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Original Article

Genotypic and phenotypic characteristics of *Candida parapsilosis* bloodstream isolates: Health Care Associated Infections in a teaching Hospital in Italy

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ABSTRACT

Background: Candidemia is the most common healthcare associated invasive fungal infection. Over the last few decades, candidemia caused by Candida species other than *Candida albicans*, particularly the *Candida parapsilosis* complex, has emerged worldwide. The aims of this study were: to analyze the genotypic and phenotypic characteristics of *C. parapsilosis* strains isolated from blood cultures and the environment in a hospital in southern Italy, to study the possible source of infection and to correlate the isolated strains.
Methods: From April to October 2022, cases of candidemia due to *C. parapsilosis* in patients admitted to a hospital in the Apulia region were investigated. However, 119 environmental samples from the intensive care unit were collected for identification of the likely environmental reservoir of infection. Routine antifungal (amphotericin B, anidulafungin, fluconazole) susceptibility was performed on all isolates. Whole genome sequencing was performed to study the genotypic correlation of the isolates. Biofilm biomass and metabolic activity were also quantified for all isolates.
Results: A total of 43 *C. parapsilosis* isolates were cultured from the bloodstream of each patient in different departments, and seven surface samples were positive for *C. parapsilosis*. Most of the isolated yeasts (41/50; 85 %) were resistant to fluconazole and were genetically related to each other, suggesting an ongoing clonal outbreak of this pathogen. The fluconazole-susceptible isolates produced significantly more biofilm than did the resistant isolates. Metabolic activity was also higher for fluconazole-susceptible than resistant isolates.
Conclusion: Cross-transmission of the microorganisms is suggested by the phenotypic similarity and genetic correlation between clinical and environmental strains observed in our study.
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Abbreviations: HCAIs, Health care associated infections; ECDC, European Center for Disease Prevention and Control; BSI, bloodstream infection; ICU, Intensive Care Unit; NAC, non-albicans Candida; MALDI-TOF MS, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry; MICs, Minimum inhibitory concentrations; FR, Fluconazole-Resistant; FS, Fluconazole-Susceptible; SNPs, single nucleotide polymorphisms

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1. Introduction

Health care associated infections (HCAIs) represent one of the most frequent adverse events in healthcare, often responsible of outbreaks [1,2]. US Center for Disease Control and Prevention estimates nearly 1.7 million hospitalized patients annually acquire HCAIs during hospitalization and one in 17 these patients die due to HCAIs [3]. ICUs are the hospital settings with the highest prevalence of HCAIs due to the presence of patients particularly susceptible as they are seriously ill and immunosuppressed. In fact, European Center for Disease Prevention and Control (ECDC) reports in 2019 that of all patients staying in an ICU for more than two days, 4 % developed with pneumonia, 3 % with bloodstream infection (BSI), and 2 % with urinary tract infection [4]. However, a high percentage of these infections are preventable through effective infection prevention measures [5].

Among microorganisms responsible for HCAIs, an ever more important role is played by the genus *Candida* spp. In Europe the incidence of candidemia in 23 ICUs was averagely 5.52 episodes per 1000 ICU admissions [6] and the EPIC II study previously reported a prevalence of 6.87 episodes of candidemia per 1000 ICU patients [7].

Although *Candida albicans* is identified as the main cause of invasive infection, an increase of infections caused by non-*albicans Candida* (NAC) species has emerged during last decades [8].

The international epidemiology of *Candida* spp. infections is variable [9]. In some European countries prevails *Candida glabrata* as NAC after *C.albicans*, while in others *C. parapsilosis* is the most widespread NAC species [10,11]. *Candida parapsilosis* is a commensal microorganism of human skin but human-to-human transmission can occur through external contaminated sources. This transmission is supported by its propensity to colonize hands, to form biofilms on inert substrates such as intravascular catheters, especially in the presence of parenteral nutrition, making it one of the most concerning species in the ICU [8,12]. The remarkable ability to form biofilms on medical devices is thought to allow *C. parapsilosis* to cause of severe clonal epidemics [13]. *C. parapsilosis* infections can cause tissue seeding, resulting in deep infections. Furthermore, *C. parapsilosis* is reported to be the predominant disease-associated species in patients treated with Continuous Ambulatory Peritoneal Dialysis [14,15]. However, rare cases of endocarditis [16] and meningitis are documented [15].

In addition, during last decades lower susceptibility to echinocandins and azole-resistant *C. parapsilosis* strains are emerged [17]**,** making the antifungal treatment more complicated [8].

