Isolation and Species Distribution of Staphylococci from Animal and Human Skin

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ABSTRACT. From April 1999 to December 2000, a survey was made on the distribution of *Staphylococcus* species on the skin of 7 kinds of animals and humans. Staphylococci were isolated from 12 (100%) of 12 pigs, 17 (89.5%) of 19 horses, 30 (100%) of 30 cows, 73 (90.1%) of 81 chickens, 10 (40%) of 25 dogs, 23 (76.7%) of 30 laboratory mice, 20 (52.6%) of 38 pigeons, and 80 (88.9%) of 90 human beings. The predominant staphylococci isolated from a variety of animal species were novobiocin-resistant species, *S. xylosus* and *S. sciuri* regardless of the animal host species. The novobiocin-resistant species including *S. xylosus* and *S. sciuri* were only occasionally isolated from human skin. The predominant staphylococci found on human skin were novobiocin-sensitive species, *S. epidermidis* (63.8%), followed by *S. warneri* (28.8%) and *S. hominis* (13.8%). The results suggest that the staphylococcal flora inhabiting animal skin are different from those of human skin in regard to the predominant species isolated. In this study, we used pulsed-field gel electrophoresis to examine the chromosomal polymorphisms of *S. epidermidis* isolated most frequently from human skin. Strains of *S. epidermidis* showed the greatest genomic diversity in their fragment patterns.

KEY WORDS: animal and human skin, pulsed-field gel electrophoresis, *Staphylococcus* species.

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Recently, taxonomic studies have shown that the genus *Staphylococcus* can be divided into at least 36 species and 17 subspecies, consisting of 6 coagulase-positive or coagulase-variable species and 31 coagulase-negative species.

Staphylococci are widespread in nature and commonly found living on the skin, in skin glands, and on mucous membranes of humans and other mammals or birds. They are sometimes found in the mouth, blood, mammary glands, and intestinal, genitourinary, and upper respiratory tracts of these hosts.

Knowledge of the distribution of the normal staphylococcal flora on the skin is an important factor in understanding the epidemiology of staphylococcal skin diseases in humans and animals. However, ecological studies on staphylococci inhabiting human [1, 12–14, 19, 21] and animal [6, 12, 16, 26] skin have been reported infrequently in the literature. We [26] previously investigated *Staphylococcus* species on the skin of animals and humans during the period from 1987 to 1990, and found novobiocin-resistant staphylococci to be the predominant species in animals, whereas novobiocin-sensitive staphylococci were abundantly present on human skin.

In this report, we re-examined the distribution of *Staphylococcus* species on the skin of healthy animals and humans. We also compared the present results with those of our previous report [26] in terms of the isolation rate of staphylococci from the skin and the dominant species of staphylococci isolated.

Among the coagulase-negative staphylococci species, *S. epidermidis* has been isolated most frequently from the anterior nares [1, 9, 13] or the skin [13, 14] of healthy humans. Many reports have concerned the phenotypic characterization and drug-resistance of *S. epidermidis* from humans, but

information regarding the molecular genetic characterization of the organism is lacking [7, 9, 17]. In this study, pulsed-field gel electrophoresis (PFGE) was used to examine the chromosomal polymorphisms among strains of *S. epidermidis* isolated from humans and animals.

MATERIALS AND METHODS

Sampling: The samples of animal skin examined were taken from 7 kinds of apparently healthy animals during the period from April 1999 to December 2000. During the same period, samples of human skin were taken from students. Details regarding the source and sampling site of animals and humans surveyed are tabulated in Table 1.

Sampling procedures: The samples were taken by vigorously swabbing healthy skin sites with a sterile cotton swab moistened with sterile physiological saline. The swabs were directly inoculated onto heart infusion agar (Nissui) supplemented with 7.5% NaCl, and were incubated aerobically for 48 hr at 37°C with a further two or three days incubation at room temperature (20°C to 25°C). Colony characteristics (morphology and pigment) were recorded for each different staphylococcal isolate, and one representative colony of each colony type per specimen was picked with a needle and transferred to heart infusion agar. Subcultures were stored at 4°C.

