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Short Communication

Is methicillin-susceptible *Staphylococcus aureus* (MSSA) CC398 a true animal-independent pathogen?

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ABSTRACT

Objectives: Methicillin-resistant *Staphylococcus aureus* (MRSA) belonging to ST398 has been widely described in animals. In parallel, methicillin-susceptible (MSSA) ST398 isolates causing severe infections in humans have recently emerged as animal-independent pathogens. This study aimed at characterising MSSA CC398 from different animal species in France in comparison with MSSA CC398 genomes, mostly of human origin.

Methods: CC398 were detected by clone-specific PCR. Antimicrobial susceptibility testing was performed using the disk diffusion method. Whole genome sequencing (WGS) was performed on 47 MRSA and MSSA isolates, of which *spa*-types as well as resistance and virulence genes were extracted. A maximum likelihood phylogenetic tree based on SNPs was performed on all sequenced isolates and 51 additional MRSA and MSSA data found on publicly available databases.

Results: From 275 MSSA isolates studied, 28 (10.18%) belonged to the CC398 lineage (26 ST398 and two single-locus variants) and mainly originated from cats (n=12/44, 27.3%) and dogs (n=8/55, 14.6%). Five different *spa*-types were identified, t571 (n=18, 64.3%) and t1451 (n=5, 17.9%) being the most frequent ones. Out of the 28 MSSA isolates, 26 carried the *scn* gene, whereas 24 carried the *erm(T)* gene, and all were genetically similar to human isolates.

Conclusion: This study challenges the current scientific opinion that human infections due to MSSA CC398 should only be considered an animal-independent issue.

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1. Introduction

Clonal complex 398 (CC398) methicillin-resistant *Staphylococcus aureus* (MRSA) was first reported in 2005 in pigs, and subsequently in numerous animal species and humans in contact, especially in high-density pig farming regions. Despite their harmlessness in pigs and their reputation as colonisers, MRSA CC398 can cause various minor to severe infections in humans. However, most of the deaths attributed to CC398 has been due to methicillin-susceptible *Staphylococcus aureus* (MSSA) isolates [1].

In the 2010s, human colonisation and infection with MSSA ST398 were frequently reported in Europe, China and the United States [2–6], mostly due to *spa*-type t571. In France, the proportion of bloodstream infections due to MSSA CC398 increased

from 3.6% in 2010 to 20.2% in 2017 [7], an increasing trend also observed internationally [6]. MSSA ST398 are considered animal-independent and differ from MRSA by the global absence of the *tet(M)* tetracycline-resistance gene and the presence of the *erm(T)* macrolide-resistance gene and the immune evasion cluster (IEC) *scn* gene [6,8]. This latter gene, together with increased capacities to bind to keratinocytes and nasal epithelial cells, might have led to a specific adaptation to the human host [8].

Since MSSA ST398 have been considered livestock-independent, very limited information is available on their prevalence and characteristics in animals. MSSA ST398 were sporadically reported from dogs in Spain and Germany [9,10], pigs in Germany, Ireland, China and Korea [11–14], or meat in Tunisia and Denmark [15,16]. Most animal isolates displayed *spa*-types t034, t108, t899, unrelated to the major human *spa*-type t571, and no animal-human transmission has been evidenced yet. Our goal was thus to investigate the presence of MSSA CC398 in a wide range of food-producing and domestic animals in France, a country where MSSA ST398 isolates have widely been reported in humans in both hospital settings and

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Table 1
Characteristics of MSSA isolates obtained from different animal species

Animal	Total MSSA	No. of CC398 (%)	Spa-types (n)
Cattle	69	3 (4.4)	t571 (1), t1451 (1), t15810 (1)
Goat	56	1 (1.8)	t571 (1)
Dog	55	8 (14.6)	t571(5), t1451 (2), t18379 (1)
Cat	44	12 (27.3)	t571(9), t1451(2), UN (1)
Horse	37	2 (5.4)	t571 (1), t3625 (1)
Other	14	2 (5.4)	t571 (1), UN (1)
Total	275	28 (10.2)	t571(18), t1451 (5), t15810 (1), t18379 (1), t3625 (1), UN (2)

UN, unknown.

the community, and then to compare these isolates with human MSSA CC398 ones.

