

Antibiotic Resistance of Staphylococci from Bulk-Tank Milk of Sheep Flocks: Prevalence, Patterns, Association with Biofilm-Formation, Effects on Milk Quality, Risk Factors

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Table S1. Characterisation of staphylococcal strains recovered from milk samples of ewes classified according to biofilm-formation, by combination of results of culture appearance on Congo Red agar and microplate adhesion method.

		Sum of results of each of three separate testing lots for each strain in the microplate adhesion method ¹			
		0	1	2	3
Culture appearance on Congo Red agar	-	non BF ²	non BF	non BF	weak BF
	±	non BF	non BF	BF	BF
	+	weak BF	BF	BF	BF

¹ sum ranged from 0 (i.e., in all three testing lots median, of three triplicates, OD was < [0.85 × median OD of the positive control strain]) to 3 (i.e., in all three testing lots median, of three triplicates, OD was ≥ [0.85 × median OD of the positive control strain]) (OD: optical density) [Vasileiou et al 2018]; ² biofilm-forming.

Reference

Vasileiou, N.G.C.; Chatzopoulos, D.C.; Gougoulis, D.A.; Sarrou, S.; Katsafadou, A.I.; Spyrou, V.; Mavrogianni, V.S.; Petinaki, E.; Fthenakis, G.C. Slime-producing staphylococci as causal agents of subclinical mastitis in sheep. *Vet. Microbiol.* **2018**, *224*, 93–99

Table S2. Details of multivariable models employed for the evaluation of the isolation of resistant staphylococcal isolates from the bulk-tank milk of 325 sheep flocks in Greece.

Outcome	Variables offered to the multivariable models (<i>n</i>)	Variables required in the final models
Isolation of an oxacillin-resistant staphylococcal isolate from the bulk-tank milk	7	(a) routine administration of antimicrobials in newborns, (b) vaccination against mastitis, (c) length of previous animal farming experience of the farmer
Isolation of a resistant staphylococcal isolate from the bulk-tank milk	10	(a) management system applied in the flock, (b) month into the lactation period at sampling, (c) annual frequency of systemic disinfections in the farm, (d) length of previous animal farming experience of the farmer, (e) presence of working staff in the flock
Isolation of a multi-resistant staphylococcal isolate from the bulk-tank milk	7	(a) management system applied in the flock, (b) length of previous animal farming experience of the farmer

Table S3. Frequency of susceptibility / resistance to individual antibiotics ¹ of staphylococcal isolates recovered from bulk-tank milk of 325 sheep flocks in Greece

