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A Study of Phage- and Ribotype Patterns of *Staphylococcus aureus* Isolated from Bovine Mastitis in the Nordic Countries

By *F.M. Aarestrup*[1], *H.C. Wegener*[1], *N.E. Jensen*[1], *O. Jonsson*[2], *V. Myllys*[3], *B.M. Thorberg*[4], *S. Waage*[5] and *V. Thamdrup Rosdahl*[6]

[1]Danish Veterinary Laboratory, Copenhagen, Denmark, [2]Mjolkursamlag KEA, Akureyri, Iceland, [3]National Veterinary and Food Research Institute, Helsinki, Finland, [4]Swedish University of Agricultural Sciences, Uppsala, Sweden, [5]Central Veterinary Laboratory, Oslo, Norway, [6]Statens Serum Institut, Copenhagen, Denmark.

Aarestrup, F.M., H.C. Wegener, N.E. Jensen, O. Jonsson, V. Myllys, B.M. Thorberg, S. Waage, V. Thamdrup Rosdahl: A study of phage- and ribotype patterns of *Staphylococcus aureus* isolated from bovine mastitis in the Nordic countries. Acta vet. scand. 1997, 38, 243-252. – This study was conducted to investigate the geographical distribution of phage and ribotypes of *Staphylococcus aureus* causing bovine mastitis in the 5 Nordic countries. A total of 403 isolates of *S. aureus* was isolated from 403 different dairy herds. One hundred five strains were isolated in Denmark, 81 in Finland, 17 in Iceland, 96 in Norway and 104 in Sweden. The isolates were phage typed and characterized for their *Eco*RI restriction fragment length polymorphisms of the genes encoding ribosomal RNA (ribotyping). A total of 351 (87%) of the 403 isolates could be typed by phages assigning them to 25 different phage types. Two to 3 different phage types predominated within each country. One type (29/52) accounted for 36% of all the isolates and was found in 4 of the countries. A total of 87 different ribotypes was found among the isolates investigated. As for phage typing 2 to 3 different types predominated within countries. However, except for one type (ribotype 1), which was commonly found in Denmark, Sweden and Finland, different ribotypes predominated within each country. The combination of phage and ribotyping assigned the isolates to 178 different types. Ninety-six percent of the isolates of ribotype 1 belonged to phage type 29/52. This combined type accounted for 17% of all the 403 isolates. These findings show that a large number of different types of *S. aureus* can be isolated from cases of bovine mastitis. However, few types predominate within different countries. These predominating types seem to be specific in each country, however, a single type was common for both Denmark, Sweden and Finland. This could suggest differences in the virulence or in modes of transmission of predominating and rare types of S. aureus associated with bovine mastitis.

Introduction

Mastitis is the most important disease in dairy production worldwide. Before 1945 the most commonly isolated mastitis pathogen was *Streptococcus agalactiae* in almost all countries (*King* 1988). Control programmes aiming at eradicating mastitis caused by *S. agalactiae* have resulted in a decrease in infections caused by *S. agalactiae.* This has been followed by a relative or absolute increase in the importance of infections caused by *S. aureus* (*Aarestrup* 1995, *Booth* 1988, *Bramley & Dodd* 1984, *Myllys et al.* 1994). Intense research and control programmes for reducing *S. aureus* mastitis

have been conducted since the early fifties, but despite of these efforts it has not been possible to reduce the prevalence of *S. aureus* mastitis to an acceptable level. One reason might be that the epidemiology of *S. aureus* is not sufficiently understood. Typing of bacteria has proven useful in epidemiological investigations of bacterial infections, and several typing techniques are available. A number of different techniques including phage typing, plasmid profiling, ribotyping, antibiogram typing, coagulase gene typing and random amplification of polymorfic DNA has been used to study types of *S. aureus* causing bovine mastitis (*Aarestrup et al.* 1995a, *Aarestrup et al.* 1995c, *Baumgartner et al.* 1984, *Davidson* 1961, *Holmberg* 1975, *Lam et al.* 1996, *Parisi & Baldwin* 1963). All of these studies have shown that a large number of different *S. aureus* types exists. None-the-less some types appear to predominate within individual herds as well as within countries. Predominant types might reflect differences in the virulence of different types or that some types harbour other factors enabling them to spread more effectively in the bovine environment.

