Candida DNA typing in burn care unit

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Different *Candida* species isolates were obtained both from 40 patients with burns, ranging from 10%–90% total body surface area (TBSA), and from the surrounding environment of burns and plastic surgery ward. *Candida* species isolated from eight patients with burns showed 4 isolates of *C. parapsilosis* followed by 3 isolates of *C. albicans* and 1 isolate of *C. tropicalis*. Sixteen isolates from the environment around the ward for patients with burns showed 3 isolates of *C. parapsilosis*, 1 isolate of *C. albicans*, 6 isolates of *C. krusei*, 3 isolates of *C. tropicalis*, 2 isolates of *C. guillermondii* and 1 isolate of *C. pseudotropicalis*. These isolates were characterized by DNA restriction pattern to identify the source of infection. From the DNA restriction pattern, it was observed that, in two separate periods the *C. albicans* and *C. parapsilosis* isolates of dressing room tap may be the source of infection in patients with burns. A strain of *C. parapsilosis* was isolated from four patients simultaneously, indicating cross infection with the single strain among these patients. Similarly, the patient's attendant harbouring *C. tropicalis* strain may have been responsible for *Candida* infection in one of the patients.

THE incidence of burn wound sepsis is very high in the Indian population due to poor socio-economic conditions and poor standards of personal hygiene coupled with the hot moist climatic conditions prevailing in most parts of India. All these factors encourage microbial growth[1]. Disseminated fungal infections have represented an increasingly frequent cause of septic death in many areas of medicine. The incidence of yeast infection in patients with burns has steadily increased, and is caused by multiple factors[2,3]. This problem is aggravated by the widespread use of topical and systemic antibiotics which, while leading to better control of bacterial infection, predisposes the patient to the development of fungal infection. Thus the use of techniques such as hyperalimentation, which are of importance in burn management, adds another portal of entry for systemic infection[4].

The poor prognosis and the increasing frequency of *Candida* septicemia have modified therapeutic and prophylactic approaches. Diagnosis of *Candida*infection is hampered due to lack of reliable tests for the detection of soluble antigens or antibodies. Treatment is often unsuccessful because of the underlying condition of the patients. Although in most of the cases *Candida* infections are due to the endogenous flora in the patients, in some cases the infections have been caused by isolates of *Candida* coming from environment[5]. The contamination of infusions or the hands of the health care workers has been shown to represent potential exogenous source of *Candida* spp[6,7]. For cases related to the endogenous flora, risk for translocation of isolates from the gut can be minimized by the prescription of prophylactic antifungal agents or at least surveying the digestive flora. For infections related to exogenous contamination, reinforcement of sanitary measures is mandatory. Nosocomial infection cannot be completely avoided, and their occurrence in clusters always raises question of a common source[8,9]. Earlier reports indicated that the DNA typing methods demonstrated the involvement of four different *Candida* strains, cross infection with one *C. albicans* strain and one *C. parapsilosis* strain, and identity between some of the *Candida* strains from the patients and nurses of the burn care unit[10,11]. DNA typing methods have revealed a great potential for simple, reproducible and powerful discrimination of strains of *Candida* species[12], eventually demonstrating the extraordinary genetic diversity and heterogeneity of both clinical and environmental isolates. Therefore, we planned to study the *Candida* incidence in patients with burns, and possible source of infection from the burn care unit environment using DNA typing methods.

