 Chilean J. Agric. Anim. Sci., ex Agro-Ciencia (2022) 38(2):234-240.
DOI: https://doi.org/10.29393/CHJAA38-23ARVP50023

ISSN 0719-3890 online

ANTIBIOTIC-RESISTANCE PROFILE OF *Staphylococcus aureus* STRAINS IN THE PORK SUPPLY CHAIN

Valeria Velasco[1]*, Alejandra Mallea[1], Ana María Bonilla[1], Jorge Campos[1], and Pedro Rojas-García[2]

[1] Universidad de Concepción, Facultad de Agronomía, Departamento de Producción Animal, Av. Vicente Méndez 595, 3812120, Chillán, Chile
[2] Universidad de Concepción, Facultad de Ciencias Veterinarias, Departamento de Ciencia Animal, Av. Vicente Méndez 595, 3812120, Chillán, Chile
* Corresponding author: vvelasco@udec.cl
https://orcid.org/my-orcid?orcid=0000-0002-7190-2672

ABSTRACT

***Staphylococcus aureus* can develop antimicrobial resistance (AMR), which is one of the global health care concerns. It can colonize skin and nares of animals and humans, with the risk of entering the food supply chain. The aim of this study was to determine the antibiotic-resistance profile of *Staphylococcus aureus* strains through the pork supply chain. The epsilon test (Etest) and the disk diffusion method were used to determine the antibiotic susceptibility of fifty-five *S. aureus* strains isolated from pigs (n=28, nasal and skin), carcasses (n=12, surface of carcasses), and meat (n=15, pork chop and leg). Ten antibiotics of seven classes were used to assess the susceptibility of *S. aureus* isolates. An 83.6% of the isolates exhibited AMR, with a higher prevalence in pigs and a high rate of penicillin resistance. Multidrug-resistance (MDR) was detected in 38.2% of the isolates, being PEN-ERY-CIP-TET the most common resistance profile. Strains exhibiting oxacillin- and cefoxitin-resistance were *mecA*-negative, while one strain was identified as vancomycin-intermediate *S. aureus* (VISA). The results confirm that measures to control and mitigate AMR need to be implemented, particularly during the different animal production stages.**

Keywords: Antimicrobial resistance (AMR); multidrug resistance (MDR); *mecA* gene; methicillin-resistant *S. aureus* (MRSA); vancomycin-intermediate *S. aureus* (VISA).

INTRODUCTION

Antimicrobial resistance (AMR) is a global public health concern, which involves human, animal, plant, and environmental health. Overuse and misuse of antibiotics are major contributors to the emergence of antimicrobial-resistant pathogens (FAO, 2020). Antimicrobial-resistant bacteria and genes associated with AMR can be transmitted between humans, animals, and the environment through the food supply chain, which represents a potential exposure route and a risk to public health (Bennani et al., 2020).

One of the pathogens that can develop AMR is *Staphylococcus aureus*, which can cause from mild to life-threatening skin and soft tissue infections in humans (Tong et al., 2015). This pathogen can also cause food poisoning through the production of enterotoxins (Argudín et al., 2010). According to the World Health Organization (WHO), the increasing rates of methicillin-resistant *S. aureus* (MRSA) infection is a major concern worldwide, being classified as Priority 2 (high) in the global priority pathogens list of antibiotic-resistant bacteria (WHO, 2017). The infections caused by MRSA are classified as health-care-associated MRSA (HA-MRSA), community-associated MRSA (CA-MRSA), and livestock-associated

