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Original article

High prevalence of avian adapted CC5 *Staphylococcus aureus* isolates in poultry meat in Spain: food chain as vehicle of MRSA and MSSA CC398-t1451

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Summary *Staphylococcus aureus* can cause food poisoning and human infections and CC5 and CC398 are relevant lineages in the animal-human interface. The objective was to determine the *S. aureus* prevalence in chicken-derived food, and to study the diversity of lineages, and their antimicrobial resistance and virulence genotypes. Sixty poultry-food samples were processed, and the *S. aureus* isolates obtained were characterised. Antibiotic susceptibility was performed and the presence of resistance/virulence genes, and avian mobile genetic elements (MGEs) was studied. Forty-four non-repetitive *S. aureus* isolates were obtained of 28/60 samples (46.7%), and 43 methicillin-susceptible (MSSA) and one methicillin-resistant *S. aureus* (MRSA) isolates were detected. Five *S. aureus* isolates were multidrug-resistant and 50% of isolates showed susceptibility to all tested antibiotics. Eighteen *spa*-types, 11 sequence-types and 8 clonal-complexes were identified in the *S. aureus* collection. Three CC398 isolates (2 MSSA/1 MRSA) of *spa*-type-t1451 were detected, and MSSA-CC398 isolates harboured the *scn*-gene (absent in MRSA-CC398). CC5 was the most frequent lineage identified (56.8%), all MSSA, and 56.7% of them contained avian-MGEs. A high prevalence of avian-adapted MSSA-CC5 isolates was detected. Poultry meat has been shown to be a vehicle for CC398-t1451 isolates, both MRSA and MSSA, showing characteristics of the animal and human clades, respectively.

Keywords CC398, CC5, food chain, food safety, MRSA, multidrug-resistance, poultry, t1451.

Introduction

The genus *Staphylococcus* is one of the most common bacterial communities found on the skin and mucous membranes of humans and animals. This genus is formed by a large number of species, divided into two main groups; coagulase-positive (CoPS) and coagulase-negative staphylococci (CoNS), according to their ability to coagulate blood plasma (Heo *et al.*, 2020). *Staphylococcus aureus* belongs to the CoPS group, is able to colonise a large number of hosts and is considered the species of this genus more related with infectious diseases. It is found as commensal in the nasal microbiota of 20–40% of general population, and less frequently on healthy people's skin, especially on the hands and perineum (Lozano *et al.*, 2011; Lee *et al.*, 2018). In livestock animals, the percentage differs depending on the host colonised; from 15 to 35% in cattle, 30% in sheep, 42% in pigs, to 90% in poultry (Haag *et al.*, 2019). Moreover, *S. aureus* is an important opportunistic pathogen for both humans and animals, it can cause multiple diseases such as skin and soft tissue infections, toxic shock syndrome, septic arthritis, endocarditis, and bacteraemia, among others (Zhou *et al.*, 2018; Cheung *et al.*, 2021).

Staphylococcus aureus is an ubiquitous bacterium and it is able to contaminate food during its preparation and processing stages. In meat foods or dairy products, the presence of *S. aureus* could come from the colonisation of the animal itself. The food chain is one of the transmission ways for bacteria with importance in public health. Food derivates can be a vehicle for the dissemination of antibiotic-resistant bacteria, which can be transmitted by the consumption or handling of contaminated food (Koutsoumanis *et al.*, 2022). However, the presence of this species in food

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can be also due to human contamination, for example: by skin lesions on workers or by coughing or sneezing since this bacterium is a commensal microorganism on human mucous membranes (Jia *et al.*, 2020; Mahros *et al.*, 2021).

Antibiotic-resistant *S. aureus* (and mainly methicillin resistant) is a global health problem. Methicillin resistant *S. aureus* (MRSA) is an important cause of morbidity and mortality throughout the world (Kwiecinski & Horswill, 2020). Methicillin resistance is mostly conferred by the genes *mecA* or *mecC* that are integrated in the Staphylococcal Chromosomal Cassette (SCC*mec*). These genes codify a modified penicillin binding protein (PBP2a) with low affinity for most of β-lactam antibiotics (with some few exceptions as ceftaroline and ceftobiprole), limiting the therapeutic options.

