Section: Miscellaneous

Original Research Article

ORAL CANDIDIASIS: AN INTEGRATED CLINICAL, PATHOLOGICAL, AND PHARMACOLOGICAL INVESTIGATION OF CANDIDA SPECIES DISTRIBUTION, ANTIFUNGAL SUSCEPTIBILITY, AND CDR1 EFFLUX-PUMP GENE DETECTION IN PAKISTAN

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ABSTRACT

Background: Oral candidiasis is a common opportunistic infection in patients with underlying disease, and rising azole resistance makes it harder to treat. Local data on species distribution, antifungal susceptibility and efflux-gene carriage from Pakistan remain limited. **Materials and Methods:** We carried out this study at a tertiary care hospital in Peshawar, Pakistan, from March 2025 to November 2025. Oral ulcer swabs (n = 257) were collected from patients with underlying disease and suspected secondary oral thrush. Candida species were identified by colony morphology on Sabouraud dextrose agar, colony colour on chromogenic agar and germ-tube formation. Susceptibility to fluconazole, itraconazole, voriconazole, nystatin and amphotericin B was classified as susceptible, susceptible-dose-dependent or resistant following CLSI guidance. Genomic DNA was extracted from all isolates, and the efflux-pump gene CDR1 was screened for by PCR. Proportions are reported with 95% Wilson confidence intervals, and groups were compared with chi-square or Fisher's exact tests. **Result:** Of the 257 swabs, 140 (54.5%; 95% CI 48.4-60.4) were culture-positive for Candida; 77 patients (55.0%) were male and 63 (45.0%) were female. Candida albicans accounted for 130 isolates (92.9%), followed by C. glabrata (n = 5), C. tropicalis (n = 4) and C. krusei (n = 1). Most cases occurred between 41 and 70 years (68.6%), and the age profile did not differ by sex (p = 0.55). Among C. albicans isolates, resistance was 63.1% to fluconazole, 53.8% to voriconazole and 33.1% to itraconazole, compared with 8.5% to nystatin and 10.0% to amphotericin B. CDR1 was detected in 38 of 140 isolates (27.1%), comprising 36 C. albicans and 2 C. glabrata. **Conclusion:** C. albicans was the dominant cause of oral candidiasis in this cohort. Azole resistance was high, while resistance to nystatin and amphotericin B was low, and CDR1 explained only part of the resistance. These findings support susceptibility-guided azole use, continued local surveillance and further molecular studies of resistance mechanisms.

INTRODUCTION

Oral candidiasis is one of the most common opportunistic infections of the oral mucosa, and it is seen most often in people whose local or systemic defenses are weakened.[1,2] Candida species are harmless commensals in many people, but poor oral hygiene, decreased salivary flow, removable dentures, uncontrolled diabetes mellitus and immunosuppressive diseases can shift the balance toward disease.[2] Although the infection is considered superficial, it may indicate underlying immune deficiency and in the very sick patient can develop into systemic infection.[2,3]

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Candida albicans is still the most common cause of oral infection, representing approximately 50% to 40% of reported series.[2] Non-albicans Candida (NAC) species, such as C. glabrata, C. tropicalis and C. krusei, however, are increasingly reported and have been associated with prolonged antifungal exposure and better diagnostics.[2] This is clinically important because the immune-evasion mechanisms and drug susceptibility of NAC species are different from that of C. albicans.[2,4] The World Health Organization recognised the weight of this problem in 2022, when its first fungal priority pathogens list placed C. albicans in the critical group, C. glabrata and C. tropicalis in the high-priority group, and C. krusei (Pichia kudriavzeveii) in the medium-priority group.[5]

Antifungal resistance adds to the difficulty. In a recent retrospective study of oral candidiasis from Poland, resistance of C. albicans to azoles reached up to 30%, whereas most NAC strains showed azole resistance above 40%; polyenes were the most effective agents.[2] Data from Pakistan point in a similar direction, although most of them come from invasive disease. The first descriptive report of invasive Candida isolates from the country found C. tropicalis to be the most common species in adults and neonates,[6] and a later analysis of invasive C. glabrata linked fluconazole resistance to prior antifungal use.[7] In Khyber Pakhtunkhwa, a study of ICU patients with oral candidiasis reported high resistance of C. albicans to voriconazole (70.2%) and fluconazole (58.5%), with resistance to nystatin and amphotericin B of around 10-12%.[3]

The biology behind azole resistance is multifactorial. Mutations in or overexpression of ERG11, which encodes the drug target, are well described, as is overexpression of the efflux pump genes CDR1, CDR2 and MDR1, which are in turn controlled by the transcription factors TAC1 and MRR1.[8] CDR1 encodes an ATP-binding cassette transporter, and its overexpression has been associated with reduced intracellular drug accumulation in azole-resistant clinical isolates of C. albicans.[9] Screening for CDR1 by PCR is therefore a practical first step towards linking phenotypic resistance to a molecular mechanism, although detecting the gene does not by itself prove that it is overexpressed.

