**Supplemental Tables and Figures**

**Table S1.** Medical conditions and medication use characteristics of 41 mother-child dyads.

|  |  |  |  |
| --- | --- | --- | --- |
| **Category** |  | **Mother****n (%)** | **Child****n (%)** |
| **Mother medication use (Y)** | \*Antifungal during pregnancy | 9 (21.9) |  |
| Oral antifungal during pregnancy | 6 (14.6) |  |
|  | \*Antifungal 6m after pregnancy | 5 (12.2) |  |
|  | Oral antifungal 6m after pregnancy | 3 (7.3) |  |
|  | \*Antibiotic during pregnancy | 18 (43.9) |  |
|  | Oral antibiotics during pregnancy | 16 (39.0) |  |
|  | \*Antibiotics 6m after pregnancy | 11 (26.8) |  |
|  | Oral antibiotics 6m after pregnancy | 9 (22.0) |  |
| **Child medical conditions/medication (Y)** | Nasal congestion |  | 4 (9.8) |
| Otitis media |  | 12 (29.3) |
| 1st Viral URI |  | 17 (41.5) |
| 2nd Viral URI |  | 7 (17.1) |
|  | Pharyngitis |  | 2 (4.9) |
|  | Cough |  | 11 (26.8) |
|  | Multiple vomiting, diarrhea constipation |  | 15 (36.6) |
|  | Covid-19 |  | 3 (7.3) |
|  | Diaper rash |  | 16 (39) |
|  | Oral thrush |  | 8 (19.5) |
|  | Eczema |  | 8 (19.5) |
|  | Poor weight gain |  | 4 (9.8) |
|  | Viral illness |  | 5 (12.2) |
|  | Abnormal hemoglobin |  | 9 (22) |
|  | High lead |  | 6 (14.6) |
|  | Oral amoxicillin |  | 10 (24.4) |
|  | Oral Nystatin |  | 7 (17.1) |
|  | Nystatin cream/ointment |  | 8 (19.5) |
|  | Oral fluconazole |  | 1 (2.4) |
|  | Clotrimazole cream |  | 3 (7.3) |

\*Includes any oral suspension, cream/ointments/suppository, or shampoo.

URI: upper respiratory infection

**Table S2.** Classification of clinical oral *C. dubliniensis* isolates susceptibility to fluconazole according to CLSI guidelines using epidemiological cutoff values (ECV)

|  |  |  |
| --- | --- | --- |
| Species and Isolation timepoint (number of isolates) | Susceptible (S)MIC ≤0.5 μg/mln (%) | Resistant (R)MIC >0.5 μg/mln (%) |
| Prenatal (2) | 2 (100) | 0 (0) |
| 24 months (2) | 2 (100) | 0 (0) |

MIC: minimum inhibitory concentration.

No clinical breakpoints were established for *C. dubliniensis*; alternatively, the ECVs [1] was used as a cut-off value to separate susceptible and resistant isolates.

**Table S3.** Classification of clinical oral *C. lusitaniae* isolates susceptibility to fluconazole according to CLSI guidelines using epidemiological cutoff values (ECV)

|  |  |  |
| --- | --- | --- |
| Species and Isolation timepoint (number of isolates) | Susceptible (S)MIC ≤2 μg/mln (%) | Resistant (R)MIC 2μg/mln (%) |
| Prenatal (1) | 1 (100) | 0 (0) |
| 24 months (1) | 1 (100) | 0 (0) |

MIC: minimum inhibitory concentration.

No clinical breakpoints were established for *C. lusitaniae*; alternatively, the ECVs [1] was used as a cut-off value to separate susceptible and resistant isolates.

**Table S4**.Classification of clinical oral *C. parapsilosis* susceptibility to caspofungin according to CLSI guidelines using clinical breakpoints (CBP)

|  |  |  |  |
| --- | --- | --- | --- |
| Timepoint (Number of isolates) | Susceptible (S)MIC ≤2μg/mln (%) | Intermediate (I)MIC =4μg/mln (%) | ResistantMIC ≥8 μg/mln (%) |
| Prenatal (3) | 3 (100) | 0 (0) | 0 (0) |
| 4 months (1) | 1 (100) | 0 (0) | 0 (0) |
| 12 months (2) | 2 (100) | 0 (0) | 0 (0) |

MIC: minimum inhibitory concentration.

**Table S5.** Classification of clinical oral *C. dubliniensis* isolates susceptibility to caspofungin according to CLSI guidelines using epidemiological cutoff values (ECV)

|  |  |  |
| --- | --- | --- |
| Species and Isolation timepoint (number of isolates) | Susceptible (S)MIC ≤0.12 μg/mln (%) | Resistant (R)MIC >0.12 μg/mln (%) |
| Prenatal (2) | 2 (100) | 0 (0) |
| 24 months (2) | 2 (100) | 0 (0) |

MIC: minimum inhibitory concentration.

