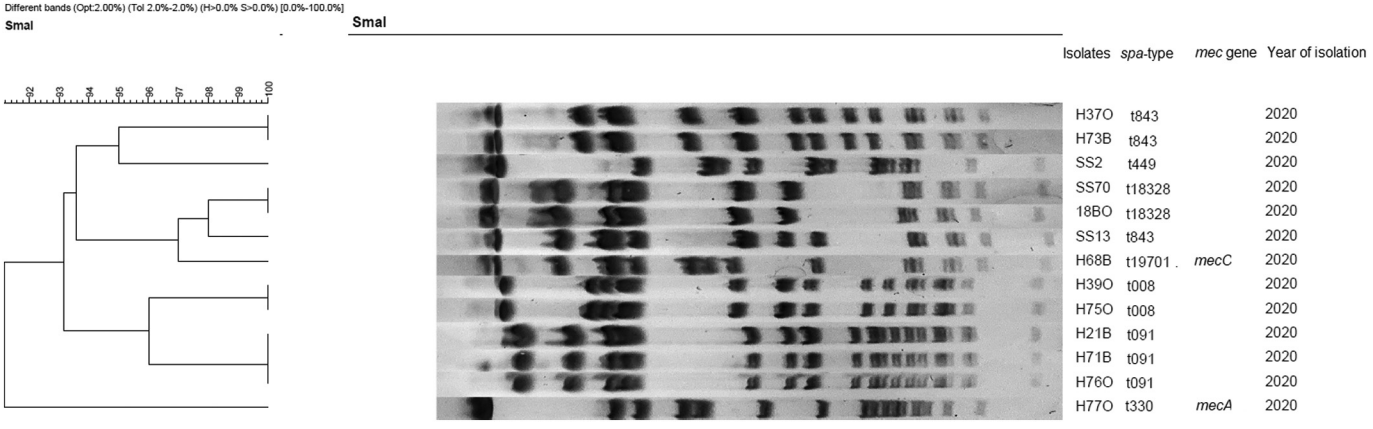

Fig. 1. PFGE dendrogram of the *S. aureus* isolates.

binding protein (*efb*), extracellular adherence protein (*map*), elastin binding protein (*ebp*), fibronectin binding protein A (*fnbA*) and Ser-Asp-rich proteins (*sdrC, sdrD, sdrE*) (http://www.mgc.ac.cn/VFs/). The isolated *mecC*-MRSA carried capsule genes (*cap8H, cap8I, cap8J, cap8K*) promoting capsule production, thus enabling the bacteria to avoid opsonization and phagocytosis. Of the immune evasion factors, the immunoglobulin G-binding protein A (*spa*) and staphylococcal immunoglobulin-binding protein gene (*sbi*) were also detected (http://www.mgc.ac.cn/VFs/).

Based on the *SCCmec* cassette analysis, the *mecC* carrying H68B1 isolate contained *ccrB3* and *ccrA1*, hence belonging to *ccr* type 8. An arsenic-resistance operon could be found in the J1 region as well, consisting of three genes: *arsR*, a transcriptional repressor; *arsB*, an arsenite efflux pump; and *arsC*, an arsenate reductase (Shore et al., 2011). In view of these characteristics, the SCCmecFinder assigned the *mecC*-MRSA strain to *SCCmec* type XI.

4. Discussion

Wildlife can act as a reservoir for a wide variety of pathogens, including multidrug-resistant microorganisms like MRSA, VRE or Enterobacteriales (Oravcova et al., 2016; Heaton et al., 2020; Grünzweil et al., 2021). Although hedgehogs in Northern Europe are known to be reservoirs of *mecC* (Bengtsson et al., 2017; Rasmussen et al., 2019), in the present work we found that the prevalence in the Budapest region was found to be very low. The MRSA carriage rate among hedgehogs in our study was just 1%. The *mecC* prevalence however seems to show significant geographical differences within Europe. The majority of *mecC*-positive *S. aureus* samples have been found in Western and Northern Europe, both in animals and humans (García-Álvarez et al., 2011; Shore et al., 2011; Paterson et al., 2014; Bengtsson et al., 2017; Rasmussen et al., 2019; Heaton et al., 2020), whereas *mecC* has a low prevalence in this region of Europe. For instance, very high prevalence has been reported among hedgehogs in Denmark (61%, 114/188) (Rasmussen et al., 2019) and Sweden (64%, 35/55) (Bengtsson et al., 2017). Only a few studies have been carried out in the countries bordering Hungary, but these seem to support our findings. In Croatia, 237 milk samples were examined from cows with mastitis, and only five *mecC*-MRSAs were detected (2.1% prevalence) (Cvetnić et al., 2021). In Austria, 767 wild animals and 723 ruminants were screened for coagulase-negative Staphylococcus (CoNS) species, and only 15 *mecC*-CoNS isolates were found (Loncaric et al., 2019). In Slovakia, small mammals such as striped field mice and yellow-necked mice were screened in two sampling areas. 61 animals were sampled in total and only seven *S. aureus* strains were found - none of them carrying the *mecC* gene (Kmeť et al., 2018). The first reports of human *mecC*-MRSA strains in these countries were published in 2015. In Austria, 295 MRSA strains, deriving from both clinical and screening samples, were examined and six *mecC*-MRSAs (CC130, CC599,t843, t1535, t3256, t5930) were detected (Kerschner et al., 2015). In Slovenia, six *mecC*-MRSAs (ST130-t843, t10009, t9397) were found among a total of 395 CA-MRSA strains (Dermota et al., 2015).

