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Enterotoxigenic Potential of *Staphylococcus intermedius*

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Staphylococcal food poisoning (SFP) caused by enterotoxigenic staphylococci is one of the main food-borne diseases. In contrast to *Staphylococcus aureus*, a systematic screening for the enterotoxins has not yet been performed on the genomic level for the coagulase-positive species *S. intermedius*. Therefore, the enterotoxigenic potential of 281 different veterinary (canine, $n = 247$; equine, $n = 23$; feline, $n = 9$; other, $n = 2$) and 11 human isolates of *S. intermedius* was tested by using a multiplex PCR DNA-enzyme immunoassay system targeting the staphylococcal enterotoxin genes *sea*, *seb*, *sec*, *sed*, and *see*. Molecular results were compared by in vitro testing of enterotoxin production by two immunoassays. A total of 33 (11.3%) *S. intermedius* isolates, including 31 (12.6%) canine isolates, 1 equine isolate, and 1 human isolate, tested positive for the *sec* gene. In vitro production of the respective enterotoxins was detected in 30 (90.9%) of these isolates by using immunological tests. In contrast, none of 65 veterinary specimen-derived isolates additionally tested and comprising 13 (sub)species of coagulase-negative staphylococci were found to be enterotoxigenic. This study shows on both molecular and immunological levels that a substantial number of *S. intermedius* isolates harbor the potential for enterotoxin production. Since evidence for noninvasive zoonotic transmission of *S. intermedius* from animal hosts to humans has been documented, an enterotoxigenic role of this microorganism in SFP via contamination of food products may be assumed.

Staphylococcal enterotoxins (SEs) are members of a subgroup of related protein exotoxins in the pyrogenic toxin (PT) family exhibiting a diverse number of shared (e.g., superantigenicity) and unique biological activities (7, 18, 42). SEs are distinguishable from other members of the PT family by their ability to induce emesis and diarrhea when ingested (17). Thus, they are causative agents in staphylococcal food poisoning (SFP), one of the main food-borne diseases having major economic and public health impacts (5, 6, 62). For *Staphylococcus aureus*, the production of SEs, such as SEA, SEB, SEC, SED, and SEE, is well documented (7, 8, 30, 40).

Unlike the situation for *S. aureus*, only limited and conflicting data are available regarding enterotoxin production in non-*S. aureus* staphylococcal species (10, 23, 25, 46, 52, 57, 63, 65). One of these species, the coagulase-positive *S. intermedius*, is the predominant staphylococcal species isolated from healthy and diseased dogs and has been isolated from a number of other carnivores, horses, and birds (16, 36, 54). In single cases, this species appears to be also responsible, as a zoonotic pathogen, for canine-inflicted human wound infections and invasive infections in immunocompromised patients (43, 45, 59, 64). Occasionally, the possession of enterotoxins has been reported for *S. intermedius*, and its involvement in an outbreak of SFP has been considered previously (4, 24, 37, 41). Previous reports regarding *S. intermedius* enterotoxins were restricted particularly to immunological procedures, which are generally limited in sensitivity and known for nonspecific results (14, 38, 53). DNA hybridization- and amplification-based approaches offer alternatives to detect staphylococcal isolates bearing the genetic sequences to produce enterotoxins irrespective of their toxin expression (56). Hence, there is an obvious need for a systematic verification of the enterotoxigenic potential of *S. intermedius* on a genomic level. For this purpose, a large panel of veterinary *S. intermedius* isolates and a control group of different animal-derived isolates of coagulase-negative staphylococci (CNS) were tested for the presence of SE-encoding genes by a multiplex PCR DNA-enzyme immunoassay (DNA-EIA).

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MATERIALS AND METHODS

Bacterial strains. From September 1999 to August 2000, 1,669 consecutive animal specimens (canine, $n = 590$; equine, $n = 896$; feline, $n = 150$; other, $n = 33$) from the skin or mucosal surfaces of animals with skin or mucocutaneous infections were investigated (Table 1). The specimens were submitted by veterinary care centers in northwestern Germany to the Department of Microbiology and Infectious Diseases, School of Veterinary Medicine, Hannover, Germany. No duplicate isolates were included. In addition, 11 human *S. intermedius* isolates previously collected were studied (49).