The purpose of this study was to determine the distribution and diversity of phage and *Eco*RI ribotypes of *S. aureus* from bovine mastitis in the 5 Nordic countries (Denmark, Finland, Iceland, Norway, Sweden). These countries are geographically close but the cattle populations in the Nordic countries are relatively separate, because very little trading of animals takes place between the Nordic countries. In the present study collections of *S. aureus* isolates from 5 Nordic countries were compared.

Materials and methods

Bacterial isolates

A total of 403 *S. aureus* isolates was isolated from 403 different dairy farms in the 5 Nordic countries from 1993 to 1994. One hundred and five isolates were from Denmark (*Aarestrup et al.* 1995c), 96 from Norway, 104 from Sweden, 81 from Finland and 17 from Iceland. All isolates were identified as *S. aureus* as previously described (*Aarestrup et al.* 1995c) using the following scheme: Gram-positive, catalase positive, oxidase negative, coagulase positive, hyaluronidase positive cocci, which were sensitive to furazolidon and novobiocin, resistant to bacitracin and produced acid from sucrose, maltose, mannitol, mannose and trehalose and did not produce acid from xylose.

Phage- and EcoRI ribotyping

All isolates were examined for their phage- and ribotype as previously described (*Aarestrup et al.* 1995c). Phage typing was performed using the international set of typing phages for human strains. All strains were initially typed at routine test dilution (RTD). Strains that were not typable at RTD were typed at 100×RTD and if still not typable, at 1000×RTD. Strains showing phage patterns with 2 or more strong reaction differences were regarded as belonging to different phage types.

For ribotyping DNA was obtained as previously described (*Aarestrup et al.* 1995c). DNA was digested with *Eco*RI and the fragments separated by agarose gel electrophoresis, Southern transferred and the genes encoding rRNA detected by hybridization with a Dig labelled rDNA probe. A single band difference was used to discriminate between different ribotypes.

Results

A total of 350 (87%) out of the 403 isolates could be typed by phages assigning the strains to 25 different phage types (Table 1). Two to 3 types predominated within each country. The number of isolates from Iceland was too limited to determine predominancy among the different types. The most prevalent phage type (29/52) accounted for 36% of all the typable strains and was commonly observed among isolates from

Table 1. Distribution of phage types among 403 isolates of *Staphylococcus aureus* isolated from bovine mastitis in 403 different herds in the 5 Nordic countries.

Phage type	Total	Denmark	Finland	Iceland	Norway	Sweden
29/52	127	33	35		22	37
83A	36	1	20	1		14
96	43	3		2	37	1
6/47/75/84/85	33	27	2	4		
3A/3C	29	7	7			15
6/81	16	1	8	3	1	3
85/89	16			1	7	8
95	8		1	1	1	5
29/52/80/84	9	7			2	
47/53/54/75/81/83A/85/95	5		3		2	
All phages	4	1		2		1
77	3	2				1
29/79/42E/47/77/81	4	4				
29/52/6/47/54/75/81	2	2				
29/52/80/83A/84/93	2	2				
29/52/42E/77/95	2	2				
80	1	1				
53	1	1				
52/3A/3C/55/6/42E/47/54/77/81/94	1	1				
29/6/47/75/84/85/89	1	1				
29/52/80/77/84/85/89	1	1				
6/42E/47/75/77/81/83A/84/93	1	1				
79/54/75/95/93	2					2
54	2			2		
80/53/84/85/89	1				1	
Non-typable	53	7	5	1	23	17
Total number of isolates	403	105	81	17	96	104

Denmark, Finland, Norway and Sweden. Phage type 83A was common in Finland and Sweden and was also found in Denmark and Iceland. Phage type 96 predominated in Norway (39%) and was recorded in Denmark, Iceland and Sweden also, whereas phage type 6/47/75/84/85 predominated in Denmark and was recorded in Finland and Iceland as well. Phage type 3A/3C was relatively commonly found in Denmark, Finland and Sweden. The other phage types were observed in relatively low frequencies. One type (6/81) was recorded in all 5 countries, phage type 95 in 4 countries, phage type 85/89 in 3 countries, a few isolates from 3 countries were susceptible to all phages, 3 phage types were found in 2 countries, and the remaining 13 types were found only in one country (Table 1).