Presumably *mecC*-MRSA prevalence is correspondingly low in farm animals in Hungary. Albert et al. examined several hundred milk samples throughout the country between 2003 and 2018, and found 31 MRSA isolates in total - but none of them was carrying the *mecC* gene (Albert et al., 2020). No *mecC*-MRSA has yet been reported in human specimens in Hungary.

The presence of the virulence factor *cna* in *S. aureus* is lineage-specific and an association between *cna* and MLST CC1, CC12, CC22, CC30, CC45, CC51 and CC239 has been observed (Deurenberg et al., 2009). Therefore, *cna* can be used to differentiate clonal complexes within *spa* types (Deurenberg et al., 2009). The *cna*-positive *S. aureus* hedgehog sample in this study (SS2) had the *spa* type t449 (26–23–13-23-31-05-05-17-25-17-25-16-28), which is related to *spa* type t005 (26–23–13-23-31-05-17-25-17-25-16-28), and t005 is associated with ST22, ST23, ST60 (https://spa.ridom.de/). Based on this relationship between *spa* type and *cna* positivity, the SS2 isolate in this study very probably belongs to MLST CC22, which is a common human epidemic lineage (Tinelli et al., 2009; Xiao et al., 2019). In addition, we have identified ST22 as the major MLST clone among blood stream MRSA isolates in Hungary (Horváth et al., 2020).

The *mecC*-MRSA isolate from this study (ST6736) is a single-locus variant of ST130 with a single nucleotide difference in the *arcC* gene and it also shares similarities with ST130 regarding the virulence genes. According to the literature, the most common MLST clonal complex in *mecC*-positive *S. aureus* isolates in hedgehogs is CC130 with the following *spa* types: ST130-t3256, ST130-t843, ST130-t5771, CC130-t843, t10755, 10,893, t11015, CC130-t528, t843, t1048, t3256, t3570, t6220, t17133, (Loncaric et al., 2013; Monecke et al., 2013; Bengtsson et al., 2017; Rasmussen et al., 2019). However, CC1943-t978, t2345, t3391, t8835, t16868 and CC2361-t3391,t15312, t9111, t978 have also been described in these animals (Rasmussen et al., 2019). The *mecC*-positive strain isolated in the present study has a new *spa* type, t19701 (repeats: 04–82–17-25-17-25-25-110), although it seems to be very similar to t843 (repeats: 04–82–17-25-17-25-25-16-17), which has been described in hedgehogs in Sweden and Austria (Monecke et al., 2013; Bengtsson et al., 2017).

However, ST130 has also been described in other animals as well (Fig. 2), such as the European rabbit (ST130-t843), red deer (ST130-tNT) (L. Ruiz-Ripa et al., 2019a, 2019b; Heaton et al., 2020), European brown hare (ST130-t843, ST130-t10513) (Loncaric et al., 2013; Monecke et al., 2016; Heaton et al., 2020), common chaffinch (ST130-t6293) (Paterson et al., 2012), yellow-necked mouse and house mouse (ST130-t843) (Mrochen et al., 2018), wild boar (ST130-t6220) and Iberian ibex (ST130-t1736) (Porrero et al., 2014a). Livestock animals are also carriers of ST130, such as cattle (ST130-t843) (Jørgensen et al., 2005; Sung et al., 2008; Cvetnić et al., 2021) and sheep (ST130-t843) (Paterson et al., 2012). Isolates belonging to clonal complex (CC) 130, have additionally been described in brown rat, European fallow deer, red fox (Monecke et al., 2016), common seal, domestic dog (Paterson et al., 2012), European otter (Loncaric et al., 2013), white stork (Gómez et al., 2016), magpies and cinereous vultures (Laura Ruiz-Ripa et al., 2019a, 2019b). The genetic relatedness of these European CC130 isolates, based on the number of allele differences, is shown in Fig. 2. The related data are summarized in Supplementary Table 4.

The *mecA*-MRSA strain identified in the current study (H77O1) belongs to ST3060-t330. According to the pubMLST database (https://pubmlst.org/) only one ST3060 isolate has been isolated so far, and that was obtained in 2014 in Spain from a white stork (Gómez et al., 2016) which had t015 *spa* type. These two *spa* types are again closely related to one another (t330: 09–02–16-34-34-17-34-16-34 and t015: 08–16–02-16-34-13-17-34-16-34) (https://spaserver.ridom.de/). The ST3060 belongs to CC45 which we have previously described in human carriage in Hungary (Laub et al., 2018).

Among the *mecC*-MRSA isolates, resistance to non-ß-lactam antibiotics is currently rare (Monecke et al., 2013; Rasmussen et al., 2019) and the minimal inhibitory concentrations for oxacillin and cefoxitin are generally lower than those seen among *mecA*-MRSA (Paterson et al., 2014). These statements are consistent with the findings of our present work. The *blaZ* gene in *mecC*-MRSA is responsible for ß-lactamase enzyme production while *mecC* is responsible for the mutation in the penicillin binding protein.

The *mecC*-MRSA isolate lacks the *pvl, chp, sak, scn* genes, which are thought to be indicators of human origin (Sung et al., 2008; Monecke et al., 2016; Ahmadrajabi et al., 2017). The absence of markers for human adaptation, such as Panton-Valentine leukocidin (*pvl*) and genes associated with beta-haemolysin-converting phages (*sea, sep, chp, sak* and *scn*), in the majority of the *mecC* isolates (Table 4) strongly suggests that *mecC* originates from animal-adapted strains (Sung et al., 2008; Monecke et al., 2013).