In our large teaching hospital in Southern Italy, *C.albicans* still represents the most frequently isolated species from blood while *C. parapsilosis* ranked second with the highest prevalence in the oncohaematologic population [18]. The same hospital, during last years we observed an increasing number of invasive infections due to *C. parapsilosis* in patients hospitalized in our ICUs.

Indeed, in the first trimester of 2022 we noted a total number of 48 *Candida* BSIs of which 23 (47.9 %) were caused by *C. parapsilosis*. In particular in ICU we noted a total number of 12 *Candida* BSIs of which 10 (83 %) were caused by *C. parapsilosis*.

Therefore, we surveyed the cases of candidemia due to *C. parapsilosis* in patients hospitalized in our institution during a seven-month period (from April to October 2022). To detect the possible source of infections, human and environmental samples were collected in the same time period. The primary aim of this study was to analyse the genotypic and phenotypic characteristics of the isolated strains, the secondary aim was to investigate the possible infection source and correlation among the isolated strains.

2. Materials and methods

2.1. Hospital settings

During period April - October 2022, we carried out a survey of candidemia cases due to *C. parapsilosis* in patients hospitalized in our university hospital in Apulia region, Southern Italy. This hospital attends to cases of high complexity in medicine and surgery, including solid organ and hematopoietic stem cell transplants and there are 5 medical and 4 surgery intensive care units. This large teaching hospital has approximately 1400 beds and it is composed of 33 physically separate buildings. Given the high number of *C. parapsilosis* candidemia particularly in ICU and according to internal hospital protocols (i.e.: mandatory to carry out an epidemiological investigation when more than two episodes of illness caused by the same microorganism in the same ward is occurring within a month), we started a prospective investigation. This ICU ward has 32 beds and carries out over 600 admission a year.

2.2. Microbiological clinical investigation

Blood cultures were made using BactAlert (Biomèrieux, Marcy l'Etoile, France). Samples reported as positive by the instrument were grown on selective media for bacteriology. For fungal detection, samples were plated onto Sabouraud dextrose agar with 0.05 % chloramphenicol (Biomèrieux, Marcy-l'Etoile, France) and incubated at 36 °C ± 1 for 5 days.

Matrix-assisted desorption/ionisation-time-of-flight mass spectrometry (MALDI-TOF-MS, Biomérieux, Marcy-l'Etoile, France) was used to identify all yeast isolates. All isolates of *C. parapsilosis* were stored at −80 °C.

In this study, an episode of candidemia was defined as a Candida infection in which at least one blood culture was obtained. Only the first episode of BSI caused by *C. parapsilosis* was recorded and analyzed for each patient.

2.3. Environmental investigation

The surfaces of rooms and equipment as well as hands of healthcare workers were sampled to evaluate the possible presence of the same microorganism in the environment. In total 119 samples were collected, of which 72 on high-touch surfaces and 47 on the healthcare personnel hands. The following surfaces were sampled: 14 touch screen multiparametric monitors, 13 touch screen monitors for assisted ventilation, 13 bed rails, 12 syringe infusion pumps, 5 hand disinfectant dispensers, 3 computer keyboards, 2 cardiac probes, 2 sink tap handle, 2 ultrasound keyboards, 1 vascular access trolley, 1 vascular access needle storage trolley, 1 medicine refrigerator handle, 1 glove box, 1 drawer handle, 1 telephone.

The surfaces sampling was performed using sterile swabs containing 10 ml of transport medium (Liofilchem Srl, Roseto degli Abruzzi, Italy), according to the indications of UNI EN 17141:2021 [19]. Flat and large surfaces (regular surfaces, i.e.: carts, workstations, monitors), were sampled with a swab over a well-defined area (10 ×10 cm), using a delimiter, while for small and curved surfaces (surfaces e.g. probes, infusion pumps) the available area was sampled. For each healthcare worker a single swab was used for both hands. All swabs were transported to the laboratory in refrigerated containers at +4 °C and immediately tested.

Swabs were seeded on plates of Sabouraud dextrose agar supplemented with 0.05 % chloramphenicol (Biomèrieux, Marcy l'Etoile, France), incubated at 30 °C ± 1, and examined daily for 5 days. The yeast isolates were identified as described above.

2.4. Antifungal susceptibility testing

The routine antifungal susceptibility to amphotericin B, caspofungin, micafungin, 5-fluorocytosine, fluconazole and voriconazole was carried out by VITEK®2 YST 07 card (Biomèrieux, Marcy l'Etoile, France.