2. Materials and methods

2.1. Bacterial collection

Nonduplicate MSSA isolates (n=275) originating from different animal species were collected in 2014–2019 through the RESAPATH network (<https://resapath.anses.fr/>) and confirmed by a triplex PCR targeting the 16S rRNA, *mecA* and *nuc* genes. CC398 isolates were detected as already published [17].

2.2. Antimicrobial susceptibility testing

Susceptibility testing was performed using the disk diffusion method according to the guidelines of the Antibiogram Committee of the French Society for Microbiology (<https://www.sfm-microbiologie.org/>). *S. aureus* ATCC 25923 was used as quality control. Penicillin G, cefoxitin, cefovecin, kanamycin, gentamicin, tobramycin, tetracycline, erythromycin, spiramycin, lincomycin, chloramphenicol, florfenicol, fusidic acid, enrofloxacin, tigecyclin and linezolid (Mast Diagnostics, Amiens, France) were tested.

2.3. Whole genome sequencing (WGS)

Total genomic DNA was extracted from 47 CC398 isolates (28 MSSA, 19 MRSA) using a NucleoSpin Microbial DNA kit (Macherey-Nagel, Hoerd, France). Libraries were prepared using the Nextera XT technology, and sequencing was performed on a NovaSeq instrument (Illumina, San Diego, CA, USA). Reads were quality trimmed and assembled using SPAdes v. 3.14.0. Quality controls are provided in Supplementary Table S1. Resistance genes and virulence markers were detected using the CGE online tools ResFinder v. 3.2 and VirulenceFinder v. 2.0 (<https://www.genomicepidemiology.org/>). Data are available under the BioProject accession number PRJNA763220.

2.4. Phylogenetic analysis

In addition to our sequenced isolates, 51 CC398 strains (48 MSSA of human (n=15), food (n=17) and animal (n=16) origin and three MRSA of human origin) were retrieved from publicly available databases (Supplementary Table S2). A phylogenetic tree was constructed based on single nucleotide polymorphism (SNP). SNPs were identified by mapping reads against the ST398 S0385 reference genome (GenBank AM990992). The maximum-likelihood phylogenetic tree was established using the CSI Phylogeny with default settings (<https://www.genomicepidemiology.org/>). The phylogenetic tree was visualised using iTOL v6 [18].

3. Results

The 275 MSSA isolates originated from cattle (n=69), goat (n=56), dogs (n=55), cats (n=44), horses (n=37) and diverse animals (n=14; four rabbits, six monkeys, two sheep, one bird and one rat). MSSA CC398 were detected by PCR in 28 (28/275, 10.2%) isolates collected from cats (n = 12/44, 27.3%), dogs (n=8/55, 14.6%), cattle (n=3/69, 4.4%), horses (n=2/37, 5.4%), goat (n=1/56, 1.8%) and others (n=2/14, 5.4%) (Table 1, Supplementary Table S1). These 28 presumptive isolates were confirmed as MSSA CC398 by WGS, with 26 isolates belonging to ST398 and the two remaining ones belonging to two different single-locus variants (SLV) (Supplementary Table S1). They belonged to five *spa* types (t571, t1451, t3625, t15810 and t18379), with the predominance of t571 (n=18, 64.3%) and t1451 (n=5, 17.9%) (Table 1). *Spa*-type t571 was particularly prevalent in cats (n=9/12, 75%) and dogs (n=5/8, 62.5%). For comparison purposes, 19 MRSA CC398, concomitantly isolated from horses (n=8), dogs (n=6), cattle (n=4) and goat (n=1), were also whole genome sequenced (Supplementary Table S1). They belonged to *spa*-types t011 (n=15, 78.9%), t899 (n=2) and t1939 (n=2).

Among MSSA CC398, only resistances to penicillin G (*blaZ*; 15/28, 53.6%) and erythromycin (*erm*(T); 23/28, 82.1%) were identified. MRSA isolates presented more associated resistances, especially to tetracyclines (*tet*(M), n=19/19), trimethoprim (*dhfrK*, n=14) and aminoglycosides (*aac*(6′)-*aph*(2′′), n=13). In terms of MSSA-associated virulence factors, only *scn* was identified in 26 MSSA isolates (92.9%).