Sixty-five CNS of 13 different (sub)species obtained from veterinary specimens (*S. capitis* subsp. *capitis*, $n = 2$; *S. chromogenes*, $n = 3$; *S. cohnii* subsp. *cohnii*, $n = 4$; *S. epidermidis*, $n = 5$; *S. equorum*, $n = 1$; *S. felis*, $n = 1$; *S. haemolyticus*, $n = 1$; *S. hominis* subsp. *hominis*, $n = 1$; *S. lentus*, $n = 1$; *S. sciuri* subsp. *sciuri*, $n = 9$; *S. simulans*, $n = 11$; *S. warneri*, $n = 3$; *S. xylosus*, $n = 22$) were included as controls.

Identification. Identification was based on growth characteristics on Columbia agar with 5% (vol/vol) sheep blood at 37°C and positive catalase production. The staphylococcal isolates were identified biochemically by the automated ID 32 Staph system (bioMérieux, Marcy l'Etoile, France). The presence of bound or free coagulase was confirmed by latex slide agglutination for clumping factor,

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TABLE 1. Origin of *S. intermedius* isolates studied

Origin	No. of *S. intermedius* isolates[a]/no. of specimens studied (%)				
	Skin	Ear	Eye	Other	Total
Dog	189/354 (53.4)	49/179 (27.4)	2/13 (15.4)	7/44 (15.9)[b]	247/590 (41.9)[c]
Horse	20/401 (5.0)	0/1	1/23 (4.3)	2/471 (0.4)[d]	23/896 (2.6)
Cat	6/120 (5.0)	0/13	0/4	3/13 (23.1)[e]	9/150 (6.0)
Rabbit	1/14 (7.1)	0/1	0/0	0/0	1/15 (6.7)
Guinea pig	1/17 (5.9)	0/1	0/0	0/0	1/18 (5.6)
Total	217/906 (24.0)	49/195 (25.1)	3/40 (7.5)	12/528 (2.3)	281/1,669 (16.8)

[a] Each isolate was recovered from a separate individual.
[b] Canine specimens consisting of 21 vaginal swabs (*S. intermedius*, $n = 2$), 12 nasal swabs (*S. intermedius*, $n = 3$), and 11 nail bed swabs (*S. intermedius*, $n = 2$).
[c] Difference significant compared with isolation of *S. intermedius* from specimens of other animal origin ($P < 0.0001$).
[d] Equine specimens consisting of 258 cervical swabs (*S. intermedius*, $n = 2$) and 213 respiratory tract specimens.
[e] Feline specimens consisting of 13 nasal swabs (*S. intermedius*, $n = 3$).

protein A, and capsular polysaccharides (Pastorex Staph-Plus; Sanofi Diagnostics Pasteur, Marnes-la-Coquette, France). In addition, an in-house slide coagulase test using rabbit and human citrate plasma and a coagulase tube test using rabbit plasma (bioMérieux) were performed. Concerning the *S. intermedius* isolates, a possible misidentification regarding *S. aureus* was excluded by PCR for detection of *S. aureus*-specific *nuc* and *coa* genes; both PCR procedures were done with primer pairs as described previously (22, 33).

Media and chemicals. Brain heart infusion broth from Merck (Darmstadt, Germany) was used for cultivation of staphylococcal strains. Chemicals were purchased from Amersham Buchler (Braunschweig, Germany) or from Serva (Heidelberg, Germany). All PCR reagents and kits were purchased from Boehringer (Mannheim, Germany). The primers and 5′-biotinylated probes were prepared by MWG Biotech (Ebersberg, Germany).

DNA isolation procedures. Staphylococcal cells were collected from 0.5 ml of an overnight brain heart infusion broth culture. Cells were pelleted by centrifugation at 5,000 × *g* for 20 min, resuspended in 185 μl of TE buffer (20 mM Tris chloride, 2 mM EDTA [pH 8.0]) with 15 μl of recombinant lysostaphin (15 mg/ml) (Sigma, St. Louis, Mo.), and incubated at 37°C for 30 min. DNA was subsequently extracted with a QIAamp tissue kit (Qiagen, Hilden, Germany) according to the manufacturer's recommendations. Nucleic acid samples were eluted with distilled water and adjusted to a final concentration of 1 μg/ml according to A_{260} values.

