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## Chapter

# *Staphylococcus aureus* in the Meat Supply Chain: Detection Methods, Antimicrobial Resistance, and Virulence Factors

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## Abstract

*Staphylococcus aureus* (*S. aureus*) can cause a wide variety of infections in humans, such as skin and soft tissue infections, bacteremia, pneumonia, and food poisoning. This pathogen could be carried on the nares, skin, and hair of animals and humans, representing a serious problem at the hospital and the community level as well as in the food industry. The pathogenicity of *S. aureus* is given by bacterial structures and extracellular products, among which are toxins, which could cause staphylococcal diseases transmitted by food (SFD). *S. aureus* has the ability to develop resistance to antimicrobials (AMR), highlighting methicillin-resistant strains (MRSA), which have resistance to all beta-lactam antibiotics, except to the fifth-generation cephalosporins. Methicillin resistance is primarily mediated by three mechanisms: production of an altered penicillin-binding protein PBP2' (or PBP2a), encoded by the *mecA* gene; high production of  $\beta$ -lactamase in borderline oxacillin-resistant *Staphylococcus aureus* (BORSA); and mutations in the native PBPs, called modified *S. aureus* (MODSA). Emerging strains have been isolated from meat-producing animals and retail meat, such as MRSA, MRSA ST398 (associated with livestock), multidrug-resistant (MDR) *S. aureus*, and enterotoxin-producing *S. aureus*. Therefore, there is a risk of contamination of meat and meat products during the different processing stages of the meat supply chain.

**Keywords:** meat-producing animals, raw meat, antimicrobial resistance (AMR), methicillin-resistant *S. aureus* (MRSA), livestock-associated methicillin-resistant *S. aureus* (LA-MRSA), multidrug-resistant (MDR), enterotoxins, *mecA* gene

## 1. Introduction

In animal production, the emergence and the spread of antimicrobial-resistant pathogens have been associated with the misuse or overuse of antibiotics [1]. Those pathogens or the genes associated with antimicrobial resistance (AMR) could enter into the food supply chain through the food-producing animals and food handlers [2] and be transmitted to humans, threatening the effective treatments of infectious diseases [3].

*Staphylococcus aureus* has the ability to develop resistance to many commonly used antimicrobials. The first resistant *S. aureus* strains were isolated 2 years after

the introduction of penicillin; in this case the mechanism of resistance was the production of the enzyme  $\beta$ -lactamase. Subsequently, in 1959, the antibiotic methicillin was introduced, and the first strain of methicillin-resistant *S. aureus* (MRSA) was clinically identified in 1960 [4]. These strains are resistant to penicillins, cephalosporins, and all  $\beta$ -lactam antibiotics, except ceftaroline and ceftobiprol.

Methicillin resistance is caused primarily by three mechanisms. The classical mechanism implies the production of an altered penicillin-binding protein, PBP2' (also called PBP2a), which is encoded by the *mecA* gene. This protein has a lower affinity for  $\beta$ -lactam antibiotics, resulting in normal cross-linking of peptidoglycan strands during bacterial cell wall synthesis [5]. Currently, new *mecA* gene homologs have been described, such as *mecB*, *mecC*, and *mecD*, which may not be detected by conventional methods [6–8]. The borderline oxacillin-resistant *S. aureus*: (BORSA) is other mechanism in which the resistance to oxacillin is mediated by an increase of the  $\beta$ -lactamase production. The third mechanism is exhibited by modified *S. aureus* (MODSA), in which the resistance to methicillin is a consequence of modifications in their native PBPs, apparently by accumulation of mutations in the transpeptidase domains [9].

Different clones of MRSA have been recognized, such as health care-associated MRSA (HA-MRSA) [10], community-associated MRSA (CA-MRSA) [11], and livestock-associated MRSA (LA-MRSA) [12].

This pathogen can cause different diseases, such as skin and soft tissue infections, bacteremia, pneumonia, and food poisoning [13, 14].

*Staphylococcus aureus* can colonize the nares, skin, and hair of animals and humans [15]. The transmission can occur either through direct contact with infected animals or humans or with asymptomatic carriers [16]. In addition, MRSA strains have been isolated from different animals, such as pigs, cattle, and poultry [1, 17, 18] and from retail raw meat [19, 20]. In recent years, raw meat has been considered as an important means by which people who have no contact with livestock can be colonized with *S. aureus* from animals, therefore, act as a vehicle of transmission of this bacteria [21].

Moreover, multidrug-resistant (MDR) *S. aureus* strains have been detected in animals and meat [20, 22, 23], and MRSA serotype (ST) 398 has been detected primarily in pigs associated also with infections in humans [12, 24].

The food poisoning is caused by eating foods contaminated with heat-stable enterotoxins produced by *Staphylococcus aureus*. Enterotoxin-producing *S. aureus* strains have been isolated from different food samples [23, 25, 26].

Therefore, the ability of *S. aureus* to colonize humans and animals and the detection of MRSA, MDR, enterotoxin-producing, and other emerging *S. aureus* strains in meat-producing animals and retail meat have increased the concern about the spread of those strains into the food supply chain [23, 26, 27]. At present, the international trade of products of animal origin is an important aspect to consider in the global dissemination of this pathogen. Thus, the countries have different regulations that tend to achieve a high level of food safety, in order to protect the health of consumers [28].

The aim of this chapter is to provide information about the detection, prevalence, characteristics, molecular typing, antimicrobial susceptibility, and the mechanisms of antimicrobial resistance of *Staphylococcus aureus* strains isolated from the meat supply chain.

