Epidemiol. Infect. (1994), **112**, 393–398

A study of a hospital cluster of systemic candidosis using DNA typing methods

F. ROMANO[1], G. RIBERA[2] AND M. GIULIANO[2]

[1] *Institute of Hygiene, Medical School, University of Chieti 'G. D'Annunzio', Via dei Vestini, 66013 Chieti Scalo, Italy*

[2] *Institute of Hygiene and Preventive Medicine, Medical School, University of Naples 'Federico II', Italy*

(*Accepted 18 October 1993*)

SUMMARY

A cluster of disseminated *Candida albicans* infections, which occurred at the Intensive Care Unit of the Department of Heart Surgery, was investigated. Ten patients became infected and seven died. A wide microbiological surveillance was carried out. A total of 14 isolates of *Candida albicans*, four environmental and ten human, were examined using the Restriction Endonuclease Analysis (REA) of DNA. The isolates were classified into five different main groups. Five of the clinical isolates had the predominant pattern Ab and two more clinical strains were very closely related. Two more isolates from the emergency kit desk and the hands of a nurse gave the same REA profile. Such a relationship proved the epidemic nature of the cluster, with most of the patients cross-infected, and strongly suggested transmission on the hands of the staff as a determinant of the epidemic. Thus, REA has the potential to address many important questions in the study of nosocomial epidemiology of *Candida albicans*.

INTRODUCTION

Candida albicans is an ubiquitous human pathogen, causing localized, invasive or disseminated disease in normal or immunocompromised hosts, promoted by such common factors as invasive procedures, catheters, immunosuppressive therapy, malignancy and broad spectrum antimicrobial agents. Invasive candidosis is usually due to autoinfection by yeasts colonizing the gastrointestinal tract, representing a potential gateway, particularly when antibiotics have eliminated bacterial competition. This concept has recently been challenged by the description of nosocomial systemic candidosis [1–4]. Systemic candidosis has a mortality of approximately 70% even when treated with amphotericin B, the drug of choice [2]. It is thus important to identify outbreaks of disseminated candidosis as these can be curtailed by stringent cross-infection measures. The key to proving cross-infection lies in the ability to type yeast isolates. This can only be done by a reproducible typing system which has adequate discriminative power. It must also be able to deliver the answer within a timescale of value to clinicians [2]. Phenotypic schemes for typing *Candida albicans* have been

extensively used, but recent advances in molecular biology now provide better methods for strain delineation for epidemiologic purposes [4–6].

In this paper we used the Restriction Endonuclease Analysis (REA) to investigate a cluster of disseminated *Candida albicans* infections.

MATERIALS AND METHODS

The outbreak

Over a 2-week period in April 1992 ten patients became infected with *Candida albicans* on the Intensive Care Unit of the Department of Heart Surgery of the Medical School of the University of Naples 'Federico II' (Table 1). Seven patients died of systemic candidosis despite treatment with amphotericin B.

The ICU was temporarily closed to new admissions and then re-opened after thorough cleaning; handwashing was changed to fungicidal agents; an intensive educational programme on hospital infection control was started.

Sources of Candida albicans *isolates*

Clinical isolates were obtained from diagnostic and surveillance cultures. Blood cultures were taken from systemically ill patients, while all the patients were screened with urine samples, mouth, nasal and pharyngeal swabs, and endotracheal tube aspirates as appropriate. These were cultured on Sabourauds glucose agar (Difco, Detroit, Michigan) at 37 °C overnight and confirmed by the germ-tube test and the API 20C system (Bio-Merieux, Lyon, France).

A wide environmental surveillance was carried out at the Unit. Air and surfaces, including beds and linens, were sampled. Mouth, nasal, pharyngeal and hand swabs were taken from all the members of the staff (four doctors and eight nurses). Doctors' and nurses' clothing was also sampled.

Isolation of total genomic DNA

Colonies of *Candida albicans* were cultured at 37 °C overnight in 5 ml of YPD broth (1% yeast extract, 2% peptone, 2% dextrose). Packed cells from 1·5 ml of medium were resuspended in 1 M sorbitol and recentrifuged. Spheroplasts were prepared, and whole cell DNA was isolated according to the method described by Scherer and Stevens [5].

