



## Ecological Studies on Coagulase-negative Staphylococci in and around Bovine Udder

メタデータ	言語: eng 出版者: 公開日: 2009-08-25 キーワード (Ja): キーワード (En): 作成者: BABA, Eiichiro, FUKATA, Tsuneo, MATSUMOTO, Haruyasu メールアドレス: 所属:
URL	<a href="https://doi.org/10.24729/00009398">https://doi.org/10.24729/00009398</a>

## Ecological Studies on Coagulase-negative Staphylococci in and around Bovine Udder

Eiichiro BABA\*, Tsuneo FUKATA\*, and Haruyasu MATSUMOTO\*\*

\*Laboratory of Veterinary Internal Medicine, College of Agriculture, \*\*Laboratory of Veterinary Internal Medicine, College of Agriculture, Yamaguchi University

(Received October 31, 1979)

### Abstract

The purpose of this work is to investigate the distribution of coagulase-negative staphylococci isolated from milk and skin samples of 35 cows. These strains were identified mainly according to the scheme described by Kloos and Schleifer. The results are summarized as follows:

1) A total of 243 strains of coagulase-negative staphylococci were isolated. These were classified into 9 species according to Kloos and Schleifer's method. From the abnormal milk samples in which coagulase-negative staphylococci were predominant, 4 species (35 strains) were isolated and 66 percent of the strains were *S. epidermidis*. From the other abnormal milk samples and the normal samples, 6 and 8 species were isolated, respectively. In both cases, *S. epidermidis* occupies one-third of the strains. Nine species were detected in the skin samples. One-third of the strains were *S. haemolyticus* and only a few strains were *S. epidermidis*. It was recognized that the distribution pattern in terms of species in milk samples differed from that in skin samples.

2) From these results, it seems likely that *S. epidermidis* play more significant role than the other species as a cause of abnormal milk.

3) A fewer number of species than the Kloos and Schleifer's method was obtained by the other methods for classification of staphylococci conducted at the same time; i.e., the biotype method by Baird-Paker, the modified method by Pelzer *et al.* and phage-typing method.

### Introduction

Bacteria causing bovine mastitis are commonly widespread in the premises of the dairy cow. Although several species of bacteria are known to be involved in the complexity of mastitis, staphylococci and streptococci were give first rank as causative agents. Until recently, it has been emphasized that streptococci were the primary pathogen of mastitis. At present, however, staphylococci become the most popular pathogens that can readily be isolated from clinical mastitis. It is considered that this phenomenon was caused by the extensive use of antibiotics to control the bovine mastitis.

Staphylococci include coagulase-positive and -negative cocci. The former are pathogenic and the latter have been commonly regarded as nonpathogenic. In the series of our studies, coagulase-negative staphylococci were isolated as readily as coagulase-positive staphylococci from the cows of clinical or subclinical mastitis<sup>1)</sup>. Reports have indicated that relatively common biochemical properties exist in various strains of coagulase-positive cocci, while the properties of coagulase-negative cocci are different depending upon strains<sup>2)-5)</sup>. It is of great interest to know whether all or any particular strains of coagulase-negative staphylococci play an important role as causative agents of mastitis.



where severe infections with coagulase-positive staphylococci or streptococci were rare. The quarter samples included normal and abnormal milk. The quality of milk was scored by the California mastitis test. The skin samples were made of swabs on the lateral udder surface and upper flank. In expecting recovery of various species of cocci from a specimen, a large number of colonies were picked from each sample. Coagulase-negative staphylococci obtained were classified by four methods. The first method was conducted using the scheme for identification of human staphylococci reported by Kloos and Schleifer<sup>6)</sup>. This scheme includes 9 strains of coagulase-negative staphylococci, and was shown in Fig.1. If more than one strain of the same species were recovered from one sample, they were regarded as a single strain.

The other three classifications were the biotypes reported by Baird-Paker<sup>7)</sup>, the modification by Pelzer *et al.*<sup>8)</sup> which is a modified method of the Baird-Paker's classification system, and the phage-typing set for coagulase-negative staphylococci characterized by differences in bacterolytic reactions prepared in our laboratory<sup>9)</sup>.