## **2. Methods of detection and identification of *Staphylococcus aureus* and methicillin-resistant *Staphylococcus aureus* (MRSA) in animals and meat**

Different culture methods have been used to detect *S. aureus*, and although conventional microbiological procedures are laborious, they are still considered standard methods for the detection and confirmation of the presence of *S. aureus*.

The test API® Staph has been shown to be a reliable method for phenotypic characterization, as other methods have had a lower precision [29]. In addition, the biochemical identification of *S. aureus* using the Sensititre™ automated system had a 100% agreement with the PCR technique by the detection of the 16S rRNA-encoding gene [20], using two selective enrichment steps preceding plating in selective agars, which seems to enhance the detection rate of MRSA [27].

According to Kateete et al. [30], there is no only phenotypic test (including the coagulase test) that can guarantee reliable results in the identification of *Staphylococcus aureus*.

In the past decades, methodologies, such as phage typing and genotyping were used. However, these techniques have disadvantages since they are time-consuming and can only be performed in specialized laboratories by trained professionals. Nowadays, more simple and precise techniques are being used, such as the detection by PCR technique, which has been used as the “gold standard” method to identify pathogens. *Staphylococcus aureus* could be confirmed by the detection of the *nuc* gene, which encodes a species-specific extracellular thermostable nuclease protein of *S. aureus*. Brakstad et al. [31] demonstrated that the detection of the *nuc* gene allows the identification of 100% of the isolates of *S. aureus*, using less than 0.69 pg of chromosomal DNA or 10 bacterial CFU cells. In the study carried out by Velasco et al. [23], an agreement of 75% between the biochemical test API® Staph and the PCR technique (detection of *nuc* gene) was determined in confirmation of *S. aureus*. A higher agreement could be reached considering a criterion of a higher probability of detection in API® Staph test.

In relation to the detection and identification of MRSA, there are different methods that have been used, mainly, in clinical laboratories. Among these tests one can mention the determination of minimum inhibitory concentrations (MIC) (dilution in agar or dilution in broth and Etest), oxacillin detection agar (OSA) [32–34], and detection of the protein PBP2' by the latex agglutination test [32, 35, 36]. This last test has an accuracy as high as the PCR method and greater than susceptibility testing method to confirm MRSA [37]. Currently, ceftiofur, a potent inducer of the *mecA* gene regulatory system, is used for the detection of heterogeneous MRSA populations [38]. Rostami et al. [39] compared the sensitivity and specificity of phenotypic reactions with the molecular detection of methicillin resistance. For the ceftiofur disk diffusion test, 100% sensitivity and specificity was obtained. In contrast, the disk oxacillin was 91.7 and 92.8%, respectively. The authors conclude that in the absence of molecular techniques, the ceftiofur disk is the best detector of MRSA, in accordance with the recommendation given by the CLSI [38].

The isolation and identification of *S. aureus* and MRSA, including selective enrichment and plating, followed by confirmation using biochemical testing and/or PCR assays, require 3–7 days approximately [20, 27, 40]. Therefore, the development of a rapid method for detection has become an important need in the microbiological analysis of samples especially when there is a potential risk of exposure for humans.

Real-time PCR technology has been used as an alternative to culture methods for the rapid detection of *S. aureus* and MRSA. However, most studies have been applied in clinical samples, and a few studies have used real-time PCR for the detection of MRSA in animals [35, 41] and meat [27, 36, 42].

The real-time PCR assay carried out by Velasco et al. [43] used a primary and a secondary enrichment of samples from meat-producing animals and retail raw meat in order to detect *S. aureus* and MRSA.

**Table 1** shows the agreement between the detection of *S. aureus* obtained by real-time PCR using primary and secondary enrichments compared with a conventional culture/PCR method.

Comparison within each sample type	No. samples	No. positive by culture/ PCR method	No. (%) of samples*			kappa statistic
			Positive agreement (sensitivity)	Negative agreement (specificity)	Total agreement	
Real-time PCR first enrichment						
Animals	77	32	32 (100.0)	34 (75.6)	66 (85.7)	0.72
Meat	112	58	52 (89.7)	42 (77.8)	94 (83.9)	0.68
Deli meat	45	5	4 (80.0)	40 (100.0)	44 (97.8)	0.88
Real-time PCR second enrichment						
Animals	77	32	32 (100.0)	36 (80.0)	68 (88.3)	0.77
Meat	112	58	52 (89.7)	46 (85.2)	98 (87.5)	0.75
Deli meat	45	5	5 (100.0)	26 (65.0)	31 (68.9)	0.29

\*Positive agreement: number positive as the denominator. Negative agreement: number negative as the denominator. Total agreement: the sum of the positive and negative agreement divided by the total sample size within each sample type  
Data from Velasco et al. [43].

**Table 1.**

Raw agreement indices among conventional culture/PCR method and real-time PCR assay, with two-step enrichment procedure for the detection of *S. aureus* from animals and retail meat.