Multiplex PCR DNA-EIA. The multiplex PCR DNA-EIA to detect simultaneously the SE-encoding genes (*sea*, *seb*, *sec*$_{1-3}$, *sed*, and *see*) was performed as previously described (9). For carryover prevention, the uracil DNA glycosylase system (Boehringer) was used. The amplification was performed in an automated thermocycler with a hot bonnet (Hybaid, Teddington, United Kingdom). For hybridization of amplified DNA from the multiplex PCR, a generic DNA-EIA (GEN-ETI-K DEIA; Sorin, Saluggia, Italy) was used according to the manufacturer's recommendations. The strips were incubated with an optimized concentration of the biotinylated probes (20 pg/μl for each probe). Reactions were judged as positive if the signal reached or exceeded the cutoff value of 0.150 absorbance units above the mean value of determinations of toxin-negative reference strains. Positive controls included DNA from toxin-producing *S. aureus* strains (SEA, KN543; SEB, ATCC 14458; SEC, ATCC 19095; SED, ATCC 23235; and SEE, ATCC 27664). In addition to standard PCR controls for contamination events, *S. aureus* Cowan 1 and *S. epidermidis* DSM 20044 served as negative controls.

Sequencing of the *sec* fragment. A 271-bp *sec* PCR amplification fragment of the *S. intermedius* strain K1144 was purified by gel filtration (Centri-Sep columns; Princeton Separations, Adelphia, N.J.) followed by direct sequence determination. After thermal cycling with fluorescent dye-labeled terminators using the primers SEC-3 and SEC-4 (9), the nucleotide sequence was determined on an automated DNA sequencer (ABI PRISM 310; Applied Biosystems Division, Perkin-Elmer Corp., Foster City, Calif.). The sequences were analyzed by alignment with known sequences of *sec* genes of *S. aureus* (20, 21, 26) and *S. intermedius* (32) by using OMIGA 2.0 software (Oxford Molecular, Oxford, United Kingdom).

Detection of toxin production. Culture filtrates of the *S. intermedius* isolates were tested for the presence of SEA to SEE by an EIA (Ridascreen Set A, B, C, D, E; R-Biopharm, Darmstadt, Germany) and, except for SEE, by semiquantitative reversed passive latex agglutination (RPLA; Unipath, Basingstoke, Hampshire, United Kingdom) assay. For EIA, a cutoff value of 0.150 absorbance units above the mean value of determinations of negative controls was defined as a positive reaction. The RPLA assay was interpreted visually as recommended by the manufacturer.

Statistical analysis. The chi-square test was used to analyze differences between frequencies of isolation of *S. intermedius* from canine specimens and those for other veterinary specimens. Fisher's exact test (one tailed) was used to assess differences regarding the presence of enterotoxin genes between canine *S. intermedius* isolates and other animal isolates.

RESULTS

Testing of *S. aureus* reference strains. The multiplex PCR with primer pairs specific for *sea*, *seb*, *sec*, *sed*, and *see* genes successfully amplified fragments from the DNAs of *S. aureus* reference strains (Fig. 1). PCR amplification was confirmed by respective hybridization reactions in a DNA-EIA system for all reference strains used. Furthermore, 100% correlation between the results of the enterotoxin analysis and the detection of the corresponding gene was observed, as shown for SEC in Table 3. The sensitivity of the multiplex PCR-EIAs reached the expected range as previously described (9). None of the primer pairs and the respective hybridization probes reacted with any of the negative control strains which also tested negative in both immunoassays.

Isolation of *S. intermedius*. The frequency of isolation of *S. intermedius* from various specimens of different animals is shown in Table 1. Overall, 281 (16.8%) *S. intermedius* isolates were collected over 1 year from 1,669 consecutive animal specimens; they consisted of 247 (41.9%) *S. intermedius* isolates from 590 canine specimens and 34 (3.2%) isolates from 1,079 specimens of other animals ($P < 0.0001$). Most of the canine *S. intermedius* isolates ($n = 189$) were derived from skin specimens.

Detection of enterotoxin genes in *S. intermedius* and CNS. A total of 292 *S. intermedius* isolates were tested for the possession of SE-encoding genes. Thirty-three (11.3%) of these isolates were found to be *sec* positive by PCR amplification (Tables 2 and 3). Figure 1 shows a representative agarose gel electrophoresis pattern of *sec*-positive *S. intermedius* isolates. All positive results were confirmed by the *sec*-specific DNA-EIA hybridization procedure (Table 3). Except for one equine isolate, all animal *S. intermedius* isolates with positive *sec* gene amplification were derived from canine specimens (31 of 247 [12.6%]) ($P = 0.07$). In addition, 1 of 11 human *S. intermedius* isolates was *sec* positive (Table 2). None of the *S. intermedius* isolates tested showed positive amplification results for the genes encoding enterotoxins SEA, SEB, SED, and SEE.