## Results

Coagulase-negative staphylococci were isolated from 31 of 43 normal milk samples, 54 of 90 abnormal milk samples and 63 of 66 skin swabs.

Under the classification by the Kloos and Schleifer's system, a total of 243 strains of coagulase-negative staphylococci including 4 untyped strains were isolated. These strains were divided into 4 groups (A, B, C, and D) according to their sources. Group A was designated by 35 strains isolated from abnormal milk where coagulase-negative staphylococci were predominant in the samples. Group B consisted of 33 strains isolated from abnormal milk where the other bacteria were predominant. Group C composed of 48 strains isolated from normal milk samples. Group D included 127 strains from skin samples. Frequency of occurrence of the Kloos and Schleifer's 9 species in each of the 4 group is presented in Fig.2. There were only 4 species in group A. *S. epidermidis* was the highest, 66%, followed by *S. haemolyticus*, *S. xylosus*, and *S. capitis*. Group B consisted of 6 species. They were *S. epidermidis*, 33%, followed by *S. cohnii*, *S. haemolyticus*, *S. xylosus*, *S. hominis*, and *S. saprophyticus*. In group C only *S. simulans* was not present. The percentage of *S. epidermidis* was the highest. *S. haemolyticus*, *S. xylosus*, *S. cohnii*, and *S. hominis* were present. In group D, all of 9 species were detected. Percentage for *S. haemolyticus* was 34 percent and that for each of *S. xylosus* and *S. cohnii* was 24 percent. *S. epidermidis* was detected only at 3 percent.

Only 4 percent of all strains were classified by biotypes of Baird-Paker's method. In milk samples, there were a few strains belonging to biotype 1 or 2, and none to the other biotypes. Each strain belonging to 3 biotypes except biotype 3 was isolated from skin samples.

Seventy-three percent of all strains were classified by the modification of Pelzer's method. The results of this method are shown in Fig.3 using frequency of occurrence in 8 subtypes. It is apparent that the distribution patterns of the strains in milk samples were different from those in the skin samples. Especially, the strains belonging to the pelzer's subgroup IIb were only 3 percent in skin samples (group D), while they were approximately 40 percent in abnormal milk samples (group A). Most of these strains belonged to *S. epidermidis* under the classification of Kloos and Schleifer.