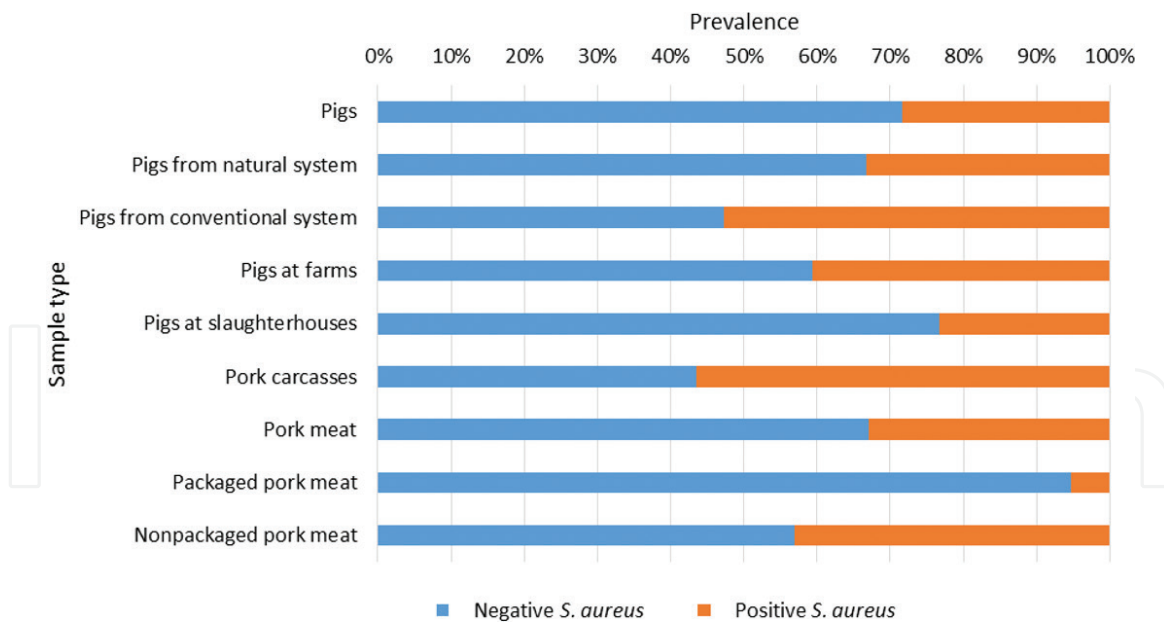
The kappa statistic for detection of *S. aureus* using the primary enrichment in real-time PCR was 0.68–0.88 (**Table 1**), which indicates a good agreement (substantial to almost perfect agreement) with the conventional culture/PCR method. Using the secondary enrichment and real-time PCR, the kappa statistic for detection of *S. aureus* was 0.29–0.77, resulting in a fair agreement when deli meat was tested. This is due to the significantly higher recovery of *S. aureus* from the secondary enrichment samples by real-time PCR. This observation suggests that small concentration of *S. aureus* could be missed when the primary enrichment alone is used in real-time PCR and that the recovery of potentially injured or nonviable strains appears to be enhanced when a secondary enrichment is used. Therefore, including a secondary selective enrichment step could improve the odds of detection of *S. aureus*.

The total agreement on the detection of the *mecA* gene between the real-time PCR using primary and secondary enrichment compared with a conventional culture/PCR method ranged from 86.7 to 98.7%. The kappa statistic for both enrichments in real-time PCR was 0–0.49. The  $k = 0$  indicates no agreement beyond that expected by chance, because the real-time PCR assay detected the *mecA* gene probably from bacteria other than *S. aureus*. This may be due to the fact that either coagulase-negative staphylococci or non-*S. aureus* species can also carry the *mecA* gene [44–46]. In this study, the DNA extraction was carried out from selective enrichments, which could contain DNA from other species that may carry the *mecA* gene.

The real-time PCR assay can decrease the total time for detection of *S. aureus* and the presence of the *mecA* gene in animal and meat samples. Using the two-step selective enrichment, the total time was <2 days by the real-time PCR method, compared with a total time of 6–7 days using the conventional/culture method. However, the presence of MRSA should be confirmed by a phenotypic and genetic method.

## 2.1 Prevalence of *Staphylococcus aureus* strains in the meat supply chain

**Figure 1** shows the prevalence of *S. aureus* in the pork meat supply in a study carried out in Chile [23]. The overall prevalence of *S. aureus* was 33.9%, with a higher prevalence on carcasses (56.5%) than pigs and pork meat ( $P \leq 0.05$ ).



**Figure 1.** Prevalence of *S. aureus* in the meat supply chain in Chile. Data from Velasco et al. [23].

The type of production system, natural or conventional, did not affect the prevalence ( $P > 0.05$ ). A higher prevalence of *S. aureus* might be expected in conventional pig production system than natural pig-farming system, due to a higher risk of spread of microorganisms between pigs by direct contact when animals are confined in a limited indoor area [47]. In addition, naturally raised pigs spend time outdoor and have access to larger pen areas, which can reduce infection intensity [48].

