STUDIES ON THE SEROLOGICAL TYPING OF STAPHYLOCOCCI ISOLATED FROM BOVINE MILK II

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A number of reports have been made on the serological type differentiation of staphylococci by the agglutination^{1,2,4-9,11-13} or precipitation^{3,10,14-17} technique. The agglutination technique, particularly that applied to slides, has generally been accepted to be preferable because of its simplicity, speed, and sharp end-points⁶. Those reports, however, deal mainly with the typing of the strains of human origin. Accordingly, there has been little information on the typing of staphylococcal strains isolated from cow's milk.

In the serological typing of coagulase-positive strains derived from milk, it was found⁸ that the antigenic patterns of these strains differed apparently from those of the strains of human origin, and that the factor sera of Oeding were yet unsatisfactory, but that those prepared from the strains of milk origin might be available for this purpose.

The present investigation, therefore, was conducted on antisera prepared from 7 strains of staphylococci which had been selected from among the strains derived from cow's milk. Antigenic analysis was carried out on the 7 strains. In addition, type division was performed on coagulase-positive staphylococci isolated from milk samples, by using absorbed sera derived from the 7 strains.

MATERIALS AND METHODS

Strains employed: A total of 162 strains of coagulase-positive staphylococci isolated from cow's milk were employed. Of them, 7 strains which had been isolated from normal (406, Y52, and H161), abnormal (Y96 and H121), and mastitis milk (H98 and H148), were used for antigenic analysis. In Tables 1 and 2 are shown their biological characters.

In addition, 115 strains of coagulase-negative staphylococci isolated from the nose, tonsil, udder skin, intestine, and uterus of cows, horses, sheep, and pigs were used to compare the antigenic pattern with the strains mentioned above. Their biological characters are indicated in Tables 3 and 4.

Both the type strains of staphylococci and the type sera of Oeding were used in this experiment.

Table 1. Biological Characters of Strains Examined

Number of strains																	
Pigment			Nitrates reduction		NH ₄ H ₂ PO ₄		Gelatin liquefaction		Fermentation of				Coagulase		Hemolysis		Total
Yellow	White	Lemon							Mannitol		Lactose						
			+	—	+	—	+	—	+	—	+	—	+	—			
144	18	0	158	4	3	159	161	1	160	2	153	9	162	0	162	0	162

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Table 2. Biological Characters of 7 Strains Examined

Strain No.	Pigment	Nitrates reduction	NH ₄ H ₂ PO ₄	Gelatin liquefaction	Fermentation of		Coagulase	Hemolysis	Quality of milk sample
					Mannitol	Lactose			
406	Yellow	+	—	+	+	+	+	β	Normal
Y52	White	+	—	+	+	+	+	$\alpha\delta$	Normal
H161	Yellow	+	—	+	+	+	+	$\alpha\beta\delta$	Normal
Y96	White	+	—	+	+	—	+	$\alpha\beta\delta$	Abnormal
H121	White	+	—	+	+	+	+	$\alpha\delta$	Abnormal
H98	Yellow	+	—	+	+	+	+	$\alpha\beta$	Mastitis
H148	Yellow	+	—	+	+	+	+	$\alpha\beta\delta$	Mastitis

Table 3. Biological Characters of Strains of Coagulase-Negative Staphylococci Isolated from Cow's Milk

Number of strains																		
Pigment			Nitrates reduction		NH ₄ H ₂ PO ₄		Gelatin liquefaction		Fermentation of				Coagulase		Hemolysis		Total	
Yellow	White	Lemon							Mannitol	Lactose								
			+	—	+	—	+	—	+	—	+	—	+	—	+	—		
49	61	5	4	111	0	115	3	112	60	55	115	0	0	115	18	97	115	

Table 4. Biological Characters of Strains of Animal Origin

Number of strains																		
Source	Pigment			Nitrates reduction		NH ₄ H ₂ PO ₄		Gelatin liquefaction		Fermentation of				Hemolysis		Coagulase		
	Yellow	White	Lemon							Mannitol		Lactose						
				+	—	+	—	+	—	+	—	+	—	+	—	+	—	
Cow	4	1	0	5	0	0	5	5	0	5	0	5	0	5	0	5	0	
Horse	14	3	0	17	0	0	17	17	0	14	3	15	2	17	0	17	0	
Sheep	10	0	0	10	0	0	10	10	0	10	0	10	0	10	0	10	0	
Pig	10	2	0	12	0	0	12	12	0	9	3	12	0	8	4	12	0	
	38	6	0	44	0	0	44	44	0	38	6	42	2	40	4	44	0	
Cow	7	2	0	9	0	9	0	9	0	6	3	4	5	0	9	0	9	
Horse	13	1	0	14	0	0	14	14	0	13	1	9	5	1	13	0	14	
Sheep	6	3	0	9	0	9	0	9	0	6	3	6	3	0	9	0	9	
Pig	6	8	0	14	0	0	14	14	0	2	12	13	1	1	13	0	14	
	32	14	0	46	0	18	28	46	0	27	19	32	14	2	44	0	46	

