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MOLECULAR TYPING OF *CANDIDA ALBICANS* STRAINS ISOLATED FROM NOSOCOMIAL CANDIDEMIA

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SUMMARY

Yeasts of the genus *Candida* have been recognized as important microorganisms responsible for nosocomial fungemia. Six blood-stream and two intravenous central catheter *C.albicans* strains were isolated from eight patients and studied by electrophoretic karyotyping of chromosomal DNA by pulsed-field gel electrophoresis. Seven chromosomal DNA profiles were identified. Two patients showed isolates with the same profile, suggesting nosocomial transmission. Karyotyping of *C.albicans* revealed an excellent discriminatory power among the isolates and may therefore be useful in the study of nosocomial candidemia.

KEYWORDS: *Candida albicans*; Nosocomial candidemia; Pulsed-field gel electrophoresis; Molecular typing.

INTRODUCTION

Fungi have recently emerged as an important cause of nosocomial infections. Based on the National Nosocomial Infections Surveillance (NNIS) [1] published document, systemic infections due to *Candida* spp. represent 10% of the nosocomial infections in the USA. *Candida albicans* is responsible for 76% of these fungal infections [21].

Fungemia caused by *Candida* spp. are reported to be related to previous digestive tract colonization or exogenous contamination [9, 25]. Recently, however outbreaks of cross-transmission nosocomial infections caused by *C.albicans* have been described, particularly in intensive care units [3, 4, 16, 27].

Various typing methods based on phenotypic or genomic markers have been used to investigate the epidemiology of nosocomial infections caused by *Candida* spp. [7, 12, 20, 23]. Of these methods, genomic typing has demonstrated the highest reproducibility and discriminatory power for *Candida* spp. [5, 8, 19, 22] and *Cryptococcus neoformans* [18].

We have few data regarding the epidemiology of nosocomial *Candida* infections in Brazilian hospitals, and on the use of genomic typing in this context. In the present study, we have used the technique of pulsed-field gel electrophoresis (PFGE) to analyze the chromosomal DNA of *C.albicans* strains isolated from patients with fungemia.

PATIENTS AND METHODS

From July 1993 to January 1994, we analyzed the

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C.albicans isolates from seven patients with fungemia hospitalized at the Hospital São Paulo. One strain isolated from blood culture of a patient hospitalized at Hospital 9 de Julho, also located in the city of São Paulo, was included in the study as an unrelated epidemiologic control. Fungemia was defined as the growth of the microorganism in one or more blood cultures obtained during the period of hospitalization, from patients without signs of a fungal infection at the time of admission [28]. The criteria for an intravenous catheter-related infection was the growth of more than 15 *C.albicans* colonies forming units on a blood agar plate incubated for 24-48 hours at 36°C [13, 15].

The identification of *C.albicans* was based on the germ tube test and clamidoconidia production [10]. Following identification, the isolates were stored on Sabouraud dextrose agar at room temperature, until the typing procedure.

The isolates were submitted to electrophoretic karyotyping (EC) by pulsed-field gel electrophoresis technique modified by DOEBBELING et al. [5]. Briefly, colonies from the stored isolates were cultured on Sabouraud dextrose agar plates and incubated for 48 hours. Ten to 20 *C.albicans* colonies recently cultured were inoculated on broth containing 1% yeast extract, 2% peptone and 2% dextrose, then incubated again for 12 h at 37°C under agitation. After centrifugation for 15 min at 3,000 rpm and 4°C, the supernatant was discarded. One hundred and fifty microliters of the cells were transferred to Eppendorf tubes and washed twice with 200 µl of 50 mM EDTA, pH 8.0. A solution of lyticase (Sigma Chemical, St. Louis, USA) 1,250 U/ml in glycerol (50% vol/vol in 0.01 M $NaPO_4$, pH 7.5), was added to the cells and the suspension incubated at 37°C for 20 min. Agarose models were prepared with 560 µl of 1% low melt agarose (BioRad Richmond, CA, USA) in 50 mM EDTA, pH 8.0. The models were incubated for 12 hours at 50°C in 1.5 ml of 0.01 M Tris buffer, pH 7.5, containing 0.45 M EDTA, 1% laurylsarcosine and 1 mg of Proteinase K (Gibco, BRL, Gaithersburg, USA) per milliliter. After washing three times with 3 ml of 50 mM EDTA, pH 8.0, the suspension was incubated overnight at room temperature. The washing procedure was repeated three times the next day and 2 mm of each agarose model were inserted in each well of a 0.8% chromosomic grade agarose gel (BioRad), For the separation of the chromosomic DNA of different molecular weights, we used a contour clamped homogeneous electric field system from BioRad Laboratories (CHEF DR-III). The electrophoretic conditions were: 150 volts, 13°C, a switch time of 120 sec for 24 h and then of 240 sec for 36 h. The gel was photographed under UV light after staining with ethidium bromide. *Saccharomyces cerevisiae* chromosome/DNA size standards (BioRad) was included in the gel as standard. As, it was the first experiment using the new CHEF apparatus, an agarose model of a previously studied *Candida albicans* genomic DNA from the Special Microbiology Laboratory, University of Iowa, USA, was included in the gel as a quality control for the method.

