In-vitro activity of 5-fluorocytosine against 1,021 Spanish clinical isolates of *Candida* and other medically important yeasts

Guillermo Quindós[1], María Teresa Ruesga[1], Estrella Martín-Mazuelos[2], Ricardo Salesa[3], Rocío Alonso-Vargas[1], Alfonso J. Carrillo-Muñoz[4], Sonia Brena[1], Rosario San Millán[1], and José Pontón[1]

[1]Departamento de Inmunología, Microbiología y Parasitología, Facultad de Medicina y Odontología, Universidad del País Vasco, Bilbao, Spain; [2]Servicio de Microbiología, Hospital Universitario Valme, Sevilla, Spain; [3]Servicio de Microbiología, Hospital Clínico Universitario Marqués de Valdecilla, Santander, Spain; [4]Departamento de Microbiología, ACIA, Barcelona, Spain

Summary

The aim of this study was to determine the prevalence of primary resistance to 5-fluorocytosine (5FC) among clinical isolates of yeasts in Spain where this drug is not currently available for therapy. We have tested the in vitro activity of 5FC against 1,021 recent yeast clinical isolates, including 522 *Candida albicans*, 140 *Candida parapsilosis*, 68 *Candida glabrata*, 41 *Candida dubliniensis*, 50 *Candida guilliermondii*, 34 *Candida tropicalis*, 28 *Candida krusei*, 20 *Candida famata*, 11 *Cryptococcus neoformans*, 5 *Cryptococcus albidus*, 43 *Rhodotorula* spp., 24 *Trichosporon* spp., 5 *Saccharomyces cerevisiae*, 9 *Pichia* spp., and 21 isolates from other 11 yeast species. The MICs were determined by the ATB Fungus agar microdilution test (bioMérieux, France) and the following interpretive breakpoints were used: susceptible, ≤ 4 µg/ml; intermediate, 8 to 16 µg/ml; resistant, ≥ 32 µg/ml. 5FC was very active against *Candida* spp. and other medically important yeasts as 852 (83.4%) of the studied isolates were susceptible (MIC ≤ 4 µg/ml). The species most susceptible to 5FC were *C. dubliniensis* (100% of isolates; MIC_{90}, 0.25 µg/ml), *C. famata* (100% of isolates; MIC_{90}, 0.25 µg/ml), *C. guilliermondii* (98% of isolates; MIC_{90}, 0.25 µg/ml), *C. glabrata* (95.5% of isolates; MIC_{90}, 0.25 µg/ml), and *C. neoformans* (90.9% of isolates; MIC_{90}, 2 µg/ml). Primary resistance to 5FC was very uncommon, and a MIC ≥ 32 µg/ml, indicator of in vitro resistance, was observed in 106 isolates (10.4%): 77 *C. albicans* (16.5% of isolates; MIC_{90}, > 128 µg/ml), 9 *C. parapsilosis* (6.4% of isolates; MIC_{90}, 8 µg/ml), 4 *C. albidus* (80% of isolates, MIC_{50}, > 128 µg/ml), 3 *C. glabrata* (4.4% of isolates; MIC_{90}, 0.25 µg/ml), 3 *C. tropicalis* (8.8% of isolates; MIC_{90}, 4 µg/ml), 2 *C. krusei* (7.1% of isolates; MIC_{90}, 8 µg/ml), 2 *Rhodotorula* spp. (4.6% of isolates, MIC_{90}, 1 µg/ml), 8 *Trichosporon* spp. (33.3% of isolates; MIC_{90}, 64 µg/ml), and 1 *C. lipolytica* (50% of isolates). Interestingly, most *C. albicans* (67 out of 77 isolates) resistant to 5FC were serotype B isolates.