Species identification: Staphylococci were identified on the basis of colony characteristics, Gram stain and morphology, catalase reaction, colony pigmentation, and anaerobic acid production from glucose. *Staphylococcus* species were identified using the coagulase test and the API-Staph system (Bio Merieux, S.A., France). In addition, conventional biochemical tests reported by Kloos and Schleifer [15],

Table 1. Sources and sampling sites of animals and humans surveyed in this study

Host	Breeding			Sampling		
	District	Farm	Condition	Period	Age	Site
Pig	Hyogo	A	good	Apr. 1999	30–90 days	back (approx. 10 cm ²)
Horse ^{a)}	Hyogo			May 1999	Adult	back (approx. 10 cm ²)
Cow ^{b)}	Tottori	A	good	Jul. 2000	2–3 years	back (approx. 10 cm ²)
		B	good	Jul. 2000	2–3 years	back (approx. 10 cm ²)
		C	good	Jul. 2000	2–3 years	back (approx. 10 cm ²)
Chicken ^{c)}	Hyogo	A	good	Jun. 2000	1–3 weeks	underwing
	Fukushima	A	good	May 1999	5 months	underwing
Dog ^{d)}	Hyogo			Apr.–Jun. 2000	3–7 years	back (approx. 10 cm ²)
Laboratory mouse ^{e)}	Hyogo			May 2000	3–5 weeks	whole body
Pigeon ^{f)}	Hyogo			Dec. 2000		underwing
Human	Hyogo Himeji			Jun. 1999	Adult	arm
	Kobe			Apr. 1999	Adult	arm

a) Horses were reared at 2 riding clubs.

b) Holstein dairy cows.

c) Layer chickens.

d) Household dogs.

e) Male Std:ddy mice were reared for experimental use at our laboratory.

f) Wild birds in the town of Kobe.

Devriese *et al.* [6], and Hajek & Balusek [8] were carried out to confirm the identification results using API-Staph. Novobiocin resistance was determined on P-agar plates (peptone, 10 g; yeast extract, 5 g; sodium chloride, 5 g; glucose, 1 g; agar, 15 g; distilled water, 1,000 ml) containing 1.6 µg/ml novobiocin.

PFGE typing: The preparation of the chromosomal DNA of *S. epidermidis* strains and the fragmentation of their genomic DNA with *Sma*I (New England BioLabs, Beverly, Mass.) were performed as described previously [7]. PFGE was performed with a 1% agarose gel (Seakem GTG, FMC Bioproducts, Rockland, Me.) using a CHEF-DR II system (Bio-Rad Laboratories Inc, Hercules, Calif.) in a 0.5 × TBE buffer (Tris base, boric acid, EDTA; pH 8.0) maintained at 14°C. The running parameters used were as follows: initial pulse, 5 sec; final pulse, 40 sec; voltage, 6 V/cm; time, 22 hr. After PFGE, the gel was stained with ethidium bromide (2 µg/ml) for 25 min, washed with distilled water for 2 hr, and then photographed under UV light (302 nm). Lambda DNA concatemers (New England BioLabs) for determining the size of *Sma*I-digested fragments were used as molecular size standards.

RESULT

Frequency of isolation: The rates of detection of staphylococci on the skin samples are shown in Table 2. Staphylococci were detected on the skin of pigs (12/12, 100%), horses (17/19, 89.5%), cows (30/30, 100%), chickens (73/81, 90.1%), dogs (10/25, 40%), laboratory mice (23/30, 76.7%), pigeons (20/38, 52.6%), and humans (80/90, 88.9%).

Staphylococcus species identified: The occurrence and distribution of *Staphylococcus* species are shown in Table 3.

Table 2. Isolation of staphylococci from the skin of animals and humans

Host	Sampling district	No. of samples	No. of positive (%)
Pig	Hyogo	12	12 (100)
Horse	Hyogo	19	17 (89.5)
Cow	Tottori	A farm	10 (100)
		B farm	10 (100)
		C farm	10 (100)
Chicken	Hyogo	36	28 (77.8)
	Fukushima	45	45 (100)
Dog	Hyogo	25	10 (40)
Laboratory mouse	Hyogo	30	23 (76.7)
Pigeon	Hyogo	38	20 (52.6)
Human	Hyogo Himeji	32	27 (84.4)
	Hyogo Kobe	58	53 (91.4)

The detailed results regarding each animal species are as follows.

Pig: The predominant species was *S. aureus* (41.7% of the 12 carriers), followed by *S. xylosus* (25%). Nine other species were present in the range 8.3 to 16.7%. Ten (83.3%) of the 12 carriers yielded 2 to 4 *Staphylococcus* species together.

Horse: The predominant species was *S. sciuri* (76.5% of the 17 carriers), followed by *S. xylosus* (23.5%). Four other species were present in the range 5.9 to 11.8%. Seven (41.2%) of the 17 carriers yielded 2 to 3 *Staphylococcus* species together.

Cow: The predominant species was *S. sciuri* (83.3% of the 30 carriers), followed by *S. xylosus* (36.7%) and *S. aureus* (23.3%). Four other species were present in the range 3.3 to 10%. Eighteen (60%) of the 30 carriers yielded 2 to 4 *Staphylococcus* species together.