No clinical breakpoints were established for *C. dubliniensis*; alternatively, the ECVs [1] was used as a cut-off value to separate susceptible and resistant isolates.

**Table S6.** Classification of clinical oral *C. lusitaniae* isolates susceptibility to caspofungin according to CLSI guidelines using epidemiological cutoff values (ECV)

|  |  |  |
| --- | --- | --- |
| Species and Isolation timepoint (number of isolates) | Susceptible (S)MIC ≤1 μg/mln (%) | Resistant (R)MIC 1μg/mln (%) |
| Prenatal (1) | 1 (100) | 0 (0) |
| 24 months (1) | 1 (100) | 0 (0) |

MIC: minimum inhibitory concentration.

No clinical breakpoints were established for *C. lusitaniae*; alternatively, the ECVs [1] was used as a cut-off value to separate susceptible and resistant isolates.

**Table S7.** List of mutations conserved among nystatin-resistant *C. albicans* strains (MIC> 2 μg/ml; n=6).

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Gene** | **Gene length** | **Gene position** | **Nucleotide substitution** | **Aminoacid substituition** | **Allele** | **Mutation type** |
| FKS1 | 5694 | chr 1 | 2955A>G |  | hetero | synonymous mutation |
| FKS1 | 5694 | chr 1 | 1929T>A |  | homo/hetero | synonymous mutation |
| FKS1 | 5694 | chr 1 | 1065A>G |  | homo/hetero | synonymous mutation |
| FKS1 | 5694 | chr 1 | 756T>C |  | homo/hetero | synonymous mutation |
| CDR2 | 4500 | chr 3 | 531A>T |  | homo/hetero | synonymous mutation |
| CDR2 | 4500 | chr 3 | 1572C>T |  | homo/hetero | synonymous mutation |
| CDR2 | 4500 | chr 3 | 1894C>T |  | homo/hetero | synonymous mutation |
| CDR2 | 4500 | chr 3 | 2048G>A | Arg683Lys | homo/hetero | missense mutation |
| CDR2 | 4500 | chr 3 | 2169T>C |  | homo/hetero | synonymous mutation |
| CDR2 | 4500 | chr 3 | 4011G>C | Leu1337Phe | homo/hetero | missense mutation |
| CDR1 | 4506 | chr 3 | 69T>C |  | homo/hetero | synonymous mutation |
| CDR1 | 4506 | chr 3 | 423A>T |  | homo/hetero | synonymous mutation |
| CDR1 | 4506 | chr 3 | 435C>T |  | homo/hetero | synonymous mutation |
| CDR1 | 4506 | chr 3 | 537A>T |  | homo/hetero | synonymous mutation |
| CDR1 | 4506 | chr 3 | 663C>T |  | hetero | synonymous mutation |
| CDR1 | 4506 | chr 3 | 2712A>G |  | homo/hetero | synonymous mutation |
| CDR1 | 4506 | chr 3 | 3225A>G |  | homo/hetero | synonymous mutation |
| CDR1 | 4506 | chr 3 | 3237A>T |  | homo/hetero | synonymous mutation |
| CDR1 | 4506 | chr 3 | 3267G>A |  | homo/hetero | synonymous mutation |
| CDR1 | 4506 | chr 3 | 3660T>C |  | homo/hetero | synonymous mutation |
| CDR1 | 4506 | chr 3 | 3717C>T |  | homo/hetero | synonymous mutation |
| MDR1 | 1695 | chr 6 | 1041A>G |  | homo/hetero | synonymous mutation |
| MDR1 | 1695 | chr 6 | 909C>T |  | homo/hetero | synonymous mutation |
| MDR1 | 1695 | chr 6 | 843A>G |  | homo/hetero | synonymous mutation |
| MDR1 | 1695 | chr 6 | 645A>G |  | homo/hetero | synonymous mutation |
| MDR1 | 1695 | chr 6 | 624A>G |  | homo/hetero | synonymous mutation |
| MDR1 | 1695 | chr 6 | 612A>T |  | homo/hetero | synonymous mutation |
| MDR1 | 1695 | chr 6 | 597A>G |  | homo/hetero | synonymous mutation |
| MDR1 | 1695 | chr 6 | 471T>A |  | homo/hetero | synonymous mutation |
| MDR1 | 1695 | chr 6 | 468C>T |  | homo/hetero | synonymous mutation |

