

Original Article

PFGE-Based Epidemiological Study of an Outbreak of *Candida tropicalis* Candiduria: The Importance of Medical Waste as a Reservoir of Nosocomial Infection

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SUMMARY: Between November 2002 and March 2003, an outbreak of candiduria occurred in the surgical intensive care unit (SICU) of a university-affiliated hospital in South Korea. This outbreak affected 34 patients and was caused by *Candida tropicalis*. To determine the source of the epidemic and the risk factors, surveillance cultures from the SICU, genotyping of *Candida* isolates by pulsed-field gel electrophoresis (PFGE), and a case-control study were performed. The surveillance cultures revealed that 6 environmental samples related to the urine disposal route were positive for *C. tropicalis*. The PFGE analysis of genomic DNA demonstrated identical band patterns for all of the *C. tropicalis* isolates obtained from SICU patients and the 6 environmental samples during the outbreak period, while epidemiologically unrelated strains showed unique PFGE band patterns. Although no risk factors were identified by the case-control study, this epidemiological investigation involving the use of molecular techniques suggests that improper disposal of infectious medical waste led to the cross-transmission of a single clone that was responsible for the outbreak of *C. tropicalis* candiduria in this SICU. After implementing a better urine disposal system and thorough hand washing procedures, no further clusters of candiduria were detected in the SICU.

INTRODUCTION

Although rare in healthy people, candiduria is common in hospitalized patients (1). In tertiary care facilities, as many as 10% of positive urine cultures yield *Candida* isolates (2), which reflects the cumulative pressure of contributing factors such as urinary instrumentation and prolonged use of broad-spectrum antibiotics (3,4). *Candida tropicalis* is the second most common *Candida* spp. colonizing humans (5) and the third most frequently isolated *Candida* spp. from urine cultures (6,7).

Although there have been several nosocomial outbreaks of candidiasis, few have specifically involved *C. tropicalis*. In addition, the nosocomial transmission of *Candida* strains through medical waste and equipment has rarely been reported. Recently, we observed an apparent nosocomial outbreak of *C. tropicalis* candiduria in a surgical intensive care unit (SICU) in Chosun University Hospital, Gwang-Ju, South Korea. We performed an epidemiological investigation of the outbreak including surveillance cultures, DNA typing and a case-control study to find the source of the epidemic, and to determine the risk factors and appropriate control measures. We used a pulsed-field gel electrophoresis (PFGE) method for DNA typing to determine whether single

or multiple clones caused the epidemic. The present study shows that poor management of medical waste can be a contributing factor to the outbreak of *C. tropicalis* candiduria in SICUs.

MATERIALS AND METHODS

Description of the outbreak: Chosun University Hospital is a 650-bed tertiary care center located in Gwang-Ju City, South Korea. An apparent nosocomial outbreak of *C. tropicalis* candiduria occurred in the SICU between November 2002 and March 2003. During this period, a total of 34 cases of *C. tropicalis* candiduria were documented (Fig. 1). In this SICU, only three patients had been identified as having *C. tropicalis* candiduria during the 4 months prior to the outbreak. In addition, these fungi have seldom been isolated from ICU patients in this cluster, and only two patients were found to harbor *C. tropicalis* between April and June 2003.

The SICU is an open room with 19 contiguous patient beds. At the time of the outbreak, the hospitalization periods of the 34 patients with *C. tropicalis* candiduria overlapped, and the patients were often cared for by the same health care workers (HCWs). The team of HCWs in the SICU consisted of 18 nurses and 3 doctors.

