


Original Article

A cluster of *Candida parapsilosis* displaying fluconazole-trailing in a neonatal intensive care unit successfully contained by multiple infection-control interventions

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Abstract

Objective: This study aimed to investigate and contain a cluster of invasive candidiasis cases caused by fluconazole-resistant *Candida parapsilosis* (FRC) in a neonatal intensive care unit.

Methods: Active surveillance was initiated. Direct observations of hand-hygiene compliance (HHC) among staff were conducted before and after the implementation of hand-hygiene (HH) education. Thirty-five environmental cultures were obtained. Phylogenetic analysis of FRC was performed using Fourier-transform infrared spectroscopy and microsatellite genotyping.

Results: A total of 14 patients (mean birth weight = 860 g, gestational age = 25 weeks) infected with FRC were identified using the fully automated analyzer, including 5 with clinical infection (three with catheter-related bloodstream infection, one with cutaneous infection, and one with fatal peritonitis) and 9 with colonization. The HHC rate in nurses before performing a sterile or aseptic procedure significantly improved after the HH education ($P < .05$). Sinks near the patients were contaminated with FRC. All FRC strains were confirmed to be susceptible to fluconazole using the CLSI method, and the microdilution procedure indicated a trailing effect. Phylogenetic analysis showed that all the fluconazole-trailing isolates from patients were clustered together and had the same genotype. Sinks were successfully decontaminated using accelerated hydrogen peroxide and drainage pipes were replaced. Ultraviolet-C decontamination was applied in the milk preparation room. No new cases were detected after the education and disinfection interventions.

Conclusions: Sinks are an important reservoir of *C. parapsilosis*. Active surveillance, environmental hygiene, and constant staff education on maintaining a high level of HHC are necessary to limit the spread of *C. parapsilosis*.

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Introduction

Invasive candidiasis (IC) is a leading cause of morbidity and mortality in neonatal intensive care units (NICU).¹ The primary risk factor for IC is prematurity, complemented by the disruption of epithelial barriers caused by procedures and equipment, such as endotracheal intubation and central venous catheters.¹ Advancements in these therapeutic procedures have significantly improved survival in premature infants; however, there has been a noticeable increase in the incidence of IC in NICU.²

Candida parapsilosis is the most common *Candida* species responsible for IC in pediatric patients.³ Fluconazole is one of the main therapeutic options for infections caused by *C. parapsilosis* since this pathogen exhibits an intrinsically reduced susceptibility to echinocandins.⁴ Thus, the emergence of fluconazole-resistant strains poses a significant clinical concern worldwide.⁴ Between April 2019 and March 2020, we encountered a cluster of IC cases caused by fluconazole-resistant *C. parapsilosis* (FRC) in the NICU of a tertiary hospital in Japan. This study aimed to investigate and contain a cluster of IC cases caused by FRC in the NICU.

Methods

The clinical setting, population characteristics, and infection-control interventions associated with this cluster are summarized in the Supplementary Table 1.⁵

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Active surveillance

The tertiary hospital had an NICU with 15 beds comprising 9 in NICU 1 and 6 in NICU 2, a growing care unit (GCU) with 17 beds, and a milk preparation room. Patients newly admitted to the NICU are initially placed in NICU 1 and then transferred to NICU 2 upon stabilization from the acute phase. A cluster was first suspected in April 2019 with two cases of IC from a catheter-related bloodstream infection (BSI) caused by FRC (minimum inhibitory concentration [MIC], 32 µg/mL). This detection was made using a fully automated analyzer (RAISUS® S4, Nissui, Tokyo, Japan). The incident of unusual cases of FRC infection, which had never previously occurred in the hospital, led to the initiation of active surveillance screening for FRC among all NICU patients. This screening included admission and weekly cultures from rectal, nasal, and skin samples because these sites are commonly colonized by *Candida* species.^{1,6} The surveillance involved an epidemiologic investigation including a detailed review of medical records of patients infected or colonized with FRC. Additionally, a comparative analysis of the incidence rates of IC caused by *Candida* species other than FRC, defined as the number of IC cases per total inpatients during the same period, was conducted for three distinct periods: before the cluster (April 2015 to March 2019), during the cluster (April 2019 to March 2020), and after the cluster (April 2020 to March 2021).

