Characteristics of *Staphylococcus aureus* Isolated from Lesions of Horses

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ABSTRACT. Seventy-six *Staphylococcus aureus* strains isolated from various lesions of horses were characterized. All of the 76 strains were identified as biotypes B (38.2%) and C (61.8%). Of 55 strains tested, 42 (76.4%) were differentiated into 7 coagulase types. Coagulase types V and VII were predominant in the metritis strains. Coagulase type II was found most frequently in the strains from phlegmon, dermatitis, sinusitis, empyema sinus, and nasal catarrh. Forty-two (55.3%) of the 76 strains were differentiated into 24 phage patterns. Twenty (58.8%) of 34 typable strains from metritis were lysed by the human group I phage 52, and group II phages 3A, 3C, 55 and 71. Forty-five (59.2%) of the 76 strains were resistant to 1 or more of 6 antibiotics. Strains resistant to penicillin G, irrespective of source, were most frequent (95.6%). Forty (93.0%) of 43 strains resistant to penicillin G alone or in combination with other antibiotics produced beta-lactamase. Only 8 (10.5%) of the 76 strains produced enterotoxins A (n=2), B (n=1) or C (n=5), and they all were isolated from metritis. Only 1 strain isolated from phlegmon and 2 from metritis produced exfoliative toxin (ET) and toxic shock syndrome toxin-1 (TSST-1), respectively. The latter 2 strains also produced enterotoxin C. The results of the present study showed the first evidence of the presence of both ET- and TSST-1-producing *S. aureus* isolated from horses.—**KEY WORDS:** exfoliative toxin, horse, *Staphylococcus aureus*, toxic shock syndrome toxin-1.

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Staphylococcus aureus has been implicated in a variety of infections in horses [3, 14, 20], such as arthritis, dermatitis, abscesses, bacteremia, cellulitis, and wound infections. This organism can also be isolated from the nares, tonsils, and intestinal contents of healthy horses [7, 23].

In the past decade, several studies on the biological and serological properties, and phage typing of *S. aureus* isolated from horses [7, 19, 23] have been published. Most of these studies were carried out on the isolates from the nares, tonsils, and intestinal contents of healthy horses. Only Devriese *et al.* [3] have examined biotypes and phage types of 45 strains of *S. aureus* isolated from different lesions of horses.

This paper deals with the biotyping, coagulase typing, phage typing, enterotoxin production, antibiotic resistance patterns, and beta-lactamase production of *S. aureus* strains isolated from various lesions of horses. In addition, the presence of exfoliative toxin (ET) and toxic shock syndrome toxin-1 (TSST-1) of the strains was demonstrated.

MATERIALS AND METHODS

Source of strains: A total of 76 *S. aureus* strains were used. They had been isolated from a variety of

clinical disease situations, including metritis (n=44, Hokkaido Prefecture), phlegmon (n=3, Ibaraki Prefecture), dermatitis (n=8, Ibaraki Prefecture), diarrhea (n=11, Hokkaido Prefecture), sinusitis (n=1, Ibaraki Prefecture), empyema sinus (n=3, Ibaraki and Shiga Prefectures), and nasal catarrh (n=6, Ibaraki and Shiga Prefectures) of Thoroughbred racehorses in 3 prefectures of Japan during May, 1985 and November, 1988.

Biotyping: Biotyping was carried out according to the scheme established by Hájek and Maršálek [5]. The identification of biotypes was performed by means of the following key criteria: coagulase activity in human and bovine plasma, fibrinolysin, pigmentation, type of growth on crystal violet agar, and production of alpha and beta hemolysins according to the methods described elsewhere [24].

Coagulase typing: Coagulase typing was performed according to the method of Ushioda *et al.* [26]. Coagulase-type-specific rabbit antisera (I to VIII) were purchased from Denka Seiken Co., Ltd., Tokyo, Japan.

Phage typing: Phage typing was conducted by the method described by Shimizu [21, 22] using 21 phages of the international set for typing human *S. aureus* (phages 29, 52, 52A, 79, 80, 3A, 3C, 55, 71, 6, 42E, 47, 53, 54, 75, 77, 83A, 84, 85, 81, 187). A

phage concentration of the routine test dilution (RTD) $\times 100$ was used. Strains showing strong lysis (ranging from more than 50 plaques to confluent lysis) were recorded as typable.