Forty patients with burns were studied over a six-month period. The clinical samples were collected at weekly intervals from these patients until their discharge from hospital (Figure 1). Swabs were also collected from the ward environment. For clinical specimens the swabs were collected from wounds of the patients with burns

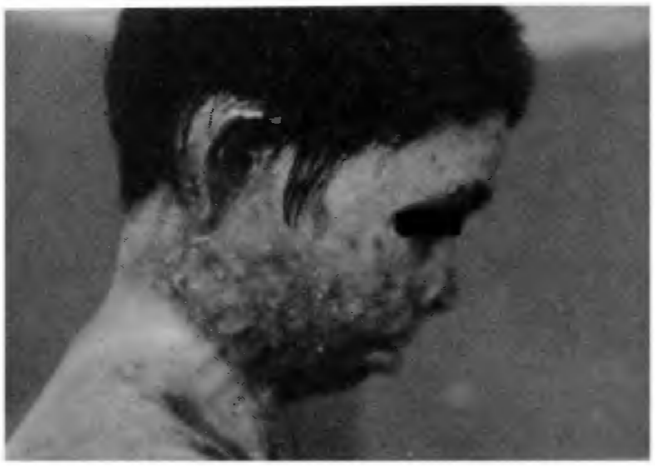

Figure 1. Patient with burns. Percentage of burn TBSA 60%.

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at the burns and plastic surgery ward, Madras Medical College Hospital, Madras. The specimens were inoculated on sabauraud glucose agar for the isolation of *Candida*. The isolated organisms were speciated by standard methods[13]. *Candida* isolates from patients' attendants and nurses were included in the study. The samples were also collected from inanimate objects like patient's bed, dressing room tap, and floor and walls of the burn ward.

For preparation of *Candida* genomic DNA, 5 ml tubes of YPD broth were inoculated with a loopful of stored cells and cultured at 37°C overnight. Packed cells from 1.5 ml of medium were prepared by centrifugation, mixed with 1 ml of 1 M sorbitol, and recentrifuged. The packed cells were suspended in 1 ml of a solution (pH 7.5) of 1 M sorbitol plus 50 mM potassium phosphate buffer with 0.2 mg of lyticase (Sigma, USA). This was incubated for 30 min at 30°C. The resultant spheroplasts were packed by centrifugation and suspended in 0.5 ml of 50 mM sodium EDTA buffer (pH 8.5) with 2 mg of sodium dodecyl sulphate per ml to which was added 0.003 ml of diethyl pyrocarbonate. This was mixed and then incubated at 70°C for 30 min. A 50 µl portion of 5 M potassium acetate was added followed by mixing and then incubation at 0°C for 30 min. The mixture was clarified by centrifugation, and the supernatant was decanted into 1 ml of ethanol and mixed. This was then clarified by centrifugation and the supernatant was discarded. To the precipitate was added several volumes of 70% ethanol, and subsequently the ethanol was decanted. The precipitate was dried and then suspended in 0.1 ml of 10 mM Tris chloride buffer (pH 7.5)–1 mM EDTA (TE solution) containing 10 µg of RNAase. 200 µl of 2-propyl alcohol was added, the mixture was clarified by centrifugation, and the supernatant decanted. An excess volume of ethanol was added to the precipitate, which was then decanted. The precipitate was dried and resuspended in 0.05 ml of TE solution. A 10 µl portion of the DNA sample prepared as described above was mixed with an equal volume of double-strength buffer (100 mM Tris chloride, pH 7.5, 20 mM $MgCl_2$, 200 mM NaCl). 0.1 µl of *Eco*RI restriction enzyme containing 50 units was added, and the mixture was incubated at 37°C (ref. 14).

Table 1. Occurrence of *Candida* species in burn patients

Sl no.	Patient	Isolates no.	Age/sex	% TBSA	Species
1	A	B1	17/F	90	*C. tropicalis*
2	B	B3	30/F	90	*C. albicans*
3	C	B5	35/F	60	*C. albicans*
4	D	B6	21/F	60	*C. albicans*
5	E	B13	27/F	50	*C. parapsilosis*
6	F	B14	30/F	40	*C. parapsilosis*
7	G	B15	30/F	40	*C. parapsilosis*
8	H	B17	5/F	30	*C. parapsilosis*

Digestion was stopped by heating the mixture to 70°C. For gel electrophoresis, gels of 0.8% agarose with a Tris acetate-EDTA buffer system were used. Electrophoresis was performed at 1 to 3 V/cm. For gel photography, 300-nm transillumination and Polaroid film was used.

