ARTIGO ORIGINAL

MOLECULAR EPIDEMIOLOGICAL ANALYSIS OF BLOODSTREAM ISOLATES OF *Candida albicans*

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ABSTRACT

Candidemia is generally related to the endogenous flora, however exogenous infection originated from hospital staff or from the environment has occurred. The randomly amplified polymorphic DNA (RAPD) method can reveal strain specific variation. In this study, we used a RAPD assay to assess genetic diversity among *Candida albicans* isolates to find the relatedness between DNA patterns of the strains recovered from clinical and environment samples from the Intensive Care Unit (ICU) in the Hospital das Clínicas of the Universidade Federal de Goiás. The primers named Cnd3 (5´-CCAGATGCAC-3`) and Cnd4 (5'-ACGGTACACT-3`) were used as single primers in the PCR. RAPD profiles from both blood and urine from the same patients were identical in almost all the samples studied, except in one patient. The bed of this patient had the same genotype from his blood. Although most of *C. albicans* isolates probably had had an endogenous origin, the finding of isolates from the patients with the same profile as the environment isolates suggest that the candidemia may have resulted from an exogenous source.

KEY WORDS: *Candida albicans.* RAPD. Nosocomial infection.

INTRODUCTION

Candidemia is considered the major fungal nosocomial infection and it is largely associated with at least 50% mortality rate (10, 12, 19). Intensive Care Unit (ICU) patients are particularly susceptible to systemic infection because they are seriously ill and subjected to a number of therapeutic and supportive interventions

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Received for publication in: 24/6/2008. Accepted in: 18/11/2008.

(central venous catheters, mechanical ventilations and tracheostomy) which breach physiological barriers to infection (14, 22).

Although the yeast species are considered important nosocomial pathogens, it is not known much about their epidemiology. *Candida* infections are generally related to the endogenous flora, but exogenous infection, originated from hospital staff or from the environment, has occurred (22). In cases in which an exogenous source is involved, the sanitary measures are mandatory to prevent the cross-transmission.

Molecular typing system can be employed to characterize the pathogen to the subspecies level or still to determine whether the infections studied are due to the same strain or to different strains (2, 11, 19). The randomly amplified polymorphic DNA (RAPD) method, that uses specific short oligonucleotides which may be arbitrarily primed at multiple positions of the yeast genome, can reveal strain specific variation. This method has been used to characterize the genetic relatedness among *Candida* species isolates (6, 15).

In this study, we used a RAPD assay to assess genetic diversity among *C. albicans* isolates to find the relationship between DNA patterns of the strains recovered from clinical and environment samples from the ICU in a tertiary hospital.

PATIENTS AND MATERIALS

Isolates and patients

A total of 10 *C. albicans* isolates recovered from blood specimens of 10 patients of the ICU in a tertiary hospital, between September 2003 and April 2005, were included in this study. Isolates of the same species recovered from other sources related to the same patients such as urine, catheter tip (central venous catheter), and surfaces of bed and tray collected on the same day were also included in this study. The sources of the isolates studied are related in Table 1.

The isolates were identified by germ tube production, chlamydospore formation, sugar assimilation and fermentation, and, when necessary, they were confirmed by the API 20C identification test, commercially available (API Laboratory Products Ltd., Grafton Way, Basingstoke, Hants, England). All the isolates were maintained in sterile water at room temperature and, before use, they were subcultured twice onto yeast-peptone dextrose agar (1% yeast extract peptone, 2% dextrose, 1.2% agar) to ensure viability and purity.

Genotypic characterization

Preparation of DNA – Genomic DNA extraction was based on the method described by Del Poeta et al. (7), and modified by Casali et al. (4). Briefly, a heavy inoculum of *C. albicans* strains grown in YEPD agar at 37ºC for 24 to 48 hours was suspended in 0.5mL TENTS (10mM Tris, pH 7.5, 1mM EDTA pH 8.0, 200mM

NaCl, 2% Triton, 1% SDS), containing 0.2mL of 0.45mm glass beads and 0.5mL of phenol:chloroform and vortexed, for 2min. After the centrifugation for 10min at 13.000g, the aqueous phase was transferred to a new tube and the same volume of 100% ethanol was added and incubated at -20°C for 1h for the DNA precipitation. The precipitated DNA was resuspended in 0.5mL TE (10mM Tris HCl pH 8.0, 1mM EDTA pH 8.0), containing 50µg/mL RNAse A, and it was incubated at 37°C for 30min. The yeast DNA was deproteinated and extracted from the sample by adding equal volume of phenol and chloroform. Finally, the DNA was precipitated with 70% ethanol and, after dried, it was stored at -20°C in 100µL of TE buffer until further processing for PCR.