The techniques employed for preparation of antisera, absorption of antisera, and agglutination reaction were the same as described in the previous part of this investigation⁸⁾.

RESULTS

1. Antigenic analysis on 7 strains of staphylococci

Of the 7 strains analyzed, only three (406, Y52, and H148) were typable with the factor sera of Oeding, as shown in Table 5.

First, cross-agglutination reaction was carried out between the 7 strains and the type sera, between the type strains and antisera of the 7 strains, and between the 7 strains and their antisera in order to find out whether any antigenic correlation existed between them.

Table 5. Agglutination Reactions of 7 Strains with Factor Sera of Oeding

Strain No.	Factor serum							
	ac	a	b	c	e	h	i	k
406	+	—	—	—	+	—	—	—
Y52	+	—	—	—	—	—	—	—
H161	—	—	—	—	—	—	—	—
Y96	—	—	—	—	—	—	—	—
H121	—	—	—	—	—	—	—	—
H98	—	—	—	—	—	—	—	—
H148	+	+	+	—	—	—	—	—

Table 6. Agglutination Reactions of 7 Strains with Antisera of Type Strains

Strain No.	Antiserum								
	1503	2095	2253	3189	17A	F21	S365	28	3647
406	+++	—	+++	—	+	+	—	+++	+
Y52	+++	+++	+++	+++	+++	+++	+++	+++	+++
H161	+++	—	++	—	+	—	—	++	—
Y96	+++	+++	++	++	++	+++	+++	+	—
H121	+++	+++	+++	+++	+++	+++	++	++	++
H98	++	++	+++	+++	+	++	+++	++	+
H148	+++	+++	+++	+++	+++	+++	+++	+++	+++

Table 7. Agglutination Reactions of Type Strains with Antisera of 7 Strains

Strain No.	Antiserum						
	406	Y52	H161	Y96	H121	H98	H148
1503	++	—	+	—	—	—	—
2095	++	—	—	+	—	—	—
2253	++	—	+	—	+	+	+
3189	++	—	—	+	—	—	—
17A	—	—	—	—	+	+	—
F21	++	—	—	+	—	+	—
S365	++	—	—	—	—	—	—
28	++	—	—	—	+	—	—
3647	++	—	—	—	—	—	—

Table 8. Agglutination Reactions of 7 Strains with Their Antisera

Strain No.	Antiserum						
	406	Y52	H161	Y96	H121	H98	H148
406	+++	+	+	++	+	+	+
Y52	+++	++	+++	+++	+++	+++	++
H161	—	—	++	—	—	—	—
Y96	+++	—	++	+++	+	—	+
H121	+++	+++	+++	+++	+++	++	++
H98	+++	+	—	+	+	++	+
H148	+++	++	+++	+++	+++	+++	+++

The results of these experiments are given in Tables 6, 7, and 8.

From the results obtained, it was presumed that the 7 strains might probably contain a few reciprocal antigens, because marked cross-reaction was observed between the 7 strains and their antisera, in spite of the fact that 4 of the 7 strains were untypable with the factor sera of Oeding.

There are, in common, 42 kinds to absorb each of the 7 antisera with one of the 7 strains, except the homologous strain. Table 9 indicates the results of cross-absorption experiments between the 7 strains and the 42 absorbed sera. If it is assumed that one of the 42 absorbed sera, which failed to react with some of the 7 strains, except the homologous one, has a specific factor, the strains which agglutinated such serum are considered to contain several antigens specific for the serum. As a result of the experiments, 5 of the 42 absorbed sera were regarded as factor sera because of their having only one factor in agglutinin absorption. These 5 factor sera were designated P, T, V, W, and X. The 7 strains were presumed to contain 4 to 8 antigens responsible for the factor sera respectively, e.g., 406, VWXYZ; Y52, QSTXYZ and so forth as shown in the same table. Accordingly, the antigenic structure of the 7 strains can be summarized as shown in Table 10. Since the 5 factor sera, except V, were obtained in pair at a time, each one of the pairs containing agglutinins of high titer was employed as listed in Table 11. A set of these factor sera was called factor sera (1) in this experiment.