Received: November 9, 2021 Accepted: July 26, 2022

MRSA (LA-MRSA) (Junnila et al., 2020). Methicillin resistance is primarily mediated by the following mechanisms: production of an altered penicillin-binding protein, PBP2′ (also called PBP2a, encoded by the *mecA* gene), which has a lower affinity for β-lactam antibiotics (Alipour et al., 2014); an increase of the β-lactamase production in borderline oxacillin-resistant *S. aureus* (BORSA); and modifications in the native PBPs, apparently by mutations in the transpeptidase domains in modified *S. aureus* (MODSA) (Argudín et al., 2018). *Staphylococcus aureus* can colonize skin, nares, and other mucosal membranes of animals and humans (Haag et al., 2019; Ajoke et al., 2012), and thus can be spread and transmitted to humans through the food supply chain (Bouchami et al., 2020). In fact, Multidrug-resistant (MDR) *S. aureus* and MRSA strains have been detected in food-producing animals, and food of animal origin (Buyukcangaz et al., 2013; Friese et al., 2013; Rajkhowa et al., 2016; Normanno et al., 2020). Pork production has been primarily associated with LA-MRSA ST398 in humans exposed to animals, with clones found in pigs and pork meat (Bouchami et al., 2020; Kim et al., 2020).

One of the most important steps to develop measures to mitigate AMR is to determine the prevalence of antimicrobial-resistant pathogens in different stages of the food supply chain. Therefore, the aim of this study was to determine the antibiotic-resistance profile of *Staphylococcus aureus* strains though the pork supply chain.

MATERIAL AND METHODS

Staphylococcus aureus isolates

A total of fifty-five *S. aureus* strains were isolated from independent samples between August 2015 and January 2016 in central Chile (between the Metropolitan Region and the Ñuble Region). Samples were taken from pigs (n=28, nasal and skin) from four farms and two slaughterhouses, carcasses (n=12, surface of carcasses) from two slaughterhouses, and retail meat (n=15, pork chop and leg) from three supermarkets and seven butcher's shops. *S. aureus* strains were isolated using selective enrichments and culture methods, while confirmation of presumptive strains was carried out by biochemical testing (Api® Staph, bioMérieux, Marcy-l'Etoile, France) and PCR (identification of the *nuc* and *mecA* genes) according to Velasco et al. (2018). Primers used for targeting the *nuc* and *mecA* genes are shown in Table 1. The PCR reactions were carried out in a thermocycler (MultigeneTM OptiMaxc, Labnet International, Inc., Edison, NJ) as follows: 94°C for 10 min (initial denaturation); 40 cycles with 94°C for 1 min (denaturation), 51°C for 1 min (annealing), 72 °C for 2 min (extension); and 72°C for 5 min (final extension).

The protein PBP2′ was determined by latex agglutination test (Oxoid Ltd., Hants, United Kingdom).

Antibiotic susceptibility testing

Ten antibiotics of seven classes were used to assess the susceptibility of *S. aureus* isolates. The epsilon test (Etest) (Liofilchem® SRL, Italy) with strips with gradient concentrations from 0.016 μg L^{-1} to 256 μg L^{-1} was used to determine the minimum inhibitory concentration (MIC) of oxacillin, penicillin, tetracycline, erythromycin, vancomycin, gentamicin, kanamycin, ciprofloxacin, and quinupristin/dalfopristin. The disk diffusion method was used to determine the susceptibility to cefoxitin (disks containing 30 μg).

Briefly, 0.1 mL of bacterial solution (saline solution 0.85% of NaCl) with a concentration of 10^8 CFU mL^{-1} approximately (0.5 McFarland turbidity standard) was uniformly distributed on Mueller-Hinton agar (MHA) plates using a cotton swab. MHA supplemented with 2% NaCl was used to test oxacillin and cefoxitin. The antibiotic strips and disks were placed onto the surface of inoculated plates and incubated at 35 ± 2°C during 24 h, with two replicates.

Reference strains were used as positive or negative controls according to their registered resistance profile: ATCC 43300 (MRSA), ATCC 25923 (*S. aureus*), and ATCC 13076 (*Salmonella enterica* subsp. *enterica*).

The breakpoints were interpreted according to the Clinical Laboratory Standards Institute guidelines (CLSI, 2018).

Table 1 Primers used for the identification of *nuc* and *mecA* genes.