Table 1. *Candida albicans* isolates recovered from 10 ICU patients with candidemia and from other clinical and environment materials related to those patients.

Patients	Isolation date (mm/dd/yy)	Number of isolates	Sources of isolates	Genotype (patterns)	
				Cnd3	Cnd4
1	09/12/2003	4	Catheter	A	1
	09/26/2003	5	Blood	A	1
	09/19/2003	6	Urine	A	1
	09/23/2003	7	Tray	A	2
2	12/18/2003	10	Blood	B	3
	12/12/2003	11	Urine	B	3
3	12/15/2003	12	Tray	C	4
	12/15/2003	13	Blood	C	4
	12/15/2003	14	Urine	C	4
4	01/06/2004	15	Tray	C	4
	01/06/2004	17	Blood	D	5
	01/06/2004	18	Urine	D	5
	01/06/2004	19	Catheter	E	6
5	05/31/2004	23	Blood	F	7
	05/17/2004	24	Urine	F'	8
	05/31/2004	25	Bed	F	7
6	04/05/2004	30	Catheter	G	9
	04/05/2004	31	Blood	G	9
	04/05/2004	32	Bed	E	6
7	08/11/2004	34	Tray	B'	10
	08/18/2004	35	Blood	H	11
	08/04/2004	36	Urine	H	11
8	10/29/2004	37	Blood	I	12
	11/19/2004	38	Urine	I	12
	10/29/2004	39	Catheter	J	12
	10/29/2004	40	Bed	I	12
9	11/10/2004	42	Catheter	E	6
	11/10/2004	43	Blood	K	13
	11/10/2004	44	Urine	K	13
10	05/04/2005	45	Blood	L	14
	05/04/2005	46	Urine	L	14

RAPD analysis

The primers named Cnd3 (5´-CCAGATGCAC-3`) and Cnd4 (5'-ACGGTACACT-3`) were used as single primers in the PCR. Amplification reactions as described by Ergon and Gülay (9) were performed in volumes of 25μL including about 25ng of the DNA template, 10mM Tris-HCl, pH 8.3, 50mM KCl, 1.5mM MgCl, 0.2mM of each of the dATP, dCTP, dGTP and dTTP, 30ng of primer and 2.5U Taq DNA polymerase (Invitrogen). Amplification was performed in a PCR MJ Research Thermal Cycler model PTC-100 ™ programmed for denaturation at 94°C for 3min; 45 cycles of 1min at 94°C, 1min at 36°C and 2min at 72°C, and an extension at 72°C for 7min. Amplification products were separated by electrophoresis in 1.2% agarose gel containing 1x tris-borate-EDTA (TBE) buffer, stained with ethidium bromide at 0.5μg/mL and visualized under UV light.

PCR profile analysis

The banding profiles for each isolate were compared visually. Bands were recorded as present (1) or absent (0). Simple matching´s similarity coefficient (SM) values for each pair-wise comparison between isolates were calculated. A SM value of 1.00 represented the same genotype, SM values between 0.80 and 0.99 represented clonally related isolates and SM under 0.80 represented distinct strains (9). Clonally related isolates are presented as of the same pattern added by apostrophe (e.g. A' and A" for Cnd3 and 1' and 1" for Cnd4).

RESULTS

A total of 31 *C. albicans* isolates (10 of blood, 9 of urine, 5 of catheter tip, 4 of tray and 3 of bed) were submitted to molecular typing using the RAPD method. Both primers (Cnd3 and Cnd4) used in this study were successful in eliciting banding profiles for each isolate. Amplifications obtained products that were specific to each primer and ranged from 3 to 7 bands with fragment size of approximately 200bp to 1800bp for Cnd3 and from 3 to 6 bands with fragment size of approximately 300bp to a 3500bp for Cnd4. Most of the major bands were present in all the isolates studied and almost all the strains had a conserved fragment ~ 1800bp for Cnd3 and ~ 600bp for Cnd4 (Figures 1 and 2).