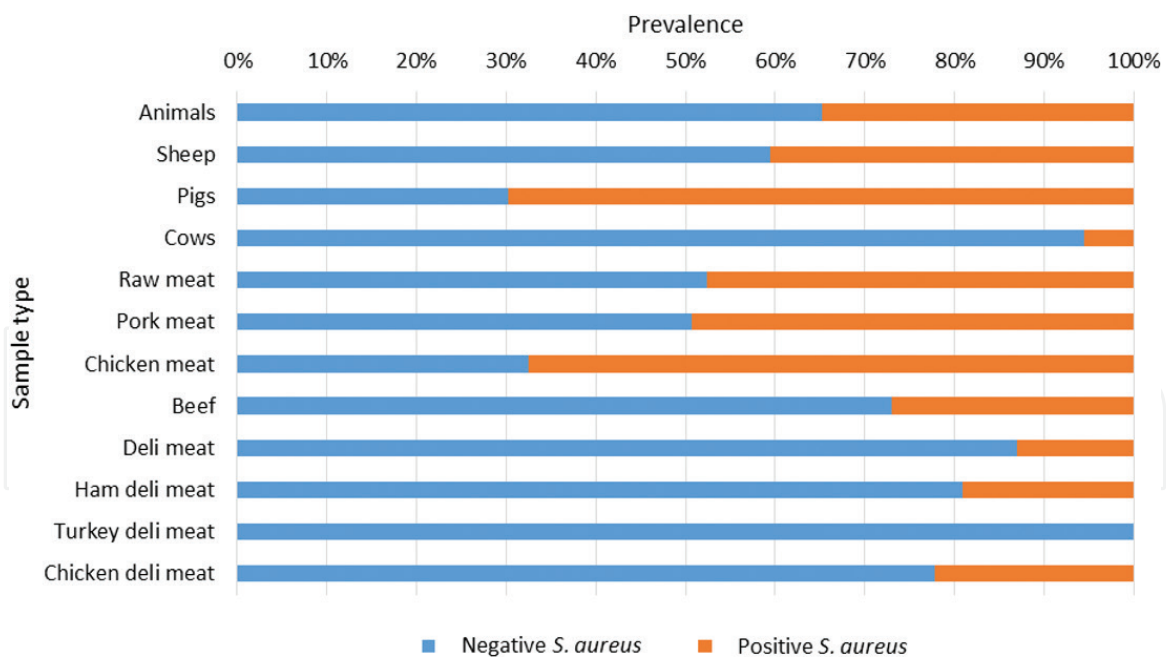
A higher prevalence of *S. aureus* was found in pigs sampled at farms (40.6%) than in pigs sampled at slaughterhouses (23.3%) ( $P \leq 0.05$ ). It might be thought that the prevalence of *S. aureus* in animals could be higher in slaughterhouses due to the risk of transmission during transportation or in resting pens, where animals from different herds could have contact [1, 49, 50]. In this study, nasal and skin swabs were taken after the stunning; however, live animals were rinsed by shower to remove external solid waste before the entrance to the process, which could reduce the impurities in the skin.

As expected, non-packaged meat was more contaminated (43.1%) than packaged meat (5.3%) ( $P \leq 0.05$ ), since non-packaged meat is more exposed to bacterial contamination, during processing and commercialization in meat counter at supermarkets and retail stores.

A higher prevalence of *S. aureus* in pigs and pork meat has been determined in other studies, with values ranging from 45 to 65% [20, 51, 52]. However, Tanih et al. [53] detected a prevalence of *S. aureus* in carcasses around 13.0%, which is much lower than the prevalence found in this study.

In addition, the *mecA* gene and the protein PBP2' were not detected in any sample from the pork meat supply. However, three *mecA*-negative *S. aureus* strains exhibited resistance to oxacillin and/or cefoxitin and were also negative for the *mecC* gene. Those strains were isolated from a skin, a carcass, and a packaged meat sample.

In a study carried out in Fargo, ND, USA [20], the overall prevalence of *S. aureus* was 37.2%. A prevalence of 34.7% was obtained in animals, with the highest proportion in pigs (50.0%) and sheep (40.6%) ( $P \leq 0.05$ ). A total of 47.6% of raw meat samples were contaminated with *S. aureus*, with the highest prevalence in chicken (67.6%) and pork (49.3%) ( $P \leq 0.05$ ). In deli meat, a prevalence of 13.0% of *S. aureus* was determined (Figure 2). Five pork samples (7.0%) were positive for MRSA.



**Figure 2.** Prevalence of *S. aureus* in the meat-producing animals and retail raw meat in North Dakota. Data from Buyukcangaz et al. [20].

Other studies have detected a higher prevalence of *S. aureus* in sheep (57%) and cattle (14%) [54]; however, the prevalence in pigs has been reported to vary widely (6–57%) [55, 56]. The recovery of *S. aureus* in meat in this study was higher than previous studies (39.2 and 14.4%) [26, 51]. The prevalence of *S. aureus* in ham was 19%, which was considerably lower than the prevalence reported by Atanassova et al. [57].

In this study, MRSA was not detected in animals; however, a prevalence of MRSA in swine ranging from 6 to 71% has been detected previously [55, 58]. In pork meat, the prevalence of MRSA has also been reported to be less than 10% in other studies [27, 51, 52].

### 3. Characterization of *Staphylococcus aureus* isolated from the meat supply chain

#### 3.1 Molecular characterization of *Staphylococcus aureus* strains in meat-producing animals and retail meat

Different molecular techniques have been used for typing *S. aureus* strains, such as pulsed-field gel electrophoresis (PFGE) based on macro-restriction patterns of genomic DNA, multilocus sequence typing (MLST) that determines the allelic profile of seven housekeeping genes, and *spa* typing based on the sequencing of the polymorphic X region of the gene encoding the protein A. A greater discriminatory power has been found with PFGE than MLST, *spa* typing, and *SCCmec* typing [59]. However, a combination of two typing methods may be most accurate for strain differentiation [60]. Conversely, it is not possible to obtain a macro-restriction pattern for ST398 strains by PFGE using the restriction enzyme *SmaI*, since the DNA of those strains cannot be digested with *SmaI*, maybe due to the methylation of the *SmaI*-recognition site caused by a methylation enzyme [61]. There is a *Cfr9I* PFGE, a new tool for studying non-typeable ST398 strains, which use *Cfr9I*: a neoschizomer of the *SmaI* enzyme [62] and specific PCRs for detection of *S. aureus* ST398 [63]. Restriction patterns with the same number of bands represent the same strain, patterns that differ up to three fragments represent strains that are closely

related, and isolates that differ at four to six bands may have the same genetic lineage [64]. Nonetheless, BioNumerics software (applied maths) allows restriction patterns of PFGE images to be normalized and to be compared within and between local laboratories with high reproducibility. The band position tolerance and optimization must be set at 1.0 and 0.5%, respectively, and a similarity coefficient of 80% to define the clusters [65].