Gene	Primer	Oligonucleotide sequence (5′-3′)	Amplicon size (bp)
nuc	*nuc-1*	GCGATTGATGGTGATACGGTT	279
	nuc-2	AGCCAAGCCTTGACGAACTAAAGC	
mecA	*mecA-1*	AAAATCGATGGTAAAGGTTGGC	533
	mecA-2	AGTTCTGCAGTACCGGATTTGC	

Fuente: Velasco et al. (2018).

Statistical analysis

The Chi-square test was used to determine significance in prevalence of antibiotic-resistant *S. aureus* between sample types only if no more than 20% of the expected counts were less than 5 and all individual expected counts were 1 or greater. On the contrary, Fisher's exact test was used with two-sided P-values. The statistic software Infostat® was used to assess significance (P≤0.05).

RESULTS AND DISCUSSION

An 83.6% (46/55) of the *S. aureus* strains isolated from the pork supply chain exhibited resistance to one or more classes of antibiotics (Table 2). Significant differences (P≤0.05) in prevalence of antibiotic-resistant *S. aureus* were observed between the types of samples for erythromycin, ciprofloxacin and tetracycline, with a greater resistance in animals compared to that observed in carcasses and retail meat. Previous studies have also reported a high prevalence of AMR and MDR *S. aureus* strains in pigs (Buyukcangaz et al., 2013; Rajkhowa et al., 2016). In the present study, a high prevalence in animals was expected because livestock production has been considered as the initial source of AMR in the food supply chain, resulting from the use of antimicrobial agents in animals (Bennani et al., 2020). In Chile, those antibiotics are currently used in pork production only for therapeutic, prophylaxis or methaphylaxis purposes. At the country level, the use of antibiotics as growth promoters in animal production has been banned since 2007 (Res. SAG N°3447, 2006), same as in other countries of the European Union (Regulation (EC) No 1831/2003). However, despite efforts to prevent resistance, misuse or overuse of antibiotics could have caused the emergence of resistant strains isolated today.

The presence of oxacillin- and cefoxitin-resistant *S. aureus* could suppose MRSA strains. However, those strains were *mecA*-, and PBP2'-negative (data not shown). Therefore, other resistance mechanisms could be involved, such as the production of modified PBPs (different *mecA* gene homologues) or an increase in β-lactamase production (Argudín et al., 2018).

Most of the strains isolated in this study (81.8%) were penicillin-resistant. This could be due to the action of the penicillinase enzyme, which hydrolyses the β-lactam ring and inactivates the drug, being the main cause for rendering penicillin useless (Peacock and Paterson, 2015). In addition, resistance to other antibiotics such as erythromycin and tetracycline was common in pigs. In fact, pork production has been associated with major antibiotic-resistant *S. aureus* strains (Bouchami et al., 2020; Kim et al., 2020), and a significantly higher resistance to penicillin, erythromycin, and tetracycline in *S. aureus* of swine origin than other type of animals has been detected (Rubin et al., 2011). Accordingly, the higher resistance to those antibiotics observed in the present study was expected.

Among the penicillin-resistant *S. aureus* strains, 46.7% showed a MIC between 0.5 and 2 μg mL^{-1} (Table 3). For erythromycin and ciprofloxacin, most of the resistant strains had a MIC higher than 256 μg mL^{-1}, and for tetracycline ≥ 32 μg mL^{-1}.

Table 2. Prevalence of antibiotic-resistant *S. aureus* strains isolated from the pork supply chain.