Key words 5-fluorocytosine, *Candida*, Serotypes, Yeasts, ATB Fungus

Address for correspondence:
Dr. Guillermo Quindós
Departamento de Inmunología,
Microbiología y Parasitología
Facultad de Medicina y Odontología
Universidad del País Vasco
Aptdo. 699, E-48080 Bilbao, Spain
Tel.: +34 94 601 28 54
Fax: +34 94 601 34 00
E-mail: oipquang@lg.ehu.es

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Actividad in vitro de la 5-fluorocitosina sobre 1.021 aislamientos españoles de *Candida* y otras levaduras de importancia médica

Resumen

El objetivo de este estudio ha sido determinar la prevalencia de la resistencia primaria a la 5-fluorocitosina (5FC) entre los aislamientos clínicos de levaduras de importancia médica en España, donde este antifúngico no está comercializado. Hemos evaluado la actividad antifúngica in vitro de la 5FC contra 1.021 aislamientos clínicos recientes de levaduras de interés médico, que incluían 522 *Candida albicans*, 140 *Candida parapsilosis*, 68 *Candida glabrata*, 41 *Candida dubliniensis*, 50 *Candida guilliermondii*, 34 *Candida tropicalis*, 28 *Candida krusei*, 20 *Candida famata*, 11 *Cryptococcus neoformans*, 5 *Cryptococcus albidus*, 43 *Rhodotorula* spp., 24 *Trichosporon* spp., 5 *Saccharomyces cerevisiae*, 9 *Pichia* spp., y otros 21 aislamientos de otras 11 especies fúngicas. Las CMIs se determinaron empleando el método de microdilución ATB Fungus (bioMérieux, Francia). Se adoptaron como puntos de corte para interpretar la sensibilidad o resistencia de los aislamientos, los siguientes: sensible, ≤ 4 µg/ml; sensibilidad intermedia, 8 a 16 µg/ml; resistente, ≥ 32 µg/ml.

La 5FC fue muy activa contra las diferentes especies de *Candida* y otras levaduras de interés médico evaluadas: 852 (83,4%) de los aislamientos clínicos eran sensibles a este antifúngico (CMI ≤ 4 µg/ml). Las especies más sensibles fueron *C. dubliniensis* (todos los aislamientos fueron sensibles; CMI_{90}, 0,25 µg/ml), *C. famata* (todos los aislamientos fueron sensibles; CMI_{90} = 0,25 µg/ml), *C. guilliermondii* (98% de los aislamientos fueron sensibles; CMI_{90} = 0,25 µg/ml), *C. glabrata* (95,5% los aislamientos; CMI_{90} = 0,25 µg/ml), y *C. neoformans* (90,9% de los aislamientos; CMI_{90}, 2 µg/ml). La resistencia primaria a la 5FC fue muy rara y una CMI ≥ 32 µg/ml, indicadora de resistencia in vitro, se observó únicamente en 106 aislamientos (10,4 %): 77 *C. albicans* (16,5% de los aislamientos; CMI_{90} > 128 µg/ml), 9 *C. parapsilosis* (6,4 % de los aislamientos; MIC_{90} = 8 µg/ml), 4 *C. albidus* (80 % de los aislamientos, CMI_{50} > 128 µg/ml), 3 *C. glabrata* (4,4% de los aislamientos; CMI_{90} = 0,25 µg/ml), 3 *C. tropicalis* (8,8% de los aislamientos; CMI_{90}, 4 µg/ml), 2 *C. krusei* (7,1% de los aislamientos; CMI_{90} = 8 µg/ml), 2 *Rhodotorula* spp. (4,6% de los aislamientos; CMI_{90} = 1 µg/ml), 8 *Trichosporon* spp. (33,3% de los aislamientos; CMI_{90} = 64 µg/ml), y una *C. lipolytica* (50% de los aislamientos). Destacaba el hecho de que la mayoría de los aislamientos de *C. albicans* (67 de 77 aislamientos) resistentes a la 5FC pertenecían al serotipo B.

Palabras clave

5-fluorocitosina, *Candida*, Serotipos, Levaduras, ATB Fungus

Invasive mycoses caused by emerging fungal pathogens are increasing in immunocompromised patients [31]. Amphotericin B continues to be the gold standard for serious mycoses treatment, and fluconazole and itraconazole are commonly used as treatment for mild or moderate fungal infections. Although not used as monotherapy, 5-fluorocytosine (5FC) may be a useful adjunct to amphotericin B or azoles in the treatment of systemic candidiasis [36]. Despite a general consensus regarding the clinical efficacy of 5FC when used in combination therapy, clinicians are often hesitant to use 5FC due to concerns about toxicity and either primary or secondary resistance [7,31,34,38]. Recent development of several new agents, such as voriconazole, caspofungin and micafungin, for the treatment of invasive candidiasis and meningeal cryptococcosis promises to offer improved coverage of these increasing mycoses [1,11,21]. However, new approaches that take into account the in vitro susceptibility of contemporary isolates determined by standardized methods [20], together with rational dosing that optimizes efficacies and limits toxicities of currently licensed agents may provide an effective, low-cost 5FC therapy with more immediate impact [24].

