

Genotypic relatedness of staphylococcal strains isolated from pustules and carriage sites in dogs with superficial bacterial folliculitis

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Objective—To determine whether staphylococcal isolates cultured from pustules and carriage sites in dogs with superficial bacterial folliculitis were genotypically the same strain by use of pulsed-field gel electrophoresis (PFGE).

Animals—40 dogs with superficial bacterial folliculitis.

Procedures—Samples were obtained from 3 pustules and 3 carriage sites (anus, axillary skin, and nasal mucosa). Bacterial culture, morphologic identification, Gram staining, catalase and coagulase tests, speciation, and PFGE were performed.

Results—Of 246 isolates, 203 were *Staphylococcus intermedius*, 5 were *Staphylococcus aureus*, 15 were *Staphylococcus* spp, and 22 were coagulase-negative staphylococcal isolates. No dog had an isolate with the same PFGE pattern as an isolate from another dog. Coagulase-positive isolates from multiple pustules and multiple carriage sites had the same PFGE pattern in 37 of 39 (94.9%) and 22 of 39 (56.4%) dogs, respectively. Coagulase-positive staphylococcal isolates from at least 1 pustule had the same PFGE pattern as an isolate from at least 1 carriage site in 34 of 36 (94.4%) dogs. Ninety-seven of 116 (83.6%) coagulase-positive staphylococcal isolates from pustules had the same PFGE pattern as an isolate from at least 1 carriage site. Sixty-nine of 91 (75.8%) coagulase-positive staphylococcal isolates from carriage sites had the same PFGE pattern as an isolate from at least 1 pustule.

Conclusions and Clinical Relevance—Coagulase-positive staphylococcal strains were heterogeneous among dogs with superficial bacterial folliculitis. In individual dogs, strains from multiple pustules were genotypically the same, and strains from pustules were genotypically the same as strains from carriage sites. (*Am J Vet Res* 2006;67:1337–1346)

Superficial bacterial folliculitis is a common bacterial skin infection in dogs.¹ In 1976, Hajek² characterized *Staphylococcus intermedius* isolates from ani-

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ABBREVIATIONS

PFGE	Pulsed-field gel electrophoresis
NCTC	National Culture Type Collection
CI	Confidence interval

mals, and Berg et al³ subsequently determined that the major coagulase-positive staphylococcal species in dogs was *S intermedius*. This opportunistic pathogen continues to be the principal organism isolated from dogs with superficial bacterial folliculitis.^{1,3-7} *Staphylococcus aureus* is occasionally isolated,^{3,4,8} and *Staphylococcus schleiferi* has recently been isolated from dogs with superficial bacterial folliculitis.⁹⁻¹³

Staphylococci may be identified on the basis of various phenotypic and genotypic characteristics. Morphologic characteristics, catalase production, coagulase activity, and reactions to biochemical tests are used for speciation of clinical isolates. Several commercial identification systems that incorporate differentiating biochemical tests are available for speciation,¹⁴ and multiple molecular methods have been used for strain typing.¹⁵⁻²⁰

Healthy dogs harbor staphylococci within hair follicles,²¹ on hair shafts,²¹⁻²⁴ on skin²⁴⁻²⁸ and mucosal surfaces,^{21,26,29,30} and at mucocutaneous junctions.^{21,31} The anal region is an important carriage site for *S intermedius*,^{26,29} and carriage of staphylococci on skin of the ventral portion of the abdomen and in the nasal vestibule has been reported.²⁵ It has been suggested that *S intermedius* populations residing on mucosal surfaces of the oral cavity, nares, perineum, and anus are the origin of the population found on the haircoat and skin surface,^{29,30} and transmission to those sites is presumed to be via skin contamination from carriage sites during grooming and pruritic behaviors.^{21,25,29,31}

Dogs with superficial bacterial folliculitis also harbor staphylococci on skin and mucosal surfaces. Dogs with superficial bacterial folliculitis have larger populations of *S intermedius* on nonlesional skin²⁵ and mucosal surfaces.^{32,33} Reported nonlesional carriage sites of staphylococci in dogs with superficial bacterial folliculitis include the skin of the ventral abdomen, anal region, oral cavity, and nasal mucosa.^{25,32} As suggested by results of studies^{21,25,29,31} in healthy dogs, carriage sites are implicated as the source of bacteria causing lesions in dogs with superficial bacterial folliculitis.

Pulsed-field gel electrophoresis is a useful technique for determining genotypic relatedness among bacterial strains within a species because it enables the delineation of strains. A bacterial strain is an isolate that can be distinguished from other isolates of the

same species by means of phenotypic characteristics, genotypic characteristics, or both.³⁴ A strain represents a clonal population in which each member is genetically identical and has the same phenotypic characteristics.³⁵ Random genetic events within a population may lead to clonal variation; therefore, a strain may also be defined as a clonal population in which members may differ by 1 or a few mutations or by loss or acquisition of an extrachromosomal element.³⁶ Epidemiologically related isolates (ie, isolates cultured from specimens collected during a specified time frame as part of an epidemiologic investigation, suggesting that the isolates may be derived from a common source) that have indistinguishable PFGE patterns are considered to be the same strain, genetically identical, and clonally related.¹⁹ Thus, epidemiologically related isolates that have the same PFGE pattern are considered to be genotypically related strains derived from a common source.³⁴

By use of *Sma*I-digested DNA, PFGE has been used to delineate staphylococcal strains¹⁹ and to characterize *S aureus*,³⁷ *S intermedius*,^{18,20,38,39} and coagulase-negative staphylococci.^{37,41} However, to the authors' knowledge, no studies have been published in which it was established whether staphylococcal isolates obtained from primary lesions were the same strain as isolates obtained from carriage sites in dogs with superficial bacterial folliculitis. If strains isolated from lesions and carriage sites are genotypically related, staphylococcal populations at the carriage site may be a source of the staphylococci that are causing lesions in dogs with superficial bacterial folliculitis.

The purpose of the study reported here was to determine whether staphylococcal isolates cultured concurrently from pustules and carriage sites were genotypically the same strain by use of PFGE pattern analysis. We hypothesized that staphylococcal strains isolated from pustules in dogs with superficial bacterial folliculitis would have the same PFGE pattern as strains isolated concurrently from carriage sites on the same dog.

Materials and Methods

Dogs—From August 2004 through August 2005, dogs examined by the clinical dermatology service at The Ohio State University College of Veterinary Medicine Teaching Hospital were evaluated for inclusion in the study if they had lesions consistent with superficial bacterial folliculitis⁴¹ and had not been treated with topical antimicrobial drugs or received parenterally administered antimicrobials within 7 days of examination. Approval by the institutional review committee on the care and use of client-owned animals at The Ohio State University was obtained prior to enrolling dogs in the study. Dogs were enrolled if at least 3 pustules were detected during dermatologic examination and cytologic evaluation of a specimen from a lesion (eg, a papule, pustule, epidermal collarette, scale, or area under a crust) revealed coccoid organisms. If these initial inclusion criteria were satisfied, informed consent and permission for sample collection were obtained from the owner.

