Virulence and in vitro antifungal susceptibility of Candida albicans and Candida catenulata from laying hens

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Abstract
In spite of evidence that domestic and wild birds may act as carriers of human pathogenic fungi, data on the role of laying hens as reservoirs of drug resistant and virulent yeasts is lacking. Here, we assess several virulence factors (phospholipase and haemolysin activity) and the antifungal susceptibility profiles of 84 Candida albicans and 17 Candida catenulata strains isolated from cloacae (group A), faeces (group B) and eggs (group C) of laying hens. Of these strains, 95% C. albicans and 23% C. catenulata strains displayed phospholipase and haemolytic activities. For C. albicans, the highest values of phospholipase (Pz = 0.62) and haemolytic activities (Hz = 0.49) were recorded among the strains from group C whilst for C. catenulata (Pz = 0.54; Hz = 0.49) among those from group A. High minimum inhibitory concentration (MIC) values for azoles and amphotericin B (AmB) were recorded irrespective of their sources in all C. albicans strains. A total of 22 C. albicans strains were multidrug resistant, displaying resistance to fluconazole, itraconazole (ITZ), voriconazole (VOR) and posaconazole (POS). All C. catenulata strains from group C were resistant to ITZ, POS, micafungin and anidulafungin and susceptible to AmB. In this study, C. albicans and C. catenulata isolated from the cloacae, faeces and eggs of laying hens produced phospholipase and haemolysin and might be multidrug resistant. In the environment (faeces) or in eggs, C. albicans and C. catenulata strains might acquire pathogenic virulence traits and/or show multidrug resistance profiles. Based on these results, breeding and handling of laying hens and/or eggs may have implications for human and animal health.

Keywords Candida albicans · Candida catenulata · Laying hens · Virulence factors · Antifungal susceptibility

Introduction
Candida species are among the most common causes of infections, with overwhelming mortality rates in immunocompromised patients (Antinori et al. 2018; Tscherner et al. 2019). Candida spp. belong to the resident microbiota of the skin, mucosa and gastrointestinal tract of mammals including humans; in addition, domestic (Subramanya et al. 2017; Cafarchia et al. 2018) and wild birds (Brilhante et al. 2010; Brilhante et al. 2013) harbour and spread these yeasts in the environment via their faeces (Brilhante et al. 2010; Elhariri et al. 2015). Indeed, Candida albicans has been isolated from the gut, cloaca and droppings of domestic (e.g. laying hens, broiler chickens, pigeons) and wild (e.g. rhea, psittacinae, rap-tors, cockatiels) birds (Subramanya et al. 2017; Cafarchia et al. 2018; Brilhante et al. 2010). In addition, bird droppings act as a suitable substrate for the growth of Candida spp. due to intrinsically high concentrations of nitrogenous compounds (Elhariri et al. 2015). Yeasts such as Candida, Cryptococcus, Geotrichum, Rhodotorula and Trichosporon have also been isolated from environment, avian sources and eggs (Cafarchia et al. 2018). Interestingly, it has been demonstrated that laying hens might disseminate yeasts, mainly C. albicans and Candida catenulata, in the environment through eggs other than faeces (Cafarchia et al. 2018). At present, there is a growing concern that these yeasts species might acquire virulence...
determinants and antimicrobial resistance when moving between different niches (Tsiodras et al. 2008). Virulence factors such as secreted hydrolases (i.e. aspartyl proteases, phospholipases and haemolysins) play a pivotal role in the pathogenesis of Candida spp. infections (Guntang et al. 2017); the occurrence of such factors has been demonstrated in yeast strains, mainly C. albicans, isolated from the gut, cloaca and/or faeces of different avian species of synanthropic and pet birds or poultry (Cafarchia et al. 2008; Sidrim et al. 2010). Some of these yeasts also displayed a multidrug resistance profile (Brilhante et al. 2013; Sidrim et al. 2010; Lord et al. 2010; Brilhante et al. 2011; Brilhante et al. 2012), which has been associated, for fluconazole resistance in particular, to the secretion of phospholipases (Ying and Chunyang 2012). In spite of the likely contribution of domestic and wild birds as carriers of human pathogenic fungi, no data is yet available on the role of laying hens as reservoirs of drug-resistant and virulent yeasts. Therefore, the present study aimed to assess (i) the production of hydrolytic enzymes such as phospholipase and haemolysin and (ii) the antifungal susceptibility profiles of C. albicans and C. catenulata isolated from the cloacae, faeces and eggs of laying hens.