This resistance in *Candida albicans* had a great variation between countries. It has been considered very important in the Americas, and Africa, and less in Europe [31,37]. The high prevalence of resistance to 5FC has been related to the high isolation of *C. albicans* serotype B from clinical specimens [13]. In Europe, serotype B has been isolated with a low frequency (< 10% of isolates) [6,26]. However, there is very little recent data involving large collection of contemporary clinical isolates of *Candida*, *Cryptococcus* and other medically important yeasts [4,14,15,24,33]. Moreover, this prevalence of 5FC resistance is not well-known in countries, like Spain [10], where this antifungal drug is not marketed and its use is very restricted. In this study, we have analyzed the frequency of 5FC resistance in 1,021 medical important Spanish yeast isolates from patients not previously treated with 5FC.

Materials and methods

Microorganisms. We tested 1,021 recent yeasts clinical isolates, that included 522 *Candida albicans*, 140 *Candida parapsilosis*, 68 *Candida glabrata*, 50 *Candida guilliermondii*, 41 *Candida dubliniensis*, 34 *Candida tropicalis*, 28 *Candida krusei*, 43 *Rhodotorula* spp, 24 *Trichosporon* sp., 20 *Candida famata*, 11 *Cryptococcus neoformans*, 9 *Pichia* spp., 5 *Cryptococcus albidus*, 5 *Saccharomyces cerevisiae*, 5 *Candida rugosa*, 2 *Candida lambica*, 2 *Candida lipolytica*, 2 *Candida lusitaniae*, 2 *Candida pelliculosa*, 2 *Candida sake*, 2 *Cryptococcus laurentii*, 1 *Candida globosa*, 1 *Candida intermedia*, 1 *Candida kefyr*, and 1 *Candida viswanatthi*.

These clinical isolates were obtained from patients attending the Spanish University Hospitals of Valme (Seville), Marqués de Valdecilla (Santander) and Cruces (Barakaldo), and the Odontological Clinics at the Universidad del País Vasco (Bilbao). Yeast isolates included 75 isolates from blood, deep tissues and normally sterile sites of patients suffering deep fungal infections caused by *Candida*, *Cryptococcus* or other medically important yeasts: 22 *C. albicans*, 18 *C. parapsilosis*, 10 *C. neoformans*, 9 *C. guilliermondii*, 7 *C. tropicalis*, 4 *C. glabrata*, 2 *C. krusei* and one each of *C. famata*, *C. pelliculosa* and *Trichosporon asahii*. Moreover, 946 mucosal isolates from HIV-infected patients and from immunocompetent persons with oropharyngeal candidiasis, *Candida* vulvovaginitis or colonized by yeasts, were also tested. The clinical samples were taken before the antifungal treatment was started and none of the patients included in the study have received 5FC previously. Each strain represented a unique consecutive incident (first isolate from each patient episode) clinical isolate. Prior to testing each isolate was cultured on Sabouraud glucose agar and CHROMagar Candida (CHROMagar, France) to ensure purity and viability. Isolates were identified by standard methods, such as germ tube test, chlamydospore formation and morphology on Corn meal agar-Tween 80, and carbohydrate assimilation by means of API ID32C (API-bioMérieux, France), and stored as water suspensions until they were used. *Candida dubliniensis* was identified by an indirect immunofluorescence assay with a polyclonal antiserum against this species as described by Bikandi *et al*. [8]. Three hundred and twenty three *C. albicans* isolates were randomly chosen to determine their serotype by an indirect immunofluorescence assay with the IgM monoclonal antibody B9E according to Barturen *et al*. [5].