The total number of 40 patients included patients with burns ranging from 10–90% total body surface area (TBSA). Among the 40 patients, 22 had 10–30% burns, 11 had 21–60%, and 7 had burns above 60% TBSA. The average hospital stay was maximum for patients with 21–60% of burns (57 ± 49 days) followed by patients with 10–30% burns (29 ± 18 days). Patients with more than 60% burns had a shorter average stay in hospital (9 ± 5 days) because of death from septicemia and hypovalemic shock.

Candida species were isolated from 8/40 patients (Table 1) and the species isolated were: *C. parapsilosis* (4 isolates), *C. albicans* (3 isolates), and *C. tropicalis* (1 isolate). A total of 16 environmental isolates of *Candida* species were obtained, of which 3 were *C. parapsilosis*, 1 was *C. albicans*, 6 were *C. krusei*, 3 were *C. tropicalis*, 2 were *C. guillermondii*, and 1 was *C. pseudotropicalis*. *Candida* species were isolated from 8/40 patients and 16/50 environmental swabs, and wherever the same species was isolated from the environment and clinical sample, DNA typing was carried out for those isolates.

The three clinical isolates of *C. albicans* (B3, B5, B6) and one environmental isolate of *C. albicans* showed similar DNA restriction pattern, using restriction enzyme *Eco*RI (Figures 2 and 3). A similar pattern was observed in the clinical isolate (B1) of *C. tropicalis* and environmental isolate (B2). A different pattern was observed in only one environmental isolate (B8) of *C. tropicalis*, that showed an extra band at 6.5 kb. The clinical and environmental isolates of *C. parapsilosis* isolate showed similar DNA restriction pattern. One environmental isolate (B23) of *C. parapsilosis* showed a different pattern. The DNA patterns of environmental isolates of *C. krusei*, *C. pseudotropicalis*, *C. guillermondii* are also shown in Figures 2 and 3. There were no clinical isolates of these three species from patients with burns.

Nosocomial infection of *Candida* in the burns unit is shown in Figure 4. One isolate of *C. tropicalis* was obtained from the patient A attendant in the 1st week of January and the same isolate was isolated from patient A subsequently. An isolate of *C. albicans* was obtained from the dressing room tap during the II week of January and similarly from the patient B a few days later. Both the patients died in the last week of January.

Patients C and D admitted in February were also infected with *C. albicans*, and all the three isolates of *C. albicans* were found to be similar to those from the dressing room tap. Patients E, F, G and H who were

admitted to the hospital during March and April were found to be infected with *C. parapsilosis* in May. These clinical isolates were analysed by DNA restriction pattern and they revealed similar DNA pattern with the isolate from dressing room tap.

Increasing frequency of systemic candidiasis outbreaks in patients with burns deserves more attention. Prevention and greater control of further epidemics depend on the increasing awareness of health care personnel[4,6]. When a case of systemic candidiasis occurs where endogenous source has been excluded, exogenous source should be clinically suspected and epidemiologically investigated