Antibiotic susceptibility tests: Antibiotic susceptibility tests were performed by the disk diffusion method with Tridisks (Eiken Chemical Co., Ltd., Tokyo). The disks and concentrations used were as follows: penicillin G (PC: 0.5, 2, 10 units), erythromycin (EM: 0.5, 2, 10 μ g), lincomycin (LCM: 2, 5, 15 μ g), chloramphenicol (CM: 5, 10, 30 μ g), tetracycline (TC: 5, 10, 30 μ g), kanamycin (KM: 5, 10, 30 μ g), gentamicin (GM: 2, 5, 10 μ g), and cephaloridine (CER: 5, 10, 25 μ g). For broth and agar media, Sensitivity Test Broth Medium (Nissui Pharmaceutical Co., Ltd., Tokyo) and Sensitivity Test Agar Medium (Nissui), respectively, were used. The results were interpreted according to Eiken's directions for the use of disks.

Beta-lactamase production: All of the strains resistant to PC alone or in combination with other antibiotics were tested for beta-lactamase production by the paper disk method with cefinase (Baltimore Biological Laboratories, Cockeysville, Md).

Enterotoxin production: Production and typing of enterotoxins were tested by reversed-passive latex agglutination using sensitized latex with type specific anti-enterotoxins A, B, C, or D immunoglobulin (Denka Seiken).

Detection of ET: For the newborn mouse bioassay, which was performed by the method described by Melish and Glasgow [15], 0.1 ml of a 24 hr culture of the test organism grown in brain heart infusion (Difco Laboratories, Detroit, Mich) was inoculated subcutaneously into 3 days old newborn Std: ddY mice. The organism, which caused a positive Nikolsky sign within 16 hr, was regarded as an ET-

producing strain. When the Nikolsky sign was tested, the mice were thoroughly asleep after treatment with ether anesthesia. For the double immunodiffusion test, 5-fold concentrates of the culture supernatant of the positive organism were tested against anti-ET-A and anti-ET-B sera on an agar plate. Antisera against ET-A and ET-B were obtained by immunizing BALB/c mice with the respective purified antigens which were originated from the ZM (ET-A) and J-sETB-8 (ET-B) strains, according to the method described by Kondo *et al.* [12, 13].

Detection of TSST-1: Production of TSST-1 was tested by the reversed-passive latex agglutination method, using sensitized latex with specific anti-TSST-1 immunoglobulin (Denka Seiken) [9].

RESULTS

Biotyping: All of the 76 strains were biotyped (Table 1). Twenty-nine (38.2%) and 47 (61.8%) strains were identified as biotypes B and C, respectively. Of 44 strains from metritis, 25 (56.8%) belonged to biotype B and 19 (43.2%) to biotype C.

Table 1. Biotypes of *S. aureus* strains isolated from lesions of horses

Source	No. of strains	Biotype ^{a)}	
		B	C
Metritis	44	25	19
Phlegmon	3	0	3
Dermatitis	8	2	6
Diarrhea	11	2	9
Sinusitis	1	0	1
Empyema sinus	3	0	3
Nasal catarrh	6	0	6

a) Hájek and Maršálek biotyping [5].

Table 2. Coagulase types of *S. aureus* strains isolated from lesions of horses

Source	No. of strains	Coagulase type									
		I	II	III	IV	V	VI	VII	VIII	II/III	UT ^{a)}
Metritis	30		3	2	2	8		10		1	4
Phlegmon	3		3								
Dermatitis	8	1	3					1		1	2
Diarrhea	5							1			4
Sinusitis	1		1								
Empyema sinus	3		1								2
Nasal catarrh	4		3								1
Total	54	1	14	2	2	8		12		2	13

a) Untypable.

