



Article

Phenotypic and molecular traits of *Staphylococcus coagulans* associated with canine skin infections in Portugal

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Table S1. Distribution of inhibition zone diameters of 21 antibiotics for the 27 *S. coagulans* studied.

Antibiotic	Distribution of Inhibition zone diameter (mm)																																													
	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46					
Penicillin **																																														
no. isolates	0	0	0	1	0	0	1	0	0	0	0	0	1	1	3	3	1	1	0	0	3	0	0	0	0	0	1	0	1	0	0	0	0	0	0	1	5	1	1	1	1	1	1			
frequency (%)	0	0	0	3.7	0	0	3.7	0	0	0	0	0	3.7	3.7	11.1	11.1	3.7	3.7	0	0	11.1	0	0	0	0	0	3.7	0	3.7	0	0	0	0	0	0	0	3.7	18.5	3.7	3.7	3.7	3.7	3.7			
Oxacillin **																																														
no. isolates	0	0	0	0	0	0	0	1	0	1	0	0	0	0	1	7	5	0	1	3	6	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
frequency (%)	0	0	0	0	0	0	0	3.7	0	3.7	0	0	0	0	3.7	25.9	18.5	0	3.7	11.1	22.2	3.7	3.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
Enrofloxacin *																																														
no. isolates	4	0	1	0	0	0	1	0	2	0	1	1	0	0	1	0	0	3	3	0	0	0	2	0	4	1	2	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
frequency (%)	14.8	0	3.7	0	0	0	3.7	0	7.4	0	3.7	3.7	0	0	3.7	0	0	11.1	11.1	0	0	0	7.4	0	14.8	3.7	7.4	0	3.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
Pradofloxacin																																														
no. isolates	0	0	0	0	0	0	0	0	0	0	0	1	3	0	0	0	0	1	2	0	1	2	1	0	4	1	6	0	3	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
frequency (%)	0	0	0	0	0	0	0	0	0	0	0	3.7	11.1	0	0	0	0	3.7	7.4	0	3.7	7.4	3.7	0	14.8	3.7	22.2	0	11.1	7.4	0	0	0	0	0	0	0	0	0	0	0	0	0	0		

Cells are colored according to the breakpoints established by respective recommendations; S: green; I: yellow; R: red. * Breakpoint established by CLSI for staphylococci isolated from animals, document VET01S ED5 [1]; ** Breakpoint established by CLSI for staphylococci isolated from humans, document M100-S30 [2]; *** Breakpoint established by EUCAST [3].

Table S1. (Continuation). Distribution of inhibition zone diameters of 21 antibiotics for the 27 *S. coagulans* studied.

Antibiotic	Distribution of Inhibition zone diameter (mm)																																															
	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46							
Ciprofloxacin **																																																
no. isolates	4	0	0	0	0	0	0	0	2	0	0	2	1	1	0	0	0	0	0	1	3	2	2	1	0	1	3	1	2	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0			
frequency (%)	14.8	0	0	0	0	0	0	0	7.4	0	0	7.4	3.7	3.7	0	0	0	0	0	3.7	11.1	7.4	7.4	3.7	0	3.7	11.1	3.7	7.4	3.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0			
Moxifloxacin **																																																
no. isolates	0	0	0	0	0	0	0	0	0	0	1	0	0	3	0	1	0	1	0	3	0	1	1	1	6	2	2	0	2	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
frequency (%)	0	0	0	0	0	0	0	0	0	0	3.7	0	0	11.1	0	3.7	0	3.7	0	11.1	0	3.7	3.7	3.7	22.2	7.4	7.4	0	7.4	11.1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Erythromycin **																																																
no. isolates	1	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	3	1	12	2	4	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
frequency (%)	3.7	0	0	0	0	0	0	0	3.7	0	3.7	0	0	0	0	0	0	0	0	0	11.1	3.7	44.4	7.4	14.8	0	3.7	0	3.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Clindamycin *																																																
no. isolates	1	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	1	1	4	1	12	1	3	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
frequency (%)	3.7	0	0	0	0	0	0	3.7	3.7	0	0	0	0	0	0	0	0	0	3.7	3.7	14.8	3.7	44.4	3.7	11.1	0	3.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Quinupristin – dalfopristin ***																																																
no. isolates	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	5	8	4	6	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
frequency (%)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	11.1	18.5	29.6	14.8	22.2	3.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Tetracycline *																																																
no. isolates	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	2	3	7	4	4	4	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
frequency (%)	0	0	0	0	0	0	0	0	0	3.7	0	0	0	0	0	0	0	0	0	0	0	7.4	11.1	25.9	14.8	14.8	14.8	0	7.4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Minocycline **																																																
no. isolates	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	4	7	2	5	3	2	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
frequency (%)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3.7	3.7	3.7	14.8	25.9	7.4	18.5	11.1	7.4	0	0	3.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Tigecycline ***																																																
no. isolates	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	11	5	3	1	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
frequency (%)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	18.5	40.7	18.5	11.1	3.7	0	7.4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Fusidic acid ***																																																
no. isolates	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	1	0	0	0	1	2	8	7	2	0	3	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
frequency (%)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3.7	3.7	0	0	0	0	3.7	0	0	0	3.7	7.4	29.6	25.9	7.4	0	11.1	3.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Cells are colored according to the breakpoints established by respective recommendations; S: green; I: yellow; R: red. * Breakpoint established by CLSI for staphylococci isolated from animals, document VET01S ED5 [1]; ** Breakpoint established by CLSI for staphylococci isolated from humans, document M100-S30 [2]; *** Breakpoint established by EUCAST [3].