To confirm the susceptibility of organisms, all *C. parapsilosis* isolates were tested for amphotericin B, fluconazole and anidulafungin (Sigma-Aldrich) by broth microdilution method. The experiments were conducted in triplicates and were performed following the EUCAST methodology [20]. *C. parapsilosis* ATCC 22019 was used as quality control.

2.5. DNA extraction, WGS, and sequence analysis

Genomic DNA was extracted using the commercial E.Z.N.A. Soil DNA kit (Omega bio-tek) following the manufacturer's instructions. Libraries were prepared with the Illumina DNAprep Kit and sequenced on Illumina MiSeq platform using a 2 x 250 bp paired-end approach. Raw reads were assembled by SPAdes- software v. 3.13.1) (https://github.com/ablab/spades). Genetic relatedness among *C. parapsilosis* isolates was carried out using CSIPhylogeny [21] and the *C. parapsilosis* FDAARGOS_650 whole genome (Acc. no. JABVZZ010000005.1) used as reference genome in phylogenetic assays and *ERG*11 BLAST analyses (BLAST: Basic Local Alignment Search Tool (nih.gov)). Isolates were clustered as group of three or more strains that originated from a common ancestor with a distance threshold of less than 0.01 from the origin.

2.6. Growth curves

Growth curves were performed by measuring $OD_{450\ nm}$ every hour for 24 h with a multiplate reader (Multiscan Ascent, Thermo). Briefly, 2–5 × 10^5 CFU/ml were seeded in RPMI-1640 and incubated at 35 °C.

2.7. Quantification of biofilm biomass

The yeast inoculum (1–5 ×10^6) was incubated in flat-bottom 96 well plates at 35 °C for 24 h. After three washes with PBS, the plates were incubated 1 h at 60 °C for fixation. 100 µl of 1 % crystal violet (CV) was added to each well to stain the fixed biofilm. The plates were incubated at room temperature for 10 min. The wells were washed with PBS three times to remove the excess of CV and 100 µl of ethanol 80 % was added to solubilize the dye. The plates were incubated and shaken at room temperature for 10 min. The absorbance was read at 570 nm with a multiplate reader (Multiscan Ascent, Thermofisher).

2.8. Determination of metabolic activity

Briefly, the yeast inoculum (1–5 ×10^6) was incubated in flat-bottom 96 well plates at 35 °C for 24 h. Two hours before the end incubation time, 100 µl of a 2.5 mg/ml XTT and 1 µM menadione solution were added to each well. Plates were incubated in the dark for 2 h at 35 °C. The absorbance for each well was read at 450 nm with a multiplate reader [22].

3. Results

During the survery period (April-October 2022), 124 patients developed *Candida* spp. sepsis, of which 54 (43,5 %; 54/124)caused from *C.parapsilosis*. In this period 43 clinical strains of *C. parapsilosis* isolated from the first positive blood culture were available and studied,of which 16 (37.20 %) from ICU patients, 25 (58.14 %) from patients in other departments of the same hospital; 1 (2.33 %) strain isolated from a blood culture from the day hospital and 1 (2.33%) strain isolated from a child patient in a pediatric ward of same hospital but located in a different area, about 3 km to the central hospital.

Seven of 72 high-touch surfaces samples (9.7 %) were positive of *C. parapsilosis*, in particular: 3/13 (23 %) from touch screen monitors for assisted ventilation, 2/12 (16 %) from syringe infusion pumps and 2/3 (66 %) from the reception stations computer keyboards. All 47 swabs performed on the hands of as many healthcare workers resulted negative. Therefore, a total of 50 isolates of *C. parapsilosis* (43 from humans and seven from the environment) were considered for further analysis.

All isolates were susceptible to amphotericin B and anidulafungin with MICs ranges of 0.06–0.5 and 0.25–1 µg/ml, respectively (Table 1). Only 9/50 isolates (18%) were fluconazole susceptible with MICs range of 0.5–1 µg/ml, the other isolates 41/50 (82 %) were resistant to the antifungal with MICs of 64–128 µg/ml. All Fluconazole-Resistant (FR) strains harbored the amino acid substitution Y132F in the protein ERG11, in addition *C. parapsilosis* n. 19 also harbored D355N. Finally, 5/7 Fluconazole-Susceptible (FS) strains harbored the amino acid substitution R398M in the protein ERG11.

The Table 1 shows how all *C. parapsilosis* environmental strains were fluconazole resistant exactly like clinical strains isolated from patients recoverd in ICU, reporting the same amino acid substitution Y132F in the protein ERG11.