**Table S8.** List of mutations conserved among fluconazole wild-type *C. albicans* strains (MIC≤ 0.5 μg/ml; n=60).

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Gene** | **Gene length** | **Gene position** | **Nucleotide substitution** | **Aminoacid substituition** | **Allele** | **Mutation type** |
| FKS1 | 5694 | chr 1 | 1065A>G |  | homo/hetero | synonymous mutation |
| CDR1 | 4506 | chr 3 | 3717C>T |  | homo/hetero | synonymous mutation |
| MDR1 | 1695 | chr 6 | 1308G>A |  | homo/hetero | synonymous mutation |
| MDR1 | 1695 | chr 6 | 1287T>C |  | homo/hetero | synonymous mutation |
| MDR1 | 1695 | chr 6 | 1272T>C |  | homo/hetero | synonymous mutation |

 **Table S9.** List of mutations conserved among caspofungin borderline high MIC values (MIC= 0.25 μg/ml) *C. albicans* strains (n=7).

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Gene** | **Gene length** | **Gene position** | **Nucleotide substitution** | **Aminoacid substituition** | **Allele** | **Mutation type** |
| FKS1 | 5694 | chr 1 | 5657C>G | Ser1886Thr | homo/hetero | missense mutation |
| FKS1 | 5694 | chr 1 | 4230A>G |  | homo/hetero | synonymous mutation |
| FKS1 | 5694 | chr 1 | 1359G>A |  | homo/hetero | synonymous mutation |
| FKS1 | 5694 | chr 1 | 1350A>G |  | homo/hetero | synonymous mutation |
| FKS1 | 5694 | chr 1 | 1065A>G |  | homo/hetero | synonymous mutation |
| CDR2 | 4500 | chr 3 | 531A>T |  | homo/hetero | synonymous mutation |
| CDR2 | 4500 | chr 3 | 1527C>T |  | homo/hetero | synonymous mutation |
| CDR2 | 4500 | chr 3 | 1894C>T |  | homo/hetero | synonymous mutation |
| CDR2 | 4500 | chr 3 | 2048G>A | Arr683Lys | homo/hetero | missense mutation |
| CDR2 | 4500 | chr 3 | 2169T>C |  | homo/hetero | synonymous mutation |
| CDR2 | 4500 | chr 3 | 4011G>C | Leu1337Phe | homo/hetero | missense mutation |
| CDR2 | 4500 | chr 3 | 4410T>C |  | homo/hetero | synonymous mutation |
| CDR1 | 4506 | chr 3 | 423A>T |  | homo/hetero | synonymous mutation |
| CDR1 | 4506 | chr 3 | 435C>T |  | homo/hetero | synonymous mutation |
| CDR1 | 4506 | chr 3 | 438A>T |  | homo/hetero | synonymous mutation |
| CDR1 | 4506 | chr 3 | 3225A>G |  | hetero | synonymous mutation |
| CDR1 | 4506 | chr 3 | 3237A>T |  | hetero | synonymous mutation |
| CDR1 | 4506 | chr 3 | 3267G>A |  | homo/hetero | synonymous mutation |
| ERG11 | 1587 | chr 5 | 1470G>A |  | homo/hetero | synonymous mutation |
| ERG11 | 1587 | chr 5 | 658G>A |  | hetero | synonymous mutation |
| ERG11 | 1587 | chr 5 | 411A>G |  | homo/hetero | synonymous mutation |
| ERG11 | 1587 | chr 5 | 357C>T |  | homo/hetero | synonymous mutation |
| ERG11 | 1587 | chr 5 | 348T>A | Glu116Asp | homo/hetero | missense mutation |
| MDR1 | 1695 | chr 6 | 1308G>A |  | homo/hetero | synonymous mutation |
| MDR1 | 1695 | chr 6 | 1287T>C |  | homo/hetero | synonymous mutation |
| MDR1 | 1695 | chr 6 | 1272T>C |  | homo/hetero | synonymous mutation |
| MDR1 | 1695 | chr 6 | 1041A>G |  | homo/hetero | synonymous mutation |
| MDR1 | 1695 | chr 6 | 645A>G |  | homo/hetero | synonymous mutation |
| MDR1 | 1695 | chr 6 | 624A>G |  | homo/hetero | synonymous mutation |
| MDR1 | 1695 | chr 6 | 612A>T |  | homo/hetero | synonymous mutation |
| MDR1 | 1695 | chr 6 | 597A>G |  | homo/hetero | synonymous mutation |
| MDR1 | 1695 | chr 6 | 471T>A |  | homo/hetero | synonymous mutation |
| MDR1 | 1695 | chr 6 | 468C>T |  | homo/hetero | synonymous mutation |



**Figure S1**. Proportions of child feeding (breast, bottle, and both breast and bottle feeding), night breast and bottle feeding, solid food intake, daycare attendance, mother and father as care provider measured at 1-, 2-, 4-, 6-, 12-, 18-, and 24-months.