Table S1. (Continuation). Distribution of inhibition zone diameters of 21 antibiotics for the 27 *S. coagulans* studied.

Antibiotic	Distribution of Inhibition zone diameter (mm)																																																
	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46								
Linezolid **																																																	
no. isolates	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	6	6	3	5	2	0	0	0	2	0	0	0	0	0	0	0	0	0	0					
frequency (%)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3.7	3.7	3.7	22.2	22.2	11.1	18.5	7.4	0	0	0	7.4	0	0	0	0	0	0	0	0	0	0	0					
Chloramphenicol **																																																	
no. isolates	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	9	3	7	4	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0				
frequency (%)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3.7	3.7	33.3	11.1	25.9	14.8	7.4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0			
Trimethoprim – sulphamethoxazol **																																																	
no. isolates	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	8	2	5	7	2	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0			
frequency (%)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3.7	3.7	29.6	7.4	18.5	25.9	7.4	3.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
Rifampicin **																																																	
no. isolates	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	2	5	5	7	3	1	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0			
frequency (%)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	7.4	7.4	18.5	18.5	25.9	11.1	3.7	0	3.7	0	3.7	0	3.7	0	0	0	0	0	0	0	0	0	0	0		
Gentamicin **																																																	
no. isolates	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	5	7	8	4	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
frequency (%)	0	0	0	0	0	0	0	0	0	0	0	0	3.7	0	0	0	3.7	0	0	18.5	25.9	29.6	14.8	0	3.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Amikacin ***																																																	
no. isolates	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	2	3	8	6	4	0	2	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
frequency (%)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3.7	0	7.4	11.1	29.6	22.2	14.8	0	7.4	3.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Tobramycin ***																																																	
no. isolates	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	3	8	5	8	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
frequency (%)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3.7	0	3.7	11.1	29.6	18.5	29.6	0	3.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Kanamycin ***																																																	
no. isolates	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	3	7	7	7	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
frequency (%)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	7.4	0	11.1	25.9	25.9	25.9	0	3.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Cells are colored according to the breakpoints established by respective recommendations; S: green; I: yellow; R: red. * Breakpoint established by CLSI for staphylococci isolated from animals, document VET01S ED5 [1]; ** Breakpoint established by CLSI for staphylococci isolated from humans, document M100-S30 [2]; *** Breakpoint established by EUCAST [3].

Table S2. Distribution of inhibition zone diameters of six antibiotics with no breakpoint or epidemiological cut-off value for the 27 *S. coagulans* studied.