In-vitro susceptibility testing. 5FC susceptibility was tested by means of the ATB fungus system (API-bioMérieux, France) which consists of ready-to-use strips with 11 concentrations of 5FC (0.125 to 128 µg/ml), four concentrations of amphotericin B (1 to 8 µg/ml), two concentrations of nystatin (4 and 8 µg/ml), and two concentrations of the imidazole compounds (1 and 8 µg/ml), miconazole, econazole and ketoconazole. For this study only the susceptibility to 5FC was evaluated. Briefly, the ATB fungus system consists of a strip with 16 pairs of cupolas containing the six dehydrated antifungal agents. For testing 5FC, the cupolas were inoculated with a yeast suspension in a semi-solid medium which required no prior heating. For inoculum, a distilled water suspension from an overnight subculture on Sabouraud Glucose Agar (Difco, USA) was adjusted visually or by means of a densitometer (ATB densitometer 1150, API-bioMérieux) to a McFarland 2 standard. Each cupola was inoculated with 135 µl of the cellular suspension in semi-solid medium and incubated at 30°C for 48 h with humidity. The growth in each well was evaluated by automated lecture using the ATB 1520 apparatus (API-bioMérieux). Two quality control isolates, *C. krusei* ATCC 6258 and *C. parapsilosis* ATCC 22019 were tested simultaneously with the rest of isolates. The categorization of each strain for 5FC susceptibility was done according to the instructions of the manufacturer [27], that are coincident to those published by the NCCLS [20]: susceptible, ≤ 4 µg/ml; intermediate, 8 to 16 µg/ml; resistant, ≥ 32 µg/ml.

Results

Tables 1 and 2 summarize the in vitro susceptibility of 1,021 *Candida* isolates to 5FC. Table 1 present geome-

Table 1. In vitro antifungal activity of 5-fluorocytosine against 1,021 clinical isolates of *Candida* spp. and other medically important yeasts.

Species (No of isolates)	MIC (µg/ml)			
	Range	50%	90%	GM
Candida albicans				
Total (522)	0.25- ≥ 128	0.25	≥ 128	0.69
Serotype A (247)	0.125- ≥ 128	0.25	0.25	0.35
Serotype B (76)	0.125- ≥ 128	≥ 128	≥ 128	16.15
Candida parapsilosis (140)	0.25- ≥ 128	0.25	8	0.60
Candida glabrata (68)	0.25- ≥ 128	0.25	0.25	0.34
Candida guilliermondii (50)	0.25-8	0.25	1	0.36
Candida dubliniensis (41)	0.25	0.25	0.25	0.25
Candida tropicalis (34)	0.25- ≥ 128	0.25	4	0.55
Candida krusei (28)	0.25- ≥ 128	8	8	5.89
Candida famata (20)	0.25-0.5	0.25	0.25	0.26
Candida rugosa (5)	0.25-16	0.25	–	0.57
Candida lambica (2)	8	–	–	8
Candida lipolytica (2)	2->128	–	–	16
Candida lusitaniae (2)	0.25	–	–	0.25
Candida pelliculosa (2)	0.25	–	–	0.25
Candida sake (2)	0.25	–	–	0.25
Candida globosa (1)	0.25	–	–	0.25
Candida intermedia (1)	0.25	–	–	0.25
Candida kefyr (1)	0.25	–	–	0.25
Candida viswanatthi (1)	0.25	–	–	0.25
Cryptococcus neoformans (11)	0.25-8	0.25	2	0.53
Cryptococcus albidus (5)	0.25- ≥ 128	0.5	–	36.76
Cryptococcus laurentii (2)	0.25	–	–	0.25
Pichia spp. (9)	0.25-16	0.25	–	0.58
Rhodotorula spp. (43)	0.25- ≥ 128	0.25	1	0.41
Saccharomyces cerevisiae (5)	0.25-4	–	–	0.43
Trichosporon spp. (24)	0.25- ≥ 128	8	64	14.25

GM, geometric mean.

Table 2. Cumulative percentages of susceptible organisms, from the most frequent species studied, at each concentration of 5-fluorocytosine.

