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Robert Mugabi

Staphylococcus mastitis, biofilms, and antibiotic resistance: Barriers to milk quality and food safety on artisanal and farmstead cheese producing farms in Vermont

R. Mugabi, J. Barlow

University of Vermont, Burlington, Vermont, USA

Methods

Sample collection from 25 different sources on dairy farms

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Bacterial isolation and DNA extraction

↓

rpoB or tuf PCR amplification

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Sequencing and NCBI blast search

↓

In vitro antibiogram and Biofilm formation

Results

Table 1: Distribution of *Staphylococcus* species by farm

	No. of isolates per farm					
Staphylococci	A	B	C	D	E	Total (%)
S. arlettae	-	1	-	-	-	1 (0.1)
S. agnetis	-	-	-	-	3	3 (0.3)
S. aureus	14	10	31	5	8	68 (6.5)
S. auricularis	**47**	3	29	2	-	81 (7.8)
S. capitis	3	1	3	-	-	7 (0.7)
S. caprae	6	-	-	-	-	6 (0.6)
S. chromogenes	14	**36**	51	6	34	**141 (13.4)**
S. cohnii	12	5	-	-	2	19 (1.8)
S. gallinarum	-	2	5	1	-	8 (0.8)
S. epidermidis	9	4	6	2	6	27 (2.7)
S. equorum	14	4	25	9	52	**104 (10)**
S. fleurettii	-	-	-	-	36	36 (3.4)
S. haemolyticus	19	9	**152**	**71**	27	**278 (26.5)**
S. hominis	3	3	8	-	4	18 (1.7)
S. kloosii	-	-	1	-	-	1 (0.1)
S. lugdunensis	-	1	-	-	1	2 (0.2)
S. saprophyticus	1	-	-	1	2	4 (0.4)
S. pasteuri	3	1	1	1	1	7(0.8)
S. pseudintermedius	-	-	-	-	1	1 (0.1)
S. simulans	21	5	-	-	1	27 (2.6)
S. sciuri	5	-	6	-	-	11 (1)
S. succinus	1	-	-	7	**56**	64 (6)
S. vitulinus	-	-	-	7	8	15 (1.4)
S. warneri	7	1	2	7	1	18 (1.8)
S. xylosus	2	3	15	43	21	84 (8)
S. sp. C035	1	-	-	-	-	1 (0.1)
S.sp. 020902-022-273	-	1	6	4	5	16 (1.5)
Total	182	90	341	166	269	1048 (100)