To our knowledge, locally generated data from Pakistan that combine species identification, antifungal susceptibility testing and efflux-gene detection in oral isolates are limited. We therefore conducted this study at a tertiary care hospital in Peshawar, Pakistan, between March 2025 and November 2025. Our aims were to determine the prevalence and species distribution of Candida in oral lesions of patients with underlying disease and clinically suspected secondary oral thrush, to describe the age and sex profile of culture-positive patients, to assess susceptibility to fluconazole, itraconazole, voriconazole, nystatin and amphotericin B, and to detect CDR1 by PCR in the isolates.

MATERIALS AND METHODS

Study design and setting: This study was carried out at a tertiary care hospital in Peshawar, Pakistan, from March 2025 to November 2025, using a non-probability convenience sampling technique. Laboratory work was performed in the microbiology laboratory of the respective hospitals. The study followed the principles of the Declaration of Helsinki,[10] and only patients willing to participate were enrolled in the study.

Participants and sample collection: Patients with oral thrush and suspected oral candidiasis were enrolled if they had an oral ulcer, an immunocompromising condition, or a secondary viral and bacterial infections that could possibly lower the immune system of the host. Patients who were already receiving antifungal treatment or immunosuppressant or corticosteroid therapy, and those unwilling to participate, were excluded. Oral swabs were collected aseptically and transferred to the laboratory. In total, 257 oral ulcer swabs were collected, of which 140 were culture-positive for Candida species and formed the study cohort. The sample size was justified as 140.

Isolation and identification of Candida: Swabs were streaked onto Sabouraud dextrose agar (SDA) and incubated at 37 °C for 24 hours, and single colonies were subcultured to obtain pure growth. Isolates were identified by colony morphology on SDA and by germ-tube formation, which was used to distinguish pathogenic C. albicans from commensal Candida.[3] Species-level identification, including that of the non-albicans species, relied on the characteristic colony colour each species produces on chromogenic agar. Pure isolates were stored in glycerol stocks at -80 °C.

Antifungal susceptibility testing: Susceptibility to fluconazole, itraconazole, voriconazole, nystatin and amphotericin B was tested by Disc diffusion method on Muller Hinton agar following CLSI guidance,[11,12] with C. albicans ATCC 90028 as the quality-control strain. Isolates were classified as susceptible (S), susceptible-dose-dependent (SDD) or resistant (R). Susceptibility results were analysed for 139 isolates, excluding the single C. krusei isolate.

DNA extraction and detection of the CDR1 gene: Genomic DNA was extracted from all 140 isolates using the Thermo Scientific Yeast DNA extraction kit and checked on a 1% agarose gel alongside a 100 bp DNA ladder. We screened for CDR1, an efflux-pump gene linked to azole resistance,[8,9] by PCR using the primers [Table 1] and cycling conditions as initial denaturation at 94 °C for three minutes followed by 35 cycles of denaturation at 94°C for 45 seconds. The annealing temperature was kept 62Co for 55 sec then an initial extension of 72°C for 50 sec, followed by a final extension at 72°C for 5 minutes, in a PCR machine (applied thermo cycler catalog 2700), and end the reaction at 12°C ∞. Products were

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resolved on a 1.5% agarose gel with a 100 bp ladder, and CDR1 was considered present when a band of the expected size (740 bp) was seen.

Table 1: CDR1 gene primer sequence annealing temperature and product size

Target DNA	Primer	Primer Sequence 5-3	Products Size	Annealing Temperature	Reference
CDR1	F R	TGTGTACTATCCATCAACCATCAGC CACCAAAATAAGCCGTTCTTCCA	650bp	62CO	[30]

Statistical analysis: Data were analysed in SPSS (31.0.2.). Categorical variables are presented as counts and percentages with 95% Wilson score confidence intervals [13,14]. Chi-square goodness-of-fit tests assessed departures from an even distribution of sex, species and age groups, and Pearson's chi-square test of independence assessed the association between sex and age group. Fisher's exact test was used when expected counts were below 5, including the comparisons of fluconazole resistance and CDR1 detection between C. albicans and C. glabrata. All tests were two-sided, and $p < 0.05$ was considered statistically significant.

