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ORIGINAL ARTICLE

Morphological and Molecular Characterization of Nasal Fungal Isolates from Restaurant Workers and Detection of *Candida* PLB1 as a Virulence Marker

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ABSTRACT

Background: Restaurant workers may be exposed to fungal bioaerosols that can colonize the upper respiratory tract and pose potential occupational and public health risks. Opportunistic fungi, particularly non-*albicans Candida*, are clinically important because of their virulence traits and possible antifungal resistance. This study aimed to identify nasal fungal isolates from restaurant workers and assess selected phenotypic and molecular virulence markers.

Methods: Ninety-three nasal swabs were collected from restaurant workers in Kirkuk, Iraq, between December 2024 and July 2025. Yeasts were identified using Sabouraud dextrose agar, Candida chromogenic agar, and microscopic examination. Molds were identified according to macroscopic and microscopic characteristics. Phospholipase activity was evaluated phenotypically using egg yolk agar. Molecular identification was performed by PCR targeting the ITS region for yeasts and the β-tubulin gene for molds, while the *PLB1* gene was amplified as a virulence marker in *Candida* isolates.

Results: Fungal growth was detected in 30 samples (32.26%). A total of 32 fungal isolates were recovered, with *Candida krusei* being the most frequent species (20/32; 62.50%), followed by *Candida glabrata* (7/32; 21.88%). Other isolates included *Penicillium chrysogenum* (6.25%), *Candida parapsilosis*, *Penicillium oxalicum*, and *Aspergillus niger* (3.13% each). The highest culture positivity was observed among workers aged 31–40 years, but age was not significantly associated with fungal positivity. Phospholipase activity was generally low. The *PLB1* gene was detected in 75% of *Candida* isolates, most commonly in *C. krusei*.

Conclusion: Nasal fungal colonization was detected in nearly one-third of restaurant workers, predominantly by non-*albicans Candida*. Molecular methods improved species-level identification and showed frequent *PLB1* detection despite low phenotypic phospholipase activity.

Key words: *Candida*; non-*albicans Candida*; Fungal colonization; Restaurant workers; Nasal swab; *PLB1*; Virulence factors.

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INTRODUCTION

Nasal fungal colonization among restaurant workers represents an important intersection between occupational health and food safety, particularly because these workers may be exposed to airborne bioaerosols, including fungal spores, while handling ready-to-eat foods. Foodborne diseases are estimated to cause 600 million illnesses and 420,000 deaths annually worldwide, demonstrating how emerging and often hidden infections in small food-service establishments may become serious public health threats [1]. Invasive and opportunistic fungal infections have also emerged as an increasing public health problem, mainly because of the growing number of immunocompromised individuals. This burden is further exacerbated by the declining effectiveness of treatment due to the development and spread of antifungal resistance. In response, the World Health Organization (WHO) issued the Fungal Priority Pathogens List (FPPL) [2].

The nasal cavity is a major entry site that continuously receives particles from inhaled air. Recent mycobiome studies support the role of fungi as detectable components of upper respiratory tract colonization. This is particularly relevant in occupational settings, where food-service facilities may generate complex microbial aerosols due to humidity, dust resuspension, and cooking activities. A cross-sectional study involving 339 individuals reported significant differences in several indices of nasal fungal composition and structure between patients with allergic rhinitis or asthma and healthy individuals. Notably, approximately 28.7% of fungal species were unique to healthy individuals, supporting the presence of stable commensal fungi, while up to 71% of dominant genera differed significantly between healthy and diseased groups. These findings indicate that the nasal cavity may act as a dynamic reservoir for opportunistic fungi and highlight the need for precise, high-resolution methods for fungal identification [3]. Furthermore, restaurant-associated sources, such as kitchen chimneys and grease-associated microbiota, may contribute to the release of bioaerosols containing fungal communities, thereby increasing exposure among workers and in the surrounding environment [4].