Different clones of methicillin-susceptible *S. aureus* (MSSA) and MRSA have been detected in humans, animals, and meat. The most common clones that cause CA-MRSA infections have been identified as USA300 and USA400 and those causing HA-MRSA infections as USA100 and USA200 [66]. Some sequence types (ST) of *S. aureus* strains have been determined, such as ST5, ST8, ST22, ST36, and ST45, among others, associated to HA-MRSA [67], ST30 and ST80 associated to CA-MRSA [68], and ST398 linked with animals [69, 70].

The SCCmec typing is based on the genetic characteristics of a mobile genetic element called staphylococcal cassette chromosome *mec* (SCCmec) that carries the *mecA* gene. The emergence of MRSA is due to the acquisition of the SCCmec element into the chromosome of MSSA strains. SCCmec elements are highly diverse and have been classified into types and subtypes as shown in **Table 2** [4, 71, 72].

The *SmaI* macro-restriction fragment profiles of some *S. aureus* strains isolated from the pork chain supply in Chile are shown in **Figure 3**. The *S. aureus* strains were genetically diverse, identifying only two clusters: ST1 from meat and carcass and ST433 from natural raised pigs. Genetic diversity among *S. aureus* strains of swine origin could suggest different source of contamination at different stages of the pork chain supply.

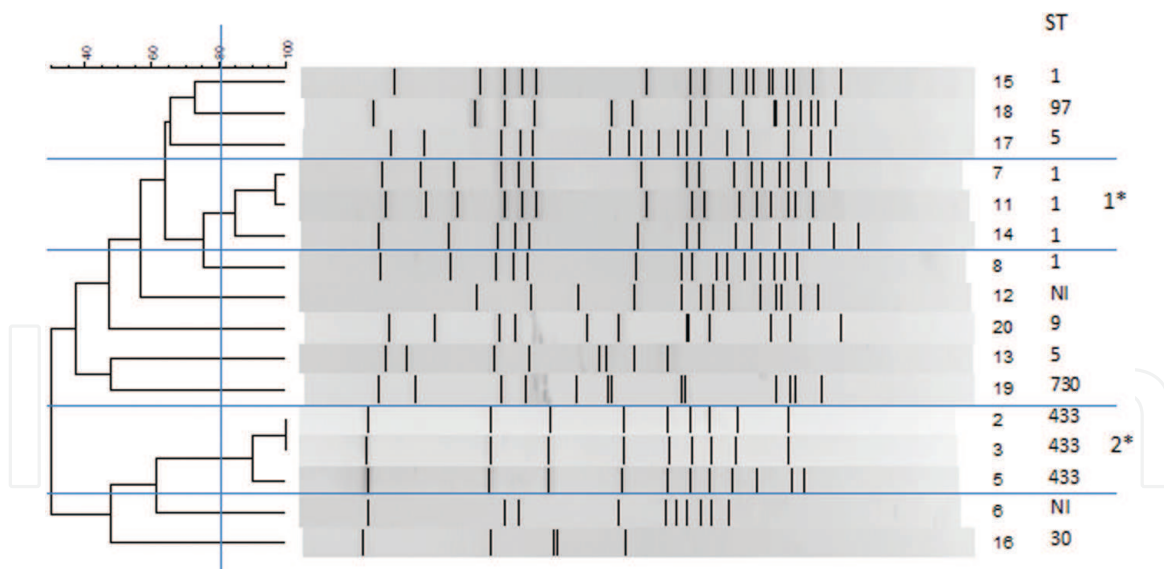
In the study carried out by Buyukcangaz et al. [20], five pork samples were positive for MRSA, of which three were ST398 and two were ST5. The most common clones in sheep were ST398 and ST133, in pigs and pork both ST398 and ST9, and in chicken ST5. The clustering of isolates obtained by PFGE agreed well with the MLST types, i.e., the identical restriction patterns or patterns that differed at two to six bands had an identical ST. A total of 34 *S. aureus* isolated from animals