Phage-typing of coagulase-negative staphylococci was performed by the use of the

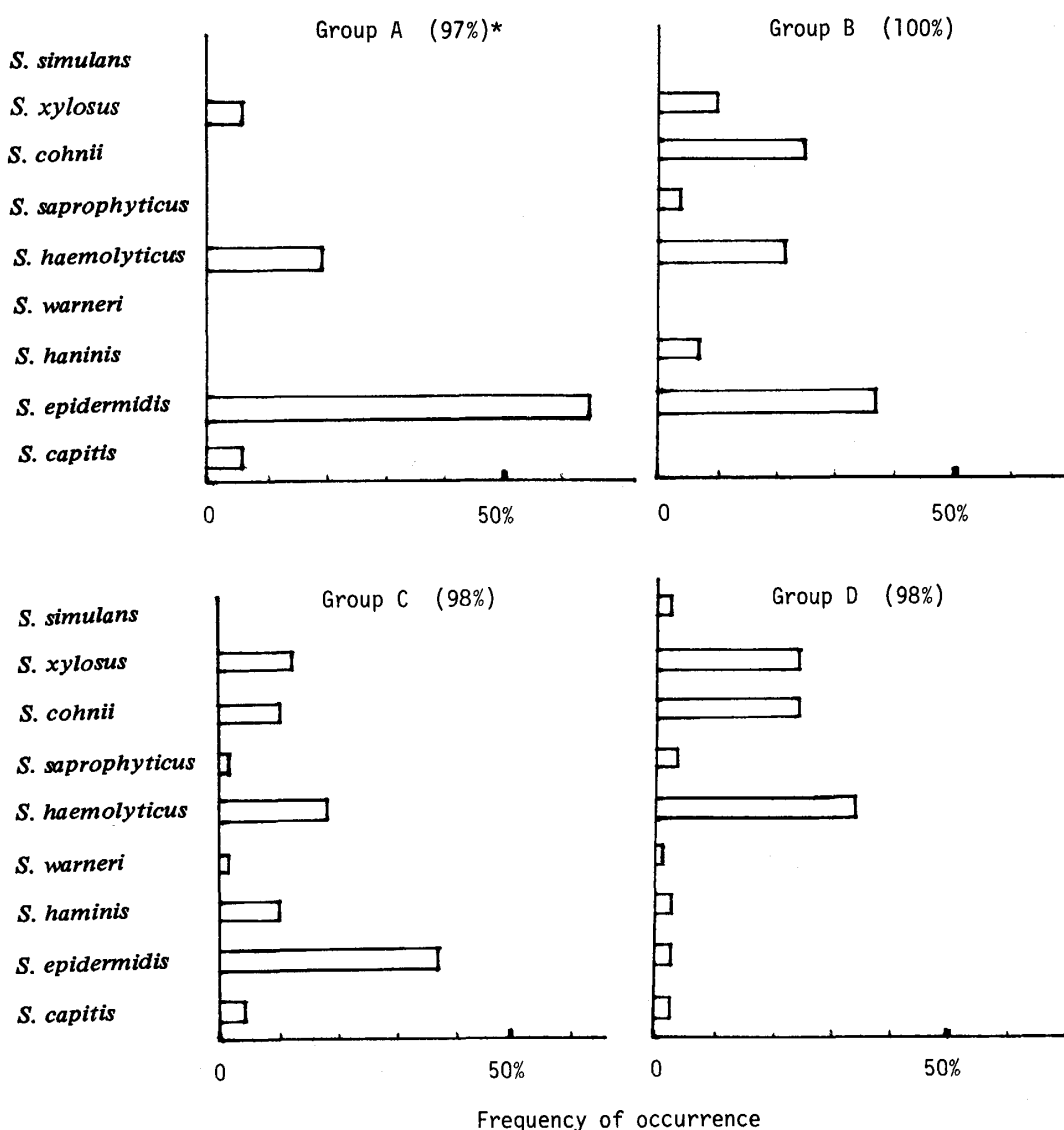


Fig. 2 Frequency of Occurrence of Coagulase-negative Staphylococci in Bovine Milk and Skin Samples according to the Kloos and Schleifer's Classification Method

Group A : Abnormal milk samples in which coagulase-negative staphylococci were predominant.

Group B : Abnormal milk samples in which bacteria other than coagulase-negative staphylococci were predominant.

Group C : Normal milk samples

Group D : Skin swabs from udder and upper flank

\* : Percentage of strains classified

provisional phage set which had been prepared for coagulase-negative staphylococci in our laboratory. The results were presented in Fig.4. Fifty-two percent of the strains were typed by this phage set. Differences were evident in the phage typing patterns between strains isolated from milk samples and those from skin samples. Most of the strains classified in our phage type III plus IV belonged to *S. epidermidis* under the classification of Kloos and Schleifer.