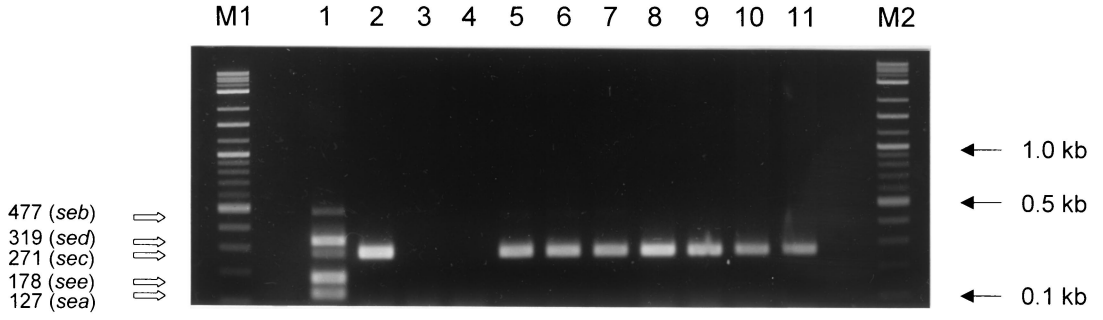

FIG. 1. Agarose gel electrophoresis pattern showing PCR-amplified products in multiplex PCR for the SE genes for *S. aureus* control strains and animal-derived *sec*-positive *S. intermedius* isolates. Lanes M1 and M2, DNA molecular size markers (1-kb/100-bp DNA ladder); lane 1, positive control with multiplex PCR of all enterotoxin genes tested (*sea*, *seb*, *sec*, *sed*, and *see*); lane 2, SEC-positive *S. aureus* ATCC 19095 with amplification product of *sec* gene; lane 3, negative control strain *S. aureus* Cowan 1; lane 4, *S. epidermidis* negative control strain DSM 20044; lanes 5 to 11, representatives of *sec*-PCR-positive *S. intermedius* isolates. Sizes are marked in base pairs on the left.

In the control group consisting of 65 animal-derived CNS isolates, multiplex PCR and subsequent DNA-EIA hybridization gave no positive results for any of the five different enterotoxin genes tested.

Sequencing results. Representative of the *sec*-positive amplification results of canine isolates, a specific 271-bp amplification fragment of *S. intermedius* strain K1144 was sequenced directly in both directions (Fig. 2). Subsequently, the nucleotide sequence of this fragment was aligned with published nucleotide sequences of different SEC subtypes. For the sequenced fragment, 100% identity to the sequence of the canine type of enterotoxin C (SEC_{canine}) was found (32). Sequencing of the *sec*-positive isolates N940276, derived from human bronchoalveolar lavage fluid, and K822, derived from an equine skin specimen, likewise showed identity with the sec_{canine} nucleotide sequence (Fig. 2).

TABLE 2. Detection of *sec* gene by multiplex PCR-DNA-EIA compared with analysis of SEC production by two different immunoassays

Origin	No. of isolates tested	No. or percentage of positive samples detected by:				No. of differences
		PCR amplification and DNA-EIA hybridization		Both immunoassays[a]		
		n	%	*n*	%	
Study isolate						
Canine	247	31[b]	12.6	29	11.7	2
Equine	23	1	4.3	0	0.0	1
Feline	9	0	0.0	0	0.0	0
Other[c]	2	0	0.0	0	0.0	0
Total no. of study isolates tested	281	32	11.4	29	10.3	3
Collection strain						
Human	11	1	9.1	1	9.1	0
Total	292	33	11.3	30	10.3	3

[a] EIA and RPLA assay for the detection of the enterotoxin.
[b] Difference not significant compared with frequency of *sec* detection in non-canine animal *S. intermedius* isolates ($P = 0.07$).
[c] Including one isolate from a rabbit and one isolate from a guinea pig.