Hand hygiene education

Education for NICU staff to improve hand-hygiene compliance (HHC) was implemented in July 2019. These included informing all the NICU staff of the IC cluster caused by FRC; conducting formal lectures on the proper utilization of hand-cleaning agents, proper handwashing techniques, and the importance of hand hygiene (HH), implementing a brainstorming session to enhance adherence to appropriate HH practices. In addition, training was conducted to ensure the implementation of standard precautions⁷; however, patient/staff cohorting was not feasible due to spatial constraints and a lack of adequate staffing in the wards. Throughout the periods before, during, and after the cluster, the alcohol-based hand rub was recommended, and the NICU staff was consistently equipped with and utilized alcohol hand sanitizers. Concurrently, the wearing of artificial nails was strictly prohibited.

At the tertiary hospital, direct observations of HHC among the NICU staff, following the World Health Organization (WHO)'s five moments for HH,⁸ had been routinely conducted twice a year by four trained infection-control nurses. The assigned nurses document every HH opportunity, identifying how many medical personnel (eg, physicians or nurses) performed HH and whether the procedure was performed. The HHC rate was calculated by dividing the number of observed HH actions by the total number of opportunities. During the study period, these observations were made before (July 28, 2019) and after (November 27, 2019) implementation of the education, and after the cluster (May 19, 2020, and November 19, 2020). Feedback on HHC was provided to the NICU staff on July 5, 2019. HHC rates observed at each time point were compared using the data recorded on May 18, 2018, as a baseline.

Environmental investigation

Despite the implementation of HH education, cases of FRC carriage and infection continued to be detected. A case-control

study conducted on all patients admitted to the NICU from April to August 2019 revealed that the consumption of donor milk was associated with an increased risk of infection or colonization with FRC (odds ratio 7.20; 95% confidence interval, 1.28–40.37) (Supplementary Table 2). Based on the results, the infection-control team identified the milk preparation room, used for donor milk preparation, as a potential high-risk site for cross-contamination and subsequently conducted an environmental investigation. Overall, 35 environmental cultures were obtained from 35 sites using eSwab collection kits (Copan, Brescia, Italy). Ten of these cultures were from 5 high-touch surfaces (medical cart, infusion preparation table, refrigerated drug storage, garbage container, and computer keyboard) and 5 medical devices (ultrasound probe, weighing scale, diaper scale, medical tape, and radiation protection apron) in the NICU; 11 were from 2 high-touch surfaces (vital signs monitor and infusion pump control panel), 5 medical devices (stethoscope, axillary thermometer, bag valve mask, and ventilator monitor/tube and water trap) and an infant incubator (access port door handle, internal wall, humidifier reservoir, and external tray) in the immediate environment of a patient with spontaneous intestinal perforation; 2 were from 2 high-touch surfaces (formula preparation counter and breastmilk refrigerator) in the milk preparation room; and 12 were from 12 sink surfaces and drains (6 in the NICU, 3 in the GCU, 1 in the nurse station, and 2 in the milk preparation room). Samples were collected according to the manufacturer's instructions.

Before the cluster, nurses in the NICU routinely cleaned high-touch environmental surfaces with disinfectant wipes containing quaternary ammonium compounds every 8 hours, although the cleaning was not performed thoroughly. The standard sinks with splash guards were used exclusively for washing hands and were cleaned daily using a neutral detergent (Supplementary Figure). Infusions, including total parenteral nutrition, are prepared exclusively on the infusion preparation table, and discarded in a sink used only for infusion disposal at the nurse station after use or upon expiration.

Identification of fungal strains and antifungal susceptibility evaluation

Fungal strains were detected from the clinical and environmental samples using CHROMagar™ *Candida* media (Kanto Chemical Co., Tokyo, Japan) (Supplementary Method 1). Species identification was performed using a VITEK-MS (Sysmex-bioMérieux Japan, Tokyo, Japan). The antifungal susceptibility of *C. parapsilosis* isolates was determined using an automated analyzer. After the cluster, this susceptibility was further confirmed using the Clinical and Laboratory Standards Institute (CLSI) broth micro-dilution method, as described in CLSI document M27-E4.⁹

Phylogenetic analysis

Phylogenetic analysis of FRC isolated from patients and environmental samples, along with one standard strain of *C. parapsilosis* (ATCC 22019) and two epidemiologically unrelated clinical isolates from the tertiary hospital, was performed using the IR Biotyper system with IR Biotyper client software Ver.3.1 (Bruker Daltonics GmbH & Co. KG), which is a typing machine based on Fourier-transform infrared (FT-IR) spectroscopy. The samples were prepared according to the manufacturer's instructions.¹⁰ Same strains were measured in two independent biological replicates. A dendrogram was generated using single spectrum with the Exploration method: Euclidean_average_linkage. The