Restriction endonuclease analysis (REA)

Whole cell DNA was digested with 50 units of *Eco*RI (Boehringer Mannheim, Germany) in 40 μl of the reaction mixture (50 mM-NaCl, 100 mM Tris hydrochloride, 10 mM $MgCl_2$, pH 7·5) at 37 °C for 3 h. Electrophoresis was performed in a 0·8% agarose gel at 100 V for 4 h. The gels were photographed under 254 nm UV light through an orange filter on Polaroid 57 film.

RESULTS

A total of 14 strains, 4 environmental and 10 human, of *Candida albicans* were examined. Among the 4 environmental strains 2 were isolated from the floor of the Intensive Care Unit; 1 from the side-wall of the elevator leading to the Unit; the

Table 1. *Details of infected patients on the Intensive Care Unit of the Department of Heart Surgery of the Medical School of the University of Naples 'Federico II'*

Patient	Date of admission to ICU	Underlying diagnosis	Date of isolation	Sites of isolation	Outcome
1	27/3	Coronary artery by-pass	3/4	Blood	Died
2	31/3	Aortic dissection	3/4	Endotracheal tube aspirate and blood	Survived
3	27/3	Mitral valve replacement	4/4	Blood	Died
4	30/3	Mitral valve replacement	4/4	Endotracheal tube aspirate	Died
5	1/4	Coronary artery by-pass	5/4	Blood	Died
6	3/4	Coronary artery by-pass	6/4	Blood	Survived
7	6/4	Mitral valve replacement	10/4	Blood	Died
8	7/4	Mitral valve replacement	13/4	Blood	Died
9*	30/3	Aortic dissection	6/4	Blood	Survived
10*	3/4	Mitral valve replacement	8/4	Blood	Died

* Isolates not available.

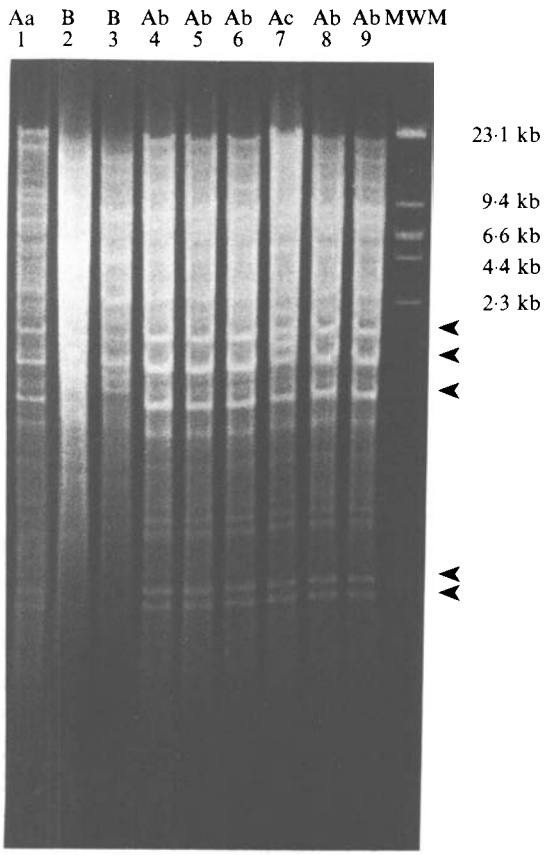

Fig. 1. REA patterns of clinical isolates. Phage λ digested with *Hin*dIII was used as molecular weight marker. The intensely stained bands that designate the main REA patterns are marked with arrows. Lanes: 1 from case 1; 2 and 3 from case 2; 4–9 from cases 3–8 respectively.

https://doi.org/10.1017/S0950268800057800 Published online by Cambridge University Press