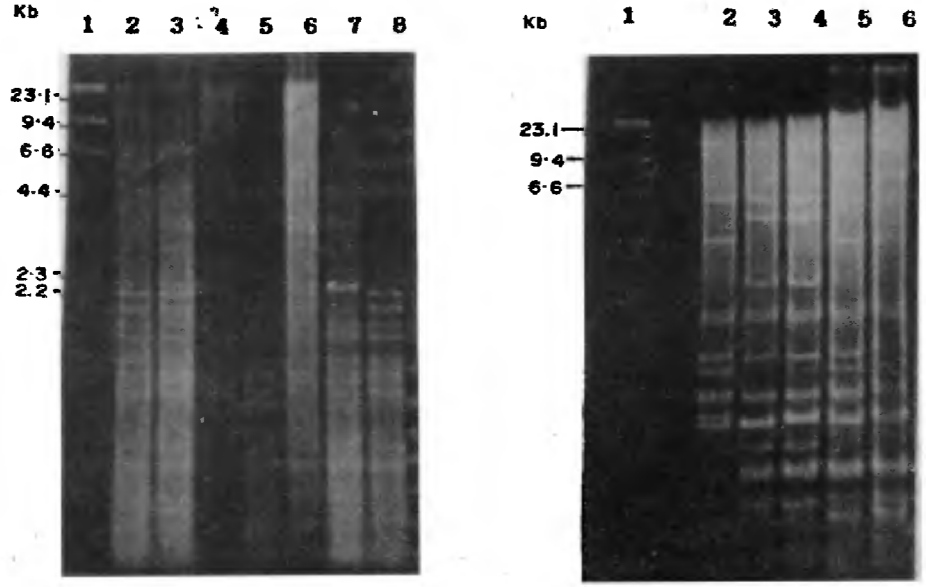

Figure 2. Gel electrophoresis of *Eco*RI restriction digests of DNA from *Candida* isolates from a burns unit.

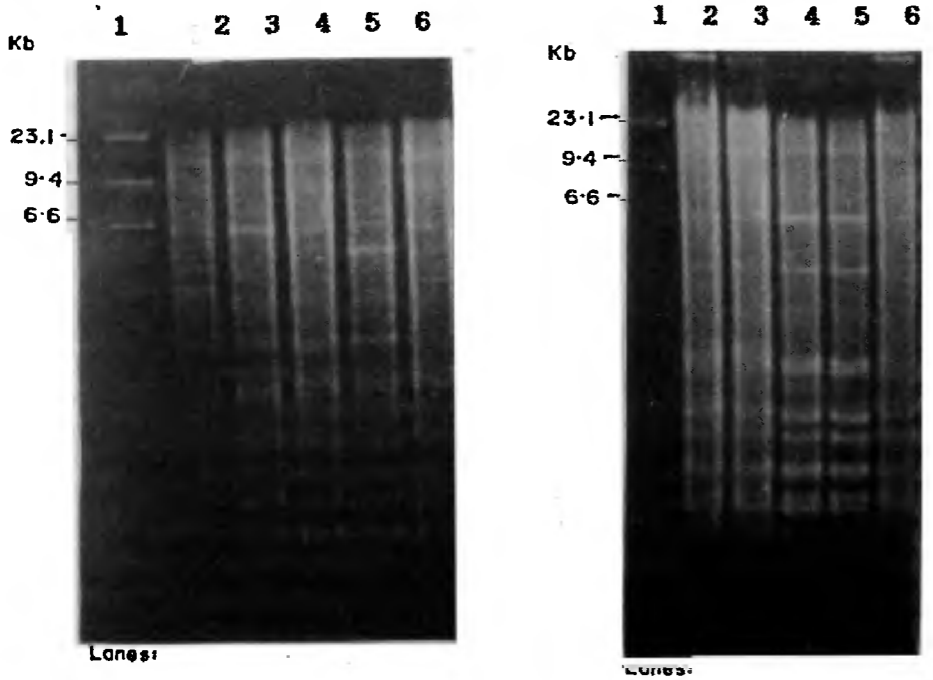

Figure 3. Gel electrophoresis of *Eco*RI restriction digests of DNA from *Candida* isolates from a burns unit.

