

Supplementary Material

Carvacrol Essential Oil: A Natural Antibiotic against Zoonotic Multidrug-Resistant *Staphylococcus* Species Isolated from Diseased Livestock and Humans

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Table S1. Types of haemolysis and biofilm formation produced by different *Staphylococcus* spp. isolated from different sources.

Species	Source of Isolates	No. of Tested Isolates	Type of Haemolysis on Sheep Blood Agar								Biofilm Formation			
			α		β		γ		Strong		Intermediate		Total Positive	
			No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
<i>S. aureus</i>	Milk	35	0	0	24	68.6	11	31.4	14	40	5	14.3	19	54.3
	Sheep	7	0	0	5	71.4	2	28.6	4	57.1	0	0	4	57.1
	Human	24	0	0	20	83.3	4	16.7	9	37.5	2	8.3	11	45.8
<i>S. schleiferi</i>	Milk	27	16	59.3	5	18.5	6	22.2	6	22.2	5	18.5	11	40.7
	Sheep	2	2	100	0	0	0	0	0	0	1	50	1	50
	Human	10	5	50	2	20	3	30	3	30	1	10	4	40
<i>S. intermedius</i>	Milk	8	5	62.5	3	37.5	0	0	3	3.5	1	12.5	4	50
	Sheep	2	2	100	0	0	0	0	0	0	0	0	0	0
	Human	9	6	66.7	3	33.3	0	0	3	33.3	1	11.1	4	44.4
<i>S. xylosus</i>	Milk	2	1	50	0	0	1	50	1	50	1	50	2	100
	Sheep	1	0	0	1	100	0	0	0	0	0	0	0	0
	Human	15	3	20	5	33.3	7	46.7	5	33.3	2	13.3	7	46.7
<i>S. haemolyticus</i>	Milk	2	0	0	2	100	0	0	1	50	0	0	1	50
	Human	5	0	0	5	100	0	0	3	60	0	0	3	60
<i>S. epidermidis</i>	Milk	1	0	0	1	100	0	0	0	0	0	0	0	0
	Sheep	1	0	0	1	100	0	0	0	0	0	0	0	0
	Human	1	0	0	1	100	0	0	0	0	1	100	1	100
<i>S. aureolaris</i>	Human	2	0	0	0	0	2	100	0	0	0	0	0	0
Total	Milk	75	22	29.3	35	46.7	18	24	25	33.3	12	16	37	49.3
	Sheep	13	4	30.8	7	53.9	2	15.3	4	30.8	1	7.7	5	38.5
	Human	66	14	21.1	36	54.6	16	24.3	23	34.8	7	10.6	30	45.5
Overall total		154	40	26	78	50.6	36	23.4	52	33.8	20	13	72	46.8

?: was calculated according to the corresponding number (No.) of tested isolates.

Table S2. Antimicrobial susceptibility of different *Staphylococcus* spp. isolated from cows' milk samples.

Antimicrobial Disc	Disc Content (µg)	<i>S. aureus</i> (n=35)			<i>S. schleiferi</i> (n=27)			<i>S. intermedius</i> (n=8)			<i>S. xylosus</i> (n=2)			<i>S. haemolyticus</i> (n=2)			<i>S. epidermidis</i> (n=1)		
		R	I	S	R	I	S	R	I	S	R	I	S	R	I	S	R	I	S
Ampicillin	10	100	0	0	100	0	0	100	0	0	100	0	0	100	0	0	100	0	0
Cefoxitin	30	100	0	0	100	0	0	100	0	0	100	0	0	100	0	0	100	0	0
Vancomycin	30	69	0	31	77	0	23	100	0	0	50	0	50	50	0	50	100	0	0
Imipenem	10	0	0	100	0	0	100	0	0	100	0	0	100	0	0	100	0	0	100
Amoxicillin-clavulanic acid	5	100	0	0	100	0	0	100	0	0	100	0	0	100	0	0	100	0	0
Kanamycin	30	29	57	14	33	52	14	25	75	0	100	0	0	100	0	0	0	0	100
Clindamycin	2	86	6	8	86	7	7	100	0	0	100	0	0	100	0	0	100	0	0
azithromycin	15	31	23	46	18	37	44	25	25	50	50	0	50	0	100	0	0	100	0
Cefuroxime	30	91	6	3	77	15	7	100	0	0	100	0	0	100	0	0	010	0	0
Chloramphenicol	30	74	6	20	74	7	18	100	0	0	50	0	50	50	0	50	100	0	0
Sulfamethoxazole-trimethoprim	25	9	11	80	18	4	77	0	25	75	0	0	100	0	0	100	0	100	0
Ciprofloxacin	5	3	14	82	0	4	96	0	25	75	0	0	100	0	0	100	0	100	0

?: was calculated according to the corresponding number (No.) of tested isolates.

Table S3. Antimicrobial susceptibility of different *Staphylococcus* spp. isolated from Sheep abscesses samples.