Table 2: Distribution of *Staphylococcus* species by niche

	No. (%) of isolates per niche							
Staphylococci	Quarter Milk[1]	Milking System and Bulk Tank Milk[2]	Teat Skin[3]	Extra Mammary Cow Skin[4]	Environm ent[5]	Human hand and nose	Dogs and pigs	Total (%)
S. arlettae	-	-	1 (100)	-	-	-	-	1 (0.1)
S. agnetis	2 (66.7)	-	1 (33.3)	-	-	-	-	3 (0.3)
S. aureus	**35 (51.5)**	**10 (14.7)**	12 (17.6)	-	-	**10 (14.7)**	1	68 (6.5)
S. auricularis	6 (7.3)	3	**43 (53.7)**	**27 (32.9)**	2 (2.4)	-	-	81 (7.8)
S. capitis	2 (28.6)	-	4 (57.1)	-	1 (14.3)	-	-	7 (0.7)
S. caprae	1 (16.7)	-	1 (16.7)	4 (66.7)	-	-	-	6 (0.6)
S. chromogenes	**82 (58.2)**	**22 (15.6)**	18 (12.8)	15 (10.6)	2 (1.4)	-	2 (1.4)	141 (13.4)
S. cohnii	-	3 (15.8)	10 (52.6)	2 (10.5)	4 (21.1)	-	-	19 (1.8)
S. gallinarum	4 (50)	3 (37.5)	-	1 (12.5)	-	-	-	8 (0.8)
S. epidermidis	7 (25)	-	3 (14.3)	1 (3.8)	-	**16 (57.1)**	-	27 (2.7)
S. equorum	11 (10.6)	5 (4.8)	21 (20.2)	**28 (26.9)**	**29 (27.9)**	7 (6.7)	3 (2.9)	104 (10)
S. fleurettii	9 (25)	6 (16.7)	12 (33.3)	6 (16.7)	1 (2.8)	2 (5.6)	-	36 (3.4)
S. haemolyticus	37 (13.26)	10 (3.6)	**109 (39.4)**	109	7 (2.5)	5 (1.8)	1 (0.4)	278 (26.5)
S. hominis	5 (27.8)	3 (16.7)	4 (22.2)	2 (11.1)	-	4 (22.2)	-	18 (1.7)
S. kloosi	-	-	1 (100)	-	-	-	-	1 (0.1)
S. lugdunensis	-	-	-	-	-	2 (100)	-	2 (0.2)
S. saprophyticus	1 (25)	-	-	1 (25)	-	2 (50)	-	4 (0.4)
S. pasteuri	1 (12.5)	1 (12.5)	-	1 (12.5)	-	4(62.5)	-	7 (0.8)
S. pseudintermedius	-	-	-	-	-	-	1 (100)	1 (0.1)
S. simulans	13 (48)	2 (7.4)	9 (33.3)	3	-	-	-	27 (2.6)
S. sciuri	-	-	-	1 (9.1)	7 (63.6)	-	3 (27.3)	11 (1)
S. succinus	3 (4.8)	7 (11.1)	**27 (42.9)**	**15 (23.8)**	6 (9.5)	5 (6.3)	1 (1.6)	65 (6)
S. vitulinus	2 (13.3)	1 (6.7)	3 (20)	6 (40)	2 (13.3)	1 (6.7)	-	15 (1.4)
S. warneri	4 (21.1)	4 (21.1)	1 (5.3)	2 (10.5)	-	6 (36.8)	1 (5.3)	18 (1.8)
S. xylosus	9 (10.7)	4 (4.8)	21 (25)	21 (25)	**25 (29.8)**	2 (2.4)	2 (2.4)	84 (8)
S. sp. C035	-	-	-	-	-	-	1 (100)	1 (0.1)
S.sp. 020902-022-273	5 (31.3)	1 (6.3))	5 (31.3)	5 (31.3)	-	-	-	16 (1.5)
Total	239 (22.8)	85 (8.1)	306 (29 2)	250 (23.9)	86 (8.2)	66 (6.3)	16 (1.5)	1048 (100)

Bold indicates dominant species per farm (Table 1) and (niche Table 2), [1]Includes quarter and cannulated milk, [2]Includes bulk tank milk, milk filter, inflation pre-milking, and teat cup post-milking samples, [3]Includes streak canal, teat end, teat barrel, and teat laceration samples, [4]Includes perineum, hock, vagina, cow nose, and udder cleft samples, Includes stall partition rail, grain, water bowl rim, used sawdust bedding, feed trough and water trough samples.

Results

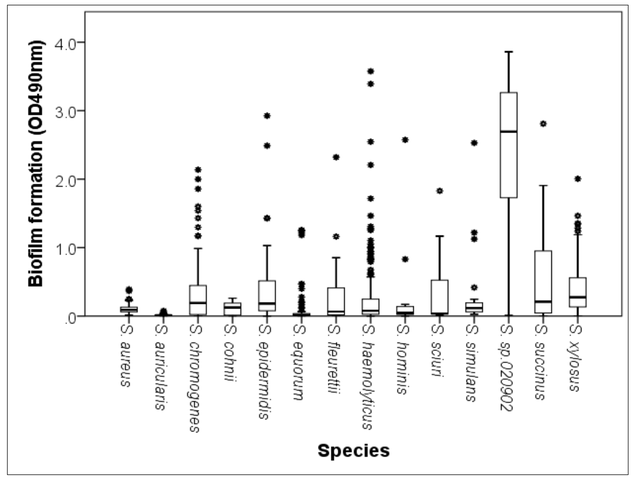

Figure 1: Box plot of the overall Biofilm formation of Staph species

(*) Indicate outlier data points. There was great variation of common CNS (*S. chromogenes* and *S. haemolyticus*) species causing IMI. Isolates from IMI cases formed stronger biofilm