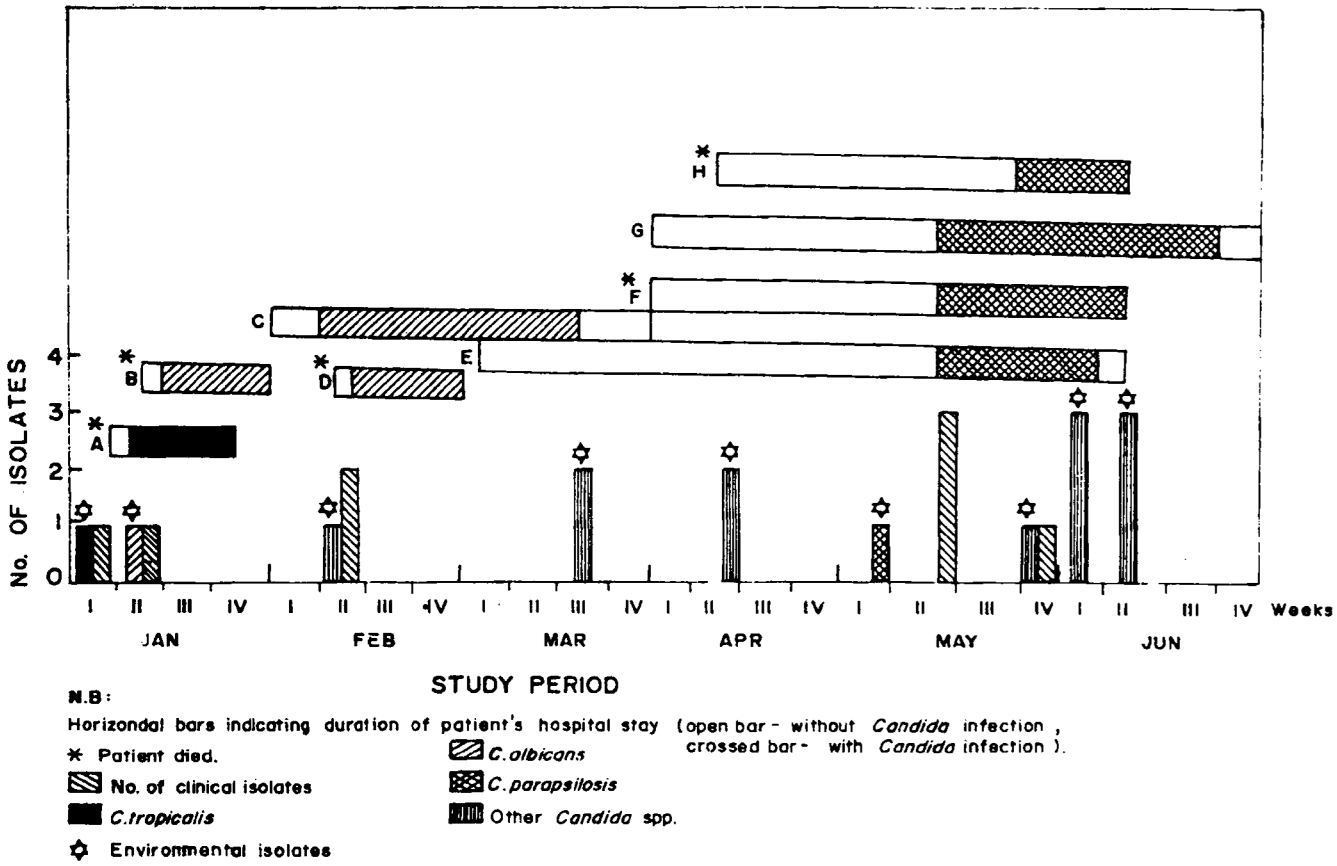

Figure 4. Nosocomial infection of *Candida* in the burns unit.

with particular respect to the hospital environment[15,16]. Given the high case fatality rate associated with fungemia, adherence to standard protocol for drug treatment and clean environment should get priority in each hospital.

Moro *et al*[17] described an outbreak of *C. albicans* systemic infection involving eight patients in an Italian geriatric hospital. They investigated the outbreak by the DNA restriction pattern method and showed that a single strain was responsible for infection in all the eight patients and a nurse was found to harbour the same strain. We obtained from the attendant of patient A an isolate of *C. tropicalis* during the 1st week of January. Subsequently, the same isolate was obtained from the patient A as well. Moreover we obtained isolates of *C. albicans* and *C. parapsilosis* from dressing room tap, and the similar DNA pattern strains were isolated from patients subsequently. The DNA pattern results revealed that a single isolate of *C. albicans* may have been responsible for infection in two cases, and a single isolate of *C. parapsilosis* may have been responsible for infection in four cases. Thus all the four patients had the *Candida* infection simultaneously with the same isolate of *C. parapsilosis*, indicating the possible source of cross infection among these patients. DNA analysis discriminated better than any of the other typing methods used, including serotyping[18] and biotyping[19]. Reproducibility was high and all isolates were typed within 48 h (with restriction enzyme *Eco*RI)[20]. Based on our experience and the aforementioned studies, DNA analysis provides a reliable means of delineating strains of clinical and environmental *Candida* isolates.