Traditionally, MRSA has been associated with hospital (HA-MRSA), and community (CA-MRSA) related infections, and lately with livestock animals (LA-MRSA). The most frequent LA-MRSA lineage in Europe is CC398. It is mainly associated with colonisation of pigs and other production animals (Monecke *et al.*, 2018), although it has been also detected in companion animals, humans, and wildlife (Bal *et al.*, 2016; Ruiz-Ripa *et al.*, 2019; Mama *et al.*, 2021). The MRSA-CC398 lineage has a high zoonotic potential. Contact with livestock is a risk factor of LA-MRSA colonisation and, also of infections for farmers, veterinarians, or slaughterhouse personnel, as well as for inhabitants of areas with a high rate of swine farms (Lozano *et al.*, 2011; Ceballos *et al.*, 2019). This clonal lineage has been detected in different types of foods such as pork meat (Lozano *et al.*, 2009; Mama *et al.*, 2020), chicken meat, lamb meat (de Boer *et al.*, 2009) or sushi (Li *et al.*, 2019).

Currently, the rapid growth of poultry farms increases the potential for zoonotic infections. *S. aureus* is one of the bacterial species that causes the highest number of diseases in poultry, being the CC5 complex the most relevant genetic lineage implicated (Murray *et al.*, 2017; Haag *et al.*, 2019). It has been observed that adaptation to the avian host is accompanied by the loss of genes involved in human pathogenesis and the acquisition of new mobile genetic elements (MGEs) specific to the new host (Lowder *et al.*, 2009; Murray *et al.*, 2017).

In a previous study carried out by our research group, a high degree of MRSA- and MSSA-CC398 contamination in pork-derived food samples was reported (Mama *et al.*, 2020). The aim of the present study was to determine the prevalence of *S. aureus* in chicken meat products in La Rioja as well as to analyse the resistance and virulence determinants of the *S. aureus* isolates obtained, identifying their lineages and their possible human or animal origin.

Materials and methods

Bacterial isolation and identification

A total of 60 samples of chicken-derived raw food were collected from 16 supermarkets and 15 local butcher retail shops in La Rioja, Spain, between July–December 2020, and February–March 2023.

Samples were enriched into 5 mL of Brain Hearth Infusion broth (BHI, Conda) supplemented with NaCl 6.5% and incubated for 24 h at 37 °C. Ten microlitres aliquots were seeded on Mannitol Salt Agar (MSA, Conda) and Oxacillin Resistance Screening Agar Base (ORSAB, Oxoid) supplemented with 2 mg L^{-1} oxacillin for 24 h at 37 °C, for *S. aureus* and MRSA recovery, respectively. Up to six colonies per plate with staphylococcal morphologies were chosen and identified by MALDI-TOF mass spectrometry (matrix-assisted laser desorption-ionisation time-of-flight) using the standard protein extraction protocol recommended by manufacturers (Bruker Daltonik, Bremen, Germany). Isolates identified as *S. aureus* were maintained and characterised.

Antimicrobial resistance phenotype and genotype

Antimicrobial susceptibility was determined in *S. aureus* isolates by disk-diffusion method for the following antibiotics (μg/disk): penicillin (10), cefoxitin (30), erythromycin (15), clindamycin (2), gentamicin (10), tobramycin (10), tetracycline (30), ciprofloxacin (5), chloramphenicol (30), linezolid (30), and trimethoprim-sulfamethoxazole (1.25 + 23.75). The disk diffusion results were interpreted according to the European Committee of Antimicrobial Susceptibility Testing (EUCAST, 2023).