Interestingly, we could detect a novel exfoliative toxin in the *mecC*-MRSA isolate, which was first described in a paper by Harrison et al. (2013a) and initially called *etD2* and *etD*-like protein but later renamed as *etE* in 2019 (Yamaguchi et al., 2002; Monecke et al., 2013; Mariutti et al., 2015; Imanishi et al., 2019). This hydrolyses desmoglein 1 in human, swine, murine and ovine tissues in vitro (Imanishi et al., 2019) and has so

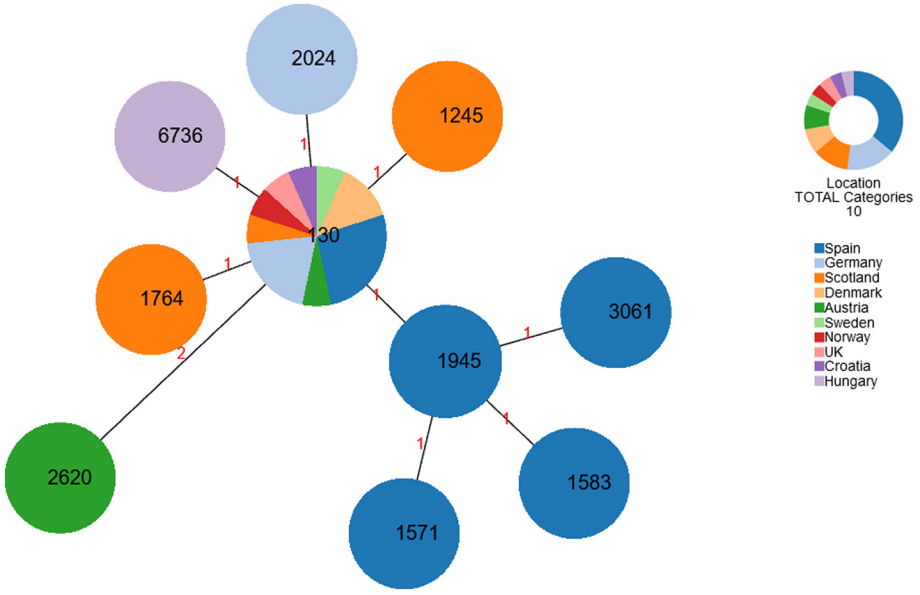

Fig. 2. Minimum spanning tree of different CC130 isolates from animals published in the literature. Nodes represent ST of the isolates, numbers in nodes denote the multilocus sequence type (7-MLST) as defined by the scheme at https://pubmlst.org/, and colours represent the country of origin. Numbers on the lines between isolates indicate differences in alleles between isolates. The figure was prepared with Phyloviz Online.

far been detected in samples from ewe mastitis, humans and hedgehogs (Harrison et al., 2013a; Monecke et al., 2013; Paterson et al., 2014; Imanishi et al., 2019). This exfoliative toxin is thought to be localized on a pathogenicity island together with the epidermal cell differentiation inhibitor gene (*edinB*) (Yamaguchi et al., 2002). The *mecC*-MRSA isolate contained this latter gene as well.

5. Conclusions

We report here the first *mecC*-MRSA strain detected in Hungary, which was isolated from a hedgehog. In addition, to the best of our knowledge, this is also the first study in Hungary investigating the prevalence of staphylococci in wildlife.

Table 4
Characterization of reported *mecC*-MRSA isolates from European hedgehogs.

	n = 1 (H68B1)	**n** = 2	**n** = 1	**n** = 114	**n** = 35
	This study	Monecke et al., 2013	Loncaric et al., 2013	Rasmussen et al., 2019	Bengtsson et al., 2017
Country	Hungary	Sweden	Austria	Denmark	Sweden
hlg[b] locus	+	+	NT[a]	NT	NT
lukD/E[c]	+	+	NT	NT	NT
PVL[d]	−	−	−	−	−
lukM/lukF-P83[e]	−	−	NT	NT	NT
edinB[f]	+	+	NT	NT	NT
etE (etD2)[g]	+	+	NT	NT	NT
chp[h]	−	−	NT	NT	NT
sak[i]	−	−	NT	NT	NT
scn[j]	−	−	NT	−	NT

[a] NT, not tested.
[b] γ-Hemolysin.
[c] Leukocidin D/E.
[d] Panton-Valentin leukocidin.
[e] Leukocidin M/P83.
[f] Epidermal cell differentiation inhibitor B.
[g] Exfoliative toxin E.
[h] Chemotaxis inhibitory protein.
[i] Staphylokinase.
[j] Staphylococcal complement inhibitor.

The *mecC*-MRSA isolate obtained in the current study (H68B1) was very similar to those found in other European countries, but the carriage rate seems to be much lower than in the northern regions. The majority of *mecC*-MRSA samples from farm animals have also been detected in Northern Europe: in the UK, Denmark and Sweden (García-Alvarez et al., 2011; Petersen et al., 2013). This finding seems to further support the idea that, in a certain geographical area, *mecC* carriage rate in wild and domesticated animal populations follow a similar pattern. Furthermore, this suggests a probable clonal spread of *mecC*-positive MRSA strains. The *mecC*-MRSA isolate described here carries a novel exfoliative toxin called *etE*, which hypothetically can also damage human tissue (Imanishi et al., 2019), so it could potentially lead to toxin-mediated staphylococcal skin diseases in humans, although there is no firm evidence to support this theory.

This isolate, like other *mecC*-MRSA isolates, was susceptible to all of the examined antibiotics except the ß-lactams. Nonetheless, monitoring antibiotic resistance in *mecC*-MRSAs is important as they may acquire resistance genes from *mecA*-MRSAs or other staphylococci.

