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RESEARCH NOTE

Clonality of slime-producing methicillin-resistant coagulase-negative staphylococci disseminated in the neonatal intensive care unit of a university hospital

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ABSTRACT

Methicillin-resistant coagulase-negative staphylococci (MR-CNS) (n = 132), isolated from pre-term neonates, were analysed to determine their antibiotic resistance patterns, clonal distribution, biofilm production and the presence of the *ica* operon. All MR-CNS were multiresistant, and 89% produced slime. A major clone was identified (77 isolates) among 115 *Staphylococcus epidermidis* isolates. Ten of 16 *Staphylococcus haemolyticus* isolates also belonged to a single clone. Most (80%) slime-positive isolates possessed all the *ica* genes tested, while the remaining 23 (20%) had a variety of gene combinations. The entire *ica* cluster was detected in three of 15 slime-negative isolates. One major and two minor slime-positive, multiresistant MR-CNS clones had disseminated among hospitalised pre-term neonates.

Keywords Coagulase-negative staphylococci, epidemiology, *ica* gene cluster, neonates, slime production, typing

Original Submission: 17 February 2006; **Revised Submission:** 22 June 2006; **Accepted:** 26 June 2006

Clin Microbiol Infect 2006; 12: 1230–1233
10.1111/j.1469-0691.2006.01565.x

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Biochemical and molecular typing studies have demonstrated that clusters of coagulase-negative staphylococci (CNS) may be distributed among both neonates and hospital staff, while isolates associated with sepsis may be more homogeneous [1–3]. CNS are recognised as potential pathogens because they produce slime and form biofilms on polymeric surfaces [4–6]. Several reports have described the chemical composition of slime [5–7]. Production of an extracellular polysaccharide intercellular adhesin is encoded by the genes of the *ica* operon (*icaA*, *icaD*, *icaB* and *icaC*), regulated by *icaR* [4–6]. Absence of biofilm formation has been associated with inactivation of either *icaA* or *icaC* following insertion of IS*256*, which resides in multiple copies on the chromosome of staphylococci [6]. The present study investigated the clonal dissemination of multiresistant, methicillin-resistant (MR)-CNS isolates in the neonatal intensive care unit (NICU) of the Patras University Hospital during 2003–2004, and correlated the presence of the *ica* operon and IS*256* with biofilm production and disease.

In total, 132 MR-CNS isolates were collected from the pre-term NICU, which has 25 beds and admits 350 patients annually. Thirty-one isolates were from blood cultures of different patients with CNS sepsis, as defined by well-established criteria [8], 18 were from intravascular catheter tips with systemic evidence of infection [9,10], 28 were recovered as the only microorganism from skin lesions in which Gram-positive cocci and polymorphonuclear leukocytes were observed upon microscopic examination, and 55 were collected from ventilation tubes; the latter were

considered to be colonising strains, as no apparent signs of infection were present. *Staphylococcus epidermidis* strains ATCC 35984 (RP62A) (a biofilm-forming strain [11,12]) and ATCC 12228 (biofilm-negative) were used as controls.

CNS were identified to the species level using the API Staph System (bioMérieux, Marcy l'Etoile, France) and by restriction fragment length polymorphism analysis of the amplified *tuf* gene [13]. Oxacillin MICs were determined by the agar dilution method [14]. Penicillin-binding protein 2a production was tested with a latex agglutination test (Slidex MRSA Detection; bioMérieux). Susceptibilities to erythromycin, clindamycin, tobramycin, netilmicin, amikacin, gentamicin, linezolid and quinupristin–dalfopristin were determined by Etest (AB Biodisk, Solna, Sweden), according to CLSI (NCCLS) guidelines [14]. Resistance to vancomycin and teicoplanin was determined by agar dilution [14].

Slime production was tested by a qualitative method in glass test tubes [11], and by a quantitative method [15,16], modified by the use of Hucher Crystal Violet (Kristal Violet; Riedel-de Haën, Seelze, Germany) for staining and by filling the wells with 100 μL of distilled H_2O and 10 μL of 1 M HCl. Absorption at 589 nm was detected with a DV 990 Microplate Reader (Gio De Vita, Roma, Italy). The cut-off optical density value was defined as three standard deviations above the mean optical density of the negative control [16]. Samples yielding optical densities >0.07 were considered to be positive for slime production.

DNA extraction and Southern blot hybridisation of *Cla*I digests with *mecA* DNA probes, and pulsed-field gel electrophoresis (PFGE) of *Sma*I digests of chromosomal DNA, were performed as described previously [17]; clonal types were designated according to well-defined criteria [18,19]. Amplification of the four genes of the *ica* operon and the transposase gene of IS*256* was performed by PCR with specific primers [5,6].

There were 255 episodes of CNS sepsis during 2003, and 329 during 2004, with methicillin resistance rates of 74% and 78%, respectively. In the NICU, the corresponding figures were 80% and 91%, respectively, based on oxacillin MICs and penicillin-binding protein 2a production. Among 132 *mecA*-positive CNS (MR-CNS) isolates, there were 115 *S. epidermidis* (MRSE), 16 *Staphylococcus haemolyticus* and one *Staphylococcus hominis*. PFGE analysis revealed that 77 MRSE

Table 1. Characteristics of slime-positive and slime-negative methicillin-resistant coagulase-negative staphylococci

				Genes detected by PCR					
Slime production	**Species**	**PFGE types**	**Clinical specimens**	**All[a]**	***ica*[b]**	***ica*/IS*256*[c]**	***ica*/IS*256*[c]**	**None**	**Total**
Positive	*Staphylococcus*	z (*n* = 74)	VT	33	–	–	1	–	34
(*n* = 117)	*epidermidis*		B	22	–	–	–	–	22
			C	8	–	–	–	–	8
			SL	9	1	–	–	–	10
		g (*n* = 4)	C	1	2	–	–	–	3
			SL	–	–	–	1	–	1
		Others (*n* = 28)	VT	5	–	3	–	–	8
			B	6	2	–	–	–	8
			C	2	1	–	1	–	4
			SL	6	2	–	–	–	8
	Staphylococcus	y (*n* = 7)	VT	–	–	2	3	–	5
	haemolyticus		C	1	–	1	–	–	2
		Others (*n* = 3)	B	–	–	1	–	–	1
			SL	–	–	–	2	–	2
	Staphylococcus	v (*n* = 1)	VT	1	–	–	–	–	1
	hominis								
Total (%)				94 (80)	8 (7)	7 (6)	8 (7)	–	117
Negative	*S. epidermidis*	z (*n* = 3)	SL	2	–	–	1	–	3
(*n* = 15)		g (*n* = 2)	VT	–	–	–	1	–	1
			SL	–	–	–	–	1	1
		Others (*n* = 4)	VT	–	–	–	–	2	2
			C	1	–	–	–	–	1
			SL	–	–	–	1	–	1
	S. haemolyticus	y (*n* = 3)	VT	–	–	–	3	–	3
		Others (*n* = 3)	VT	–	–	–	1	–	1
			SL	–	–	–	2	–	2
Total (%)				3 (20)	–	–	9 (60)	3 (20)	15

VT, ventilation tubes; B, blood; C, catheters; SL, skin lesions.
[a]Positive for all *ica* genes and IS*256*.
[b]Positive for all *ica* genes and negative for IS*256*.
[c]Positive for two or three *ica* genes and IS*256*.
[d]Positive for one *ica* gene and/or IS*256*.