Moreover, the following resistance genes were analysed by PCR and sequencing of obtained amplicons: beta-lactams (*mecA*, *mecB*, *mecC*, and *blaZ*), erythromycin [*msr*(A) and *mph*(C)], erythromycin-clindamycin [*erm*(A), *erm*(B), *erm*(C), *erm*(T), and *erm*(43)], clindamycin [*lnu*(A), *lnu*(B), *lsa*(B), and *vga*(A)], tetracycline [*tet*(K), *tet*(L), and *tet*(M)], gentamicin [*aac*(6′)-Ie-*aph*(2″)-Ia], tobramycin [*ant*(4′)-Ia], chloramphenicol (*fexA*, cat_{pC194}, cat_{pC221}, and cat_{pC223}), linezolid (*cfr*, *optrA*, *poxtA*), and trimethoprim-sulfamethoxazole (*dfrA*, *dfrD*, *dfrG* and *dfrK*; Tables S1 and S2).

Molecular typing of isolates

All *S. aureus* isolates were characterised by *spa*-typing, and the obtained sequences were analysed using Ridom Staph Type software (Ridom GmbH, Münster, Germany). Seventeen representative isolates, one of each *spa*-type detected, were typed by Multilocus sequences typing (MLST). For this purpose, PCR and

sequencing of seven housekeeping genes were performed to determine the sequence type (ST) and the clonal complex (CC). A specific PCR was carried out for the CC398 *S. aureus* lineage determination, studying *sau-1-hsdS1* DNA fragment. In addition, the *agr*-typing was performed in all *S. aureus* isolates, and the isolate carrying *mecA* gene was subjected to SCC*mec* typing by PCR (Tables S1 and S2).

Virulence profile and host adaptation markers

The presence of the genes encoding the virulence factors Panton-Valentine leucocidin (*lukF/S-PV*), toxic shock syndrome toxin (*tst*), and exfoliative-toxins A, B and D (*eta, etb, etd*, respectively), was studied by PCR. The human Immune Evasion Cluster (IEC) genes (*scn*, *chp*, *sak, sea*, and *sep*) were investigated by PCR, using *scn* gene as marker of IEC system. Depending on the IEC genes detected, seven IEC types were identified (A–G). The IEC system has been considered as a human adaptation marker. The presence of avian mobile genetic elements (MGEs) was determined by PCR using avian MGE-specific primers (φAv1, φAvβ, SaPIAv, pAvY, pAvX), and later confirmed by Sanger sequencing (Tables S1 and S2).

Positive and negative controls from the collection of the Universidad de La Rioja were used in each PCR assay.

Results

Prevalence of *S. aureus* and MRSA in chicken-derived food

A total of 315 *Staphylococcus* spp. isolates from different species were obtained in the 60 poultry samples analysed. Among them, 98 *S. aureus* isolates were identified from 28 of the 60 samples analysed (47%), detecting between 1 and 6 *S. aureus* isolates per sample. After antimicrobial resistance phenotype and *spa*-type determination, 44 *S. aureus* isolates were considered as non-repetitive and selected for further characterisation corresponding to one isolate per each of the 28 positive samples or more than one if they presented different Antimicrobial Resistance Phenotype (AMR) phenotype and/or *spa*-type (Table S3). One single MRSA isolate was recovered (representing 1.6% of tested samples, and 2.3% of non-repetitive *S. aureus* isolates). The remaining 217 isolates were CoNS, and were not further characterised in this study.

Resistance phenotype and genotype of *S. aureus* isolates

Among the 44 non-repetitive *S. aureus* isolates, 11.4% ($n = 5$) were multidrug-resistant (MDR) (resistant to at least three different classes of antimicrobial agents);

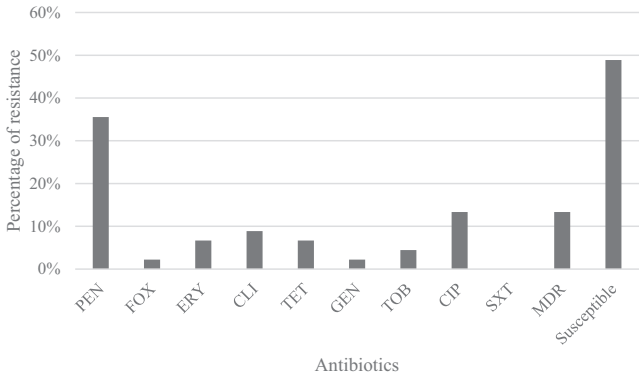

Figure 1 Antimicrobial resistance rates detected among the 44 *S. aureus* isolates detected of poultry meat samples. CIP, ciprofloxacin; CLI, clindamycin; ERY, erythromycin; FOX, cefoxitin; GEN, gentamicin; MDR, multi-drug resistant; PEN, penicillin; SXT, trimethoprim-sulfamethoxazole; TET, tetracycline; TOB, tobramycin.

