PULSED FIELD GEL ELECTROPHORESIS (PFGE) AS AN EPIDEMIOLOGICAL TYPING METHOD IN DETECTING NOSOCOMIAL OUTBREAKS OF CANDIDA PARAPSILOSIS

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SUMMARY

Nosocomial infections with Candida species have emerged as an increasingly important cause of mortality and morbidity in the intensive care units. We investigated apparent outbreaks of serious C. parapsilosis infection from intensive care units of two hospitals. C parapsilosis was also recovered from the hands of two nurses from one of the units. DNA subtyping was performed by using the novel technique of Pulsed-field gel electrophoresis (PFGE). The results of this study indicate genetic variation among the isolates and we were able to discriminate isolates that were indistinguishable by the conventional methods such as biotyping and antibiotic susceptibility typing. It revealed three genetically distinct groups of C. parapsilosis isolates. Electrophoretic karyotyping by PFGE appear to be more useful molecular tool for discriminating among strains of C. parapsilosis and will be a useful marker for evaluating the epidemiology of future C. parapsilosis infections.

INTRODUCTION

Nosocomial infections with Candida species are recognised as a significant cause of mortality and morbidity in both seriously ill and immuo-compromised patients.[1] Although C. albicans is considered the most prevalent species recovered from humans during the last 20 years, a mark shift in the spectrum of Candida infection has been observed among hospitalized patients.[2] In a greater population (35-45%) of patients with systemic candidiasis, other Candida species has replaced C. albicans as the etiological agent of infection. These include Candida parapsilosis, C. glabrata, C. tropicalis, C. krusei and C. lusitanie.[3]

Until recently, the development of invasive candidiasis was thought to be due to autoinfection by endogenous Candida strains which initially colonized the patient.[4] However, exogenous source of infection are being reported increasingly. These include cross-infection among intensive care unit patients, attributed to hand carriage among health care workers,[5] contaminated parenteral nutrition and blood pressure monitoring devices.[6]

In a outbreak, the study of epidemiological marker is important in order to distinguish between individual or epidemic strains, thus differentiating independent acquisition from cross-infection. By this approach, nosocomial transmission should be revealed by the widespread occurrence of group of genetically similar strains of hospitalized.[7] Moreover, a rapid and accurate identification of disease causing species is crucial for the clinical treatment and epidemiological studies.[8]

Conventional methods used for the identification and typing of clinical isolates of Candida species are based on a variety of physiological and morphological marker. These include reaction to a panel of biochemical tests, resistogram typing and serotyping.[9] These techniques are time consuming and rely on phenotypic expression which makes them potentially unreliable.[10] Hence these culture based diagnostic methods are often limited to the detection of organisms at the advance stage of disease.[11] Earlier detection of infection permits prompt initiation of antifungal therapy with a greater likelihood of improved survival and reduced mortality.[12]

To investigate the possibility of single source outbreaks, the technique of pulsed field gel electrophoresis[13] was used to analyze C. parapsilosis isolates from intensive care unit patients of two hositals with distinct geographic locations to determine the relationship of isolates within an apparent outbreak and the predominantPFGE type in each outbreak. Electrophoretic karyotying with PFGE appears superior than other molecular typing methods[14] and useful tool for epidemiological studies of Candida species.[15]

MATERIAL AND METHODS

Isolates:

Nine isolates of C. parapsilosis from two different geographic locations were evaluated. Six of these were from St Bartholomew's Hospital, London. The other three isolates were from Neonatal Surgical Unit, St Mary's Hospital, manchester. Identification was performed by a negative germ tube formation and the yeast API 20C method. Table-I shows the source and site of these isolates.

Growth of the isolates:

The isolates from the stock cultures on slopes were subcultured on Sabouraud's dextrose agar plates, and incubated at 30°C for 48 hours. A single colony from each plate was resuspended and grown in 25 ml of yeast peptone dextrose (1% yeast extract, 2% dextrose, 2% bactopeptone) broth in a flask for 48 hours at 30°C with shaking. The cells cultures were transferred to glass universal bottles and chilled on ice for 10 minutes. The cells were collected by centrifugation at 4,000 rpm for 10 minutes, washed in 10 ml of 40 mM EDTA (pH 7.5) and pelleted again by centrifugation at 4,000 rpm for 10 minutes. The pellets were resuspended in 50 nM EDTA (pH 7.5) at a ratio of 3 volumes of EDTA to 2 volumes of cells.