Sample collection—Samples were collected at 6 sites (from 3 pustules and 3 carriage sites [ie, anus, nonlesional axillary skin, and nasal mucosa]) by use of a sterile swab technique. The investigator wore sterile surgical gloves while

collecting samples. For collection of exudate from pustules, hair surrounding the pustule was clipped to a length of approximately 0.25 cm with a steam-sterilized blade^a attached to electric clippers.^b Clipped hairs were plucked with steam-sterilized tweezers to create a 1-cm-diameter site free of hair. A sterile adhesive template with a 4-mm-diameter opening (created with a 4-mm sterile punch biopsy tool) was placed over the site to isolate the pustule. A sterile 25-gauge needle was used to puncture the pustule, exudate was collected with a dry sterile swab,^c and the swab was placed in an empty steam-sterilized glass transfer tube. The pustules from which samples were obtained were most commonly located on the ventral and lateral areas of the thorax, ventral portion of the abdomen, and medial aspect of the thighs.

Carriage sites were sampled in the following order: nasal mucosa, nonlesional axillary skin, and anus. To obtain the sample from the nasal mucosa, a dry sterile swab was rolled against the mucosa at a point approximately 1 cm inside the left nasal vestibule for 5 seconds and placed in an empty steam-sterilized glass transfer tube. For the axillary skin sample, nonlesional skin in the left axilla was prepared as described for collection of a sample from a pustule. The sample was obtained by rolling a dry sterile swab for 5 seconds over the entire template opening, after which the swab was placed in an empty steam-sterilized glass transfer tube. For the anal sample, the site was prepared as described. The sterile template was placed over the anus just medial or dorsal to the junction of haired and nonhaired skin of the anus. The sample was obtained by rolling a dry sterile swab for 5 seconds over the entire template opening and placing the swab in an empty steam-sterilized glass transfer tube. The 6 samples collected from each dog were transported to the microbiology laboratory within 1 hour, where they were transferred immediately to culture plates.

Bacterial cultures, morphologic descriptions, and catalase and coagulase tests—Standard methods⁴² for aerobic bacterial culture were used.⁴ Swab specimens were plated on trypticase soy agar with 5% sheep's blood^c and MacConkey medium^f and incubated at 35°C for 18 to 24 hours. From samples that resulted in growth of colonies with morphologic characteristics of staphylococci, a representative colony of each different morphologic type (referred to hereafter as staphylococcal colony type) was isolated.⁴⁴ For each isolate, colony morphology and Gram-stain characteristics were recorded. Catalase production was determined by use of hydrogen peroxide, and coagulase activity was determined by use of rabbit plasma.⁸ Isolates were transferred to brain heart infusion media^h and stored at -80°C until characterized. For inclusion of a given dog and its bacterial isolates in the study, at least 1 of 3 samples obtained from pustules had to result in the growth of at least 1 staphylococcal colony type.

Species identification—A commercial identification systemⁱ was used to speciate each isolate that had been morphologically identified as staphylococcal. Tests were performed and interpreted according to manufacturer's instructions by a single investigator (LRP). Briefly, a fresh culture of each isolate was used to prepare a 0.5 McFarland standard suspension. Aliquots (55 µL) of the suspension were pipetted into each of 26 test-strip cupules containing dried test substrate. After incubation of cupules at 34° to 38°C for 24 hours in a humidified chamber and addition of revealing reagents to some cupules, reaction results were interpreted as positive or negative. From the results of these biochemical tests, a profile number was calculated and entered into the manufacturer's database to speciate the isolate.⁴⁵ For isolates that could not be speciated because of an unacceptable profile number, the test was repeated. If the isolate again had biochemical test results that resulted in an unacceptable profile

number, the organism was classified as coagulase-positive or coagulase-negative *Staphylococcus* spp on the basis of colony morphology and coagulase test results. *Staphylococcus aureus* (American Type Culture Collection 25923) was used as an internal quality control standard.

PFGE—DNA from all isolates underwent PFGE for strain typing.^k Preparation of casting plugs, cell lysis, deproteinization, plug washing, restriction digestion of DNA in agarose plugs, casting of agarose gels, and preparation of the electrophoresis unit were performed according to the CDC Pulse Net protocol.⁴⁵

The following reagents were prepared by use of the standardized protocol methods: Tris-EDTA buffer, cell lysis buffer, proteinase K solution (20 mg/mL), plug agarose, 20% SDS, 1M Tris (pH, 8.0), 0.5M EDTA (pH, 8.0), EC lysis buffer (ie, 6 mM C₄H₁₁NO₃·HCl,^l 1M NaCl, 0.1M EDTA, 0.5% polyoxyethylene cetyl ether,^m 0.2% deoxycholic acid, and 0.5% N-laurylsarcosine; pH, 7.5), lysostaphin stock solution (10 mg/mL), N-laurylsarcosine sodium salt (10%),ⁿ and 0.5 × Tris-borate-EDTA buffer.

The stored frozen isolates were thawed, and cells were streaked onto trypticase soy agar^o and incubated for 18 to 24 hours at 37°C. To prepare casting plugs, bacterial cells were suspended in cold Tris-EDTA (10 mM Tris and 1 mM EDTA; pH, 8.0) buffer solution and mixed with lysostaphin (10 mg/mL stock solution), and the solution was mixed with an equal volume of melted 1% genetic technology grade agarose^p:1% SDS agarose, pipetted into a plug mold, and allowed to harden. For cell lysis and deproteinization, plug pieces were placed in EC lysis buffer and incubated at 37°C for a minimum of 3 hours. Plugs were then transferred to a cell lysis-proteinase K (50 mM Tris:50 mM EDTA [pH, 8.0], 1% N-laurylsarcosine sodium salt, and 0.1 mg/mL proteinase K) buffer solution and incubated 18 to 24 hours at 55°C. Plug pieces were washed 2 times for 15 minutes in water and 4 times for 15 minutes in Tris-EDTA buffer, and plug pieces were stored at 4°C after the final wash. For DNA restriction, plug pieces were restricted for 3 hours in 20 units of *Sma*I according to the manufacturer's instructions^q and placed into a 1% genetic technology grade agarose gel.

Pulsed-field gel electrophoresis was performed on gels for 19 hours in Tris-borate-EDTA buffer at 14°C by use of 1 of 2 commercial systems.¹⁵ Standardized run parameters for 1 system^r were as follows: 10 kilobase, low molecular weight; 700 kilobase, high molecular weight; an initial pulse time of 5.0 seconds; and a final pulse time of 40.0 seconds. Standardized run parameters for the second system^r were as follows: voltage, 6V; angle, 120; initial pulse time of 5.0 seconds; final pulse time of 40.0 seconds; and linear pulse ramp. *Staphylococcus aureus* (NCTC 8325)^s was used as the normalized molecular size standard, and run times were optimized so that the lowest band of the *S aureus* standard migrated 1.0 to 1.5 cm from the bottom of the gel. After electrophoresis, gels were stained in ethidium bromide for 20 minutes and destained in distilled water for 60 minutes.