RESULTS

Study population and prevalence of Candida infection: Of the 257 oral ulcer swabs collected from patients with underlying systemic disease and clinically suspected secondary oral candidiasis, 140 (54.5%; 95% CI 48.4-60.4) were culture-positive for Candida on morphological and physiological identification. These 140 isolates formed the final study cohort. Seventy-seven patients (55.0%; 95% CI 46.7-63.0) were male and 63 (45.0%; 95% CI 37.0-53.3) were female, a split that did not differ significantly from parity ($\chi^2 = 1.40$, df = 1, $p = 0.24$).
Species distribution: Candida albicans was by far the most common isolate (n = 130; 92.9%; 95% CI 87.4-96.1). Non-albicans Candida (NAC) species accounted for only 10 isolates (7.1%; 95% CI 3.9-12.6): C. glabrata (n = 5; 3.6%), C. tropicalis (n = 4; 2.9%) and C. krusei (n = 1; 0.7%). The species distribution departed strongly from an even spread ($\chi^2 = 344.1$, df = 3, $p < 0.001$; [Figure 1]).

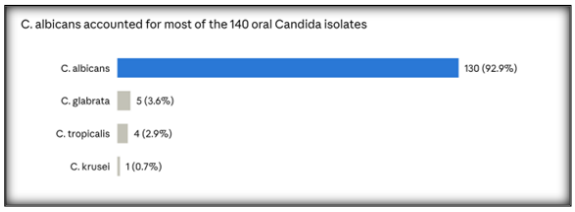


Figure 1: Species-wise distribution of Candida isolated from patients with oral candidiasis (n = 140).

Age distribution: Patients ranged from 10 to 90 years, and culture-positive cases clustered in middle and older age [Table 2, Figure 2]. The 56-70-year group contributed the largest share (n = 55; 39.3%; 95% CI 31.6-47.6), followed by the 41-55-year group (n = 41; 29.3%). Together, patients aged 41-70 years accounted for 96 of 140 cases (68.6%; 95% CI 60.5-75.7). The youngest (10-25 years; 10.7%) and oldest (71-90 years; 9.3%) groups contributed the fewest cases. The age distribution was significantly non-uniform ($\chi^2 = 51.3$, df = 4, $p < 0.001$; [Figure 2]).

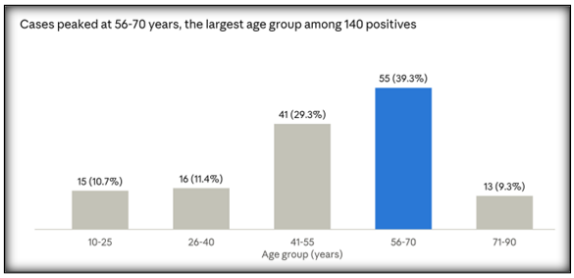


Figure 2: Age-wise distribution of Candida-positive patients with oral candidiasis (n = 140).

Age and sex: The age profile was similar in both sexes [Table 3, Figure 3]. Cases peaked at 56-70 years (males 41.6%, females 36.5%), followed by 41-55 years (28.6% and 30.2%), while the 10-25 and 71-90-year groups contributed the fewest. Males outnumbered females in four of the five age groups (10-25, 41-55, 56-70 and 71-90 years), whereas females predominated at 26-40 years (10 vs 6). These differences were not statistically significant: the age distribution did not differ between sexes ($\chi^2 = 3.07$, df = 4, $p = 0.55$; all expected counts ≥ 5.85; [Figure 3]).

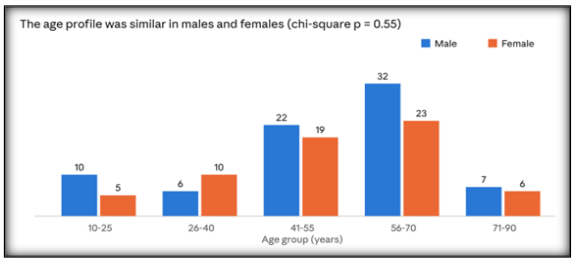


Figure 3: Age- and sex-wise distribution of Candida-positive patients (males, n = 77; females, n = 63).