Antibiotic	Distribution of Inhibition zone diameter (mm)																																													
	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46					
Apramycin																																														
no. isolates	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	3	4	7	4	6	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
frequency (%)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	7.4	11.1	14.8	25.9	14.8	22.2	3.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Bacitracin																																														
no. isolates	0	0	0	0	0	0	0	0	0	0	5	7	4	5	4	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
frequency (%)	0	0	0	0	0	0	0	0	0	0	18.5	25.9	14.8	18.5	14.8	7.4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Florfenicol																																														
no. isolates	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	4	9	8	1	1	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
frequency (%)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3.7	3.7	14.8	33.3	29.6	3.7	3.7	0	3.7	0	3.7	0	3.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Mupirocin																																														
no. isolates	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	2	0	5	7	2	4	0	3	0	1					
frequency (%)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	11.1	7.4	0	18.5	25.9	7.4	14.8	0	11.1	0	3.7					
Neomycin																																														
no. isolates	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	7	7	7	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
frequency (%)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3.7	0	3.7	25.9	25.9	25.9	3.7	3.7	3.7	3.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Novobiocin																																														
no. isolates	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	4	3	5	8	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
frequency (%)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	11.1	14.8	11.1	18.5	29.6	14.8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table S3. Inhibition zone diameters of 27 antibiotics for the single *S. schleiferi* isolate studied.

Antibiotic	ZD (mm)	Antibiotic	ZD (mm)	Antibiotic	ZD (mm)	Antibiotic	ZD (mm)
Penicillin	11	Clindamycin	28	Chloramphenicol	28	Kanamycin	28
Oxacillin	19	Quinupristin - dalfopristin	29	Florfenicol	30	Neomycin	23
Enrofloxacin	15	Tetracycline	35	Trimethoprim - sulfamethoxazole	29	Apramycin	25
Pradofloxacin	26	Minocycline	34	Rifampicin	36	Bacitracin	20
Ciprofloxacin	17	Tigecycline	29	Gentamicin	25	Mupirocin	42
Moxifloxacin	25	Fusidic acid	17	Amikacin	28	Novobiocin	36
Erythromycin	30	Linezolid	34	Tobramycin	27		

ZD: inhibition zone diameter.



Table S4. Primers used in this study.

Target Gene	Primers	Nucleotide Sequence (5'-3')	Amplicon Size (bp)	Reference
<i>S. coagulans</i> and <i>S. schleiferi</i> identification				
<i>nuc</i>	nuc-fw	AATGGCTACAATGATAATCACTAA	526	[4]
	nuc-rv	CATATCTGTCTTTCGGCGCG		
Screening of resistance genes and mutations				
<i>mecA</i>	mecA_Fw	GGTCCCATTAACTCTGAAG	1040	[5]
	mecA_Rv	AGTTCTGCAGTACCGGATTTCG		
<i>blaZ</i>	blaZ_Fw	GATAAGAGATTTGCCTATGC	533	[6]
	blaZ_Rv	GCATATGTTATTGCTTGACC		
<i>erm(A)</i>	erm(A)_Fw	AAGCGGTAAACCCCTCTGAG	442	[7]
	erm(A)_Rv	TCAAAGCCTGTCGGAATTGG		
<i>erm(B)</i>	erm(B)_Fw	TGGAACAGGTAAAGGGCATT	433	[8]
	erm(B)_Rv	TGTGGTATGGCGGGTAAGTT		
<i>erm(C)</i>	erm(C)_Fw	TCGTAAC TGCCATTGAAATA	348	[8]
	erm(C)_Rv	TCACTTTAGGTTTAGGATGAAA		
<i>msr(A)</i>	msr(A)_Fw	GATTGTCCCAAGCCAGTAAA	445	[9]
	msr(A)_Rv	GCCATTTGCACTTTAGGAGA		
<i>mph(C)</i>	mph(C)_Fw	ATGACTCGACATAATGAAAT	900	[10]
	mph(C)_Rv	CTACTCTTTCATACCTAACTC		
<i>vga(A)</i>	vga(A)_Fw	ACCCGAGACATCTTCACCAC	400	[11]
	vga(A)_Rv	GGAAATTGACGAGGGGAGA		
<i>vga(C)</i>	vga(C)_Fw	ACGAATAAAGGGATCGAAGC	510	[9]
	vga(C)_Rv	AGCACATGCACAGGTTTGTA		
<i>fusB</i>	fusB_Fw	ATTCAATCGGAAACCTATAATGATA	292	[12]
	fusB_Rv	TTATATATTTCCGATTTGATGCAAG		
<i>fusC</i>	fusC_Fw	GATATTGATATCTCGGACTT	128	[13]
	fusC_Rv	AGTTGACTTGATGAAGGTAT		
<i>tet(K)</i>	tet(K)_Fw	GTAGCGACAATAGGTAATAGT	361	[14]
	tet(K)_Rv	GTAGTGACAATAAACCTCCTA		
<i>tet(M)</i>	tet(M)_Fw	GTTAAATAGTGTTCTTGAG	657	[15]
	tet(M)_Rv	CTAAGATATGGCTCTAACAA		
<i>tet(L)</i>	tet(L)_Fw	GTCGGTAATTGGGTTTGTTG	421	This study
	tet(L)_Rv	TGACAGCACGCTAACGATAA		
<i>grlA</i>	grlA_Fw	CAAGAGCGTGCTTTRCCT	300	This study
	grlA_Rv	CTGACTYAATTTTCGCTTCAG		
<i>gyrA</i>	gyrA_Fw	ATGAGTGTTATYGTRTCTCGT	261	This study
	gyrA_Rv	CATMGAACCRAAGTTACCTTG		

