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DEVELOPMENT OF FUNGAL IDENTIFICATION TOOLS AND EVALUATION OF MICROBIOLOGICAL AND CLINICAL PROFILES OF CANDIDA AND RELATED OPPORTUNISTIC YEAST SPECIES: A REVIEW OF A DOCTORAL THESIS

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ABSTRACT

Invasive and superficial fungal infections caused by Candida and related opportunistic yeasts remain a significant, and frequently under-recognised, cause of morbidity and mortality worldwide, a burden that is disproportionately felt in resource-limited settings where reference-standard identification platforms such as MALDI-TOF mass spectrometry and Sanger sequencing remain largely inaccessible. The thesis under review compiles sixteen linked studies — spanning novel multiplex and real-time PCR assay development, comparative diagnostic evaluation, and multicentre clinical-epidemiological profiling of Candida species and rare yeast pathogens in Iran — into a single body of work culminating in an integrative discussion. This review synthesises the thesis across two thematic strands, namely (i) the development and validation of low-cost molecular identification tools, and (ii) the clinical, epidemiological, and antifungal-susceptibility profiling of Candida species complexes and emerging yeasts, before offering a critical appraisal of the work's methodological strengths, limitations, and implications for diagnostic microbiology in developing countries.

KEYWORDS: Candida; opportunistic yeasts; multiplex PCR; antifungal susceptibility; molecular diagnostics; developing countries; candidemia.

1. INTRODUCTION

Fungal pathogens, once regarded as peripheral to infectious-disease practice, are now understood to cause an estimated 1.5 million deaths annually, with opportunistic yeasts of the genus Candida accounting for a large share of invasive disease in hospitalised and immunocompromised patients. Global surveillance efforts have documented a marked epidemiological shift away from Candida albicans toward non-albicans Candida (NCAC) species and intrinsically drug-resistant organisms such as Candida auris, a shift attributed to widespread antifungal prophylaxis and the expansion of at-risk patient populations (1,2). This transition matters clinically because many NCAC species and cryptic sibling species are difficult to distinguish using conventional phenotypic methods, and because reference molecular platforms remain concentrated in a small minority of laboratories even within middle-income countries (3).

It is against this backdrop that the reviewed thesis situates its contribution. Structured as a general introduction followed by fifteen linked research chapters and a concluding discussion, the thesis addresses two interlocking problems: first, how accurate, affordable molecular tools can be engineered for yeast identification in settings lacking mass-spectrometry infrastructure; and second, what the resulting clinical, genotypic, and antifungal-susceptibility profile of circulating Candida and related yeast isolates looks like in a large multicentre Iranian cohort. The purpose of the present review is to draw together the thesis's constituent studies into a coherent narrative, to identify the cumulative contribution of the work relative to the existing literature, and to offer a critical assessment of its methodology and scope.

2. Scope and Structure of the Thesis

The thesis opens with a general introduction that frames fungal disease as an under-resourced area of biomedical research relative to its biodiversity and clinical footprint, before reviewing the phylogenetic complications that arise when morphologically similar yeasts are taxonomically misclassified (for example, the historical grouping of the genetically distinct Nakaseomyces clade under Candida). The subsequent chapters can be grouped into two broad categories. The first, comprising roughly half of the research chapters, concerns the design, optimisation, and validation of novel PCR-based assays for yeast identification. The second comprises multicentre observational studies that apply these and comparable molecular tools to characterise the prevalence, genotypic diversity, and antifungal-resistance profile of clinically important Candida species complexes and rarer opportunistic yeasts in Iranian

hospitals, closing with a case report of a previously unreported human pathogen and a synthesising discussion chapter.

3. Development and Validation of Molecular Identification Tools

A central and recurring contribution of the thesis is the design of multiplex PCR assays intended to substitute for costly reference platforms. The YEAST PANEL multiplex PCR, validated against 804 clinical isolates from three countries alongside more than 800 reference strains, is reported to identify 21 pathogenic yeast genera directly from pure colonies without a DNA-extraction step, a design choice aimed squarely at reducing per-test cost and turnaround time in under-resourced laboratories. In a related comparative study, a 21-plex PCR assay was benchmarked head-to-head against API 20C AUX, MALDI-TOF MS, and rDNA sequencing across 301 isolates; while MALDI-TOF achieved the highest raw accuracy, the thesis reports that combining the 21-plex PCR with the API biochemical system yielded a substantially improved identification rate, illustrating a pragmatic 'hybrid' diagnostic strategy for laboratories that already own basic biochemical kits but lack mass spectrometry.

The thesis then addresses a persistent limitation of standard identification schemes: their inability to resolve cryptic sibling species within the C. albicans, C. glabrata, and C. parapsilosis species complexes. A one-step 9-plex PCR targeting HWP1, an intergenic spacer region, and ITS rDNA is reported to distinguish nine cryptic species across 167 type strains and 280 patient isolates with complete specificity, including cases where MALDI-TOF misidentified C. africana as C. albicans. A parallel line of work targets Candida auris and its phylogenetically related multidrug-resistant congeners (C. haemulonii, C. duobushaemulonii, C. pseudohaemulonii): a tetraplex PCR validated on 821 isolates is reported to have produced no false positives, while a subsequent multiplex quantitative real-time PCR extended detection directly to spiked serum samples, reportedly detecting as few as one to ten genome copies per millilitre without cross-reactivity — a sensitivity level intended to support culture-independent, direct-from-blood screening for this globally spreading pathogen.