	n	AMP	AZI	CXI	CIP	CLA	CLI	ERY	FOS	FUS	GEN	MOX	MUP	OXA	PEN	RIF	TEI	TET	TOB	SXT
<i>S. aureus</i>	54	7	0	0	1	0	2	1	0	1	2	0	1	2	7	0	0	6	1	2
<i>S. simulans</i>	35	1	0	0	0	0	0	0	3	0	0	0	0	1	1	0	0	0	0	0
<i>S. equorum</i>	23	15	0	0	0	0	11	14	12	1	0	0	0	1	15	0	0	2	0	0
<i>S. haemolyticus</i>	22	8	0	0	0	0	1	0	2	0	0	0	0	0	8	0	0	3	1	0
<i>S. chromogenes</i>	13	3	0	0	0	0	1	1	0	0	0	0	0	0	3	0	0	3	0	0
<i>S. lentus</i>	12	11	0	0	1	0	11	3	1	2	0	1	0	11	11	0	0	1	0	0
<i>S. lugdunensis</i>	11	6	0	0	0	0	1	0	1	0	0	0	0	0	6	0	0	2	0	0
<i>S. warneri</i>	9	3	0	0	0	0	1	0	2	0	0	0	0	0	3	0	0	1	0	0
<i>S. kloosii</i>	7	5	0	0	0	0	4	0	4	0	0	0	0	4	5	0	0	1	0	0
<i>S. capitis</i>	6	2	0	0	0	0	0	0	3	0	0	0	0	0	2	0	0	1	0	0
<i>S. intermedius</i>	6	2	0	0	0	0	0	0	0	0	0	0	0	1	2	0	0	1	0	0
<i>S. cohnii</i>	4	2	0	0	0	0	2	1	0	2	0	0	0	1	2	0	0	1	0	0
<i>ssp. cohnii</i>																				
<i>S. epidermidis</i>	4	3	0	0	0	0	0	1	0	0	0	0	0	1	3	0	0	1	0	0
<i>S. saprophyticus</i>	4	2	0	0	0	0	0	0	2	2	0	0	0	0	2	0	0	2	0	0
<i>S. xylosus</i>	4	2	0	0	0	0	3	0	0	3	0	0	0	1	2	1	0	0	0	0
<i>S. auricularis</i>	3	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	1	0	0
<i>S. cohnii</i>	3	2	0	0	0	0	1	0	0	1	0	0	0	1	2	0	0	0	0	0
<i>ssp. urealyticum</i>																				
<i>S. sciuri</i>	3	1	0	0	0	0	1	0	0	1	0	0	0	1	1	0	0	0	0	0
<i>S. vitulinus</i>	3	1	0	0	0	0	1	0	0	1	0	0	0	1	1	0	0	1	0	0
<i>S. hominis</i>	2	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>S. pasteurii</i>	2	2	0	0	0	0	0	0	1	0	0	0	0	0	2	0	0	1	0	0
<i>S. carnosus</i>	2	1	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0

Total	232	79	0	0	2	0	41	21	31	14	2	1	1	27	79	1	0	28	2	2
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¹ as established by use of VITEK 2; ² AMP: ampicillin, AZI: azithromycin, CXI: ceftiofur, CIP: ciprofloxacin, CLA: clarithromycin, CLI: clindamycin, ERY: erythromycin, FOS: fosfomicin, FUS: fusidic acid, GEN: gentamicin, MOX: moxifloxacin, MUP: mupirocin, OXA: oxacillin, PEN: penicillin, RIF: rifampicin, TEI: teicoplanin, TET: tetracycline, TOB: tobramycin, SXT: trimethoprim-sulfamethoxazole.

Table S4. Details of associations of antibiotic resistance with biofilm-formation by staphylococcal isolates from the bulk-tank milk of 325 sheep flocks in Greece.

Antibiotic	Proportion of biofilm-forming resistant isolates	<i>p</i>
All antibiotics	77/107 (72.0%)	0.79
Ampicillin, penicillin	56/79 (70.9%)	0.95
Clindamycin	33/41 (80.5%)	0.14
Fosfomycin	30/33 (90.9%)	0.007
Tetracycline	21/28 (75.0%)	0.63
Oxacillin	21/27 (77.8%)	0.42
Erythromycin	16/21 (76.2%)	0.59

Table S5. Details of associations of milk quality with isolation of resistant or multi-resistant staphylococcal isolates from the bulk-tank milk of 325 sheep flocks in Greece.