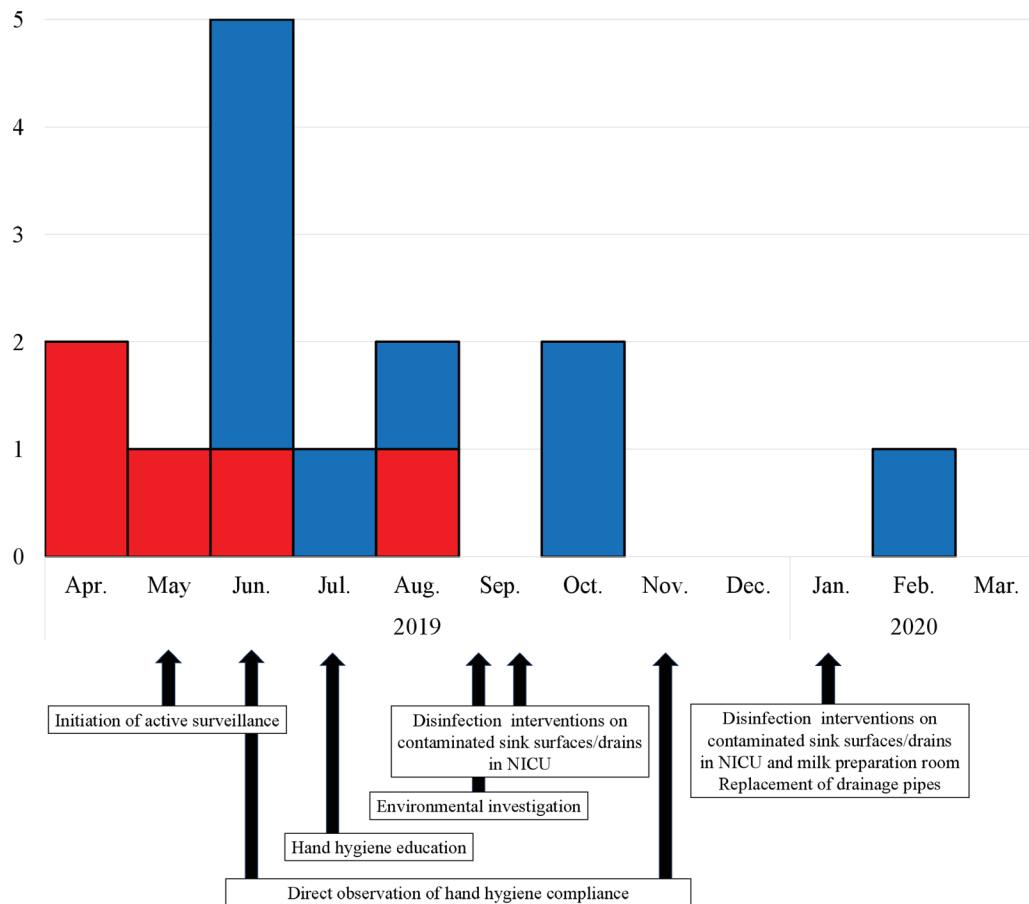


Figure 1. Epidemic curve of patients newly infected/colonized with fluconazole-resistant *Candida parapsilosis* (FRC) in the neonatal intensive care unit from April 2019 to March 2020. The red and blue bars indicate patients infected and colonized with FRC, respectively.

clustering cutoff value was calculated automatically using the software, which was the result of Simpson's index of diversity. Microsatellite genotyping was performed on all FRC isolates from patient and environmental samples as described previously (Supplementary Method 2).¹¹

Statistical analysis

Fisher's exact test was used to compare the categorical variables. Analysis was performed using JMP Pro 16 statistical analysis software (SAS Institute, 2021). A P -value $<.05$ was considered statistically significant.

Results

Active surveillance

Among the 201 newborns (mean birth weight = 2,134 g; gestational age = 34 weeks) admitted to the NICU between May 2019 and March 2020, a total of 14 patients were identified with FRC, including 5 with clinical infection and 9 with colonization detected through active surveillance (Figure 1). Among the 14 patients infected with FRC (mean birth weight = 860 g, gestational age = 25 weeks), 5 extremely low birthweight infants were infected with FRC strains, including 3 cases of catheter-related BSI, 1 of cutaneous infection, and 1 of fatal peritonitis with intestinal perforation despite surgical and antifungal treatment with a fluconazole plus amphotericin B combination (Figure 2).