2. Serological typing by means of factor sera (1)

From the results of agglutination reactions of the 9 type strains of Oeding with the set of factor sera (1), it seemed difficult to find any definite correlation between the

Table 9. Cross-Absorption Tests between 7 Strains and Their Antisera

Antiserum	Absorbed with	Strains and their antigens							Antigen* assumed	Remaining factors
		406	Y52	H161	Y96	H121	H98	H148		
		VWXYZ	QSTXYZ	TVWYZ	PQST	PQSX	PQSTVXYZ	QTVXY		
406	Y52	+	—	+	—	—	+	+	V	VW
Y52	406	—	+	+	+	+	+	+		QST
406	H161	+	+	—	—	+	+	+	X	X
H161	406	—	+	+	+	—	+	+	T	T
406	Y96	+	+	+	—	+	+	+		VWXYZ
Y96	406	—	+	+	+	+	+	+		PQST
406	H121	+	+	+	—	—	+	+	Y	VWYZ
H121	406	—	+	—	+	+	+	+	Q	PQS
406	H98	+	—	+	—	—	—	—	W	W
H98	406	—	+	+	+	+	+	+		PQST
406	H148	+	+	+	—	—	+	—	Z	WZ
H148	406	—	+	+	+	+	+	+		QT
Y52	H161	+	+	—	+	+	+	+		QSX
H161	Y52	+	—	+	—	—	+	+		VW
Y52	Y96	+	+	+	—	+	+	+		XYZ
Y96	Y52	—	—	—	+	+	+	—	P	P
Y52	H121	+	+	+	+	—	+	+		TYZ
H121	Y52	—	—	—	+	+	+	—		P
Y52	H98	—	—	—	—	—	—	—		—
H98	Y52	+	—	+	+	+	+	+		PV
Y52	H148	+	+	+	+	+	+	—		SZ
H148	Y52	+	—	+	—	—	+	+		V
H161	Y96	+	+	+	—	—	+	+		VWYZ
Y96	H161	—	+	—	+	+	+	+		PQS
H161	H121	+	+	+	+	—	+	+		TVWYZ
H121	H161	+	+	—	+	+	+	+		PQSX
H161	H98	+	—	+	—	—	—	—		W
H98	H161	+	+	—	+	+	+	+		PQSX
H161	H148	+	+	+	—	—	+	—		WZ
H148	H161	+	+	—	+	+	+	+		QX
Y96	H121	—	+	+	+	—	+	+		T
H121	Y96	+	+	—	—	+	+	+		X
Y96	H98	—	—	—	—	—	—	—		—
H98	Y96	+	+	+	—	+	+	+		VXYZ
Y96	H148	—	+	—	+	+	+	—	S	PS
H148	Y96	+	+	+	—	+	+	+		VXY
H121	H98	—	—	—	—	—	—	—		—
H98	H121	+	+	+	+	—	+	+		TVYZ
H121	H148	—	+	—	+	+	+	—		PS
H148	H121	+	+	+	+	—	+	+		TVY
H98	H148	+	+	+	+	+	+	—		PSZ
H148	H98	—	—	—	—	—	—	—		—

* Name given by the author.

Table 10. Antigenic Structure of 7 Strains

Strain No.	Antigen*							
406				V	W	X	Y	Z
Y52	Q	S	T			X	Y	Z
H161				T	V	W	Y	Z
Y96	P	Q	S	T				
H121	P	Q	S				X	
H98	P	Q	S	T	V		X	Y Z
H148		Q		T	V		X	Y

* Name given by the author.

Table 11. Preparation of Factor Sera

Factor serum	Antiserum	Absorbed with
P	{Y96 H121	Y52 Y52 *
T	{H161 Y96	406 H121 *
V	H148	Y52
W	{406 H161	H98 H98 *
X	{406 H121	H161 Y96 *

* Factor serum which contains agglutinin of high titer.