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Antitumour agent coralyne: A guanine-cytosine specific DNA-binding alkaloid

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The base and sequence specificity of coralyne–DNA interaction were investigated by spectrophotometric, spectrofluorimetric, circular dichroic, thermal melting and viscometric studies. The strong binding of this alkaloid to various natural and synthetic DNAs was characterized by remarkably large changes of hypochromism and bathochromism in the absorption bands, quenching of steady state fluorescence intensity, decrease in quantum yield, perturbations in circular dichroic spectrum, stabilization of DNA against thermal denaturation and increase in contour length of sonicated rod-like duplex DNA. Binding of coralyne to various DNAs was dependent upon the base pair composition and sequence of DNA, being larger for GC-rich DNA and alternating GC polymer as observed from the above techniques. These studies demonstrate the GC base pair specificity in the intercalative DNA binding of coralyne.

THE molecular mechanism of the sequence-specific recognition of DNA by small molecules has received considerable attention in the last several years. A detailed understanding of the various factors that govern the binding of small molecules to specific nucleotide sequences in DNA is essential for the rational design of new anticancer drugs for gene targeting[1,2]. Extensive studies are therefore currently directed to delineate the molecular mechanism, site and regiospecificity by which these molecules bind to DNA targets[3,4]. In this context, our laboratory has been studying the binding aspects of natural alkaloids to different DNA polymers[5–11]. Alkaloids are known to have important role in medicinal chemistry due to their extensive biological activity. Especially noteworthy is the isoquinoline group of alkaloids for their wide distribution in several botanical families with myriad therapeutic applications[12,13]. The most important members of the protoberberine group are berberine and its synthetic analogue coralyne. Both of them are reported to possess promising antitumour activities, with the latter having very low cytotoxicity[14–16]. Coralyne has attracted recent attention for its ability to inhibit the topoisomerase I function[17]. The topoisomerase I poisoning of several coralyne derivatives has been reported very recently where mixed modes of DNA interaction have been envisaged depending on the state of saturation in the ring system for stabilizing the enzyme–DNA–drug ternary complex[18]. Coralyne has been earlier reported to form a complex with duplex DNA by mechanism of intercalation[19,20], but the details of the nature and base specificity in its interaction still remains obscure. In order to understand the molecular nature of its specificity towards sequence of base pairs, we have carried out a series of physico-chemical measurements on the interaction of coralyne with several natural and synthetic DNAs of varying base composition and sequences.

Coralyne chloride (Figure 1) was obtained from Aldrich Chemical Co., St. Louis, MO, USA and its purity was checked by thin-layer chromatography and melting point determinations. We have estimated a molar extinction coefficient (ε) of 17,500 M^{-1} cm^{-1} at 424 nm for determining the concentration of coralyne in our solution conditions. The four natural DNAs, namely *Clostridium perfringens* (CP), calf thymus (CT), *Escherichia coli*

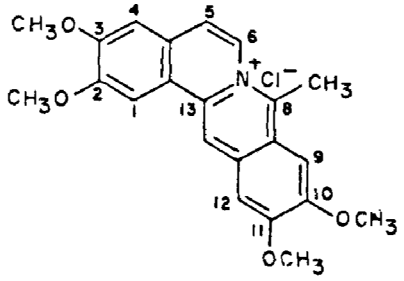

Figure 1. Chemical structure of coralyne chloride.

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