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Candida albicans in a neonatal intensive care unit: antifungal susceptibility and genotypic analysis

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SUMMARY

Invasive candidiasis in neonates has become an increasing problem over the past decade in Neonatal Intensive Care Units (NICUs).
From August 2005 to January 2006, six invasive candidiasis occurred in neonates in NICU of the S. Matteo hospital of Pavia. The study focused on the species involved and their *in vitro* antifungal susceptibility. Genotyping was conducted to determine clonal relatedness. A total of 22 yeasts were isolated from different biological samples of neonates during six months. The infants were infected with or colonized by *Candida albicans* and six patients developed *C. albicans* deep infections. The genotyping of the transposable intron region of *C. albicans* strains showed that they belonged to the genotype A (17 isolates) and genotype B (5 isolates). The RAPD confirmed these results. These data suggest that nosocomial transmission of *C. albicans* could be take into account as a mode of acquisition by neonates in NICUs at this hospital.

KEY WORDS: Candida albicans, NICU, Genotyping

Invasive candidiasis has become an increasingly important problem in the neonatal intensive care unit (NICU), resulting in significant morbidity and mortality of low-birth-weight infants. Premature neonates often have compromised skin integrity, gastrointestinal tract disease, chronic malnutrition, central venous catheters, long-term endotracheal intubation, and other factors that lead to increased risk of acquiring such infections. Infections with fungi (particularly candidal species) and with coagulase-negative staphylococci (CoNS) are especially prevalent (Benjamin *et al.,* 2000).

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Candida spp. are the third most common pathogen of nosocomial acquired blood stream infections in premature infants, and they are associated with the second highest mortality rate. Despite antifungal treatment, mortality from all *Candida* species in premature infants has been consistently reported at 20% by large multicenter studies. Prematurity and low birth weight are strongly associated with the development of neonatal nosocomial bloodstream infections (Chapman *et al.*, 2003; Roilides *et al.,* 2004; Smiths *et al.,* 2005).
C. albicans has been the species most often associated with neonatal infections (Benjamin *et al.,* 2000), but recently there has been a changing pattern in the isolates recovered from neonates with invasive candidiasis. Although C. *albicans* remains the most frequently isolated species in many centers, infections due to non-*abicans Candida* spp. have increased in frequency in recent years. In some centers, *Candida parapsilosis* has reported as the predominant

pathogen, causing clusters and common-source outbreaks (Roilides *et al.,* 2004). *C. albicans* is thought to be more virulent than other non-*albicans Candida* species and has been associated with increased rates of end-organ damage and a higher attributable mortality than other species (Smiths *et al.,* 2005).

Development of neonatal candidiasis depends on several broad factors including: susceptibility of the infant, size of inoculum, and virulence of the *Candida* spp. Specific risk factors for invasive disease tend to fall in one of two categories: those that increase colonization of mucosal surfaces and those that disrupt or impair immune function. Infants are initially colonized by vertical transmission during birth or horizontally by parents or caregivers (Smiths *et al.,* 2005).

We performed this study to determine the molecular epidemiology and drug susceptibility of *Candida* isolates causing superficial and invasive candidiasis in a NICU during a 6-month period. A total of 22 *Candida* strains isolated from 14 neonates admitted to the NICU of S. Matteo hospital Pavia, Italy, from August 2005 to January

TABLE 1 - *Clinical data of the fourteen neonates; source and genotype of C. albicans isolates.*

Neonate	*Sample*	*Date of Culture*	*Patient's age (Days)*	*Identification*	*Genotype*
1	blood	12-08-05	28	Candida albicans	A
2	blood	19-08-05	75	Candida albicans	A
3	cutaneous swab rectal swab	05-09-05	31	Candida albicans	A A
4	rectal swab	05-09-05	31	Candida albicans	A
5	blood CVC rectal swab cutaneous swab cutaneous swab cutaneous swab	28-09-05 30-09-05 04-10-05 06-10-05 06-10-05	26 28 32 34 34	Candida albicans	A A A A A A
6	rectal swab	06-10-05	31	Candida albicans	B
7	urine	10-10-05	22	Candida albicans	B
8	urine	13-10-05	29	Candida albicans	A
9	CUC CUC swab	08-11-05	6	Candida albicans	B B
10	blood	13-12-05	13	Candida albicans	B
11	CSF	15-12-05	60	Candida albicans	A
12	ocular swab	16-12-05	39	Candida albicans	A
13	urine	19-12-05	24	Candida albicans	A
	stool	02-01-06	42	Candida albicans	A
14	urine	04-01-06	18	Candida albicans	A

CVC = central venous catheter; CUC = central umbilical catheter; CSF = cerebrospinal fluid.