A total of 87 different ribotypes were found among the isolates examined. Representative types are shown in Fig. 1. As for phage typing 2 to 3 different types predominated within each country (Table 2). Sixteen ribotypes, accounting for 55% of all the isolates, were found in

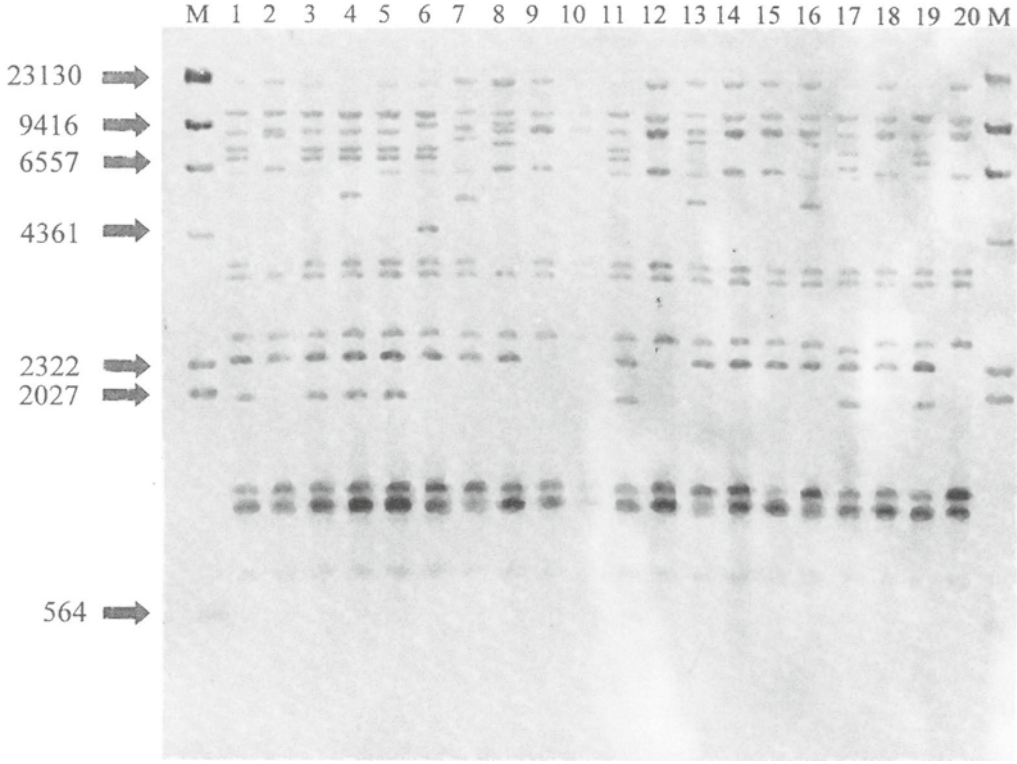

Figure 1. Representative *Eco*RI restriction fragment length polymorphisms of the gene encoding ribosomal RNA (ribotypes) of *Staphylococcus aureus* strains isolated from bovine mastitis in the 5 Nordic countries. Marker: *Hind*III digested Lambda-DNA.

more than one country. One type (ribotype 1) was common in Denmark, Sweden and Finland and found in one isolate from Norway also. This type accounted for 17% of all the isolates and is found in lanes 14, 15 and 18 in Fig. 1. Otherwise different types predominated within each country. Four types were recorded in 3 countries, 11 types in 2 countries, and the remaining 71 types were found only in one country (Table 2).