In order to determine whether the event was a true outbreak, we examined the techniques of urine collection, transport and culture, and we retrospectively reviewed the medical records of patients who had *C. tropicalis* candiduria

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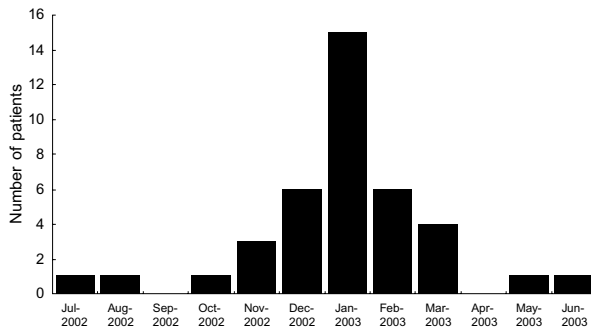


Fig. 1. Epidemic curve showing the number of SICU patients with *C. tropicalis* in their urine cultures at 1-month intervals from July 2002 to June 2003.

during the study period. At this SICU, all urine samples were correctly obtained from an indwelling catheter by syringe aspiration after disinfection of the area where the needle puncture was made. Urine samples were not obtained from urine collection bags, and all the collected urine samples were transported in a fresh state to the laboratory. In addition, we found that there had been no changes or defaults in the laboratory techniques of urine culture that might have accounted for the increase in positive results.

C. tropicalis was repeatedly isolated from urine samples in 82.4% (28/34) of the patients on separate days. After verification of the adequacy of the urine sampling and culture techniques at the early stage of the epidemiological investigation, *C. tropicalis* was repeatedly isolated in most patients. In these 28 patients, *C. tropicalis* was isolated from urine cultures 2 to 5 times in 16 patients, 6 to 10 times in 10 patients, and 14 times from 2 patients. The isolation interval of urinary *C. tropicalis* in the same patient ranged from 2 to 145 days.

Surveillance cultures: Surveillance cultures were initially performed in the SICU on February 4th, and were repeated on February 6th and February 13th, 2003. Extensive environmental cultures of the SICU were performed for the presence of *C. tropicalis* and focused on various inanimate surfaces and items in frequent contact with patients and HCWs. Swabs pre-moistened with sterile normal saline were used to collect the environmental samples. The survey included the following items: bed sheets, surfaces of Foley catheters, skin near the urethra of the patient, washing stands, dressing cart surfaces, utility carts, floors, lubricants, upper lids of disinfectant sponge cans, handles of doors, waste water disposal sink surfaces, and urinals for collecting urine from the collection bags of patients. The inner and outer surfaces of the urine collection buckets and the disinfectant solution used for disinfecting the patients' collected urine were also cultured. Hand swabs and nares scrapings were obtained from 15 of the 21 HCWs working in the SICU and were cultured. During the swabbing of hands, nails were scraped to check for the carriage of *Candida* spp. A total of 76 environmental samples, including 30 samples from HCWs, were obtained during the study period. The same surveillance cultures were performed after the outbreak disappeared.

***C. tropicalis* isolates and identification:** A total of 147 strains from the urine cultures of 34 patients and 6 strains from environmental cultures were identified as *C. tropicalis* in the clinical microbiology laboratory of the hospital. *C. tropicalis* was identified by assimilation tests using a YBC test kit with the VITEK system (BioMerieux, Durham, N. C.,

USA) or an API 20C following the manufacturer's instructions.

Molecular typing: A total of 50 isolated strains, including 22 urinary outbreak isolates from patients, 6 isolates from environmental cultures, and 22 epidemiologically unrelated *C. tropicalis* strains, were analyzed by PFGE analysis. All of the epidemiologically unrelated strains were obtained from Chonnam National University Hospital (CNUH), which is located near Chosun University Hospital in the same city.

The isolates of *C. tropicalis* were characterized by PFGE using previously described methods (8-11) with certain modifications. In brief, *C. tropicalis* isolates were collected from Sabouraud dextrose agar (SDA), and genomic DNA was prepared in an agarose plug. The plugs of genomic DNA were subjected to cell lysis by lyticase at 37°C in lysis buffer for 2 h, treated with proteinase K in 50 mM EDTA buffer, and incubated for 16 h at 50°C. The macro-sized genomic DNA was then digested with *Bss*HIII in restriction enzyme buffer for 16 h at 37°C. Electrophoresis was performed with a CHEF-DR II system (Bio-Rad, Hercules, Calif., USA) in 0.5× TBE buffer. The electrophoretic conditions were 6 V/cm at 14°C, with alternating pulses at an angle of 120 degrees in a 5-50 s pulse-time gradient for 20 h. A ladder of *Saccharomyces cerevisiae* chromosomal DNA was used as a molecular weight marker. The gel was stained with ethidium bromide and photographed under UV light. Strains were considered to be different if they displayed one or more band differences according to the interpretive criteria for DNA restriction patterns previously described (8,12).