Species (No. of isolates)	No. (%) of susceptible isolates at MIC (µg/ml) of:									
	≤ 0.25	0.5	1	2	4	8	16	32	64	≥ 128
Candida albicans (522)	416 (79.7)	420 (80.5)	429 (82.2)	434 (83.1)	436 (83.5)	443 (84.9)	445 (85.2)	449 (86)	452 (86.6)	522 (100)
serotype A (247)	231 (93.5)	231 (93.5)	232 (93.9)	233 (94.3)	233 (94.3)	234 (94.7)	236 (95.5)	237 (96)	237 (96)	247 (100)
serotype B (76)	19 (25)	22 (28.9)	22 (28.9)	24 (31.6)	25 (32.9)	28 (36.8)	28 (36.8)	29 (38.2)	30 (39.5)	76 (100)
Candida parapsilosis (140)	103 (73.6)	107 (76.4)	114 (81.4)	118 (84.3)	120 (85.7)	129 (92.1)	131 (93.6)	131 (93.6)	131 (93.6)	140 (100)
Candida glabrata (68)	63 (92.6)	64 (94.1)	64 (94.1)	65 (95.6)	65 (95.6)	65 (95.6)	65 (95.6)	65 (95.6)	65 (95.6)	68 (100)
Candida guilliermondii (50)	38 (76)	43 (86)	45 (90)	49 (98)	49 (98)	50 (100)				
Candida dubliniensis (41)	41 (100)									
Candida tropicalis (34)	25 (73.5)	27 (79.4)	28 (82.4)	30 (88.2)	31 (91.2)	31 (91.2)	31 (91.2)	32 (94.1)	32 (94.1)	34 (100)
Candida krusei (28)	3 (10.7)	3 (10.7)	3 (10.7)	4 (14.3)	10 (35.7)	26 (92.9)	26 (92.9)	26 (92.9)	27 (96.4)	28 (100)
Candida famata (20)	19 (95)	20 (100)								
Cryptococcus neoformans (11)	7 (63.6)	7 (63.6)	9 (81.8)	10 (90.9)	10 (90.9)	11 (100)				
Cryptococcus albidus (5)	1 (20)	1 (20)	1 (20)	1 (20)	1 (20)	1 (20)	1 (20)	1 (20)	1 (20)	5 (100)
Rhodotorula spp. (43)	36 (83.7)	38 (88.4)	39 (90.7)	40 (93)	40 (93)	41 (95.3)	41 (95.3)	41 (95.3)	41 (95.3)	43 (100)
Trichosporon (24)	1 (4.2)	1 (4.2)	1 (4.2)	1 (4.2)	2 (8.3)	14 (58.3)	16 (66.7)	18 (75)	22 (91.7)	24 (100)
Pichia spp. (9)	7 (77.8)	7 (77.8)	7 (77.8)	7 (77.8)	7 (77.8)	8 (88.9)	9 (100)			
Saccharomyces spp. (5)	4 (80)	4 (80)	4 (80)	4 (80)	5 (100)					
Total (1000)	764 (76.4)	783 (78.3)	806 (80.6)	828 (82.8)	842 (84.2)	894 (89.4)	903 (90.3)	912 (91.2)	921 (92.1)	1000 (100)

tric mean MIC, MIC_{50}, MIC_{90} and MIC range for each yeast species. Results in table 2 are presented by species as cumulative percentages of susceptible organisms at each concentration of 5FC throughout the full dilution series. Overall, 852 out of 1,021 (83.4%) of the isolates were susceptible to 5FC, but 63 (6.2%) and 106 (10.4%) isolates were intermediate and resistant, respectively.

5FC was very active against *Candida* spp. and other medically important yeast isolates as 852 (83.4%) of the studied isolates were susceptible (MIC ≤ 4 µg/ml). The most susceptible species to 5FC were *C. dubliniensis* (100% of isolates; MIC_{90}, 0.25 µg/ml), *C. famata* (100% of isolates; MIC_{90}, 0.25 µg/ml), *C. guilliermondii* (98% of isolates; MIC_{90}, 0.25 µg/ml), *C. glabrata* (95.5% of isolates; MIC_{90}, 0.25 µg/ml), and *C. neoformans* (90.9% of isolates; MIC_{90}, 2 µg/ml).