Table 12. Agglutination Reactions of Type Strains with Factor Sera

Strain No.	Factor serum					Serological type of Oeding
	P	T	V	W	X	
1503	—	—	—	+	+	ae
2095	—	+	—	+	+	abc
2253	—	—	—	+	—	ace
3189	—	+	—	+	+	bf
17A	—	—	—	—	—	h
F21	—	—	—	—	—	abci
S365	—	—	—	+	—	abk
28	—	—	—	+	+	ae
3647	—	—	—	+	+	abc

Table 13. Distribution of Serological Types in Strains of Milk and Animal Origin

Serological type	Number of strains isolated from			
	Cow's milk		Animals	
	Coagulase		Coagulase	
	+	—	+	—
TWX	32 (19.8)*		3 (6.8)	
T	26 (16.0)	13 (11.3)	3 (6.8)	
X	25 (15.5)	13 (11.3)	2 (4.5)	3 (6.6)
TX	19 (11.7)	8 (6.9)	1 (2.3)	2 (4.3)
TW	9 (5.6)	2 (1.7)	5 (11.5)	
W	8 (4.9)	1 (0.9)	2 (4.5)	
WX	8 (4.9)		1 (2.3)	
P		3 (2.6)	1 (2.3)	4 (8.7)
PT	1 (0.62)			2 (4.3)
PTV	1 (0.62)	1 (0.9)		
PTVWX		2 (1.7)		1 (2.2)
PTVX	1 (0.62)			
PTW			2 (4.5)	1 (2.2)
PTWX	1 (0.62)		7 (15.9)	
PTX	2 (1.24)	1 (0.9)	3 (6.8)	2 (4.3)
PVWX	1 (0.62)	1 (0.9)		2 (4.3)
PVX			1 (2.3)	
PW			2 (4.5)	
PX		2 (1.7)	4 (9.1)	
TV		1 (0.9)		
TVWX	1 (0.62)			
TVX		1 (0.9)		
V	1 (0.62)	1 (0.9)		
VW				1 (2.2)
—	26 (16.0)	65 (56.5)	7 (15.9)	28 (60.9)
Total	162(100.0)	115(100.0)	44(100.0)	46(100.0)

* In parentheses is shown percentage.

antigenic patterns of the 9 type strains with the factor sera of Oeding and those with the factor sera of the present author shown in Table 12.

Comparison was made between the distribution of antigens of the strains employed and that of the other strains used as control materials in agglutination with factor sera (1). The results are given in Table 13 and Fig. 1 which follows it. Of the 162 strains of coagulase-positive staphylococci derived from cow's milk, 136 (84.0%) were proved typable with the factor sera. Of the 136 strains, 127 (93.4%) reacted with neither P nor V, but 9 (6.6%) did with either of them. On the other hand, of 50 typable strains of coagulase-negative staphylococci derived from cow's milk, 37 (74.0%) reacted with neither P nor V, but 13 (26.0%) did with both of them.

As for the strains of staphylococci of animal origin which were typed with the same factor sera, 20 (54.1%) of 37 coagulase-positive strains and 13 (72.0%) of 18 coagulase-

negative strains agglutinated with either P or V.

From the data herein presented, it was clarified that much more strains agglutinated with either P or V from among the coagulase-negative staphylococci of milk origin and those of animal origin than from among the coagulase-positive staphylococci derived from milk samples. It was also found that the antigenic patterns of the coagulase-negative strains were evidently different from those of the coagulase-positive ones derived from milk samples.

It must also be mentioned that the typable strains of coagulase-positive staphylococci derived from cow's milk was much higher in proportion than those of coagulase-negative ones of milk and animal origin.

Fig. 1. Distribution of Serological Types in Strains of Milk and Animal Origin

DISCUSSION

As described above, the strains of coagulase-positive staphylococci isolated from cow's milk were typable, in a high percentage, with factor sera (1) prepared from the strains of the same origin, although no marked reactions between the 9 type strains and the factor sera were observed (Table 12). It became clear, therefore, that the antigens of the staphylococcal strains isolated from cow's milk were heterogeneous and as complex as those of human origin, being shared with almost all strains.

Generally speaking, there may occur as many types as the number of sera used in the typing of organisms, especially when a considerable number of antigens are distributed in these organisms. OEDING¹²⁾ divided 459 strains of coagulase-positive staphylococci of human origin into 12 types; however, he pointed out in his later report¹³⁾ that there were 4 main groups and a limited number of additional groups of minor importance with only qualitative and quantitative differences in distribution of antigens.