Gel images were captured by use of a commercial documentation system.^u All images were normalized, analyzed, and stored with gel analysis software.^v Analyses were performed by a single investigator (SY) by use of the gel analysis software and visual inspection. Random genetic events including point mutations, insertions, and deletions alter PFGE patterns. Patterns without fragment differences are considered to be genetically the same strain and genotypically related. One random genetic event may lead to 2 or 3 fragment differences between related isolates.³⁴ For the present study, isolates with fewer than 4 band differences were considered to be the same strain and genotypically related.

Statistical analysis—Descriptive statistics were used to define the population of dogs. Electrophoretic patterns were

analyzed to determine the percentage of dogs that had coagulase-positive staphylococcal isolates with the same PFGE pattern in multiple pustules and multiple carriage sites. In individual dogs, PFGE patterns were analyzed to determine the percentage of dogs that had coagulase-positive staphylococcal isolates from at least 1 pustule and at least 1 carriage site with the same PFGE pattern. In addition, PFGE patterns were analyzed to determine the percentage of coagulase-positive staphylococcal isolates from pustules that had the same PFGE pattern as a coagulase-positive staphylococcal isolate from at least 1 carriage site and to determine the percentage of coagulase-positive staphylococcal isolates from carriage sites that had the same PFGE pattern as a coagulase-positive staphylococcal isolate from at least 1 pustule. Finally, PFGE patterns were analyzed to determine the relatedness between coagulase-negative staphylococcal isolates from pustules and carriage sites. For relevant proportions, exact binomial 95% CIs and *P* values were calculated for testing a null hypothesis that the proportion is 0.5 (ie, that distribution of genetically related isolates is random). A χ^2 test for equal proportions was performed to test the probability of equal proportions among carriage sites. Analyses were performed with commercial software.^w All *P* values were 2-sided.

Results

Dogs—Forty dogs met the inclusion criteria and were included in the study. Dogs ranged from 3 months to 15 years of age (mean, 4.6 years) and included 18 neutered males, 9 sexually intact males, 10 spayed females, and 3 sexually intact females. Ten mixed-breed dogs and 21 purebreds were included, with Labrador Retrievers (*n* = 7), Golden Retrievers (5), Boxers (3), German Shepherd Dogs (2), Lhasa Apsos (2), and Cairn Terriers (2). The following breeds were represented by 1 dog each: Border Collie, Boston Terrier, English Bulldog, Cocker Spaniel, Newfoundland, Pug, Scottish Terrier, Shar Pei, and Chihuahua.

Sample submission, results of bacterial culture, and colony morphology—Six sites (3 pustules and 3 carriage sites [eg, anus, axillary skin, and nasal mucosa]) were sampled in each of the 40 dogs, resulting in submission of 240 samples for bacterial culture. Of those, 207 (86.3%) had at least 1 staphylococcal colony type (from 118 pustule, 33 anus, 26 axillary skin, and 30 nasal mucosa samples). In 33 (13.8%) samples (from 2 pustule, 7 anus, 14 axillary skin, and 10 nasal mucosa samples), no staphylococcal colonies were identified.

In samples that resulted in the growth of staphylococcal colony types, a single colony type was identified in 171 of the 207 (82.6%) samples and more than 1 colony type was identified in 36 (17.4%) samples. In those 36 samples, 2 colony types were identified in 33 samples (from 13 pustule, 7 anus, 5 axillary skin, and 8 nasal mucosa samples) and 3 colony types were identified in 3 samples (from 1 anus and 2 nasal mucosa samples). Thus, 246 staphylococcal colonies were isolated from 207 of 240 samples submitted.

At least 1 staphylococcal colony type was identified in samples from all 3 pustules in 38 of 40 (95.0%; 95.0% CI, 83.1% to 99.4%; *P* < 0.001) dogs and in samples from 2 of 3 pustules in the remaining 2 (5.0%) dogs. Thus, staphylococcal colonies were isolated from at least 2 of the 3 pustules in all 40 dogs.

Staphylococcal colony types were not identified in 2 samples from pustules, each of which came from a different dog.

At least 1 staphylococcal colony type was identified in samples from all 3 carriage sites in 18 of 40 (45.0%) dogs, in samples from 2 of 3 carriage sites in 15 (37.5%) dogs, and in samples from 1 carriage site in 5 (12.5%) dogs. Thus, staphylococcal colonies were isolated from at least 2 of 3 carriage sites in 33 of 40 (82.5%; 95.0% CI, 67.2% to 92.7%; $P < 0.001$) dogs. Staphylococcal colony types were not identified in samples from any carriage site in 2 dogs.

Gram stain and catalase and coagulase tests—All 246 isolates were Gram positive and catalase positive. Of the 246 isolates, 224 (91.1%) were coagulase positive (128 pustule, 35 anus, 26 axillary skin, and 35 nasal mucosa isolates) and 22 (8.9%) were coagulase negative (3 pustule, 7 anus, 5 axillary skin, and 7 nasal mucosa isolates). One coagulase-positive isolate from the anus was lost during storage at -80°C after bacterial culture, morphologic description, Gram staining, and catalase and coagulase testing. Therefore, 245 isolates (223 coagulase-positive isolates and 22 coagulase-negative isolates) were available for speciation and PFGE.

Species identification—Two hundred twenty-three coagulase-positive isolates were cultured from 40 dogs. Of those, 203 (91.0%) were *S. intermedius* (117 pustule, 32 anus, 24 axillary skin, and 30 nasal mucosa isolates), 5 (2.2%) were *S. aureus* (3 pustule, 1 anus, and 1 nasal mucosa isolates), and 15 (6.7%) were coagulase-positive *Staphylococcus* spp (8 pustule, 1 anus, 2 axillary skin, and 4 nasal mucosa isolates).

Staphylococcus intermedius isolates were cultured from all 3 pustules in 36 of 40 (90.0%; 95.0% CI, 76.3% to 97.2%; $P < 0.001$) dogs and from at least 1 of 3 pustules in 39 of 40 (97.5%; 95.0% CI, 86.8% to 99.9%; $P < 0.001$) dogs. Four of the 5 *S. aureus* isolates were cultured from a single dog (from 3 pustules and the anus), and *S. intermedius* was always isolated concurrently. The fifth *S. aureus* isolate was cultured from the nasal mucosa in another dog. The 15 coagulase-positive *Staphylococcus* spp isolates were cultured from 6 of 40 (15.0%) dogs (from all 3 pustules in 2 dogs and from 1 pustule in 2 dogs). Coagulase-positive *Staphylococcus* spp isolates were cultured only from carriage sites in 2 dogs (from all 3 carriage sites in 1 dog and from the nasal mucosa in the second dog).

Twenty-two coagulase-negative isolates were cultured from 13 of 40 (32.5%) dogs. A single coagulase-negative isolate was cultured from 8 dogs, 2 were cultured from 1 dog, and 3 were cultured from 4 dogs. Sixteen of 22 (72.7%) isolates were speciated, 5 (22.7%) were classified as coagulase-negative *Staphylococcus* spp, and 1 (4.5%) from the nasal mucosa was not recognized as a member of genus *Staphylococcus* and was identified as *Stomatococcus mucilaginosus*.