Materials and methods

Fungal strains

A total of 101 strains of C. albicans (n = 84) and of C. catenulata (n = 17), previously isolated from the cloacae, faeces and eggs of laying hens (Cafarchia et al. 2018), were employed in this study. In particular, two or three Candida spp. strains per group in each positive shed were selected. C. catenulata was chosen since it has also been isolated from yolks and albumens of eggs (Cafarchia et al. 2018). The strains were assigned to three groups based on their origin. Group A included C. albicans (n = 67) and C. catenulata (n = 5) strains from cloacal swabs of laying hens; group B, C. albicans (n = 13) and C. catenulata (n = 4) strains from pools of faeces; and group C. C. albicans (n = 4) and C. catenulata (n = 8) strains from egg samples. The strains were isolated and identified based on colonial morphology, microscopic features, and by matrix-assisted laser desorption/ionisation time of flight mass spectrometry (MALDI-TOF MS), as previously reported (Cafarchia et al. 2018). Strains were stored at −80 °C at the Department of Veterinary Medicine, University of Bari (Italy). Prior to testing, each strain was sub-cultured at least twice onto Sabouraud dextrose agar (SDA, Liofilchem, Italy) plates at 30 °C for 24–48 h to ensure strain purity and viability. All strains were molecularly confirmed by amplification and sequencing of the nuclear ribosomal ITS1-5.8S-ITS2 region.

In particular, genomic DNA was isolated from each strain using the DNeasy Blood & Tissue Kit (QIAGEN, Hilden, Germany), following manufacturer’s instructions. The nuclear ribosomal ITS1-5.8S-ITS2 region was amplified using ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS4 (5′-TCCTCCGCTATTGATATGC-3′) primers. The PCR reaction consisted of 4 μl genomic DNA (~100 ng) and 46 μl of PCR mix containing 2.5 mM MgCl2, 10 mM Tris-HCl (pH 8.3) and 50 mM KCl, 250 μM of each dNTP, 100 pmol of each primer and 1.25 U of AmpliTaq Gold (Applied Biosystems, Foster City, CA, USA), with an initial denaturation step of 10 min at 95 °C, followed by 35 cycles of denaturation for 1 min at 95 °C, annealing for 45 s at 55 °C, extension for 1 min 30 s at 72 °C and a final extension at 72 °C for 5 min. PCR products were examined on a 2% agarose gel stained with GelRed (VWR International PBI, Milano, Italy) and visualised on a Gel Logic 100 gel documentation system (Kodak, New York, USA). The amplicons were purified and sequenced in both directions using the Big Dye Terminator v.3.1 chemistry in a 3130 genetic analyser (Applied Biosystems, CA, USA). Sequences were aligned using the ClustalW program (Larkin et al. 2007) and compared with those available in GenBank by basic local alignment search tool (BLAST—http://blast.ncbi.nlm.nih.gov/Blast.cgi).

Preparation of the yeast suspensions for the enzymatic activities

A loopful of the stock culture was streaked onto SDA and incubated at 37 °C for 24–48 h. Colonies were transferred into sterile saline solution and adjusted to an optical density of 1.3 using a turbidimeter (DEN-1 McFarland Densitometer, Biosan) that was equivalent to 1.5 × 107 CFU/ml as validated by quantitative plate count of colony forming unit (CFU) in SDA.

Phospholipase activity

The production of phospholipase for Candida spp. was assessed using the egg-yolk plate method as previously described (Cafarchia et al. 2008; Price et al. 1982). A total of 10 μl yeast suspension was transferred onto egg-yolk plates and incubated at 32 °C for 5 days. The formation of a precipitation zone surrounding the colonies was considered as an indicator for enzyme production. The production of phospholipase (Pz) was expressed as a ratio of colony diameter by the total diameter of the colonies plus zone of precipitation (Price et al. 1982). Each strain was tested in duplicate, and the experiments were repeated over two consecutive days. The Pz values were reported as average of the values registered. The following ranges of activity according to the Pz index were...
established as follows: high, \( Pz \leq 0.69 \); moderate, \( Pz = 0.70-0.89 \); weak, \( Pz = 0.90-0.99 \); none, \( Pz = 1 \) (Price et al. 1982).