Table 3. Phage patterns of *S. aureus* strains isolated from lesions of horses

Source	No. of typable strains/ No. of strains tested(%)	Phage pattern (No. of strains)
Metritis	34/44(77.3)	52+(8), 3C+(1), 3A/3C/55/71+(6), 3A/3C/71+(4), 3C/55/71(1), 85(1), 6/54/83A+(1), 6/75(1), 53/54/75/77/85/84(1), 6(1), 83A(1), 29/54+(1), 29/52/53(1), 29/52/79/80/53/54/75/77/85/84/83A+(1), 6/47/53/54/77/81+(1), 75/81(1), 6/47/53/77/85/83A/81(1), 29/3C/6/75+(1), 81(1)
Phlegmon	2/ 3(66.7)	6/75(2)
Dermatitis	2/ 8(25)	52/80+(1), 29/52/80/54/75+(1)
Diarrhea	1/11(9.1)	52+(1)
Sinusitis	0/ 1(0)	
Empyema sinus	2/ 3(66.7)	54+(1), 29/3C/6/54+(1)
Nasal catarrh	1/ 6(16.7)	52/80+(1)
Total	42/76(55.3)	

Table 4. Antibiotic resistance patterns of *S. aureus* strains isolated from lesions of horses

Source	No. of antibiotic resistant strains/ No. of strains tested (%)	Resistance pattern										
		PC ^{a)}	EM	PC TC	PC CM	EM KM	PC TC	PC TC EM	PC TC CM	PC TC KM	PC LCM EM KM	PC TC LCM EM KM
Metritis	29/44(65.9)	27						1				1
Phlegmon	3/ 3(100)						1				1	1
Dermatitis	6/ 8(75.0)	1	1		2	1					1	
Diarrhea	1/11(9.1)	1										
Sinusitis	1/ 1(100)	1										
Empyema sinus	2/ 3(66.7)	1		1								
Nasal catarrh	3/ 6(50)	2					1					
Total	45/76(59.2)	33	1	1	2	1	1	1	1	2	2	

a) Abbreviations are shown in Materials and Methods.

All or most of the strains from skin and nasal lesions or from diarrhea, respectively, belonged to biotype C.

Coagulase typing: Twenty-two of the 76 strains could not be tested for coagulase typing because of insufficient coagulase production in their culture supernatantes. Of 54 strains tested, 41 (75.9%) were typed and differentiated into 7 coagulase types (Table 2). Coagulase types V and VII were predominant in the metritis strains. Coagulase type II was found most frequently in the strains from skin lesions of phlegmon and dermatitis, and sinusitis, empyema sinus, and nasal catarrh of respiratory tract infections. All of 8 coagulase type V strains were lysed by group II phages. Eight of 14 coagulase type II, 7 of 12 coagulase type VII, and 7 of 13

coagulase-untypable strains were resistant to the phages used.

Phage typing: Forty-two (55.3%) of the 76 strains were typable at 100 × RTD (Table 3). A total of 24 different patterns were detected. Twenty (58.8%) of 34 typable strains from metritis were lysed by group I phage 52, and group II phages 3A, 3C, 55 and 71.

Antibiotic resistance: Forty-five (59.2%) of the 76 strains were resistant to 1 or more of the 6 antibiotics, except for GM and CER (Table 4). Overall, resistance to PC (95.6%), irrespective of source, was most frequent, whereas strains were least resistant to EM (15.6%), TC (13.3%), CM (6.7%), KM (13.3%), and LCM (8.9%).

Beta-lactamase production: Forty (93.0%) of 43 strains resistant to PC alone or in combination with

Table 5. Enterotoxin types of *S. aureus* strains isolated from lesions of horses

Source	No. of strains	No. of enterotoxigenic strains	Enterotoxin type		
			A	B	C
Metritis	44	8(18.2%)	2	1	5
Pneumon	3	0			
Dermatitis	8	0			
Diarrhea	11	0			
Sinusitis	1	0			
Empyema sinus	3	0			
Nasal catarrh	6	0			
Total	76	8(10.5%)	2	1	5

other antibiotics were beta-lactamase producers.

Enterotoxin production: Of the 76 strains, only 8 (10.5%) were enterotoxigenic, and they were isolated from metritis (Table 5). Four of 5 enterotoxin C type strains belonged to biotype C. Enterotoxin types A (n=2) and B (n=1) strains belonged to biotype B. No correlation between enterotoxin type and coagulase type, phage group, or antibiotic resistance pattern was found.

Detection of ET: Of the 76 strains, only one isolated from phlegmon was found to produce ET by the newborn mouse bioassay. The properties of the strain were biotype C, coagulase type II, phage pattern 6/75, and antibiotic resistance pattern PC/LCM/EM/KM. The culture supernatant of the strain that was positive for ET formed no precipitin line against the known anti-ET-A and anti-ET-B sera by the double immunodiffusion test.