Only 3 (13.6%) coagulase-negative isolates (1 *Staphylococcus epidermidis*, 1 *Staphylococcus sciuri*, and 1 coagulase-negative *Staphylococcus* sp) were cultured from pustules. Each was from a different dog and was isolated

concurrently with *S. intermedius* in the same pustule. Nineteen of 22 (86.4%) coagulase-negative isolates were cultured from carriage sites (7 from the anus, 5 from the axillary skin, and 7 from the nasal mucosa) in 13 dogs. Five of those 19 isolates were the only staphylococcal isolate cultured from the carriage site, and 14 were isolated concurrently with *S. intermedius*.

The coagulase-negative staphylococcal species identified from the anus were *Staphylococcus chromogenes* ($n = 2$), *Staphylococcus haemolyticus* (1), *Staphylococcus capitis* (1), *Staphylococcus caprae* (1), and coagulase-negative *Staphylococcus* spp (2). The coagulase-negative staphylococcal spp identified from the nonlesional axillary skin were *S. chromogenes* (1), *S. epidermidis* (1), *S. sciuri* (1), *Staphylococcus cohnii* (1), and coagulase-negative *Staphylococcus* spp (1). The coagulase-negative staphylococcal spp identified from the nasal mucosa were *S. chromogenes* (1), *S. haemolyticus* (1), *Staphylococcus warneri* (1), *Staphylococcus lentus* (1), *Staphylococcus hominis* (1), and coagulase-negative *Staphylococcus* spp (1).

PFGE

Untypeable isolates—Of the 223 coagulase-positive isolates submitted for PFGE, a PFGE pattern could not be obtained for 7 *S. intermedius* isolates (from 3 pustules and 4 carriage sites). Five of these untypeable isolates included all the isolates cultured from 1 dog; therefore, this dog was excluded from PFGE

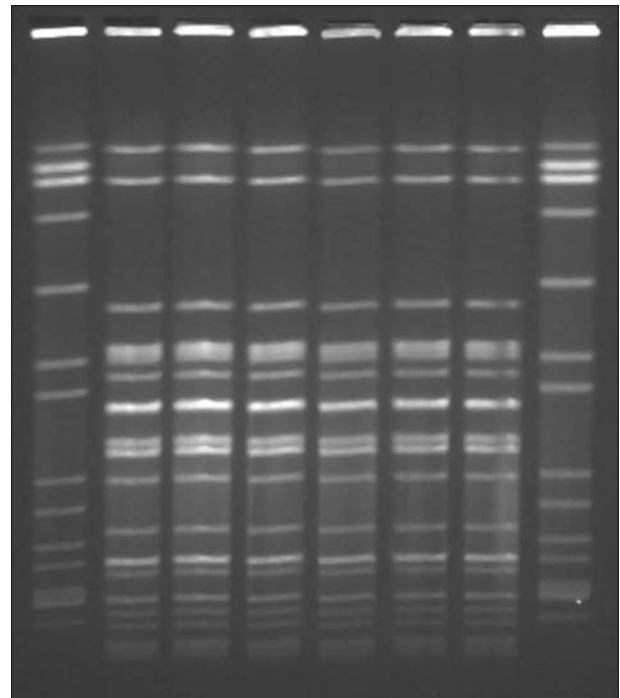


Figure 1—Pulsed-field gel electrophoresis patterns for 6 *Staphylococcus intermedius* isolates after digestion with the restriction enzyme *Sma*I. Isolates were cultured from 3 pustules and 3 carriage sites on a dog with superficial bacterial folliculitis. Lanes 1 and 8 (from left to right) = *Staphylococcus aureus* NCTC 8325 standards. Lanes 2 through 4 = Isolates from pustule 1, pustule 2, and pustule 3, respectively. Lanes 5 through 7 = Isolates from the anus, nonlesional axillary skin, and nasal mucosa, respectively. Notice that all 6 isolates had the same PFGE pattern.

analyses. Of the 22 coagulase-negative isolates submitted for PFGE, a PFGE pattern could not be obtained for 7 isolates (from 1 pustule and 6 carriage sites). Thus, 216 coagulase-positive isolates (196 *S. intermedius*, 5 *S. aureus*, and 15 *Staphylococcus* spp) and 15 coagulase-negative isolates cultured from 39 dogs were successfully strain typed by use of PFGE.

PFGE patterns—Sixty-six PFGE patterns were observed among the 196 *S. intermedius* isolates, 2 patterns were observed among the 5 *S. aureus* isolates, 7 patterns were observed among the 15 coagulase-positive *Staphylococcus* spp isolates, and 14 patterns were observed among the 15 coagulase-negative isolates. None of the 39 dogs had any isolate with the same PFGE pattern as an isolate from another dog.

PFGE patterns for coagulase-positive staphylococcal isolates from pustules—In 29 of 39 (74.4%) dogs, *S. intermedius* isolates from all 3 pustules had the same PFGE pattern (Figure 1). In addition to having the same PFGE pattern for the *S. intermedius* isolates from all 3 pustules, in 1 of those 29 dogs, *S. aureus* was also isolated from all 3 pustules, and all 3 *S. aureus* isolates had the same PFGE pattern (Figure 2). Another 1 of those 29 dogs also had coagulase-positive *Staphylococcus* spp isolated from all 3 pustules, and all 3 coagulase-positive *Staphylococcus* spp isolates had the same PFGE pattern.

In 1 of 39 (2.6%) dogs, coagulase-positive *Staphylococcus* spp alone were isolated from all 3 pustules. All 3 of the coagulase-positive *Staphylococcus* spp isolates had the same PFGE pattern.

In an additional 7 (17.9%) dogs, isolates from 2 pustules had the same PFGE pattern. In 6 of those 7 dogs, *S. intermedius* was isolated from 2 of 3 pustules and both had the same PFGE pattern (Figure 3). In 1 of those 7 dogs, a coagulase-positive *Staphylococcus* sp isolate from 1 pustule had the same PFGE pattern as an *S. intermedius* isolate from another pustule. Thus, 37 of 39 (94.9%; 95.0% CI, 82.7% to 99.4%; $P < 0.001$) dogs had coagulase-positive staphylococcal isolates with the same PFGE pattern cultured from multiple (ie, at least 2) pustules.

In 1 of the remaining 2 dogs, *S. intermedius* was isolated from all 3 pustules and each isolate had a different PFGE pattern (Figure 4). In the last dog, *S. intermedius* was isolated from 2 pustules and a coagulase-positive *Staphylococcus* sp was isolated from the third pustule; each of the 3 isolates had a different PFGE pattern.