TABLE 2 - *Neonate risk factors for invasive disease.*

Neonate	*Gestational age*	*Birth Weight*	*Mechanical ventilation*	*Abdominal surgeries*	*Catheters*	*Parenteral nutrition*	*Intralipid emulsion*	*H_2 blockers*	*Antibiotic therapy*
1	33 wg+ 6d	2150 gr	-	-	-	-	-	-	AK+ TEC
2	26 wg	1930 gr	Yes	Yes	CVC	Yes	Yes	Yes	AMP+ AK
3	29 wg + 2d	V	-	-	-	-	-	-	-
4	29 wg + 6d	V	-	-	-	-	-	-	-
5	25 wg + 6d	E	Yes	Yes	CVC	Yes	Yes	-	AMP-sulb+AK
6	23 wg	E	Yes	-	-	Yes	Yes	-	AK+ TEC
7	25 wg+ 3d	E	Yes	-	-	Yes	Yes	-	OXA+ AK
8	24wg+ 3d	E	-	-	-	Yes	Yes	Yes	OXA+ AK
9	28 wg + 2d	E	Yes	-	CUC	Yes	Yes	-	AMP+ GEN
10	25 wg	E	Yes	Yes	CVC	Yes	Yes	-	AK+ CAZ
11	33 wg	4240 gr	-	Yes	-	-	-	Yes	VAN + AK+CAZ
12	27 wg + 4d	V	-	-	-	-	-	-	-
13	27 wg	V	-	-	CVC	Yes	Yes	-	-
14	28 wg + 2d	V	-	-	CVC	Yes	-	Yes	VAN+ CAZ

Wg = Week gestation; d = days; V = Very Low Birth Weight (<1500 g); E = Extremely Low Birth Weight (<1000 g); CVC = central venous catheter; CUC = central umbilical catheter; AK = amikacin; AMP-sulb = ampicillin – sulbactam; CAZ = ceftazidime; GEN = gentamicin; OXA = oxacillin; TEC = teicoplanin; VAN = vancomycin.

2006 were identified to the species level by the ability to form germ tubes and by the Vytek System (BioMérieux). Susceptibility to antifungal drugs was determined by colorimetric method Sensititre YeastOne (Trek Diagnostic System, East Grinstead, UK), based on microdilution methodology with RPMI 1640 medium supplemented with a pH indicator (Alamar blue), in accordance with the CLSI method M27-A2 (Clinical and Laboratory Standards Institute, 2002). Minimal inhibitory concentrations (MICs) of amphotericin B, fluconazole, itraconazole, ketoconazole, 5-flucytosine, and voriconazole were determined using control isolates of C. *albicans* ATCC 90028 and C. *parapsilosis* ATCC 22019.
Genotyping of the isolates was determined based on the amplification of a transposable intron region of the *Candida* 25 S rRNA gene. The primer pairs used were CA-INT-L (5'-ATAAGGGAAGTCGGCAAAATAGATCCGTAA-3') and CA-INT-R (5'-CCTTGGCTGTGGTTTCGCTAGATAGTAGAT-3') (Tay, 2005).
Random amplification of polymorphic DNA was employed to confirm the 25 S rRNA gene analyse. The two random primers utilized were number 4 (5'-GGTGACGCAG- 3') and 7 (5'-ACCCGACCTG- 3') considered by Lian *et al.* 2004 the only ones showing a good pattern with a high percentage of polymorphism.
The strains recovered from the neonates during the study period were isolated from deep and superficial sites of infection. Out of 14 patients, six developed an invasive candidiasis (Table 1). The infants with a positive sample for *C. albicans* had certain risk factors for invasive disease including prematurity, low or extremely low birth weight, presence of CVC, parenteral nutrition, long-term usage of hyperalimentation, intralipid

emulsion and broad-spectrum antibiotic therapy. Medical records obtained to determine risk factors for the acquisition of invasive candidiasis are summarized in Table 2.

All the 22 yeasts isolated belonged to the species *Candida albicans* and were sensitive to amphotericin B, itraconazole, ketoconazole, 5-flucytosine, fluconazole and caspofungin. The PCR products of representative genotype of *C. albicans*, obtained with the primer CA-INT, were demonstrated in Figure 1. The PCR resulted in the generation of a single product for *C. albicans* genotypes A (~450 bp) and B (~840 bp). The genotype distribution of the 22 isolates is demonstrated in Table 1. Genotype A was the predominant *C. albicans* type that was isolated from the NICU during the six months considered (17 isolates). The RAPD assay confirmed the analyses of the transposable intron region of the *Candida* 25 S rRNA gene: the isolates belonging to the same genotype presented an identical pattern of bands with the random primers used (Figure 2).

Prematurity and low birth weights are strongly associated with the development of neonatal nosocomial bloodstream infections and *Candida* spp. are increasingly important hospital-acquired pathogens in neonatal intensive care units (NICU) causing considerable mortality in preterm infants.