Differentiation among the isolates was achieved by visual comparison of the electrophoretic karyotypes (EK) [26]. Isolates were considered the same profile if all the bands in one isolate matched the bands in another. To simplify the analysis among the isolates, letters in alphabetic order were used to designate the EK profiles.

RESULTS

The clinical and epidemiological data of the eight *C.albicans* isolates and the results of the electrophoretic karyotyping (EK) are presented in Table 1.

The molecular typing revealed seven different EK profiles that were named from "A" to "G" (Fig 1). Isolates 3 (catheter) and 4 (blood) both collected on the same day from different patients had the same EK profile "C" (Fig 1). The *C.albicans* isolates showed six to seven chromosomal DNA bands with molecular weights of 1.0 to > 2.2 Kilobases (Kb) (Fig 1). The variations in the molecular weight of EK profiles

Table 1
Molecular typing of *Candida albicans* strains isolated from patients with fungemia.

Isolate	Underlying disease	Site	Date	Karyotype
1	Diabetes mellitus	blood	15/07/93	A
2	no record	catheter	21/07/93	B
3	AIDS	catheter	31/08/93	C
4	Breast cancer	blood	31/08/93	C
5	Iowa (USA)	blood	-/-/93	QC*
6	Renal transplant	blood	13/09/93	D
7	Chronic liver disease	blood	28/10/93	E
8	no record	blood	13/03/93	F
9	Hospital 9 Julho	blood	01/01/94	G

* *C.albicans* agarose model used as quality control of the method.

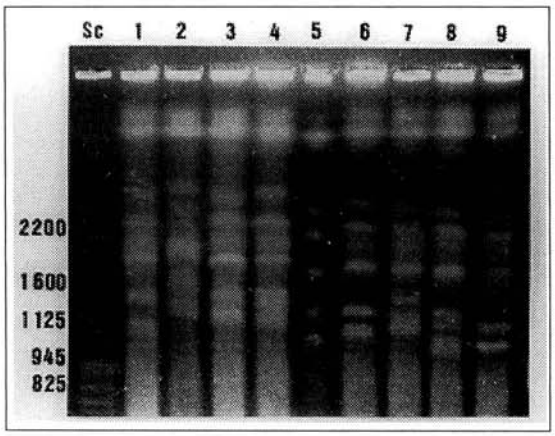

Fig. 1. - Electrophoretic Kariotypes (EK) of *Candida albicans* strains isolated from nosocomial candidemia. Sc - *Saccharomyces cerevisiae* chromosome DNA size standards (Kb = kilobases). Lanes 1, 2, 6, 7 and 8 are karyotype: A, B, D, E and F, respectively Lanes 3 and 4 are karyotype C. Lane 5 is *C.albicans* from the Special Microbiology Lab., Iowa, USA. Lane 9- karyotype G from Hospital 9 de Julho, São Paulo.

occurred mainly in the central and lower segments of the gel and were responsible for the differentiation among the isolates. The high molecular weight bands were common to all isolates.

The number of bands and respective molecular weights of the Iowa isolate (Lane 5; Fig 1) were previously known and confirmed in this study (two bands over 2.2 Kb, a 2.2 Kb band and three bands between 2.2 and 1.0 Kb).

DISCUSSION

Recent studies revealed that systemic infections due to *Candida* spp. are not a rare and not only seen in patients presenting terminal diseases. Candidemia has been reported to occur in 5 to 10 out of every 10,000 patients hospitalized [29] with a crude mortality rate of up to 50% [30].

The detection of *Candida* spp. in blood cultures is a key step in the diagnosis of invasive disease [24, 28]. In our study, the isolates from blood cultures or vascular catheters were considered to be responsible for the nosocomial infections since most of the patients presented a serious underlying disease and had the risk factors for candidemia [6] (Table 1).