One limitation of our study is that only hedgehogs from Budapest and its suburbs were studied, therefore no firm conclusions on the prevalence and geographic distribution of *S. aureus*/MRSA in hedgehogs can be drawn for the country as a whole. Furthermore, other potential animal hosts, including domestic and wild animals, should also be investigated in the future.

Based on our results and the data in the literature (Albert et al., 2020), the risk of zoonotic transmission of *mecC*-MRSA, either from wild or domestic animals, is currently very low in Hungary. Nevertheless, awareness of the presence of *mecC*-MRSA is important, as these strains could be overlooked and misidentified in clinical microbiology laboratories where *mecC*-MRSA may not be correctly identified as MRSA by PCR (Paterson et al., 2014) and *mecC*-MRSAs could potentially emerge as novel human pathogens.

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CRediT authorship contribution statement

Judit Sahin-Tóth: Investigation, Writing – original draft, Visualization. **Ervin Albert:** Writing – review & editing. **Alexandra Juhász:** Resources, Writing – review & editing. **Ágoston Ghidán:** Writing – review & editing. **János Juhász:** Data curation, Writing – review & editing. **Andrea Horváth:** Writing – review & editing. **Martin C. Steward:** Writing – review

& editing. **Orsolya Dobay:** Conceptualization, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

Ahmadrajabi, R., Khavidaki, S.L., Kalantar-Neyestanaki, D., Fasihi, Y., 2017. Molecular analysis of immune evasion cluster (IEC) genes and intercellular adhesion gene cluster (ICA) among methicillin-resistant and methicillin-sensitive isolates of Staphylococcus aureus. J. Prev. Med. Hyg. 58, E308–E314. https://doi.org/10.15167/2421-4248/jpmh2017.58.4.711.

Albert, E., Sipos, R., Jánosi, S., Kovács, P., Kenéz, Á., Micsinai, A., Noszály, Z., Biksi, I., 2020. Occurrence and characterisation of methicillin-resistant Staphylococcus aureus isolated from bovine milk in Hungary. Acta Vet. Hung. 68, 236–241. https://doi.org/10.1556/004.2020.00040.

Al-Talib, H., Yean, C.Y., Al-Khateeb, A., Hassan, H., Singh, K.K.B., Al-Jashamy, K., Ravichandran, M., 2009. A pentaplex PCR assay for the rapid detection of methicillin-resistant Staphylococcus aureus and panton-valentine leucocidin. BMC Microbiol. 9, 1–8. https://doi.org/10.1186/1471-2180-9-113.

Atshan, S.S., Shamsudin, M.N., Karunanidhi, A., van Belkum, A., Lung, L.T.T., Sekawi, Z., Nathan, J.J., Ling, K.H., Seng, J.S.C., Ali, A.M., Abduljaleel, S.A., Hamat, R.A., 2013. Quantitative PCR analysis of genes expressed during biofilm development of methicillin-resistant Staphylococcus aureus (MRSA). Infect. Genet. Evol. 18, 106–112. https://doi.org/10.1016/j.meegid.2013.05.002.

Becker, K., Roth, R., Peters, G., 1998. Rapid and specific detection of toxigenic Staphylococcus aureus: use of two multiplex PCR enzyme immunoassays for amplification and hybridization of staphylococcal enterotoxin genes, exfoliative toxin genes, and toxic shock syndrome toxin 1 gene. J. Clin. Microbiol. 36, 2548–2553. https://doi.org/10.1128/jcm.36.9.2548-2553.1998.

Becker, K., Ballhausen, B., Köck, R., Kriegeskorte, A., 2014. Methicillin resistance in staphylococcus isolates: the "mec alphabet" with specific consideration of mecC, a mec homolog associated with zoonotic S. aureus lineages. Int. J. Med. Microbiol. 304, 794–804. https://doi.org/10.1016/j.ijmm.2014.06.007.

Bengtsson, B., Persson, L., Ekström, K., Unnerstad, H.E., Uhlhorn, H., Börjesson, S., 2017. High occurrence of mecC-MRSA in wild hedgehogs (Erinaceus europaeus) in Sweden. Vet. Microbiol. 207, 103–107. https://doi.org/10.1016/j.vetmic.2017.06.004.

Campbell, S.J., Deshmukh, H.S., Nelson, C.L., Bae, I.G., Stryjewski, M.E., Federspiel, J.J., Tonthat, G.T., Rude, T.H., Barriere, S.L., Corey, R., Fowler, V.G., 2008. Genotypic characteristics of Staphylococcus aureus isolates from a multinational trial of complicated skin and skin structure infections. J. Clin. Microbiol. 46, 678–684. https://doi.org/10.1128/JCM.01822-07.

Cvetnić, L., Samardžija, M., Duvnjak, S., Habrun, B., Cvetnić, M., Tkalec, V.J., Đuričić, D., Benić, M., 2021. Multi locus sequence typing and spa typing of Staphylococcus aureus isolated from the milk of cows with subclinical mastitis in Croatia. Microorganisms 9. https://doi.org/10.3390/microorganisms9040725.

Dalla Serra, M., Coraiola, M., Viero, G., Comai, M., Potrich, C., Ferreras, M., Baba-Moussa, L., Colin, D.A., Menestrina, G., Bhakdi, S., Prévost, G., 2005. Staphylococcus aureus bicomponent γ-hemolysins, HlgA, HlgB, and HlgC, can form mixed pores containing all components. J. Chem. Inf. Model. 45, 1539–1545. https://doi.org/10.1021/ci050175y.