SCCmec type	<i>mec</i> gene complex	Structure of <i>mec</i> gene complex	<i>ccr</i> gene complex	<i>ccr</i> genes
I	Class B	IS1272- $\Delta$ <i>mecR1-mecA-IS431</i>	Type 1	<i>ccrA1, ccrB1</i>
II	Class A	<i>mecI-mecR1-mecA-IS431</i>	Type 2	<i>ccrA2, ccrB2</i>
III	Class A	<i>mecI-mecR1-mecA-IS431</i>	Type 3	<i>ccrA3, ccrB3</i>
IV	Class B	IS1272- $\Delta$ <i>mecR1-mecA-IS431</i>	Type 2	<i>ccrA2, ccrB2</i>
V	Class C2	IS431- <i>mecA</i> - $\Delta$ <i>mecR1-IS431</i>	Type 5	<i>ccrC1</i>
VI	Class B	IS1272- $\Delta$ <i>mecR1-mecA-IS431</i>	Type 4	<i>ccrA4, ccrB4</i>
VII	Class C1	IS431- <i>mecA</i> - $\Delta$ <i>mecR1-IS431</i>	Type 5	<i>ccrC1</i>
VIII	Class A	<i>mecI-mecR1-mecA-IS431</i>	Type 4	<i>ccrA4, ccrB4</i>
IX	Class C2	IS431- <i>mecA</i> - $\Delta$ <i>mecR1-IS431</i>	Type 1	<i>ccrA1, ccrB1</i>
X	Class C1	IS431- <i>mecA</i> - $\Delta$ <i>mecR1-IS431</i>	Type 7	<i>ccrA1, ccrB6</i>
XI	Class E	<i>blaZ-mecA-mecR1-mecI</i>	Type 8	<i>ccrA1, ccrB3</i>
XII	Class C2	IS431- <i>mecA</i> - $\Delta$ <i>mecR1-IS431</i>	Type 9	<i>ccrA1, ccrC2</i>
XIII	Class A	IS431- <i>mecI-mecR1-mecA-IS431</i>	Type 9	<i>ccrC2</i>

Adapted from Aguayo-Reyes et al. [4], Wu et al. [71], and Baig et al. [72].

**Table 2.**  
 Genetic structure of the different SCCmec types described in *Staphylococcus aureus*.





**Figure 3.**

Dendrogram showing the genetic similarity and sequence types (ST) of *S. aureus* isolates from pork production chain in Chile.

(sheep and pigs) and from pork meat, which were ST398, could not be restricted with *SmaI* or *XmaI* during PFGE analysis. The high prevalence of ST398 indicates a potential risk for humans to acquire this emerging sequence type which has potential for causing infection. The MRSA isolates had the same MLST allelic profile and indistinguishable PFGE patterns than two MSSA strains, all obtained from pork. The close genetic similarity of the MRSA and MSSA isolates may be due to the acquisition of *mecA* gene by horizontal transfer of SCC*mec* from MRSA strains to MSSA lineages [1, 22, 73, 74].

In addition, contamination of meat with *S. aureus* strains from animals and humans could occur during slaughtering or processing. In fact, the genetic relatedness between *S. aureus* strains ST9 from pigs and pork meat may suggest the possible contamination of meat during slaughtering [20], and the genetic similarity between clones isolated from humans and meat suggests the spread of *S. aureus* into the food chain supply [75].

### 3.2 Antimicrobial resistance in *Staphylococcus aureus* from meat-producing animals and meat

Methicillin and other  $\beta$ -lactam antibiotics affect the cell wall synthesis in gram-positive bacteria inhibiting the last stage of the peptidoglycan synthesis called transpeptidation. During the transpeptidation the linkage between N-acetylmuramic acid and the cell wall takes place, catalyzed by transpeptidases and carboxypeptidases, called penicillin-binding proteins (PBPs). These proteins are able to bind penicillin in their active sites through a covalent bond between a serine and the  $\beta$ -lactam ring, resulting in the inhibition of the transpeptidation [76].

Methicillin resistance in *S. aureus* is primarily mediated by the production of an altered penicillin-binding protein, PBP2' (also called PBP2a), encoded by the *mecA* gene, which is carried on the staphylococcal cassette chromosome *mec* (SCC*mec*). This protein has a lower affinity for  $\beta$ -lactam antibiotics, resulting in a normal cross-linking of peptidoglycan strands during cell wall synthesis [77].

Some studies have isolated *S. aureus* strains from humans and livestock that are phenotypically resistant to methicillin, but they do not harbor the *mecA* gene. The phenotypic methicillin resistance has been associated with variations of the *mecA* gene, such as the *mecA*<sub>LGA251</sub> renamed as *mecC* [7, 78], the *mecB* gene [6], and others

that are not as well-known [75]. The *mecC* gene is located on the staphylococcal cassette chromosome *mec* type XI (SSC*mec* XI) and exhibits 70% sequence homology with the *mecA* gene [7, 79, 80]. Additionally, MRSA lacking the *mec* genes (MRLM) may have uncommon phenotypes, such as the  $\beta$ -lactamase hyperproduction (BHP), which partially hydrolyzes the  $\beta$ -lactam ring, usually known as borderline oxacillin-resistant *S. aureus* (BORSA), with an intermediate resistance level to oxacillin [81]. Different nucleotide mutations in *pbp* genes, the *pbp4* promoter, and genes involved in penicillin-binding protein 4 overproduction have also been associated with MRLM, called as modified *S. aureus* (MODSA) [9, 81, 82].

In March 2017, Schwendener et al. [83] reported a new *mec* gene called *mecD*, which confers resistance to all  $\beta$ -lactams antibiotics, including anti-MRSA cephalosporins, ceftobiprole, and ceftaroline. The gene was found in strains of *Macrococcus caseolyticus* isolated from bovines and canines. Alarmingly, the *mecD* gene was in an island of resistance associated with a site-specific integrase, which implies a risk of transmission by horizontal gene transfer to other species.

Other *S. aureus* strains with significant importance have also been detected in the meat supply chain, such as multidrug-resistant (MDR) *S. aureus*, which exhibit resistance to at least three classes of antibiotics [22].