Enterotoxin production analysis. Overall, there was a complete agreement between enterotoxin gene detection and enterotoxin production for 289 (99.0%) of the 292 *S. intermedius* isolates. Except for three *S. intermedius* isolates, the amplification of *sec*-specific sequences was confirmed for 30 (90.9%) isolates on a protein level by semiquantitative detection of corresponding toxin production using two different immunological tests (Tables 2 and 3). Two of three isolates with non-corresponding results (equine isolate K822 and canine isolate E865) were negative in both EIA and RPLA, whereas the third isolate (canine isolate E826) showed a weak SEC production value near the cutoff in the EIA but was negative in RPLA (Table 3).

One of the canine *S. intermedius* isolates (K861) showed, in addition to *sec* gene amplification and SEC production, a positive expression of SEA in EIA (ratio of optical density at 450 nm to that at 630 nm [$OD_{450/630}$], 1.135) as well as positive agglutination in SEA-RPLA, but the isolate was found to be negative by *sea* PCR amplification and respective DNA-EIA hybridization ($OD_{450/630}$, 0.013). All other *S. intermedius* isolates tested negative for SEA. None of the *S. intermedius* isolates showed production of SEB, SED, or SEE.

All clinical CNS isolates tested as well as the *S. epidermidis* reference strain, which was included as a control, were negative by both EIA and RPLA for all enterotoxins tested.

DISCUSSION

Whereas for *S. aureus* the possession of enterotoxins is known to be a cause of food poisoning and septic shock-like illness (7, 19, 47), only scant and contradictory information is available concerning the production of enterotoxins by *S. intermedius* and other non-*S. aureus* staphylococci and their pathogenic role. Since some studies showed evidence for the production of exotoxins such as β-toxin and enterotoxins A and C by *S. intermedius* (4, 24, 31), an enterotoxigenic potential of this species may be assumed. Furthermore, a unique molecular variant of type C SEs was described elsewhere as SEC_{canine} for pyoderma isolates (32). Also, Hirooka et al. described enterotoxigenic canine *S. intermedius* isolates (37). In addition, an

TABLE 3. Results of testing enterotoxin positive *S. intermedius* strains

S. intermedius strain	Origin of clinical isolate	Result by:			
		Agarose gel analysis for *sec* gene[a]	Colorimetric hybridization assay[b]	Immunoassay	
				EIA[c]	RPLA[a,d]
Isolates					
62579/1	Dog, skin	+	0.855	1.996	+
E789	Dog, nose	+	0.810	2.184	+
E826	Dog, skin	+	0.996	0.319	−
E865	Dog, skin	+	0.679	0.022	−
E1827/2	Dog, skin	+	0.876	2.151	+
K149	Dog, skin	+	0.455	1.180	+
K166	Dog, skin	+	0.600	1.644	+
K184/1	Dog, skin	+	0.791	1.609	+
K372/1	Dog, skin	+	0.469	0.576	+
K738	Dog, ear	+	2.101	>2.200	+
K798	Dog, skin	+	0.736	2.103	+
K813	Dog, skin	+	0.445	>2.200	+
K817	Dog, skin	+	0.674	2.116	+
K822	Horse, skin	+	2.393	0.006	−
K823	Dog, skin	+	0.522	>2.200	+
K841	Dog, skin	+	0.908	0.756	+
K844	Dog, skin	+	0.390	>2.200	+
K861[f]	Dog, skin	+	1.156	1.488	+
K864	Dog, skin	+	0.610	>2.200	+
K879	Dog, skin	+	1.082	2.053	+
K892	Dog, skin	+	0.593	1.602	+
K939	Dog, skin	+	1.818	>2.200	+
K1039	Dog, skin	+	0.264	>2.200	+
K1112	Dog, skin	+	1.476	2.069	+
K1114/1	Dog, skin	+	2.083	>2.200	+
K1118/1	Dog, nail bed	+	2.424	>2.200	+
K1144	Dog, skin	+	1.213	>2.200	+
K1155	Dog, ear	+	0.880	>2.200	+
K1182/C3	Dog, skin	+	1.034	2.112	+
K1185	Dog, skin	+	1.331	1.993	+
K1245/1	Dog, skin	+	0.962	1.910	+
K1248/1	Dog, skin	+	1.391	1.968	+
N940276	Human, BAL[g]	+	1.320	>2.200	+
Control strains[e]					
ATCC 19095		+	>2.200	1.755	+
Cowan 1		−	0.024	0.116	−
DSM 20044		−	0.028	0.014	−