The phylogenetic tree grouping all CC398 isolates (from this study plus 51 CC398 publicly available genomes) indicated a clear divergence between MRSA and MSSA isolates (Fig. 1), but also showed two subgroups among MSSA isolates. One subgroup contained all t571 and t1451 isolates (mostly originating from humans and domestic animals), while the second subgroup mostly encompassed t034 isolates (mainly coming from livestock and food samples).

4. Discussion

Genomic-based studies showed that the CC398 population originally emerged as a human-adapted animal-independent lineage characterised by methicillin-susceptibility, the presence of the *erm*(T) gene and the prophage ϕ Sa3 containing the IEC gene cluster. This lineage then evolved as an animal-dependent lineage through the loss of ϕ Sa3 and the acquisition of the *tet*(M) and *mecA* resistance genes [6,19].

MRSA CC398 have been recurrently reported from animals, but there was a lack of data on MSSA CC398. In this study, we showed that, though considered animal-independent, MSSA CC398 were surprisingly frequent (10.2%, 28/275) in sick animals, especially in cats and dogs (27.3% and 14.6%, respectively). Whether they may also colonise healthy animals would be important to know since MSSA CC398 were shown to circulate in households [20].

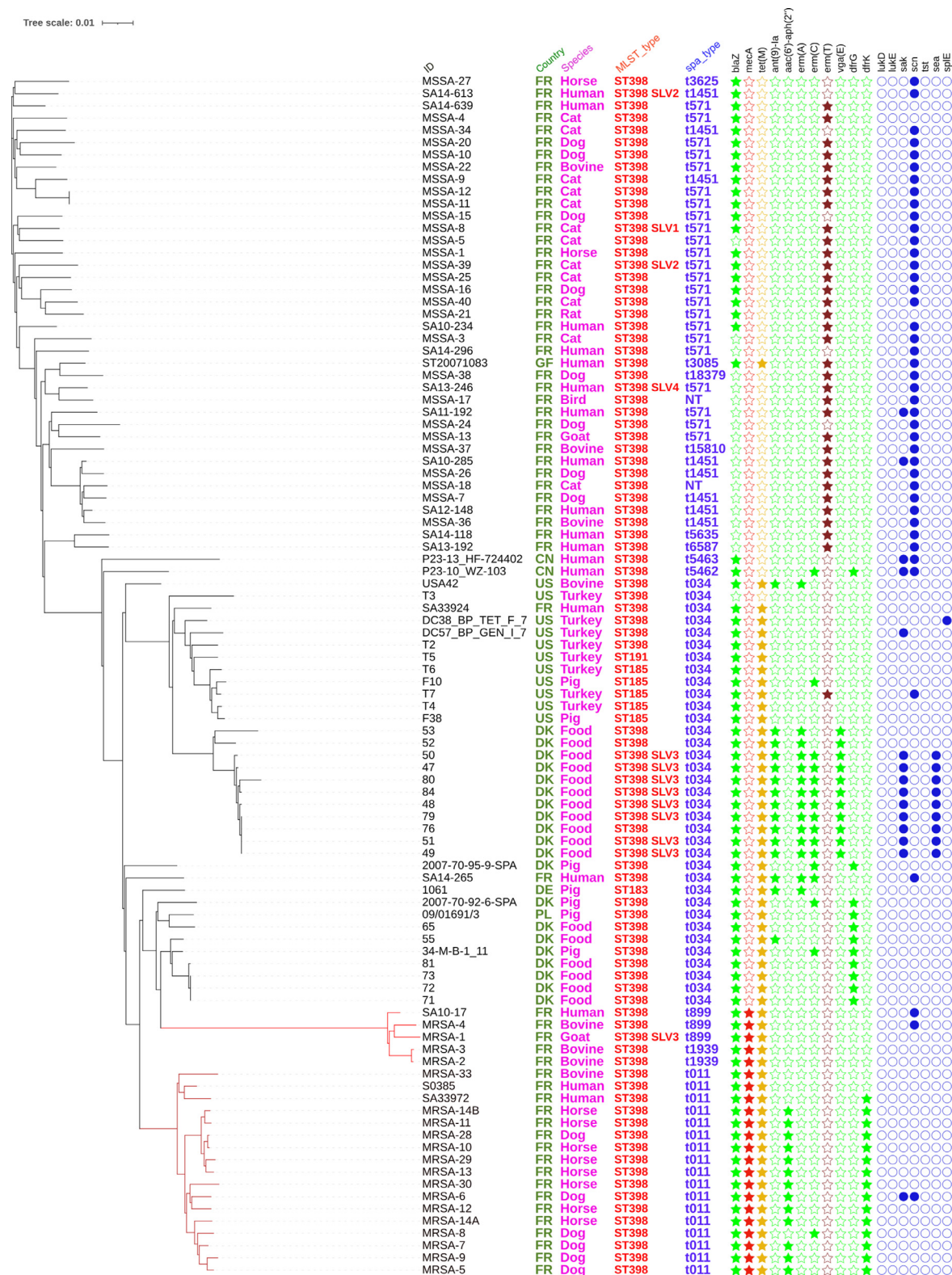


Fig. 1. Maximum-likelihood phylogenetic tree based on SNP analyses of 98 CC398 strains obtained by CSI Phylogeny from the Center for Genomic Epidemiology Web-based platform (<https://www.genomicsequencing.org/>). *Spa* type (*spa*), isolate origin (CN, China; DE, Germany; DK, Denmark; FR: France; GF: French Guiana; PL, Poland; US, United States), the presence of antimicrobial resistance, and virulence markers are indicated. MRSA is indicated with red branches in the tree. SLV1-SLV4 indicate ST398 single-locus variants (see Supplementary Tables S1 and S2). The filled stars and circles indicate confirmed antimicrobial resistance and virulence markers, respectively, as extracted from ResFinder and VirulenceFinder. Visualisation was realised using iTOL v6. Isolates named with prefix MSSA and MRSA are unique to this study. Genes encoding resistance