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Use of Genotypic Identification by *sodA* Sequencing in a Prospective Study To Examine the Distribution of Coagulase-Negative *Staphylococcus* Species among Strains Recovered during Septic Orthopedic Surgery and Evaluate Their Significance

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A total of 212 coagulase-negative *Staphylococcus* strains recovered prospectively during 119 surgeries for proven or suspected bone and joint infection (BJI) were identified by *sodA* sequencing. These strains were identified as 151 *Staphylococcus epidermidis* isolates, 15 *S. warneri* isolates, 14 *S. capitis* isolates, 9 *S. hominis* isolates, 6 *S. lugdunensis* isolates, 5 *S. haemolyticus* isolates, 4 *S. caprae* isolates, 4 *S. pasteuri* isolates, 3 *S. simulans* isolates, and 1 *S. cohnii* isolate. Only *S. epidermidis*, *S. lugdunensis*, *S. capitis*, and *S. caprae* were found to be infecting organisms and were involved, respectively, in 35 (81.4%), 3 (7.0%), 3 (7.0%), and 2 (4.6%) cases of BJI.

Coagulase-negative *Staphylococcus* (CoNS) strains are a leading cause of arthroplastic infections, accounting for 15 to 37.5% of isolates recovered from peroperative samples (13). *Staphylococcus epidermidis* is the main species responsible for these infections and for other device-related bone and joint infections (BJI). There are some reports of true BJI caused by CoNS species other than *S. epidermidis*, including *S. caprae* (1), *S. lugdunensis* (24), and *S. simulans* (18). However, no prospective study has specifically addressed the species distribution of CoNS strains in BJI. Identification of CoNS strains to the species level has long been an obstacle preventing this question from being answered satisfactorily. PCR-based sequencing methods can now accurately identify a wide spectrum of *Staphylococcus* species (7, 11, 17). In this 4-year prospective study, we used *sodA* sequencing to identify CoNS species associated with BJI.

All surgical procedures performed in the orthopedic department of Raymond Poincaré hospital (Garches, France) between January 1999 and December 2002 for proven or suspected BJI were studied prospectively. We included all procedures in which at least three independent samples were collected during the same operative procedure, at least one sample was positive for a CoNS strain, and no samples were positive for organisms other than CoNS strains. The samples were processed in a class 2 laminar-flow safety cabinet. A portion of the sample was Gram stained, and the remainder was used to inoculate 10 ml of Schaedler broth (BioMérieux, Marcy l'Etoile, France). After agitation, aliquots were used to inoculate chocolate agar plates (incubated in 5% CO_2) and blood agar plates (incubated aerobically and anaerobically). The plates were examined daily for 7 days. Broths were subcultured after 1 and 5 days. The "CoNS group" was identified by Gram staining, catalase testing, Slidex latex agglutination testing (BioMérieux), and tube coagulase testing (Bio-Rad, Marnes la Coquette, France). Antibiotic susceptibility was evaluated by the disk diffusion method on Mueller-Hinton agar (Bio-Rad). Strains were identified to the species level by partial *sodA* sequencing (17) as described recently by our group (21). We used the definitions and criteria recommended by the OSIRIS (Oxford Skeletal Infection Research and Intervention Service) group (2). CoNS isolates recovered from multiple samples and belonging to the same species (identical *sodA* sequence) were considered to be the same strain if they had the same colony morphology and identical antibiotic susceptibility patterns. A strain was defined as "significant" if it was recovered from ≥ 3 distinct peroperative samples. The chi-square test (with Yates' correction for expected frequencies of <5)

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TABLE 1. Prevalences of *S. epidermidis* and other CoNS species

Species and no. of positive samples per procedure	No. (%) of strains		
	Arthroplasties[a] (n = 132)	Others (n = 80)	Total (n = 212)
S. epidermidis			
1 or 2 samples	73 (73)	38 (74.5)	111 (73.5)
≥3 samples	27 (27)	13 (25.5)	40 (26.5)
Total	100 (100)	51 (100)	151 (100)
Other CoNS species			
1 or 2 samples	26 (81.2)	25 (86.2)	51 (83.6)
≥3 samples	6 (18.8)	4 (13.8)	10 (16.4)
Total	32 (100)	29 (100)	61 (100)

[a] Revision arthroplasties.

was used to compare values, and P values of <0.05 were considered to be statistically significant.