while 50% (22/44) of isolates were susceptible to all the antimicrobial agents tested. Figure 1 shows the antimicrobial resistance percentages detected among the 44 *S. aureus* isolates. Resistance to linezolid and chloramphenicol was not found among the *S. aureus* isolates.

The antimicrobial resistance mechanisms detected for each antibiotic were (gene/n° of isolates): penicillin (*blaZ*/16), cefoxitin (*mecA*/1), erythromycin (*mrs*(A)/2), erythromycin-clindamycin (*erm*(A)/1; *erm*(B)/1; *erm*(C)/4; *erm*(T)/2; *erm*(43)/1), clindamycin (*lnu*(A)/2, *lnu*(B)/1), gentamicin-tobramycin (*aac*(6′)-Ie-*aph*(2″)-Ia/1) tobramycin (*ant*(4′)-Ia/1), and tetracycline (*tet*(K)/2; *tet*(M)/2).

One *mec*A-carrying MRSA isolate was detected carrying the SCC*mec* type V. Moreover, this isolate was MDR and presented resistance to tetracycline, gentamicin, tobramycin, streptomycin, and ciprofloxacin.

Molecular characterisation of *S. aureus* isolates

A high genetic diversity was detected among the *S. aureus* isolates, with the identification of 18 different *spa*-types (t002, t084, t105, t148, t230, t304, t491, t504, t548, t1204, t1451, t1901, t2162, t2461, t3047, t3478, t7392, t21532), being the most frequent: t304 (20.4%), t3478 (20.4%), t148 (9.1%), and t1451 (6.8%). Different *spa*-types were detected in isolates of the same sample in 10 of the 28 positive samples. In this respect, between 3 and 4 different *spa*-types were detected in isolates of three of the samples while two *spa*-types were identified in isolates of the remaining eight samples (Table S3).

The *S. aureus* isolates were ascribed to 11 sequences types (STs) belonging to eight clonal complexes (CCs).

Table 1 Molecular characteristics of the 44 *S. aureus* isolates recovered of poultry meat samples

CC	ST	*spa*-type (number of isolates)	*agr*-type	IEC	Resistance phenotype[a,b]	Resistance genotype[a]	Virulence content	Aviar MGEs[c]
5	5	t002 (2)	II	–	–	–	–	Avβ, PAvX, SAPIAv
		t105 (1)	II	F	–	–	–	–
		t504 (1)	II	–	TET	*tet*(M)	*eta*	–
		t548 (2)	II	–	PEN[1], ERY[1], CLI[1], CIP[1]	*blaZ*, *erm*(A), *erm*(C)	–	pAvX[1], Avβ
		t2162 (1)	II	F	–	–	–	–
		t3478 (9)	II	–	CLI[1], CIP[1]	*erm*(C)[1], *lnu*(B)[1]		Avβ[5], PAvX[3], SAPIAv[2]
	6	t304 (9)	I	–	PEN[1], CIP[1]	*blaZ*[1]	–	pAvX[1], Avβ[1]
		t21532 (1)	I	–	–	–	–	–
7	7	t1204 (2)	I	G	PEN	*blaZ*	–	–
8	72	t148 (4) t2461 (1)	I	B	PEN	*blaZ*	–	–
	789	t1901 (1)	I	G	PEN, TET, TOB, CIP	*blaZ*, *tet*(K), *ant*(4′)-Ia	–	–
15	15	t491 (1)	II	–	–	–	–	–
	582	t084 (1)	II	B	–	–	–	–
22	22	t7392 (2)	I	B	PEN	*blaZ*	–	–
45	45	t230 (2)	I	B	PEN	*blaZ*	–	–
133	133	t3047 (1)	I	–	CIP	–	*tst*	–
398	398	t1451 (3)	I	C[2]/–[1]	PEN, FOX[1], TET[1], ERY[2], CLI[2], GEN[1], TOB[1], CIP[1]	*blaZ*, *mecA*[1], *tet*(K)[1], *tet*(M)[1], *mrs*(A)[2], *erm*(B)[1], *erm*(C)[2], *erm*(T)[2], *erm*(43)[1], *lnu*(A)[2], *aac*(6′)-Ie-*aph*(2″)-Ia	–	–