**Haemolytic activity**

The haemolytic activity of *Candida* spp. was assessed by using SDA supplemented with 7% sheep blood and 3% glucose and adjusted to a pH of 5.6 ± 0.29. A total of 10 μL using SDA supplemented with 7% sheep blood and 3% glucose was inoculated in duplicate onto the media and incubated at 37 °C for 2 and 5 days, respectively. After incubation, a distinct translucent halo around the inoculation site indicated positive haemolytic activity (Elfeky and Gohar 2016). The ratio of the diameter of the colony (a) by that of the translucent zone (b) was used as value of the haemolytic index (Hz value) which represents the extent of haemolysin activity by the different *C. albicans* strains tested. Each strain was tested in duplicate, and the experiments were repeated over two consecutive days. The Hz values were reported as average of the values registered.

**Antifungal susceptibility test**

The sensitivity to antifungal drugs of *C. albicans* and *C. catenulata* strains was evaluated using broth microdilution (BMD) testing in accordance with the guidelines available in the CLSI document M27-A3 (CLSI 2008). The following antifungal drugs were supplied by the manufacturers as pure standard compounds: amphotericin B (AmB) and itraconazole (ITC) (Sigma-Aldrich Milan, Italy), fluconazole (FLC) and voriconazole (VOR) (Pfizer Pharmaceuticals; Groton, Connecticut, USA), posaconazole (POS) (Schering-Plough Corporation, Kenilworth, NJ, USA), anidulafungin (ANI) (Sigma-Aldrich, Milan, Italy) and micafungin (MCF) (Sigma-Aldrich; Milan, Italy). FLC was dissolved in sterile water, whereas the remaining drugs were solubilised in dimethyl sulfoxide-DMSO (Sigma-Aldrich, Milan, Italy). The concentration of each antifungal drug ranged from 0.016 to 32 g/mL, except for FLC (i.e. from 0.06 to 128 mg/L). Visual reading of plates was performed after 48-h incubation at 35 °C. All strains were tested in duplicate. For azoles, the minimum inhibitory concentration (MIC) endpoint was defined as the lowest concentration that produced a significant decrease in turbidity (≥ 50%) compared with that of drug-free control. For AmB, the MIC endpoint was defined as the minimum concentration at which no visible growth was detected. Control strains (*Candida parapsilosis* ATCC 22019 and *Candida krusei* ATCC 6258 of the American Type Culture Collection, Manassas, VA, USA) were included to evaluate the accuracy of drug dilutions and reproducibility of results (CLSI 2008). Data were reported as MIC range, mean MIC value (MICm) and MIC to which 50% (MIC50) and 90% (MIC90) strains were inhibited. The following breakpoints or epidemiological cut-off values (ECV) were used to categorise these *Candida* strains as sensitive (S), dose-dependent sensitive (SDD) or resistant (R) (Pfaller 2012a; CLSI 2018): FLC (S MIC ≤ 2 μg/mL, SDD MIC = 4–32 μg/mL, R MIC ≥ 8 μg/mL); ITZ (S MIC = 0.125 μg/mL, SDD MIC = 0.25–0.5 μg/mL, R MIC ≥ 1 μg/mL); POS (ECV MIC ≤ 0.06 μg/mL, R MIC ≥ 0.06 μg/mL); VOR (S MIC ≤ 0.125 μg/mL, SDD MIC = 0.25–0.5 μg/mL, R MIC ≥ μg/mL); MCF and ANI (S MIC ≤ 0.25 μg/mL, SDD MIC = 0.5 μg/mL, R MIC ≥ 1 μg/mL) and AmB (ECV S MIC ≤ 2 μg/mL, R MIC > 2 μg/mL).

**Statistical analyses**

The Chi-square test (\( \chi^2 \)) and the Student’s \( t \) test were used to compare the virulence data, resistance occurrence and mean MIC values of azoles, echinocandins, and AmB of *C. albicans* and *C. catenulata* from the three groups, respectively. A value of \( P < 0.05 \) was considered statistically significant.

**Results**

The PCR assay produced species-specific bands of the expected size (550 bp) for both *C. albicans* and *C. catenulata* strains, and sequence analysis confirmed the initial identification, based on colonial morphology, microscopic features and MALDI identification as *C. albicans* (accession numbers: KY101860, MH061333, MN419374) for 84 yeast isolates and *C. catenulata* (accession number: KY103373) for 17 yeast isolates.

A total of 80 *C. albicans* (95%) and 4 *C. catenulata* (23.5%) strains showed phospholipase and haemolytic activities that differed according to the sample source, with the highest values recorded for *C. albicans* (Pz = 0.62, Hz = 0.49) in strains from group C and for *C. catenulata* (Pz = 0.54; Hz = 0.49) in strains from group A (Table 1).