bp: base pair; Fw: "forward"; Rv: "reverse"; R: A + G; Y: C + T; M: A + C.



Table S5. Control strains used in the screening of antibiotic resistance genes.

Target Gene	Control strain	Reference	Target Gene	Control strain	Reference
<i>mecA</i>	<i>S. aureus</i> SM1	[16]	<i>vga(A)</i>	<i>S. epidermidis</i> FMV-51	[18]
<i>blaZ</i>	<i>S. aureus</i> SM1	[16]	<i>vga(C)</i>	<i>S. aureus</i> 49.1	[11]
<i>erm(A)</i>	<i>S. aureus</i> SM39	[8]	<i>fusB</i>	<i>S. epidermidis</i> FMV-97	[19]
<i>erm(B)</i>	<i>S. pseudintermedius</i> 4877/10	[17]	<i>fusC</i>	<i>S. epidermidis</i> FMV-34	[19]
<i>erm(C)</i>	<i>S. aureus</i> SM26	[8]	<i>tet(K)</i>	<i>S. epidermidis</i> ATCC12228	[20]
<i>msr(A)</i>	<i>S. epidermidis</i> FMV-51	[18]	<i>tet(M)</i>	<i>S. aureus</i> H4/09	[11]
<i>mph(C)</i>	<i>S. epidermidis</i> FMV-51	[18]	<i>tet(L)</i>	<i>S. aureus</i> 25.1	[11]