Isolation of resistant staphylococcal isolates from the bulk-tank milk (<i>n</i> = 99)	No isolation of resistant staphylococcal isolates from the bulk-tank milk (<i>n</i> = 226)	<i>p</i>
Somatic cell counts (cells mL ⁻¹)		
0.527 × 10 ⁶ (95% CI: 0.461 × 10 ⁶ - 0.600 × 10 ⁶)	0.473 × 10 ⁶ (95% CI: 0.430 × 10 ⁶ - 0.518 × 10 ⁶)	0.21
Flocks with increased somatic cell counts (> 1.000 × 10 ⁶ cells mL ⁻¹)		
22/99 (22.2%)	32/226 (14.2%)	0.07
Total bacterial counts (cfu mL ⁻¹)		
499 × 10 ³ (95% CI: 363 × 10 ³ - 692 × 10 ³)	360 × 10 ³ (95% CI: 288 × 10 ³ - 447 × 10 ³)	0.10
Flocks with increased total bacterial counts (> 1500 × 10 ³ cfu mL ⁻¹)		
18/99 (18.2%)	40/226 (17.7%)	0.92
Fat (%)		
6.03 ± 0.08	6.22 ± 0.06	0.05
Protein (%)		
4.46 ± 0.03	4.41 ± 0.02	0.10
Isolation of multi-resistant staphylococcal isolates from the bulk-tank milk (<i>n</i> = 39)	No isolation of multi-resistant staphylococcal isolates from the bulk-tank milk (<i>n</i> = 286)	<i>p</i>
Somatic cell counts (cells mL ⁻¹)		
0.502 × 10 ⁶ (95% CI: 0.393 × 10 ⁶ - 0.638 × 10 ⁶)	0.487 × 10 ⁶ (95% CI: 0.448 × 10 ⁶ - 0.529 × 10 ⁶)	0.93
Flocks with increased somatic cell counts (> 1.000 × 10 ⁶ cells mL ⁻¹)		
8/40 (20.0%)	46/285 (16.1%)	0.54
Total bacterial counts (cfu mL ⁻¹)		
639 × 10 ³ (95% CI: 380 × 10 ³ - 1096 × 10 ³)	373 × 10 ³ (95% CI: 309 × 10 ³ - 447 × 10 ³)	0.07
Flocks with increased total bacterial counts (> 1500 × 10 ³ cfu mL ⁻¹)		
7/40 (17.5%)	51/285 (17.9%)	0.95
Fat (%)		
5.99 ± 0.14	6.19 ± 0.05	0.16
Protein (%)		
4.48 ± 0.05	4.42 ± 0.01	0.17

Table S6. Results of univariable analysis for association with isolation of oxacillin-resistant staphylococcal isolates from the bulk-tank milk of 325 sheep flocks in Greece.

Isolation of oxacillin-resistant staphylococcal isolates from the bulk-tank milk (<i>n</i> = 26)				No isolation of resistant staphylococcal isolates from the bulk-tank milk (<i>n</i> = 299)				<i>p</i>
Management system applied in the flock								
Intensive 3	Semi-intensive 17	Semi-extensive 6	Extensive 0	Intensive 40	Semi-intensive 134	Semi-extensive 101	Extensive 24	0.17
Month into the lactation period at sampling								
0–1st 4	2nd–5th 12	6th–9th 9	After 9th 1	0–1st 19	2nd–5th 126	6th–9th 138	After 9th 16	0.30
Machine- or hand-milking								
Machine-milking 23		Hand-milking 3		Machine-milking 232		Hand-milking 67		0.19
No. of ewes in the flock								
≤ 165 ewes 4	166-330 ewes 11	331-500 ewes 8	> 500 ewes 3	≤ 165 ewes 84	166-330 ewes 109	331-500 ewes 58	> 500 ewes 48	0.48
Total milk quantity per ewe obtained during the preceding milking period								
≤ 200 L 11	201-400 L 14		> 400 L 1	≤ 200 L 163	201-400 L 126		> 400 L 10	0.49
Average number of lambs born per ewe								
≤ 1.50 22		> 1.50 4		≤ 1.50 257		> 1.50 42		0.85
Collaboration with a veterinarian								
Yes 23		No 3		Yes 254		No 45		0.63
Total visits made annually by veterinarians to the flock during the preceding season								
≤ 4 9	5 - 7 7		> 7 10	≤ 4 130	5 - 7 79		> 7 90	0.61
Clinical mastitis annual incidence risk in the flock								
≤ 0.50% 2		> 0.50% 24		≤ 0.50% 54		> 0.50% 245		0.18
Age of lamb removal from their dams								
< 45 days 12	45–60 days 12		> 60 days 2	< 45 days 107	45–60 days 158		> 60 days 34	0.55