High MIC values for azoles and AmB were recorded in all *C. albicans* strains from groups B and C (Table 2). *C. catenulata* showed high ITZ, POS ad VOR MIC values in strains from group B (faeces). ANI and MCF displayed the lowest MICs for both *C. albicans* and *C. catenulata* (Table 2). For *C. catenulata*, high MIC values for MCF (MIC\(_{90} = 8\)) and ANI (MIC\(_{90} = 8\)) were registered in strains from group C.

A relatively large number of *C. albicans* strains from groups B and C were resistant to azoles (i.e. from 30.7% for VOR to 92.3% for POS in group B and from 50 to 100% for all azoles in group C). MCF resistance was observed in *C. albicans* strains from group A (6%) and group C (50%) (Table 3). The drugs with the highest number of resistant *C. albicans* strains was POS (i.e. 83.6% strains in group A, 100% strains in groups B and C) followed by FLZ and ITZ (Table 3). All *C. albicans* strains were susceptible to ANI and AmB. A total of 21 *C. albicans* strains (15 from group A, 4
from group B and 2 from group C) were multidrug resistant, displaying resistance to FLC, ITZ, VOR, and POS. All C. catenulata strains from the group C were resistant to FLZ, ITZ, POS, MCF and ANI but susceptible to AmB (Table 3). C. catenulata strains from group A (40%) and group B (25%) were resistant to AmB.

**Discussion**

The results of this study demonstrate that C. albicans and C. catenulata isolated from cloacae, faeces and eggs of laying hens produce phospholipase and haemolysin and are resistant to azoles or AmB and echinocandin, thus highlighting the role

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**Table 1** Phospholipase activity and haemolysin activity of Candida albicans and Candida catenulata strains from cloaca swabs (group A), faeces (group B) and eggs (group C)

| Group       | Candida albicans | Candida catenulata |
|-------------|------------------|--------------------|
|             | Phospholipase    | Haemolysin         | Phospholipase    | Haemolysin         |
|             | Number of isolates (%) | Mean Pz (sd) | Number of isolates (%) | Mean Hz (sd) | Number of isolates (%) | Mean Pz (sd) | Number of isolates (%) | Mean Hz (sd) |
| Group A     | 65/67 (97.0) | 0.65 (0.11) | a, b | 66/67 (98.5) | 0.58 (0.12) | 5/5 (100) | 0.54 (0.03) | d | 2/5 (40) | 0.49 (0.001) |
| Group B     | 11/13 (84.6) | 0.73 (0.03) | b | 11/13 (84.6) | 0.52 (0.08) | 2/4 (50) | 0.72 (0.03) | d | 24 (50) | 0.59 (0.002) |
| Group C     | 3/4 (75.0) | 0.62 (0.12) | b | 4/4 (100) | 0.49 (0.02) | 1/8 (12.5) | 0.67 (0.04) | d | 0/8 (0) | 0 |
| Total       | 80/84 (95.2) | 0.66 (0.05) | 81/84 (96.4) | 0.53 (0.04) | 8/17 (47) | 0.64 (0.09) | 4/17 (23.5) | 0.54 (0.07) |

sd, standard deviation; the statistically significant differences were reported with the same superscript letters

**Table 2** Minimum inhibitory concentration (MIC, μg/mL) for the antifungal drugs tested of Candida albicans and Candida catenulata isolated from cloacal swabs (group A), faeces (group B) and eggs (group C) of laying hens