A total of 119 surgical procedures (104 patients) yielding only CoNS isolates were included. The patients (64 males and 40 females) were between 21 and 86 years of age (mean, 56 years). Ninety-one patients underwent a single procedure, 11 underwent two procedures, and 2 underwent three procedures. About 60% (71 of 119) of surgical procedures were revision arthroplasty surgery (hip, 46; knee, 23; other, 2), and 40% (48 of 119) were for fracture nonunions, contiguous osteitis, or other BJI. Over 50% (62 of 119) of procedures yielded three or more positive samples. A single species was recovered in 84.9% of procedures, two species were recovered in 12.6% of procedures, and three or more species were recovered in 2.5% of procedures.

A total of 212 CoNS strains were recovered peroperatively. All but one were unambiguously identified, and they belonged to 10 species: *S. epidermidis* (151 strains), *S. warneri* (15 strains), *S. capitis* (13 strains), *S. hominis* (9 strains), *S. lugdunensis* (6 strains), *S. haemolyticus* (5 strains), *S. caprae* (4 strains), *S. pasteuri* (4 strains), *S. simulans* (3 strains), and *S. cohnii* (1 strain). The unidentifiable strain had phenotypic features of *S. capitis*, but the *sodA* fragment shared only 95.1% identity with *S. capitis* subsp. *capitis* CIP 81.53 T and 94.9% identity with *S. capitis* subsp. *urealyticus* CIP 104192 T. The next closest species was *S. caprae* (92.1% identity with *S. caprae* CIP 104000 T). Thus, this strain is likely to be a novel subspecies of *S. capitis* or an *S. capitis*-like organism. This strain was included in the *S. capitis* group.

Of the 212 strains recovered during the study, 151 (71.2%) were *S. epidermidis* and 61 (28.8%) were other CoNS species (Table 1). Irrespective of the type of surgery performed, about 25% of *S. epidermidis* strains (40 of 151 [26.5%]) were significant (isolated from ≥3 distinct peroperative samples). The overall proportion of significant strains was lower with CoNS species other than *S. epidermidis* (11 of 51 [21.6%]), but this difference was not significant (P = 0.12). There was also no difference according to the type of surgery performed. The proportion of significant strains differed markedly among the non-*S. epidermidis* organisms: *S. caprae*, *S. lugdunensis*, and *S. capitis* strains were significant and nonsignificant, whereas strains of *S. warneri*, *S. hominis*, *S. haemolyticus*, *S. pasteuri*, *S. simulans*, and *S. cohnii* were never significant. The difference between these two groups was highly significant in our study ($P < 0.0001$). The same level of significance was obtained by comparing the group formed by *S. epidermidis*, *S. capitis*, *S. caprae*, and *S. lugdunensis* to all other CoNS species. Overall, the bacteriological criteria for BJI (isolation of at least one CoNS strain from ≥3 peroperative samples) were met for 43 procedures, involving 42 patients. These 43 cases of BJI were each caused by a single species (only one significant CoNS species): 81.4% (35 of 43) were due to *S. epidermidis* and 18.6% (8 of 43) to *S. capitis*, *S. caprae*, or *S. lugdunensis* (three, two, and three cases, respectively).

Our study confirms the high prevalence of *S. epidermidis* in orthopedic surgery, irrespective of its involvement in a pathological process (6, 15, 16). Over 70% of isolated strains belonged to this species. The predominance of *S. epidermidis* in human infections has been linked to its overrepresentation in the skin flora, its resistance to multiple antibacterial agents, and its ability to adhere and to form biofilms on materials (9, 22, 23). Numerous CoNS species other than *S. epidermidis* were encountered throughout this study, but only *S. capitis* (including one "*S. capitis*-like" strain), *S. caprae*, and *S. lugdunensis* were found to be causative agents of BJI. Their involvement was marginal relative to that of *S. epidermidis* (~4.5 to 7% of BJI caused by CoNS strains for each of these species versus ~81% for *S. epidermidis*). These three species have previously been reported as agents of BJI (1, 3, 6, 8, 16, 19, 20, 24). Five of the species that were never significant in our study have also been reported to have little or no pathogenicity: *S. warneri*, *S. hominis*, *S. haemolyticus*, *S. pasteuri*, and *S. cohnii* (4, 6, 10, 12, 14, 16, 25). Finally, *S. simulans* and *S. schleiferi* deserve further examination, as both have been involved in a few cases of BJI (5, 18). Their low prevalence may explain their absence from our study.

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