[a] +, positive; −, negative (judged by eye).
[b] DNA-EIA with *sec*-specific 5′-biotinylated oligonucleotide probe; $OD_{450/630}$ ratio.
[c] EIA for the detection of SEC; $OD_{450/630}$ ratio.
[d] RPLA assay for the detection of SEC.
[e] Control strains: ATCC 19095, *S. aureus* SEC reference positive strain; Cowan 1, *S. aureus* enterotoxin-negative strain; DSM 20044, *S. epidermidis* enterotoxin negative strain.
[f] *S. intermedius* isolate K861 showing in addition to SEC-*sec* detection positive results in EIA and in RPLA assay testing SEA production but failing in *sea* gene amplification and hybridization, respectively.
[g] BAL, bronchoalveolar lavage fluid.

outbreak of food poisoning with a butter blend due to *S. intermedius* has been reported previously (41). However, valid molecular epidemiology data concerning *S. intermedius* enterotoxin genes are still lacking. Thus, the aim of the study reported here was to investigate systematically the enterotoxigenic potential of *S. intermedius* by using a multiplex PCR-DNA-EIA compared with the results of two different immunoassays.

In this study, *S. intermedius* was isolated from more than 50% of canine cutaneous specimens (41.9% of all specimens) tested. This is in agreement with previous findings of carrier rates of 40 to nearly 100% for specimens of different cutaneous and mucocutaneous origin of healthy and diseased dogs (2, 3, 11, 27). The isolation of *S. intermedius* in other animal hosts compared to dogs was significantly lower (3.2%), which is in agreement with the results of previous observations (29, 35, 48).

Positive PCR amplification and DNA-EIA hybridization results were detected in 31 (12.6%) of 247 canine *S. intermedius* isolates, all possessing *sec* gene sequences, whereas no sequences of other enterotoxin genes studied were found. Regarding *S. intermedius* isolates of other animals tested, only one *sec*-positive isolate was identified from an equine specimen. Canine isolates tend to have a higher prevalence of the *sec* gene but not to a statistically significant extent. Thus, in contrast to equine, feline, and other animal hosts, a substantial portion of the canine population tested harbors enterotoxigenic *S. intermedius*. The quite frequent detection of the *sec* gene in *S. intermedius* isolates studied here (overall, 11.3%) was comparable with the SEC prevalence in *S. aureus* of about 5 to 15% (1, 44, 50).

Because the *sec* primers and the *sec*-specific probe of the multiplex PCR-EIA anneal in homologous regions of the different *S. aureus sec* gene variants (sec_1, sec_2, and sec_3) as well as of the *S. intermedius* sec_{canine} gene (except for the 1-bp difference in SEC-5B probe), the assay used here covered the *sec* gene variants of both species. Concerning slight differences in the nucleotide sequences of the *sec* subtypes, sequencing was performed to subtype the amplified *sec* gene fragments. All *sec* amplicons sequenced were represented by sec_{canine} type (32). This applied not only to the *sec*-positive canine isolates but also to the equine and human isolates.

A comparison of the molecular detection of the enterotoxin genes was performed between the results obtained from two immunological assays for analysis of enterotoxin production in vitro. Overall, there was a complete agreement for 289 (99.0%) of the 292 *S. intermedius* isolates regarding all enterotoxins tested. Concordant positive results for SEC production and the respective toxin gene amplification-hybridization were obtained from 30 (90.9%) of 33 *sec*-positive isolates, thus resulting in three *sec* gene-possessing *S. intermedius* isolates without, or with only weak, enterotoxin production in vitro. Regarding *S. aureus*, it is known that a number of enterotoxigenic strains with positive reactions in biological assays (monkey-feeding assay) tested negative in immunological assays as described previously for the optimum sensitivity plate, a modified Ouchterlony method (13, 28, 58). With the advent of the EIA methods, it was shown that several of these strains produced only very small amounts of enterotoxin (10 to 15 ng) (58). Thus, negative or very weak results of immunoassays might be due to expression that is nonexistent or too weak to be detected even by the more sensitive modern immunological assays, or they could be explained by variations in antigenic composition of the toxin protein.

Because to date the amount of enterotoxin required to cause illness in humans is not known (12, 13), the molecular-based detection of staphylococci that produce only minimal enterotoxin is of particular importance. In principle, differences between toxin gene expression in food products or artificial culture and that in the natural habitat have to be considered (40). In addition, enterotoxin production in food is influenced by many factors that do not influence DNA-based methods (34).