After November 2019 following the introduction of HH education and disinfection interventions on sink surfaces and drains and in the milk preparation room, only one additional case occurred in February 2020. Fluconazole-resistant *Candida* isolates have not been identified from clinical specimens in other units during and following this cluster.

During the period from April 2015 to March 2021, a total of eleven cases of IC caused by *Candida* species other than FRC were identified, comprising six cases caused by fluconazole-susceptible *C. parapsilosis*, four caused by *C. albicans*, and one caused by *C. tropicalis*. The incidence rates of the IC before the cluster, during the cluster, and after the cluster were not significantly different (Supplementary Table 3).

Hand hygiene education

Before the implementation of the HH education, the overall HHC rate was comparable to baseline (94/128 [73%] vs 82/116 [71%] opportunities, $P \geq .05$) (Table 1). However, the HHC rate before performing a sterile or aseptic procedure was significantly lower than the baseline (11/14 [79%] vs 3/12 [25%] opportunities, $P < .05$). After the education, the rate was increased, reaching levels comparable to that at the baseline (11/14 [79%] vs 8/12 [67%] opportunities, $P \geq .05$).

The HHC rate in physicians was significantly worse than that in nurses both before and after the HH education (12/25 [48%] vs 69/89 [78%] and 10/19 [53%] vs 95/105 [90%] opportunities, respectively; both $P < .05$). After the HH education, the HHC rate

(A)

ID	BBW (g)	GA	Basal disease	Positive culture	Period of detection*	Diagnosis	MIC (µg/mL)				Treatment	Outcome	Bed number											
							FLCZ	AMPH-B	MCFG	FLCZ (CLSI)			Apr.	May	Jun.	Jul.	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.
1	488	23w2d	RDS	Blood	12D	CRBSI	32	2	1	≤2	AMPH-B +FLCZ	Survived	①	①	⑤	⑩	①	⑤	⑤	⑩	⑭			
2	257	23w1d	RDS	CVC	21D	CRBSI	32	2	1	≤2	MCFG	Survived	③											
3	535	23w2d	PPHN	Blood	10D	CRBSI	32	2	2	≤2	AMPH-B	Survived		③										
4	995	29w6d	RDS	Skin	7D	Colonization	32	2	1	≤2	None	Survived			③	⑫	⑭							
5	1555	32w2d	RDS	Stool	15D	Colonization	32	2	1	≤2	None	Survived			⑥	⑧								
6	523	23w1d	RDS	CVC	14D	Colonization	32	2	1	≤2	None	Survived			①	①	①	⑮	⑪	⑭	⑩	⑩	⑬	⑬
7	1007	27w0d	RDS	Skin	60D	Colonization	32	2	1	≤2	None	Survived	④	④	⑪									
8	557	23w0d	RDS	Skin	8D	Cutaneous infection	32	2	1	≤2	AMPH-B	Survived			②	②	⑥	⑫	⑮	⑮	⑩			
9	965	27w3d	RDS	Skin	7D	Colonization	32	2	1	≤2	None	Survived				③	⑪	⑥						
10	457	23w0d	RDS	Blood	8D	SIP	32	1	2	≤2	AMPH-B +FLCZ	Dead				③	③							
11	525	26w3d	RDS	Sputum	20D	Colonization	32	1	1	≤2	None	Survived				②	⑥	⑫	③					
12	729	25w0d	RDS	Skin	5D	Colonization	32	2	1	≤2	None	Survived						①	①	①	⑨	⑨	⑨	
13	641	24w0d	RDS	Skin	18D	Colonization	32	2	1	≤2	None	Survived						③	③	③	⑧			
14	501	23w6d	RDS	Sputum	13D	Colonization	32	2	1	≤2	FLCZ	Survived											②	⑧

(B)