Both primers had high discriminatory power. Among 31 *C. albicans* isolates, 14 patterns were detected with both primers (Table I). The similarity coefficients for Cnd3, between profiles A-L showed that some strains had closely related patterns. For example, the isolate 7 (A') had high similarity with the isolates 4, 5 and 6 (A), the isolate 24 (F') with isolates 23 and 25 (F) and the isolate 34 (B') with the isolates 10 and 11 (B). The similarity coefficients for Cnd4 showed that the strains described in each profile (1-14) had the same genotype (Table 1).

Reproducibility of RAPD patterns was assessed by repetition of RAPD analysis on the type strains in at least two experiments [no differences were found within each type strain profile (data not shown)].

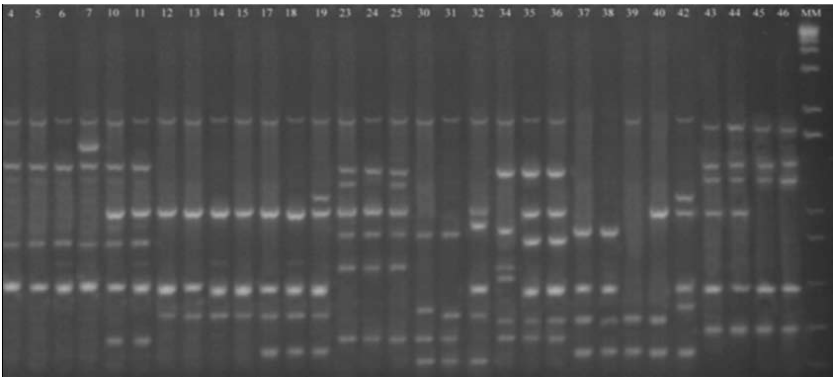

Figure 1. RAPD Profiles of 31 *Candida albicans* isolates obtained with primer Cnd3. Profile A (lines 1-3: catheter tip, blood and urine related to patient 1); profile A´(line 4: tray related to patient 1); profile B (lines 5, 6: blood and urine related to patient 2); profile C (lines 7, 8, 9 and 10: tray, blood and urine related to patient 3 and tray related to patient 4); profile D (lines 11, 12 blood and urine related to patient 4); profile E (lines 13 ,19, 27: catheter related to patient 4, 19 bed related to patient 6, 27 catheter related to patient 9, respectively); profile F (lines 14, 16: blood and bed related to patient 5); profile F' (line 15: urine related to patient 5); profile G (lines 17, 18: catheter and blood related to patient 6); profile H (lines 21, 22: blood and urine related to patient 7); profile I (lines 23, 24, 26: blood, urine and bed related to patient 8); profile J (line 25: catheter related to patient 8); profile K (lines 28, 29: blood and urine related to patient 9), and profile L (lines 30, 31: blood and urine related to patient 10).

RAPD profiles from both blood and urine from the same patient were identical in almost all samples studied, except for patient 5, where the two clinical samples were different by using the two primers. However, it was observed that the isolate from the bed of this patient (5) had the same genotype as the one isolated from his blood.

Interesting facts were verified with isolates 12 (surface of tray of the patient 3) and 40 (bed of patient 8) of *C. albicans* that showed the same genotype from blood and urine from these patients and, with isolates 19 (catheter of the patient 4), 32 (bed of the patient 6) and 42 (catheter of the patient 9) which had the same genotype.

The analysis between the two primers showed highly similar RAPD patterns among different isolates. Exceptions occurred between tray isolate from patient 1, urine isolate from patient 5, tray isolate from patient 7 (SM between 0,80 and 0,99 with Cnd3 and under 0,80 with Cnd4) and also with the catheter tip isolate from patient 8 (distinct strain with Cnd3 and same genotype of blood and urine of this patient with Cnd4).