The Fig. 1 shows the amount of biomass (A) and the metabolic activity (B) of the biofilm of all isolates. FS isolates produced a significantly higher amount of biofilm than FR isolates (Fig. 1**A**). Similarly, the metabolic activity was higher in FS than FR isolates (Fig. 1**B**). FS isolates produced absorbance ranges of 0.500–1.126 OD_{570} and 0.203–1.07 OD_{450}, by CV staining and XTT assay, respectively. Conversely, FR isolates produced absorbance ranges of 0.128–0.380 OD_{570} and 0.017–0.116 OD_{450} by CV staining and XTT assay, respectively. The only exception were the isolates 22 and 33 which were weak biofilm producers.

Growth curves of individual isolates are presented in Fig. 2A-B. While a wide growth variation among FR clinical isolates was detected, there was a trend towards of a lower growth rate among susceptible isolates compared to environmental isolates. Two representative growth curves are shown in Fig. 2B by comparing *C. parapsilosis* No. 37 (highest growth rate among FS isolates) to *C. parapsilosis* No. 44 (lowest growth rate among environmental strains).

Fig. 3 shows the phylogenetics relationship among 50 isolates of *C. parapsilosis*. SNPs differences between all isolates were minimum 171 and maximum 3245. In particular, FR isolates showed SNPs range of 171–723 whereas FS strains showed more differences with SNPs range of 190–2747. *C. parapsilosis* No. 20, 22, 25, 37 and 38 were further phylogenetically distant than the other yeasts. The phylogenetic three highlighted six groups/clusters. Group I contained *C. parapsilosis* No. 4, 11, 12, 16, 23, 27, 28, 29, 40, 46; Group II *C. parapsilosis* No. 6, 13, 14, 48, 49, 50; Group III *C. parapsilosis* No. 2, 10, 15, 24, 34, 36, 40; Group IV *C. parapsilosis* No. 1, 3, 18, 21, 32, 41, 42, 44, 45, 47; Group V *C. parapsilosis* No. 5, 8, 19, 26, 31, 35, 39, 43 and Group VI *C. parapsilosis* No. 7, 9, 17 and 33.

Group I, II and IV showed SNPs range of 171–275, 209–438 and 181–382, respectively. Furthermore, in all 3 groups there was at least one isolate of environmental origin. Group III and V did not contain any environmental isolates and showed SNPs range of 194–651 and 181–5250 respectively. Finally, Group VI was formed only by fluconazole-susceptible clinical strains and showed SNPs range of 190–267 (Fig. 3).

Table 1
Phenotypic and genotypic characteristics of 50 isolates of *C.parapsilosis* considered in the study.

Isolate No	MIC (μg/ml)			Biofilm	Amino acid substitutions
	AMB[a]	FLU[b]	ANID[c]	CV[d]	ERG 11p[e]
1	0.25	64	0.25	NP[f]	Y132F
2	0.125	64	0.5	NP	Y132F
3	0.125	64	0.5	NP	Y132F
4	0.25	64	0.5	NP	Y132F
5	0.25	128	0.5	NP	Y132F
6	0.125	128	0.25	NP	Y132F
7	0.25	0.5	1	WP[g]	WT[h]
8	0.125	64	0.25	NP	Y132F
9	0.125	1	0.5	MP[i]	WT
10	0125	128	0.25	WP	Y132F
11	0.125	128	0.25	WP	Y132F
12	0.125	128	0.25	WP	Y132F
13	0.125	64	0.5	WP	Y132F
14	0.25	128	0.5	WP	Y132F
15	0.125	64	0.25	WP	Y132F
16	0.125	64	0.25	WP	Y132F
17	0.125	0,5	1	MP	WT
18	0.125	64	0.5	WP	Y132F
19	0.125	128	0.25	WP	Y132F, D355N
20	0.25	0,5	0.25	SP[j]	R398M
21	0.125	128	0.25	NP	Y132F
22	0.125	0,5	0.5	WP	R398M
23	0.125	64	1	NP	Y132F
24	0.125	128	1	WP	Y132F
25	0.125	0.5	1	SP	R398M
26	0.125	128	0.5	NP	Y132F
27	0.125	128	0.5	NP	Y132F
28	0.5	64	0.5	NP	Y132F
29	0.125	64	0.5	NP	Y132F
30	0.125	128	0.5	WP	Y132F
31	0.06	64	0.5	WP	Y132F
32	0.125	64	0.5	WP	Y132F
33	0.125	0.5	1	WP	WT
34	0.125	128	0.5	NP	Y132F
35	0.125	128	0.5	WP	Y132F
36	0.5	128	0.5	NP	Y132F
37	0.06	0.5	0.25	MP	R398M
38	0.25	0.5	1	SP	R398M
39	0.25	128	0.5	WP	Y132F
40	0.125	128	0.5	WP	Y132F
41	0.125	128	0.5	NP	Y132F
42	0.125	128	0.5	NP	Y132F
43	0.25	128	0.5	WP	Y132F
44[k]	0.25	128	0.5	WP	Y132F
45	0.25	128	0.5	WP	Y132F
46	0.25	128	0.5	WP	Y132F
47	0.25	128	0.5	WP	Y132F
48	0.25	128	0.5	WP	Y132F
49	0.25	128	0.5	NP	Y132F
50	0.125	128	0.5	WP	Y132F
C. parapsilosis ATCC 22019	0.25	1	0.5	-	-