Detection of TSST-1: Of the 76 strains, only 2 isolated from metritis produced TSST-1. They also produced enterotoxin C. One strain showed biotype C, coagulase type II, phage pattern 29/54, and resistance to PC, and the other showed biotype B, coagulase type III, phage pattern 29/52/79/80/53/54/75/77/85/84/83A, and sensitivity to the antibiotic.

DISCUSSION

In the present study, all of the *S. aureus* strains isolated from various lesions of horses were classified as biotypes B and C according to Hájek and Maršálek [5]. Such strains of biotypes B and C had also been frequently found in nares, tonsils, and intestinal contents of healthy horses [7, 19, 23, 24]. Biotypes B and C, characteristics of the swine and poultry *S. aureus* ecovar and the bovine ecovar, respectively, seem to be widely distributed in horses.

In a previous report [11], it was shown that 2 of 8 strains from the nares of healthy horses were coagulase-typable, and were placed in coagulase types I and III. In the present study, 41 (75.9%) of 54 strains examined were typed and differentiated into 7 types. Most strains from metritis, the skin and nasal lesions belonged to coagulase types V, VII and II, respectively. The results suggest a correlation between these coagulase types and types of diseases.

In the present study, about 60% of the 76 strains were resistant to 1 or more of 6 antimicrobial agents (PC, EM, TC, CM, KM, LCM), and 96% of them were resistant to PC, regardless of their different sources. No strain was resistant to CER and GM. These results were similar to those of Hájek *et al.* [7] and Markel *et al.* [14].

S. aureus strains isolated from horses are mostly sensitive to the set of human *S. aureus* phages. Phage patterns of the isolates found in the nares, tonsils, and intestinal contents of healthy horses [19, 23] and various lesions of diseased horses [3] are known to be very heterogeneous. Similar results were obtained in the present study.

Melish and Glasgow [15, 16] demonstrated that all *S. aureus* strains isolated from patients with staphylococcal scalded skin syndrome (SSSS) belonged to phage group II, and when the organisms were inoculated into newborn mice, all the mice showed a positive Nikolsky sign. Afterwards, it was found that the *S. aureus* strains isolated from such patients elaborate an ET, which is regarded to be important in the pathogenesis of SSSS [17]. Phage group II strains were found only in the metritis, and not in the skin lesions of horses in this study. These phage group II strains were negative for ET. However, it is interesting to note that one strain isolated from phlegmon, phage type 6/75, produced ET. The culture supernatant of the organism, however,

formed no precipitation line against the known anti-ET-A and anti-ET-B sera on agar plate. This suggests that the strain may possess an unknown antigen of ET. Further studies are required to investigate whether or not ET plays an important role in the pathogenesis of skin infections, including phlegmon of horses.

Phage typing studies [2] indicated that the main phage types of *S. aureus* strains from patients associated with toxic shock syndrome (TSS) were types 29, 52, and 29/52, and that these phage types also had a high capability of producing TSST-1. Also, Hayes *et al.* [8] found a significant association between toxic-shock-associated protein (s)-positive strains and phage types 29, 52, and 29/52. In the present study, phage type 52 strains were also found in the metritis and diarrhea of horses, but none produced TSST-1. However, out of 44 metritis strains, 2 belonging to mixed groups were found to produce TSST-1. These also produced an enterotoxin C. Interestingly, both enterotoxin C and TSST-1 producing strains of *S. aureus* have also been isolated from a goat with severe staphylococcal dermatitis of the udder [18] and healthy goats [4], or cows with mastitis [Yokomizo *et al.*, 109th Annual Meeting of the Japanese Society for Veterinary Science, Tokyo, April, 1990]. In addition, such strains producing both enterotoxins and TSST-1 are found in patients that are or are not TSS [2, 9]. The role of TSST-1, including enterotoxins, in the pathogenesis of metritis of horses has not been clarified.

Hájek and Maršálek [7] reported that 4 of 16 horse strains belonging to biotypes A, B, or C were enterotoxigenic and all produced enterotoxin C exclusively. In the present study, it was found that 8 strains from metritis were enterotoxigenic and 5 strains produced enterotoxin C. Such enterotoxin C-producing strains were also frequently found in bovine mastitis udders in Japan [10, 25].

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