| Groups         | Antifungal agents | Candida albicans | Candida catenulata |
|----------------|-------------------|------------------|--------------------|
|                | Range | MIC<sub>50</sub> | MIC<sub>90</sub> | MIC m (dev.st) | Range | MIC<sub>50</sub> | MIC<sub>90</sub> | MIC m (dev.st) |
| Group A: cloacal swab | FLC     | 0.008-128 | 0.25 | 128 | 33.8 (55.4) | 4-128 | 8 | 8 | 31.2 (54.1) |
|                | ITZ     | 0.06-32 | 0.25 | 32 | 7.6 (12.9) | 0.25-32 | 0.5 | 1 | 6.9 (14.0) |
|                | VOR     | 0.06-32 | 0.015 | 32 | 7.5 (13.1) | 0.03-32 | 0.06 | 0.125 | 6.4 (14.2) |
|                | POS     | 0.03-32 | 0.125 | 32 | 7.2 (12.7) | 0.032-32 | 0.5 | 0.5 | 6.7 (14.1) |
|                | MCF     | 0.03-1 | 0.03 | 0.125 | 0.1 (0.2) | 0.016-0.125 | 0.032 | 0.125 | 0.07 (0.05) |
|                | ANI     | 0.03-0.5 | 0.06 | 0.5 | 0.1 (0.1) | 0.016-0.25 | 0.06 | 0.25 | 0.1 (0.1) |
|                | AmB     | 0.016-2 | 1 | 1 | 1.3 (0.5) | 0.5-2 | 0.5 | 2 | 1.2 (0.75) |
| Group B: faeces | FLC     | 0.25-128 | 8 | 128 | 52.9 (61.8) | 4-128 | 8 | 128 | 67 (70.4) |
|                | ITZ     | 0.25-32 | 1 | 32 | 10.3 (15.1) | 0.25-32 | 0.5 | 32 | 16.1 (18.2) |
|                | VOR     | 0.03-32 | 0.125 | 32 | 9.9 (15.3) | 0.125-32 | 16 | 32 | 20.0 (15.2) |
|                | POS     | 0.06-32 | 0.5 | 32 | 7.6 (13.9) | 0.5-32 | 16 | 32 | 20.1 (15.1) |
|                | MCF     | 0.03-0.125 | 0.06 | 0.125 | 0.06 (0.04) | 0.016-0.125 | 0.016 | 0.03 | 0.2 (0.4) |
|                | ANI     | 0.03-0.125 | 0.125 | 0.25 | 0.1 (0.1) | 0.06-0.5 | 0.06 | 0.06 | 0.1 (0.2) |
|                | AmB     | 0.125-2 | 1 | 2 | 1.2 (0.6) | 1-2 | 1 | 1 | 1.2 (0.5) |
| Group C: eggs | FLC     | 4-128 | 128 | 128 | 67 (70.4) | 8-32 | 32 | 32 | 19 (11.3) |
|                | ITZ     | 0.25-16 | 16 | 16 | 8.3 (8.8) | 1-8 | 2 | 8 | 3.8 (3.4) |
|                | VOR     | 0.03-16 | 16 | 16 | 8.0 (9.2) | 0.06-2 | 0.125 | 0.25 | 0.3 (0.7) |
|                | POS     | 0.125-16 | 16 | 16 | 8.1 (9.1) | 1-4 | 2 | 4 | 2.6 (1.2) |
|                | MCF     | 0.06-2 | 2 | 2 | 1.0 (1.1) | 2-16 | 2 | 8 | 4.7 (5.0) |
|                | ANI     | 0.25-0.5 | 0.25 | 0.25 | 0.3 (0.1) | 1-8 | 4 | 8 | 3.6 (2.1) |
|                | AmB     | 0.5-2 | 0.5 | 0.5 | 0.8 (0.8) | 0.5-1 | 0.5 | 1 | 0.68 (0.3) |

FLC, fluconazole; ITZ, itraconazole; VOR, voriconazole; POS, posaconazole; MCF, micafungin; ANI, anidulafungin; AmB, amphotericin. The statistically significant differences were reported with the same superscript letter.
of laying hens in harbouring and spreading pathogenic organisms in the environment. This finding is similar to that previously reported for strains from wild (Brilhante et al. 2013; Lord et al. 2010) or domesticated/pet birds (Subramanya et al. 2017; Ying and Chunyang 2012).

The production of phospholipase and proteinase has been shown to play a significant role in the pathogenesis and virulence of *Candida* species and related diseases (Dabiri et al. 2018). In addition, multidrug resistant yeasts often cause fatal infection in immunocompromised hosts (Mellinghoff et al. 2018). In particular, even if > 10^6 CFU/gr have been detected in Australian Camembert or other European cheese (Roostita 1996), the yeast was identified as the causative agent in onychomycosis or vulvovaginal infections and in only three cases of candidaemia (i.e. in one patient with gastric cancer, in one hospitalised premature infant and in one cirrhotic patient with endocarditis) (Crozier and Coats 1977; Sârbu et al. 2013; Çakır et al. 2019; Ha et al. 2018; Radosavljevic et al. 1999).

