Detection and Identification of *Staphylococcus lugdunensis* Are Not Hampered by Use of Defibrinated Horse Blood in Blood Agar Plates[▽]

Staphylococcus lugdunensis is a coagulase-negative staphylococcus with pathogenic properties similar to those of *Staphylococcus aureus* (4). Identification of *S. lugdunensis* is not necessarily straightforward, resulting in considerable variation in detection rates in different laboratories. Böcher and coworkers recently published data to increase awareness of this pathogen and described some basic criteria for identification to the species level (2). These criteria were (i) *Eikenella corrodens*-like odor, (ii) colony pleomorphism, (iii) prominent β -hemolysis after 2 days of incubation, and (iv) ID32Staph identification (bioMérieux). These criteria seemed, however, to be accurate only when the samples were cultured on Columbia sheep blood agar and not on agar containing 5% horse blood. They reported less-prominent odor and hemolysis on horse blood agar than on sheep blood agar. The identification problems led to differences in detection rates, and they reported an incidence (or in our view detection rate) of 53 infections per 100,000 inhabitants per year in a county in Denmark using Columbia sheep blood agar and zero to four *S. lugdunensis* infections per 100,000 habitants per year in neighboring counties using 5% horse blood.

At the Department of Clinical Microbiology, Central Hospital, Växjö, Sweden, we have identified between 40 and 90 cases of *S. lugdunensis* per year over the last 10 years in our laboratory (Table 1). We have used *Eikenella corrodens*-like (bleach-like) odor, colony pleomorphism, and prominent β -hemolysis after 2 days of incubation combined with negative tube coagulase (with horse plasma; Statens Serum Institut, Copenhagen, Denmark), positive reactions with ornithine decarboxylase and pyrrolidonyl aminopeptidase (1, 3), and resistance to deferroxamine (250 μ g) (ROSCO; A/S Rosco, Taastrup, Denmark) (1) as standard criteria for identification of *S. lugdunensis* during this time. This simple strategy was validated by comparison to results obtained with API-Staph (bioMérieux) and Staph-Zym (Rosco) in 1996 (G. Kahlmeter, L. Bieber, and R. Smyth, unpublished data).

In February 2009 we changed the composition of standard

blood agar plate (blood agar base [Merck]) with 5% human blood to 5% defibrinated horse blood (National Veterinary Institute, Uppsala, Sweden) at our clinical laboratory. We validated the “new” plate when the change was made but were now concerned by the statement of Böcher et al. (2) Using the same criteria for the identification of *S. lugdunensis* before and after the change of medium, we revalidated the medium (Table 1). The detection rates of *S. aureus* and *S. lugdunensis* in samples from skin and soft tissue infections (SSTI) were calculated.

Although the β -hemolysis and odor were slightly less distinct, there was no change in the isolation rate of *S. lugdunensis* or *S. aureus* or in the ratio between the two bacteria (20 to 40 times as many *S. aureus* were seen per year) after the shift to 5% defibrinated horse blood in the agar. The detection rate the last year in our laboratory was almost identical to the level in the recent Danish study using Columbia sheep blood agar, 55 isolates per 100,000 inhabitants per year. The true incidence of *S. lugdunensis* infections may be higher, since not all wound infections are cultured. Our isolates were not genotyped, and we cannot exclude minor outbreaks. On the other hand, the number of cultures and number of positive patients in relation to cultures have been very stable over many years (Table 1).

Thus, in contrast to Böcher et al., we did not find that the use of defibrinated horse blood influenced the identification rate of *S. lugdunensis*. However, all horse blood is not necessarily the same, but then neither is all sheep blood. Other factors influencing the isolation rate of *S. lugdunensis* are probably more important than the use of human, sheep, or horse blood in the agar. In our experience it is important to educate staff on the clinical relevance and importance of this rather unobtrusive coagulase-negative staphylococcus and not to ignore “coagulase-negative staphylococci” as probable skin contaminants. It is important to stress that *S. lugdunensis* looks decidedly more “impressive” with larger colony size, more β -hemolysis, and with a more distinct odor after 36 to 48 h of incubation.

TABLE 1. Summary of results of finding *S. lugdunensis* and *S. aureus* in cultures from skin and soft tissue infections (SSTI) at the Department of Clinical Microbiology, Växjö, Sweden, from February through October 2001 to 2009

Species or parameter	Bacterial source	No. (%) of finding <i>S. lugdunensis</i> or <i>S. aureus</i> in cultures ^a in the following yr:								
		2001	2002	2003	2004	2005	2006	2007	2008	2009 ^b
<i>Staphylococcus lugdunensis</i>	Isolates	42 (1.1)	59 (1.5)	46 (1.2)	53 (1.4)	56 (1.3)	75 (1.7)	93 (2.0)	80 (1.7)	85 (1.8)
	Patients	41 (1.5)	55 (1.8)	45 (1.6)	48 (1.7)	51 (1.7)	70 (2.2)	88 (2.7)	75 (2.2)	80 (2.2)
<i>Staphylococcus aureus</i>	Isolates	1,417 (36.3)	1,523 (37.4)	1,382 (36.8)	1,285 (33.8)	1,459 (35.1)	1,643 (36.6)	1,736 (37.5)	1,661 (35.8)	1,774 (36.7)
	Patients	1,094 (39.5)	1,232 (41.4)	1,096 (40.0)	1,050 (38.1)	1,144 (39.2)	1,276 (40.6)	1,312 (39.8)	1,279 (37.9)	1,363 (38.3)
Total no.	SSTI samples	3,908	4,071	3,758	3,804	4,153	4,490	4,629	4,635	4,832
	Patients sampled	2,770	2,973	2,738	2,757	2,922	3,145	3,300	3,373	3,561

^a Number (percentage) of finding *S. lugdunensis* and *S. aureus* in culture unless specified otherwise.

^b The composition of standard blood agar plate was changed in February 2009.

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