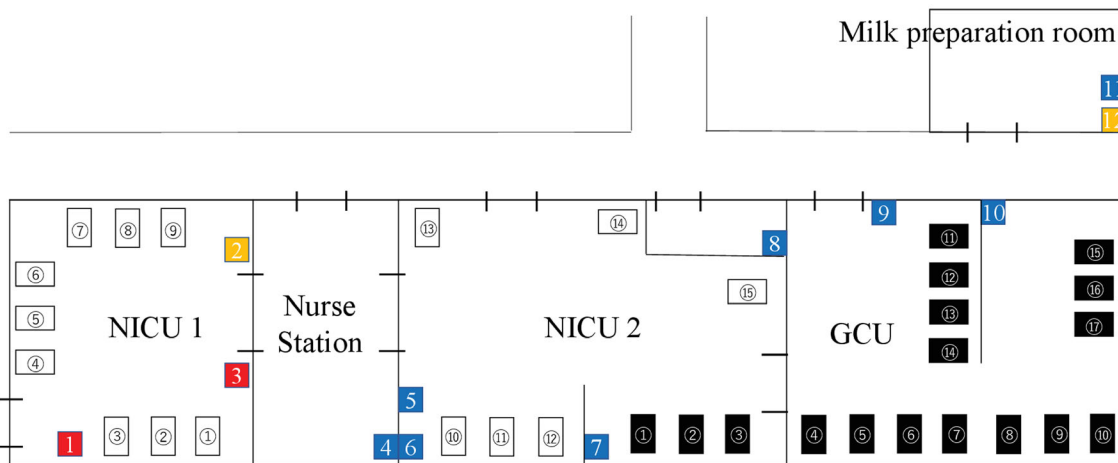


Figure 2. (A) Patient characteristics and locations and (B) schematic map of the neonatal intensive care unit (NICU). The circled numbers in white and black in A and B correspond to one another. In B, white and black rectangles represent the beds in the NICU and the growing care unit (GCU), respectively. Red, yellow, and blue squares in B represent fluconazole-resistant *Candida parapsilosis*-positive, fluconazole-susceptible *C. parapsilosis*-positive, and negative sinks, respectively. Note: *The period of detection, quantified in days (D), between the patient’s admission to the NICU and the initial detection of fluconazole-resistant *C. parapsilosis* in either clinical specimens or through active surveillance. Abbreviations: NICU, Neonatal intensive care unit; GCU, growing care unit; CVC, central venous catheter; CRBSI, Catheter-related bloodstream infection; SIP, spontaneous intestinal perforation; AMPH-B, Amphotericin B; FLCZ, fluconazole; MCFG, micafungin; CLSI, the Clinical and Laboratory Standards Institute

in nurses significantly improved (83/108 [77%] vs 95/105 [90%] opportunities, $P < .05$), whereas that in physicians remained unchanged (10/14 [71%] vs 10/19 [53%] opportunities, $P \geq .05$).

On the direct observation on November 19, 2020, after the cluster, the overall HHC rate was observed to be similar to that at the baseline level (94/128 [73%] vs 103/147 [71%] opportunities, $P \geq .05$). However, there was a noticeable decline in the HHC rate among nurses, reverting to baseline levels (83/108 [77%] vs 92/127 [72%] opportunities, $P \geq .05$).

Environmental investigation

C. parapsilosis was detected in 5 of the 35 environmental cultures, which included samples from a medical device (diaper scale) in the NICU, three sinks (Nos. 1 to 3) in the NICU, and one sink (No.12)

in the milk preparation room (Figure 2). It was not detected in sink No.4 used for infusion disposal. As shown in Figure 2, FRC strains were isolated from two sink surfaces/drains (sink No.1 and 3) near NICU beds No.1 through 4, which were occupied by 13 out of 14 patients (93%) at admission. Other *Candida* species including *C. albicans*, *C. guilliermondii*, *C. intermedia*, and *C. lusitanae* were detected in 9 out of 12 cultures (75%) from sink drains and were not detected in 9 cultures from high-touch surfaces, 10 from medical devices or 4 from an incubator.

Antifungal susceptibility testing

The MICs of all the FRC strains, comprising 2 isolated from the environment and 14 derived from patients, as determined by the fully automated analyzer, were further confirmed using the CLSI

Table 1. Hand hygiene compliance rates of the staff of the neonatal intensive care unit