Antibiotics		No (%) resistant *S. aureus* strains							
Sub-Classes	Agents	Animal (n=28)		Carcass (n=12)		Meat (n=15)		Total (n=55)	
Penicilins	PEN	21	(75.0)	11	(91.6)	13	(86.7)	45	(81.8)
	OXA	0	(0.0)	1	(8.3)	1	(6.7)	2	(3.6)
	CEF	0	(0.0)	1	(8.3)	1	(6.7)	2	(3.6)
Macrolides*	ERY	18	(64.3) a	2	(16.6) b	4	(26.7) b	24	(43.6)
Glycopeptides	VAN	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)
Aminoglycosides	GEN	0	(0.0)	1	(8.3)	1	(6.7)	1	(1.8)
	KAN	6	(21.4)	1	(8.3)	2	(13.3)	9	(16.4)
Quinolones**	CIP	15	(53.6) a	1	(8.3) b	1	(6.7) b	17	(30.9)
Streptogramins	QDA	0	(0.0)	1	(8.3)	2	(13.3)	3	(5.5)
Tetracyclines**	TET	17	(60.7) a	2	(16.6) b	0	(0.0) b	19	(34.5)

OXA – Oxacilin, PEN – Penicilin, CEF – Cefoxitin, ERY – Erytromycin, VAN – Vancomycin, GEN – Gentamicin, KAN – Kanamycin, CIP – Ciprofloxacin, QDA - Quinupristin/Dalfopristin, TET – Tetracycline.

*Different letters indicate significant differences between type of samples using Chi-square test (P≤0.05).

**Different letters indicate significant differences between type of samples using Fisher's exact test (P≤0.05).

The MIC provides the concentration of antibiotic required to inhibit the growth of a pathogen. Therefore, determination of the MIC in antibiotic-resistant strains is based on the possibility to choose more effective infection therapies (Kowalska-Krachmal and Dudek-Wicher, 2021). In this study, the MICs of antibiotic-resistant *S. aureus* isolated from the pork supply chain could also be considered for characterization and for the development of future actions to mitigate AMR.

Resistance to vancomycin was not observed. However, one *S. aureus* strain from an animal exhibited a MIC of 4 μg mL^{-1} for vancomycin, which is considered as vancomycin-intermediate-resistant *S. aureus* (VISA) (CLSI, 2018).

Vancomycin has been used as a gold standard to treat severe MRSA and other resistant Gram-positive infections in humans. Nevertheless, the increasing *S. aureus* MICs and high level of vancomycin-resistant *S. aureus* (VRSA), first reported in 2002, have been informed (Kest and Kaushik, 2019). More concern is added with the findings of VISA strains of swine origin reported in other studies (Kwok et al., 2013; Moreno et al.,

Table 3. Minimal inhibitory concentrations (MIC) of antibiotic-resistant *S. aureus* strains isolated from the pork supply chain.

Antibiotics	Total	No (%) resistant *S. aureus* strain by MIC (μg mL^{-1})																			
		0.5-1.5		2		>2		4		8		>8		16		>16		32		>32		>256	
OXA	2									1												1	
										(50)												(50)	
PEN	45	16		5						3										4		3	
		(35.6)		(11.1)		3	(6.7)	1	(2.2)	(6.7)		3	(6.7)	1	(2.2)	3	(6.7)	3	(6.7)	(8.9)		(6.7)	
ERY	24																			1		23	
																				(4.2)		(95.8)	
VAN	1							1*															
								(100)															
GEN	2																			2			
																				(100)			
KAN	9																			6		3	
																				(66.7)		(33.3)	
CIP	17							1				1										15	
								(5.9)				(5.9)										(88.2)	
QDA	3							1						1						1			
								(33.3)						(33.3)						(33.3)			
TET	19															2		7		10			
																(10.5)		(36.8)		(52.6)			

OXA – Oxacilin, PEN – Penicilin, CEF – Cefoxitin, ERY – Erytromycin, VAN – Vancomycin, GEN – Gentamicin, KAN – Kanamycin, CIP – Ciprofloxacin, QDA - Quinupristin/Dalfopristin, TET – Tetracycline.

2016; Sineke et al., 2021 .

All aminoglycosides' breakpoints were deleted from CLSI (2018), except gentamicin. Thus, the former breakpoint for kanamycin was used as interpretive criteria in this study (CLSI, 2016). Resistance to kanamycin is primarily caused by the production of APH(3')-I-3, ANT(4') (4'')-I or bifunctional APH(2')-AAC(6) enzymes, which have been related to a loss of synergism of kanamycin with β-lactams and glycopeptides (Leclercq et al., 2013).