PFGE patterns for coagulase-positive staphylococcal isolates from carriage sites—In 8 of 39 (20.5%) dogs, *S. intermedius* isolates from all 3 carriage sites had the same PFGE pattern (Figure 1). In 1 of 39 dogs, coagulase-positive *Staphylococcus* spp isolates from all 3 carriage sites had the same PFGE pattern. In 14 of 39 (35.9%) dogs, isolates from 2 carriage sites had the same PFGE pattern. In 13 of these 14 dogs, both isolates were *S. intermedius* (Figure 4); in the remaining dog, an *S. intermedius* isolate from the axillary skin had the same PFGE pattern as a coagulase-positive *Staphylococcus* sp isolate from the nasal mucosa. That dog was also 1 of the 8 dogs in which the *S. intermedius* isolates from all 3 carriage sites had the same PFGE pattern. Thus, 22 of 39 (56.4%; 95.0% CI,

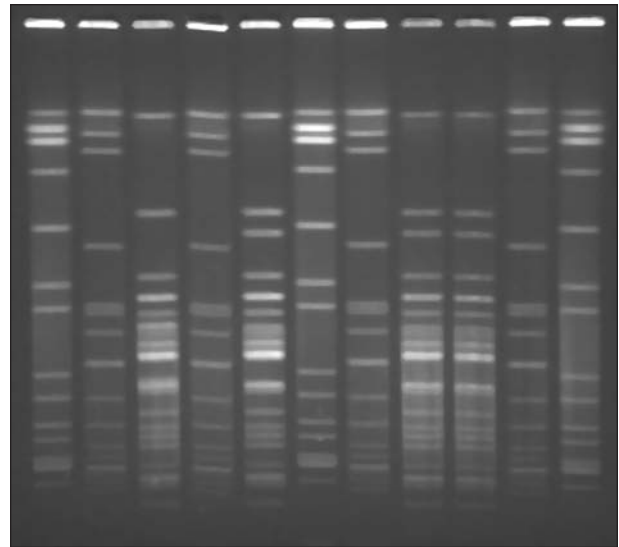


Figure 2—Pulsed-field gel electrophoresis patterns for 8 coagulase-positive staphylococcal isolates after digestion with the restriction enzyme *Sma*I. Isolates were cultured from 3 pustules and 1 carriage site on a dog with superficial bacterial folliculitis. Lanes 1, 6, and 11 = *S. aureus* NCTC 8325 standards. Lanes 2, 4, 7, and 10 = *S. aureus* isolates from pustule 1, pustule 2, pustule 3, and the anus, respectively (notice that those isolates had the same PFGE pattern). Lanes 3, 5, 8, and 9 = *S. intermedius* isolates from pustule 1, pustule 2, pustule 3, and the anus, respectively (notice that those isolates had the same PFGE pattern).

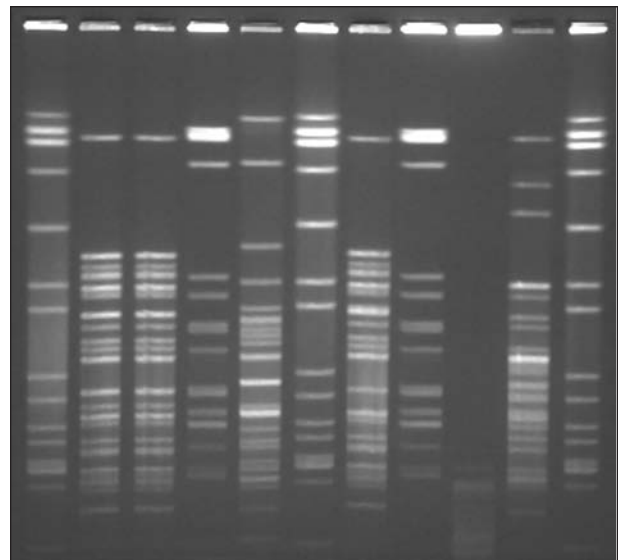


Figure 3—Pulsed-field gel electrophoresis patterns for 8 staphylococcal isolates after restriction enzyme digestion with *Sma*I. Isolates were cultured from 3 pustules and 3 carriage sites on a dog with superficial bacterial folliculitis. Lanes 1, 6, and 11 = *S. aureus* NCTC 8325 standards. Lanes 2, 3, and 7 = *S. intermedius* isolates from pustule 1, pustule 2, and the anus, respectively (notice that those isolates had the same PFGE pattern). Lanes 4 and 8 = *Staphylococcus epidermidis* isolates from pustule 3 and nonlesional axillary skin, respectively (notice that both isolates had the same PFGE pattern). Lane 5 = *S. intermedius* isolate from pustule 3 (notice that the PFGE pattern was different from that of the other isolates). Lane 9 = Untypeable isolate from nasal mucosa (notice that the PFGE pattern was different from that of the other isolates). Lane 10 = *S. intermedius* isolate from nasal mucosa (notice that the PFGE pattern was different from that of the other isolates).

39.6% to 72.2%; $P = 0.423$) dogs had coagulase-positive staphylococcal isolates with the same PFGE pattern cultured from multiple (at least 2) carriage sites.

In 8 additional dogs, *S. intermedius* was isolated from 2 carriage sites and each had a different PFGE pattern (Figure 5). In 6 dogs, coagulase-positive staphylococci were isolated from only 1 carriage site (Figure 2), and in 3 dogs, coagulase-positive staphylococci were not isolated from any carriage site.

Genotypic relatedness between coagulase-positive staphylococcal isolates from pustules and carriage sites—The PFGE patterns for isolates from pustules and carriage sites within each of 36 dogs were analyzed; 3 dogs were excluded because coagulase-positive staphylococci were not isolated from any carriage site in these dogs. In 26 of 36 (72.2%; 95.0% CI, 54.8% to 85.8%; $P = 0.008$) dogs, isolates from all 3 pustules had the same PFGE pattern as an isolate from at least 1 carriage site (Figures 1 and 2). In 4 of 36 (11.1%) dogs, isolates from 2 of 3 pustules had the same PFGE pattern as an isolate from at least 1 carriage site (Figure 3). In 4 of 36 dogs, an isolate from 1 of 3 pustules had the same PFGE pattern as an isolate from at least 1 carriage site (Figure 4). In 2 of 36 (5.6%) dogs, isolates from all pustules had different PFGE patterns than isolates from all carriage sites (Figure 5). Thus, 34 of 36 (94.4%; 95.0% CI, 81.3% to 99.3%; $P <$

