Isolation of *Staphylococcus* Species from the Tonsils of Healthy Pigs and Phage Patterns of Isolates

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ABSTRACT. Between April and June 1986, the tonsils of 126 healthy pigs were examined for the presence of staphylococci. All of the pigs examined harboured staphylococci in the tonsils. The most predominant *Staphylococcus* species was *S. hyicus* subsp. *hyicus* (92.9%), followed by *S. aureus* (57.1%), *S. hominis* (40.5%), *S. simulans* (22.2%), *S. epidermidis* (3.2%), *S. xylosus* (2.4%), *S. haemolyticus* (1.6%) and *S. hyicus* subsp. *chromogenes* (0.8%). Unidentifiable species were isolated from 10 (7.9%) of the 126 tonsils examined. The combinations of *S. hyicus* subsp. *hyicus* and *S. aureus* with or without other species were most frequently encountered, finding in 53.2% of all the tonsils examined. The main phage patterns of 69 *S. hyicus* subsp. *hyicus* strains typable with *S. hyicus* subsp. *hyicus* phage set were S13 (n=27), S9/S39/S188 (n=12), S9/S188 (n=11) and S9 (n=6). The main phage patterns of 63 *S. aureus* strains typable with the human *S. aureus* phage set were 3A/3C (n=36) and 3C (n=16). Sixty-one coagulase-negative staphylococcal strains typable with Pulverer's phage set were differentiated into 25 phage patterns.—**KEY WORDS:** phage type, porcine tonsil, *staphylococcus*.

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Staphylococci are not only members of the normal flora of human skin, respiratory and gastrointestinal tracts, but also found commonly in air and human environments. Similarly, many pigs harbour staphylococci on the skin, in the nares and in the gastrointestinal tract [2, 4, 6, 10, 12, 20]. However, there has not been enough research regarding the distribution and carriage of these organisms in porcine tonsils [21]. The purpose of the present study was to investigate the state of distribution of staphylococci in the tonsils of apparently healthy pigs and, in addition, to study phage patterns of the isolates.

MATERIALS AND METHODS

Specimens: During the period between April and June, 1986, a total of 126 tonsils were collected from 126 apparently healthy pigs at an abattoir in Hyogo Prefecture. A

portion of each tonsil was placed in a sterile dish for transportation to the laboratory. The tonsils were rapidly prepared for isolation of staphylococci.

Isolation of staphylococci: After sterilizing the surface of the tonsils by boiling, small portions were cut off aseptically. Different surfaces of the tissue blocks were randomly stamped repeated several times directly onto nutrient agar supplemented with 7.5% NaCl and Tween 80-containing selective medium for isolation of *S. hyicus* [2]. After aerobic incubation for 48 hours at 37°C, two or more colonies resembling staphylococci were picked from each selective plate and subjected to biochemical tests. Staphylococci were identified on the basis of colony characteristics, Gram stain morphology, catalase reaction and anaerobic acid production from glucose (GF medium, Nissui) [22].

Identification of *Staphylococcus* species:

The species were identified according to the methods for staphylococci identification of Kloos and Schleifer [9], Hájek and Balusek [5], and Akatov *et al.* [1]. Characteristics used for the identification of *Staphylococcus* species were as follows: coagulase activity in rabbit plasma, hemolysis on bovine blood agar, nitrate reduction, acetylmethylcarbinol production, phosphatase and urease activities, novobiocin susceptibility, and aerobic acid production from arabinose, cellobiose, fructose, galactose, glucose, glycerol, lactose, maltose, mannitol, mannose, raffinose, ribose, sucrose, trehalose, turanose, xylitol and xylose.

Phage typing: Phage typing was carried out using the same method as previously described by Shimizu [16, 17] with 21 phages of the international set for typing human *S. aureus* (29, 52, 52A, 79, 80, 3A, 3C, 55, 71, 6, 42E, 47, 53, 54, 75, 77, 83A, 84, 85, 81, 187), 5 phages of the Kawano's set for typing *S. hyicus* subsp. *hyicus* (S9, S13, S39, S188, CH11) [8, 19], and 15 phages of the Pulverer's set for typing human *S. epidermidis* (Ph5, Ph6, Ph9, Ph10, Ph12, Ph13, Ph14, Ph15, Ph16, U4, U14, U15, U16, U20, U46) [7, 15]. Strains showing strong lysis (ranging from more than 50 plaques to confluent lysis) were recorded as typable.