TABLE - I

DETAILS OF C. PARAPSILOSIS ISOLATES FROM THE TWO HOSPITALS

Isolate	Site of Isolate	PFGE type
	St. Bartholomew's Hospital, London	
67	Nurse's hands	I
471	Blood Culture	III
272	Blood Culture	II
258	Blood Culture	II
44	Nurse's hands	I
45/R	Blood Culture	I
	St. Mary's Hospital, Manchester	
11/P	Intra-Abdominal Abscess	III
12/K	Umbilical Swab	I
13/S	Gastrostomy	I

Preparation of chromosomal DNA:

The cells samples were prepared by a modification of the method of Schwartz and Canto[13] described previously.[16]

Pulsed field gel electrophoresis (PFGE):

Electrophoresis was conducted in a Bio-Rad CHEF-DR II system. The plugs were loaded into 1% agarose gels in 0.5% of TBE

buffer (45 mM Tris, 45 mM boric acid, 1 mM sodium EDTA {pH 8.0}). For the analysis of Candida species, gels were electrophoresed for 72 hours at 12° C with a two step pulse time; initial pulse time of 300 seconds for 24 hours at an electric field strength of 4.2 V/cm followed by 1200 seconds for 48 hours and a field strength of 2.4 V/cm. Gels were stained with ethidium bromide (0.5 ug/ml ind distilled water) for 15 minutes and de-stained in distilled water for 3 hours. DNA bands were visualized with a UV light transilluminator and photographed.

RESULTS

Six isolates of C. parapsilosis from St Bartholomew's Hospital, London and three isolates from St Mary's Hospital were examined in this study (table-I). In order to determine relatedness of the isolates which had previously been characterized as outbreaks by conventional agarose gel electrophoresis were analysed using Pulsed-field gel electrophoresis.

Six to seven bands with approximate molecular sized ranging from 940 to 2900 kilobases were observed. Each band was identified and the distance from the origin of the gel relative to the molecular weight standards was measured. Isolates were considered different if any readily detectable band did not match. Variation in the electrophoretic karyotype occurred primarily in the middle and lower segments of the gels. Differentiation was based on variation in bands at 1550 and 1020 kilobases. Bands at 2900,2200,2050,1300,1200 and 940 kilobases were conserved (Fig-I, Table-II).

Analysis of the electrophoretic karyotype pattern showed three classes of chromosome variation. Among the six isolates from St Bartholomew's Hospital London (Fig-I, lanes 2-6), three isolated were PFGE type I (lanes 2,6, and 7), whereas type II was found in two isolates (lanes 4 and 5). One isolate produced type III (lane 3). Among the isolates from St Mary's Hospital, Manchester, two ware PFGE type I (lanes 9 and 10), whilst one isolate produced type III (lane 8).

DISCUSSION

The principal underlying the technique of PFGE is that isolate of the same strain produce indistinguishable DNA fingerprint patterns.[2] The nine isolates of C. parapsilosis from two separate hospitals (Table I) produced indistinguishable DNA fingerprint patterns when digested with EcoR I (JP Burine, personal communication). Similar study carried out in the past on whole cell DNA and mitochondrial DNA of C. parapsilosis used a range of restriction enzyme, such as EcoR I, BamH I, kpn I, Bgl II, Hpa II, Pua II and Hind III. This produced uniform and reproducible fragment patterns with each enzyme.[17] In contrast, DNA fingerprinting C. albicans isolates with these enzyme proved helpful,[18] but the degree of discrimination was lower compared to PFGE.[15]

