Capsular Polysaccharide Serotypes Among *Staphylococcus aureus* Isolates from Cases of Bovine Mastitis and Dogs

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젖소 유방염과 개에서 분리된 황색포도상구균에 대한 Capsular Polysaccharide형의 동정

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요 약 : 본 연구는 임상형 및 준임상형 유방염에 이환된 젖소의 유즙에서 분리된 24주의 황색포도상구균에 대한 capsular polysaccharide(CP)형을 확인하고, 1% 토끼 혈청이 함유된 serum-soft agar 배지에서 집락의 모양을 관찰하였다. 또한 동물에 따라 우세한 CP형의 차이가 있는지를 비교하고자 개에서 분리된 13주에 대하여 동일한 실험을 수행하여 다음과 같은 결과를 얻었다. 집락모양을 관찰한 결과 젖소 유래 24주 중 16주(66.7%)는 diffuse, 5주(20.8%)는 compact, 나머지 3주(12.5%)는 분류가 불가능(indeterminate)하였다. CP형 확인결과 9주(37.5%)는 type 5, 2주(8.3%)는 type 8, 13주(54.2%)는 분류가 불가능(non-typeable)하였다. 한편, 개에서 분리된 균주 중 1주(type 8)를 제외한 12주(92.3%)는 type 5로 분류되었으며, 13주 중 8주(61.5%)가 diffuse형의 집락을 보였다. 본 실험에 사용한 균주의 수가 충분하지 못한 문제가 있지만 동물에 따라 우세한 CP형이 다를 수 있으며 분류 불가능한 균주가 상대적으로 높은 비율을 차지하였는데 이는 새로운 CP형의 분류형이 필요함을 시사하는 것으로 판단된다. 또한 type 5와 type 8만을 포함한 유방염 백신은 한계가 있을 것으로 사료된다.

Key words : Bovine mastitis, Dog, *Staphylococcus aureus*, Capsular polysaccharide, Serotype

Introduction

Staphylococcus aureus was and still is a major bacterial pathogen in bovine mastitis, and the importance of outermost cell surface properties as virulence factors has gained considerable attention[10,16,17]. Surface molecules determine adherence to host tissues and colonization, and through them the organism makes its first contact with cellular and humoral host factors. A major obstacle to producing a protective immune response to *S aureus* is *in vivo* development of an extracellular capsular polysaccharides (CPs), sometimes described as a microcapsule or slime layer[3,19]. This characteris-

tic masks recognition of antibody by neutrophils[8,24] because 95 to 100% of *S aureus* isolated from cows with mastitis are encapsulated[17,28].

The involvement of CP in the invasiveness of many bacteria is well established[32,34,35]. Although there have been many different studies as to the nature, occurrence, and pathogenic importance of capsular structures in *S aureus* from bovine mastitis[17,21,27,28], CP are significant immunogenic structures[8,14,24] and mediators of bacterial adherence[18,27]. In an *in vitro* assay as a correlate of virulence, nonencapsulated *S aureus* organisms are readily opsonized and killed[12]. The enhanced virulence of encapsulated bacteria, in contrast, resist opsonophagocytosis[5,12,29,33] and lysis by phages[37].

Eleven distinct CP serotypes have been identified

in *S aureus* [13, 31]. By using specific polyclonal or monoclonal antibodies, it has been shown that two CP types, 5 and 8, account for 70 to 80% of human isolates [1, 2, 6, 9, 31]. Types 5 and 8 were previously reported to represent 69% of the *S aureus* capsular serotypes isolated from cows with mastitis [25]. These two types also constituted about 80% of the *S aureus* isolated from goats, sheep, and cows with mastitis [25,33]. For these reasons, the work reported here is part of an investigation undertaken attempts to determine the serotypes of *S aureus* isolated from bovine mastitis milk and to examine the morphology of the colony in relation to virulence factors. In addition, the distribution of CP types among isolates from cow and dog was explored. This comparison allows to understand the predominant type by animals, and previous reports on serotyping is reviewed.

Materials and Methods

Bacteria, culture media and colony morphology in serum soft agar

Strains used in this study were isolated from both milk of clinical or subclinical cases of bovine mastitis in a farm located at Kyonggi province and dogs that admitted to the Veterinary Medical Teaching Hospital of Seoul National University. Identification of *S aureus* was based on colony morphology, Gram stain, catalase, hemolytic pattern. All isolates gave positive results in tests for coagulase, DNAse, and mannitol fermentation. Modified 110 medium was prepared by the method of Yoshida and Ekstedt [36]. Serum soft agar (SSA) was prepared from tryptic soy or modified 110 broth by addition of 1% (vol/vol) normal rabbit serum and 0.15% (wt/vol) agar according to the method of Opdebeeck *et al* [19].