Antimicrobial Disc	Disc Content (µg)	<i>S. aureus</i> (n=7)			<i>S. schleiferi</i> (n=2)			<i>S. intermedius</i> (n=2)			<i>S. xylosus</i> (n=1)			<i>S. epidermidis</i> (n=1)		
		R	I	S	R	I	S	R	I	S	R	I	S	R	I	S
Ampicillin	10	100	0	0	100	0	0	100	0	0	100	0	0	100	0	0
Cefoxitin	30	100	0	0	100	0	0	100	0	0	100	0	0	100	0	0
Vancomycin	30	71	0	29	50	0	50	0	0	100	100	0	0	100	0	0
Imipenem	10	0	0	100	0	0	100	0	0	100	0	0	100	0	0	100
Amoxicillin-clavulanic acid	5	100	0	0	100	0	0	100	0	0	100	0	0	100	0	0
Kanamycin	30	71	0	29	100	0	0	50	0	50	010	0	0	0	0	100
Clindamycin	2	100	0	0	100	0	0	100	0	0	100	0	0	100	0	0
azithromycin	15	29	29	42	0	50	50	0	0	100	0	0	100	0	0	100
Cefuroxime	30	86	0	14	50	0	50	100	0	0	0	0	100	0	0	100
Chloramphenicol	30	58	0	42	50	0	50	50	0	50	0	0	100	100	0	0
Sulfamethoxazole-trimethoprim	25	0	14	86	0	0	100	0	0	100	0	0	100	0	0	100
Ciprofloxacin	5	0	14	86	0	0	100	0	0	100	0	0	100	0	0	100

?: was calculated according to the corresponding number (No.) of tested isolates.

Table S4. Antimicrobial susceptibility of different *Staphylococcus* spp. isolated from human samples.

Antimicrobial Disc	Disc Content (µg)	<i>S. aureus</i> (n=24)			<i>S. schleiferi</i> (n=10)			<i>S. intermedius</i> (n=9)			<i>S. xylosus</i> (n=15)			<i>S. haemolyticus</i> (n=5)			<i>S. epidermidis</i> (n=1)			<i>S. aureularis</i> (n=2)		
		R	I	S	R	I	S	R	I	S	R	I	S	R	I	S	R	I	S	R	I	S
Ampicillin	10	100	0	0	100	0	0	100	0	0	100	0	0	100	0	0	100	0	0	100	0	0
Cefoxitin	30	100	0	0	100	0	0	100	0	0	100	0	0	100	0	0	100	0	0	100	0	0
Vancomycin	30	54	0	46	30	0	70	44	0	56	40	0	60	60	0	40	100	0	0	100	0	0
Imipenem	10	21	8	71	0	0	100	11	33	56	13	7	80	0	0	100	100	0	0	50	0	50
Amoxicillin-clavulanic acid	5	100	0	0	100	0	0	100	0	0	100	0	0	100	0	0	100	0	0	100	0	0
Kanamycin	30	83	17	0	70	20	10	78	11	11	93	0	7	80	20	0	100	0	0	100	0	0
Clindamycin	2	100	0	0	90	10	0	89	0	11	93	0	7	80	0	20	100	0	0	100	0	0
Azithromycin	15	50	33	17	60	10	30	78	11	11	67	20	13	80	0	20	100	0	0	100	0	0
Cefuroxime	30	92	8	0	50	30	20	67	22	11	80	13	7	80	0	20	100	0	0	50	0	05
Chloramphenicol	30	50	17	33	50	10	40	56	11	33	40	20	40	20	20	60	100	0	0	100	0	0
Sulfamethoxazole-trimethoprim	25	62	13	25	80	10	10	67	0	33	87	0	13	60	20	20	100	0	0	100	0	0
Ciprofloxacin	5	54	13	33	70	20	10	67	11	22	80	0	20	40	0	60	100	0	0	100	0	0

%; was calculated according to the corresponding number (No.) of tested isolates.

Table S5. Prevalence and distribution of resistance and virulence-associated genes in the examined *Staphylococcus* isolates.

Species	Source of Isolates	No. of Tested Isolate	Resistance Genes								Virulence Genes											
			<i>mecA</i>		<i>vanA</i>		<i>vanC1</i>		<i>ermC</i>		<i>hla</i>		<i>icaA</i>		<i>icaD</i>		<i>sva</i>		<i>sea</i>		<i>sed</i>	
			No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
<i>S. aureus</i>	Milk	19	19	100	11	57.9	17	89.5	3	15.9	11	57.9	15	78.9	17	89.5	14	73.7	0	0	0	0
	Sheep	4	4	100	4	100	4	100	1	25	3	75	3	75	4	100	3	75	0	0	0	0
	Human	11	11	100	8	72.7	10	90.1	3	27.3	7	63.6	10	90.1	11	100	8	72.7	0	0	0	0
<i>S. schleiferi</i>	Milk	11	10	90.9	4	36.4	8	72.7	0	0	3	27.3	8	72.7	7	63.6						
	Sheep	1	0	0	0	0	1	100	0	0	0	0	1	100	1	100						
	Human	4	3	75	0	0	3	75	0	0	2	50	3	75	3	75						
<i>S. intermedius</i>	Milk	4	3	75	2	66.7	2	50	0	0	2	50	2	50	1	25						
	Human	4	3	75	0	0	3	75	0	0	2	50	3	75	2	50						
<i>S. xylosus</i>	Milk	2	2	100	1	50	0	0	1	50	0	0	2	100	2	100						
	Human	7	7	100	0	0	6	85.7	0	0	3	42.9	3	42.9	3	42.9						
<i>S. haemolyticus</i>	Milk	1	1	100	0	0	1	100	0	0	0	0	1	100	1	100						
	Human	3	3	100	0	0	3	100	0	0	2	66.7	3	100	3	100						
<i>S. epidermidis</i>	Human	1	1	100	0	0	1	100	1	100	1	100	0	0	1	100						
Total	Milk	37	35	94.6	18	48.6	29	78.4	4	10.8	16	43.2	28	75.7	28	75.7						
	Sheep	5	4	80	4	80	5	100	1	20	3	60	4	80	5	100						
	Human	30	28	93.3	8	26.7	26	86.7	4	13.3	17	56.7	22	73.3	23	76.7						
Overall total		72	67	93.1	30	41.7	60	83.3	9	12.5	36	50	54	75	56	77.8						