[a]A number in superscript reflects when not all isolates of the group have the referred characteristic.
[b]CIP, ciprofloxacin; CLI, clindamycin; ERY, erythromycin; FOX, cefoxitin; GEN, gentamicin; PEN, penicillin; SXT, trimethoprim-sulfamethoxazole; TET, tetracycline; TOB, tobramycin.
[c]Mobile Genetic Elements.

Most of them belonged to CC5 (59.1%), followed by CC8 (11.4%), CC398 (6.8%), and CC45 (6.7%) (Table 1). The *agr* allotypes I (60%) and II (40%) were detected among the *S. aureus* collection. Three isolates were assigned to the clonal complex CC398 and all of them presented the *spa*-type t1451. They were obtained of 3/60 samples (5%); one of them was MRSA, and the other two were MSSA. All CC398 isolates, except MRSA strain X3013, were erythromycin-resistant/clindamycin-inducible resistant, and carried the gene *erm*(T) (Table 2).

Virulence gene content

None of the isolates carried either the Panton-Valentine leucocidin (PVL)-toxin genes or the exfoliative genes *etb* or *etd*. One isolate was positive for the *tst* gene (ST133/CC133) and other isolate for *eta* gene (ST5/CC5; Table 1).

The gene *scn* (marker to the IEC system) was identified in 29 isolates (Table 1 and Fig. 2). All CC5 isolates were *scn* negative, except 2 isolates with *spa*-types t105 and t2161. Some IEC negative isolates belonging to the clonal lineages CC15, CC133 and CC398 were also identified (Fig. 2). In the case of CC398 isolates, the MRSA CC398 was negative for IEC while the two MSSA CC398 presented IEC type C (Table 2). Among the IEC positive isolates, the following IEC combinations were identified (IEC type/ %): IEC-B/20.4%, IEC-G/6.8%, IEC-C/4.5%, and IEC-F/4.5%. IEC types A, D, and E were not detected among our isolates (Table 1).

Avian-specific MGEs were detected in 15 *S. aureus* isolates, all of them belonging to the lineage CC5. Plasmid pAvX was found by PCR and sequencing in eight *S. aureus* isolates, phage φAvβ in 10 isolates, and four isolates harboured the staphylococcal pathogenicity island SaPIAv. Three isolates had combinations of two MGEs, and two isolates carried the three tested MGEs (Table 1 and Fig. 2).

Discussion

Staphylococcus aureus is a common contaminant in poultry meat and is considered an opportunistic pathogen in human medicine (Murray *et al.*, 2017). It is known that *S. aureus* isolates of animal origin can be transmitted to humans by direct contact with the animal, by human-to-human spread (from those associated with farms to the rest of the community), or by

Table 2 Characteristics of the *Staphylococcus aureus* CC398 isolates detected in poultry meat samples

Strain	ID meat sample number	Methicillin resistance	ST-*spa*-type	*agr*-type	IEC	Resistance phenotype[a]	Resistance genotype
X3013[b]	7	MRSA	ST398-t1451	I	–	PEN, FOX, TET, GEN, TOB, CIP	*blaZ, mecA, tet*(K), *tet*(M), *aac*(6′)-Ie-*aph* (2″)-Ia
X3417	29	MSSA	ST398-t1451	I	C	PEN, ERY, CLI[c]	*blaZ, mrs*(A), *erm*(C), *erm*(T), *lnu*(A)
X3427	30	MSSA	ST398-t1451	I	C	PEN, ERY, CLI[c]	*blaZ, mrs*(A), *erm*(C), *erm*(B), *erm*(T), *erm*(43), *lnu*(A)

[a]CIP, ciprofloxacin; CLI, clindamycin; ERY, erythromycin; FOX, cefoxitin; GEN, gentamicin; PEN, penicillin; TET, tetracycline; TOB, tobramycin.
[b]This strain contained the gene *mecA* in the SCC*mec* type V element.
[c]Inducible.