Antifungal susceptibility profile: Susceptibility data were available for 139 isolates: C. albicans (n = 130), C. glabrata (n = 5) and C. tropicalis (n = 4) [Table 2]. The single C. krusei isolate has no susceptibility data [Table 2].
Among C. albicans isolates, resistance was highest to fluconazole (n = 82; 63.1%; 95% CI 54.5-70.9) and voriconazole (n = 70; 53.8%; 95% CI 45.3-62.2), and intermediate for itraconazole (n = 43; 33.1%; 95% CI 25.6-41.5). Resistance to the polyenes was low:

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nystatin (n = 11; 8.5%; 95% CI 4.8-14.5) and amphotericin B (n = 13; 10.0%; 95% CI 5.9-16.4), with 88.5% and 86.9% of isolates remaining susceptible, respectively. Susceptible-dose-dependent (SDD) results were uncommon (1.5-5.4% for the azoles and 3.1% for each polyene).

The five C. glabrata isolates showed fluconazole, itraconazole and voriconazole resistance in 2 isolates each (40.0%), nystatin resistance in 1 (20.0%) and no amphotericin B resistance (one isolate SDD). Fluconazole resistance did not differ significantly between C. albicans and C. glabrata (82/130 vs 2/5; Fisher's exact test, p = 0.37), although the comparison has very low power. The four C. tropicalis isolates showed no azole or nystatin resistance (one SDD isolate for each azole) and one amphotericin B-resistant isolate (25.0%). Across all 139 isolates, resistance was 60.4% to fluconazole, 51.8% to voriconazole, 32.4% to itraconazole, 10.1% to amphotericin B and 8.6% to nystatin.

Table 2: Antifungal susceptibility of oral Candida isolates, n (%)

Agent	Category	C. albicans (n = 130)	C. glabrata (n = 5)	C. tropicalis (n = 4)	All isolates (n = 139)
Fluconazole	S	45 (34.6)	3 (60.0)	3 (75.0)	51 (36.7)
	R	82 (63.1)	2 (40.0)	0 (0.0)	84 (60.4)
	SDD	3 (2.3)	0 (0.0)	1 (25.0)	4 (2.9)
Itraconazole	S	80 (61.5)	3 (60.0)	3 (75.0)	86 (61.9)
	R	43 (33.1)	2 (40.0)	0 (0.0)	45 (32.4)
	SDD	7 (5.4)	0 (0.0)	1 (25.0)	8 (5.8)
Voriconazole	S	58 (44.6)	2 (40.0)	3 (75.0)	63 (45.3)
	R	70 (53.8)	2 (40.0)	0 (0.0)	72 (51.8)
	SDD	2 (1.5)	1 (20.0)	1 (25.0)	4 (2.9)
Nystatin	S	115 (88.5)	4 (80.0)	4 (100.0)	123 (88.5)
	R	11 (8.5)	1 (20.0)	0 (0.0)	12 (8.6)
	SDD	4 (3.1)	0 (0.0)	0 (0.0)	4 (2.9)
Amphotericin B	S	113 (86.9)	4 (80.0)	3 (75.0)	120 (86.3)
	R	13 (10.0)	0 (0.0)	1 (25.0)	14 (10.1)
	SDD	4 (3.1)	1 (20.0)	0 (0.0)	5 (3.6)

S, susceptible; R, resistant; SDD, susceptible-dose-dependent. Percentages are calculated from the total isolates of each species.

Molecular confirmation and CDR1 detection: Genomic DNA was successfully extracted from all 140 isolates using the Thermo Scientific Yeast DNA extraction kit and resolved on 1% agarose gel alongside a 100 bp ladder. PCR targeting the CDR1 efflux-pump gene yielded a product of the expected size (740 bp) on 1.5% agarose gel (Fig. 5). CDR1 was detected in 38 of 140 isolates (27.1%; 95% CI 20.5-35.0), and only among azole-resistant isolates of two species: C. albicans (36 of 130; 27.7%; 95% CI 20.7-35.9) and C. glabrata (2 of 5; 40.0%; 95% CI 11.8-76.9). The gene was not amplified in the remaining isolates. Detection rates did not differ significantly between C. albicans and C. glabrata (Fisher's exact test, p = 0.62), although the C. glabrata estimate is imprecise because of the small number of isolates (n = 5).

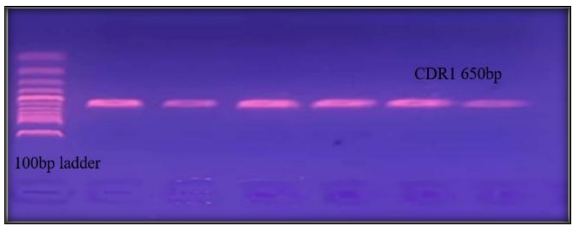


Figure 4: PCR amplification of CDR1 (740 bp) on 1.5% agarose gel. Lane L, 100 bp DNA ladder.