Another mechanism of resistance to  $\beta$ -lactam antibiotics is the production of the enzyme  $\beta$ -lactamase, which hydrolyses the  $\beta$ -lactam ring resulting in the inactivation of the antibiotic. This enzyme is encoded by *blaZ* gene located in a transposon element within a plasmid [84].

**Table 3** shows the resistance profiles of *S. aureus* strains isolated from the pork meat supply chain in Chile. A total of 16 profiles were observed, including 8 profiles

Antimicrobial resistance profile*	No. of subclasses resistant to	No. (%) of all <i>S. aureus</i> isolates with the specific profile		
		Animal N = 28	Carcass N = 12	Meat N = 15
PEN-KAN-ERY-CIP-TET	5	3 (10.7)		
PEN-CEF-KAN-ERY-TET	4	1 (3.6)		
PEN-KAN-ERY-TET	4	1 (3.6)	1 (8.3)	
PEN-ERY-CIP-TET	4	10 (35.7)	1 (8.3)	
PEN-KAN-ERY	3	1 (3.6)		
PEN-ERY-CIP	3	1 (3.6)		
PEN-GEN-QDA	3		1 (8.3)	
PEN-ERY-QDA	3			1 (6.7)
OXA-PEN-CEF-GEN-KAN	2			1 (6.7)
PEN-ERY	2	1 (3.6)		2 (13.3)
PEN-CIP	2	1 (3.6)		1 (6.7)
PEN-QDA	2			1 (6.7)
PEN-TET	2	1 (3.6)		
KAN-ERY	2			1 (6.7)
OXA-PEN-CEF	1		1 (8.3)	
PEN	1	1 (3.6)	7 (58.3)	7 (46.6)
Susceptible to all tested	0	7 (25.0)	1 (8.3)	1 (6.7)

\*OXA, oxacillin; PEN, penicillin; CEF, cefoxitin; GEN, gentamicin; KAN, kanamycin; ERY, erythromycin; CIP, ciprofloxacin; QUI/DAL, quinupristin/dalfopristin; TET, tetracycline [38].

**Table 3.** Antimicrobial resistance profiles of *Staphylococcus aureus* strains isolated from the meat chain supply in Chile.

of MDR (resistance to at least three classes of antibiotics) [26]. The most MDR *S. aureus* strains were isolated from pigs. Rubin et al. [85] determined a significant higher resistance to penicillin, erythromycin, and tetracycline in *S. aureus* of swine origin than other type of animals.

The less effective antibiotic was penicillin. The low effectiveness of penicillin could be due to the enzyme penicillinase that hydrolyzes the  $\beta$ -lactam ring and inactivates the drug [5].

Two *S. aureus* strains were both oxacillin- and ceftiofur-resistant, and one *S. aureus* strain exhibited only ceftiofur resistance. However, those strains were *mecA*- and PBP2'-negative. Currently, the ceftiofur disk diffusion method is used to detect methicillin resistance [38]; it is easier to interpret and has a higher sensitivity

Antimicrobial resistance profile*	No. of subclasses resistant to	No. (%) of all <i>S. aureus</i> isolates with the specific profile		
		Animal (n = 58)	Raw meat (n = 69)	Deli meat (n = 6)
ERY-PEN-TET-LINC-CHL-GEN-CIP-QUI/DAL	8		1 (1.4)	
ERY-PEN-TET-LINC-CHL-CIP-QUI/DAL	7		1 (1.4)	
ERY-PEN-TET-LINC-CHL-STR	6	2 (3.4)		
ERY-PEN-TET-LINC-KAN	5		1 (1.4)	
PEN-TET-LINC-CHL-STR	5	1 (1.7)		
PEN-TET-LINC-GEN	4	1 (1.7)		
PEN-TET-LINC-KAN	4		1 (1.4)	
PEN-TET-LINC-STR	4	2 (3.4)		
ERY-PEN-TET-LINC	4	1 (1.7)	13 (18.8)	
PEN-TET-LINC	3	22 (37.9)	1 (1.4)	
PEN-LINC-STR	3	1 (1.7)		
ERY-PEN-LINC	3		2 (2.9)	
ERY-TET-LINC	3		5 (7.2)	
PEN-LINC	2	4 (6.9)	1 (1.4)	1 (16.7)
PEN-TET	2	12 (20.7)	2 (2.9)	
TET-LINC	2	3 (5.2)		
ERY-LINC	2		3 (4.3)	
ERY-PEN	2		2 (2.9)	
LINC	1	1 (1.7)		
PEN	1	3 (5.2)	10 (14.5)	1 (16.7)
TET	1	3 (5.2)	4 (5.8)	
ERY	1			1 (16.7)
Susceptible to all tested	0	2 (3.4)	22 (31.9)	3 (50.0)

\*CIP, ciprofloxacin; CHL, chloramphenicol; ERY, erythromycin; GEN, gentamicin; KAN, kanamycin; LINC, lincomycin; QUI/DAL, quinupristin/dalfopristin; PEN, penicillin; STR, streptomycin, TET, tetracycline. Data from Buyukcangaz et al. [20].

**Table 4.** Antimicrobial resistance (AR) profiles of *Staphylococcus aureus* and methicillin-resistant *S. aureus* (MRSA) isolates from animals and retail meat.

[86]. Those strains did not harbor the *mecC* gene; therefore, they could carry other variations of the *mecA* gene that are not as well-known [75, 78, 82] or could present uncommon phenotypes such as BORSA [81, 87]. Therefore, the whole genome sequencing is always necessary to understand the mechanism of resistance.