Primary resistance to 5FC was very uncommon, and a MIC ≥ 32 µg/ml, indicator of in vitro resistance, was observed in 106 isolates (10.4%): 77 *C. albicans* (16.5% of isolates; MIC_{90}, > 128 µg/ml), 9 *C. parapsilosis* (6.4% of isolates; MIC_{90}, 8 µg/ml), 4 *C. albidus* (80% of isolates, MIC_{50}, > 128 µg/ml), 3 *C. glabrata* (4.4% of isolates; MIC_{90}, 0.25 µg/ml), 3 *C. tropicalis* (8.8% of isolates; MIC_{90}, 4 µg/ml), 2 *C. krusei* (7.1% of isolates; MIC_{90}, 8 µg/ml), 2 *Rhodotorula* spp. (4.6% of isolates, MIC_{90}, 1 µg/ml), 8 *Trichosporon* spp. (33.3% of isolates; MIC_{90}, 64 µg/ml), and 1 *C. lipolytica* (50% of isolates).

Interestingly, most *C. albicans* (67 out of 77 isolates) resistant to 5-fluorocytoine were serotype B isolates and there was a statistically significant difference ($p<0.05$) between the susceptibility to 5FC of *C. albicans* serotype A and serotype B isolates, being the latter more resistant to this antifungal agent.

5FC was very active against blood, spinal cord fluid, peritoneum and other normally sterile specimen isolates (Table 3). Only 6 out of 75 yeast isolates (8%) from these sources were resistant, 5 *C. albicans* and 1 *C. tropicalis*; and 3 isolates showed intermediate susceptibility to 5FC, one each isolate of *C. albicans*, *C. tropicalis*, and *T. asahii*. Of the 5 out of 22 (22.7%) *C. albicans* deep site isolates resistant to 5FC, 3 were serotype B and 2 serotype A. The intermediate susceptible isolate was not serotyped.

According to MIC geometric mean, the in vitro susceptibility to 5FC of the species should be (from more to less susceptible): *C. dubliniensis* ≈ *C. glabrata* ≈ *C. globosa* ≈ *C. intermedia* ≈ *C. kefyr* ≈ *C. lusitaniae* ≈ *C. pelliculosa* ≈ *C. sake* ≈ *C. viswanatthii* ≈ *C. laurentii* (0.3) > *C. albicans* serotype A ≈ *Rhodotorula* spp. ≈ *S. cerevisiae* (0.4) > *C. neoformans* (0.5) > *C. parapsilosis* ≈ *C. tropicalis* ≈ *C. rugosa* ≈ *Pichia* spp. (0.6) > *C. krusei* (5.9) > *C. lambica* (9) > *Trichosporon* spp. (14.3) > *C. lipolytica* (16) > *C. albicans* serotype B (16.2) > *C. albidus* (106.7).

Discussion

The antifungal compound 5FC has activity against many isolates of *Candida*, *Cryptococcus* and other medically important yeasts but is not often used to treat patients with mycoses. Its unique indication as monotherapy is in urinary candidiasis. However, its use in combined therapy together with amphotericin B, fluconazole, itraconazole or voriconazole is indicated as potentially useful for treatment of severe or refractory mycoses, including cryptococcal and candidal meningitis, candidemia and acute hematogenously or chronic disseminated candidiasis (hepatosplenic candidiasis), candidal endocarditis and endophthalmitis[24,29].

Although *C. albicans* remains the most common pathogen, non-*C. albicans* species are an increasingly frequent problem in invasive (especially *C. parapsilosis*, *C. glabrata* or *C. tropicalis*), oral (especially *C. dubliniensis*) or vaginal (especially *C. glabrata*) candidiasis. 5FC is active against yeasts, including *Candida* and *Cryptococcus*, and against the dematiaceous fungi and *Aspergillus*.

Primary resistance among *C. albicans* has been reported to range from 6.5% in Europe to 33% in the United States of America, with an overall prevalence of 7 to 9%. However, much of these data concerning primary resistance to 5FC are from studies prior to the standardization of in vitro testing methods [2,3,7,34]. 5FC susceptibility testing performed very well in commercial systems, as Sensititre Yeast One (Trek Diagnostic Systems, USA), NeoSensitabs (Rosco, Denmark), Fungitest (Bio-Rad,

France) or ATB Fungus. The ATB Fungus micromethod is very easy to perform and read clear endpoints of growth, very reproducible, with standardized inoculum, with clear control reference growth, and the possibility of both, visual and automatically readings [19,27].