to beta-lactams (*blaZ*, *mecA*), tetracycline [*tet(M)*], macrolide-lincosamide-streptogramin B [*erm(A)*, *erm(C)*, *erm(T)*], pleuromutilin-lincosamide-streptogramin A [*vgaE*], aminoglycosides [*ant(9)-Ia*, *aac(6')-aph(2'')*], and trimethoprim (*dfpK*, *dfpG*), and those encoding leukocidins (*lukD*, *lukE*), staphylokinase (*sak*

Most MSSA CC398 belonged to the *spa*-types t571 (18/28, 64.3%) and t1451 (5/28, 17.9%), which are the most prevalent *spa*-type in isolates from French hospitals [4,5]. A total of 26/28 isolates harboured the *scn* gene, while 24/26 harboured the *erm*(T) gene and were thus strongly reminiscent of the ancestral human population [6,8,19].

According to the SNP-phylogeny, all French animal isolates clustered with the human ones, while most other isolates for which genomic data were available clustered in another sub-clade mostly encompassing *spa*-type t034 isolates from food or livestock origin. Price *et al.* already suggested the presence of two sub-clades of MSSA CC398 [19], but more data from different countries and continent are needed to refine the global phylogeny of this specific clade. This phylogeny also proved that French MSSA isolates shared no common features with French MRSA isolates, so that any genetic link between animal MRSA and MSSA CC398 can be excluded.

In conclusion, this is the first report of such high proportions of MSSA CC398 in animals, and mainly in pets. Our data support the hypothesis that animal MSSA CC398 are phylogenetically linked to human strains circulating in France [4,5], and even if a human origin seems an acceptable hypothesis, more data are needed to decipher whether they were acquired from humans or vice versa. More globally, these data indicate that domestic animals—and principally pets—could contribute to the persistence—and maybe spread—of MSSA CC398 in the human population, making MSSA CC398 epidemiology less independent of the animal host than previously thought.

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Declaration of Competing Interest

None to declare.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jgar.2022.02.017.

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