Dermota, U., Zdovc, I., Strumbelj, I., Grmek-Kosnik, I., Ribic, H., Rupnik, M., Golob, M., Zajc, U., Bes, M., Laurent, F., Mueller-Premru, M., 2015. Detection of methicillin-resistant Staphylococcus aureus carrying the mecC gene in human samples in Slovenia. Epidemiol. Infect. 143, 1105–1108. https://doi.org/10.1017/S0950268814001861.

Desvars-Larrive, A., Ruppitsch, W., Lepuschitz, S., Szostak, M.P., Spergser, J., Feßler, A.T., Schwarz, S., Monecke, S., Ehricht, R., Walzer, C., Loncaric, I., 2019. Urban brown rats (Rattus norvegicus) as possible source of multidrug-resistant enterobacteriaceae and meticillin-resistant Staphylococcus spp., Vienna, Austria, 2016 and 2017. Eurosurveillance 24, 1–12. https://doi.org/10.2807/1560-7917.ES.2019.24.32.1900149.

Deurenberg, R.H., Rijnders, M.I.A., Sebastian, S., Welling, M.A., Beisser, P.S., Stobberingh, E.E., 2009. The Staphylococcus aureus lineage-specific markers collagen adhesin and toxic shock syndrome toxin 1 distinguish multilocus sequence typing clonal complexes within spa clonal complexes. Diagn. Microbiol. Infect. Dis. 65, 116–122. https://doi.org/10.1016/j.diagmicrobio.2009.07.007.

Dube, F., Söderlund, R., Lampinen Salomonsson, M., Troell, K., Börjesson, S., 2021. Benzylpenicillin-producing trichophyton erinacei and methicillin-resistant Staphylococcus aureus carrying the mecC gene on European hedgehogs – a pilot-study. BMC Microbiol. 21, 1–11. https://doi.org/10.1186/s12866-021-02260-9.

García-Álvarez, L., Holden, M.T.G., Lindsay, H., Webb, C.R., Brown, D.F.J., Curran, M.D., Walpole, E., Brooks, K., Pickard, D.J., Teale, C., Parkhill, J., Bentley, S.D., Edwards, G.F., Girvan, E.K., Kearns, A.M., Pichon, B., Hill, R.L.R., Larsen, A.R., Skov, R.L., Peacock, S.J., Maskell, D.J., Holmes, M.A., 2011. Meticillin-resistant Staphylococcus aureus with a novel mecA homologue in human and bovine populations in the UK and Denmark: a descriptive study. Lancet Infect. Dis. 11, 595–603. https://doi.org/10.1016/S1473-3099(11)70126-8.

Gómez, P., Lozano, C., Camacho, M.C., Lima-Barbero, J.F., Hernández, J.M., Zarazaga, M., Höfle, Ú., Torres, C., 2016. Detection of MRSA ST3061-t843-mecC and ST398-t011-mecA in white stork nestlings exposed to human residues. J. Antimicrob. Chemother. 71, 53–57. https://doi.org/10.1093/jac/dkv314.

Grünzweil, O.M., Palmer, L., Cabal, A., Szostak, M.P., Ruppitsch, W., Kornschober, C., Korus, M., Misic, D., Bernreiter-Hofer, T., Korath, A.D.J., Feßler, A.T., Allerberger, F., Schwarz, S., Spergser, J., Müller, E., Braun, S.D., Monecke, S., Ehricht, R., Walzer, C., Smodlaka, H., Loncaric, I., 2021. Presence of β-lactamase-producing enterobacterales and salmonella isolates in marine mammals. Int. J. Mol. Sci. 22, 1–24. https://doi.org/10.3390/ijms22115905.

Harrison, E.M., Paterson, G.K., Holden, M.T.G., Larsen, J., Stegger, M., Larsen, A.R., Petersen, A., Skov, R.L., Christensen, J.M., Bak Zeuthen, A., Heltberg, O., Harris, S.R., Zadoks, R.N., Parkhill, J., Peacock, S.J., Holmes, M.A., 2013a. Whole genome sequencing identifies zoonotic transmission of MRSA isolates with the novel mecA homologue mecC. EMBO Mol. Med. 5, 509–515. https://doi.org/10.1002/emmm.201202413.

Harrison, E.M., Paterson, G.K., Holden, M.T.G., Morgan, F.J.E., Larsen, A.R., Petersen, A., Leroy, S., De Vliegher, S., Perreten, V., Fox, L.K., Lam, T.J.G.M., Sampimon, O.C., Zadoks, R.N., Peacock, S.J., Parkhill, J., Holmes, M.A., 2013b. A staphylococcus xylosus isolate with a new mecC allotype. Antimicrob. Agents Chemother. 57, 1524–1528. https://doi.org/10.1128/AAC.01882-12.

Heaton, C.J., Gerbig, G.R., Sensius, L.D., Patel, V., Smith, T.C., 2020. Staphylococcus aureus epidemiology in wildlife: a systematic review. Antibiotics 9. https://doi.org/10.3390/antibiotics9020089.

Horváth, A., Dobay, O., Sahin-Tóth, J., Juhász, E., Pongrácz, J., Iván, M., Fazakas, E., Kristóf, K., 2020. Characterisation of antibiotic resistance, virulence, clonality and mortality in MRSA and MSSA bloodstream infections at a tertiary-level hospital in Hungary: a 6-year retrospective study. Ann. Clin. Microbiol. Antimicrob. 19, 1–11. https://doi.org/10.1186/s12941-020-00357-z.