In particular, even if > 10^6 *C. catenulata* CFU/gr have been detected in Australian Camembert or other European cheese (Roostita 1996), the yeast was identified as the causative agent in onychomycosis or vulvovaginal infections and in only three cases of candidaemia (i.e. in one patient with gastric cancer, in one hospitalised premature infant and in one cirrhotic patient with endocarditis) (Crozier and Coats 1977; Sârbu et al. 2013; Çakır et al. 2019; Ha et al. 2018; Radosavljevic et al. 1999). However, even if *C. catenulata* is less pathogenic than *C. albicans*, its high distribution and low susceptibility to ITZ, POS, MCF and ANI as well as AmB might require a specific therapeutic protocol. A case of *C. catenulata* invasive infection was reported in a cancer patient that was successfully treated with intravenous AmB, following failed FLZ treatment (Radosavljevic et al. 1999). Nosocomial infections caused by *Candida* spp. have become more common in recent years, and

### Table 3  *Candida albicans* and *Candida catenulata* strains isolated from cloacal swabs (group A), faeces (group B) and eggs (Group C) of laying hens that are resistant to at least one of the antifungal drugs tested

| Antifungal agents | Group A: cloacal swab | GROUP B: POOL OF FAECE | Group C: EGGS |
|------------------|----------------------|------------------------|--------------|
|                  | *C. albicans* (%)    | *C. catenulata* (%)    | *C. albicans* (%) | *C. catenulata* (%) |
| FLC              | 24/67 (35.8%)        | 4/5 (80%)              | 10/13 (76.9%) | 3/4 (75%) |
| ITZ              | 25/67 (37.3%)        | 3/5 (60%)              | 7/13 (53.8)  | 2/4 (50%) |
| VOR              | 18/67 (26.8%)        | 1/5 (20%)              | 4/13 (30.7)  | 1/4 (25%) |
| POS              | 56/67 (83.6%)        | 4/5 (80%)              | 12/13 (92.3%) | 4/4 (100%) |
| MCF              | 4/67 (6%)            | 0/5 (0%)               | 0/13 (0%)    | 0/4 (0%) |
| ANI              | 0/67 (0%)            | 0/5 (0%)               | 0/13 (0%)    | 0/4 (0%) |
| AmB              | 0/67 (0%)            | 2/5 (40%)              | 0/13 (0%)    | 1/4 (25%) |

FLC, fluconazole; ITZ, itraconazole; VOR, voriconazole; POS, posaconazole; MCF, micafungin; ANI, anidulafungin; AmB, amphotericin. The statistically significant differences were reported with the same superscript letter
those caused by non-albicans Candida spp. are rising proportionally more than those by C. albicans, being responsible for 35–65% of all candidaemia in the general patient population (Sharma et al. 2017). Food or beverages may act as sources of Candida spp. infection that, following ingestion, might enter the bloodstream and cause fungaemia (Benedict et al. 2016). Although clinical cases are rare and occur in unusual clinical circumstances, the presence of yeasts in food may be of significance. In the environment (faeces) or in eggs, C. albicans strains are more pathogenic and are characterised by protease and haemolysin production and azole resistance, whereas C. catenulata is characterised by a multidrug resistance profile. Interestingly, the high MIC values for AmB detected among C. albicans from faeces or eggs and in C. catenulata from cloaca swabs are in contrast with those previously reported for yeasts isolated from Agapornis birds and cockatiels that are bred under different conditions (Brilhante et al. 2018; Reis et al. 2018).

However, despite the high MIC values registered for AmB, resistance phenomena were detected only in C. catenulata from groups A and B, most likely due to the high value of ECV used (i.e. MIC > 2) or to the small number of samples tested. Since AmB and echinocandins are the first line of treatment for Candida fungaemia (Keane et al. 2018; Demir et al. 2019), resistance to these drugs should be not underestimated and needs to be further investigated using molecular approaches. The co-habitation of humans and laying hens and/or eggs may expose humans to multidrug-resistant and virulent yeasts. The breeding and handling of these animals may have consequently substantial implications for human and animal health. Therefore, high hygiene procedures in laying hens farm as well as the use of personal protective equipment might be important to prevent human (keepers/visiting) and bird exposure to infectious propagules contaminating the environment and the eggs.

**Authors’ contributions** Wafa Rhimi and Claudia Cafarchia conceptualised the study and wrote the manuscript. Wafa Rhimi and Chioma Inyang Anike performed the research. Claudia Cafarchia, Giada Annoscia, Antonio Camarda, Adriana Mosca and Domenico Otranto contributed to analysis and interpretation of the study data. Claudia Cafarchia, Cinzia Cantacessi and Otranto Domenico revised, edited and made intellectual inputs in the manuscript. Cinzia Cantacessi revised the English version of the manuscript. All authors read and approved the final manuscript.

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