Among pathogenic yeasts, *Candida* spp. are among the most clinically important opportunistic fungi because of their ability to colonize mucosal surfaces, cause infection in susceptible hosts, and exhibit antifungal resistance. *Pichia kudriavzevii*, also known as *Candida krusei*, has received particular attention because of its intrinsic resistance to fluconazole [5].

In addition to antifungal resistance, several virulence-related factors may increase the clinical significance of *Candida* isolates. Cell-wall mannan, a major surface component of *Candida*, contributes to adhesion, colonization, and modulation of the host immune response. Moreover, extracellular enzymes such as phospholipases may promote membrane disruption, adhesion, tissue invasion, and persistence. Therefore, combining phenotypic phospholipase assays with polymerase chain reaction (PCR)-based detection of phospholipase-associated genes, such as *PLB1*, may improve the assessment of virulence potential [6].

Culture-based identification remains fundamental for the detection of filamentous fungi; however, phenotypic overlap may limit accurate species differentiation, particularly among *Aspergillus* and *Penicillium* species. Consequently, molecular targets other than the internal transcribed spacer (ITS) region are often recommended for reliable species discrimination. The β-tubulin gene, commonly referred to as *BenA*, is one of the most informative targets for improving species-level identification in these genera [7].

Integrating phenotypic, molecular, and virulence-marker data is therefore particularly important in studies involving occupationally exposed groups in the food-service industry. Although ITS- and β-tubulin/*BenA*-based identification, together with *PLB1* detection, are established approaches, local data from Kirkuk on nasal fungal isolates among restaurant workers remain limited. The novelty of this study lies in providing local epidemiological data using an integrated phenotypic, molecular, and virulence-marker approach to characterize fungal isolates among food-service workers.

MATERIALS AND METHODS

Study design, setting, and participants

This cross-sectional study was conducted in Kirkuk City, Iraq, from December 2024 to July 2025. Nasal swab samples were collected from 93 male restaurant workers employed in food preparation. Participants were recruited from 20 restaurants distributed across four areas of Kirkuk City.

Eligible participants were workers directly involved in food preparation who voluntarily agreed to participate in the study. Workers not involved in food preparation were excluded. The study protocol was reviewed and approved by the competent scientific authorities at the College of Science, University of Kirkuk, according to College Council session No. *158S* dated *18 November 2024*, based on University Council Secretariat letter No. *4/4/S/170* dated *26 November 2024*. Official permission for sample collection was also obtained from the Kirkuk Health Directorate, Training and Human Development Center, under official letter No. *243*. Written informed consent was obtained from all participants before sample collection.

No specific health-related exclusion criteria were applied; however, all participants reported being in good general health at the time of sample collection, with no history of chronic illness or current antifungal medication use. Demographic and occupational data, including age, smoking sta-

tus, health status, medication use, chronic disease history, duration of employment, and work section, were recorded using a structured questionnaire. The participants were aged 16–62 years, and their duration of employment ranged from 3 months to 5 years. Of the 93 participants, 37 were non-smokers and 56 were smokers of cigarettes, hookah, or both. Nasal swabs were collected from the middle nasal meatus using sterile swabs containing Amies transport medium. Samples were transported promptly to the laboratory and processed under aseptic conditions.

Fungal isolation and phenotypic identification

Each nasal swab was inoculated onto Sabouraud dextrose agar (SDA) and incubated aerobically at 25–28 °C for 5 days. Fungal colonies growing on SDA were initially examined macroscopically according to colony color, shape, texture, elevation, surface appearance, and other morphological characteristics. Microscopic examination was performed to support phenotypic identification. Yeast isolates were examined using lactophenol cotton blue (LPCB)-stained smears, whereas mold isolates were examined using the adhesive tape preparation method [8]. Suspected *Candida* isolates were further evaluated using Candida chromogenic agar. Preliminary identification was based on combined macroscopic, microscopic, and chromogenic characteristics, followed by molecular confirmation.

