Reduction in Percentage of Clusters of *Candida albicans* and *Candida parapsilosis* Causing Candidemia in a General Hospital in Madrid, Spain

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ABSTRACT The presence of clusters in units with a high incidence of candidemia suggests the need for the prevention of candidemia. We analyzed the percentage of patients involved in clusters and its evolution over a large period of time in a tertiary hospital. We studied 432 patients admitted to Gregorio Marañón Hospital with candidemia caused by *Candida albicans* ($n = 276$) or *Candida parapsilosis* ($n = 156$) between January 2007 and December 2014. Incident isolates were genotyped. A cluster was defined as a group of ≥ 2 patients infected by an identical genotype; we considered clusters to be “tracking clusters” when the patients involved in the cluster were admitted to the same ward within a period of 24 months. The study period was split into two periods, 2007 to 2010 (period 1) and 2011 to 2014 (period 2). The number of episodes of *C. albicans* and *C. parapsilosis* candidemia ($n = 262$ versus $n = 170$, respectively), the mean incidence (1.62 versus 1.36 episodes per 1,000 admissions, respectively), and the percentage of episodes caused by clusters (overall clusters [40% versus 12%] and tracking clusters [18% versus 3%], respectively) were significantly lower in period 2 than in period 1. Linear regression analysis showed a positive correlation between the overall number of episodes of candidemia and episodes caused by clusters ($r^2 = 0.89$). We found a reduction in the number of episodes of candidemia caused by *C. albicans* and *C. parapsilosis* and a decrease in the percentage of episodes caused by clusters over time. Interestingly, the reduction was accompanied by the implementation of a campaign to reduce the number of catheter-related infections.

KEYWORDS *Candida albicans*, *Candida parapsilosis*, candidemia, cluster, genotyping, catheter-related infection

Candidemia is a major cause of morbidity and mortality in hospitalized patients. It is associated with prolonged hospitalization, multifocal colonization (including the central venous catheter tip), and high mortality and costs (1–3). Candidemia is commonly caused by the translocation of endogenous strains colonizing the gastrointestinal tract (4), although many episodes are caused by exogenous strains acquired from the environment. Exogenous strains are prone to patient-to-patient transmission, particularly in medical and postsurgical intensive care units (ICUs), oncology-hematology units, and neonatal ICUs (5).

The epidemiology and source of candidemia can be studied by fingerprinting the isolates to unravel the presence of *Candida* clusters (identical genotypes infecting various patients) that have the potential to cause outbreaks (6). We previously reported

Received 5 April 2018 Returned for modification 30 April 2018 Accepted 4 May 2018

Accepted manuscript posted online 9 May 2018

Citation Escribano P, Sánchez-Carrillo C, Muñoz P, Bouza E, Guinea J, on behalf of the COMIC Study Group. 2018. Reduction in percentage of clusters of *Candida albicans* and *Candida parapsilosis* causing candidemia in a general hospital in Madrid, Spain. J Clin Microbiol 56:e00574-18. <https://doi.org/10.1128/JCM.00574-18>.

Editor David W. Warnock

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that a high percentage of episodes of candidemia were caused by clusters of *C. albicans* and *C. parapsilosis* (7, 8). The presence of clusters in the hospital might be indirect evidence of patient-to-patient transmission.

Most studies to track the source of nosocomial *Candida* outbreaks were designed after detecting an abnormal increase in the number of new episodes of candidemia in a specific hospital ward (9, 10). However, we previously showed that genotyping of all consecutive isolates causing candidemia in a hospital is useful for detecting areas where the transmission of isolates may have been more active (7, 11). We here report the incidence of candidemia and the percentage of patients involved in clusters in a large tertiary hospital and the evolution over time by genotyping the isolates.

(This study was partially presented at the 26th European Congress of Clinical Microbiology and Infectious Diseases in Amsterdam, The Netherlands, 2016, abstr P1613 [12].)

MATERIALS AND METHODS

Hospital and isolates. The Gregorio Marañón Teaching Hospital is a 1,550-bed public hospital serving a population of 750,000 inhabitants in Madrid, Spain. The hospital covers all medical and surgical specialties and has adult and neonatal intensive care units. It is also a referral center for solid-organ transplantation, heart surgery, oncology, hematology, bone marrow transplantation, and HIV/AIDS care.