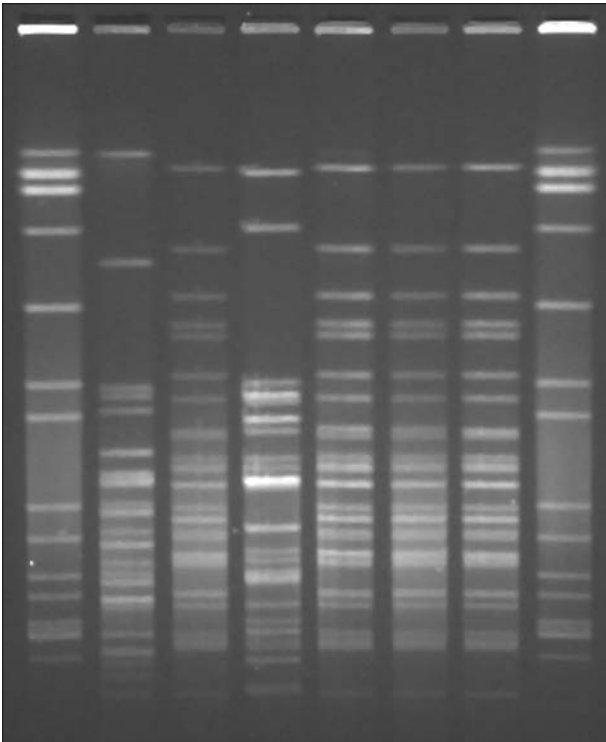


Figure 4—Pulsed-field gel electrophoresis patterns for 6 coagulase-positive *S. intermedius* isolates after restriction enzyme digestion with *Sma*I. Isolates were cultured from 3 pustules and 3 carriage sites in a dog with superficial bacterial folliculitis. Lanes 1 and 8 = *S. aureus* NCTC 8325 standards. Lanes 2, 4, and 5 = *S. intermedius* isolates from pustule 1, pustule 3, and the anus, respectively (notice that each had a different PFGE pattern). Lanes 3, 6, and 7 = *S. intermedius* isolates from pustule 2, axillary skin, and nasal mucosa, respectively (notice that each had the same PFGE pattern).

0.001) dogs had coagulase-positive staphylococcal isolates from at least 1 pustule with the same PFGE pattern as coagulase-positive staphylococcal isolates from at least 1 carriage site (Table 1).

The PFGE patterns for the 216 isolates from pustules (125 isolates) and carriage sites (91 isolates) were

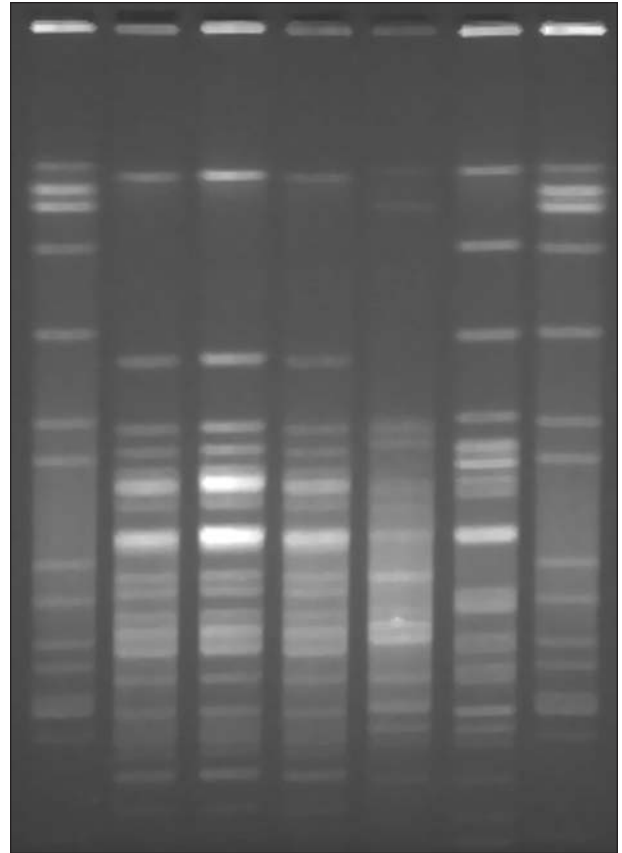


Figure 5—Pulsed-field gel electrophoresis patterns for 5 *S. intermedius* isolates after restriction enzyme digestion with *Sma*I. Isolates were cultured from 3 pustules and 2 carriage sites in a dog with superficial bacterial folliculitis. Lanes 1 and 7 = *S. aureus* NCTC 8325 standards. Lanes 2 through 4 = *S. intermedius* isolates from pustule 1, pustule 2, and pustule 3, respectively (notice that those isolates had the same PFGE pattern). Lanes 5 and 6 = *S. intermedius* isolates from the anus and nasal mucosa, respectively (notice that each had a different PFGE pattern).

Table 1—Number of dogs that had coagulase-positive staphylococcal isolates with the same PFGE pattern from the corresponding number of pustules and carriage sites, indicating the genotypic relatedness of coagulase-positive staphylococcal isolates obtained from pustules and carriage sites in 36 dogs with superficial bacterial folliculitis.

No. of carriage sites	No. of pustules			
	3	2	1	0
3	7*	0	1	0
2	9	2	1	0
1	10	2	2	0
0	0	0	0	2
Total No. of dogs	26	4	4	2

*For example, there were 7 dogs with isolates from all 3 pustules that had the same PFGE pattern as isolates from all 3 carriage sites.

Table 2—Number of isolates from sampled sites that had the same or different PFGE pattern as an isolate from at least 1 carriage site (for pustules) or at least 1 pustule (for carriage sites), indicating the genotypic relatedness of 207 coagulase-positive staphylococcal isolates from pustules (116 isolates) and carriage sites (91 isolates) in 36 dogs with superficial bacterial folliculitis.

Species	Site from which sample was obtained							
	Pustules		Anus		Axillary skin		Nasal mucosa	
	Same	Different	Same	Different	Same	Different	Same	Different
<i>Staphylococcus intermedius</i>	88	17	23	8	21	2	22	6
<i>Staphylococcus aureus</i>	3	0	1	0	0	0	0	1
<i>Staphylococcus</i> spp	6	2	0	1	0	2	2	2
Total	97*	19*	24	9	21†	4†	24	9

*For example, 97 isolates from pustules had a PFGE pattern that was the same as a PFGE pattern of an isolate from at least 1 carriage site, and 19 isolates from pustules had a PFGE pattern that was different from the PFGE patterns of all isolates from all carriage sites in the same dog. †Twenty-one isolates from an axillary skin site had a PFGE pattern that was the same as that of an isolate from at least 1 pustule, and 4 isolates from axillary skin had a PFGE pattern that was different from the pattern of all isolates from all pustules in the same dog.

analyzed. Nine pustule isolates from 3 dogs were excluded from this analysis because coagulase-positive staphylococci were not isolated from any carriage site in those dogs. Thus, the PFGE patterns for 207 isolates (116 from pustules and 91 from carriage sites) were analyzed. Ninety-seven of 116 (83.6%; 95.0% CI, 75.6% to 89.8%; $P < 0.001$) isolates from pustules had the same PFGE pattern as an isolate from at least 1 carriage site. Nineteen of 116 (16.4%) isolates from pustules had different PFGE patterns than isolates from all carriage sites (Table 2).

Sixty-nine of 91 (75.8%; 95.0% CI, 65.7% to 84.2%; $P < 0.001$) isolates from carriage sites had the same PFGE pattern as an isolate from at least 1 pustule. Of those, 24 (34.8%) were from the anus, 21 (30.4%) were from axillary skin, and 24 (34.8%) were from nasal mucosa. The isolates were equally distributed between carriage sites ($P = 0.878$). Twenty-two of 91 (24.2%) isolates from carriage sites had different PFGE patterns than isolates from all pustules. Of these, 9 of 22 (40.9%) were from the anus, 4 of 22 (18.2%) were from the axillary skin, and 9 of 22 were from the nasal mucosa (Table 2). The isolates were equally distributed between carriage sites ($P = 0.321$).