Table 3. Occurrence of *Staphylococcus* species on the skin of animals and humans

Species	Host											
	Pig	Horse	Cow			Chicken		Dog	Laboratory mouse	Pigeon	Human	
	Hyogo 12 ^{a)}	Hyogo 17	TottoriA 10	TottoriB 10	TottoriC 10	Hyogo 28	Fukushima 45	Hyogo 10	Hyogo 23	Hyogo 20	Himeji 27	Kobe 53
Novobiocin-sensitive												
<i>S. aureus</i>	5 ^{b)}		7			1	1		2		2	7
<i>S. intermedius</i>										4		
<i>S. hominis</i>		1									2	9
<i>S. hyicus</i>	1									1		
<i>S. epidermidis</i>	2	1				9	2			1	22	29
<i>S. haemolyticus</i>	1										1	1
<i>S. warneri</i>	1					1	2		1		4	19
<i>S. capitis</i>		1						1			3	7
<i>S. caprae</i>	1											
<i>S. chromogenes</i>	1							1				
<i>S. simulans</i>	1											2
<i>S. lugdunensis</i>												3
Novobiocin-resistant												
<i>S. xylosus</i>	3	4	7	3	1	17	11	1	16	8	1	1
<i>S. cohnii</i> subsp. <i>cohnii</i>	1		1				25			1		
<i>S. cohnii</i> subsp. <i>uealyticus</i>			1	2								
<i>S. lentus</i>			1		1	1	23			2		
<i>S. saprophyticus</i>		2	1			10	3	1		3	1	4
<i>S. sciuri</i>	2	13	7	9	9	3	7	5	10	3	4	
Unidentifiable	6	4	1		4	17	26	3	3	8	12	33

a) No. of carriers.

b) No. of individuals from which *Staphylococcus* species were isolated.

Chicken: The predominant species was *S. xylosus* (38.4% of the 73 carriers), followed by *S. cohnii* subsp. *cohnii* (34.2%), *S. lentus* (32.9%), *S. saprophyticus* (17.8%), *S. epidermidis* (15.1%), and *S. sciuri* (13.7%). Two other species were present in the range 2.7 to 4.1%. Fifty-seven (78.1%) of the 73 carriers yielded 2 to 4 *Staphylococcus* species together.

Dog: The predominant species was *S. sciuri* (50% of the 10 carriers), followed by *S. xylosus* (10%), *S. capitis* (10%), *S. chromogenes* (10%), and *S. lentus* (10%). One (10%) of the 10 carriers yielded 3 *Staphylococcus* species together.

Laboratory mouse: The predominant species was *S. xylosus* (69.6% of the 23 carriers), followed by *S. sciuri* (43.5%). Two other species were present in the range 4.3 to 8.7%. Nine (39.1%) of the 23 carriers yielded 2 *Staphylococcus* species together.

Pigeon: The predominant species was *S. xylosus* (40% of the 20 carriers), followed by *S. intermedius* (20%), *S. sciuri* (15%), and *S. saprophyticus* (15%). Four other species were present in the range 5 to 10%. Eight (40%) of the 20 carriers yielded 2 to 3 *Staphylococcus* species together.

Human: The predominant species was *S. epidermidis* (63.8% of the 80 carriers), followed by *S. warneri* (28.8%), *S. hominis* (13.8%), *S. capitis* (12.5%), and *S. aureus* (11.3%). Six other species were present in the range 2.5 to 6.3%. Fifty-eight (72.5%) of the 80 carriers yielded 2 to 6 *Staphylococcus* species together.

On the whole, the novobiocin-resistant species such as *S.*

xylosus and *S. sciuri* were isolated most frequently from the various animal species tested. In human, novobiocin-sensitive species such as *S. epidermidis* and *S. warneri* were predominant.

Combination of *Staphylococcus* species: As mentioned above, 10 to 83.3% of the carriers yielded 2 to 6 *Staphylococcus* species together. In humans, the combinations of *S. epidermidis* and other species were frequently found in 42 (52.5%) of the 80 carriers. However, a predominant combination was not observed for each animal host.

PFGE analysis of *S. epidermidis*: The PFGE patterns obtained after *Sma*I digestion of the genomic DNAs from human (n=44), pig (n=2), horse (n=1), and chicken (n=10) strains are shown in Fig. 1. Human strains generated 7 to 14 fragments ranging from 48 to 631 kb in size and yielding 41 different patterns. Only two fragments of 88 kb (61.4%) and 82 kb (68.2%) were highly conserved and were shared by almost all of the strains examined. The pig, horse, and chicken strains showed PFGE patterns different from those of the human strains. Furthermore, the PFGE patterns of the pig, horse, and chicken strains were quite different from each other.