A candidemia episode was defined as the isolation of *Candida* spp. from blood cultures obtained from a peripheral vein in a patient with compatible clinical signs and symptoms. We retrospectively selected first episodes of candidemia caused by *C. albicans* and *C. parapsilosis* from January 2007 to December 2014. The incident isolates from all patients were selected, and the isolates were identified by amplification and sequencing of the ITS1-5.8S-ITS2 region (13).

We recorded the number of admissions, overall episodes, and incidence of candidemia, episodes of candidemia caused by *C. albicans* and *C. parapsilosis*, and incidence of catheter-related candidemia. The incidence of candidemia was calculated as the number of episodes of candidemia (overall and catheter related) per 1,000 hospital admissions.

Genotyping. *C. albicans* and *C. parapsilosis* isolates were genotyped using a panel of 6 and 4 short tandem repeats, respectively (14–17). The electrophoretic bands were sized and scored using GeneMapper version 4.0 (Applied Biosystems-Life Technologies Corporation, CA, USA). *C. albicans* and *C. parapsilosis* strains were used as controls in each run to ensure accuracy of size and to minimize run-to-run variation.

The allelic composition was studied for each locus, and the total allelic composition was converted to binary data by scoring the presence or absence of each allele. The genetic relationships between the genotypes were studied by constructing a dendrogram in BioNumerics version 6.6 (Applied Maths, Sint-Martens-Latem, Belgium). Isolates were considered genotypically identical when they showed the same alleles for all markers. A cluster was defined as a group of ≥ 2 patients infected by an identical genotype. The number of genotypes, number of clusters, and number of patients involved in clusters were calculated. The ward of admission of the patients involved in the cluster was recorded. "Tracking clusters" were defined as clusters in which the patients were epidemiologically related (admitted to the same ward within a period of 24 months).

Data analysis. Two periods of study were established, period 1 (2007 to 2010) and period 2 (2011 to 2014). An educational program to reduce catheter-related infections was started in January 2011. Healthcare workers' knowledge of guidelines for the prevention of catheter-related bloodstream infections was assessed before and after the program, and care bundles were implemented in adult units, neonatal units, and the pediatric intensive care unit, as well as in the internal medicine department (18–20).

Epidemiological data (total admissions, number of episodes of candidemia, and incidence) were compared using the chi-square test (IBM SPSS Statistics for Windows, version 21.0; SPSS, Inc., Armonk, NY, USA). Proportions of episodes, clusters, and patients involved in clusters were compared using a standard binomial method for the calculation of 95% confidence intervals using the Epidat 3.1 software (Servicio de Información sobre Saúde Pública de la Dirección Xeral de Saúde Pública de la Consellería de Sanidade, Xunta de Galicia, Spain).

Ethical considerations. This study (protocol no. 39/15) was approved by the ethics committee of the Hospital Gregorio Marañón (CEIC-A1). The need for informed consent was waived, owing to the retrospective design of the study.

RESULTS

Epidemiology and incidence of candidemia during the study period. Data on epidemiology and incidence are shown in Table 1. The hospital recorded a mean of 54,238 admissions per year and 581 episodes of candidemia (53 to 110 per year) during the study period. Most episodes were caused by *C. albicans* ($n = 276$ [47%]) and *C. parapsilosis* ($n = 156$ [27%]). The mean incidence of candidemia during the period was 1.49 episodes per 1,000 admissions and year. The year with the highest number of candidemia episodes and incidence was 2007. The considerable difference between the

TABLE 1 Epidemiological data during each year of the study period, periods 1 and 2

Characteristic	Data by yr										Mean for period:	
	2007	2008	2009	2010	2011	2012	2013	2014	Overall (mean)		1	2
Total no. of admissions	67,882	58,724	53,433	55,137	52,911	49,336	48,247	48,238	54,238	58,794	49,683	
No. of episodes of candidemia	110	79	61	84	72	65	53	57	581	334	247	
No. (%) of episodes of <i>C. albicans</i>	52 (47.27)	39 (49.37)	26 (42.62)	50 (59.52)	33 (45.83)	29 (44.62)	23 (43.40)	24 (42.11)	276 (47)	167 (50)	109 (44)	
No. (%) of episodes of <i>C. parapsilosis</i>	40 (36.36)	27 (34.18)	15 (24.59)	13 (15.48)	18 (25.00)	15 (23.08)	14 (26.42)	14 (24.56)	156 (27)	95 (28)	61 (25)	
Incidence of candidemia	1.92	1.53	1.52	1.49	1.76	1.43	1.13	1.11	1.49	1.62	1.36	
Incidence of <i>C. albicans</i>	0.91	0.65	0.71	0.87	0.83	0.66	0.48	0.51	0.70	0.79	0.62	
Incidence of <i>C. parapsilosis</i>	0.72	0.51	0.36	0.27	0.53	0.38	0.35	0.29	0.43	0.47	0.39	
Incidence of CRC ^a	0.55	0.58	0.51	0.47	0.51	0.44	0.23	0.24	0.44	0.53	0.36	