The results of EK were satisfactory with regard to the ability to discriminate among the isolates and were similar to published data [11, 14, 17]. MAGEE & MAGEE (1987) [14] and LASKER et al. (1989) [11], utilizing PFGE, detected the presence of eight to ten chromosomal DNA bands, and reported that the high molecular weight bands (> 2.2 Kb) showed a low resolution. This situation was also observed in our gels. Some authors suggest that EK is the best method for the molecular typing of *C.albicans* and other *Candida* species [2, 8, 11, 16, 19]. EK is a highly sensitive and reproducible procedure. It has also the advantage that there is no need to use DNA probes such as ribotyping or specific conserved sequences which often requires a transfer procedure to a solid support as well as adequate detected systems. The disadvantage of EK is the running time of the gel, around 60 h, and the cost of the PFGE device. The digestion of the genomic DNA with restriction endonuclease followed by PFGE (not performed in our study) can increase the discriminatory power when combined with EK [2, 5, 26].

EK demonstrated the possible nosocomial transmission of a strain of *C.albicans*, responsible for the infection of patients 3 and 4. Both isolates were obtained on the same day but in different wards of the hospital.

The first report of a cross-transmission nosocomial infection outbreak caused by *C.albicans* was published by BURNIE et al. (1985) [4]. These authors showed that the same *C.albicans* strain responsible for an outbreak of systemic infection was present in the mouth and the hands of the health care staff. Nosocomial transmission and outbreaks of systemic infection by *C.albicans* [4, 21, 29], and other species [5], have been well described in the literature with most of the studies using molecular typing as an epidemiological tool.

The present study is the first in the Brazilian medical literature to employ molecular typing to assess the epidemiology of nosocomial candidemia. With the current increase in the prevalence of *Candida* spp. fungemia, this method proved to be helpful in understanding the epidemiology of these microorganisms.

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RESUMO

Tipificação molecular de espécies de *Candida albicans* isoladas de candidemia hospitalar

Leveduras do gênero *Candida* têm sido reconhecidas como importantes causadoras de fungemias hospitalares. Foram estudados os DNA cromossômicos de oito cepas de *C.albicans*, obtidas de oito pacientes com fungemia hospitalar, por cariotipagem eletroforética através de "pulsed-field gel electrophoresis". As cepas foram obtidas pelo isolamento da levedura em seis hemoculturas e duas infecções relacionadas ao uso de cateter intra-venoso central. Foram identificados sete perfis de DNA cromossômico. Dois pacientes mostraram cepas com o mesmo perfil de DNA sugerindo transmissão nosocomial. A cariotipagem eletroforética revelou excelente capacidade discriminatória entre os isolados sendo útil no estudo das candidemias hospitalares.

REFERENCES

1. BANERJEE, S. N.; EMORI, T. G.; CULVER, D. H. et al. - Secular trends in nosocomial primary bloodstream infections in the United States, 1980-1989. **Amer. J. Med., 91** (Suppl. 3B): 86S-89S, 1991.

2. BRANCHINI, M. L. M.; PFALLER, M. A.; RHINE-CHALBERG, J.; FREMPONG, T. & ISENBERG, H. D. - Genotypic variation and slime production among blood and catheter isolates of *Candida parapsilosis*. **J. clin. Microbiol., 32**: 452-456, 1994.

3. BURNIE, J. P.; MATTHEWS, R.; LEE, W. et al. - Four outbreaks of nosocomial systemic candidiasis. **Epidem. Infect., 99**: 201-211, 1987.

4. BURNIE, J.; ODDS, F. C.; LEE, W.; WEBSTER, C. & WILLIAMS, J. D. - Outbreak of systemic *Candida albicans* in intensive care unit caused by cross infection. **Brit. med. J., 290**: 746-748, 1985.

5. DOEBBELING, B. N.; LEHMANN, P. F.; HOLLIS, R. J.; WU, L. C. et al. - Comparison of pulsed-field gel electrophoresis with isoenzyme profiles as a typing system for *Candida tropicalis*. **Clin. infect. Dis., 16**: 377-383, 1993.

6. HARVEY, R. L. & MYERS, J. P. - Nosocomial fungemia in a large community teaching hospital. **Arch. intern. Med., 147**: 2117-2120, 1987.

7. HOWELL, S. A. & NOBEL, W. C. - Typing tools fot the investigation of epidemic fungal infection. **Epidem. Infect., 105**: 1-9, 1990.

8. KAUFMANN, C. S. & MERZ, W. G. - Electrophoretic karyotypes of *Torulopsis glabrata*. **J. clin. Microbiol., 27**: 2165-2168, 1989.

9. KOMSHIAN, S. V.; UWAYDAH, A. K.; SOBEL, J. D. & CRANE, L. R. - Fungemia caused by *Candida* species and *Torulopsis glabrata* in the hospitalized patient: frequency, characteristics and evaluation of factors influencing outcome. **Rev. infect. Dis., 11**: 379-390, 1989.

10. LACAZ, C. S. - Candidiases. São Paulo, EDUSP, 1980.

11. LASKER, B. A.; CARLE, G. F.; KOBAYASHI, G. S. & MEDOFF, G. - Comparison of the separation of *Candida albicans* chromosome-sized DNA by pulsed-field gel electrophoresis techniques. **Nucleic Acids Res., 17**: 3783-3793, 1989.

