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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

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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*.

isolates belonged to a single clone, 'z', while six additional isolates belonged to a second clone, 'g'. The remaining 32 isolates belonged to 23 different clonal types. Ten *S. haemolyticus* isolates belonged to a single clone, 'y', and the remaining six belonged to four PFGE types (Table 1). Identical isolates from catheter tips and blood cultures from the same sepsis episode in a single patient were excluded (i.e., one isolate per patient).

All isolates were sensitive to netilmicin, quinupristin–dalfopristin, linezolid and vancomycin. However, five MRSE isolates were resistant to teicoplanin (MIC >8 mg/L). A relationship was observed between antibiotic resistance and PFGE banding patterns. MRSE isolates of clone z and the unique *S. hominis* isolate were resistant to β-lactams, erythromycin, clindamycin, gentamicin, tobramycin and amikacin. Diversity among the resistance phenotypes was observed in the remaining MRSE isolates. *S. haemolyticus* strains of clone y expressed resistance to β-lactams, erythromycin, clindamycin and tobramycin.

Slime production was observed by 117 (89%) isolates with both methods (106 MRSE, ten *S. haemolyticus* and one *S. hominis*) (Table 1). The clinical origin and PCR results for the isolates are also shown in Table 1. Although most MR-CNS (117 of 132, of which 106 were slime-positive) carried IS*256*, this element was not located in the *icaC* gene, based on the size of the PCR amplicons (data not shown) [6].

S. epidermidis and *S. haemolyticus* predominated as colonising and infecting species among preterm neonates. Carriage of *mecA* was consistently high, with an increasing tendency during the study period, as reported previously for CNS isolates of NICU origin [3]. The major clonal types identified in the present study were not related to CNS clones identified in other wards of the hospital [20], and predominated in the NICU among colonising isolates, as well as among isolates associated with sepsis and catheter and skin infections. Three of 17 hospital personnel in the NICU also carried MRSE of clone z (data not shown). Antimicrobial pressure may be one factor influencing the spread of the major clones in the NICU, since β-lactams and aminoglycosides constitute the first-line treatment for potential sepsis in neonates.

Most (89%) of the CNS isolates produced biofilm and carried the *ica* operon, which are factors that contribute to CNS dissemination [4]. Three isolates were biofilm-negative, but carried the *ica* genes, which is an observation made previously [4]. Co-expression of *icaA* and *icaD* is associated with enhanced biofilm production [4]. The presence of IS*256* is probably related to the resistance of most isolates to aminoglycosides, since IS*256* is associated with the aminoglycoside resistance-mediating transposon Tn*4001* [6].

CNS strains require several properties to disseminate in an NICU, including antibiotic resistance, biofilm production and the ability to colonise multiple hosts. This study demonstrated the presence and spread of one major (z) and one minor (g) clone among MRSE strains, as well as a main clone (y) among *S. haemolyticus* strains, in the NICU during a 2-year period. Multiresistant strains producing biofilm were associated with colonisation and disease among pre-term neonates. These findings suggest a need for control programmes in order to prevent and reduce MR-CNS infections in neonates, since prolonged treatment of such infections leads to an increasing antibiotic selective pressure in NICUs.

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RESEARCH NOTE

Specific and sensitive diagnosis of syphilis using a real-time PCR for *Treponema pallidum*

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ABSTRACT

A real-time PCR assay with a *Taq*man probe was developed that targeted the *polA* gene of *Treponema pallidum*. The test was validated using an analytical panel (n = 140) and a clinical panel of genital samples (n = 112) from patients attending a sexually transmitted infections clinic. High sensitivities and specificities of 94–100% were achieved using two real-time PCR platforms, the Rotor-Gene and the iCycler. The assay can be completed within 2 h, enabling reporting in <8 h. This fast and robust assay is suitable for implementation in routine laboratories for diagnosing primary syphilis.

Keywords Diagnosis, genital samples, real-time PCR, sexually transmitted infections, syphilis, *Treponema pallidum*

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Syphilis is caused by active infection with *Treponema pallidum* [1,2]. Laboratory diagnosis can be performed by dark-field microscopy, serology and PCR [3,4]. Dark-field microscopy can identify

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