^aCRC, catheter-related candidemia.

incidence of catheter-related candidemia and the overall incidence of candidemia meant that the former could not account for the latter.

Although statistically significant differences were not found, the number of candidemia episodes was higher in period 1 than in period 2 ($n = 334$ versus $n = 247$, respectively), as well as for episodes caused by *C. albicans* and *C. parapsilosis* ($n = 262$ versus $n = 170$, respectively) (Table 1). Differences in the percentage of episodes caused by *C. albicans* (50% versus 44%, respectively) and *C. parapsilosis* (28% versus 25%, respectively) between the study periods did not reach statistical significance ($P = 0.3$). The mean incidence was significantly lower in period 2 (1.36 episodes per 1,000 admissions) than in the period 1 (1.62 episodes per 1,000 admissions) ($P < 0.05$) (Table 1). The incidence of catheter-related candidemia also decreased (0.53 versus 0.36, respectively). The number of episodes and incidence were lower in period 2, particularly from 2012 onward.

Genotyping and cluster analysis. We found 243 *C. albicans* genotypes and 108 *C. parapsilosis* genotypes, of which 11% (39/351) were clusters. We did not find statistically significant differences between the percentage of clusters of *C. albicans* ($n = 23$ [9.5%]) and *C. parapsilosis* ($n = 16$ [15%]) ($P = 0.19$). Of the 432 patients, 126 (29%) patients were involved in clusters; however, the percentage of patients infected by *C. albicans* clusters ($n = 62/276$ [22.5%]) was significantly lower than that of patients infected by *C. parapsilosis* clusters ($n = 64/156$ [41%]) ($P < 0.0001$) (Fig. 1A). The number of patients involved in each cluster ranged from 2 to 6 patients for *C. albicans* and from 2 to 15 patients for *C. parapsilosis*. The number of patients in clusters per year ranged from 2 to 22 patients for *C. albicans* and from 1 to 28 patients for *C. parapsilosis*. Patients involved in clusters were admitted mainly to surgical, adult, and neonatal intensive care units (Fig. 2A).

As not all patients involved in clusters were epidemiologically related, a refined definition of cluster, tracking clusters, was adopted when the patients were admitted to the same ward within a 2-year period. The 19 tracking clusters (13 *C. albicans* and 6 *C. parapsilosis*) represented as many as 49% of the clusters and involved 51 patients (29 and 22 patients infected by *C. albicans* and *C. parapsilosis*, respectively) (Table 2). Half of the tracking clusters were found in the neonatology unit (Table 2 and Fig. 2B).

C. albicans tracking clusters were detected within a sampling interval ranging from +1 day to +678 days (Table 2); most were detected sporadically, and the patients were admitted to the same ward at the time of blood sample collection (from +1 day to +126 days). *C. parapsilosis* tracking clusters were detected over a wider period (from +27 days to 1,023 days) (Table 2), suggesting the persistence of some genotypes. This is well illustrated by cluster CP-121, which persisted in the neonatology unit for years (Table 2).

We observed three *C. albicans* tracking clusters (CA-48, CA-162, and CA-294) and one *C. parapsilosis* tracking cluster, which involved patients who were in different wards at the time of the blood sample collection, although they had previously shared a hospital ward (Table 2).

Furthermore, the percentage of episodes caused by clusters was significantly higher in period 1 considering both overall clusters (40% versus 12%, respectively) and tracking clusters (18% versus 3%, respectively) ($P < 0.0001$) (Fig. 1A and B). The reduction was observed in all units where clusters were detected (Fig. 2A and B). Linear regression analysis revealed a positive correlation between the overall number of candidemia episodes and episodes caused by clusters ($r^2 = 0.89$).