12. LEE, W.; BURNIE, J. P. & MATTHEWS, R. - Fingerprinting *Candida albicans*. **J. immunol. Meth., 93**: 177-182, 1986.

13. LINARES, J.; STIGES-SERRA, A.; GARAN, J.; PEREZ, J. L. & MARTIN, R. - Pathogenesis of catheter sepsis: a prospective study with qualitative and semiquantitative cultures of hub and segments. **J. clin. Microbiol., 21**: 357-360, 1985.

14. MAGEE, B. B. & MAGEE, P. T. - Electrophoretic karyotypes and chromosome numbers in *Candida* species. **J. gen. Microbiol.**, 133: 425-430, 1987.

15. MAKI, D. G.; WEISE, C. E. & SARAFIN, H. W. A. - A semiquantitative culture method for identifying intravenous-catheter infection. **New Engl. J. Med., 296**: 1305-1307, 1977.

16. MATTEWS, R. & BURNIE, J. P. - Assessment of DNA fingerprint for rapid identification of outbreaks of systemic candidiasis. **Brit. med. J., 298**: 354-357, 1989.

17. MERZ, W. G.; CONNELLY, C. & HIETER, P. - Variation of electrophoretic karyotypes among clinical isolates of *Candida albicans*. **J. clin. Microbiol., 26**: 842-845, 1988.

18. PERFECT, J. R.; MAGEE, B. B. & MAGEE, P. T. - Separation of chromosomes of *Cryptococcus neoformans* by pulsed field gel electrophoresis. **Infect. Immun., 57**: 2624-2627, 1989.

19. PFALLER, M. A. - Epidemiological typing methods for mycosis. **Clin. infect. Dis.,** 14 (suppl. 1): S4-S10, 1992.

20. PFALLER, M. A.; CABEZUDO, I.; HOLLIS, R.; HUSTON, B. & WENZEL, R. P. - The use of biotyping and DNA fingerprint in typing *Candida albicans* from hospitalized patients. **Diagn. Microbiol. infect. Dis., 13**: 481-489, 1990.

21. PFALLER, M. A. & WENZEL, R. P. - Impact of the changing epidemiology of fungal infections in the 1990s. **Europ. J. clin. Microbiol. infect. Dis., 11:** 287-291, 1992.

22. REAGAN, D. R.; PFALLER, M. A.; HOLLIS, R. J. & WENZEL, R. P. - Characterization of the sequence of colonization and nosocomial candidemia using DNA fingerprinting and a DNA probe. **J. clin. Microbiol.**, 28: 2733-2738, 1990.

23. SCHERER, S. & STEVENS, D. A. - Application of DNA typing methods to epidemiology and toxonomy of *Candida* species. **J. clin. Microbiol., 25**: 675-679, 1987.

24. SOLOMKIN, J. S.; FLOHR, A. M. & SIMMONS, R. L. - Indications for therapy for fungemia in post operative patients. **Arch. Surg., 117**: 1272-1275, 1982.

25. SOLOMON, S. L.; KHABBAZ, R. F.; PARKER, R. H. et al. - An outbreak of *Candida parapsilosis* bloodstream infections in patients receiving parenteral nutrition. **J. infect. Dis., 149**: 98-102, 1984.

26. VASQUEZ, J. A.; BECKLEY, A.; DONABEDIAN, S.; SOBEL, J. F. & ZERVOS, M. J. - Comparison of restriction enzyme analysis versus pulsed-field gradient gel electrophoresis as a typing system for

Torulopsis glabrata and *Candida* species other than *Candida albicans*. **J. clin. Microbiol., 31**: 2021-2030, 1993.

27. VAUDRY, W. L.; TIERNEY, A. J. & WENMAN, W. M. - Investigation of a cluster of systemic *Candida albicans* infections in a neonatal intensive care unit. **J. infect. Dis., 158**: 1375-1379, 1988.

28. WALSH, T. J. & PIZZO, P. A. - Laboratory diagnosis of candidiasis. **In**: BODEY, J. P., ed. - *Candidiasis: pathogenesis, diagnosis and treatment.* New York, Raven Press, 1993. p. 110-135.

29. WENZEL, R. P. & PFALLER, M. A. - Candida species: emerging hospital bloodstream pathogens. **Infect. Control Hosp. Epidem., 12**: 523-524, 1991.

30. WEY,S. B.; MORI, M.; PFALLER, M. A.; WOOLSON, R. F. & WENZEL, R. P. - Hospital-acquired candidemia: the attributable mortality and excess length of stay. **Arch. intern. Med., 148**: 2642-2645, 1988.

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