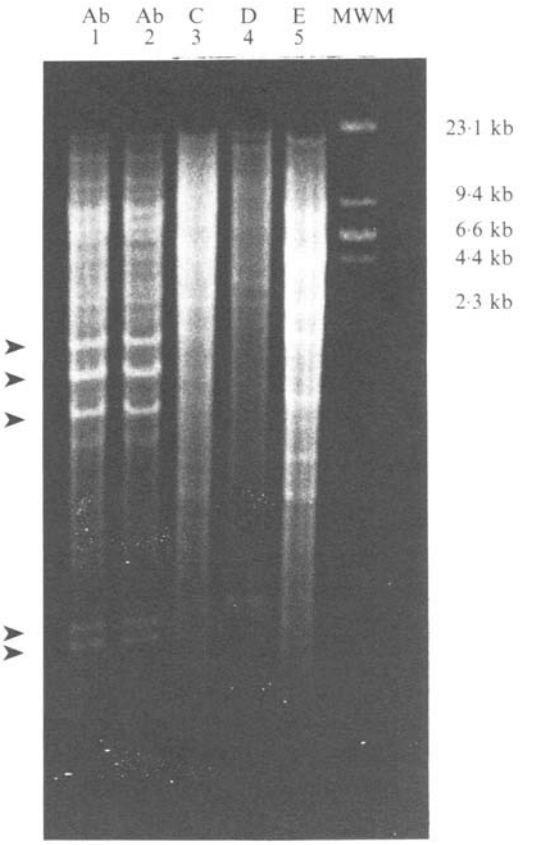

Fig. 2. REA patterns of the isolates from the staff (lane 1) and the environment (lanes 2–5). Phage λ digested with *Hin*dIII was used as molecular weight marker. The intensely stained bands that designate the main REA patterns are marked with arrows. Lanes: 1 from nurse hands; 2 from emergency kit desk; 3 and 4 from the floor; 5 from the side-wall of the elevator.

last 1 from the emergency kit desk. One of the nursing staff, appointed to the emergency kit desk, was found to be a hand carrier of *Candida albicans*. Eight out of 10 patients with *Candida albicans* supplied the remaining 9 strains. Isolates from two patients were not available from the diagnostic laboratory. All the strains were classified into five different main groups, designated with capital letters (A–E), according to their REA pattern (Figs 1 and 2). Two clinical strains (Fig. 1, lanes 2–3) came from different body sites of patient 2 and were indistinguishable on REA profile (designated pattern B). Five clinical isolates (Fig. 1, lanes 4, 5, 6, 8, 9) from different patients had the predominant fingerprint pattern (designated pattern Ab). Two strains (lanes 1 and 7) had distinct fingerprint patterns, but were very closely related to the REA A profile (assigned the letters a and c). Figure 2 shows that isolates from the emergency kit desk and the nurse's hands gave the predominant pattern (designated Ab). The REA profiles of the remaining environmental isolates (designated pattern C through E) are shown. Table 2 indicates the distinct REA patterns and their distribution among isolates.

https://doi.org/10.1017/S0950268800057800 Published online by Cambridge University Press

Table 2. *Detail of the isolates from the Intensive Care Unit of the Heart Surgery Department*

Source of isolate	Site of isolate	REA profile
Patient 1	Blood	Aa
Patient 2	Endotracheal tube aspirate	B
Patient 2	Blood	B
Patient 3	Blood	Ab
Patient 4	Endotracheal tube aspirate	Ab
Patient 5	Blood	Ab
Patient 6	Blood	Ac
Patient 7	Blood	Ab
Patient 8	Blood	Ab
Nurse	Hands	Ab
Environment	Emergency kit desk	Ab
	Floor	C
	Floor	D
	Elevator	E

DISCUSSION

A DNA typing technique has been used to address a fundamental question in nosocomial epidemiology of *Candida albicans*: coincidence or cross-infection of a cluster of cases.

In our study nearly all isolates from patients showed the same or a closely related restriction fragment pattern, designated REA profile Ab, demonstrating a clear epidemic cluster of infections due to *Candida albicans*. It was also possible to prove hand carriage of the epidemic strain. The cycle of infection thus appeared to involve transmission on the hands of staff and presumably the subsequent contamination of the emergency kit desk. Patient 2 was not infected with the epidemic strain, and could represent expression of the baseline rate of auto-infection by *Candida albicans*.

Conventional typing methods such as biotyping with API-type systems, serology or serotyping and phage typing are usually adequate for the identification purposes required by the diagnostic laboratory. However, there are occasions when these techniques are not sufficiently discriminatory for the investigation of suspected outbreaks of infection, or for the detection of a particular strain [7].

In a review of typing methods for *Candida albicans* Warnock [8] listed three desirable criteria of a typing method: that it should differentiate a sufficient number of strains, that it should give clear and reproducible results, and that it should not be too difficult to perform. The most recent reports strongly confirm that phenotypic characteristics may not be relied on absolutely to infer identity or non-identity of isolates in epidemiologic studies. Such a non-correlation is particularly marked when applied to the hospital clusters of infection [9, 10]. This underlines the importance of typing yeast isolates to differentiate between a cluster of yeast infections occurring as a coincidence and a true outbreak, which is not a matter of chemoprophylaxis strategy, but raises fundamental issues of cross-infection policies to bring outbreaks under control.