When combining phage and ribotyping a total of 178 different types was found. For some types a high degree of correspondence between ribo- and phage typing was observed, whereas for other types only limited agreement between the 2 typing methods was observed (Table 3). Agreement between the 2 methods was especially pronounced for isolates of ribotype 5, of which all isolates belonged to phage type 29/52, and for ribotype 1, of which 96% belonged to phage type 29/52. Ribotype 5 is found in lanes 9, 10, 12 and 20 in Fig. 1 and differs from ribotype 1 by one band difference only. Twelve (57%) of ribotype 60 were assigned to phage type 96. Among the isolates from Norway 8 (40%) isolates of ribotype 2 were assigned to phage type 96 and 7 (35%) to phage type 29/52. Sixteen (64%) of the 25 isolates of ribotype 3 from Finland were assigned to phage type 83A. Eighteen (58%) of the iso-

Table 2. Distribution of *Eco*RI ribotypes among 403 isolates of *Staphylococcus aureus* isolated from bovine mastitis in 403 herds in the 5 Nordic countries.

Ribotype[a)]	Total	Denmark	Finland	Iceland	Norway	Sweden
1	70	20	23		1	26
2	26				20	6
3	26	1	25			
4	19				1	18
5	18		12			6
6	14	10				4
7	9	3		5		1
8	7		1	1		5
9	8	1				7
10	7			3		4
11	5			3		2
12	3	1			1	1
13	3			2	1	
14	3	1	1	1		
15	2	1			1	
16	2		1			1
17	31	31				
18	5	5				
19	4	4				
20	4	4				
21-37	23	23				
38	4					4
39-51	19					19
52	7		7			
53-59	11		11			
60	21				21	
61	9				9	
62	5				5	
63	4				4	
64-85	34				34	
86-87	2			2		
Total	403	105	81	17	96	104

[a)] Ribotypes were discriminated by one band difference.

lates of ribotype 17 were assigned to phage type 6/47/75/84/85 and 8 (25%) to phage type 29/52. The 18 isolates from Sweden of ribotype 4 were assigned to 9 different phage types.

Discussion

S. aureus can been isolated from a wide variety of animal and human sources (*Kloos* 1980). In cattle *S. aureus* can be isolated from skin, milk, nares, vagina, perineum, hair coat, tonsils and has been isolated from several places in the cow's environment (*Davidson* 1961, *Matos et al.* 1991, *Roberson et al.* 1994). With the wide host-range of *S. aureus,* a large number of po-

Table 3. Combination of the most common ribo- and phage types among 403 isolates of *Staphylococcus aureus* isolated from bovine mastitis in the 5 Nordic countries.

Phage type	Ribotype																		Total
	1	2	3	4	5	6	7	8	9	10	11	17	18	52	60	61	62	other	
29/52	67	7		5	18			1				8			3	2		16	127
83A	1		16	3		1	1		2		1							11	36
96		8		1						1					12	3	2	16	43
6/47/75/84/85						1	3			2	1	18						8	33
3A/3C	1			1		7	1		4	1				6				8	29
6/81			7				2				1							6	16
85/89		2		1		1						1			1			10	16
95								5										3	8
29/52/80/84				1								2	1					5	9
47/53/54/75/81/83A/85/95			2											1		1		1	5
Non-typable	1	9		4		3			1	1	2	1			5	3	3	20	53
Other			1	3		1	2	1	1	2		1	4		1			12	29
Total	70	26	26	19	18	14	9	7	8	7	5	31	5	7	21	9	5	116	403

tential reservoirs for infections exists, and this may be one of the reasons for the limited success in controlling bovine mastitis. Better knowledge of the subtypes of *S. aureus* associated with bovine mastitis might make it possible to identify factors related to the bacterium or specific bacterial clones of relevance for intervention and control.