The use of antimicrobial agents in pigs is an important risk factor for increasing the prevalence of MRSA, promoting the selective pressure, and enhancing the emerging and the spread of MRSA [88]. In Holland, a high prevalence of MRSA was detected in pigs, with a resistance to different antibiotics, suggesting the spread of MRSA strains within animals in the slaughterhouses [1].

**Table 4** shows the antimicrobial resistance profiles of the 133 *S. aureus* strains isolated from animals and retail meat in the study of Buyukcangaz et al. [20]. The most common resistance profiles in isolates were penicillin-tetracycline and penicillin-tetracycline-erythromycin, in animals and raw meat, respectively. Most of the *S. aureus* strains isolated from animals exhibited resistance to the same antimicrobials reported by other authors [89, 90]. Other authors have also determined a higher occurrence of resistance to penicillin, tetracycline, and erythromycin in *S. aureus* strains isolated from retail meat and different food samples [26, 91]. Penicillin resistance has been reported to spread rapidly among *S. aureus* strains being facilitated by plasmids and is the most frequently reported resistance detected in foodborne *S. aureus* [26].

The rate of MDR strains was 41.4%, in animals was 51.7%, and in meat 36.2% (n = 25). The MDR isolates were found in pigs, pork, and sheep. MDR isolates from pork were mainly ST398 (60%) and ST9 (30%). All MDR strains from sheep were ST398.

Five pork samples that were MRSA (three ST398 and two ST5) exhibited penicillin resistance and four MDR. In addition, most of the *S. aureus* isolates susceptible to all antimicrobial agents were obtained from chicken, of which 76% were ST5.

The AMR bacteria in animals have increased over time due to the frequent use of antimicrobial agents at the farm level [1, 89]. Therefore, controlling the use of antibiotics in farming could limit the risk of transmission of AMR pathogens among animals and to humans [90].

### 3.3 Characteristics of pathogenicity of *Staphylococcus aureus* strains in meat-producing animals and meat

*S. aureus* produces different virulence factors, including bacterial structures such as capsules and adhesins, and extracellular products, such as enzymes, with activity of coagulase, catalase, hyaluronidase, and toxins such as toxin  $\alpha$ , toxin  $\beta$ , toxin leucocidin, enterotoxin, exfoliative toxin, and toxic shock syndrome toxin. These virulence factors contribute to different stages of infection from adhesion of the pathogen to the surface, to invasion, causing toxic effects, tissue damage, and distal disease. The synthesis of these virulence factors is a highly regulated process, which contributes to the production of the different human or animal diseases [92, 93].

The main regulator of virulence gene expression is the *agr* operon, which functions through a quorum sensing mechanism. The locus is autocatalytic, controlled in a manner dependent on cell density through the production and detection of self-inducing peptides (AIP). The *agr* locus has two divergent transcription units, RNAII and RNAPIII, controlled by their promoters, P2 and P3, respectively [94]. This locus exerts a negative regulation on the adhesin molecules in the colonization stage of the host during the stationary phase. However, when a high load of the autoinducer peptide (*agrD* protein) is reached in the post-exponential growth stage, RNAPIII is activated and inhibits the expression of adhesion proteins, activating the expression

of extracellular enzymes and toxins ( $\alpha$ - $\beta$  hemolysins, lipases, proteases, etc.), virulence factors related to nutrient acquisition, survival and bacterial dissemination [95, 96].

In dairy, one of the main virulence factors is the formation of biofilms, which are structured consortia of bacterial cells that are immersed in a polymeric matrix consisting of polysaccharides, proteins, extracellular DNA (eDNA), lipids, and other macromolecules. The biofilms allow bacteria to adhere to inert or living surfaces, increasing their growth rate and survival in a hostile environment [97].

Enterotoxin-producing *S. aureus* strains may cause gastroenteritis and have a significant importance due to its detection in the meat supply chain. Five classical enterotoxins have been found in *S. aureus*, which are known as SE types (SEA to SEE) encoded by the *se* genes. However, in recent years, new SEs and SE-like toxins have been detected [26]. Since enterotoxins can resist heat treatment and low pH conditions that can easily destroy the bacteria, it is important to highlight the impact of the expression of enterotoxins by *S. aureus* on human health [25]. In the study carried out by Velasco et al. [23], only 1 *S. aureus* strain of a total of 23 strains isolated from pork meat samples was positive for enterotoxin B (SEB) determined by the reversed passive latex agglutination test and for the *seb* gene detected by PCR method. The SEB-producing *S. aureus* strain was isolated from a meat sample obtained from a butcher store and was non-packaged. Therefore, contamination of meat with food-borne *S. aureus* may occur in the meat supply chain, primarily in more exposed food, such as non-packaged meat.

#### 4. Conclusions

*Staphylococcus aureus* is present in the meat supply chain, and some emerging strains, such as MRSA, MRSA ST398, MRLM, MDR, and enterotoxin-producing *S. aureus*, have been detected in animals, meat, and humans.

The genetic similarity between *S. aureus* strains isolated from humans, animals, and meat suggests the potential risk of contamination of meat during processing or handling, the spread of emerging *S. aureus* strains into the food chain, and the potential transmission to humans.

Further research is needed to expand the knowledge and comprehension of the molecular characterization and the different mechanisms of AMR in *S. aureus*.

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#### Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this book chapter.

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