Daily number of milking sessions					
1	2	3	1	2	3
0	19	7	1	245	53
Duration of the dry-period					
≤ 2 months	> 2 months	≤ 2 months	> 2 months		
10	16	97	202		0.53
Means of calculating live bodyweight for the administration of pharmaceutical products					
Weighing	Estimation	Weighing	Estimation		
5	21	68	231		0.68
Routine overdosing (compared to dose prescribed) of pharmaceuticals					
Yes	No	Yes	No		
7	19	54	245		0.27
Annual frequency of systemic disinfections in the farm					
0 – 1 occasion	2 – 10 occasions	> 10 occasions	0 – 1 occasion	2 – 10 occasions	> 10 occasions
4	20	2	75	208	16
					0.51
Routine administration of antimicrobials in newborns					
Yes	No	Yes	No		
3	23	67	232		0.19
Vaccination against mastitis					
Yes	No	Yes	No		
14	12	112	187		0.10
Administration of 'dry-ewe' treatment at the end of the lactation period					
Yes	No	Yes	No		
5	21	48	251		0.67
Use of teat disinfection after milking					
Yes	No	Yes	No		
4	22	48	251		0.93
Age of the farmer					
Up to 50 years	Over 50 years	Up to 50 years	Over 50 years		
16	10	181	118		0.92
Length of previous animal farming experience of the farmer					
≤ 5 years	> 5 years	≤ 5 years	> 5 years		
11	15	63	236		0.013

Education of the farmer				
Primary, secondary or post-secondary education	Tertiary education	Primary or secondary education	Tertiary education	
22	4	260	39	0.74
Farmer by profession				
Yes	No	Yes	No	
23	3	269	30	0.69
Family tradition in farming				
Yes	No	Yes	No	
22	4	261	38	0.69
Presence of working staff in the flock				
Yes	No	Yes	No	
13	13	110	189	0.18

Table S7. Results of univariable analysis for association with isolation of staphylococcal isolates resistant to at least one antibiotic from the bulk-tank milk of 325 sheep flocks in Greece.

Isolation of resistant staphylococcal isolates from the bulk-tank milk (<i>n</i> = 99)				No isolation of resistant staphylococcal isolates from the bulk-tank milk (<i>n</i> = 226)				<i>p</i>
Management system applied in the flock								
Intensive 17	Semi-intensive 53	Semi-extensive 26	Extensive 3	Intensive 26	Semi-intensive 98	Semi-extensive 81	Extensive 21	0.032
Month into the lactation period at sampling								
0–1st 13	2nd–5th 45	6th–9th 38	After 9th 3	0–1st 10	2nd–5th 93	6th–9th 109	After 9th 14	0.015
Machine- or hand-milking								
Machine-milking 83		Hand-milking 16		Machine-milking 172		Hand-milking 54		0.12
No. of ewes in the flock								
≤ 165 ewes 20	166-330 ewes 36	331-500 ewes 24	> 500 ewes 19	≤ 165 ewes 68	166-330 ewes 84	331-500 ewes 42	> 500 ewes 32	0.28
Total milk quantity per ewe obtained during the preceding milking period								
≤ 200 L 49	201-400 L 47		> 400 L 3	≤ 200 L 125	201-400 L 93		> 400 L 8	0.57
Average number of lambs born per ewe								
≤ 1.50 85		> 1.50 14		≤ 1.50 195		> 1.50 31		0.92
Collaboration with a veterinarian								
Yes 82		No 17		Yes 195		No 31		0.42
Total visits made annually by veterinarians to the flock during the preceding season								
≤ 4 42	5 - 7 20		> 7 37	≤ 4 97	5 - 7 66		> 7 63	0.13
Clinical mastitis annual incidence risk in the flock								
≤ 0.50% 17		> 0.50% 82		≤ 0.50% 39		> 0.50% 187		0.73
Age of lamb removal from their dams								
< 45 days 43	45–60 days 43		> 60 days 13	< 45 days 76	45–60 days 127		> 60 days 23	0.11
Daily number of milking sessions								
1 0	2 81	3 18	1 1	2 183	3 42	0.80		