Phage typing has previously been used in several investigations of the epidemiology of *S. aureus* mastitis (*Davidson* 1961, *Edwards & Smith* 1966, *Fox et al.* 1991, *Price et al.* 1954). Low typability has been reported in some studies (*Coles & Eisenstark* 1959, St. *George et al.* 1962), whereas a high typability has been reported by others: In Ireland (*Mackie et al.* 1987), South Africa (*Swartz et al.* 1985), Nigeria (*Mohan* 1980), Denmark (*Aarestrup et al.* 1995c, *Farah et al.* 1988) and Finland (*Syrjälä & Saloniemi* 1987). In the present study a relatively high typability was observed among isolates from Denmark (93%), Sweden (84%), Finland (94%), Norway (76%) and Iceland (94%). In a Swedish study from 1975 (*Holmberg* 1975), 85% of the isolates were typable with the same set of phages. The phages 6, 42D, 75 and 83A were the phages that most commonly lysed *S. aureus* isolates in that study, whereas the phages 29 and 52 rarely caused lysis. In the present study the phages most commonly associated with lysis of the isolates from Sweden were 29, 52, 83A, 3C and 3A. Thus, when compared to the present study changes have occurred in the distribution of phage patterns of *S. aureus* from bovine mastitis in Sweden during the last 20 years. A change in types of *S. aureus* causing bovine mastitis from the 1950'ties to 1992 has previously been reported in Denmark (*Aarestrup et al.* 1995d). In a Norwegian study from 1968 (*Nygård et al.* 1968) a typability as low as 51% was recorded. This could indicate a change in the pattern in Norway during the last 30 years. However, they did

not include phage 96, which in the present study typed 39%, of all Norwegian strains.

Previous studies have reported the occurrence of *S. aureus* from bovine mastitis belonging to similar phage types in several countries (*Aarestrup* 1995, *Davidson* 1972, *Frost* 1967, *Holmberg* 1975, *Lopes et al.* 1990, *Mackie et al.* 1987, *Nygård et al.* 1968, *Parisi & Baldwin* 1963, *Swartz et al.* 1985). In the present study 12 phage types accounting for a total of 94% of the typable isolates were found in more than one of the Nordic countries. One type 29/52 (group I) predominated in Denmark, Finland, Norway and Sweden, but was not recorded among the 17 isolates from Iceland. The other predominating phage types were 83A in Sweden and Finland, 96 in Norway and 6/47/75/84/85 in Denmark.

Among Danish isolates from hospitalized human patients group I was commonly observed in the 60'ties, phage type 83A complex in the 70'ties and the phage types 95 and 94, 96 complex in the 80'ties and 90'ties (*Renneberg & Rosdahl* 1992, *Rosdahl et al.* 1990). Thus, some of the most prevalent human types are also found in bovine mastitis. This could indicate some connection between the human and bovine reservoir. A recent study using multi locus enzyme electrophoresis did only find minor correspondence between human and bovine strains (*Kapur et al.* 1995).

In the present study ribotyping was found to be more discriminatory than phage typing. As for phage typing some of the most commonly found ribotypes predominated in one country only. However, ribotype 1 was among the most predominating types in both Denmark, Sweden and Finland and was recorded in one isolate from Norway also.

Combining ribo- and phage typing produced a very large number of types. However, agreement between some ribo- and phage types was observed (Table 3). This was especially pronounced for ribotype 1 and phage type 29/52. This combined type accounted for 17% of all isolates. The occurrence of this combined type in 4 countries and as a dominating type in 3 could indicate a spread of a single clone. Isolates belonging to phage type 29/52 are relatively commonly reported from humans (*Rosdahl et al.* 1990, *Renneberg & Rosdahl* 1992), and since trading with cows among the countries is rather uncommon, it is possible that human beings have acted as vectors for the spread of the bacteria. The same agreement between phage and ribotyping was found for ribotype 5, where all isolates were assigned to phage type 29/52. This type was found in Sweden and Finland and differed from ribotype 1 by a single band. This could indicate that this clone has changed in ribotype, but remained stable in the phage type.