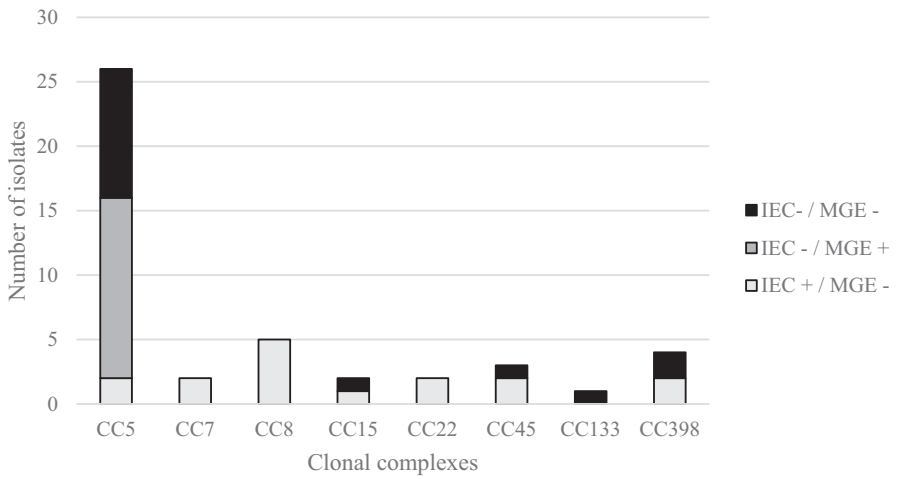

Figure 2 Clonal lineages in relation to presence/absence of Immune Evasion Cluster (IEC) and avian mobile genetic elements (MGEs) in the 44 *Staphylococcus aureus* isolates recovered of poultry meat samples.

exposure or ingestion of contaminated food (Bortolaia *et al*., 2016). Foodborne transmission can affect a larger number of people through the consumption and handling of contaminated meat, an example being cross-contamination in the kitchen.

LA-MRSA transmission has been broadly studied in pig farm environment and it has been indicated that the greatest zoonotic risk is posed for farmers, veterinarians, and slaughterhouse workers through close contact with animals (Quero *et al*., 2023). However, there is less information about the prevalence of *S. aureus* and MRSA and their genetic lineages in the poultry industry. It is very necessary to monitor this problem taking into consideration that there has been a significant increase in chicken meat production in recent decades. Chicken meat accounts for approximately one third of the world's global meat production. Over the last two decades (2000–2020), global chicken meat production has increased significantly, from 25% in 2000 to 35% in 2020. This represents a growth of 104%, making it the most produced type of meat in 2020 with an increase of approximately 61 million tonnes. Moreover, Spain is one of the leading European countries in poultry production (fourth in poultry meat and third in egg production) and one of the major poultry products exporting countries (FAO, 2022). Currently, there are 5046 poultry farms in Spain (data of July 2024), most of them (91%) correspond to production farms, being the remaining 6% to multiplication, 2% to breeding and 1% to selection farms (Ministerio de Agricultura, Pesca y Alimentación of Spain, 2024).

