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Unbiased Species-Level Identification of Clinical Isolates of Coagulase-Negative Staphylococci: Does It Change the Perspective on *Staphylococcus lugdunensis*?

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Unbiased species-level identification of coagulase-negative staphylococci (CoNS) using matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) identified *Staphylococcus lugdunensis* to be a more commonly isolated CoNS in our laboratory than previously observed. It has also highlighted the possibility of vertical transmission.

Coagulase-negative staphylococci (CoNS) have been mostly considered insignificant contaminants when isolated from clinical samples. This, in turn, has led to a lack of universal agreement on the importance of identifying them to the species level (1, 2).

The introduction of matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) to routine clinical laboratories has made it feasible to rapidly and accurately identify clinical isolates without adding significant costs (3, 4).

MALDI-TOF MS has been in routine diagnostic use at our laboratory since 2010. We identify all clinical isolates, irrespective of site or clinical details. In an attempt to assess the value of unbiased species-level identification of CoNS, we investigated the epidemiology and clinical correlates associated with the isolation of *Staphylococcus lugdunensis*, a relatively virulent CoNS that causes a spectrum of disease similar to that of *Staphylococcus aureus* (5).

S. lugdunensis isolates were identified from the laboratory information management system (LIMS). Specimen request forms, electronic clinical records, and written clinical records were reviewed (for hospital and community samples). Only the isolates with a MALDI-TOF MS spectral score of ≥2 (which reflects high confidence in identification to the species level) were included. A total of 20,806 CoNS isolates were identified using MALDI-TOF MS in a 24-month period. Of those, 559 were identified as *S. lugdunensis* (Table 1), whereas 31 isolates were identified in the preceding 24 months using conventional techniques. Duplicate results from the same patient were removed ($n = 81$). In total, 478 individual isolates were reviewed (Table 2). The clinical data we collected included demographics, sample type, sample site, clinical symptoms, diagnosis, comorbidities, antibiotic usage, surgical procedures, and indwelling and prosthetic devices.

A total of 121 isolates (25.3%) were from primary care. The male-to-female ratio was 0.84, and the median age was 42 years, with a calculated incidence of 30.5 cases per 100,000 persons in the local population. Twenty-two isolates (4.6%) were from neonates (<30 days old), with 10 isolated in the first 7 days of life. Of the 22 neonatal isolates, 5 were from sterile sites (blood, $n = 3$; cerebrospinal fluid [CSF], $n = 1$; and urine, $n = 1$) and the rest were from swabs (Table 3).

The commonest infections were skin and soft tissue infections (SSTI), with a total of 237 episodes; of these, 99 (41.8%) were abscesses, and 79 (79.8%) required drainage. Abscesses were equally distributed between the upper and lower halves of the body. The commonest abscess sites were the axilla ($n = 16$) and neck ($n = 10$) in the upper torso and the pelvis ($n = 10$) and thighs ($n = 10$) in the lower body. Vaginal abscesses and infected Bartholin cysts were diagnosed in 7 patients, in addition to 34 isolates from vaginal swabs, taken either as part of a routine pregnancy examination or with the clinical detail of vaginal discharge. A total of 35 isolates were from surgical site infections.

Nineteen episodes of *S. lugdunensis* bacteremia were identified. The clinical significance of 7 of them was doubtful, as the patients were not specifically treated for infection without an adverse outcome. Of the remaining 12 patients, 2 had endocarditis, 1 had native valve endocarditis (NVE), and 1 had prosthetic valve endocarditis (PVE). Three bacteremic patients had respiratory tract infections. One patient each had osteomyelitis, cellulitis, and a wound infection. Three blood cultures were from neonates with early neonatal sepsis. During the same period, CoNS (other species) were isolated in blood cultures from 139 neonates, predominately from preterm infants with suspected late sepsis. An isolate from the CSF of a neonate was considered a likely contaminant due to normal cell counts in the CSF. Ten urine samples, 70% of which were from women, were positive for *S. lugdunensis*. Twenty-two isolates were obtained from around the breast, 7 (32%) from abscesses, 5 from postsurgical wound swabs, and 1 from an infected breast implant; the remaining isolates had insufficient clinical details. Twenty-seven (5.6%) patients had prosthetic devices *in situ* (including intravascular catheters and orthopedic hardware). Data about the antibiotics prescribed were available for 202 patients, 136 (67.3%) of whom were receiving monotherapy; the

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TABLE 1 Frequency of staphylococcal species identified by MALDI-TOF MS in a 24-month period

Species	No. of isolates	%
Staphylococcus epidermidis	10,550	50.704
Staphylococcus haemolyticus	4,103	19.720
Staphylococcus hominis	1,787	8.589
Staphylococcus capitis	1,615	7.762
Staphylococcus warneri	702	3.374
Staphylococcus lugdunensis	559	2.687
Staphylococcus simulans	360	1.730
Staphylococcus saprophyticus	270	1.298
Staphylococcus caprae	157	0.755
Staphylococcus pettenkoferi	143	0.687
Staphylococcus pasteuri	115	0.553
Staphylococcus sciuri	103	0.495
Staphylococcus cohnii	100	0.481
Staphylococcus intermedius	47	0.226
Staphylococcus sp.[a]	40	0.192
Staphylococcus condimenti	38	0.183
Staphylococcus auricularis	33	0.159
Staphylococcus schleiferi	33	0.159
Staphylococcus sp.	19	0.091
Staphylococcus equorum	11	0.053
Staphylococcus xylosus	7	0.034
Staphylococcus carnosus	4	0.019
Staphylococcus saccharolyticus	3	0.014
Staphylococcus piscifermentans	2	0.010
Staphylococcus pseudintermedius	2	0.010
Staphylococcus arlettae	1	0.005
Staphylococcus delphini	1	0.005
Staphylococcus felis	1	0.005
Total	20,806	100