Phospholipase activity assay

Phospholipase activity of *Candida* isolates was assessed using egg yolk agar, as previously described [9]. The medium consisted of 13.0 g SDA, 11.7 g sodium chloride, 0.11 g calcium chloride, and 10% sterile egg yolk. All components, except egg yolk, were mixed and sterilized. The egg yolk was centrifuged at 500 *g* for 10 min at room temperature, and 20 mL of the filtrate was added aseptically to the sterile medium.

Fresh *Candida* cultures grown on SDA for 24 h were harvested and suspended in sterile phosphate-buffered saline (PBS) until visible turbidity was achieved. A 10 μL aliquot of each suspension was inoculated in triplicate onto egg yolk agar plates. The plates were incubated at 37 °C for 72 h.

Phospholipase activity was expressed as the Pz value, calculated as follows:

$$Pz = \frac{\text{colony diameter}}{\text{colony diameter + precipitation zone diameter}}$$

Lower Pz values indicate higher phospholipase activity. The activity was interpreted as follows: negative activity, P_z = 1.00; very low activity, P_z = 0.90–0.99; low activity, P_z = 0.80–0.89; high activity, P_z = 0.70–0.79; and very high activity, $P_z \leq 0.69$ [9].

DNA extraction and molecular identification

Genomic DNA was extracted from a single pure fungal colony using the Wizard® Genomic DNA Purification Kit (Promega, USA), according to the manufacturer's instructions. Molecular identification was performed using polymerase chain reaction (PCR).

The internal transcribed spacer region (ITS1–5.8S–ITS2) was amplified using the universal ITS1/ITS4 primer pair for yeast identification. Mold isolates were identified by amplification of the β-tubulin gene, also referred to as *BenA*, using the Bt2a/Bt2b primer pair [10]. The *PLB1* gene was amplified in *Candida* isolates as a molecular marker of phospholipase-associated virulence [10, 11]. Primer sequences, annealing temperatures, expected product sizes, and applications are presented in Table 1.

PCR reactions were performed in a final volume of 25 μL using GoTaq® G2 Green Master Mix. Each reaction contained 12.5 μL of master mix, target-specific primers, template DNA, and nuclease-free water. For ITS and *PLB1* amplification, 1 μL of each primer was used. For β-tubulin amplification, 1.5 μL of each Bt2 primer was used. Template DNA volumes were 3 μL for ITS, 1 μL for β-tubulin, and 5 μL for *PLB1*; nuclease-free water was added to complete the final reaction volume.

For ITS amplification, PCR cycling conditions consisted of initial denaturation at 95°C for 5 min, followed by 35 cycles of denaturation at 94°C for 1 min and annealing at 55°C for 1 min, with a final extension at 72°C for 7 min. For β-tubulin amplification, the cycling conditions were initial denaturation at 95°C for 10 min, followed by 40 cycles of denaturation at 95°C for 15 s, annealing at 58°C for 15 s, and extension at 72°C for 15 s. For *PLB1* amplification, PCR conditions consisted of initial denaturation at 95°C for 5 min, followed by 32 cycles of denaturation at 95°C for 45 s, annealing at 63°C for 45 s, and extension at 72°C for 45 s [11].

PCR products were visualized by agarose gel electrophoresis. A 1% agarose gel was used for ITS and β-tubulin amplicons, whereas a 1.5% agarose gel was used for *PLB1* amplicons. The expected product sizes were approximately 450–900 bp for ITS, 450–550 bp for β-tubulin, and 765 bp for *PLB1*. The β-tubulin amplification products were purified and sequenced by Macrogen Inc. (Seoul, South Korea). Sequence data were processed using MEGA software version 6 and compared with reference sequences in the NCBI database using BLAST to determine the closest species-level matches. The *PLB1* amplification products were interpreted based on the presence of the expected 765 bp band.