Genotypic relatedness between coagulase-negative staphylococcal isolates from pustules and carriage sites—Twenty-two coagulase-negative isolates were cultured from 13 dogs. During speciation, 1 of those 22 isolates could not be classified as belonging to genus *Staphylococcus*, and the DNA from 7 of the 22 coagulase-negative isolates was degraded during PFGE procedures (Figure 3). Five of those 7 isolates represented all of the coagulase-negative isolates from 2 dogs. Thus, the PFGE patterns for 14 coagulase-negative staphylococcal isolates from 11 dogs were analyzed. Of those, only 2 isolates had the same PFGE pattern. Both were *S epidermidis* isolated from a pustule and the axillary skin of a single dog. In 2 other dogs, the same species of coagulase-negative staphylococci was isolated more than once, but in neither dog did the isolates have the same PFGE pattern.

Discussion

In the present study, 94.4% of dogs had coagulase-positive staphylococcal isolates from at least 1

pustule that were the same strain as isolates from at least 1 carriage site, and 72.2% had coagulase-positive staphylococcal isolates from all 3 pustules that were the same strain as an isolate from at least 1 carriage site. In addition, 83.6% of coagulase-positive staphylococcal isolates from pustules were the same strain as an isolate from at least 1 carriage site, and a substantial number (75.8%) of coagulase-positive staphylococcal isolates from carriage sites were the same strain as an isolate from at least 1 pustule. Thus, on the basis of PFGE pattern analysis, results indicated that coagulase-positive staphylococcal isolates cultured from pustules and carriage sites in dogs with superficial bacterial folliculitis are genotypically the same strain. Because genotypically related strains were isolated from individual dogs, they may be considered to be clonally related and derived from a common source in each dog.^{19,34}

These results support the findings of a recent study³⁹ in which PFGE patterns of *S intermedius* isolates obtained from lesions in dogs with dermatologic disease were compared with isolates obtained from clinically normal sites (eg, nares, external auditory meatus, and skin). In 3 of 6 dogs, isolates from dermatitic lesions had the same PFGE pattern as isolates from at least 1 clinically normal site. However, in the present study more dogs were evaluated, both pustules and carriage sites were sampled in each dog, and the same carriage sites were sampled in all dogs. Thus, in the present study, analysis of the genotypic relatedness between staphylococcal strains cultured from pustules and carriage sites in each of 40 dogs with superficial bacterial folliculitis could be performed.

Carriage of staphylococcal organisms on non-lesional skin and mucosal surfaces in healthy dogs has been compared with carriage in dogs with superficial bacterial folliculitis in previous studies.^{25,32} However, to the authors' knowledge, no study has established whether any 1 carriage site is more likely than another to harbor the staphylococcal strain isolated from a pustule in individual dogs with superficial bacterial folliculitis. In the present study, isolates from carriage sites that were the same strain as an isolate from a pustule were equally distributed between the 3 carriage sites

sampled. This suggests that none of the carriage sites evaluated was more likely than another to harbor the staphylococcal strain that would be isolated from a pustule.

The primary lesion in canine superficial bacterial folliculitis is a pustule,⁴² the type of lesion sampled in the present study. The fact that exudate was collected directly from pustules reduced the probability that bacteria from the nonlesional skin surface would be cultured and increased the probability of isolating the pathogenic organism. The anus, nonlesional axillary skin, and nasal mucosa were the carriage sites chosen for sampling in this study because each site has been reported^{3,25,27,29,31,32} as a carriage site of staphylococci. It is known that staphylococci may be resident on hair shafts,²¹⁻²⁴ so hairs were removed from all sites prior to sampling in the present study to ensure that cultured organisms had not been obtained from the hair shafts.

Staphylococcal colony types were not identified in culture samples from only 2 pustules in 2 different dogs. This may have been the result of sampling a resolving lesion, the fact that there was another concurrent pustular disease, or an error in sampling technique. In these 2 dogs, *S intermedius* was isolated from the other 2 pustules, confirming the diagnosis of superficial bacterial folliculitis. Obtaining a sterile culture from a sample obtained from a pustule may occur on rare occasions in dogs with suspected superficial bacterial folliculitis. Thus, clinicians should consider patient history, dermatologic examination, and results of skin-surface cytologic examination, deep skin scrapings, and dermatophyte culture, as well as the response to appropriate antimicrobial treatment before eliminating superficial bacterial folliculitis as the cause of observed lesions. Staphylococcal colony types were not identified in culture samples obtained from all 3 carriage sites in only 2 dogs. This finding was in agreement with that from an earlier study²⁹ in which samples obtained from carriage sites in 2 of 50 dogs did not result in the isolation of staphylococcal organisms.

Staphylococcus intermedius was the most common (203/224 [90.6%]) species identified in the present study and was isolated from at least 1 pustule in 97.5% of dogs and from all 3 pustules in 90.0% of dogs. These results confirm that *S intermedius* is the most common staphylococcal organism isolated from primary lesions in dogs with superficial bacterial folliculitis.^{1,3-7,13} The identification of only 5 *S aureus* isolates was in agreement with results of previous studies^{3,4,8} and indicates that *S aureus* is infrequently isolated from dogs with superficial bacterial folliculitis.

More than 1 staphylococcal organism was isolated from 13 of 118 pustules sampled. *Staphylococcus intermedius* was identified in each and always concurrently with a different *S intermedius*, *S aureus*, coagulase-positive *Staphylococcus* spp, or a coagulase-negative staphylococcal organism. Other investigators have reported^{4,6,11} isolation of more than 1 staphylococcal organism from lesions in dogs with superficial bacterial folliculitis, but the clinical importance of isolating > 1 organism from a pustule warrants further investigation.

Coagulase-negative staphylococci have been isolated from healthy dogs^{10,21,27,28,30,31,41} and from lesions in

dogs with superficial bacterial folliculitis.^{6,10-13,37} In the present study, 22 coagulase-negative staphylococcal isolates were cultured from 13 of 40 dogs. Only 3 of those isolates were obtained from pustules; each isolate came from a different dog, and all were isolated concurrently with *S intermedius*. This result was in agreement with that from a recent study¹¹ in which 28 dogs with pyoderma were evaluated and *S intermedius* was isolated concurrently with a coagulase-negative staphylococcal organism in 2 of the 28 dogs. The clinical importance of isolating coagulase-negative staphylococci concurrently with *S intermedius* warrants further investigation.

One coagulase-negative isolate obtained from nasal mucosa was not recognized as a member of genus *Staphylococcus* but was identified as *S mucilaginosus*. The commercial identification test used in the present study identifies species in genera *Staphylococcus*, *Micrococcus*, *Stomatococcus*, and *Aerococcus*. This isolate had morphologic characteristics and Gram-stain, catalase test, and coagulase test results typical of staphylococci yet was not identified as a staphylococcal species. Therefore, that isolate was not evaluated further in this study.