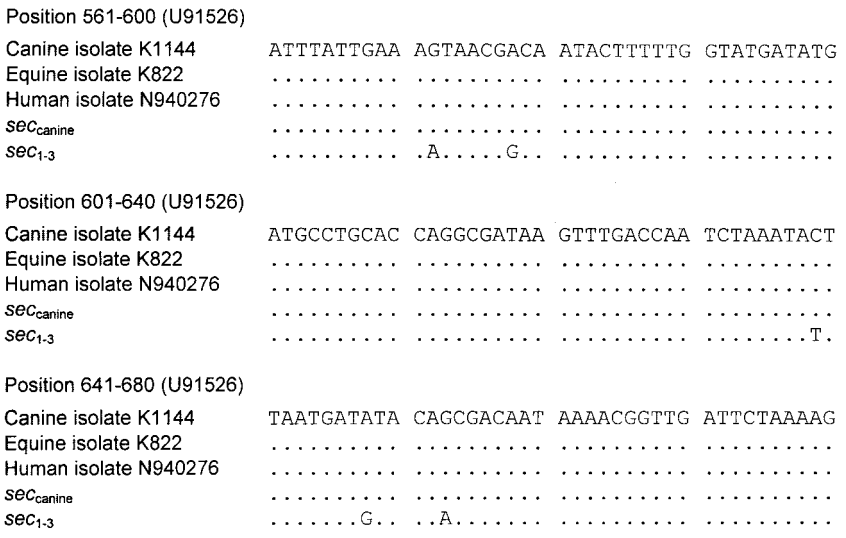

FIG. 2. Alignment of sequences coding for SEC including reference sequences of *S. aureus* subtypes, *S. intermedius* canine subtype, and clinical *sec*-positive isolates derived from canine, equine, and human specimens. The EMBL accession numbers of the *sec* nucleotide sequences used for the alignment are as follows: sec_1, X05815; sec_3, X51661; sec_{canine}, U91526. For sec_2, a sequence was used as reported by Bohach and Schlievert (21). The sequences of the three *S. aureus* subtypes were merged, since there were no differences in the aligned region as showed here. Dots indicate identity.

The positive result for SEA production but negative result by *sea*-specific PCR in one *S. intermedius* isolate might be due to nonspecific results, since immunological methods can be influenced by various factors (14, 38, 53). In addition, cross-reactions with newly described SEs (15, 39, 51, 55, 66) or still-unknown enterotoxins sharing a close antigenic relatedness could cause positive results in immunological assays but remain unrecognized in molecular tests. Recently, Jarraud et al. (39) reported on an enterotoxin gene nursery (*egc*) of *S. aureus* which was probably generated from an ancestral gene through gene duplication and variation, suggesting further, still-unknown enterotoxins in addition to previously identified members of the expanded PT family.

In general, the numbers and types of microorganisms present in a finished food product are influenced strongly by, among other things, infected or colonized persons practicing poor personal hygiene (40). Although *S. intermedius* is adapted primarily to carnivore hosts, its entry into human foods is not precluded. Both invasive and noninvasive zoonotic transmissions from dogs to humans have been documented elsewhere (43, 59–61). As supported by the sec_{canine}-positive human *S. intermedius* isolate reported here, enterotoxigenic *S. intermedius* strains may contaminate food products during handling and processing via animal-human contacts. Once enterotoxigenic *S. intermedius* strains are in foods, their growth may be expected to lead to the production of enterotoxins. Thus, such strains may play a role as a causative agent of SFP, as had already been demonstrated by a food-related outbreak in the western United States (41). Given the completely negative results of enterotoxin testing of the CNS control group, the occurrence of SEs in animal CNS isolates may be at least very rare.

In summary, this is the first study in which a large number of *S. intermedius* isolates was tested systematically for staphylococcal toxin genes. It has been demonstrated on both molecular and immunological levels that the coagulase-positive species *S. intermedius* possesses a substantial enterotoxigenic potential, particularly for SEC. Hence, in addition to the role of *S. aureus* in the etiology of SFP, we may assume a causative role particularly for canine *S. intermedius* isolates via canine-human contact. Therefore, *S. intermedius* should not be disregarded when screening food products for contamination with enterotoxin-producing pathogens or studying outbreaks of SFP. Furthermore, the application of a PCR-based method offers an alternative method for the rapid and specific detection of enterotoxigenic *S. intermedius* isolates, even for isolates with no or only very weak production of enterotoxins in vitro.

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