DISCUSSION

The reduction in the incidence of candidemia accompanied by the reduction in patients involved in clusters suggest a better control of the infection in the hospital. This may be a consequence of the implementation of measures for hospital infection control, such as the above-mentioned educative catheter-related infection control campaign.

FIG 1 (A) Graph showing the overall clusters found and the number of patients infected by *C. albicans* and *C. parapsilosis* isolates (episodes caused by clusters [yellow and cyan sections] or singleton genotypes [peach and green sections]) in each year of the study period. The percentage of patients infected by clusters is also shown. (B) Graph showing only the patients infected by tracking clusters and number of patients infected by *C. albicans* and *C. parapsilosis* isolates (episodes caused by tracking clusters [yellow and cyan sections] or singleton genotypes [peach and green sections]) in each year of the study period. The percentage of patients infected by tracking clusters is also shown.

Candidemia is mostly caused by *C. albicans* and *C. parapsilosis* because of their ability to colonize intravenous devices, health workers' hands, and the hospital environment (21). These characteristics facilitate nosocomial spread of the organism, eventually resulting in outbreaks that are not generally detected in routine monitoring of infection and therefore cannot be controlled (4). The overall mean incidence of candidemia during the study period was higher than that previously published in the CANDIPOP study (22). This difference may be a consequence of the shorter study duration of CANDIPOP, although it is well known that the incidence of candidemia varies considerably between centers, thus probably reflecting center-to-center differences in the impact of antifungal policy, central venous catheter care procedures, and training programs, as well as difficulties in the implementation of infection control programs (21).

FIG 2 (A) Ward of admission of patients with candidemia infected by clusters of *C. albicans* (black bars) and *C. parapsilosis* (white bars) during period 1 and period 2. (B) Ward of admission of patients with candidemia infected by tracking clusters of *C. albicans* (black bars) and *C. parapsilosis* (white bars) during period 1 and period 2.

We observed a reduction in the number of episodes of candidemia and in the incidence of candidemia (and catheter-related candidemia) in period 2, especially from 2012 onward. This period started at the same time as implementation of the training program for catheter-related infection control that included bedside visits, talks to nurses on all shifts, and distribution of handouts with information containing the main recommendations for catheter care in adult, neonatal, and pediatric intensive care units, as well as in internal medicine departments. The results revealed an improvement in the level of catheter care after the training program (18–20). A reduction in the frequency of nosocomial infection by means of preventive strategies is a key objective

TABLE 2 Tracking clusters of *C. albicans* and *C. parapsilosis*

Cluster code	No. of patients involved	Ward of admission	Date of blood culture collection (mo/day/yr)	Sampling interval ^a
<i>C. albicans</i>				
CA-34	3	Neonatology	08/02/2010 12/10/2010 12/16/2010	+61 +67
CA-35	2	Neonatology	06/11/2008 06/12/2008	+1
CA-39	2	Adult ICU	07/10/2014 07/12/2014	+2
CA-48 ^b	2	Pediatric ICU Pediatric hematology	04/13/2008 06/16/2008	+63
CA-50	2	Neonatology	11/23/2010 12/16/2010	+23
CA-66	2	Neonatology	02/14/2009 02/16/2010	+363
CA-162 ^b	2	Internal medicine Digestive medicine	06/28/2007 05/07/2009	+678
CA-227	2	Neonatology	03/02/2010 04/15/2010	+44
CA-258	2	Digestive medicine	12/16/2007 05/06/2008	+126
CA-285	2	Neonatology	12/09/2013 12/12/2013	+3
CA-294 ^b	2	General surgery Internal medicine	05/09/2008 06/19/2008	+41
CA-305	2	General surgery	11/25/2009 12/03/2009	+8
CA-371	4	Neonatology	09/21/2010 09/24/2010 10/05/2010 10/24/2010	+3 +13 +32
<i>C. parapsilosis</i>				
CP-85	2	Pediatrics	02/19/2007 02/13/2008	+346
CP-111	2	Adult ICU	03/15/2007 06/30/2007	+107
CP-117	2	Neonatology	08/10/2007 01/11/2009	+154
CP-121	12	Neonatology	04/10/2007 05/09/2007 05/18/2007 12/30/2007 03/13/2008 05/12/2008 05/27/2008 06/03/2008 01/09/2009 05/30/2009 10/08/2009 05/04/2011	+29 +38 +264 +337 +397 +412 +418 +543 +684 +815 +1023
CP-122	2	Neonatology	01/01/2007 07/11/2007	+192
CP-131 ^c	2	Adult ICU Oncology	02/01/2007 01/06/2007	+27