[a] AMB, amphotericin B
[b] FLU, fluconazole
[c] ANID, anidulafungin
[d] CV, cristalviolet staining
[e] ERG11p, protein ERG11
[f] NP, not producer
[g] WP, weak producer
[h] WT, wild type
[i] MP, medium producer
[j] SP, strong producer
[k] In bold, environmental isolates.

4. Discussion

Despite efforts to control the infections in healthcare setting, HCAIs continue progressively to escalate with infection rates 3–20 times higher in low-income countries. In addition to Gram-negative and Gram-positive bacteria infections, invasive *Candida* infections often complicate the clinical course of the immunocompromised patients.

C. albicans still represents the most common cause of invasive candidiasis, however infections due to *Candida* non-albicans are increasing. *C. parapsilosis* complex, which includes *C. parapsilosis sensu strictu*, *C.orthopsilosis* and *C. metapsilosis*, has emerged as the second to fourth most common species of *Candida* causing invasive infections [23–26]. Although the mortality rate due to this species is generally lower than that reported for other *Candida* species, *C. parapsilosis* complex possesses several distinct features, such as its high affinity for parenteral nutrition, the ability to develop biofilms on intravascular devices and inert surfaces and an intrinsic low susceptibility to echinocandins [8,27]. Historically this species has been considered susceptible to fluconazole (FS) [8,28], however, in the last years an increasing number of reports describing infections and outbreaks due to FR *C. parapsilosis* (also described in triazole-naïve patients) has been published [29,30].

In this study, we described our experience with the isolation of FR and FS *C. parapsilosis* in several wards of a large teaching hospital in Italy. All our patients infected with *C. parapsilosis* isolates had not been treated with fluconazole moreover, none of them were receiving antifungal prophylaxis when they developed *C. parapsilosis* BSIs.

FR isolates grouped into four clusters which were variably represented in several hospital wards. Since FR isolates were more phylogenetically related than FS isolates, the existence of a common ancestral lineage can be assumed. Moreover, a similar lineage persisted in the environment as demonstrated by the fact that three clusters (I, II and IV) contained environmental strains. This finding supports three types of transmission scenarios: human-to-human, environment-to-human, human-to-environment. Notably, FR isolates had similar or even higher fitness than FS isolates that may explain both the high pathogenic property of resistant strains and their ability to persist in the environment. We showed that all FR isolates carried the *ERG11*^{Y132F} mutation, already observed in FR isolates originating in Europe, mainly in Italy [31]. It is noteworthy that patients infected with FR isolates harboring this mutation had a higher risk of mortality than those infected with other strains [29]. During our survey, three out of 43 patients (7 %) with *C. parapsilosis* candidemia died, all infected with FR isolates, however, the lack of autopsy did not allow us to establish whether the death was due to fungal infection.

It is interesting to note that among the nine FS *C. parapsilosis* strains, the four strains belonging to Group VI had a wild-type *ERG*11. On the contrary, the remaining five strains, which were not phylogenetically related to none of the six groups, harbored the substitution of the amino acid R398M, which does not confer resistance to azoles [13] and could only be considered a compensatory alteration of the ERG11 gene, as a possible adaptation of the strain to the main clusters [32].