In this work, the presence of *S. aureus* was detected in 47% of the chicken meat samples analysed (28/60), similar to that described in other studies with samples of the same origin. In Libya, *S. aureus* was detected in 40% of chicken meat samples, while in chicken meat products, the percentage of occurrence decreased to 30% (Naas *et al*., 2019). In another recent study in Serbia, *S. aureus* was detected in 90% of the chicken

meat samples (Lika *et al.*, 2021), a higher presence than that detected in our study, but in line with what has been detected in Japan over the years, where different studies evidenced the presence of *S. aureus* in 65–80% of the chicken meat samples that they analysed (Kitai *et al.*, 2005; Hiroi *et al.*, 2012). In 2023, a new study in Egypt detected the presence of *S. aureus* in 22% of the chicken burger, fillet, luncheon, nuggets, and panne samples tested (Morshdy *et al.*, 2023) in line with the results obtained in a similar study in Iran where *S. aureus* was found in 24% of the samples (Jafarzadeh *et al.*, 2023).

A single MRSA isolate (1.6%) was found in our study carrying the *mecA* gene for methicillin resistance, similar to the 4% found in other studies in Denmark (Tang *et al.*, 2017; Li *et al.*, 2019), but remarkably lower than the 30% of MRSA detected in Nigeria in 2023 (Igbinosa *et al.*, 2023).

Although the rate of MRSA has been low, the dissemination of MRSA through food is an issue of great relevance due to the strong impact it can have on public health. Moreover, 11.4% of our isolates showed a MDR phenotype, the possible spread of these isolates through the food chain being highly worrying.

Different CCs were detected, with CC5 being the most abundant (59.1%), followed by CC8 (13.6%), and CC398 (6.8%). Isolates of the CC5 are common in human infections (Lowder *et al.*, 2009). The presence of human lineages in poultry meat indicates that slaughterhouses and meat handling are critical points where contamination can occur. However, the clonal lineage CC5 is also associated with livestock animals, and mainly with poultry. Host jumping of *S. aureus* CC5 from human to avian has been detected, facilitated by direct contact between both hosts. In the process of adaptation, it seems that CC5 isolates have lost genes related to human adaptation (IEC system) and have acquired genetic elements that allow them to adapt to the new avian host (Lowder *et al.*, 2009; Salgueiro *et al.*, 2020). MGEs genes related to avian adaptation were detected in 15 of our *S. aureus* isolates, all of them of the lineage CC5 and representing 60% of CC5 isolates.

Three *S. aureus* isolates (6.8%) belonged to CC398, one of them being MRSA and the remaining MSSA. Several genomic studies have been focused on knowing the possible origin of the clonal lineage CC398. MSSA CC398 (named human-clade), has been considered as an animal-independent lineage adapted to humans, characterised by methicillin susceptibility and presence of the human IEC system and frequently carrying the erythromycin-clindamycin inducible (ERY-CLI^{Ind}) resistance gene *erm*(T). This genetic lineage seems to have evolved into MRSA CC398 (animal-clade) due to the acquisition of methicillin (mediated by *mecA* gene) and tetracycline (by *tet*(M) gene) resistance, in addition to the loss of the φSa3 prophage containing the IEC cluster (Tegegne *et al.*, 2022). In our study, MSSA CC398 isolates possessed the IEC type C, in addition to exhibiting the characteristic ERY-CLI^{Ind} resistance phenotype, mediated by the *erm*(T) gene. The MRSA-CC398 isolate did not carry IEC, however, it harboured the *mecA*, *tet*(K) and *tet*(M) genes, in addition to the SCC*mec*-type V, characteristic of the animal-clade. Some *spa*-types have been associated with human- or animal-clades of CC398. While the *spa*-type t571 is widely found in MSSA CC398 isolates (human-clade; Bouiller *et al.*, 2020), the *spa*-type t011 is more related to MRSA-CC398 isolates (animal-clade; Tang *et al.*, 2017). In our study, all CC398 (MRSA and MSSA) isolates belonged to the *spa*-type t1451. This *spa*-type has been previously found in pig-derived food MRSA isolates (Mama *et al.*, 2020), as well as in MRSA or MSSA isolates originating from pigs (Santos *et al.*, 2020), pets (Gómez-Sanz *et al.*, 2013; Tegegne *et al.*, 2022) and cattle (Tegegne *et al.*, 2022). Moreover, t1451-isolates have also been found in MRSA and MSSA isolates of human origin causing infections (Lozano *et al.*, 2011; Pérez-Moreno *et al.*, 2017; Mama *et al.*, 2021).