A total of sixteen resistance profiles were obtained (Table 4). Multidrug-resistance (MDR), defined as the resistance to 3 or more classes of antibiotics, was found in 8 profiles, with 38.2% of the *S. aureus* strains. Most of the multidrug-resistant *S. aureus* were isolated from pigs (17/21). The most common resistance profiles were PEN-ERY-CIP-TET in pigs, and PEN in carcasses and retail meat. These results indicates that multidrug-resistant *S. aureus* strains are present in the pork supply chain with the risk of transmission to humans, and thus can cause infections that can be difficult to treat.

CONCLUSIONS

Most of the *S. aureus* strains isolated from the pork supply chain exhibited AMR, while MDR was also detected. Samples from animals presented a higher prevalence of antibiotic-resistant *S. aureus* strains than carcasses and retail meat. Oxacillin- and cefoxitin-resistant *S. aureus* strains, which were *mecA*-negative, were also detected, suggesting other mechanisms of methicillin resistance associated. These findings allow characterizing *S. aureus* strains in the food supply chain to control and reduce the use of antibiotics in farming in order to mitigate the spread of AMR.

ACKNOWLEDGEMENTS

This work was supported by the National Fund for Scientific and Technological Development (Fondecyt), Chilean Government [grant number 11140379].

Declaration of competing interest

The authors declare that they have no known

Table 4 Antimicrobial-resistance profiles of *S. aureus* strains isolated from the pork supply chain.

Antimicrobial resistance profile	No. of subclasses resistant to	No. (%) of all *S. aureus* 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)

Interpretive criteria according to CLSI (2018).

OXA – Oxacilin, PEN – Penicilin, CEF – Cefoxitin, ERY – Erytromycin, VAN – Vancomycin, GEN – Gentamicin, KAN – Kanamycin, CIP – Ciprofloxacin, QDA - Quinupristin/Dalfopristin, TET – Tetracycline.

*Interpretive criteria according to CLSI (2016) for kanamycin.

competing financial interests or personal relationships that could have influenced the work reported in this paper.

LITERATURE CITED

Ajoke, O.I., I.O. Okeke, O.A. Odeyemi, and A.E.J. Okwori. 2012. Prevalence of methicillin-resistant *Staphylococcus aureus* from healthy community individuals volunteers in Jos South, Nigeria. J. Microbiol. Biotech. Food Sci. 1(6):1389-1405.

Alipour, F., M. Ahmadi, and S. Javadi. 2014. Evaluation of different methods to detect methicillin resistance in *Staphylococcus aureus* (MRSA). J. Infect. Public. Health. 7, 186–191. https://doi.org/10.1016/j.jiph.2014.01.007.

Argudín, M.A., M.C. Mendoza, and M.R. Rodicio. 2010. Food poisoning and *Staphylococcus aureus* enterotoxins. Toxins. 2, 1751–1773. https://doi.org/10.3390/toxins2071751.

Argudín, M.A., S. Roisin, L. Nienhaus, M. Dodémont, R. De Mendonça, C. Nonhoff, et al. 2018. Genetic diversity among *Staphylococcus aureus* isolates showing oxacillin and/or cefoxitin resistance not linked to the presence of *mec* genes. Antimicrob. Agents Chemother. 62, 1–6. https://doi.org/10.1128/AAC.00091-18.

Bennani, H., A. Mateus, N. Mays, E. Eastmure, K.D.C. Stärk, and B. Häsler. 2020. Overview of evidence of antimicrobial use and antimicrobial resistance in the food chain. Antibiotics. 9, 49. https://doi.org/10.3390/antibiotics9020049.