Overall, these data lead us to make several considerations in the case of increasing isolation of *C. parapsilosis* in each hospital setting. First, active environmental surveillance followed by establishing severe infection control strategies such as hand care-workers and appropriate use of personal protective equipment monitoring should be implemented to minimize the spread of this microorganism. Second, a phenotypic characterization (i.e.: fluconazole susceptibility testing by standard methodologies) should be considered not only for isolates causing invasive diseases (i.e.: BSIs) but also for strains from non-sterile sites (i.e.: urine or respiratory secretions) and from environment. It must be noted that most fungi recovered from non-sterile sites, especially the environmental ones, are not routinely tested for susceptibility. A colonized patient by a FR *C. parapsilosis* strain, although antifungal therapy is not indicated, should be isolated as it is clearly stated for those patients colonized by *C. auris* [33]. Third, when resistance to fluconazole characterizes a

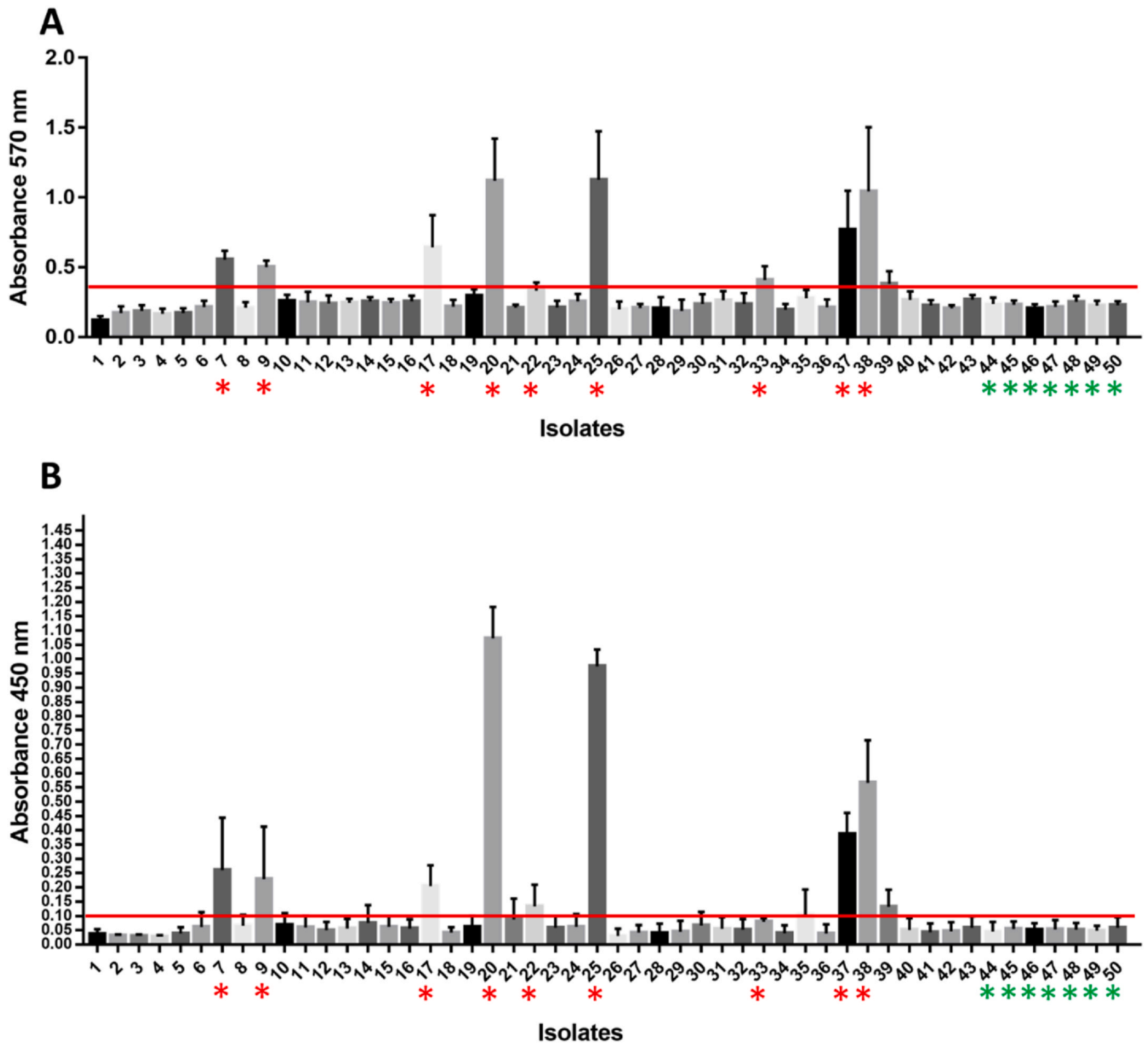

Fig. 1. Representations of the amount of biomass (A) and metabolic activity (B) of the biofilm of *Candida parapsilosis* isolates. Red asterisk highlights strain susceptible to fluconazole and green asterisk environmental isolates. The red lines showed the lowest values obtained from the fluconazole-susceptible isolates.

quite high percentage of *C. parapsilosis* strains in each hospital (i.e.: > 10 %), it would be advisable to start therapy, if clinically indicated, with drugs other than triazoles at least until the *in vitro* susceptibility tests are available.

