

BINARY TYPING AS A TOOL IN MOLECULAR AND ECOLOGICAL EPIDEMIOLOGY OF BOVINE *STAPHYLOCOCCUS AUREUS* MASTITIS

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Staphylococcus aureus remains a major cause of mastitis in dairy cattle despite widespread application of udder health programs that were introduced in the 1960s³. Those programs are based on our understanding of factors that affect presence and spread of the disease agent in the dairy environment. Lack of success in mastitis control may partially be explained by insufficient knowledge of the ecological epidemiology of *S. aureus* mastitis. The study of a disease's ecology has two objectives: firstly, to increase the understanding of the pathogenesis, maintenance and, for infectious agents, transmission of disease and, secondly, to apply this knowledge in development of control measures⁴. Molecular techniques, such as binary typing, can be used to identify *S. aureus* strains. This allows for tracing of sources and routes of pathogen spread at a more detailed level than the bacterial species level. Furthermore, association of certain *S. aureus* strains with a distinct clinical course of infection may contribute to our understanding of disease mechanisms. In this study, molecular data were combined with observational data from longitudinal studies in dairy herds to explore a possible association between specific strains of *S. aureus* and epidemiological or clinical characteristics of mastitis.

Materials and Methods

Quarter milk samples were routinely collected at 3-week (group I) or 5-week (group II) intervals from all lactating animals in 10 Dutch dairy herds in 1997-1998 (group I) and 1994-1995 (group II). Additional farmer-collected samples were available for all cases of clinical mastitis (any visual abnormality of milk and/or udder, with or without systemic signs of disease). Samples were used for bacteriologic culture according to NMC standards² and for determination of somatic cell count (SCC) by means of a Fossomatic cell counter. After identification, isolates were confirmed as *S. aureus* using the ApiStaph test and stored frozen (group I on beads at -20 °C, group II in glycerol stocks at -80°C) until use for strain typing. DNA isolation, Southern blotting and hybridization studies were performed as described previously¹, using a 16S rRNA gene probe to confirm presence of DNA, a *nuc* gene probe to confirm that isolates were *S.*

aureus, and 10 probes developed for binary typing of human *S. aureus* strains to determine binary types of bovine isolates (probes AW-1, 2, 3, 5, 6, 9, 11, 14 and 15¹).

Statistical analysis was performed using Chi-square tests (Statistix for Windows 1.0), ignoring interdependence of repeated observations within udder quarters.

Results

Among 451 isolates originating from 10 farms, 33 binary types were detected (preliminary results). The majority of isolates belonged to a limited number of predominant genotypes (8 types comprised 91% of all isolates, 3 types comprised 82 % of all isolates). Per farm, 1 to 15 types were found. Isolates belonging to one binary type originated from 1 or multiple (up to 8) farms. Frequency of isolation for the eight predominant genotypes is shown in Table 1.

Table 1. Predominant genotypes (**type**) with number of isolates found in the study (**n**) and per farm (**R1 to T7**). Total number of isolates typed per farm and number of cows and quarters from which isolates originated are shown. Columns do not add up to **total** because only predominant binary types are shown. Percentage: number of isolates with specified binary type as proportion of total number of isolates from that farm. R1 to R3: group I (sampling every 3 weeks in 1997 and 1998). T1 to T7: group II (sampling every 5 weeks in 1994 and 1995).

Type	n	R1		R2		R3		T1		T2		T3		T4		T5		T6		T7	
		n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
1105	6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	6 (6.7)	-	-	-	-
1107	186	3 (2.1)	15 (22.4)	72 (82.8)	4 (80.0)	-	9 (75.0)	6 (85.7)	72 (80.9)	-	5 (100.0)	-	-	-	-	-	-	-	-	-	
1619	10	4 (2.8)	-	1 (1.1)	-	-	-	-	-	-	-	-	-	-	-	-	5 (5.6)	-	-	-	
5203	7	4 (2.8)	-	1 (1.1)	-	1 (3.4)	1 (8.3)	-	-	-	-	-	-	-	-	-	-	-	-	-	
5715	89	86 (59.7)	3 (4.5)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
9299	8	-	8 (11.9)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
17491	10	-	-	1 (1.1)	-	-	1 (8.3)	-	-	-	-	-	-	-	-	-	7 (100.0)	-	-	-	
21587	94	34 (23.6)	36 (53.7)	1 (1.1)	-	23 (82.1)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
Total	451	144	67	87	5	28	12	7	89	7											
Cows		40	31	25	3	13	5	3	33	4	2										
Quarters		56	36	38	3	20	7	3	41	4	2										

Binary type was associated with farm of origin (farms T1, T4, T6 and T7: one predominant type only; farms R1, R2, R3, T2, T3 and T5 vs. binary type: $\chi^2=547$, $df=35$, $p=0.0000$). After stratification by farm, no association was observed between predominant binary types and subclinical vs. clinical disease (farms T1, T4, T6 and T7: one predominant type only; farms R1, R2, R3, T2, T3 and T5: $p>0.5$ for each farm).

Discussion

This study shows that binary typing can be used to characterize bovine *S. aureus* strains. The majority of intramammary infections is caused by a limited number of predominant strains per farm. This is in agreement with the contagious nature of *S. aureus* mastitis. The predominant types occurred on multiple farms throughout the country, suggesting that a limited number of *S. aureus* strains are adapted to the bovine udder as a habitat.

Analysis of preliminary results did not show a relationship between strain and outcome of infection (clinical vs. subclinical). In a previous study, a relation between genotype of *S. aureus* and clinical outcome (level of SCC elevation, severity of clinical disease) was observed⁵. At the time of submission of this abstract, final typing results for the present study were not available. Further analysis and interpretation need to be performed after definitive determination of all genotypes.

Binary typing is a simple strain typing method with high discriminatory power. Results can easily be interpreted and fully automated because they are read as a string of binary (0/1) digits. They can be used for comparison of typing results within and between studies, laboratories and host species (e.g. human strains versus bovine strains).

Identification of isolates at the strain level is a prerequisite for studies that attempt to associate specific bacterial strains with clinical or epidemiological characteristics. Molecular epidemiological tools such as binary typing can be used to enhance our understanding of the ecology of *S. aureus* mastitis and may contribute to improvement of mastitis control programs. Other possible applications of binary typing include elucidation of associations between strains and the probability of cure after treatment of mastitis (strain specific prognosis), and tracing of the origin of outbreaks of *S. aureus* infections (particularly methicillin resistant *S. aureus*) or *S. aureus* induced food poisoning.

References

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