Each PCR run included a no-template control, in which DNA was replaced with nuclease-free water, and a negative extraction control to monitor possible contamination during DNA

Table 1. PCR primers used for molecular identification of fungal isolates and detection of the *PLB1* virulence marker

Primer	Target gene/region	Sequence (5'–3')	T_a (°C)	Product size	Application
ITS1	ITS region	TCCGTAGGTGAACCTGCGG	55	450–900 bp	Yeast identification
ITS4	ITS region	TCCTCCGCTTATTGATATGC	55	450–900 bp	Yeast identification
Bt2a-F	β-tubulin/*BenA*	GGTAACCAAATCGGTGCTGCTTTC	58	450–550 bp	Mold identification
Bt2b-R	β-tubulin/*BenA*	ACCCTCAGTGTAGTGACCCTTGGC	58	450–550 bp	Mold identification
PLB1-F	*PLB1*	ATGATTTTGCATCATTTG	63	765 bp	PLB1 detection
PLB1-R	*PLB1*	AGTATCTGGAGCTCTACC	63	765 bp	PLB1 detection

T_a, annealing temperature; ITS, internal transcribed spacer; bp, base pairs.

extraction. A previously confirmed *PLB1*-positive *Candida* isolate was included as a positive control for *PLB1* amplification. Data were analyzed using SPSS version 26 (IBM Corp., Armonk, NY, USA). Categorical variables were summarized as frequencies and percentages. Associations between categorical variables were assessed using the chi-square test. Fisher's exact test or the Fisher–Freeman–Halton exact test was applied when expected cell counts were less than 5, as appropriate. A *P*-value of less than 0.05 was considered statistically significant. Ninety-five percent confidence intervals for proportions were calculated using Wilson's method.

RESULTS

Fungal isolation and species distribution

Fungal growth was detected in 30 of 93 nasal swab samples, giving an overall culture positivity rate of 32.3% (95% CI: 23.6–42.3%). Two positive samples yielded mixed fungal growth; therefore, a total of 32 fungal isolates were recovered. Yeasts were the predominant isolates, with *Candida* spp. accounting for 28 of 32 isolates (87.5%), whereas molds accounted for 4 isolates (12.5%).

Among the recovered fungal isolates, *Candida krusei* was the most frequent species, representing 20 of 32 isolates (62.5%), followed by *Candida glabrata* with 7 isolates (21.9%). Less frequent isolates included *Penicillium chrysogenum* in 2 isolates (6.3%), and *Candida parapsilosis*, *Penicillium oxalicum*, and *Aspergillus niger* in 1 isolate each (3.1%) (Table 2).

Table 2. Distribution of fungal isolates recovered from nasal swabs of restaurant workers

Fungal isolate	No.	%
Candida krusei	20	62.5
Candida glabrata	7	21.9
Candida parapsilosis	1	3.1
Penicillium chrysogenum	2	6.3
Penicillium oxalicum	1	3.1
Aspergillus niger	1	3.1
Total	**32**	**100**

Culture positivity according to age and smoking status

Culture positivity varied according to age group. The highest positivity rate was observed among workers aged 31–40 years (8/15; 53.3%), followed by those aged 41–50 years (2/4; 50.0%) and 51–60 years (2/5; 40.0%). No positive cultures were detected in the 61–70-year age group. However, the association between age group and culture positivity was not statistically significant (Fisher's exact test, $P = 0.30$).

Table 3. Culture positivity stratified by age group and smoking status

Characteristic	Total	Positive	Negative
Age group, years[a]			
≤ 20	29	10 (34.5)	19 (65.5)
21–30	39	8 (20.5)	31 (79.5)
31–40	15	8 (53.3)	7 (46.7)
41–50	4	2 (50.0)	2 (50.0)
51–60	5	2 (40.0)	3 (60.0)
> 60	1	0 (0.0)	1 (100.0)
Smoking status[b]			
Non-smokers	37	17 (45.9)	20 (54.1)
Cigarette smokers	33	8 (24.2)	25 (75.8)
Hookah smokers	13	3 (23.1)	10 (76.9)
Cigarette + hookah	10	2 (20.0)	8 (80.0)
Overall	**93**	**30 (32.3)**	**63 (67.7)**

Values are presented as *n* or *n* (%).
[a] Fisher's exact test: $P = 0.30$.
[b] Fisher–Freeman–Halton exact test: $P = 0.178$.