Many reports currently indicate that molecular biology techniques may be

applied to problems of candida epidemiology or taxonomy [11, 12]. Magee and co-workers [13], Scherer and Stevens [5], Matthews and Burnie [10], Lee and colleagues [3], analysed successfully restriction fragments of DNA of *Candida* spp. to distinguish the various isolates. The potential of using mitochondrial DNA fingerprinting [6, 14], electrophoretic karyotype analysis [14], species-specific DNA probes [4, 6, 15, 16], are still under evaluation. The goal of all these efforts is to develop a powerful epidemiological tool to be used to track *Candida albicans* within a host, between hosts, or between hosts and inanimate objects (or *vice versa*).

Our study shows that REA is currently a good approach to study nosocomial epidemiology of *Candida albicans*. Indeed it can provide a highly sensitive and widely applicable method of delineating isolates. It can be completed within no more than 24 h and the results can therefore be available in time for appropriate infection control measures to be carried out. The technique, even though it requires some additional equipment, is within the capability of experienced routine diagnostic laboratories.

It is evident that these methods have the potential to address many questions such as individual colonization, transmission, recurrent diseases (e.g. vaginitis) and epidemiology in selected disease categories or the environment.

REFERENCES

1. Burnie JP, Odds FC, Lee W, Webster C, Williams JD. Outbreak of systemic *Candida albicans* in intensive care unit caused by cross-infection. BMJ 1985; **290**: 746–8.
2. Burnie JP, Matthews R, Lee W *et al.* Four outbreaks of nosocomial systemic candidiasis. Epidemiol Infect 1987; **99**: 201–11.
3. Lee W, Burnie JP, Matthews R, Oppenheim BO, Damani N. Hospital outbreaks with yeasts. J Hosp Infect 1991; **18**: 237–49.
4. Fox BC, Mobley HLT, Wade JC. The use of a DNA probe for epidemiological studies of candidiasis in immunocompromised hosts. J Infect Dis 1989; **159**: 488–94.
5. Scherer S, Stevens DA. Application of DNA typing methods to epidemiology and taxonomy of *Candida* species. J Clin Microbiol 1987; **25**: 675–9.
6. Merz WG. *Candida albicans* strain delineation. Microbiol Rev 1990; **54**: 321–34.
7. Jordens JZ. Restriction enzyme analysis of chromosomal DNA and its application in epidemiological studies. J Hosp Infect 1991; **18** Suppl. A: 432–7.
8. Warnock DW. Typing of *Candida albicans*. J Hosp Infect 1984; **5**: 244–52.
9. Stevens DA, Odds FC, Scherer S. Application of DNA typing methods to *Candida albicans* epidemiology and correlations with phenotype. Rev Infect Dis 1990; **12**: 258–66.
10. Matthews RC, Burnie JP. Assessment of DNA fingerprinting for rapid identification of outbreaks of systemic candidiasis. BMJ 1989; **298**: 354–7.
11. Scherer S, Magee PT. Genetics of *Candida albicans*. Microbiol Rev 1990; **54**: 226–41.
12. Pfaller MA. The use of molecular techniques for epidemiologic typing of *Candida* species. Curr Top Med Mycol 1992; **4**: 43–63.
13. Magee BB, D'Souza TM, Magee PT. Strain and species identification by restriction fragment length polymorphisms in the ribosomal DNA repeat of *Candida* species. J Bacteriol 1987; **169**: 1639–43.
14. Carruba G, Pontieri E, De Bernardis F, Martino P, Cassone A. DNA fingerprinting and electrophoretic karyotype of environmental and clinical isolates of *Candida parapsilosis*. J Clin Microbiol 1991; **29**: 916–22.
15. Oren I, Manavathu EK, Lerner SA. Isolation and characterization of a species-specific DNA probe for *Candida albicans*. Nucleic Acids Res 1991; **19**: 7113–6.
16. Wilkinson BM, Morris L, Adams DJ, Evans EG, Lacey CJ, Walmsey RM. A new, sensitive polynucleotide probe for distinguishing *Candida albicans* strains and its use with a computer assisted archiving and pattern comparison system. J Med Vet Mycol 1992: **30**: 123–31.