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Mupirocin resistance in coagulase-negative staphylococci, after topical prophylaxis for the reduction of colonization of central venous catheters

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Summary: Topical mupirocin was routinely applied to insertion sites of central venous catheters (CVC) of neonates in a neonatal intensive care unit. After five years, mupirocin resistance was recorded in 42% of clinical isolates of coagulase-negative staphylococci (CNS). This decreased to 21% during a mupirocin-free interval of five months. We performed a prospective study on the significance of mupirocin use on the staphylococcal skin flora of 15 newly admitted neonates. During treatment, mupirocin-susceptible strains were replaced by highly resistant ones. After treatment, all but one neonate harboured at least one resistant strain; 29% of all strains were moderately resistant (mupirocin minimum inhibitory concentrations (MICs) 16 mg/L) and 55% were highly resistant (MICs >1024 mg/L). One CVC (7%) became colonized with a resistant strain. One year after stopping routine mupirocin application the incidence of resistance had dropped to 13%; CVC colonization was recorded in 2·4%.

Keywords: Coagulase-negative staphylococci; mupirocin-resistance; central venous catheters; neonates.

Introduction

The prophylactic application of mupirocin to reduce colonization of central venous catheters (CVC) by coagulase-negative staphylococci (CNS) was introduced in our neonatal intensive care unit (NICU) in 1988. This was associated with a decrease in the staphylococcal colonization rate of CVCs from 8·2% during 1987 to 2·9% in 1991. The isolation of mupirocin-resistant CNS from a catheter tip in January 1992, prompted us to study the development of resistance to mupirocin.

Methods

The level of resistance to mupirocin among CNS from clinical isolates of the NICU was determined during a mupirocin-free period of five months

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and then one month after reintroduction of the antibiotic. Controls were isolates from 36 patients hospitalized in an ICU ward where mupirocin had never been used. Subsequently, the influence of mupirocin on CNS from skin flora was studied prospectively in 15, newly admitted, consecutive neonates (age 2–36 days), all with CVCs *in situ*. Mupirocin ointment (2% g/g) was applied once daily to the CVC insertion site for 2–24 days (mean seven days). One skin swab was taken from each insertion site and the opposite site of the body before and during the insertion period (day 3–7) and after removal of the CVC (seven and 14 days after). Samples were cultured on 5% blood agar and mannitol-salt agar (48 h at 35°C, 5% CO_2) and in Iso-sensitest broth, which was subcultured after overnight incubation at 35°C on to blood agar and mannitol-salt agar. All CVCs were cultured after removal according to the method of Maki *et al.*[1]

Five colonies of Gram-positive, catalase-positive and coagulase-negative cocci (Staphaurex-negative) (Murex diagnostics, Utrecht, The Netherlands) were selected at random from each agar plate and identified by the API Staph system. The susceptibility to lysozyme and novobiocin (5 μg disc diffusion, cut-off diameter 16 mm) was determined. The susceptibility to mupirocin (MIC, mg/L) was determined in duplicate by microdilution in Iso-sensitest broth with an inoculum of 10^5 cfu/mL from an overnight culture. The breakpoint for susceptibility was <4 mg/L; isolates with mupirocin MICs of 4–64 mg/L were considered low-level resistant, and those with mupirocin MICs of >700mg/L as high-level resistant. Fisher's exact test and Wilcoxon's test were used for statistical analysis.

Results

Mupirocin resistance was found in clinical isolates of five out of nine patients in the NICU during December 1991. At the end of a five-month mupirocin-free period (from 1 May 1992–1 October 1992) the resistance rate was 21%, compared with 8% in patients in the mupirocin-free ICU where mupirocin was never used ($P=0{\cdot}2$).

Reintroduction of mupirocin into the NICU resulted in an in-vitro resistance rate of 42% among clinical isolates after one month (November 1992), whereas the prevalence in the comparative ICU was 5% ($P<0{\cdot}05$) (Table I).