In addition, 15 coagulase-positive and 5 other coagulase-negative isolates could not be identified with the commercial identification system used and were classified simply as *Staphylococcus* spp. The identification system incorporates 26 biochemical tests and has been used in studies of staphylococcal isolates derived from dogs.^{33,46} *Staphylococcus intermedius*, *S aureus*, *S schleiferi*, and other staphylococcal species of veterinary importance are included in the manufacturer's species database. The test has also identified *S schleiferi* in a human clinical isolate.⁴⁷ Results of 2 recent studies,^{9,48} however, suggest that *S schleiferi* isolates from dogs may be misidentified by commercial identification systems. Isolation of *S schleiferi* from dogs has only recently been reported, and the organism has been identified in isolates obtained from healthy dogs,¹⁰ dogs with otitis externa,¹⁰ and dogs with superficial bacterial folliculitis.⁹⁻¹³ Biochemical profile numbers required to identify *S schleiferi* subsp *coagulans* and *S schleiferi* subsp *schleiferi* isolates from dogs may not be included in the manufacturer's species database. If these isolates were indeed *S schleiferi*, this deficiency may explain why some of the isolates were not speciated.

The PFGE protocol used in the present study was successful in strain typing most staphylococcal strains and supports the results of a previous investigation¹⁸ establishing PFGE as a useful method of characterizing *S intermedius* isolates obtained from dogs. However, in the present study, the DNA from 14 isolates was degraded during the PFGE procedure, and the same result occurred when PFGE was repeated. With PFGE, fragments may not be delineated as a result of production of endogenous endonucleases by the bacteria, DNA digestion at inappropriate temperatures, use of inappropriate electrophoresis parameters, incorrect concentration or temperature of the running buffer, or an unsecured gel in the electrophoresis chamber.⁴⁹ Standardized methods were used, and the *S aureus* molecular weight standard was delineated on each gel

in which DNA degradation occurred. Further, on all but 1 gel in which the DNA from all clinical isolates was degraded, other staphylococcal strains on the gel were successfully typed. Therefore, the most likely explanation for the DNA degradation in some of the clinical isolates in this study was the production of endogenous endonucleases by the bacteria.⁴⁹

Results of studies^{18,20,39} in which PFGE was used suggest that the *S intermedius* population is heterogeneous and that *S intermedius* strains isolated from different dogs with superficial bacterial folliculitis are not clonally related.²⁰ The heterogeneity of staphylococcal strains isolated from dogs with superficial bacterial folliculitis and delineated with PFGE was confirmed in the present study because none of the 39 dogs evaluated had an isolate that was the same strain as an isolate from another dog, a finding that corroborated those findings in previous studies. In 1 study,³⁹ none of the PFGE patterns for *S intermedius* isolates obtained from dogs with dermatitis (with or without otitis) and healthy dogs had the same PFGE pattern. In another study²⁰ in which PFGE was used to characterize *S intermedius* isolates from healthy dogs and isolates from pustules in dogs with superficial bacterial folliculitis, each dog had different strains of *S intermedius*. Finally, in another study,¹⁸ 52 *S intermedius* isolates from diseased and healthy dogs were evaluated and found to have different PFGE patterns.

coagulase-positive staphylococcal isolates that were the same strain in at least 2 pustules in (94.9% of dogs. To the authors' knowledge, the present study is the first in which the same strain of staphylococci was identified concurrently in multiple lesions on the same dog on the basis of genotype. The same strain of coagulase-positive staphylococci was also isolated from multiple carriage sites in 22 of 39 dogs. Although this finding was not significant, it suggests that a single strain of coagulase-positive staphylococci may colonize more than 1 carriage site in a given dog.

On the basis of results of the present study, the genotypic relatedness between coagulase-positive staphylococci isolated from pustules and carriage sites in individual dogs with superficial bacterial folliculitis has been confirmed, but it has not been established whether the staphylococcal organism causing lesions was transferred to the skin surface from a carriage site prior to development of infection. It is also possible that carriage sites are colonized with staphylococci from lesions as a result of grooming and pruritic behaviors after infection is established. To establish that carriage sites are the source of bacteria causing lesions, PFGE patterns for isolates obtained from carriage sites prior to the development of lesions must be compared with isolates obtained from lesions and carriage sites when a dog is diagnosed with superficial bacterial folliculitis. If carriage sites are a source of staphylococci, strains isolated from carriage sites prior to infection would theoretically be the same as those isolated from lesions and carriage sites during infection. If carriage sites are established as the source of bacteria causing lesions in dogs with superficial bacterial folliculitis, the use of systemically and topically active antimicrobial

agents for treatment of staphylococcal organisms colonizing carriage sites warrants further investigation.

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- a. Cryogen-X size 40 clipper blade, Oster Professional Products, McMinnville, Tenn.
 - b. Golden A5 single-speed clipper, Oster Professional Products, McMinnville, Tenn.
 - c. Calgiswab type 1, Hardwood Products Co LLC, Guilford, Me.
 - d. Clinical Microbiology Laboratory, The Ohio State University Veterinary Teaching Hospital, The Ohio State University, Columbus, Ohio.
 - e. BBL Trypticase soy agar with 5% sheep blood, Becton-Dickinson & Co, Sparks, Md.
 - f. BBL MacConkey agar, Becton-Dickinson & Co, Sparks, Md.
 - g. BBL Coagulase Plasma, Rabbit tube coagulase test, Becton-Dickinson Co, Sparks, Md.
 - h. BBL brain heart infusion media, Becton-Dickinson Co, Sparks, Md.
 - i. API ID 32 Staph, bioMerieux Inc, Marcy l'Etoile, France.
 - j. *Staphylococcus aureus* ATCC 25923, MicroBioLogics Inc, St Cloud, Minn.
 - k. The Bureau of Public Health Laboratories, The Ohio Department of Health, Columbus, Ohio.
 - l. Trizma hydrochloride, Sigma-Aldrich Inc, St Louis, Mo.
 - m. Brij 58, polyoxyethylene 20 cetyl ether, Sigma-Aldrich Inc, St Louis, Mo.
 - n. Sarcosyl N-laurylsarcosine, sodium salt, Sigma-Aldrich Inc, St Louis, Mo.
 - o. Trypticase soy agar with 5% sheep blood, Remel Inc, Lenexa, Kan.
 - p. SeaKem Gold GTG agarose, FMC BioProducts, Rockland, Me.
 - q. Smal kit 20 U/μL, New England BioLabs, Beverly, Mass.
 - r. CHEF Mapper DR II Pulsed-field Electrophoresis System, Bio-Rad Laboratories Inc, Hercules, Calif.
 - s. GenePath strain typing system, Bio-Rad Laboratories Inc, Hercules, Calif.
 - t. *Staphylococcus aureus* NCTC 8325, Health Protection Agency, London, UK.
 - u. Gel Doc 2000 gel documentation system, Bio-Rad Laboratories Inc, Hercules, Calif.
 - v. BioNumerics gel analysis software, version 2.1, Applied Maths Inc, Austin, Tex.
 - w. SAS statistical software, version 9.2, SAS Institute Inc, Cary, NC.
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