Candida infections currently rank as the fourth leading cause of nosocomial bloodstream infections and, similar to the situation in the adult population, *C. albicans* is the most commonly isolated species in neonatal candidiasis (Rangel-Frausto *et al.*, 1999). *C. albicans* is thought to be more virulent than other non-*albicans Candida* species and has been associated with increased rates of end-organ damage and a higher attributable mortality than other species.

Although most *Candida* infections appear to originate from an endogenous source, several reports have described the nosocomial transmission of a single strain from one patient to another (Waggouner-Fountain *et al.*,1996; Huang *et al.*, 1998; Reef *et al.*, 1998).

Molecular typing of an infectious agent is important for epidemiological studies and for the development of appropriate infection control strategies. Because of the characteristics of *C. albicans* and the need for better understanding its epidemiology, molecular techniques are employed to provide the characterization of the strains and isolates. Such a characterization can be used to track the organism within a host, between hosts, or between host and inanimate objects, or to associate particular strains with various anatomic sites, particular disease entities, or particular host characteristics.

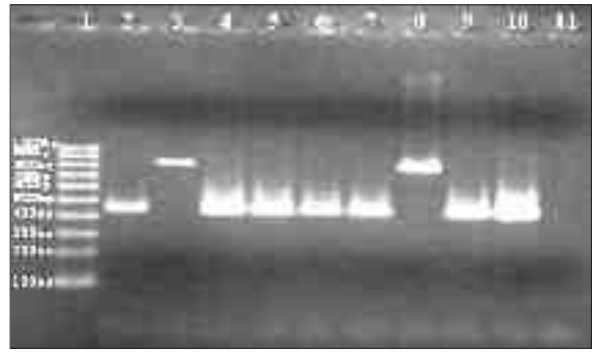

FIGURE 1 - *PCR genotyping with primer CA-INT. Lane 1, 100 bp DNA ladder; 2. genotype A, neonate 1; 3. genotype B, neonate 9; 4-7. genotype A, neonates 2,5,8,12; 8. genotype B, neonate 10; 9 and 10. genotype A, neonates 13 and 14; 11. negative control.*

In this report the genotyping of *C. albicans* strains, that involved 14 infants with very or extremely low birth weights in an NICU, showed that the majority of the clinical isolates have identical genotypes, with a single genotype (A) predominating in most isolates. There is no correlation between the *Candida* genotype and antifungal susceptibility, as indicated in literature (McCullough *et al.*, 1999) where strains of genotype A were reported as less susceptible to flucytosine than either genotype B or genotype C strains.

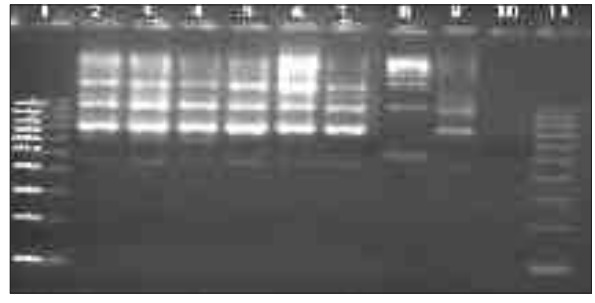

FIGURE 2 - *PCR genotyping with random primer 4. Lane 1, 100 bp DNA ladder; 2-7. genotype CA-INT A, neonate 1,2,3,8,12,13; 8. 1Kb DNA ladder; 9. genotype CA-INT B, neonate 9; 10. negative control; 11.100 bp DNA ladder.*

In a clinical care setting when isolates derived from different patients are genetically identical, it may be generally assumed that cross-infection has occurred or that the patients were infected by exposure to a common source (Pfaller, 1995). Neonates become colonized with *Candida* spp. either from their mothers, during birth, or from colonized hospital personnel during their stay in the NICU. From the epidemiology studies conducted by Hedderwick *et al.* 2000 seems that *Candida* colonized premature infants acquires yeasts more frequently from the ICU and less frequently from their mothers, even if there is still not a clear understanding of the epidemiology of yeast transmission and colonization in the clinical care setting. The same authors, as well as those of the American national epidemiology of mycoses survey –NEMIS- (Rangel-Frausto *et al.*, 1999), have focused on the role of the carriage of yeasts on the hands of healthcare workers in the transmission of infection to neonates.

In our study, the genotyping of C. *albicans* isolates recovered from different neonates who were in the same NICU suggests a clonal spread of two strains, belonging to two genotypes (A and B), that caused colonization and infection of these infants and even though horizontal transmission is the most probable explanation for this cluster of cases, environmental, hand and pharynx cultures of NICU healthcare workers samples were not obtained. At the same time, they could be assumed as a probable hypothesis to understand the source of infection and the route of transmission.

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