[a] Nontyped strain having high similarity to unpublished isolate AY953148.1, "*Staphylococcus croceolyticus*."

most commonly prescribed antibiotics were flucloxacillin (n = 51) and amoxicillin-clavulanic acid (n = 44).

During this period, antibiotic susceptibility testing was reserved for selected isolates. Of the 41 isolates for which antibiotic susceptibilities were tested, 31 had MICs available; the remaining 10 had sensitivity testing by disk diffusion methods. None of the isolates had a cefoxitin MIC of >4 mg/liter (isolates with cefoxitin MIC values of >4 mg/liter are considered methicillin resistant, mostly due to the presence of the *mecA* gene) (6, 7). Three isolates showed macrolide resistance, and one isolate was glycopeptide resistant.

With unbiased species-level identification using MALDI-TOF MS, *S. lugdunensis* is the 6th commonest CoNS isolate in our laboratory. Previous investigators either overestimated (8) or underestimated (6) the incidence of *S. lugdunensis* in clinical isolates. As previously described, it is mostly isolated from SSTI (9). We also recognized possible vaginal carriage, which may represent a source of vertical transmission, highlighted by the isolation of *S. lugdunensis* from 22 neonates in our cohort (Table 3). A recent prospective study demonstrated a doubtful clinical significance of *S. lugdunensis* isolated from a pediatric population; however, selection bias cannot be excluded, and 3 of the 6 isolates identified were from neonates (10). *S. lugdunensis* has also been isolated from sterile amniotic fluid (11). The possibility of vaginal colonization and neonatal sepsis requires further examination, as this has been demonstrated with other organisms such as group B streptococci (12). Apart from the possibility of vaginal carriage and vertical transmission, our results mirror those of previous findings by Böcher et al. (9), showing SSTI as the primary pathology and abscess formation as a common finding. Most other CoNS have not been associated with abscesses, due to the absence of the necessary virulence factors coagulase and protein A (13). Although these two factors are absent from *S. lugdunensis*, its ability to cause abscesses has been demonstrated previously (14, 15). The presence of an iron-regulated surface determinant protein, common only to *S. lugdunensis* and *S. aureus* (16, 17), and a potent

TABLE 2 Diagnosis according to the request forms

Diagnosis	No.	%
None	80	16.7
Superficial abscess	99	20.7
Cellulitis	13	2.7
Necrotizing fasciitis	1	0.2
Device infection	8	1.7
Osteomyelitis	6	1.3
Endocarditis	2	0.4
Wound infection	124	25.9
Septic arthritis	1	0.2
Urinary tract infection	1	0.2
Upper respiratory tract infection	8	1.7
Central venous catheter-related infection	4	0.8
Infected sebaceous cyst	18	3.8
Neonatal sepsis	4	0.8
Lower respiratory tract infection	7	1.5
Boil	1	0.2
Other	100	21
Pleural effusion	1	0.2
Total	478	100.0

TABLE 3 Distribution and modes of delivery for *S. lugdunensis* isolates from neonates

Age (days)	Sample type	Delivery mode
0	Swab	Spontaneous vertex
1	Blood culture	Emergency CS[a]
1	Swab	Emergency CS
1	Blood culture	Emergency CS
1	Gastric aspirate	Emergency CS
3	Blood culture	Spontaneous vertex
4	Urine	Elective CS
5	Umbilical swab	Emergency CS
5	CSF	Spontaneous vertex
7	Umbilical swab	Spontaneous vertex
12	Umbilical swab	Emergency CS
18	Nasopharyngeal swab	Unknown
19	Swab	Unknown
20	Swab	Emergency CS
25	Respiratory	Spontaneous vertex
29	Sputum	Unknown
30	Endotracheal tube tip	Emergency CS
30	Sputum	Unknown
30	Respiratory	Spontaneous breech
30	Nasopharyngeal swab	Unknown
30	Respiratory	Unknown
30	Swab	Spontaneous vertex

[a] CS, Cesarean section.

cytolytic streptolysin S-like toxin suggests the capacity for skin colonization and possible virulence (16, 18).

Despite the reputation of *S. lugdunensis* causing aggressive endocarditis, our findings support those from other reports suggesting that bacteremia does not necessarily always equate to a clinical syndrome or sepsis (5, 19, 20).

The limitations of our study include the retrospective data collection, which may not reflect accurate diagnoses and definitions. In addition, the presence of sampling bias cannot be excluded, as most samples are sent due to suspected infections. Attributing causality for CoNS, including *S. lugdunensis*, remains difficult, especially when the majority of the samples are from skin swabs, in which differentiation between pathogen and commensal is difficult. Further studies are necessary to refute or confirm these observations.

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