Regarding smoking status, non-smokers showed the highest culture positivity rate (17/37; 45.9%), followed by cigarette smokers (8/33; 24.2%), hookah smokers (3/13; 23.1%), and workers who smoked both cigarettes and hookah (2/10; 20.0%). Smoking status was not significantly associated with culture positivity (Fisher–Freeman–Halton exact test, $P = 0.178$) (Table 3).

Phenotypic characterization of fungal isolates

The recovered fungal isolates showed distinct macroscopic and microscopic characteristics that supported their preliminary phenotypic identification. On Sabouraud dextrose agar (SDA), *Candida* isolates appeared as smooth, shiny, creamy colonies. Microscopic examination of *Candida* isolates stained with lactophenol cotton blue showed oval-to-spherical yeast cells. On CHROMagar Candida, *Candida krusei* produced purple colonies, *Candida glabrata* produced cream-to-mauve colonies, and *Candida parapsilosis* produced white-to-cream colonies.

The mold isolates also demonstrated characteristic phenotypic features. *Penicillium oxalicum* colonies appeared

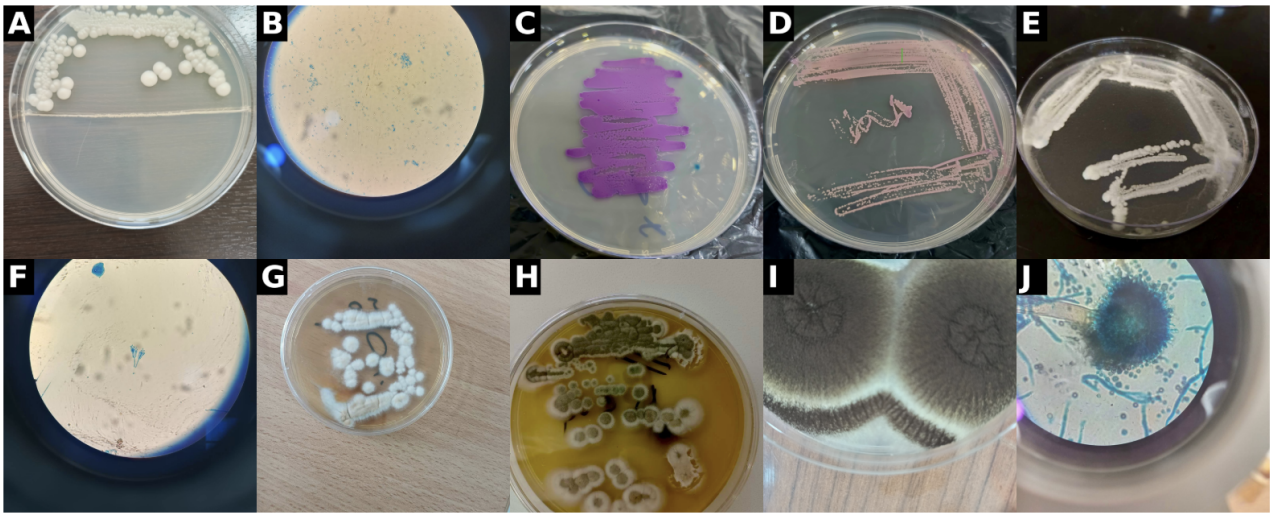

Figure 1. Phenotypic characterization of representative fungal isolates recovered from nasal swabs of restaurant workers. **A:** *Candida* spp. colonies on Sabouraud dextrose agar. **B:** Microscopic appearance of *Candida* spp. stained with lactophenol cotton blue. **C:** *Candida krusei* on CHROMagar Candida. **D:** *Candida glabrata* on CHROMagar Candida. **E:** *Candida parapsilosis* on CHROMagar Candida. **F:** Microscopic appearance of *Penicillium* spp. stained with lactophenol cotton blue. **G:** *Penicillium chrysogenum* colonies on Sabouraud dextrose agar. **H:** *Penicillium oxalicum* colonies on Sabouraud dextrose agar. **I:** *Aspergillus niger* colonies on Sabouraud dextrose agar. **J:** Microscopic appearance of *Aspergillus niger* stained with lactophenol cotton blue.

greenish-gray with relatively fluffy white margins, whereas *Penicillium chrysogenum* colonies appeared whitish to yellowish-white with a soft, cottony-to-downy surface. Microscopic examination of *Penicillium* spp. showed septate hyphae and brush-like conidiophores bearing chains of conidia. *Aspergillus niger* produced dark, densely sporulating colonies on SDA, and microscopic examination revealed characteristic conidial heads with phialides and chains of conidia. Representative macroscopic and microscopic features of the isolated fungi are shown in Figure 1.