Case-control study: We conducted a case-control study to compare the clinical variables associated with *C. tropicalis* candiduria during the outbreak period. We selected 27 case patients and 62 control patients. Case and control patients were defined according to whether or not *C. tropicalis* was isolated in the urine cultures of patients who were admitted to the same SICU during the epidemic period. The controls were 62 randomly selected, age- and sex-matched patients. The medical records of both case and control patients were reviewed, and their various clinical factors were compared for the presence of risk factors.

Statistical analysis: Frequencies and descriptive statistics about the demographic and clinical characteristics of cases and controls were determined. Univariate and multivariate logistic regression analyses were performed to identify independent risk factors of candiduria. Crude and adjusted odds ratios (ORs) and 95% confidence intervals (CIs) were calculated. An OR of >1 was taken to indicate an increased risk of candiduria, an OR of <1 indicated a decreased risk, and an OR of 1 indicated a similar risk. All statistical analyses were performed with the Statistical Package for the Social Sciences (SPSS, version 10.0) for Windows (SPSS, Chicago, Ill., USA).

RESULTS

Epidemiological survey: Six strains of *C. tropicalis* were isolated after performing surveillance cultures of 76 environmental samples. All of these six strains were recovered from apparatuses used along the urine disposal route. The environmental samples from which *C. tropicalis* was isolated included one strain from the urinal used for collecting urine from the ora of the urine collection bags of patients, three strains from the inner surface of the bucket used for collecting and transferring urine from the urinals for disposal, and

two strains from the surface of the medical waste disposal sink in the SICU. *C. tropicalis* was repeatedly isolated from these six samples related to the urine disposal route, on every sampling and in each swab culture. We found that the urinals, the urine bucket, and the medical waste disposal sink had been inadequately disinfected and used for the disposal of urine from multiple SICU patients during the outbreak period.

Other environmental sample cultures, including swab samples from various inanimate surfaces within the SICU and the hands and nares of HCWs, did not yield *C. tropicalis*. Likewise, the organism was not isolated from any of the 20 follow-up swabs of the environment.

PFGE analysis: Genotypic analysis of the strains from

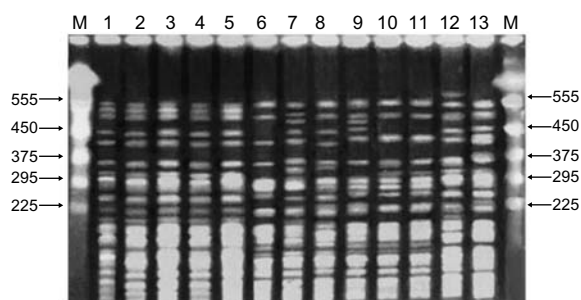


Fig. 2. Representative PFGE patterns of *BssHII*-digested genomic DNA from nosocomial outbreak and non-outbreak strains of *C. tropicalis*. Lane M, molecular size marker of *S. cerevisiae* chromosomal DNA; lane 1, an environmental strain; lanes 2 to 5, clinical outbreak isolates from the SICU; lanes 6 to 13, non-outbreak isolates from CNUH.

the outbreak was conducted by performing PFGE on *BssHII*-digested genomic DNA of *C. tropicalis*. All of the 22 clinical strains obtained from 19 patients showed identical band patterns. The environmental strains of *C. tropicalis* isolated from the urine disposal route showed a band pattern identical to that of the clinical outbreak strain. Differences in band patterns were found between outbreak and non-outbreak strains and also among the non-outbreak strains obtained from CNUH (Fig. 2).