SSA procedures

One loopful of a 2-h broth culture of the strains to be detected was used to inoculate three SSA tubes successively. After vigorous mixing, the third tube was found to give a convenient number of colonies for correct determination of morphology. The colony morphology was recorded as compact, diffuse or indeterminate after incubation at 37 for 24 h.

Capsular polysaccharide serotyping

Capsular serotyping of the isolates was performed by the method of immunodiffusion assay [11,15]. Briefly, type 5 and type 8 capsular polysaccharides were detected in bacteria grown on columbia agar slants (Difco). The bacteria were suspended in PBS and autoclaved at 121°C for 1 h. After centrifugation for 10 min at 10,000×g, the polysaccharides were detected in the supernatant by inhibition enzyme-linked immunosorbent assay [2] by using purified capsular antigen preparations and the corresponding monoclonal antibodies. Strains lacking both type 5 and 8 capsular polysaccharides were designated non-typeable. All isolates were further retested by use of colony immunoblot technique.

Statistical method

Fisher's exact test was used to evaluate the differences between serotype and colony morphology.

Results and Discussion

The capsule is defined as a polysaccharide layer covering the cell wall [35]. Capsulate *S aureus* strains produce diffuse colonies in SSA, whereas non-capsulate strains produce compact colonies [36]. Of the 24 *S aureus* isolates employed, 16 (66.7%) grew as diffuse and 5 (20.8%) were compact (Table 1). The proportion of colony morphology by capsular types was not differed significantly (p > 0.05). Two studies have demonstrated that only 5% of *S aureus* strain isolated from human infections produce a capsule as defined by the microscopic Indian ink and the SSA techniques [30,36]. Several other studies have shown that most *S aureus* strains (85~95%) from bovine intramammary infection produce diffuse colonies when inoculated directly from infected milk into SSA [17,20,22]. Of the isolates from bovine origin, 3 strains or 13% were scored as indeterminate on morphology in the regular SSA, indicating these strains would be 'pseudodiffuse' which was first observed by Chomarat *et al* [4].

Karakawa and Vann [13] first reported development of a serotyping system for the *S aureus* capsule that consisted of 8 serotypes. Sompolisky *et al.* [31] expanded this typing system to 11 serotypes but also found that types

Table 1. Capsular serotype and colony morphology in SSA of 24 *S aureus* isolates from bovine and canine origin

Capsular type	Morphology			Total
	Diffuse	Compact	Indetermi-nate	
Bovine				
5	6	2	1	9 (37.5%)
8	2	0	0	2 (8.3%)
Non-typeable	8	3	2	13 (54.2%)
Canine				
5	7	5	0	12 (92.3%)
8	1	0	0	1 (7.7%)

5 (17%) and 8 (69%) were the predominant serotypes from the 348 samples tested including 16 bovine origin, with non-typeable rate of 10%. Of the bovine isolates, 1 was type 5 (6%), 2 were type 8 (12%), and 13 (82%) were non-typeable. In this study, type 5 was predominant, accounting for about 9 or 82.8% of typeable isolates. This finding is in agreement with the work of many others[1,31,33]. Poutrel *et al.*[25] reported non-typeable rate of 31%, with the predominant type of 5 (51%). Guidery *et al.*[7] have shown that in the United States, type 5 accounted 18% of the isolates and type 8 accounted for 23% with considerable non-typeable rate; 59% of the US isolates and 31% of the French isolates were non-typeable. 54.1% of the isolates employed in the current study were non-typeable, indicating encapsulated with other than the 11 prototype capsules. These studies suggest two possible inferences: firstly, the serotypes of isolates from bovine origin could be varied by geographic regions or farms, and thus new serotypes would require to type this non-typeable strains, as indicated by Guidry *et al.*[7] showing that they further purified these non-typeable strains, and the CP was chemically and serologically characterized as serotype 336. Secondly, a vaccine containing serotypes 5 and 8, which account for only 46% of the cases of mastitis would have limited potential for comprehensively preventing *S aureus* mastitis.

On the other hand, as was described by Poutrel and Sutra[25,26], the distribution of CP types of clinical isolates could different by varied animal origin; type 8 was predominant from rabbits and horses, whereas type 5 was more frequent among isolates from bovine,

poultry and pigs. This finding was also supported by a study by Pak[23], who showed that 92.3% or 12 isolates of methicillin-resistant and methicillin-susceptible *S aureus* from canine origin were of CP type 5 (Table 1). However, the collection of isolates tested here was not large enough to enable a definitive conclusion on the distribution of types. Nevertheless, these results show that combination of CPs could be alternative in the future development of vaccines for farm animals for which *S aureus* infections result in economic losses.

Acknowledgments

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