Bouchami, O., M.J. Fraqueza, N.A. Faria, V. Alves, O.U. Lawal, H. Lencastre, et al. 2020. Evidence for the dissemination to humans of Methicillin-Resistant *Staphylococcus aureus* ST398 through the pork production chain: a study in a portuguese slaughterhouse. Microorganism. 8 (12), 1892. https://doi.org/10.3390/microorganisms8121892.

Buyukcangaz, E., V. Velasco, J.S. Sherwood, R.M. Stepan, R.J. Koslofsky, and C.M. Logue. 2013. Molecular typing of *Staphylococcus aureus* and methicillin-resistant *S. aureus* (MRSA) isolated from animals and retail meat in North Dakota, United States. Foodborne Pathog. Dis. 10, 608–617. https://doi.org/10.1089/fpd.2012.1427.

Clinical and Laboratory Standards Institute (CLSI). 2016. Performance Standards for Antimicrobial Susceptibility Testing. Twenty-sixth Edition. M100-S25. Wayne, PA, USA.

Clinical and Laboratory Standards Institute (CLSI). 2018. Performance Standards for Antimicrobial Susceptibility Testing. Twenty-eighth Edition. M100-S28. Wayne, PA, USA.

Cuny, C., F. Layer, B. Strommenger, and W. Witte. 2011. Rare occurrence of methicillin-resistant *Staphylococcus aureus* CC130 with a novel *mecA* homologue in humans in Germany. PLoS ONE. 6, e24360. https://doi.org/10.1371/journal.pone.0024360.

Food and Agriculture Organization of the United Nations (FAO). 2020. Progress report on the implementation of FAO Action Plan on Antimicrobial Resistance (AMR) 2016-2020, and the proposal for a new FAO Action Plan on AMR 2021-2025. Committee on Agriculture. Twenty-seventh Session. June, 2020.

Friese, A., J. Schulz, K. Zimmermann, B.A. Tenhagen, A. Fetsch, J. Hartung, et al. 2013. Occurrence of livestock-associated methicillin-resistant *Staphylococcus aureus* in turkey and broiler barns and contamination of air and soil surfaces in their vicinity. Appl. Environ. Microbiol. 79, 2759–2766. https://doi.org/10.1128/AEM.03939-12.

Haag, A.F., J.R. Fitzgerald, and J.R. Penadés. 2019. *Staphylococcus aureus* in animals. Microbiol. Spectr. 7, GPP3-0060. https://doi.org/10.1128/microbiolspec.GPP3-0060-2019.

Junnila, J., T. Hirvioja, K. Auranen, K. Rantakokko-Jalava, J. Silvola, L. Lindholm, K., et al. 2020. Changing epidemiology of methicillin-resistant *Staphylococcus aureus* in a low endemicity area – new challenges for MRSA control. Eur. J. Clin. Microbiol. Infect. Dis. 39, 2299-2307. https://doi.org/10.1007/s10096-020-03824-9.

Kest, H., and A. Kaushik. 2019. Vancomycin-resistant *Staphylococcus aureus*: Formidable threat or silence before the storm? Infect. Dis. Epi. 5, 093. https://doi.org/10.23937/2474-3658/1510093.

Kim, Y.H., H.S. Kim, S. Kim, M. Kim, and H.S. Kwak. 2020. Prevalence and characteristics of antimicrobial-resistant *Staphylococcus aureus* and Methicillin-Resistant *Staphylococcus aureus* from retail meat in Korea. Food Sci. Anim. Res. 40(5):758-771. https://doi.org/10.5851/kosfa.2020.e50.

Kowalska-Krochmal B., and R. Dudek-Wicher. 2021. The minimum inhibitory concentration of antibiotics: methods, interpretation, clinical relevance. Pathogen. 10, 165. https://doi.org/10.3390/pathogens10020165.

Kwok, G.M.L., M.M.O. O'Donoghue, V.C. Doddangoudar, J. Ho, and M.V. Boost. 2013. Reduced vancomycin susceptibility in porcine ST9 MRSA isolates. Front. Microbiol. 4, 316. https://doi.org/10.3389/fmicb.2013.00316.