Table 3: Distribution of MIC for Staphylococcus species

				Percentage of isolates inhibited at each concentration (µg/mL)														
		Resistant[1]	BP[2]															
Antimicrobial	Bacterial spp	(%)	(µg/mL)	≤0.12	≤1/2	2/4	0.25	0.5	1	2	4	8	16	32	64	128	256	NI[3]
Ampicillin	CNS	3.8	≤0.25	89.7	-	-	**6.4**	2.7	0.6	0.3	0	0	-	-	-	-	-	0.3
	S. aureus	14.8	≤0.25	68.9	-	-	16.4	3.3	**6.6**	0	3.3	1.6	-	-	-	-	-	-
Penicillin	CNS	8.9	≤0.12	**91.1**	-	-	5	1.9	0.8	0.3	0.1	0.4	-	-	-	-	-	0.4
	S. aureus	19.7	≤0.12	80.3	-	-	3.3	**8.2**	3.3	0	1.6	0	-	-	-	-	-	3.3
Cephalothin	CNS	0.1	≤8	-	-	-	-	-	-	**99.2**	0.5	0.1	-	-	-	-	-	0.1
	S. aureus	0	≤8	-	-	-	-	-	-	86.9	**8.2**	4.9	0	-	-	-	-	-
Ceftiofur	CNS	0.3	≤2	-	-	-	-	79.7	**15.5**	4	0.5	-	-	-	-	-	-	0.3
	S. aureus	0	≤2	-	-	-	-	42.1	47.4	**10.5**	0	-	-	-	-	-	-	-
Oxacillin	CNS	NA	≤0.25	-	-	-	-	-	-	**96.9**	1	-	-	-	-	-	-	2.1
	S. aureus	0	≤2	-	-	-	-	-	-	**100**	0	-	-	-	-	-	-	-
Erythromycin	CNS	6.3	≤0.5	-	-	-	68.5	17.4	1.5	**2.8**	3.3	-	-	-	-	-	-	6.3
	S. aureus	9.8	≤0.5	-	-	-	65.6	14.8	6.6	1.6	1.6	-	-	-	-	-	-	**9.8**
Pirlimycin	CNS	7.5	≤2	-	-	-	-	77.3	7.4	**7.8**	4	-	-	-	-	-	-	3.5
	S. aureus	1.6	≤2	-	-	-	-	78.7	**14.8**	4.9	-	-	-	-	-	-	-	1.6
Tetracycline	CNS	8.7	≤4	-	-	-	-	-	87.7	**2.6**	0.4	0.3	-	-	-	-	-	8.7
	S. aureus	0	≤4	-	-	-	-	-	77	**13.1**	9.8	0	-	-	-	-	-	-
Penicillin/Novobiocin	CNS	0	≤1/2	-	99.5	0.5	-	-	-	-	-	-	-	-	-	-	-	-
	S. aureus	0	≤1/2	-	**90.2**	9.8	-	-	-	-	-	-	-	-	-	-	-	-
Sulfadimethoxine	CNS	29.5	≤256	-	-	-	-	-	-	-	-	-	61	3.1	4.4	1.9	**29.5**	
	S. aureus	39.3	≤256	-	-	-	-	-	-	-	-	-	33	15	11.5	3.3	**39.3**	

1 Resistant = percentage of isolates classified resistant based on 2008 and 2013 CLSI break points, BP = Breakpoint, MIC at which an isolate is considered susceptible according to 2008 and 2013 CLSI guidelines, 3 Isolates that were not inhibited at the concentration of the antibiotic used, Dash indicates concentrations not tested for a given antibiotic; MIC 50 is underlined, MIC90 is bold

Discussion and Conclusion

- Staphylococci remain important mastitis pathogens though some species might be beneficial, molecular based species identification has given more insight on the true epidemiology of these bacteria.
- Dominant species varied between farms, and some species common on cow skin did not seem to cause mastitis suggesting that they're commensals.
- Species causing IMI (*S. aureus*, *S. chromogenes*), were rare in the environment or cow extramammary cow skin, which may suggest they spread from an infected cow to another, supporting the importance of cow teat end disinfection and hygiene in reducing mastitis cases.
- Improving hygiene during milking can reduce the transmission of some species that may spread contagiously.
- Biofilm formation was species specific and there was association predominant species and biofilm formation
- In general, antibiotic resistance was low apart from resistance to sulfadimethoxine and a few beta-lactam antibiotics

USDA

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