Regarding the second most prevalent CC found in our study, CC8 (13.6%), this clonal lineage is commonly related to CA-MRSA infections (Harada *et al.*, 2018). In this regards, MSSA CC8 isolates are equally common agents of diseases (Bowers *et al.*, 2018; Crandall *et al.*, 2020). All our CC8 isolates were methicillin susceptible and contained the *scn* gene which indicate a possible human origin of these isolates. The lineage CC45 has been previously described in chicken (Benito *et al.*, 2014) and pig meat samples in our country (Benito *et al.*, 2014; Mama *et al.*, 2020). In those studies, CC45 isolates carried the IEC types B or D (Benito *et al.*, 2014; Mama *et al.*, 2020). Our CC45 isolates also presented the IEC type B, suggesting their human origin and a possible contamination by meat handling.

The *scn* gene was absent in 65.9% of the isolates. The lack of the IEC system is related to the possible animal origin of the isolates. In our study, the IEC-negative isolates belonged to different CCs (CC5, CC15, CC133, and CC398). In this sense, CC133 is a clonal lineage that has been also associated with livestock animals and it has been widely described in ruminants (Gharsa *et al.*, 2012; Sheet *et al.*, 2019). In our study, one isolate belonged to CC133 and was IEC-negative. However, this MSSA isolate contained the *tst* gene. The presence of this virulence gene in this clonal complex has been described previously (Romanò *et al.*, 2020; Shittu *et al.*, 2021).

It has been observed that *S. aureus* transmission from animals to humans through the food chain as well as contamination of meat products by handling of

personnel can take place in the food industry (de Mesquita Souza Saraiva *et al.*, 2022). In this study, raw meat food products were analysed. Raw chicken meat should be cooked before consumption, which will limit the possible risk for the consumer. However, food handlers of these raw samples could be colonised and/or infected by these *S. aureus* isolates and could facilitates its spread. Food animals are recognised as major reservoirs of antibiotic-resistant bacteria, playing a significant role in the current global challenge of antimicrobial resistance. The spread of antimicrobial resistance knows no geographic boundaries, making it a worldwide concern. Molecular typing techniques can help us to know the possible human or animal origin of antimicrobial resistant isolates and can help to establish correct prevention measures. Adopting a One Health approach is crucial for ensuring food safety, effectively combating infectious diseases, and limiting the emergence and spread of antibiotic resistance. Finally, it should not be forgotten that avian staphylococcosis can cause lesions that lead to important economic losses due to decreased production (Andresen, 2020).

Conclusion

CC5 is a genetic lineage well adapted to poultry, having been identified in most of *S. aureus* isolates of the poultry samples and in many of these isolates have been detected avian adaptation genes. In addition, poultry meat has been shown to be a vehicle for MRSA- and MSSA-CC398-t1451 isolates showing characteristics of the animal- and human-clade, respectively. MRSA CC398 usually shows a multi-resistance phenotype, so its transmission through the food chain should be monitored. The detection of isolates containing virulence genes such as *eta* and *tst* genes may be a risk mainly for food handlers.

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Author contributions

Paula Eguizábal: Investigation; methodology; writing – original draft; formal analysis. **Rosa Fernández-Fernández:** Methodology; writing – review and editing. **Allelen Campaña-Burguet:** Methodology; writing – review and editing. **Carmen González-Azcona:** Methodology; writing – review and editing. **Irene Marañón-Clemente:** Methodology; writing – review and editing. **Carmen Tenorio:** Methodology; writing – review and editing. **Carmen Torres:** Conceptualization; investigation; funding acquisition; writing – review and editing; formal analysis; supervision. **Carmen Lozano:** Conceptualization; investigation; writing – review and editing; formal analysis; supervision; writing – original draft.

Ethical approval

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Primer pairs and PCR conditions used for the molecular typing and detection of antimicrobial resistance genes, and virulence determinants in the *S. aureus* isolate.

Table S2. Reactives and concentration of the PCR.

Table S3. *S. aureus* isolates detected in the 60 poultry samples.