Imanishi, I., Nicolas, A., Caetano, A.C.B., Castro, T.L. de P., Tartaglia, N.R., Mariutti, R., Guédon, E., Even, S., Berkova, N., Arni, R.K., Seyffert, N., Azevedo, V., Nishifuji, K., Le Loir, Y., 2019. Exfoliative toxin E, a new Staphylococcus aureus virulence factor with host-specific activity. Sci. Rep. 9, 1–12. https://doi.org/10.1038/s41598-019-52777-3.

Inagaki, K., Lucar, J., Blackshear, C., Hobbs, C.V., 2019. Methicillin-susceptible and methicillin-resistant Staphylococcus aureus bacteremia: nationwide estimates of 30-day readmission, in-hospital mortality, length of stay, and cost in the United States. Clin. Infect. Dis. 69, 2112–2118. https://doi.org/10.1093/cid/ciz123.

Ito, T., Hiramatsu, K., Tomasz, A., De Lencastre, H., Perreten, V., Holden, M.T.G., Coleman, D.C., Goering, R., Giffard, P.M., Skov, R.L., Zhang, K., Westh, H., O'Brien, F., Tenover, F.C., Oliveira, D.C., Boyle-Vavra, S., Laurent, F., Kearns, A.M., Kreiswirth, B., Ko, K.S., Grundmann, H., Sollid, J.E., John, J.F., Daum, R., Soderquist, B., Buistx, G., 2012. Guidelines for reporting novel mecA gene homologues. Antimicrob. Agents Chemother. 56, 4997–4999. https://doi.org/10.1128/AAC.01199-12.

Jørgensen, H.J., Mørk, T., Caugant, D.A., Kearns, A., Rørvik, L.M., 2005. Genetic variation among Staphylococcus aureus strains from Norwegian bulk milk. Appl. Environ. Microbiol. 71, 8352–8361. https://doi.org/10.1128/AEM.71.12.8352-8361.2005.

Kerschner, H., Harrison, E.M., Hartl, R., Holmes, M.A., Apfalter, P., 2015. First report of mecC MRSA in human samples from Austria: molecular characteristics and clinical data. New Microbes New Infect. 3, 4–9. https://doi.org/10.1016/j.nmni.2014.11.001.

Kmeť, V., Čuvalová, A., Stanko, M., 2018. Small mammals as sentinels of antimicrobial-resistant staphylococci. Folia Microbiol. (Praha) 63, 665–668. https://doi.org/10.1007/s12223-018-0594-3.

Lakhundi, S., Zhang, K., 2018. Methicillin-resistant Staphylococcus aureus: molecular characterization, evolution, and epidemiology. Clin. Microbiol. Rev. 31, 1–103.

Laub, K., Kardos, S., Nagy, K., Dobay, O., 2011. Detection of Staphylococcus aureus nasal carriage in healthy young adults from a Hungarian university. Acta Microbiol. Immunol. Hung. 58, 75–84. https://doi.org/10.1556/AMicr.58.2011.1.8.

Laub, K., Tóthpál, A., Kovács, E., Sahin-Tóth, J., Horváth, A., Kardos, S., Dobay, O., 2018. High prevalence of Staphylococcus aureus nasal carriage among children in Szolnok Hungary. Acta Microbiol. Immunol. Hung. 65, 59–72. https://doi.org/10.1556/030.65.2018.001.

Loncaric, I., Kübber-Heiss, A., Posautz, A., Stalder, G.L., Hoffmann, D., Rosengarten, R., Walzer, C., 2013. Characterization of methicillin-resistant Staphylococcus spp. carrying the mecC gene, isolated from wildlife. J. Antimicrob. Chemother. 68, 2222–2225. https://doi.org/10.1093/jac/dkt186.

Loncaric, I., Kübber-Heiss, A., Posautz, A., Ruppitsch, W., Lepuschitz, S., Schauer, B., Feßler, A.T., Krametter-Frötscher, R., Harrison, E.M., Holmes, M.A., Künzel, F., Szostak, M.P., Hauschild, T., Desvars-Larrive, A., Misic, D., Rosengarten, R., Walzer, C., Slickers, P., Monecke, S., Ehricht, R., Schwarz, S., Spergser, J., 2019. Characterization of mecC gene-carrying coagulase-negative Staphylococcus spp. isolated from various animals. Vet. Microbiol. 230, 138–144. https://doi.org/10.1016/j.vetmic.2019.02.014.

Mariutti, R.B., Souza, T.A.C.B., Ullah, A., Caruso, I.P., De Moraes, F.R., Zanphorlin, L.M., Tartaglia, N.R., Seyffert, N., Azevedo, V.A., Le Loir, Y., Murakami, M.T., Arni, R.K., 2015. Crystal structure of Staphylococcus aureus exfoliative toxin D-like protein: structural basis for the high specificity of exfoliative toxins. Biochem. Biophys. Res. Commun. 467, 171–177. https://doi.org/10.1016/j.bbrc.2015.08.083.