^aNumber of days after the blood sample collection of the first patient involved in the cluster.^bPatients admitted to different wards at the time of diagnosis of candidemia but who had previously shared a ward of admission: CA-48, pediatric hematology, (March 2008); CA-162, internal medicine, May 2008; and CA-294, general surgery, May 2008 (7).^cPatients admitted to different wards at the time of diagnosis of candidemia but who had previously shared a ward of admission: CP-131, oncology unit in January 2007.

in the hospital (23). The epic3 guidelines suggest implementation of continuous training programs and periodical evaluation of health care workers' knowledge in order to improve catheter care (24). Previous studies have shown that the implementation of an educational program has a positive and significant impact on health care worker

knowledge of the Centers for Disease Control and Prevention (CDC) guidelines for the prevention of catheter-related infection (25).

Hypothetically, the presence of clusters may suggest patient-to-patient transmission of *Candida* spp., which would affect the species distribution in different wards and the incidence of candidemia. Previous studies have reported the presence of clusters of *C. albicans* and *C. parapsilosis* within the hospital that were likely involved in intrahospital transmission of infection (5, 7, 11). Patient-to-patient transmission is difficult to demonstrate. However, the plummeting incidence and the significantly fewer patients in clusters in period 2 than in period 1 for both *C. albicans* (30% versus 10%, respectively) and *C. parapsilosis* (57% versus 14%, respectively) suggest that the implementation of the campaign may have played a role. The larger percentage of patients was in clusters caused by *C. parapsilosis*, even though the number of clusters of *C. albicans* was elevated. *C. parapsilosis* is frequently isolated from the hands of healthy people and health care workers, thus highlighting the importance of hand washing to prevent horizontal transmission of this pathogen (21, 26).

C. albicans and *C. parapsilosis* clusters were found mainly in surgical units, adult units, and the neonatal intensive care unit, as previously reported (5, 21, 27, 28). We observed a reduction in the proportion of patients involved in clusters over period 2 not only overall, but also in the tracking clusters (15% versus 4% for *C. albicans*, and 22% versus 1.6% for *C. parapsilosis*, respectively). It should be noted that our definition of tracking cluster is arbitrary in the absence of consensus on the period of time patients have to be in the cluster. Other authors considered the period of observation to be 15 days (29), 3 months (30), 6 months (31), or even more than 1 year (7, 11).

Our study is subject to a series of limitations. First, we did not collect isolates from the hospital environment or health care workers' hands, thus making it impossible to detect the environmental niches of the clusters or to illustrate evidence of patient-to-patient transmission. Second, the high proportion of clusters with no epidemiological data from the patients involved may be a consequence of a low typing resolution of the microsatellites (e.g., homoplasia, an intrinsic limitation of microsatellite typing analysis, leading to alleles with identical sizes but different sequences). We are currently trying to clarify this issue using whole-genome sequencing. In order to address the limitation, we added the definition of tracking cluster, although this did not substantially change the observations reported using the overall clusters. However, we cannot exclude the possibility that patients located in different wards at diagnosis previously shared a ward of admission, since patients are often moved within the hospital and information on transfers is not always available. Furthermore, some of the clusters may be actually clones frequently circulating in the community and that eventually cause nosocomial infection. Finally, we observed that the lower incidence and percentage of patients in clusters occurred simultaneously with the catheter-related infection control implementation, but we cannot prove that.

In conclusion, we found that the reduction in the number of episodes of candidemia caused by *C. albicans* and *C. parapsilosis* was accompanied by a decrease in the percentage of episodes caused by clusters. Our observations suggest that implementation of measures to reduce the number of catheter-related infections leads to better control of nosocomial candidemia.

ACKNOWLEDGMENTS

This study was supported by grants PI14/00740 and MSI15/00115 from the Fondo de Investigación Sanitaria (FIS; Instituto de Salud Carlos III; Plan Nacional de I+D+I 2013-2016) and cofinanced by the European Regional Development Fund (ERDF) 'A way of making Europe.' P.E. (CPI15/00115) and J.G. (CPI15/00006) are recipients of a Miguel Servet contract supported by FIS.

The funders had no role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript.

We declare no conflicts of interest.

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