Generally, *C. parapsilosis* displays higher echinocandin MICs compared with other *Candida* species because of naturally occurring *FKS1* mutations [34,35]. However, clinical trials have revealed that systemic infections sustained by this species and treated with echinocandins yielded outcome like infections caused by other species and treated with the same drugs [36,37]. Similarly, although tolerance to amphotericin B in *C. parapsilosis* has been previously described, resistance to polyenes has been rarely documented [38]. Thus, both drug classes might be utilized in infections due to FR *C. parapsilosis* [37]. Indeed, although the scope of our study did not include a clinical evaluation, it should be noted that all candidemia cases were treated with liposomal amphotericin B alone or associated with anidulafungin. Almost all ICU patients underwent combination therapy while patients hospitalized in other wards were often treated with the polyene alone. These data are still being analyzed and the results will be presented later.

Interestingly, we found a significant difference in biofilm production between FS and FR isolates. In particular, all FR isolates, including the environmental strains, did not produce biofilm or they were weak producers. On the opposite, a higher biofilm production was observed among FS isolates. Our data agree with recent clinical studies suggesting that FR *C. parapsilosis* isolates causing severe outbreaks produce thin biofilms [29,39]. This makes us hypothesize that since the biofilm can protect microorganisms from the action of drugs and disinfectants, mutated and therefore resistant strains have less need to produce this matrix to protect themselves. Indeed, one study showed that FR isolates of *C. parapsilosis* harboring Y132F (the only resistant genotype found in our study) had lower biofilm production capacity than isolates with the double mutation Y132F +K143R [29]. As already hypothesized by Daneshnia *et al.* [30], since