Recent studies, using standardized methods, from America [14,15,24,26, 33] and Europe [10,20,35,39] have estimated resistance to 5FC to be 0 to 0.6% for *C. albicans* and 0.6 to 6% for all *Candida* species combined. Like the findings of these studies, our results show that only a minority (less than 10%) of isolates manifested 5FC resistance. In Spain, 5FC is rarely used in the treatment of invasive mycoses and the results of the present study are indicative of primary resistance to this compound. This primary resistance has been described in non-*C. albicans* species of *Candida* and *C. neoformans* as well as *C. albicans* serotype B. However, we have observed an excellent susceptibility to 5FC in the present study for the majority of the strains with the exception of *C. krusei*, *Trichosporon* spp., *C. albidus* and the serotype B of *C. albicans*. The most common species causing hematogenous candidiasis in Spain [22] and the rest of the world [23,30] are very susceptible to 5FC (geometric mean of MIC ranging from 0.34 µg/ml for *C. glabrata* to 0.69 µg/ml for *C. albicans*. The incidence of primary resistant *C. albicans* strains (14.8%) in the present study is higher than the frequency of resistance showed by other reports. However, our results are coincident with those reported in other Spanish studies [20,26].

The relationship between serotypes in *C. albicans* and 5FC resistance has been investigated by different authors [2,9,34]. The incidence of primary resistance to 5FC among *C. albicans* serotype B isolates has been estimated to be very high (between 30 and 85%), which contrasts with the low rate (1-11%) of resistant isolates among serotype A. In the present study, 48 (63.2%) *C. albicans* serotype B clinical isolates were resistant to 5FC: three of them were blood isolates and the rest were isolated from oral cavity or vagina. Eleven of these 45 superficial isolates were from HIV infected patients but the majority was from non-immunocompromised patients. These results suggest that 5FC may be less suitable than other antifungal agents to treat oral and vaginal candidiasis.

We have found high rates of decreased susceptibility to 5FC in *C. tropicalis* (8.8%), and *C. krusei* (7.1% resistant, 57.2% with intermediate susceptibility). *C. krusei* was the least susceptible species of *Candida*: 35.7% susceptible; MIC_{90}, 8 µg/ml. Many other authors [4,10,15, 24] have also observed a reduced susceptibility in *C. krusei* isolates. A similar rate of resistance in *C. tropicalis* has also been described [14,15, 24,33]. However, in contrast to other reports [4,10,15], 5FC showed potent in vitro activity against the rest of the non-*C. albicans* species of *Candida* and *C. neoformans* isolates studied. Less than or equal to 5% of *C. glabrata*, *C. guilliermondii*, and *C. parapsilosis* isolates were resistant to 5FC, and all 41 *C. dubliniensis* isolates showed a MIC < 0.5 µg/ml. This excellent activity of 5FC against *C. dubliniensis* was observed in a previous study from our group using the M27A method of NCCLS [25].

Pfaller *et al*. [24] observed in their survey of 8,803 clinical isolates of different *Candida* species from more than 200 medical centers worldwide a pattern of 5FC susceptibility similar to that reported by us in the present study. According to these authors, we have not observed the reduced susceptibility of *C. glabrata* isolates described in other surveys [4,10]. A decreased susceptibility to 5FC has been recently reported in *Trichosporon* spp. and *C. albidus* isolates. Two recent reports have revealed that isolates from superficial and deep clinical specimens of *Trichosporon inkin* and *T. asahii* were resistant to 5FC [18,40]. *C. albidus* was resistant to the combined treatment of amphotericin B and 5FC in a patient with cryptococcaemia caused by this fungus [16].