DISCUSSION

In this study from a tertiary care hospital in Peshawar, just over half of the swabs (140 of 257; 54.5%) taken from patients with underlying disease and suspected oral thrush grew Candida. Candida albicans accounted for nearly all isolates (92.9%). Azole resistance was common in C. albicans (fluconazole 63.1%, voriconazole 53.8%, itraconazole 33.1%), resistance to nystatin and amphotericin B was low (8.5% and 10.0%), and the efflux-pump gene CDR1 was detected in 27.1% of isolates. Because many healthy adults carry Candida in the mouth,[15] isolation alone does not establish disease; however, all our patients had suspected thrush and an underlying condition, which makes these isolates clinically relevant. Collectively the current result suggests that C. albicans remains the main causative agent of oral candidiasis in this study population. Yet they cannot support the azole group is the final treatment option because of high level of resistant comparatively the nystatin is still the best therapeutic option in case of oral candidiasis.

The predominance of C. albicans is consistent with published oral candidiasis series, where it is the most common isolate.[2,3] However, the proportion of non-albicans Candida (NAC) in our cohort (7.1%) was significantly lower than that reported in a large retrospective series from Poland (33.2%),[2] and in the patients with oral candidiasis in ICU in Khyber Pakhtunkhwa (one quarter).[3] The low proportion of NAC in this population should not be interpreted as a regional norm, as reports from India indicate that C. tropicalis is playing a larger role in the country,[16] and the emergence of NAC has been described in a Karachi population.[17]

There are a number of possible explanations. Patients who were already on antifungal treatment were excluded, and extended antifungal exposure is one of

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the factors associated with the emergence of NAC,[2] which may have reduced the pre-selection of these species in our cohort. Some NAC species may also be overlooked or misidentified by colony morphology and chromogenic agar, further increasing the proportion. Although the number of NAC isolates was low, they are not insignificant.

The World Health Organization has classified C. glabrata and C. tropicalis as high priority fungi and C. krusei as medium priority fungi,[5] and fluconazole resistance in invasive C. glabrata from Pakistan has been associated with previous use of antifungals.[7] We do not make species-level comparisons, and these observations are descriptive, because there are only 10 NAC isolates. The age group of culture-positive patients ranged from 41 to 70 years, with the 56-70-year group being the largest (68.6%). This is consistent with the study that linked oral Candida positivity with increasing age,[18] and the regional ICU study that reported the peak age of 61-70 years.[3] There is one caution here: our age figures are shares of positive cases, not prevalence. It is not possible to say whether older people were more likely to be infected or more likely to be sampled without the age distribution of all 257 patients. The age profile was comparable in both sexes and the sex ratio (55.0% male, 45.0% female) was not significantly different from parity. There is mixed evidence regarding sex. Some studies did not show any sex difference after adjusting for age,[18] while others, including the regional ICU study, showed higher rates in women.[3,19] There was no sex difference in our data, but this study was not designed to test one. Host factors are likely more important than age or sex alone.

Host factors probably matter more than age or sex alone. Oral candidiasis is promoted by weakened immunity, dentures, dry mouth, malnutrition and endocrine disease, among other factors.[20] Our inclusion criteria deliberately selected immunocompromised patients and those with viral co-infections, groups in which the defenses that normally restrain Candida are impaired. We did not record diabetes or denture use, although both are established contributors,[2,21] and so cannot say how much each added to the infections we saw.

Azole resistance was the most striking finding. Fluconazole resistance in C. albicans (63.1%) was far higher than the resistance of up to 30% to azoles reported for C. albicans in oral isolates from Poland,[2] yet close to the fluconazole (58.5%) and voriconazole (70.2%) resistance reported for ICU patients with oral candidiasis in Khyber Pakhtunkhwa.[3] Large global surveillance programmes have tracked susceptibility of Candida to fluconazole and voriconazole for more than a decade,[22] and differences in testing methods and interpretive criteria make direct comparison across studies difficult, but the agreement within the region is notable.