Mehrotra, M., Wang, G., Johnson, W.M., 2000. Multiplex PCR for detection of genes for Staphylococcus aureus enterotoxins, exfoliative toxins, toxic shock syndrome toxin 1,

and methicillin resistance. J. Clin. Microbiol. 38, 1032–1035. https://doi.org/10.1128/jcm.38.3.1032-1035.2000.
Milheiriço, C., Oliveira, D.C., De Lencastre, H., 2007. Update to the multiplex PCR strategy for assignment of mec element types in Staphylococcus aureus. Antimicrob. Agents Chemother. 51, 3374–3377. https://doi.org/10.1128/AAC.00275-07.
Monday, S.R., Bohach, G.A., 1999. Use of multiplex PCR to detect classical and newly described pyrogenic toxin genes in staphylococcal isolates. J. Clin. Microbiol. 37, 3411–3414. https://doi.org/10.1128/jcm.37.10.3411-3414.1999.
Monecke, S., Gavier-Widen, D., Mattsson, R., Rangstrup-Christensen, L., Lazaris, A., Coleman, D.C., Shore, A.C., Ehricht, R., 2013. Detection of mecC-positive Staphylococcus aureus (CC130-MRSA-XI) in diseased European hedgehogs (Erinaceus europaeus) in Sweden. PLoS One 8, 4–9. https://doi.org/10.1371/journal.pone.0066166.
Monecke, S., Gavier-Widén, D., Hotzel, H., Peters, M., Guenther, S., Lazaris, A., Loncaric, I., Müller, E., Reissig, A., Ruppelt-Lorz, A., Shore, A.C., Walter, B., Coleman, D.C., Ehricht, R., 2016. Diversity of Staphylococcus aureus isolates in European wildlife. PLoS One 11, 1–27. https://doi.org/10.1371/journal.pone.0168433.
Mrochen, D.M., Schulz, D., Fischer, S., Jeske, K., El Gohary, H., Reil, D., Imholt, C., Trübe, P., Suchomel, J., Tricaud, E., Jacob, J., Heroldová, M., Bröker, B.M., Strommenger, B., Walther, B., Ulrich, R.G., Holtfreter, S., 2018. Wild rodents and shrews are natural hosts of Staphylococcus aureus. Int. J. Med. Microbiol. 308, 590–597. https://doi.org/10.1016/j.ijmm.2017.09.014.
Oliveira, D.C., de Lencastre, H., 2002. Multiplex PCR strategy for rapid identification of structural types and variants of the mec element in methicillin-resistant staphylococcus aureus. Antimicrob. Agents Chemother. 46, 2155–2161. https://doi.org/10.1128/AAC.46.7.2155.
Oravcova, V., Hadelova, D., Literak, I., 2016. Vancomycin-resistant enterococcus faecium with vanA gene isolated for the first time from wildlife in Slovakia. Vet. Microbiol. 194, 43–47. https://doi.org/10.1016/j.vetmic.2015.11.027.
Paterson, G.K., Larsen, A.R., Robb, A., Edwards, G.E., Pennycott, T.W., Foster, G., Mot, D., Hermans, K., Baert, K., Peacock, S.J., Parkhill, J., Zadoks, R.N., Holmes, M.A., 2012. The newly described mecA homologue, mecALGA251, is present in methicillin-resistant Staphylococcus aureus isolates from a diverse range of host species. J. Antimicrob. Chemother. 67, 2809–2813. https://doi.org/10.1093/jac/dks329.
Paterson, G.K., Harrison, E.M., Holmes, M.A., 2014. The emergence of mecC methicillin-resistant Staphylococcus aureus. Trends Microbiol. 22, 42–47. https://doi.org/10.1016/j.tim.2013.11.003.
Peck, S.M., Hewitt, W.L., 1945. The production of an antibiotic substance similar to penicillin by pathogenic fungi (Dermatophytes). Public Health Rep. 60, 148–153. https://doi.org/10.4135/9781412963855.n124.
Petersen, A., Stegger, M., Heltberg, O., Christensen, J., Zeuthen, A., Knudsen, L.K., Urth, T., Sorum, M., Schouls, L., Larsen, J., Skov, R., Larsen, A.R., 2013. Epidemiology of methicillin-resistant Staphylococcus aureus carrying the novel mecC gene in Denmark corroborates a zoonotic reservoir with transmission to humans. Clin. Microbiol. Infect. 19, E16–E22. https://doi.org/10.1111/1469-0691.12036.
Porrero, M.C., Mentaberre, G., Sánchez, S., Fernández-Llario, P., Casas-Díaz, E., Mateos, A., Vidal, D., Lavín, S., Fernández-Garayzábal, J.F., Domínguez, L., 2014a. Carriage of Staphylococcus aureus by free-living wild animals in Spain. Appl. Environ. Microbiol. 80, 4865–4870. https://doi.org/10.1128/AEM.00647-14.
Porrero, M.C., Valverde, A., Fernández-Llario, P., Díez-Guerrier, A., Mateos, A., Lavín, S., Cantón, R., Fernández-Garayzabal, J.F., Domínguez, L., 2014. Staphylococcus aureus carrying mecC gene in animals and urban wastewater, Spain. Emerg. Infect. Dis. 20, 899–901. https://doi.org/10.3201/eid2005.130426.
Rasmussen, S.L., Larsen, J., Van Wijk, R.E., Jones, O.R., Berg, T.B., Angen, O., Larsen, A.R., 2019. European hedgehogs (Erinaceus europaeus) as a natural reservoir of methicillin-resistant Staphylococcus aureus carrying mecC in Denmark. PLoS One 14, 1–13. https://doi.org/10.1371/journal.pone.0222031.