Resistance to 5FC in yeasts can result from loss or mutation of any of the enzymes involved in its uptake (cytosine permease), metabolism (cytosine deaminase, uracil-phosphoribosyl transferase) and incorporation into RNA, either a failure to metabolize the drug to 5-fluorouridine monophosphate and 5-fluorodeoxyuridine monophosphate, or the loss of feedback control of pyrimidine biosynthesis. Intrinsic resistance in fungi results predomi-

Table 3. Susceptible and resistant isolates to 5-fluorocytosine, from the most frequent species studied, related to their clinical origin

Species (No. of isolates)	All the isolates			Deep site isolates			Superficial site isolates		
	S (%)	I (%)	R (%)	S (%)	I (%)	R (%)	S (%)	I (%)	R (%)
Candida albicans (522)	436 (83.5)	9 (1.7)	77 (14.8)	16 (72.8)	1 (4.5)	5 (22.7)	426 (84)	8 (1.6)	72 (14.4)
serotype A (247)	233 (94.3)	3 (1.2)	11 (4.5)	9 (81.8)	0 (0)	2 (18.2)	224 (94.9)	3 (4.2)	9 (3.8)
serotype B (76)	25 (32.9)	3 (3.9)	48 (63.2)	2 (40)	0 (0)	3 (60)	23 (32.4)	3 (4.2)	45 (63.4)
Candida parapsilosis (140)	120 (85.7)	11 (0)	9 (0)	18 (100)	0 (0)	0 (0)	102 (83.6)	11 (9)	9 (7.4)
Candida glabrata (68)	65 (95.6)	0 (0)	3 (4.4)	4 (100)	0 (0)	0 (0)	61 (95.3)	0 (0)	3 (94.7)
Candida guilliermondii (50)	49 (98)	1 (2)	0 (0)	9 (100)	0 (0)	0 (0)	40 (97.6)	1 (2.4)	0 (0)
Candida dubliniensis (41)	41 (100)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	41 (100)	0 (0)	0 (0)
Candida tropicalis (34)	31 (91.2)	0 (0)	3 (8.8)	6 (85.7)	0 (0)	1 (14.3)	25 (92.6)	0 (0)	2 (7.4)
Candida krusei (28)	10 (35.7)	16 (57.2)	2 (7.1)	1 (50)	1 (50)	0 (0)	9 (34.6)	15 (57.7)	2 (7.7)
Candida famata (20)	20 (100)	0 (0)	0 (0)	1 (100)	0 (0)	0 (0)	19 (100)	0 (0)	0 (0)
Cryptococcus neoformans (11)	10 (90.9)	1 (9.1)	0 (0)	10 (100)	0 (0)	0 (0)	0 (0)	1 (100)	0 (0)
Cryptococcus albidus (5)	1 (20)	0 (0)	4 (80)	0 (0)	0 (0)	0 (0)	1 (20)	0 (0)	4 (80)
Rhodotorula spp. (43)	40 (93)	1 (2.3)	2 (4.7)	0 (0)	0 (0)	0 (0)	40 (93)	1 (2.3)	2 (4.7)
Trichosporon (24)	2 (8.3)	14 (58.3)	8 (33.4)						
Pichia spp. (9)	7 (77.8)	2 (22.2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Saccharomyces spp. (5)	5 (100)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Total (1000)	837 (83.7)	55 (5.5)	108 (10.8)	65 (89)	2 (2.7)	6 (8.2)	772 (83.3)	53 (5.7)	102 (11)

nantly from a defect in the cytosine deaminase and acquired resistance, in *C. albicans*, usually results from a defect in uracil:phosphoribosyl transferase, an enzyme involved in the synthesis of both 5-fluorouridine monophosphate and 5-fluorodeoxyuridine monophosphate, and of uridylate in the pyrimidine salvage pathway [31,37].

The excellent spectrum and potency of 5FC versus *Candida* and other species of yeasts, along with the favorable pharmacokinetic and pharmacodynamic parameters described recently [1,12,16,17,24,28,34], suggest that 5FC might be used with dosing regimens that produce lower concentrations in serum than those currently employed that could reduce the risk of toxicity to the host maintaining the overall efficacy when 5FC is coupled with amphotericin B or an azole in the treatment of invasive mycoses.

In summary, 5FC primary resistance is uncommon (<10%) among Spanish clinical isolates. By species, 5FC showed more potent in vitro activity against *C. dubliniensis*, *C. glabrata*, *C. lusitaniae*, *C. albicans*, *C. neoformans*, and *C. parapsilosis*.

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