As a result of experiment, the 162 strains of coagulase-positive staphylococci isolated from cow's milk were divided into 15 types. While a small number of strains had antigenic factor P or V, the majority of the strains had one of factors T, W, and X. Therefore, the latter 3 factors were supposed to be distributed most widely among them. Accordingly, it was considered that the strains examined could be divided into 7 main groups, which had one of factors T, W, and X, and some less important groups, which had factor P or V.

On the other hand, the antigenic patterns of both coagulase-negative strains derived from milk samples and strains of animal origin differed apparently from those of coagulase-positive strains derived from milk samples.

Owing to the presence of antigenic overlap among the 7 strains examined (Table 10), it was not possible to choose a single serum applicable to the detection of normal, abnormal, and mastitis milk.

SUMMARY

With immune sera prepared from 7 strains of coagulase-positive staphylococci derived from normal, abnormal, and mastitis milk, cross-absorption tests and antigenic analysis were performed.

Then serological typing was carried out of 162 strains of coagulase-positive staphylococci isolated from milk samples with a set of 5 factor sera prepared by the present author. The antigenic patterns of these strains were compared with those of 115 strains of coagulase-negative staphylococci derived from milk samples and 44 strains of coagulase-positive and 46 strains of coagulase-negative staphylococci of animal origin. The results obtained are as follows:

1. From the results of antigenic analysis on the 7 strains by means of cross-absorption, it was presumed that these strains might contain 4 to 8 antigens respectively.
2. A set of 5 factor sera which had a single factor specific for a given strain was obtained by cross-agglutination reactions between the 7 strains and 42 absorbed sera.
3. Of the 162 strains of coagulase-positive staphylococci isolated from cow's milk, 136 (84.0%) were classified into 7 main types and 26 (16.0%) remained untyped.
4. The antigenic pattern of the coagulase-positive strains derived from milk samples was apparently differed from that of the coagulase-negative strains of the same origin. Such difference was more obviously seen in the case of the staphylococcal strains of animal origin.
5. The coagulase-positive strains derived from milk samples could be typed in a higher percentage than the coagulase-negative ones of milk and animal origin.
6. It was difficult to find any significant correlation between the factor sera and the property of milk samples.

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牛乳由来 *Staphylococcus* の血清学的型別に関する研究 II

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牛乳から分離されたコアグラゼ陽性のブドウ球菌の型別を Oeding の方法に従い、因子血清を用いて行なった。その結果、抗原因子の分布は、人由来菌株のそれと明らかに異なることを前報で報告した。従って、人由来の菌株から作成した因子血清を、ただちに牛乳由来の菌株の型別に利用することは適当ではなく、この目的には、同じく牛乳由来の菌株から作成した抗血清を用いることが、妥当であると考えられた。

そこで今回は、供試した牛乳由来コアグラゼ陽性菌 162 株のうちから、その由来が正常乳からのもの 3 株、異常乳および乳房炎乳からのもの各 2 株、計 7 株を選んで、抗血清を作成し、これと 7 株との交差吸収試験によって、抗原分析を行なった。また、その際得られた因子血清によって、供試菌の型別を行ない、その抗原因子型を、牛乳由来のコアグラゼ陰性菌 115 株および家畜由来の菌 90 株のそれと比較した。これらの結果は、次のとおりである。

1. 7 菌株について、その抗血清との交差吸収試験によって、抗原分析を行なった結果、7 菌株は、それぞれ 4~8 個の抗原を所有するものと推定された。

2. その際に得られた吸収血清と 7 菌株との交差凝集反応によって、独自の抗体因子を有する 5 つの因子血清 (1) が得られた。

3. これら 5 種の因子血清 (1) によって、牛乳由来のコアグラゼ陽性菌 162 株の型別を行なった。その結果、136 株 (84.0%) が型別可能で、そのうち 127 株 (93.4%) は、7 つの主要な型に型別された。

4. 牛乳由来のコアグラゼ陽性菌株の抗原因子型は、同じ由来のコアグラゼ陰性菌株のそれに比べて、かなり違っていた。この差異は、家畜由来の菌株と比較した場合に、一層明らかに認められた。

5. 牛乳由来および家畜由来のコアグラゼ陰性菌株では、本因子血清によって型別が可能であったものは、きわめて低率であった。

6. 本因子血清は、7 菌株との交差凝集反応において、いずれも著明な交差反応を示した。そのため、正常乳、異常乳または乳房炎乳汁から分離した 7 菌株のいずれかに対して、特異的に反応するものを、この因子血清の中から見いだすことはできなかった。