Skin cultures of the 15 neonates admitted from 1 October 1992–1 May 1993 revealed 208 strains (eight strains of *Micrococcus* spp. and 200 strains of CNS). The strains of CNS were identified as *S. epidermidis* (82%), *S. warneri* (8%), *S. capitis* (4·5%), *S. haemolyticus* (3%), *S. hominis* (1·5%) and *S. saprophyticus* (1%). The susceptibility of these CNS to mupirocin is shown in Table II. Initially seven patients had only susceptible strains, three had both susceptible and low-resistant strains and five harboured both susceptible and highly resistant strains. The number of resistant strains increased during treatment: on days 3–7, seven of 13 neonates had

Table I. *Mupirocin resistance among clinical isolates of patients hospitalized in the neonatal intensive care unit (NICU) during periods with (M+) and without (M−) mupirocin prophylaxis compared with that found among isolates of patients in the ICU where mupirocin was never applied*

Periods		Test	No. of patients with resistance (total no. studied)	%
NICU	M+ Since 1988	December 1991	5 (9)	
	M− 1 May 1992–1 Oct. 1992	September 1992	4 (19)	21
	M+ 1 Oct. 1992–1 Apr. 1993	March 1993	5 (12)	42
	M− Since 1 April 1993	March 1994	2 (15)	13
ICU	M− Ongoing	September 1992	3 (36)	8
	M− Ongoing	March 1993	2 (39)	5

Table II. *Range of minimum inhibitory concentrations (MICs) (mg/L) of mupirocin of coagulase-negative staphylococci (CNS) isolates cultured from the skin of each patient, before (day 0), during (day 3–7) and after (7 and 14 days) topical mupirocin application*

	MIC (mg/L)		
	Before	During	After
No. of strains	75 (8)*	65 (33)	60 (35)
Patient			
1	0·25 (0)	0·25 (0)	0·25–8 (0)
2	0·12–16 (0)	0·12–>1024 (2)	16–>1024 (4)
3	0·12–0·25 (0)	0·12–>1024 (2)	>1024 (5)
4	0·12–0·25 (0)	>1024 (5)	0·5–>1024 (3)
5	0·12–16 (0)	8–>1024 (2)	16–>1024 (2)
6	0·06–>1024 (1)	>1024 (5)	0·12–>1024 (2)
7	0·12–0·25 (0)	16 (0)	16 (0)
8	0·12–16 (0)	0·5–>1024 (3)	16–>1024 (4)
9	0·12–>1024 (2)	0·12–>1024 (3)	0·12–>1024 (3)
10	0·12 (0)	0·12–>1024 (3)	16–>1024 (3)
11	0·12–>1024 (2)	>1024 (5)	>1024 (5)
12	0·06–>1024 (1)	NE	NE
13	0·12–>1024 (2)	NE	NE
14	0·06–0·12 (0)	0·06–>1024 (3)	NE
15	0·12 (0)	16 (0)	16–>1024 (4)

* The number of resistant isolates among five colonies from one sample are shown in brackets.
NE, not evaluable.

acquired high-level resistant strains; on days 7–14, 10 out of 12 neonates, still hospitalized, harboured highly resistant strains (Figure 1). Three neonates were not evaluable (one died and two were transferred to another hospital). Strains of CNS isolated from other body sites showed the same patterns of resistance. One of 15 CVC tips (7·7%) was colonized with a highly resistant *S. epidermidis*; all other tips were sterile.