Because patients on current antifungal treatment were excluded, the high rate is unlikely to reflect therapy at the time of sampling. Earlier azole exposure, empirical azole use in the community and acquisition of resistant strains in hospital are all plausible explanations, although our design cannot separate them. Azole resistance in Candida rose sharply in the 1990s when patients with AIDS received prolonged azole therapy,[23] and resistance can emerge and spread wherever drug pressure is repeated.[24] The link between prior antifungal use and fluconazole resistance seen in invasive C. glabrata from Pakistan,[7] points in the same direction and underlines the importance of exposure history.

Resistance to the polyenes was low and similar to that seen in the regional ICU study (9.9% for nystatin and 11.7% for amphotericin B),[3] and to the low polyene resistance reported in Poland.[2] In practical terms, polyenes remain a dependable option for oral disease, whereas empirical azole therapy risks failure in a majority of C. albicans infections in this setting. Our results therefore favour susceptibility-guided rather than empirical azole use. SDD results were uncommon, and the five C. glabrata and four C. tropicalis isolates are too few for firm conclusions; the comparison of fluconazole resistance between C. albicans and C. glabrata was not significant, but it had very low power.

CDR1 was detected in 27.7% of C. albicans and in 2 of 5 C. glabrata isolates. It encodes an ATP-binding cassette efflux pump whose overexpression lowers intracellular azole accumulation in resistant clinical isolates,[9] and a recent systematic review ranked CDR1 first among resistance genes by expression level in fluconazole-resistant C. albicans, particularly in patients with HIV infection or cancer.[25] In C. glabrata, azole resistance is largely driven by overexpression of CDR1 and/or CDR2,[26] so finding CDR1 in this species fits the biology. A Nigerian study detected CDR1 in 80% of multidrug-resistant C. albicans selected for molecular testing.[27] Our lower figure is calculated over all C. albicans isolates rather than only resistant ones, so the two are not directly comparable. The role of multidrug transporters, including CDR1, was recognised early in azole-resistant C. albicans from patients with AIDS,[28] and our oral isolates from patients with underlying disease extend that observation to a new setting.

Two points deserve emphasis. First, only 36 C. albicans isolates carried CDR1, whereas 82 were fluconazole-resistant and 70 voriconazole-resistant, so this efflux gene can explain only part of the resistance we observed. Other mechanisms, including ERG11 alterations, MDR1 overexpression and regulatory changes in TAC1, MRR1 and UPC2, probably contribute.[8,25] Second, CDR1 is a native gene of C. albicans, so a positive PCR should be read as a marker of the assay target rather than as proof of resistance. Quantitative expression assays and sequencing would be needed to link CDR1 activity to the resistant phenotype, and the lower detection rate seen here should be confirmed with a second primer set.

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A strength of this work is that species identification, susceptibility profiling and molecular screening were carried out on the same cohort of 140 isolates in a setting where such data are scarce. Clinically, successful management of oral candidiasis depends on accurate diagnosis, correction of predisposing factors and an adequate choice, dose and duration of antifungal therapy, since inadequate treatment contributes to poor outcomes and recurrence.[29] Our findings add that, locally, the antifungal choice should be informed by susceptibility results, with polyenes preferred when results are unavailable, and that treatment in patients with underlying disease should be followed up for response.[2,3]

CONCLUSION

In this cohort of patients with underlying disease and suspected oral thrush, C. albicans was by far the leading cause, azole resistance was high, polyene resistance was low, and CDR1 was present in a minority of isolates. These findings support susceptibility-guided azole use and continued local surveillance.

Limitations: This was a single-center study that used convenience sampling, which limits generalizability. Identification relied on phenotypic methods, and isolates were subcultured from a single colony, so misclassification of non-albicans species and mixed infections, found in about 8% of patients in a Polish series,[2] may have been missed. The non-albicans group (n = 10) and the C. glabrata group (n = 5) were too small for species-level inference. Age figures describe positive cases rather than prevalence, predisposing factors such as diabetes and denture use were not analysed, and there was no uninfected comparison group. Finally, we screened only for CDR1 and did not measure its expression or examine other resistance genes.

Future recommendations: Future work should use multicenter, adequately powered sampling that includes more non-albicans isolates and an uninfected comparison group, with recorded risk factors such as diabetes, dentures and prior antifungal exposure. Species should be confirmed by sequencing or mass spectrometry, and susceptibility testing should be complemented by minimum inhibitory concentrations. Resistance mechanisms should be explored by measuring the expression of CDR1, CDR2 and MDR1 and by sequencing ERG11 and the regulators TAC1, MRR1 and UPC2 [8,25]. At the clinical level, local antifungal surveillance and stewardship programmes are needed to guide empirical therapy and to monitor any rise in polyene resistance.

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