Characteristics of cases and controls: The mean age of the case patients with *C. tropicalis* candiduria was 56 years of age, and the ratio of males to females was 1.7:1. There was no evidence of systemic *C. tropicalis* infection in any of the cases, and cultures from other sites such as blood and wounds did not contain *C. tropicalis*. Most patients had undergone a major operation (81.5%); additionally, they were all receiving antibiotic therapy (100%) and most had been intubated (74.1%). The major underlying diseases of the patients were intracranial hemorrhage (59%), epidural hemorrhage (11%) and cerebral aneurysm (11%).

The prevalence of the various clinical factors among the 27 case patients with *C. tropicalis* candiduria was similar to that among the age- and sex-matched control patients. All of the case and control patients had urinary catheters when the outbreak of *C. tropicalis* candiduria was detected in the SICU. The results of univariate and multivariate logistic regression analyses for the possible risk factors are summarized in Table 1. The mental state, intubation, presence of a central venous catheter, and history of a preceding operation were similar in both the case and control groups. The noted risk factors by

Table 1. Univariate and multivariate analyses of possible risk factors for 27 cases of *C. tropicalis* candiduria compared with 62 matched patient controls

Factor	No. (%) of subjects		Univariate analysis	Multivariate analysis
	Case (n = 27)	Control (n = 62)	OR (95% CI) ³⁾	OR (95% CI)
Mental state				
normal~drowsy	15 (55.6)	38 (61.3)	–	–
stuporous~comatose	12 (44.4)	24 (38.7)	1.26 (0.50-3.16)	–
Duration of SICU ²⁾ stay prior to isolation of <i>C. tropicalis</i>				
< 15 days	4 (14.8)	35 (56.5)	–	–
≥ 15 days	23 (85.2)	27 (43.5)	7.45 (2.30-24.12) ¹⁾	3.42 (0.93-12.60)
Intubation				
No	7 (25.9)	27 (43.5)	–	–
Yes	20 (74.1)	35 (46.5)	2.20 (0.81-5.96)	–
Central venous catheter				
No	20 (74.1)	51 (82.3)	–	–
Yes	7 (25.9)	11 (17.7)	1.62 (0.55-4.77)	–
Prior Surgery				
No	5 (18.5)	22 (35.5)	–	–
Yes	22 (81.5)	40 (64.5)	2.41 (0.80-7.28)	–
Duration of antibiotic therapy prior to isolation of <i>C. tropicalis</i>				
< 10 days	1 (3.7)	22 (35.5)	–	–
≥ 10 days	26 (96.3)	40 (64.5)	4.25 (1.81-111.97) ¹⁾	4.16 (0.41-41.76)
No. of antibiotics per patient				
< 2	2 (7.4)	26 (41.9)	–	–
≥ 2	25 (92.6)	36 (58.1)	9.03 (1.96-41.52) ¹⁾	2.77 (0.47-16.06)

¹⁾: $P < 0.05$.

²⁾: Surgical intensive care unit.

³⁾: Odds ratio (95% confidence intervals).

univariate analysis were the length of the SICU stay, the duration of antibiotic therapy, and the number of antibiotics taken per patient. After multivariate logistic regression analysis, however, none of the risk factors was found to be independently significant.

Control measures: After the outbreak was identified in the SICU, various infection control measures were instituted in order to minimize the epidemic. Given that environmental sampling revealed the same *C. tropicalis* isolates along the route of the urine disposal system, we identified the source of the outbreak as inadequately disinfected urine disposal equipment. The urinals, the urine bucket, and the medical waste disposal sink had often been used in an inadequately disinfected state and were shared by multiple SICU patients during the outbreak period. We therefore changed the urine disposal system in this SICU. All urine disposal equipment was made as sterile as possible, and equipment for individual use was implemented. Separate urinals for the disposal of urine were distributed to each patient and both patients and HCWs were instructed in proper waste management practices. This minor change in the urine disposal system produced a significant result, rapidly reducing the isolation rate of *C. tropicalis* in the urine cultures of SICU patients.