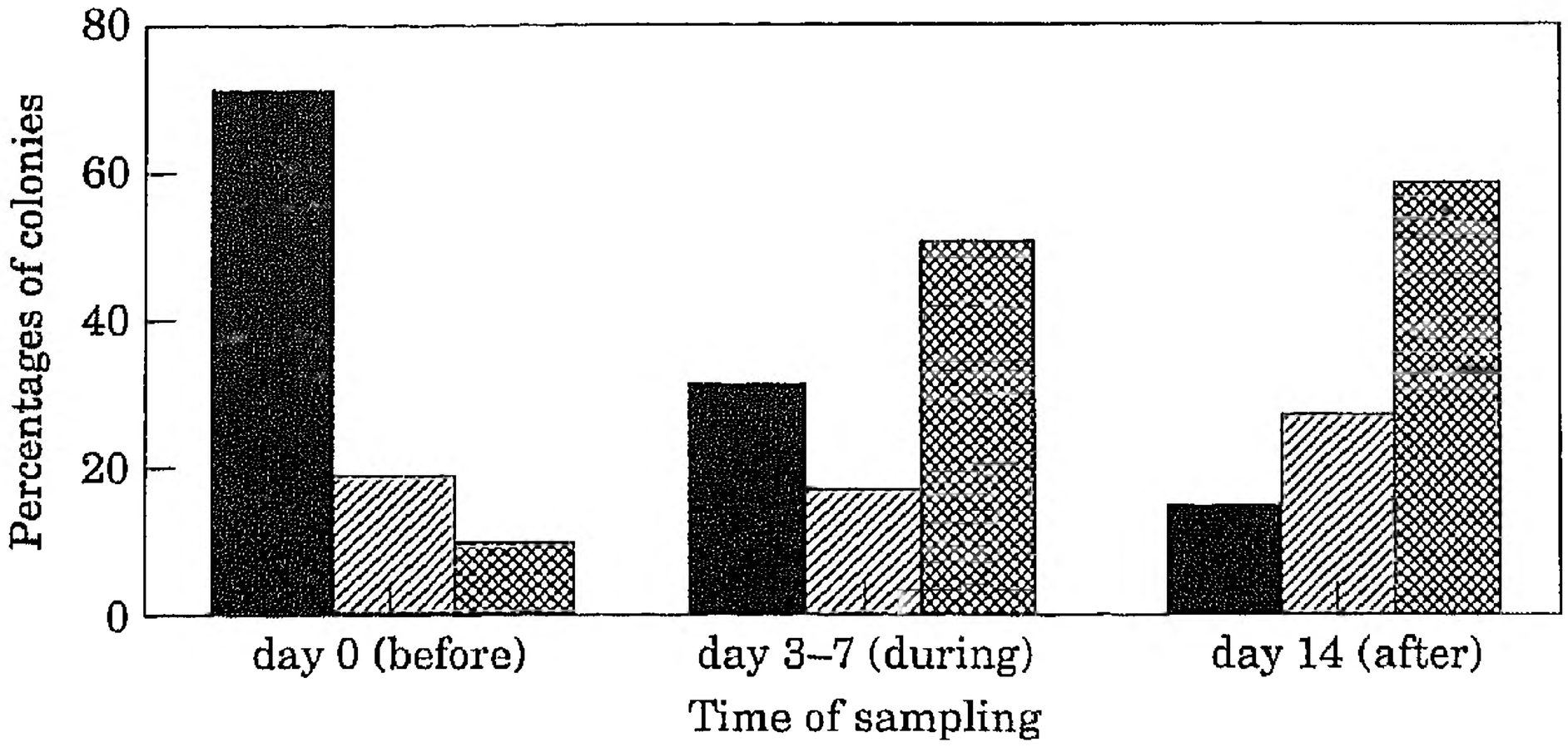

Figure 1. Selection of mupirocin resistance. Changes in coagulase-negative staphylococci of skin flora over time. (■), Susceptible; (▨), low resistance; (▧), high resistance.

After stopping prophylactic mupirocin application, we continued recording mupirocin susceptibility in CNS from skin swabs and CVCs taken from NICU patients. After one year the level of resistance had fallen to 13% and the precentage of colonized CVCs appeared to be 2·4%.

Discussion

Our results clearly show that the routine use of mupirocin in a NICU can lead to the selection of resistant strains in skin flora. The prevalence of resistant strains decreased after stopping the routine application of mupirocin, but it was still at a high level after five months. Surprisingly, we found 5–8% resistance in an ICU ward never using mupirocin. The reason for this is unexplained, but it might have resulted from cross-infection from the NICU or because of the use of mupirocin for methicillin-resistant *Staphylococcus aureus* (MRSA) prophylaxis in referring hospitals. However, we also found a mupirocin resistance rate of 10% in CNS in the skin flora of neonates on admission to the NICU suggesting an unusually high level of naturally occurring resistance in our local population. The slow decline in resistance after stopping mupirocin prophylaxis suggests that resistant strains may colonize the hospital staff and environment for long periods. Persistence of a multi-resistant *S. epidermidis* clone in a NICU for four years has been described.[3] In our study, highly resistant strains easily replaced susceptible ones in the flora during therapy, thus creating a reservoir in which high-level resistant isolates predominated. Furthermore we found that prophylaxis had little beneficial effect on patients, since the percentage of culture-positive CVCs during the study period was comparable with that observed during the period without mupirocin.

We conclude that topical mupirocin is not useful for the prophylactic

treatment of CVCs and leads to the emergence of high-level mupirocin-resistant isolates. If this resistance were to transfer to *S. aureus in vivo*, as it can *in vitro*,[4] it would make mupirocin unsuitable for treating MRSA carriers.

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