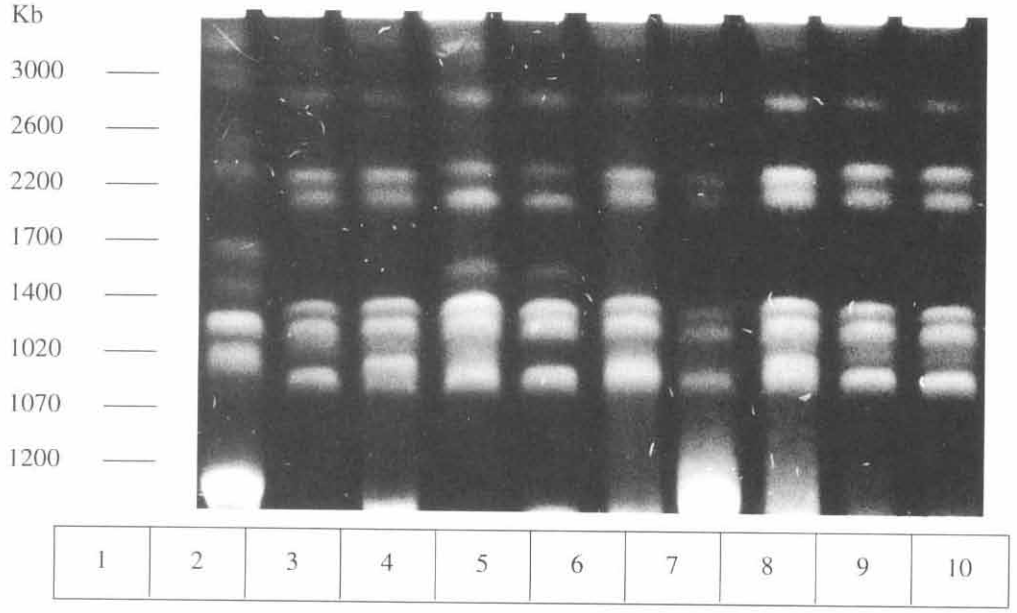
Pulsed field gel electrophoresis of C. parapsilosis isolates used in this study produced distinct, well separated chromosomal DNA bands over a wide size range from 940 to 2900 Kb. The electrophoretic karyotype of these isolates consisted of 6-7 DNA bands. Bands at 940, 1200,1300,2050, 2200, and 2900 KB were generally conserved, and will be useful in differentiating this species from other Candida species under identical running conditions. Three distinct electrophoretic types were defined on the basis of presence or absence of chromosomal DNA bands at 1020 and 1550 Kb (Table-II). Among the isolates form St Bartholomew's Hospital London, three isolates (Table-I isolated 67, 44 and 45/R) produced PFGE type I. These lacked bands at 1550 and 1020 Kb. Two isolates produced PFGE type II (Fig-I lanes 4 and 5), whereas type III was produced by a single isolate (line 3).

TABLE - II

DETAILS OF CHROMOSOMAL DNA BANDS OF THE THREE PFGE TYPES AMONG C. PARAPSILOSIS ISOLATES.

PFGE TYPE	CHROMOSOMAL DNA BANDS IN KILOBASES								TOTAL ISOLATES
	2900	2200	2050	1550	1300	1200	1020	940	
I	+	+	+	-	+	+	-	+	5
II	+	+	+	+	+	+	-	+	2
III	+	+	+	-	+	+	+	+	2

+ PRESENCE OF BAND - ABSENCE OF BAND



The three neonates from St Mary's Hospital, Manchester were admitted to the neonatal surgical unit during a single week. C. parapsilosis was isolated from the drainage of an intra abdominal abscess of the index case (11/P) on 29th October 1997. This patient remained colonized till 13th November 1997, and produced PFGE type III. The other two cases from two adjacent beds yielded C. Parapsilosis on 7th November 1997. Both of these produced type I (Figure-I, lane 9 and 10).

Following institution of strict and washing practices and other infection control measures, no further isolates of C. parapsilosis ware isolated from the units, signifying the importance of effective control measures. This study demonstrates the discrimination of PFG electrophoresis. It produced three separate Karyotype patterns for isolates, which were identical on DNA fingerprinting. This study also signifies that good hand washing practices by staff is the single most important infection control

measure and is essential to prevent spread of yeasts as well as other infection.

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