DISCUSSION

The present study was conducted in order to determine the distribution of *Staphylococcus* species on the skin of healthy animals and humans. The frequency of detection of

Fig. 1. PFGE of *Sma*I-digested genomic fragments of *S. epidermidis* isolated from humans ($n=44$), pigs ($n=2$), horse ($n=1$) and chickens ($n=10$). Human lanes 32 and 36 included 3 and 2 strains, respectively. Other lanes included only one strain each. L indicates the lambda ladder DNA concatemers as molecular size markers (kb).

staphylococcal carriers on the skin of animals and humans is to some extent dependent on the body sites selected for sampling and the kind of media used for isolation. The isolation rate of staphylococci was 40 to 100% in seven animals and 88.9% in humans, and was slightly higher than those of our previous report [26] that used the same method, i.e., isolation medium and sampling site, as used in the present study. Also, we previously reported [26] that the predominant staphylococci isolated from the skin of various animals were of novobiocin-resistant species and those from humans were of novobiocin-sensitive species. The same tendencies were recognized again in this study.

S. xylosus and *S. sciuri* were isolated most frequently from various animals. Kloos *et al.* [16] investigated *Staphylococcus* species on the normal skin of various domestic and

wild animals, and found *S. xylosus* and *S. sciuri* to be the predominant species. These two species have also been isolated frequently from teat skin of cows [3]. Interestingly, *S. xylosus* and *S. sciuri* were only occasionally isolated from the nares [1, 13, 14] in humans and the tonsils [23, 25] in pigs and cows. Taken together these results suggest that *S. xylosus* and *S. sciuri* are well-adapted to the skin environment of apparently healthy domestic and wild animals, or fowls. In chickens, other *S. cohnii* subsp. *cohnii*, *S. lentus* and *S. saprophyticus* are also abundantly present on the skin.

Compared with isolates from animals, *Staphylococcus* species isolated from humans are somewhat different with regard to the predominant species isolated and in species characteristics. The novobiocin-sensitive species were the

predominant inhabitants of human skin, whereas novobiocin-resistant species were found only occasionally in this environment. These results are in agreement with earlier findings in humans [1, 13, 14, 26]. Eight different novobiocin-sensitive species were isolated, 2 of which were predominant: *S. epidermidis* and *S. warneri*. Particularly, *S. epidermidis* was isolated from 51 (63.8%) of 80 staphylococcal carriers. Other investigators [1, 13, 14] have also reported that this organism is the major species inhabiting the skin of persons living in the United States. In studies of the distribution of staphylococci on several body areas of infant [1] and adult [13, 14] skin, *S. epidermidis* was found in greatest numbers in the forehead, axillae, arms, and legs. This species thus appears to be indigenous to human skin, regardless of body area.

The genomic DNA profile by PFGE has recently been utilized for the typing of *Staphylococcus* species, and a large degree of genomic diversity in human-origin *S. epidermidis* has been reported [7, 9, 17]. Our results clearly demonstrated the presence of genetic heterogeneity among strains of *S. epidermidis* from humans. Also, animal-origin strains displayed a variety of different patterns. As stated by George and Kloos [7], the great adaptability of this species may account for the large amounts of chromosome fragment polymorphisms observed among isolates.

The normal bacterial flora of the skin has been recognized as an important factor in understanding bacterial skin diseases in human beings, and in animals such as pigs, horses, chickens, and dogs. In human beings, *S. aureus* is considered an important primary pathogen causing skin bacterial diseases such as impetigo and staphylococcal scaled-skin syndrome. In veterinary dermatology, *S. aureus* and *S. hyicus* have been implicated in dermatitis of chickens [20, 28] and horses [5, 24, 27], and in exudative epidermitis of pigs [2], respectively. *S. intermedius* is one of the causative agents of canine bacterial skin disease such as otitis externa and pyoderma. In the present study, *S. aureus* and *S. hyicus* were either not isolated or only rarely isolated from the skin of the humans, chickens and horses, and pigs examined, respectively. The nares in humans and chickens are frequent sites of colonization by *S. aureus*. The *S. aureus*-carrying rates were 12.6 to 21.6% for humans [10, 11, 18, 22] and 49.2% for chickens [20]. Also, *S. hyicus* has been isolated at a rate of 53.1% from the nares [2] and 73.6 to 92.9% from the tonsils [25, 29] of healthy pigs. These findings suggest that the nares and the tonsils of pigs are a suitable milieu for the organism. *S. intermedius* was not isolated from the dog skin examined in the present study. Devriese and De Pelsmaecker [4] reported that *S. intermedius* was isolated more frequently from the anal and nasal sites of healthy dogs and pointed out the possibility that these two body orifices, and especially the anus, figure as sources of *S. intermedius* flora on other parts of the body.

In conclusion, this study indicates that the staphylococcal flora inhabiting animal skin is different from that of human skin in regard to the predominant species isolated. The results of the present study indicate considerable genetic

diversity within the species *S. epidermidis*.

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