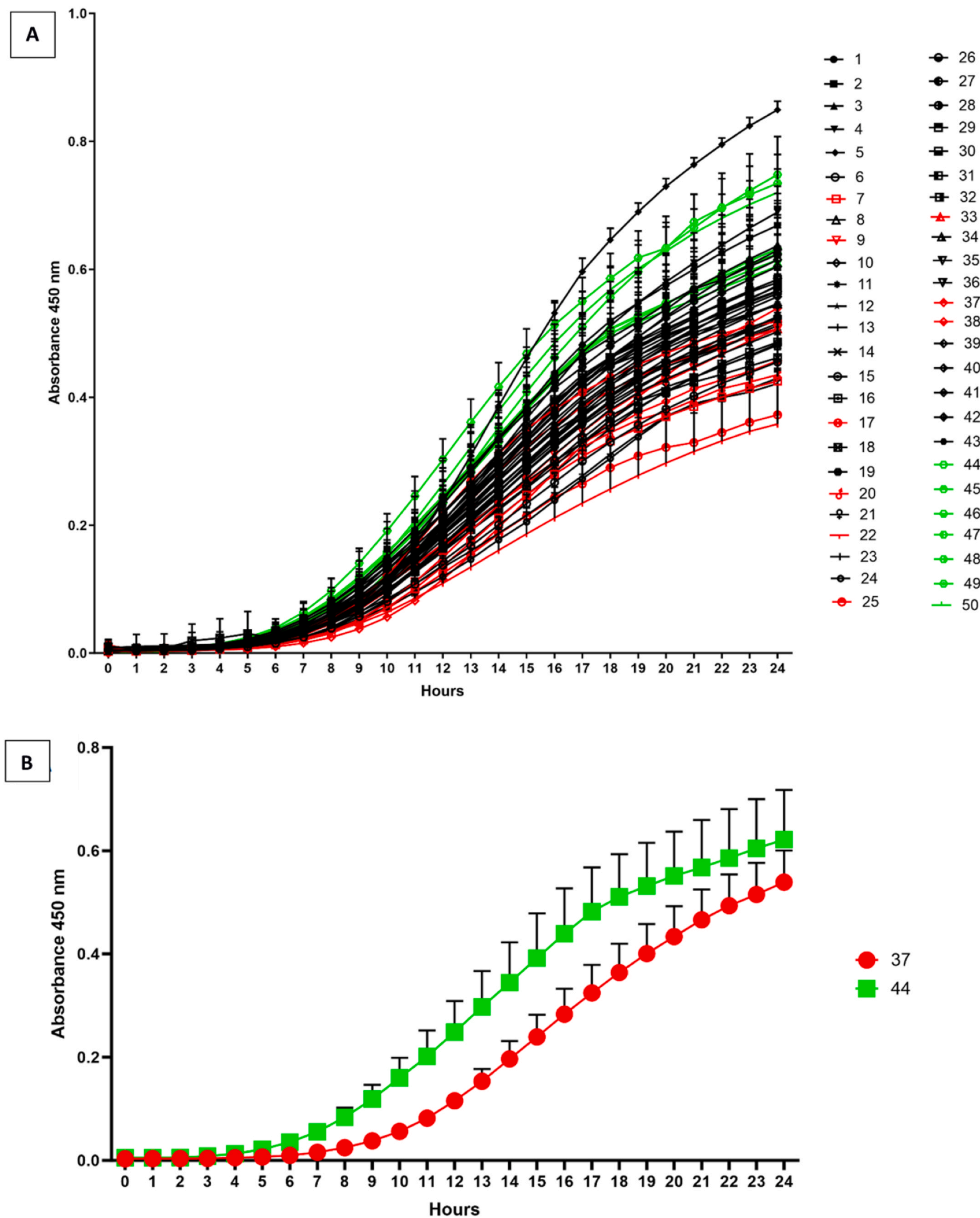

Fig. 2. Representation of the growth patterns of 50 C. parapsilosis strains. Pannell (A): Black, red and green lines indicated the fluconazole-resistant, fluconazole-susceptible and the environmental isolates, respectively. Pannell (B) comparison of the two strains for each group with highest and lowest growth rates.

Fig. 3. Phylogenetic tree of 50 C. parapsilosis strains. Names were highlighted with colours distinguish different groups: red, group I; blue, group II; grey, group III, pink, group IV; green, group V, violet, group VI. The figure was drawn using iTOL v.6 software.

biofilm production is mainly required for survival on abiotic surfaces, one can speculate that our outbreak was primarily caused by human-to-human (i.e.: skin) transmission, during patient assistance manoeuvres. The findings that samples obtained from the hands of healthcare workers were all negative while those from high-touch surfaces were positive for *C. parapsilosis*, make us to hypothesize that the protective devices were not adequately used during care practices.

We previously described an outbreak of fungemia due to FS *C. parapsilosis* occurring in oncological pediatric patients in the same hospital setting [28]. Infection control investigation showed that *C. parapsilosis* colonized the hands of two healthcare workers while the environmental samples were all negative. The genotypic analysis showed a relatedness between the strains isolated from blood cultures and those from both hands of only one of the two healthcare workers thereby suggesting the lack of PPEs use in that specific case [28]. Thus, the difference between the two outbreaks resides in the complete lack (old outbreak [colonized hands]) or incorrect use (current outbreak [colonized keyboard]) of PPEs (i.e.: gloves). Girou *et al.* [40] showed that gloves can become a 'second skin' if worn for long periods without being changed frequently, allowing pathogens to spread not only to the patient but also to the surrounding environment.

Although the transmission of pathogens from one infected patient to another frequently occurs through the hands of healthcare personnel, contaminated surfaces, and medical equipment can play an important role in the transmission of some microorganisms. The presence of these pathogens on the surfaces can represent a risk for both patients and health-care workers [41].

5. Conclusions

Due to increasing frequency of *C. parapsilosis* from the bloodstream of patients recovered in our hospital, we investigated the genotypic and phenotypic characteristics of the strains. Most of the isolates were FR and genetically related, suggesting an ongoing clonal outbreak of this pathogen. The phenotypic similarity and the genetic correlation observed in our study between clinical and environmental strains suggest a cross-transmission in which hospital surfaces and the incorrect use of the most important PPE play a crucial role. These findings emphasize the importance to implement the infection control measures to minimize the spread of these pathogens and optimize the antifungal stewardship to avoid the risk of drug-resistance. In particular, the use of fluconazole (or other triazoles) should be avoided in the presence of high prevalence of *Candida* species with reduced susceptibility to this drug class, especially those causing outbreaks.

Ethical approval statement

This study was reviewed and approved by the Institutional Review Board of the University Hospital of Bari (Study 1338/CE-Approved Prot. 490 - 14/9/2023).

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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