RESULTS

Staphylococci were isolated from 126 (100%) of the 126 pig tonsils examined. Table 1 shows the species of staphylococci isolated. The most predominant *Staphylococcus* species was *S. hyicus* subsp. *hyicus* (92.9%), followed by *S. aureus* (57.1%), *S. hominis* (40.5%), *S. simulans* (22.2%), *S. epidermidis* (3.2%), *S. xylosus* (2.4%), *S. haemolyticus* (1.6%) and *S. hyicus* subsp. *chromogenes* (0.8%). Ten unidentifiable strains were isolated from 10 (7.9%) of the 126 tonsils examined. One hundred and four (82.5%) of the 126 tonsils examined yielded

Table 1. Frequency of isolation of staphylococci from the tonsils of healthy pigs^{a)}

Species	No. of tonsils harboring staphylococci (%)
<i>S. hyicus</i> subsp. <i>hyicus</i>	117 (92.9)
<i>S. aureus</i>	72 (57.1)
<i>S. hominis</i>	51 (40.5)
<i>S. simulans</i>	28 (22.2)
<i>S. epidermidis</i>	4 (3.2)
<i>S. xylosus</i>	3 (2.4)
<i>S. haemolyticus</i>	2 (1.6)
<i>S. hyicus</i> subsp. <i>chromogenes</i>	1 (0.8)
Unidentifiable	10 (7.9)

a) One hundred and twenty-six tonsils of pigs were examined.

two to five *Staphylococcus* species together. The remaining 22 (17.5%) tonsils yielded only one species (Table 2). The combination of *S. hyicus* subsp. *hyicus* and *S. aureus* was most frequently encountered, finding in 19.8% of all the tonsils examined. The next frequent combination was *S. hyicus* subsp. *hyicus*/*S. aureus*/*S. hominis* (15.9%), followed by *S. hyicus* subsp. *hyicus*/*S. hominis* (11.9%), and *S. hyicus* subsp. *hyicus*/*S. aureus*/*S. simulans* (8.7%).

As can be seen in Table 2, the combinations of *S. hyicus* subsp. *hyicus* and *S. aureus* with or without other species were frequently encountered and found in about 53% of the tonsils examined.

One hundred and fifteen isolates from 115 tonsils were subjected to typing, employing the 5 *S. hyicus* subsp. *hyicus* phages (Table 3). Sixty-nine (60.0%) of the 115 isolates were typable with one or more of the phages; 45 being typable at routine test dilution (RTD) and 24 at 100×RTD. Although 9 different phage patterns were detected, the main phage patterns were S13 (n=27), S9/S39/S188 (n=12), S9/S188 (n=11) and S9 (n=6). About 81% of the typable isolates were divided into these 4 phage patterns.

Seventy-two isolates from 72 tonsils were subjected to typing, using the 21 phages of

Table 2. Combinations of *Staphylococcus* species isolated from the tonsils of healthy pigs^{a)}

Species	No. of tonsils harboring staphylococci (%)
hyi ^{b)} alone	16 (12.7)
aur alone	2
hom alone	2
sim alone	1
xyl alone	1
hyi+aur	25 (19.8)
hyi+aur+hom	20 (15.9)
hyi+aur+sim	11 (8.7)
hyi+aur+epi	2
hyi+aur+xyl	1
hyi+aur+UC	2
hyi+aur+sim+hom	3
hyi+aur+sim+UC	1
hyi+aur+hom+UC	1
hyi+aur+sim+hom+UC	1
hyi+hom	15 (11.9)
hyi+hom+chr	1
hyi+hom+UC	2
hyi+sim	5
hyi+sim+hom	4
hyi+sim+UC	1
hyi+epi	1
hyi+epi+UC	1
hyi+xyl	1
hyi+hae	2
hyi+UC	1
aur+hom	2
aur+sim	1

a) One hundred and twenty-six tonsils of pigs were examined.

b) Abbreviations: hyi, *S. hyicus* subsp. *hyicus*; aur, *S. aureus*; hom, *S. hominis*; sim, *S. simulans*; xyl, *S. xylosum*; epi, *S. epidermidis*; hae, *S. haemolyticus*; chr, *S. hyicus* subsp. *chromogenes*; UC, Unidentifiable.

international human *S. aureus* set (Table 4). Sixty-three (87.5%) of the 72 isolates were typable with one or more of the phages; 40 isolates at RTD and 23 at 100×RTD. The majority (92.1%) of the 63 typable isolates belonged to group II, the main phage pattern being 3A/3C (n=36) or 3C (n=16).

A total of 99 coagulase-negative staphylococci (CNS) isolates consisting of 6 species and unidentifiable isolates were subjected to typing, using the 15 phages of Pulverer set for typing human *S. epidermidis*. The results of phage typing according to staphylococcal species are shown in Table 5. Typabilities of

Table 3. Phage typing of *S. hyicus* subsp. *hyicus* by use of *S. hyicus* subsp. *hyicus* phages

Phage pattern	No. of isolates
S9	1 (5) ^{a)}
S13	21 (6)
S188	1 (3)
CH11	(1)
S9/S13	1 (2)
S9/S188	6 (5)
S9/S13/S188	2 (1)
S9/S39/S188	11 (1)
S9/S13/S39/S188	2
Untypable	46
Typability (%)	69/115 (60)

a) Data are expressed as No. of isolates typable at RTD. Data in parentheses are expressed as No. of isolates typable only at 100×RTD.