Ruiz-Ripa, L., Alcalá, L., Simón, C., Gómez, P., Mama, O.M., Rezusta, A., Zarazaga, M., Torres, C., 2019. Diversity of Staphylococcus aureus clones in wild mammals in Aragon, Spain, with detection of MRSA ST130-mecC in wild rabbits. J. Appl. Microbiol. 127, 284–291. https://doi.org/10.1111/jam.14301.
Ruiz-Ripa, Laura, Gómez, P., Alonso, C.A., Camacho, M.C., de la Puente, J., Fernández-Fernández, R., Ramiro, Y., Quevedo, M.A., Blanco, J.M., Zarazaga, M., Höfle, U., Torres, C., 2019. Detection of MRSA of lineages CC130-mecC and CC398-mecA and Staphylococcus delphini-lnu(A) in magpies and cinereous vultures in Spain. Microb. Ecol. 78, 409–415. https://doi.org/10.1007/s00248-019-01328-4.
Seilie, E.S., Bubeck Wardenburg, J., 2017. Staphylococcus aureus pore-forming toxins: the interface of pathogen and host complexity. Semin. Cell Dev. Biol. 72, 101–116. https://doi.org/10.1016/j.semcdb.2017.04.003.
Shore, A.C., Deasy, E.C., Slickers, P., Brennan, G., O'Connell, B., Monecke, S., Ehricht, R., Coleman, D.C., 2011. Detection of staphylococcal cassette chromosome mec type XI carrying highly divergent mecA, mecI, mecR1, blaZ, and ccr genes in human clinical isolates of clonal complex 130 methicillin-resistant Staphylococcus aureus. Antimicrob. Agents Chemother. 55, 3765–3773. https://doi.org/10.1128/AAC.00187-11.
Silva, J.G., Araujo, W.J., Leite, E.L., Dias, L.M., Vasconcelos, P.C., Silva, N.M.V., Oliveira, R.P., Sena, M.J., Oliveira, C.J.B., Mota, R.A., 2021. First report of a livestock-associated methicillin-resistant Staphylococcus aureus ST126 harbouring the mecC variant in Brazil. Transbound. Emerg. Dis. 68, 1019–1025. https://doi.org/10.1111/tbed.13771.
Smith, J.M.B., 1965. Staphylococcus aureus strains associated with the hedgehog, Erinaceus europaeus. J. Hyg. 63, 285–291. https://doi.org/10.1017/S0022172400045162.
Smith, J.M.B., Marples, M.J., 1964. A natural reservoir of penicillin-resistant strains of Staphylococcus aureus. Nature 201, 844. https://doi.org/10.1177/006947706400200105.
Smith, J.M.B., Marples, M.J., 1965. Dermatophyte lesions in the hedgehog as a reservoir of penicillin-resistant staphylococci. J. Hyg. 63, 293–303. https://doi.org/10.1017/S0022172400045174.
Sung, J.M.L., Lloyd, D.H., Lindsay, J.A., 2008. Staphylococcus aureus host specificity: comparative genomics of human versus animal isolates by multi-strain microarray. Microbiology 154, 1949–1959. https://doi.org/10.1099/mic.0.2007/015289-0.
Szabó, J., Dombrádi, Z., Dobay, O., Orosi, P., Kónya, J., Nagy, K., Rozgonyi, F., 2009. Phenotypic and genetic characterisation of methicillin-resistant Staphylococcus aureus strains isolated from the university hospitals of Debrecen. Eur. J. Clin. Microbiol. Infect. Dis. 28, 129–136. https://doi.org/10.1007/s10096-008-0588-1.
Térport, 2013. Közép-Magyarország. http://www.terport.hu/regiok/magyarorszag-regioi/kozep-magyarorszagi-regio.
The European Committee on Antimicrobial Susceptibility Testing, 2020. Breakpoint tables for interpretation of MICs and zone diameters.
Tinelli, M., Monaco, M., Vimercati, M., Ceraminiello, A., Pantosti, A., 2009. Methicillin-susceptible Staphylococcus aureus in skin and soft tissue infections, northern Italy. Emerg. Infect. Dis. 15, 250–257. https://doi.org/10.3201/eid1502.080010.
Velvet Assembler, .. 1.2.10 https://github.com/dzerbino/velvet/tree/master.
WHO, 2017. WHO publishes list of bacteria for which new antibiotics are urgently needed. https://www.who.int/news/item/27-02-2017-who-publishes-list-of-bacteria-for-which-new-antibiotics-are-urgently-needed.
Worthing, K.A., Coombs, G.W., Pang, S., Abraham, S., Saputra, S., Trott, D.J., Jordan, D., Wong, H.S., Abraham, R.J., Norris, J.M., 2016. Isolation of mecC MRSA in Australia. J. Antimicrob. Chemother. 71, 2348–2349. https://doi.org/10.1093/jac/dkw138.
Xiao, N., Yang, J., Duan, N., Lu, B., Wang, L., 2019. Community-associated Staphylococcus aureus PVL+ ST22 predominates in skin and soft tissue infections in Beijing, China. Infect. Drug Resist. 12, 2495–2503. https://doi.org/10.2147/IDR.S212358.
Yamaguchi, T., Nishifuji, K., Sasaki, M., Fudaba, Y., Aepfelbacher, M., Takata, T., Ohara, M., Komatsuzawa, H., Amagai, M., Sugai, M., 2002. Identification of the Staphylococcus aureus etd pathogenicity island which encodes a novel exfoliative toxin, ETD, and EDIN-B. Infect. Immun. 70, 5835–5845. https://doi.org/10.1128/IAI.70.10.5835-5845.2002.
Zhen, X., Lundborg, C.S., Zhang, M., Sun, X., Li, Y., Hu, X., Gu, S., Gu, Y., Wei, J., Dong, H., 2020. Clinical and economic impact of methicillin-resistant Staphylococcus aureus: a multicentre study in China. Sci. Rep. 10, 1–8. https://doi.org/10.1038/s41598-020-60825-6.