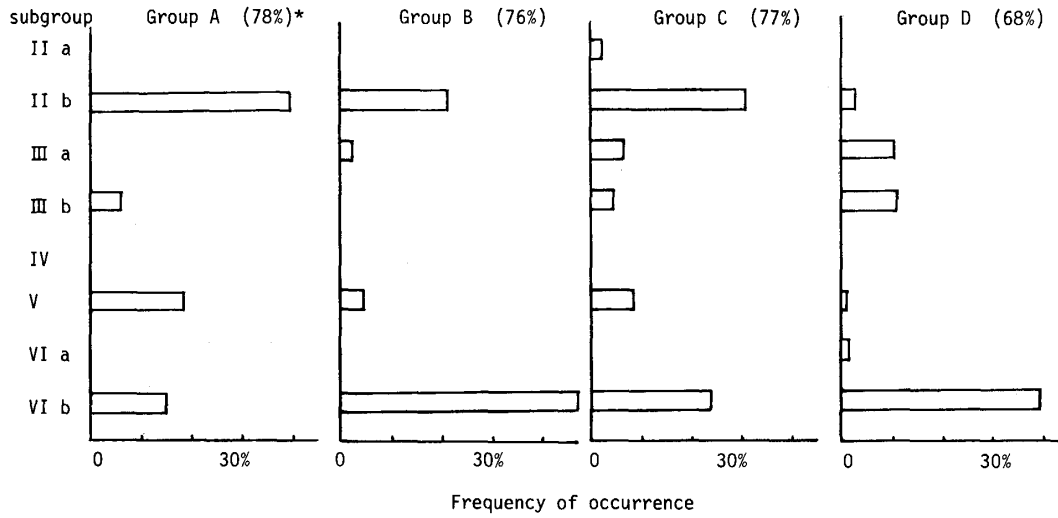


Fig. 3 Frequency of Occurrence of Staphylococci in each Subgroup according to the Pelzer's Method  
\* : Percentage of strains classified

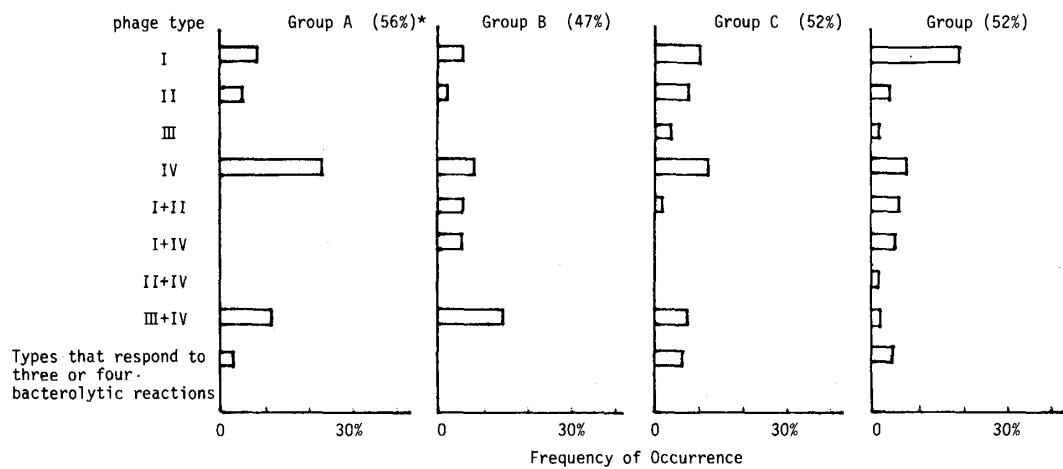


Fig. 4 Frequency of Occurrence of Staphylococci in each Phage type according to Our typing Set  
\* : Percentage of strains classified

### Discussion

The importance of coagulase-positive staphylococci as a cause of acute or chronic mastitis has been emphasized for many years. Economic importance of subclinical mastitis caused by coagulase-negative staphylococci should be also recognized due to reduction in milk production and degradation in milk quality. In the present survey, coagulase-negative staphylococci were detected in 64 percent of milk samples studied, and they were predominant in 19 percent of abnormal milk samples.

Kloos and Schleifer made a scheme which can be easily used for the classification of

human staphylococci. In the present study, this scheme has been used for classification of bovine staphylococci. Kume *et al.*<sup>10)</sup> conducted a similar work. There were some differences in frequency of occurrence between our results and Kume's. For instance, Kloos' *S. epidermidis* was detected in 44 percent of milk samples in this survey. On the other hand, it was detected in 11 percent of their samples. *S. warneri* was isolated from 1 percent in our study and 12 percent in their samples. These differences might be attributed to differences in samples studied.