%; was calculated according to the corresponding number (No.) of tested isolates.

Not investigated

Table S6. Primers sequences, target genes, amplicon sizes and cycling conditions for PCR and RT-PCR assays.

Primers	Primers Sequences	Amplified Product	Primary Denaturation	Amplification (35 Cycles)			Final Extension	References
				2ry Denaturation	Annealing	Extension		
<i>16S rRNA</i>	CCTATAAGACTGGGATAACTTCGGG CTTTGAGTTTCAACCTTGCGGTCTG	791 bp	94°C 5 min.	94°C 30 sec.	55°C 40 sec.	72°C 45 sec.	72°C 10 min.	[61]
<i>mecA</i>	GTAGAAATGACTGAACGTCCGATAA CCAATTCCACATTGTTTCGGTCTAA	310 bp	94°C 5 min.	94°C 30 sec.	50°C 30 sec.	72°C 30 sec.	72°C 7 min.	[62]
<i>vanA</i>	CATGACGTATCGGTAAAATC ACCGGGCAGRGTATTGAC	885 bp	94°C 5 min.	94°C 30 sec.	50°C 40 sec.	72°C 45 sec.	72°C 10 min.	[63]
<i>vanC1</i>	GGTATCAAGGAAACCTC CTTCCGCCATCATAGCT	822 bp	94°C 5 min.	94°C 30 sec.	54°C 40 sec.	72°C 45 sec.	72°C 10 min.	[64]
<i>ermC</i>	ATCTTTGAAATCGGCTCAGG CAAACCCGTATTCCACGATT	299 bp	94°C 5 min.	94°C 30 sec.	51°C 30 sec.	72°C 30 sec.	72°C 7 min.	[65]
<i>hla</i>	GAAGTCTGGTGAAAACCTGA TGAATCCTGTCGCTAATGCC	704 bp	94°C 5 min.	94°C 30 sec.	53°C 40 sec.	72°C 45 sec.	72°C 10 min.	[66]
<i>icaA</i>	CCTAACTAACGAAAGGTAG AAGATATAGCGATAAGTGC	1315 bp	94°C 5 min.	94°C 30 sec.	49°C 1 min.	72°C 1 min.	72°C 12 min.	[67]
<i>icaD</i>	AAACGTAAGAGAGGTGG GGCAATATGATCAAGATA	381 bp	94°C 5 min.	94°C 30 sec.	49°C 30 sec.	72°C 30 sec.	72°C 7 min.	
<i>spa</i>	TCAACAAAGAACAACAAAATGC GCTTTCGGTGCTTGAGATT	226 bp	94°C 5 min.	94°C 30 sec.	55°C 30 sec.	72°C 30 sec.	72°C 7 min.	[68]
<i>sea</i>	TTGGAAACGGTTAAAACGAA GAACCTTCCCATCAAAAACA	120 bp	94°C 5 min.	94°C 30 sec.	50°C 30 sec.	72°C 30 sec.	72°C 7 min.	[69]
<i>sed</i>	CCAATAATAGGAGAAAATAAAAAG ATTGGTATTTTTTTTCGTTC	278 bp	94°C 5 min.	94°C 30 sec.	57°C 40 sec.	72°C 45 sec.	72°C 10 min.	[70]

Table S7. Cycling conditions of the different primers during RT-PCR.

Target Gene	Reverse Transcription	Primary Denaturation	Amplification (40 Cycles)			Dissociation Curve (1 Cycle)		
			Secondary Denaturation	Annealing (Optics on)	Extension	Secondary Denaturation	Annealing	Final Denaturation
16S <i>rRNA</i>				55°C/ 30 sec.			55°C/ 1 min.	
<i>mecA</i>	50°C/ 30 min.	94°C/ 15 min.	94°C/ 15 sec.	50°C/ 30 sec.	72°C/ 40 sec.	94°C/ 1 min.	50°C/ 1 min.	94°C/ 1 min.
<i>vanC1</i>				54°C/ 30 sec.			54°C/ 1 min.	