Fingerprinting of bloodstream isolates of *Candida parapsilosis* suggests horizontal transmission of infections among neonatal intensive care unit patients in Kuwait

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Introduction and Objectives:

Candida parapsilosis is increasingly being associated with invasive candidiasis in critically ill patients. *C. parapsilosis* also causes candidemia in ~35% of all cases in neonates. Nosocomial outbreaks are facilitated by spread of *C. parapsilosis* from health care workers (HCWs) to susceptible patients via hand carriage, its affinity for glucose-containing parenteral nutrition and ability to form biofilms on intravascular devices. This study determined genotypic heterogeneity among *C. parapsilosis* isolates from candidemia patients in neonatal intensive care units (NICUs) of Maternity Hospital (MH), Kuwait. Isolates from two other hospitals were also included for comparison purposes.

Materials and Methods

A total of 88 (52 bloodstream and seven colonizing strains from 50 NICU-MH patients, 10 colonizing strains from nine non-candidemia NICU-MH patients, two isolates from MH-HCWs and 18 bloodstream isolates from two other hospitals) *C. parapsilosis sensu stricto* isolates identified by multiplex PCR were used. Fingerprinting was performed by 6-loci-based microsatellite typing and isolates were assigned a numerical microsatellite genotype (MSG). Phylogenetic relationship was determined by minimum spanning tree (MST) and dendrogram was generated by using BioNumerics with UPGMA settings.

Results

- All isolates were identified as *C. parapsilosis sensu stricto* by multiplex PCR, species-specific PCR/PCR-sequencing of rDNA and were uniformly susceptible to amphotericin B, fluconazole and caspofungin by Etest.
- Fifty bloodstream isolates from 50 neonates yielded 37 MSGs; 30 unique MSGs and 7 MSGs from 20 isolates (clusters). Duplicate isolates had identical MSG. MSG of 5 colonizing strains was same as bloodstream isolate while one strain yielded a unique MSG (MSG13) that differed in only one marker (Fig. 1).
- Ten strains from 9 non-candidemia neonates yielded 7 MSGs; two unique MSGs, one (MSG39) for 3 strains from 2 neonates (cluster) and 5 isolates sharing MSG with bloodstream isolates (Fig. 1). Two strains from 2 HCWs and all 9 isolates from nine patients at Hospitals A and B were unique (Fig. 1).
- Thus 88 isolates exhibited 61 MSGs with 29 isolates from 26 patients from MH clustering into 9 MSGs (Fig. 1). All cluster isolates in MST were either from NICU-1 patients or mostly included isolates from patients from NICU-1 (Fig. 2).
- Four clonal complexes (A to D) (MSGs differing in one marker were detected in MH (Table 1). There was overlap in time of stay for some or all isolates from neonates in NICU-1 for MSG11, MSG7, MSG25 and MSG39 and for some isolates in different NICUs for MSG11, MSG2, and MSG38. Endemic genotypes (MSG3 & MSG11) persisted in NICUs of MH for several years.

CONCLUSIONS

- All *C. parapsilosis* isolates from HA and HB hospitals belonged to different MSGs showing high discriminatory power of the typing scheme.
- Our data from MH showed that 33 isolates from 29 preterm neonates with/without candidemia belonged to the same or very closely-related MSGs.
- The findings suggest that some strains have persisted in MH environment, particularly in NICU-1, over several years and these endemic genotypes were likely transmitted to other patients in NICU-1 and other nearby NICUs in MH in Kuwait.

Fig. 1. Dendrogram based on allele numbers of six diploid microsatellite markers from 88 *C. parapsilosis* isolates. Similarity (scale bar on upper left corner) is presented in percentages.

Fig. 2. Minimum spanning tree of 70 *C. parapsilosis* isolates from MH. Circles represent genotypes (MSG), lines represent relative distance between isolates and circle size denotes no. of isolates.

Table 1. *C. parapsilosis* genotypes from Maternity Hospital forming clonal complexes

Microsatellite genotype (MSG)	Patient no. ^a	Microsatellite fragment length-based allelic profile ^b												Clonal complex
		CanpaSTR3A/a	CanpaSTR3A/b	CanpaSTR3B/a	CanpaSTR3B/b	CanpaSTR3C/a	CanpaSTR3C/b	CanpaSTR6A/a	CanpaSTR6A/b	CanpaSTR6B/a	CanpaSTR6B/b	CanpaSTR6C/a	CanpaSTR6C/b	
MSG2	MH2, MH41, MH44, MH47, MH47D, MH51C	26	26	37	46	39	52	7	7	6	6	7	7	A
MSG36	MH46	26	26	37	46	39	52	7	7	9	14	7	7	
MSG3	MH3, MH4, MH30, MH55C	26	26	47	47	40	40	7	7	6	6	7	7	B
MSG34	MH43	26	26	47	47	39	39	7	7	6	6	7	7	
MSG7	MH9, MH10, MH10C	25	27	32	32	38	42	11	11	6	6	7	7	C
MSG8	MH11, MH31	25	27	32	32	36	42	11	11	6	6	7	7	
MSG1	MH1	25	25	32	32	27	27	11	11	6	6	7	7	D
MSG12	MH15, MH15C ₁	26	32	32	32	44	44	7	7	6	6	7	7	
MSG13	MH15C ₂	26	32	32	32	44	44	12	12	6	6	7	7	

^aMH: Patient from Maternity Hospital; MHC: colonizing isolate from Maternity Hospital patient
^bBold numbers in red font indicate marker differences within the clonal complex