Table 4. Phage typing of *S. aureus* by use of the international human *S. aureus* phages

Group	Phage pattern	No. of isolates
I	29	1
	29/52	1
	29/52A/79	(1) ^{a)}
II	3A	2 (1)
	3A/3C	18 (18)
	3A/3C/55	2 (1)
	3C	15 (1)
III	85	1
Mixed	3A/3C/79	(1)
	Untypable	9
Typability (%)		63/72 (87.5)

a) See the footnotes on Table 3.

S. hominis, *S. simulans*, *S. epidermidis*, *S. xylosum*, *S. haemolyticus* and *S. hyicus* subsp. *chromogenes* isolates were 64.7% (33/51), 60.7% (17/28), 50.0% (2/4), 33.3% (1/3), 100% (2/2) and 0% (0/1), respectively. Six (60%) of 10 unidentifiable isolates were successfully typed with the phages used. Although 25 different phage patterns were detected, the phage patterns commonly found were Ph5/Ph9/Ph10/Ph12/Ph13/U4/U14/U16/U20/U46 (n=12), Ph5/Ph10/Ph12/U4/U16 (n=8), Ph5/Ph10/Ph12/U4 (n=7), and Ph5/Ph10/Ph12/Ph13/U4/U16/U20/U46 (n=5). There was no significant relation between the phage patterns and staphylo-

Table 5. Phage patterns of 61 coagulase-negative staphylococcal strains typable with Pulverer's phage set^{a)}

Phage pattern											Staphylococcal species ^{b)} (No. of isolates)
Ph5	Ph9	Ph10	Ph12	Ph13	U4	U14	U16	U20	U46		hom (5), sim (3), epi (2), xyl (1), UC (1)
Ph5	Ph9	Ph10	Ph12	Ph13	U4	U14	U16	U20			hom (1), sim (1)
Ph5	Ph9	Ph10	Ph12	Ph13	U4	U16	U46				hom (1)
Ph5	Ph9	Ph10	Ph12	U4	U16						sim (2)
Ph5	Ph9	Ph10	Ph12	U4							UC (1)
Ph5	Ph9	Ph10	Ph12	U4							sim (1)
Ph5	Ph10	Ph12	Ph13	U4	U16	U20	U46				sim (5)
Ph5	Ph10	Ph12	Ph13	U4	U16						hom (1)
Ph5	Ph10	Ph12	Ph13	U4	U20						hom (1)
Ph5	Ph10	Ph12	Ph13								hom (1)
Ph5	Ph10	Ph12	U4	U16							hom (7), UC (1)
Ph5	Ph10	Ph12	U4								hom (7)
Ph5	Ph10	Ph12	U14								hom (1), hae (1)
Ph5	Ph10	Ph12									hom (3)
Ph5	Ph10	Ph13	U4	U16	U20	U46					sim (2)
Ph5	Ph10										UC (1)
Ph5	Ph12										UC (2)
Ph5											hom (2)
Ph10	Ph12	Ph16									hom (1)
Ph10	Ph12	U16									hae (1)
Ph10											hom (1)
Ph12											sim (1)
Ph16											hom (1)
U4											sim (1)
U16											sim (1)

a) Phage typing was performed at 1×RTD.

b) Abbreviations are listed in Table 2.

coccal species.

DISCUSSION

The results of the present study indicate that porcine tonsils provide suitable environments for the harbour of staphylococci. The predominant *Staphylococcus* species found in the tonsils were *S. hyicus* subsp. *hyicus*, *S. aureus*, *S. hominis* and *S. simulans*. About 83% of the carriers yielded two to five species together. The combinations of *S. hyicus* subsp. *hyicus* and *S. aureus* with or without other species were frequently encountered.

S. hyicus subsp. *hyicus* [3] is not only isolated from pigs with exudative epidermitis [18] and with septic polyarthritis [14], but also a common inhabitant of skin or nares in

healthy pigs [2, 20]. Devriese [2] reported that the isolation rate of *S. hyicus* subsp. *hyicus* was 53.1% in the nares, 54.8% in the skin of snout, and 54.4% in the external ears of 228 healthy pigs. Furthermore, he said that this organism was detected at especially high rates in younger pigs. Takeuchi *et al.* [20] also isolated *S. hyicus* subsp. *hyicus* from the nares and/or the external ears in 408 pigs at the isolation rate of 23.8%. Moreover, in young pigs below three weeks of age, the rate rose to as high as 46.2%. In 1984, although we [21] reported that *S. hyicus* subsp. *hyicus* was very frequently isolated from the tonsils of healthy pigs with a rate of 73.6%, the rate in the present study was higher, being 92.9%. The reasons for the difference are not known. In any case, it seems that the porcine tonsil is a suitable

milieu for *S. hyicus* subsp. *hyicus*.