The remaining dominating types were almost specific to one country only, and only rarely found in other countries. This suggests that some clones have spread within the different countries, perhaps due to divergence in the sources of infection or differences in the capability of the different clones to infect the various breeds of cows.

The bovine mammary gland is highly sensitive to infection with *S. aureus,* and only a limited number of colony forming units is necessary to cause infection (*Klastrup* 1956). Already 30 years ago *Newbould* (1967) observed differences among strains of *S. aureus* in their ability to multiply in the presence of milk leucocytes. Since then differences in strains capability to adhere to bovine udder epithelial cells or to resist phagocytosis and killing by PMN have been reported (*Aarestrup et al.* 1994, *Opdebeeck et al.* 1988, *White et al.* 1980) and the predominating types have been shown to be most refractory to killing by PMN (*Aarestrup et al.* 1994). Variations among cows in their ability to eliminate *S. aureus* have also been observed (*New-*

bould 1967, *Paape et al.* 1978, *White et al.* 1980), indicating some genetic differences between cow breeds in their ability to resist infection of *S. aureus*. Recently, some bovine leucocyte antigen types have been found associated with susceptibility or resistance to infections with *S. aureus* (*Aarestrup et al.* 1995b, *Schukken et al.* 1994). These observations could indicate that the epidemiology of mastitis depends on both factors related to the genotype of the bacteria and of the genotype of the host. Better understanding of the interplay between different types of *S. aureus* and the different host genotypes may improve our possibilities to control or prevent bovine mastitis.

In conclusion, this study showed that a large number of *S. aureus* types can cause bovine mastitis, but that some types predominate. Predominating types were in most cases specific to each country indicating unique sources of *S. aureus,* or that some types might more easily infect and survive in the bovine environment in each country, maybe as a consequence of racial differences among the cattle population. However, this study also indicated that a single *S. aureus* clone has spread among cows in most of the Nordic countries.

Sammendrag

Fag- og ribotyper blandt Staphylococcus aureus isoleret fra bovin mastitis i de nordiske lande.

Med det formål at undersøge den geografiske fordeling og diversitet blandt *Staphylococcus aureus* isolater fra bovin mastitis i de 5 nordiske lande blev 403 isolater indhentet fra 403 forskellige besætninger undersøgt for deres fag- og ribotype. Fra Danmark blev 105 isolater undersøgt, fra Finland 81, fra Island 17 isolater, fra Norge 96 og fra Sverige 104. I alt kunne 351 (87%) af *S. aureus* isolaterne fagtypes, og blev inddelt i 25 forskellige typer. To til 3 forskellige fagtyper dominerede inden for hvert land. Én type (29/52) udgjorde 36% af alle de typbare isolater og blev fundet i 4 af de 5 nordiske lande. Ribotypning inddelte isolaterne i 87 forskellige typer. Som for fag typning dominerede 2 til 3 forskellige typer inden for hvert land, og med undtagelse af én meget almindelig type (ribotype 1) var der tale om forskellige typer, der dominerede inden for hvert land. Ved at kombinere fag- og ribotypning kunne isolaterne inddeles i 178 forskellige typer. De fleste (96%) af de isolater, der tilhørte ribotype 1, var af fag type 29/52. Denne kombinerede fag- og ribotype udgjorde 17% af alle isolaterne og var en dominerende type i Danmark, Sverige og Finland. Denne undersøgelse viste, at mange forskellige *S. aureus* typer kan forårsage bovin mastitis; men nogle typer er mere almindelige end andre. De fleste af disse dominerende typer er kun almindelige i ét land. Den mest almindelige type afveg dog ved at kunne findes i 4 lande. Dette kunne indicere, at der er forskelle i virulens eller transmission af dominerende og sjældne typer af *S. aureus* associeret med bovin mastitis.

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Reprints may be obtained from: F.M. Aarestrup, Danish Veterinary Laboratory, Bülowsvej 27, DK-1790 Copenhagen V, Denmark. E-mail: faa@svs.dk, tel: +45 35 30 01 00, fax: +45 35 30 01 20.