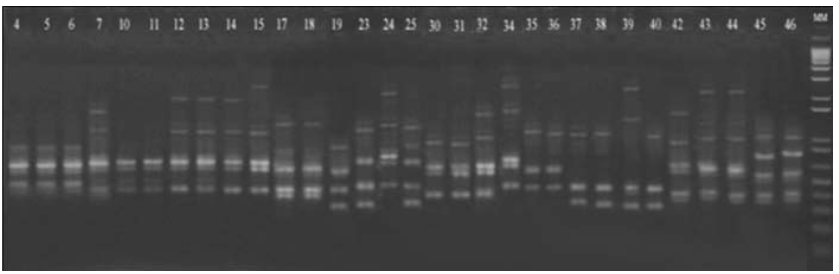

Figure 2. RAPD Profiles of 31 *Candida albicans* isolates obtained with primer Cnd4. Profile 1 (lines 1, 2, 3: catheter tip, blood and urine related to patient 1), profile 2 (line 4: Tray related to patient 1); profile 3 (lines 5, 6: blood and urine related to patient 2); profile 4 (lines 7, 8, 9 and 10: Tray, blood and urine related to patient 3 and Tray related to patient 4); profile 5 (lines 11, 12: blood and urine related to patient 4), profile 6 (line 13, 19, 27: catheter related to patient 4, bed related to patient 6; catheter related to patient 9, respectively); profile 7 (lines 14, 16: blood and bed related to patient 5), profile 8 (line 15: urine related to patient 5); profile 9 (lines 17, 18: catheter and blood related to patient 6); profile 10 (line 20: Tray related to patient 7); profile 11 (lines 21, 22: blood and urine related to patient 7); profile 12 (lines 23, 24, 25, 26 blood, urine, catheter and bed related to patient 8); profile 13 (lines 28, 29: blood and urine related to patient 9); and profile 14 (lines 30, 31: blood and urine related to patient 10).

DISCUSSION

This is the first time that a molecular epidemiology *Candida* bloodstream infection (BSI) research in the population of Goias, Brazil, has been done. Although nosocomial candidemia constitutes a growing issue (19) associated with significant mortality among critically ill patients, it is quite difficult to achieve a precise understanding of its epidemiology. Colonization precedes candidemia and it is considered to be an important risk factor in endogenous infections (8). Tortorano et al. (21), showed a previous colonization of the alimentary tract by the same *Candida* species causing fungaemia. Candidemia has also been related to previous colonization of the urinary tract (3). Urinary tract colonization deserves

consideration because it is a common event in hospitalized patients affecting 6.5-20% of the patients (13). In this study, molecular typing demonstrated that the paired isolates from blood and urine were identical for 93% patients with *C. albicans*, suggesting an endogenous origin of candidemia in these cases.

Although less prevalent than endogenous infections, it is well known that exogenous sources of *C. albicans* may be involved in the development of nosocomial candidiases (17). In this study, it was found that strains of *C. albicans* isolated from the blood of patients 5 and 8 were identical to the isolated from the surface of their beds. Besides, identical strains were isolated from the blood and surface of tray of the patient 3 (Table 1). These samples from the environment (surfaces of bed and tray) were collected on the same day that the blood collection of patients with candidemia was. Robert et al. (16), characterized strains of *C. albicans* colonized on admission as identical to other patient that was culture negative on admission and acquired the yeast after 25 days, suggesting cross infection. The epidemiology of nosocomial *C. albicans* isolates infection is complex and the mechanism by which the patients in our study acquired their strains remains not totally clear. However, the finding of three isolates from the blood of patients with the same molecular profile as the ones recovered from the environment isolates suggests that the candidemia could be resulted from an exogenous source.

Exogenous acquisition of candidemia is also postulated to be associated with intravascular devices and parenteral nutrition. The candidemia related to catheter has been suggested by some researchers (19, 20). Studies of Almirante et al. (1) showed a high relevance of catheter related sources in cases of candidemia described in Barcelona, Spain, 2002 to 2003. In our study the isolates from catheter tip and blood of patient 6 had the same genotype, suggesting that in these cases the portal of entry of *C. albicans* was via catheter. Besides, interestingly, molecular typing demonstrated that isolates of bed from patient 6 (isolate 32) and of catheter from patients 4 and 9 (isolates 19 and 42) had also the same genotypes. It is possible that *C. albicans* strains recovered in catheter tip or surface of bed had been carried by the hands of hospital coworkers. Analyses performed from hospital coworkers hands, indicated that lapses with hygiene practices may allow the transmission from hands to central venous catheter (5). In this study samples from the coworkers hands were not collected because this was not its aim.