In the present study, it was possible to type 60% of the isolates tested by employing *S. hyicus* subsp. *hyicus* phages, showing 9 phage patterns. These phage patterns were also frequently detected in isolates from pigs with exudative epidermitis in Japan [8].

Up to the present time, several researchers have been studied on the isolation and the properties of *S. aureus* from healthy pigs [4, 6, 10, 12, 13]. The strains studied by them were isolated from the nares, skin or fecal sources. Hájek and Maršálek [6], and Mori *et al.* [12] reported so high nasal carrier rates as 78.9% and 85%, respectively. Dimitracopoulous *et al.* [4] examined 377 rectal swabs from pigs and found *S. aureus* in 44% of them. In the present study, about half of the pigs examined harboured *S. aureus* in the tonsils. The tonsils may be as important as the nares for localization of *S. aureus*.

It has been well known that most of *S. aureus* strains isolated from the nares of healthy pigs are usually susceptible to group II phages, 3A, 3C or 55, of the human phage set [12, 13]. In the present study, 87.5% of 72 isolates from the tonsils were typable, and 92.1% of them belonged to group II. From these results, it was suggested that human phage group II strains were widely distributed among apparently healthy pigs.

The taxonomy of the genus *Staphylococcus* has been revised several times over the last 30 years. Recent classification divided the genus *Staphylococcus* into 23 species, including 4 subspecies. There are 20 coagulase-negative species. Studies on characterization of animal CNS and their distribution on animal skin have been infrequently reported in the literature [10]. However, natural populations of CNS living in the nares or the tonsils of pigs have not yet been studied in much detail. CNS isolated from the tonsils of pigs in this study were *S. hominis*, *S. simulans*, *S. epidermidis*, *S.*

xylosus, *S. haemolyticus* and *S. hyicus* subsp. *chromogenes*. Novobiocin resistant species were rarely found in the tonsils.

With the human *S. epidermidis* phage set of Pulverer, it was possible to type 61.6% of 99 CNS isolates from porcine tonsils. The typable isolates were differentiated into 25 phage patterns. These phage patterns have never been detected in isolates from the nostrils, throat or skin of healthy humans and from clinical materials (acne lesions, pus and urine) [7, 11]. According to Heczko *et al.* [7] and Kurosaka [11], most of human typable isolates showed phage patterns consisting of lytic reactions with one or more of the phages Ph6, Ph14, Ph15, Ph16 and U15. In the present study, phage patterns which included anyone of these phages were detected with only 2 of the porcine isolates. These facts seem to suggest that phage susceptibilities of porcine CNS isolates are significantly different from those of human isolates. Further, experiments will be carried out to clarify this point.

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要 約

豚扁桃からのブドウ球菌の分離とフェージ型別：清水 晃・河野潤一・寺西 永・葉杖真二・藤波哲也・木村重・杉原一三¹⁾（神戸大学農学部家畜衛生学教室，¹⁾家畜管理学教室）——1986年4～6月に，1と畜場に搬入された豚126頭の扁桃からブドウ球菌の分離を試みるとともに，分離菌のフェージ型について調べた。全例から菌が分離されたが，分離頻度の最も高かった菌種は *S. hyicus* subsp. *hyicus* 92.9%，ついで *S. aureus* 57.1%，*S. hominis* 40.5%，*S. simulans* 22.2%，*S. epidermidis* 3.2%，*S. xylosus* 2.4%，*S. haemolyticus* 1.6%，*S. hyicus* subsp. *chromogenes* 0.8%の順であり，ノボピオシン耐性の菌種はまれであった。同定不能菌株は10例7.9%から分離された。104例（82.5%）の同一扁桃からは2～5種類のブドウ球菌が同時に分離され，他の22例（17.5%）では1菌種のみが生息していた。菌種の組み合わせで最も多くみられたのは，*S. hyicus* subsp. *hyicus* と *S. aureus*（他菌種との共存を含める）のそれで，全検索例の約53%を占めた。分離菌種の主要フェージ型は，*S. hyicus* subsp. *hyicus* では S9, S13, S9/S188, S9/S39/S188, *S. aureus* では 3A/3C, 3C であり，コアグラゼ陰性ブドウ球菌では多彩なフェージ型を示した。以上から，鼻粘膜や皮膚とともに扁桃も *S. hyicus* subsp. *hyicus* および *S. aureus* の重要な生息部位であることが明らかにされた。