	HHC rate (the number of observed HH actions/the total number of opportunities)							
	Overall	Physician	Nurse	WHO's five moments for HH				
				Before touching a patient	Before performing a sterile or aseptic procedure	After potential exposure to body fluids	After touching a patient	After touching the patient's immediate environment
May 18, 2018 (Baseline)	94/128 (73%)	10/14 (71%)	83/108 (77%)	28/34 (82%)	11/14 (79%)	3/7 (43%)	21/28 (75%)	20/25 (80%)
June 28, 2019 (Before HH education)	82/116 (71%)	12/25 (48%)	69/89 (78%)	32/42 (76%)	3/12 (25%) ^a	4/7 (57%)	27/33 (82%)	16/22 (73%)
November 27, 2019 (After HH education)	107/132 (81%)	10/19 (53%)	95/105 (90%) ^a	42/48 (88%)	8/12 (67%)	3/3 (100%)	36/45 (80%)	18/24 (75%)
May 19, 2020 (After the cluster)	104/131 (79%)	8/13 (62%)	96/118 (81%)	33/40 (83%)	14/23 (61%)	4/6 (67%)	31/37 (84%)	22/25 (88%)
November 19, 2020 (After the cluster)	103/147 (70%)	10/19 (53%)	92/127 (72%)	37/53 (70%)	19/28 (68%)	5/7 (71%)	18/28 (64%)	24/31 (77%)

Note. Abbreviations: WHO, World Health Organization; HHC, hand-hygiene compliance; HH, hand hygiene.

^aStatistically significant difference from the baseline ($P < .05$).

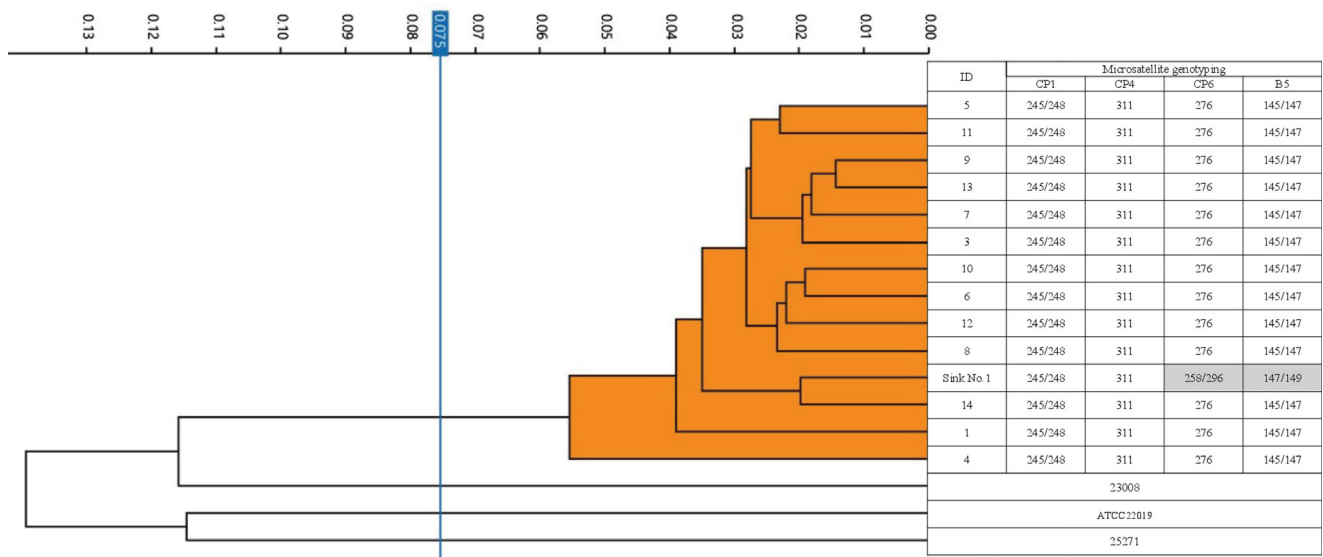


Figure 3. Fourier-transform infrared spectroscopy-based dendrogram of the one standard strain of *C. parapsilosis* (ATCC22019) and two epidemiologically unrelated clinical isolates from the university hospital (23008 and 25271), microsatellite alleles and fluconazole susceptibility of the *Candida parapsilosis* trailing strain. The blue line represents the automatic cutoff value (0.075). Clusters composed of the trailing strains are shaded orange. Gray boxes indicate the allele numbers that differ from the others.

method. These strains with MIC values of ≤ 2 were found to be susceptible to fluconazole (Figure 2). Although no quantitative definition exists, the trailing effect is often defined as the presence of residual growth in wells containing antifungal agents at concentrations above the MIC.^{12,13} These isolates showed residual growth in wells containing 32 $\mu\text{g/mL}$ of fluconazole using broth microdilution procedures, indicating the trailing effect.¹² All *C. parapsilosis* isolates with MIC values of ≤ 2 , including the fluconazole-trailing strains, were susceptible to amphotericin B and micafungin, as determined by the automated analyzer.