References

1. Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals, 5th Edition. CLSI Supplement VET01S. USA, 2020.
2. Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing, 30th Edition. CLSI Supplement M100. USA, 2020.
3. European Committee on Antimicrobial Susceptibility Testing (EUCAST). Breakpoint tables for interpretation of MICs and zone diameters, v11.0. 2021. <http://www.eucast.org>
4. Sasaki, T.; Tsubakishita, S.; Tanaka, Y.; Sakusabe, A.; Ohtsuka, M.; Hirota, S.; Kawakami, T.; Fukata, T.; Hiramatsu, K. Multiplex-PCR method for species identification of coagulase-positive staphylococci. *J Clin Microbiol* **2010**, *48*, 765–769. doi: 10.1128/JCM.01232-09.
5. Petinaki, E.; Arvaniti, A.; Dimitracopoulos, G.; Spiliopoulou, I. Detection of *mecA*, *mecR1* and *mecI* genes among clinical isolates of methicillin-resistant staphylococci by combined polymerase chain reactions. *J Antimicrob Chemother* **2001**, *47*, 297–304. doi: 10.1093/jac/47.3.297
6. Milheirico, C.; Portelinha, A.; Krippahl, L.; de Lencastre, H.; Oliveira, D.C. Evidence for a purifying selection acting on the β -lactamase locus in epidemic clones of methicillin-resistant *Staphylococcus aureus*. *BMC Microbiol* **2011**, *11*, 76. doi: 10.1186/1471-2180-11-76
7. Jensen, L.B.; Hammerum, A.M.; Bager, F.; Aarestrup, F.M. Streptogramin resistance among *Enterococcus faecium* isolated from production animals in Denmark in 1997. *Microb Drug Resist* **2002**, *8*, 369–374. doi: 10.1089/10766290260469642
8. Costa, S.S.; Palma, C.; Kladec, K.; Fessler, A.T.; Viveiros, M.; Melo-Cristino, J.; Schwarz, S.; Couto, I. Plasmid-borne antimicrobial resistance of *Staphylococcus aureus* isolated in a hospital in Lisbon, Portugal. *Microb Drug Resist* **2016**, *22*, 617–626. doi: 10.1089/mdr.2015.0352
9. Ferreira, C.; Costa, S.S.; Serrano, M.; Oliveira, K.; Trigueiro, G.; Pomba, C.; Couto, I. Clonal Lineages, Antimicrobial Resistance, and PVL Carriage of *Staphylococcus aureus* associated to skin and soft-tissue infections from ambulatory patients in Portugal. *Antibiotics* **2021**, *10*, 345. <https://doi.org/10.3390/antibiotics10040345>
10. Schnellmann, C.; Gerber, V.; Rossano, A.; Jaquier, V.; Panchaud, Y.; Doherr, M.G.; Thomann, A.; Straub, R.; Perreten, V. Presence of new *mecA* and *mph(C)* variants conferring antibiotic resistance in *Staphylococcus* spp. isolated from the skin of horses before and after clinic admission. *J Clin Microbiol* **2006**, *44*, 4444–4454. doi: 10.1128/JCM.00868-06
11. Couto, N.; Belas, A.; Kadlec, K.; Schwarz, S.; Pomba, C. Clonal diversity, virulence patterns and antimicrobial and biocide susceptibility among human, animal and environmental MRSA in Portugal. *J Antimicrob Chemother* **2015**, *71*, 1479–1487. doi: 10.1093/jac/dkv141
12. O'Neill, A.J.; Larsen, A.R.; Henriksen, A.S.; Chopra, I. A fusidic acid-resistant epidemic strain of *Staphylococcus aureus* carries the *fusB* determinant, whereas *fusA* mutations are prevalent in other resistant isolates. *Antimicrob Agents Chemother* **2004**, *48*, 3594–3597. doi: 10.1128/AAC.48.9.3594-3597.2004
13. Castanheira, M.; Watters, A.A.; Bell, J.M.; Turnidge, J.; Jones, R.N. Fusidic acid resistance rates and prevalence of resistance mechanisms among *Staphylococcus* spp. isolated in North America and Australia, 2007–2008. *Antimicrob. Agents Chemother* **2010**, *54*, 3614–3617. doi: 10.1128/AAC.01390-09
14. Strommenger, B.; Kettlitz, C.; Werner, G.; Witte, W. Multiplex PCR assay for simultaneous detection of nine clinically relevant antibiotic resistance genes in *Staphylococcus aureus*. *J Clin Microbiol* **2003**, *41*, 4089–4094. doi: 10.1128/jcm.41.9.4089-4094.2003
15. Aarestrup, F.; Agerso, Y.; Gerner-Smidt, P.; Madsen, M.; Jensen, L. Comparison of antimicrobial resistance phenotypes and resistance genes in *Enterococcus faecalis* and *Enterococcus faecium* from humans in the community, broilers, and pigs in Denmark. *Diagn Microbiol Infect Dis* **2000**, *37*, 127–137. doi: 10.1016/s0732-8893(00)00130-9.



16. Costa, S.S., Falcão, C., Viveiros, M., Machado, D., Martins, M., Melo-Cristino, J., Amaral, L., Couto, I. Exploring the contribution of efflux on the resistance to fluoroquinolones in clinical isolates of *Staphylococcus aureus*. *BMC Microbiology*. 2011;11:241. doi: 10.1186/1471-2180-11-241
17. Couto, N., Monchique, C., Belas, A., Marques, C., Gama, L.T., Pomba, C. Trends and molecular mechanisms of antimicrobial resistance in clinical staphylococci isolated from companion animals over a 16 year period. *J Antimicrob Chemother* **2016**, *71*, 1479-1487. doi: 10.1093/jac/dkw029.
18. Holtreman, F. D. Characterization of plasmids of *Staphylococcus epidermidis* and correlation with efflux-mediated resistance. 2018. MSc Thesis in Biomedical Sciences, Universidade NOVA de Lisboa. <http://hdl.handle.net/10362/53495>
19. Rosa, M.S.R. O. Contribution of efflux to antimicrobial resistance in *Staphylococcus epidermidis*. 2017. MSc Thesis in Biomedical Sciences, Universidade NOVA de Lisboa. <http://hdl.handle.net/10362/20443>
20. MacLea, K., Trachtenberg, A. Complete genome sequence of *Staphylococcus epidermidis* ATCC 12228 chromosome and plasmids, generated by long-read sequencing. *Genome Announc*. 2017;5: e00954-17. doi: 10.1128/genomeA.00954-17