The feeding patterns, including breastfeeding, bottle feeding (exclusively), night feeding, and consumption of solid food, daycare attendance and care provider are illustrated. Exclusively, breastfeeding gradually decreased from 32% at one month to 0% at 18 months. On the other hand, exclusively bottle feeding nearly doubled from 36% at one month to 61% at six months, remained stable between six to 12 months, and sharply decreased at 18 months. The breast and bottle-fed portion remained stable at approximately 30% from one to six months, then reduced to 0% at 24 months. Night breastfeeding was high during the first two months (58%), gradually decreasing as the children got older. Moreover, children who had night bottle feeding were high (73%) during the first six months, with a sharp drop after six months, reaching 3% at the age of two years. Consumption of solid food started as early as two months and peaked at 100% at 12 months. Daycare attendance was low and remained below 20% for all study points. Mother was involved in the care of their child approximately 100% of the time. However, the father was involved in the care of his/her child between 39% - 62% of the time.



**Figure S2.** Distribution of MIC values of 3 antifungal drugs tested in 4 *C.* *dubliniensis* clinical isolates from mother-child dyads**.**

The MIC values for nystatin, fluconazole, and caspofungin are illustrated separately in A1-3, B1-3, and C1-3. For nystatin and fluconazole, 100% of the mothers’ and children's isolates had MIC values of 0.5 μg/ml (A1-B2). For caspofungin, all the isolates had MIC value of 0.06 except one mother’s isolate had a value of 0.12 μg/ml (C1-3).

****

**Figure S3.** Distribution of MIC values of 3 antifungal drugs tested in of 6 *C. parapsilosis* clinical isolates from mother-child dyads.

The MIC values for nystatin, fluconazole, and caspofungin are illustrated in A1-3, B1-3, and C1-3 separately. For nystatin (A1-3), each one of the mother isolates had a different MIC value ranging between 0.5 - 4 μg/ml (A1). All children’s *C. parapsilosis* isolates had MIC values ≤2 μg/ml (A2-3). Regarding fluconazole (B1-3), mothers and children had the same frequency distribution of MIC values centered around 2-4 μg/ml. For caspofungin (C1-3), all the isolates had a MIC value of 0.25 μg/ml except one mother’s isolate, with a higher value of 0.5 μg/ml.



**Figure S4.** Heatmaps representing the relative growth of *Candida* isolates in the presence of different drug concentrations.

Abbreviations; RPMI: Roswell Park Memorial Institute Medium, NYS: nystatin, FLC: fluconazole, CAS: caspofungin, MIC: minimum inhibitory concentration, OD600: optical density measured at 600nm, v: study visit number, S: saliva, C.a: *C. albicans*,C.d: *C. dubliniensis*,C.p: *C.**parapsilosis*.

MIC assays were performed in RPMI medium in the presence of nystatin, fluconazole, or caspofungin. Optical densities were measured after 24 hours; the values were averaged for triplicate measurements and normalized to the no-drug (growth) controls. Data were displayed using GraphPad Prism version 9.4.1. see the color bar representing the relative growth.

Example of one mother-child dyad with *C. albicans* displaying the same MIC values for the mother and the two visits for the child for all tested drugs; this also indicates no change in the child's MIC values over time (A1-3). Cases of nystatin resistance where *C. albicans* from mom-child dyad had MIC values >2 μg/ml (B). Situations of susceptible dose-dependent of *C.**parapsilosis* to fluconazole, MIC= 4 μg/ml (C). A pair with *C. dubliniensis*, where the child’s isolate had a lower MIC value for caspofungin, MIC=0.06 μg/ml, compared to the mother’s isolate, MIC= 0.12 μg/ml (D).

**References**

1. Pfaller, M.A.; Diekema, D.J. Progress in antifungal susceptibility testing of Candida spp. by use of Clinical and Laboratory Standards Institute broth microdilution methods, 2010 to 2012. *J Clin Microbiol* **2012**, *50*, 2846-2856, doi:10.1128/JCM.00937-12.

2. Epstein, J.B.; Pearsall, N.N.; Truelove, E.L. Quantitative relationships between Candida albicans in saliva and the clinical status of human subjects. *J Clin Microbiol* **1980**, *12*, 475-476, doi:10.1128/jcm.12.3.475-476.1980.