Phylogenetic analysis

Phylogenetic analysis utilizing FT-IR spectroscopy was performed on a total of 14 fluconazole-trailing strains, comprising 13 isolates

derived from patients and one isolated from sink No.1 (Figure 3). Strains derived from patient No.2 and sink No.3 were excluded due to lack of preservation. The phylogenetic analysis showed that all the included isolates were clustered together and separated from epidemiologically unrelated strains, including ATCC 22019 (Figure 3). A cutoff value of 0.075 was determined for cluster attribution. Microsatellite genotyping also indicated that all pseudo-resistant isolates from patients had the same genotype; however, an isolate from sink No.1 was assigned to a genotype different from the other patient-derived isolates (Figure 3).

Environmental infection control

To enhance environmental infection control, the following comprehensive interventions were implemented. Infection control

nurses checked the cleaning practices of high-touch environmental surfaces to ensure thorough compliance. In September 2019 and January 2020, sinks with *Candida* species were disinfected using a commercially available 0.5% accelerated hydrogen peroxide (AHP)-based disinfectant (Hyprox accele™, Virox Technologies Inc., Oakville, ON, Canada). Hydrogen peroxide, including AHP, has been demonstrated to be safe for both humans and the environment.¹⁴ Its germicidal efficacy against fungi including *Candida* species has been widely documented.^{15–17} The Centers for Disease Control and Prevention guidelines include AHP as an effective agent for disinfecting environmental surfaces.¹⁷ The sink bowls were covered with Virox-soaked gauze for 5 min,¹⁷ then wiped with clean gauze. The drains were flushed with Virox and drainage pipes (tailpieces, traps, and drain extensions) were replaced. After disinfection, the resampled cultures from the sink drains showed no fungal species. Additionally, three 10-min cycles of Ultraviolet-C (UV-C) irradiation using MoonBeam™3 (Diversey, Fort Mill, SC, USA) were applied in the milk preparation room in January 2020.

Discussion

This study describes the successful containment of a cluster of IC caused by fluconazole-trailing *C. parapsilosis* in the NICU using multiple infection-control interventions. The study highlights the role of sink drains as important reservoirs for *C. parapsilosis*, underlining the importance of maintaining HHC in healthcare providers, environmental hygiene, and regular monitoring to prevent nosocomial transmission in the NICU.

According to a previous systematic review, lower incidence rates of hospital-acquired infection can be achieved with HHC rates of over 60%.¹⁸ During the study period, the overall HHC rate consistently exceeded 70%, although the possibility of overestimation due to the Hawthorne effect cannot be disregarded.¹⁹ Notably, the rate before performing a sterile or aseptic procedure was significantly lower at 25% before the HH education. The observed low HHC rate before performing a sterile or aseptic procedure might have been a contributing factor to the cluster, including three cases of catheter-related BSI. Furthermore, the HHC rates in physicians were markedly lower than those in nurses as previously reported,^{20,21} averaging around 50%, and showed no significant improvement after HH education. Conversely, the HHC rate in nurses improved significantly to 90% following the education but regressed to 72% after 1 year, returning to the baseline level. Despite the lower number of observed HH opportunities for physicians compared to nurses, and the variability in the number of opportunities among the WHO's five moments for HH, this study demonstrated that the HH education should be prioritized for physicians and the appropriate moments for HH, and should be implemented regularly to maintain high HHC rate.

Environmental investigations revealed that only sinks near the incubators used by patients infected or colonized with a fluconazole-trailing *C. parapsilosis* strain at admission were contaminated with that strain, suggesting that the sinks were the source of the cluster. Sinks are a major reservoir of pathogenic microorganisms.^{22,23} Previous studies have indicated a correlation between nosocomial infection of *C. parapsilosis* and contaminated water environments.^{2,24} *Candida* species that colonize sink drains are frequently dispersed through the splattering of water, and this dispersion may lead to the contamination of medical devices or the hands of healthcare providers.²⁵ *C. parapsilosis* is a common cause

of outbreaks in NICUs because it forms biofilms that adhere to environmental surfaces and medical devices and is spread via the hands of healthcare providers.^{2,26,27} Therefore, in addition to monitoring and preventing contamination of sinks with *C. parapsilosis*, maintaining HHC in healthcare providers is crucial in preventing the acquisition of *C. parapsilosis* and the occurrence of IC in the NICU.