RAPD–PCR, Southern blotting, PFGE, microsatellite multiplex PCR and MLST are methods recommended and commonly used to type microorganisms as *Candida*. The 14 different patterns presented by both primers, Cnd3 and Cnd4, confirm the discriminatory value of RAPD, which is considered a powerful genotyping method (18, 22). The RAPD assay using primers Cnd3 and Cnd4 may be an important tool to identify the intra-specific genetic variability among *C. albicans* isolates.

In conclusion, in this study, although candidemia was strongly associated to endogenous sources, such as candiduria, it was indicated that catheter, surface of bed and tray were probably important exogenous sources of infection.

ACKNOWLEDGMENTS

Financial support: Universidade Paulista

RESUMO

Análise epidemiológica molecular de *Candida albicans* da corrente sanguínea

Candidemia está geralmente relacionada à microbiota endógena, porém infecções exógenas originadas do *staff* ou do meio ambiente podem ocorrer. O método de DNA polimórfico amplificado aleatoriamente (RAPD) pode revelar variações específicas do isolado. Neste estudo, foi utilizada a análise de RAPD para avaliar a diversidade genética entre isolados de *C. albicans*, buscando-se encontrar similaridade entre os padrões de DNA dos isolados obtidos de amostras clínicas e ambientais da unidade de terapia intensiva (UTI) no Hospital das Clínicas da Universidade Federal de Goiás. Os *primers* denominados Cnd3 (5´-CCAGATGCAC-3`) e Cnd4 (5'-ACGGTACACT-3`) foram usados como *primer* na PCR. Perfis de RAPD do sangue e da urina, provenientes dos mesmos pacientes, mostraram-se idênticos em quase todas as amostras estudadas, exceto na de um paciente. O isolado da cama deste paciente tinha o mesmo genótipo da amostra obtida de seu sangue. Embora a maioria dos isolados de *C. albicans* provavelmente tivesse origem endógena, o encontro de isolados de pacientes com o mesmo perfil dos isolados do meio ambiente sugere que a candidemia pode resultar de uma fonte exógena.

DESCRITORES: *Candida albicans*. RAPD. Infecções nosocomiais.

REFERENCES

1. Almirante B, Rodriguez D, Park BJ, Estrella MC, Planes AM, Almela M, Mensa J, Sanchez F, Ayats J, Gimenez M, Saballs P, Fridkin SK, Morgan J, Rodriguez-Tudela JL, Warnock DW, Pahissa A. and Barcelona Candidemia Project Study Group. Epidemiology and predictors of mortality in cases of *Candida* bloodstream infection: results from population-based surveillance, Barcelona, Spain, from 2002 to 2003. *J Clin Microbiol 43*: 1829-1835, 2005.
2. Andrighetto C, Psomas E, Tzanetakis N, Suzzi G, Lombardi A. Randomly amplified polymorphic DNA (RAPD) PCR for the identification of yeasts isolated from dairy products. *Lett Appl Microbiol 30*: 5-9, 2000.
3. Binelli CA, Moretti ML, Assi RS, Sauaia N, Meneses PR, Ribeiro E, Geiger DCP, Mikami Y, Miyaii M, Oliveira MS, Barone AA, Levin AS. Investigation of the possible association between nosocomial candiduria and candidaemia. *Eur Clin Microbiol Infect Dis 12*: 538-543, 2006.
4. Casali AK, Goulart L, Silva LKR, Ribeiro AM, Amaral AA, Alves SH, Schrank A, Meyer W, Vainstein MH. Molecular typing of clinical and environmental *Cryptococcus neoformans* isolates in the Brazilian state Rio Grande do Sul. *FEMS Yeast Res 33*: 405-415, 2003.
5. Clark TA, Slavinski SA, Morgan J, Lott T, Arthingtons-Skaggs BA, Brandt ME, Webb RM, Currier M, Flowers R, Fridkin SK, Hajjeh RA. Epidemiologic and molecular characterization of an outbreak of *Candida parapsilosis* bloodstream infections in a community hospital. *J Clin Microbiol 42*: 4468-4472, 2004.