There was a difference in the distribution pattern of each Kloos' species among the 4 groups. The number of the Kloos' species detected in group A was less than that in group B or C. *S. epidermidis* was detected more frequently in group A than the other milk samples. Although in skin samples there were all Kloos' 9 species, only a limited number of *S. epidermidis* was detected. It is expected that the Kloos' *S. epidermidis* should be predominant species in skin samples, if organisms in a bovine udder are originated from skin. This finding suggests that *S. epidermidis* being a few in skin samples propagates readily in udder when invades concurrently with the other species. Isolation of *S. epidermidis* in high frequency from abnormal milk samples in which coagulase-negative cocci were predominant suggests that this organism is responsible for causing abnormal milk. Much attention should now be directed to the pathogenicity of Kloos' *S. epidermidis*, though special attention has been given mainly to coagulase-positive staphylococci or streptococci as pathogens causing bovine mastitis.

Baird-Paker's method was capable of typing only percent of the strains in this study. It became evident, therefore, that this method was inadequate to type coagulase-negative cocci isolated from bovine milk.

By the use of Pelzer's classification system, 73 percent of the strains were classified. Kume *et al.* also reported that 83 percent of coagulase-negative cocci were classified by this system. These figures, however, will demonstrate that the Kloos' method was more reliable for classification than the Pelzer's.

About a half of the test strains in each group were typed by our phage set. Therefore the phage typing method is seemed to be unsatisfactory. It is hoped that the method for typing of coagulase-negative staphylococci applied has to be improved.

From the results of this survey, it is suggested that an experimental study is required to demonstrate if *S. epidermidis*, being a few in skin samples, would become a causative agent of bovine mastitis in udder.

### References

- 1) MATSUMOTO, H., BABA, E., FUKATA, T., and NAKAMA, S. (1974). The Correlation between Coagulase Production and the Other Biochemical Properties of Staphylococci Isolated from Bovine Mastitis. *Bull. Univ. Osaka Pref.*, **26**, 17-32.
- 2) SHARPE, M. E., MEAVE, F. K., and REITER, B. (1962). Staphylococci and Micrococci Associated with Dairying. *J. appl. Bact.*, **25**, 403-415.
- 3) DE WAART, L., MOSSEI, D. A. A., TEN BROEKE, R., and VAN DE MOOSDIJK, A. (1968). Enumeration of *Staphylococcus aureus* in Foods with Special Reference to Egg-yolk Reaction and Mannitol Negative Mutants. *J. appl. Bact.*, **31**, 276-285.
- 4) FORBES, D. (1968). Taxonomy of some Staphylococci and Micrococci Isolated from Bovine Teat Canals and Foremilks. *J. appl. Bact.*, **31**, 426-435.
- 5) SOVADINA, M. (1975). Classification of Coagulase-negative Staphylococci of

- Human Origin. *Zbl. Bakt. Hyg., I. Abt Orig.*, A 230, 313-319.
- 6) KLOOS, W. E. and SCHLEIFER, K. H. (1975). Simplified Scheme for Routine Identification of Human *Staphylococcus* Species. *J. clin. Microbiol.*, 1, 82-88.
  - 7) BAIRD-PAKER, A. C. (1963). A Classification of Micrococci and Staphylococci Based on Physiological and Biochemical Tests. *J. gen. Microbiol.*, 30, 409-427.
  - 8) PELZER, K., PULVERER, G., JELJASZEWICZ, J., and PILLICH, J. (1973). Modification of Baird-Pakers's Classification System of *Staphylococcus albus*. *Med. Microbiol. Immunol.*, 158, 249-257.
  - 9) FUKATA, T. (1973). Bacteriophage Typing of Coagulase-negative Staphylococci Isolated from Bovine Mastitis. MS. Thesis, University of Osaka Prefecture.
  - 10) KUME, T., KASHIWAZAKI, M., MITANI, K., and SINOWEAY, H. (1977). Identification of Coagulase-negative Staphylococci and Isolated from Bovine Milk. The 83rd Meeting of the Japanese Society of Veterinary Science, (Kanagawa).