Duration of the dry-period					
≤ 2 months		> 2 months	≤ 2 months	> 2 months	
33		66	74	152	0.92
Means of calculating live bodyweight for the administration of pharmaceutical products					
Weighing		Estimation	Weighing	Estimation	
15		84	58	168	0.037
Routine overdosing (compared to dose prescribed) of pharmaceuticals					
Yes		No	Yes	No	
18		81	43	183	0.86
Annual frequency of systemic disinfections in the farm					
0 – 1 occasion	2 – 10 occasions	> 10 occasions	0 – 1 occasion	2 – 10 occasions	> 10 occasions
4	32	3	75	196	15
					0.09
Routine administration of antimicrobials in newborns					
Yes		No	Yes	No	
20		79	50	176	0.70
Vaccination against mastitis					
Yes		No	Yes	No	
39		60	87	139	0.88
Administration of 'dry-ewe' treatment at the end of the lactation period					
Yes		No	Yes	No	
20		79	33	193	0.21
Use of teat disinfection after milking					
Yes		No	Yes	No	
20		79	32	194	0.17
Age of the farmer					
Up to 50 years		Over 50 years	Up to 50 years	Over 50 years	
61		38	136	90	0.81
Length of previous animal farming experience of the farmer					
≤ 5 years		> 5 years	≤ 5 years	> 5 years	
30		69	44	182	0.032
Education of the farmer					
Primary, secondary or post-secondary education		Tertiary education	Primary or secondary education	Tertiary education	
89		10	193	33	0.27

Farmer by profession				
Yes	No	Yes	No	
89	10	203	23	0.98
Family tradition in farming				
Yes	No	Yes	No	
84	15	199	27	0.43
Presence of working staff in the flock				
Yes	No	Yes	No	
48	51	75	151	0.009

Table S8. Results of univariable analysis for association with isolation of multi-resistant staphylococcal isolates from the bulk-tank milk of 325 sheep flocks in Greece.

Isolation of multi-resistant staphylococcal isolates from the bulk-tank milk (<i>n</i> = 40)				No isolation of multi-resistant staphylococcal isolates from the bulk-tank milk (<i>n</i> = 286)				<i>p</i>
Management system applied in the flock								
Intensive 9	Semi-intensive 23	Semi-extensive 7	Extensive 1	Intensive 34	Semi-intensive 128	Semi-extensive 100	Extensive 23	0.030
Month into the lactation period at sampling								
0–1st 6	2nd–5th 17	6th–9th 14	After 9th 3	0–1st 17	2nd–5th 121	6th–9th 133	After 9th 14	0.14
Machine- or hand-milking								
Machine-milking 35		Hand-milking 5		Machine-milking 220		Hand-milking 65		0.14
Total visits made annually by veterinarians to the flock during the preceding season								
≤ 4 19	5 - 7 6	> 7 15	≤ 4 120	5 - 7 80	> 7 85			0.21
Age of lamb removal from their dams								
< 45 days 22	45–60 days 13	> 60 days 5	< 45 days 97	45–60 days 157	> 60 days 31			0.021
Means of calculating live bodyweight for the administration of pharmaceutical products								
Weighing 9		Estimation 31		Weighing 64		Estimation 221		0.99
Annual frequency of systemic disinfections in the farm								
0 – 1 occasion 18	2 – 10 occasions 74	> 10 occasions 8	0 – 1 occasion 61	2 – 10 occasions 154	> 10 occasions 10			0.12
Use of teat disinfection after milking								
Yes 11		No 29		Yes 41		No 244		0.034
Length of previous animal farming experience of the farmer								
≤ 5 years 15		> 5 years 25		≤ 5 years 59		> 5 years 226		0.018
Presence of working staff in the flock								
Yes 18		No 22		Yes 105		No 180		0.32