Beyond Candida, the thesis develops assays for two under-recognised opportunistic pathogens. A dual-function multiplex PCR is presented for Cyberlindnera fabianii, a biofilm-forming, fluconazole-resistant yeast frequently confused with Wickerhamomyces anomalus on biochemical testing, and is accompanied by a review of previously published cases. Similarly, a multiplex real-time PCR targeting the genus Madurella — the principal causative agents of eumycetoma, a neglected tropical disease — is reported to differentiate four morphologically

similar species with high specificity and analytical sensitivity directly from biopsy material, avoiding the two-week delay associated with culture-based diagnosis.

4. Clinical, Epidemiological, and Antifungal-Susceptibility Profiling

The second thematic strand applies these and comparable molecular tools to describe the clinical and microbiological landscape of yeast infection in Iran across several linked multicentre cohorts. Among haemato-oncology patients, oral yeast carriage was dominated by C. albicans and Kluyveromyces marxianus, with a reported nystatin treatment-failure rate of approximately 70 percent and a significant association between prior oral candidiasis and treatment failure, a finding the thesis uses to question the continued reliance on topical nystatin in this population. In bloodstream isolates of C. glabrata, the thesis reports a mortality rate of roughly 35 percent together with elevated minimum inhibitory concentrations to caspofungin and voriconazole, resistance mutations concentrated in PDR1 and ERG11 rather than the FKS genes, and genotype-dependent differences in outcome using AFLP fingerprinting.

Similar multicentre profiling was extended to the C. parapsilosis species complex (including the less-studied siblings C. orthopsilosis and C. metapsilosis), C. tropicalis, and the rare species C. nivariensis. Despite comparatively low measured resistance to first-line azoles in several of these cohorts, mortality rates were reported to be strikingly high (in the range of 45–60 percent for C. parapsilosis and C. tropicalis bloodstream isolates), a pattern the thesis attributes substantially to inappropriate or absent antifungal therapy rather than to laboratory-confirmed drug resistance, alongside AFLP evidence suggestive of possible nosocomial or horizontal transmission in several clusters. The C. tropicalis cohort additionally reported the emergence of fluconazole and pan-azole resistance in antifungal-naive patients, linked to mutations in MRR1, TAC1, and UPC2. The clinical section concludes with a case report describing the first documented human bloodstream and catheter-associated infection caused by Wickerhamomyces myanmarensis, an organism previously known only from an environmental (palm-sugar) source, identified only after biochemical and MALDI-TOF platforms failed and molecular sequencing and species-specific qPCR succeeded.

5. Critical Appraisal

5.1 Strengths

The thesis's principal strength lies in its consistent orientation toward diagnostic equity: each molecular assay is explicitly designed and benchmarked with reference to cost, turnaround time, and infrastructural feasibility in developing-country laboratories, rather than in the abstract. The validation samples are also comparatively large and multicountry for assay-development studies of this kind (several hundred isolates per assay in some chapters), and the pairing of assay-development chapters with real-world clinical-epidemiological chapters allows the diagnostic tools to be assessed against an actual multicentre disease burden rather than in isolation. The identification of a previously unreported human pathogen (W. myanmarensis) further demonstrates the practical diagnostic yield of the molecular approaches developed elsewhere in the thesis.

5.2 Limitations

Several limitations, largely acknowledged implicitly within the thesis's own discussion chapter, merit note. First, the clinical-epidemiological cohorts are drawn predominantly from a limited number of Iranian tertiary centres, which constrains the generalisability of prevalence and resistance figures to other developing-country settings with different antifungal-prescribing practices and patient populations. Second, several of the reported resistance and mortality associations are drawn from retrospective, non-randomised cohorts in which the contribution of confounding clinical variables (severity of underlying illness, delay to appropriate therapy) to observed mortality is difficult to fully disentangle from species-or genotype-specific virulence. Third, while the multiplex assays are repeatedly reported to achieve very high or complete specificity and sensitivity in validation panels, independent external validation in laboratories other than those involved in assay development would strengthen confidence in real-world performance, particularly for assays intended for direct use on complex clinical specimens such as serum or biopsy tissue. Finally, the reliance on AFLP fingerprinting, while useful for local outbreak and transmission inference, is a lower-resolution typing method than whole-genome sequencing approaches now increasingly used to track organisms such as C. auris internationally (2).

6. Significance and Implications

Taken as a whole, the thesis makes a cumulative case that carefully engineered multiplex PCR assays can substitute for, or usefully supplement, mass-spectrometry-based identification in laboratories that cannot afford the latter, while simultaneously generating

clinically actionable epidemiological and resistance data. This dual contribution is consistent with broader calls in the literature for diagnostic strategies tailored to the realities of laboratories in Asia and other developing regions, where surveys have repeatedly found that only a small minority of centres have access to advanced identification platforms (3). The recurring finding across the clinical chapters — that mortality from Candida bloodstream infection remains high even where laboratory-confirmed drug resistance is comparatively low — also carries a practical implication that extends beyond diagnostics: improving access to accurate, rapid species identification is unlikely by itself to improve patient outcomes unless it is paired with parallel improvements in antifungal stewardship and timely treatment initiation.

7. CONCLUSION

This thesis assembles a substantial and internally coherent body of work on the development of low-cost molecular diagnostic tools for opportunistic yeast pathogens and their application to describing the clinical and antifungal-susceptibility landscape of candidemia and related infections in Iran. Its principal contribution is methodological and translational: demonstrating, across a series of validated multiplex and real-time PCR assays, that accurate species- and even cryptic-species-level identification is achievable without recourse to mass spectrometry, while its clinical chapters add meaningful multicentre data on resistance mechanisms, genotypic diversity, and treatment outcomes that are of direct relevance to antifungal stewardship in resource-limited settings. Future work building on this thesis would benefit from prospective, multi-country validation of the assays described and from linking genotypic and resistance data more directly to standardised treatment and outcome protocols.

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