Leclercq, R., R. Cantón, D.F.J. Brown, C.G. Giske, P. Heisig, A.P. MacGowan, et al. 2013. EUCAST expert rules in antimicrobial susceptibility testing. Clin. Microbiol. Infect. 19, 141-160. https://doi.org/10.1111/j.1469-0691.2011.03703.x.

Moreno, L.Z, M.C. Dutra, M. Moreno, T.S.P. Ferreira, G.F.R. da Silva, C.E.C. Matajira, et al. 2016. Vancomycin-intermediate livestock-associated methicillin-resistant *Staphylococcus aureus* ST398/t9538 from swine in Brazil. Mem. Inst. Oswaldo Cruz, Río de Janeiro. 111, 659-661. https://doi.org/10.1590/0074-02760160276.

Normanno, G., E. Spinelli, M. Caruso, R. Fraccalvieri, L. Capozzi, A. Barlaam, et al. 2020. Occurrence and characteristics of methicillin-resistant *Staphylococcus aureus* (MRSA) in buffalo bulk tank milk and the farm workers in Italy. Food Microbiol. 91, 103509. https://doi.org/10.1016/j.fm.2020.103509.

Peacock, S.J., and G.K. Paterson. 2015. Mechanisms of methicillin resistance in *Staphylococcus aureus*. Annu. Rev. Biochem. 84, 577–601. https://doi.orgr/10.1146/annurev-biochem-060614-034516.

Rajkhowa, S., D.K. Sarma, and S.R. Pegu. 2016. SCC *mec* typing and antimicrobial resistance of methicillin-resistant *Staphylococcus aureus* (MRSA) from pigs of Northeast India. Vet. Res. Commun. 40, 117-122. https://doi.otg/10.1007/s11259-016-9661-x.

Regulation (EC) No 1831/2003 of the European Parliament and of the council of 22 September 2003 on additives for use in animal nutrition. (2003). European Food Safety Authority (EFSA). Off. J. Eur. Union. 268, pp. 29-43

Res. SAG No. 3447. (2006). Modifica resolución No. 1992 de 2006. Agriculture Department of Chile. https://www.sag.gob.cl/sites/default/files/RES_3447_MODIF_1992_INSUMOS.pdf (accessed 05 March 2021).

Rubin, J.E., K.R. Ball, and M. Chirino-Trejo. 2011. Antimicrobial susceptibility of *Staphylococcus aureus* and *Staphylococcus pseudintermedius* isolated from various animals. Can. Vet. J. 52, 153-157.

Sineke, N., J. Asante, D.G. Amoako, A.L.K. Abia, K. Perrett, L.A. Bester, et al. 2021. *Staphylococcus aureus* in intensive pig production in South Africa: antibiotic resistance, virulence determinants, and clonality. Pathogens. 10, 317. https://doi.org/10.3390/pathogens10030317.

Tong, S.Y.C., J.S. Davis, E. Eichenberger, T.L. Holland, and V.G. Fowler. 2015. *Staphylococcus aureus* infections: Epidemiology, pathophysiology, clinical manifestations, and management. Clin. Microbiol. Rev. 28, 603–661. https://doi.org/10.1128/CMR.00134-14.

Velasco, V., J.L. Vergara, A.M. Bonilla, J. Muñoz, A. Mallea, D. Vallejos, et al. 2018. Prevalence and characterization of *Staphylococcus aureus* strains in the pork chain supply in Chile. Foodborne Pathog. Dis. 15, 262-268. https://doi.org/10.1089/fpd.2017.2381.

World Health Organization (WHO). 2017. Global priority list of antibiotic-resistant bacteria to guide research, discovery, and development of new antibiotics. Geneva: 2017. Available from: https://www.who.int/news/item/27-02-2017-who-publishes-list-of-bacteria-for-which-new-antibiotics-are-urgently-needed.