In addition, HCWs were educated to increase awareness about nosocomial transmission within the unit. Frequent hand washing was strictly carried out by SICU HCWs before handling any equipment. The hospital infection control team regularly carried out inspections to ensure compliance with guidelines for the prevention of nosocomial infection. After these control measures were instituted, no further clusters of *C. tropicalis* candiduria were detected in the SICU.

DISCUSSION

Although most instances of candiduria arise from an endogenous source (13-16), exogenous acquisition of candiduria can occur among a minority of ICU patients (16,17). In the present study, a thorough epidemiological investigation with surveillance cultures and PFGE-based molecular biology techniques coupled with proper management enabled the identification of the source of the epidemic and the prompt curtailment of the *Candida* outbreak. To the best of our knowledge, this is the first study making use of molecular biology techniques to prove that inadequately disinfected urinary disposal equipment can be the source of an outbreak of nosocomial *C. tropicalis* candiduria.

The presence of candiduria may signal diverse pathological states, including invasive renal parenchymal disease, fungal balls in obstructed ureters, superficial lower urinary tract infection, and lower urinary tract *Candida* colonization. Accordingly, a wide spectrum of clinical disease occurs; however, the majority of patients present with asymptomatic candiduria, probably in association with *Candida* colonization due to urinary catheterization (18). In this study, it was difficult to determine whether the candiduria originated from infection or colonization. In the majority of our patients, *C. tropicalis* was repeatedly isolated from urine cultures. However, none of the patients with *C. tropicalis* candiduria developed systemic infection, and all candiduria improved without antifungal therapy after removal of the urinary catheter.

The most frequently noted risk factors for nosocomial candiduria include urinary instrumentation, recent antibiotic therapy and advanced age (6,7). In the present study, various clinical factors, including the state of urinary instrumenta-

tion, were identical in both the case and control groups. Some risk factors associated with antibiotic therapy were recognized by univariate analysis, but none of them was found to be independently significant by multivariate logistic regression analysis.

The restriction endonuclease analysis of genomic DNA using *Bss*HIII followed by PFGE is highly effective in discriminating among isolates of *C. tropicalis* (8,12). Hence, we used PFGE typing to evaluate clonal relatedness among the obtained *C. tropicalis* isolates. The identification of identical banding patterns among the clinical outbreak strains and environmental isolates of *C. tropicalis* suggested that the dissemination of a single clone of the fungus was responsible. Other epidemiologically unrelated strains demonstrated different unique PFGE patterns. These results confirm that restriction endonuclease analysis by PFGE using the restriction enzyme *Bss*HIII is a useful molecular typing method for identifying the source of a *Candida* outbreak and for discriminating *C. tropicalis* strains.

The exogenous acquisition of infections may be accounted for by hospital-related activities such as manual procedures performed by HCWs, contaminated infusates and other biomaterials, and the inanimate environment (8,12). According to our investigation, this outbreak of *C. tropicalis* candiduria was attributable to a single epidemic strain which colonized urinary disposal equipment and spread among patients. The isolation of *C. tropicalis* of the same PFGE type along the route of urine disposal prompted us to seek a faulty method of emptying urine drainage bags as the primary factor contributing of the epidemic. Although we did not detect *C. tropicalis* on the hands of HCWs by surveillance cultures, we speculate that the *C. tropicalis* strain was transmitted to other patients from the hands of the HCWs who manipulated urinary catheters. The epidemic strain of *C. tropicalis* may have been transmitted when nursing staff emptied drainage bags into the urinals, as some of SICU patients are known to have shared inadequately disinfected urinals during the outbreak period. After adopting sterile urine disposal methods accompanied by thorough hand washing practices and hygiene education, the epidemic in our SICU was brought under control.

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