6. Dassanayake RS, Samaranayake YH, Samaranayake LP. Genomic diversity of oral *Candida krusei* isolates as revealed by DNA fingerprinting and electrophoretic karyotyping. *Acta Pathol Microbiol Immun Scand 108*: 697-704, 2000.
7. Del Poeta M, Toffaletti DL, Rude TH, Dykstra CC, Heitman J, Perfect JR. Topoisomerase I is essential in *Cryptococcus neoformans*: role in pathology and as an antifungal target. *Genetics 152*: 176-178, 1999.
8. Eggimann P, Garbino J, Pitter D. Epidemiology of *Candida* species infections in critically ill non-immunosuppressed patients. *Lancet Infect Dis 3*: 685-702, 2003.
9. Ergon MC, Gülay Z. Molecular epidemiology of *Candida* species isolated from urine at an intensive care unit. *Mycoses 48*: 126-131, 2005.
10. Giammanco GM, Lopes MM, Coimbra RS, Pignato S, Grimont PAD, Grimont F, Freitas G, Giammanco G. Value of morphotyping for the characterization of *Candida albicans* clinical isolates. *Mem Inst Oswaldo Cruz 100*: 483-490, 2005.
11. Mehta SK, Stevens DA; Mishra S, Feroze F, Pierson DL. Distribution of *Candida albicans* genotypes among family members -demonstration by sequential DNA fingerprinting with probes Ca3, C1 and CARE2. *Diagn Microbiol Infect Dis 34*: 19-25, 1999.
12. Meunier F, Aoun M, Bitar. Candidemia in immunocompromised patients. *Clin Infect Dis 14*: 120-125, 1992.
13. Pagano L, Antinori A, Ammassari A, Mele L, Nosari A, Melillo L, Martino B, Sanguinetti M, Quitani F, Nobile F, Cartotenuto M, Morra E, Morace G, Leone G. Retrospective study of candidaemia in patients with hematological malignancies. *Eur J Haematol 63*: 77-85, 1999.
14. Pfaller MA. Epidemiology of Nosocomial candidiasis: the importance of molecular typing. *Brazilian J Infect Dis 4*: 161-167, 2000.
15. Pinto PM, Resende MA, Koga-Ito CY, Tendler M. Genetic variability analysis among clinical *Candida* spp. isolates using random amplified polymorphic DNA. *Mem Inst Oswaldo Cruz 99*: 147-152, 2004.
16. Robert F, Lebreton F, Bougnoux ME, Paugam A, Wassermann D, Schlotterer M, Tourte-Schaefer C, Dupouy-Camet J. Use of random amplified polymorphic DNA as a typing method for *Candida albicans* in epidemiological surveillance of a burn unit. *J Clin Microbiol 33*: 2366-2371, 1995.
17. Schonian G, Meusel O, Tietz HJ, Meyer W, Graser Y, Tausch I, Presber W, Mitchell TG. Identification of clinical strains of *Candida albicans* by DNA fingerprinting with the polymerase chain reaction. *Mycoses 36*: 171-179, 1993.
18. Shin JH, Og YG, Cho D, Kee SJ, Shin MG, Suh SP, Ryang DW. Molecular epidemiological analysis of bloodstream isolates of *Candida albicans* from a University Hospital over a Five-Year Period. *J Microbiol 43*: 546-554, 2005.
19. Shin JH, Park MR, Song JW, Shin DH, Jung SI, Cho K, Kee SJ, Shin MG, Suh SP, Ryang DW. Microevolution of *Candida albicans* strains during catheter-related candidemia. *J Clin Microbiol 42*: 4025-4031, 2004.
20. Torturano AM, Kibbler C, Peman J, Bernhardt H, Klingspor L, Grillot R. Candidaemia in Europe: epidemiology and resistance. *Int J Antimicrob Agents 27*: 359-366, 2006.
21. Tortorano AM, Peman J, Bernhardt H, Klingspor L, Kibbler CC, Faure O, Biraghi E, Canton E, Zimmermann K, Seaton S, Grillot R. Epidemiology of candidaemia in Europe: results of 28-month European Confederation of Medical Mycology (ECMM) hospital-based surveillance study. *Eur J Clin Microbiol Infect Dis 23*: 317-322, 2004.
22. Vrioni G, Matsiota-Bernard P. Molecular typing of *Candida* isolates from patients hospitalized in an intensive care unit. *J Infect 42*: 50-56, 2001.