While it can be challenging to entirely eradicate pathogens from contaminated sinks, previous studies have demonstrated that implementing risk mitigation measures, such as sink cleaning and disinfection, is effective in interrupting clusters.²⁸ In this study, sink surfaces contaminated with *Candida* species were successfully decontaminated using AHP. Furthermore, UV-C irradiation was applied in the milk preparation room, predicated on the hypothesis of potential environmental contamination. The milk preparation room, utilized for both formula and donor milk preparation by nursing staff and expression of milk by mothers, presents a risk for pathogen transmission to infants through milk, milk preparation equipment, and environmental surfaces when milk handling procedures and HH practices are inadequate.²⁹ UV-C devices are frequently used as an adjunct to standard cleaning in healthcare settings and are effective against *Candida* species on environmental surfaces.^{30,31} Meanwhile, the most effective cluster mitigation strategy involves the replacement of sink components, despite the costs and labor-intensive nature.²⁸ In this study, the drainage pipes, which are particularly challenging to decontaminate using disinfectants, were replaced.

Owing to the trailing effect, the automated analyzer can occasionally misread the MIC and report pseudo-resistance.^{13,32} Thus, confirmation might be required when high MICs are observed, particularly in regions where resistant strains are uncommon. To date, knowledge regarding the molecular mechanisms responsible for the trailing effect is limited, and the clinical implications of the trailing effect remain unclear.^{12,33} In this study, four out of five infants infected with the fluconazole-trailing *C. parapsilosis* strain were successfully treated with amphotericin B or micafungin. One case of peritonitis resulted in fatality despite combined treatment with amphotericin B and fluconazole, although no definitive evidence indicated that fungal infection directly contributed to the cause of death. In premature infants, who are particularly susceptible to IC, the potential influence of the trailing effect should be considered when selecting antifungal agents, as it may affect their efficacy.¹²

Strain typing of *Candida* species is useful for identifying nosocomial transmission by elucidating the clonality among strains, and for determining the infection route by linking clinical isolates with environmental reservoirs.² Although several discriminating molecular typing methods have been developed for *Candida* spp., including random amplified polymorphic deoxyribonucleic acid typing and amplified fragment length polymorphism fingerprinting, these methods require highly trained staff and are time-consuming, expensive, and unsuitable for real-time surveillance.^{24,34} In this study, phylogenetic analysis based on FT-IR spectroscopy helped detect the clonal spread of a fluconazole pseudo-resistant strain among patients. This finding was substantiated through microsatellite genotyping. While not considered the gold standard, this method represents the most widely used high-resolution approach for typing *C. parapsilosis*.³⁵ FT-IR spectroscopy has previously proven to be useful for typing bacterial and fungal species (including *C. albicans* and *C. auris*) to investigate hospital outbreaks.^{34,36} To our knowledge, however, this is the first study to examine its application to *C. parapsilosis*.

In this study, the results obtained through FT-IR spectroscopy and microsatellite genotyping were consistent for the patient-derived isolates, whereas discordance was observed for the environment-derived isolates. FT-IR spectra can fluctuate depending on the conditions during cultivation and sample preparation, and a standard method for the phylogenetic analysis of *C. parapsilosis* has not yet been established.^{34,36} Although further optimization and evaluation are needed to enhance its discriminatory power, FT-IR spectroscopy is rapid, easy to use, and inexpensive compared to other typing methods.³⁴ FT-IR spectroscopy is a promising tool for the early detection of nosocomial transmission of *C. parapsilosis*.

Our study had some limitations. First, owing to the simultaneous implementation of multiple measures in the study, it was challenging to ascertain which measure exhibited the highest efficacy. Second, the cause of the trailing presented by *C. parapsilosis* in this study is unknown because molecular investigations related to antifungal susceptibility have not been conducted. Third, the transmission of *C. parapsilosis* via the hands of healthcare providers remains unconfirmed because we did not examine the contamination of healthcare providers. Nevertheless, a cluster of invasive candidiasis caused by fluconazole-trailing *C. parapsilosis*, presenting with fluconazole trailing, in the NICU was successfully treated by implementing targeted infection-control interventions for contaminated sinks, identified using an environmental investigation, and the milk preparation room, and by improving HHC among NICU staff. Ensuring environmental hygiene and constant staff education to maintain a high HHC level is necessary to limit the spread of *C. parapsilosis* strains in the NICU.

Supplementary material. The supplementary material for this article can be found at <https://doi.org/10.1017/ash.2024.77>.

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