Phenotypic phospholipase activity and molecular detection of *PLB1*

Phenotypic phospholipase activity was detected in 5 of 28 *Candida* isolates (17.9%). Activity was observed in 3 of 20 *Candida krusei* isolates (15.0%) and 2 of 7 *Candida glabrata* isolates (28.6%), whereas the single *Candida parapsilosis* isolate showed no detectable phospholipase activity.

Molecular detection of the *PLB1* gene showed a higher positivity rate than the phenotypic assay. Overall, *PLB1* was detected in 21 of 28 *Candida* isolates (75.0%). The gene was detected in 17 of 20 *C. krusei* isolates (85.0%), 3 of 7 *C. glabrata* isolates (42.9%), and the single *C. parapsilosis* isolate. Although *PLB1* detection was more frequent among *C. krusei* isolates, the association between *Candida* species and *PLB1* positivity did not reach statistical significance (Fisher–Freeman–Halton exact test, $P = 0.08$) (Table 4).

Molecular identification of yeasts and molds

Molecular identification supported the phenotypic identification of the recovered fungal isolates. Amplification of the internal transcribed spacer (ITS1–5.8S–ITS2) region produced species-associated amplicon sizes among the *Candida* isolates. *Candida krusei* isolates showed bands of approximately 510 bp, *Candida glabrata* isolates showed bands of approximately 871 bp, and the single *Candida parapsilosis* isolate showed a band of approximately 520 bp (Table 5).

For mold identification, amplification and sequencing of the β-tubulin gene, also referred to as *BenA*, enabled species-level confirmation of selected *Penicillium* isolates. BLAST analysis showed high sequence similarity with reference sequences, confirming one isolate as *Penicillium oxalicum* and two isolates as *Penicillium chrysogenum*. All sequenced isolates showed 99.0% identity and 100% query coverage with their closest reference matches (Table 6). The *Aspergillus niger* isolate was identified phenotypically and was not included in the β-tubulin sequencing results.

Table 4. Phenotypic phospholipase activity and *PLB1* gene detection among *Candida* isolates

Species	Total	Phospholipase-positive	*PLB1*-positive
C. krusei	20	3 (15.0)	17 (85.0)
C. glabrata	7	2 (28.6)	3 (42.9)
C. parapsilosis	1	0 (0.0)	1 (100.0)
Total	**28**	**5 (17.9)**	**21 (75.0)**

Values are presented as *n* or *n* (%). *PLB1* association by species: Fisher–Freeman–Halton exact test, $P = 0.08$.

Table 5. ITS PCR amplification profile of *Candida* isolates

Species	Isolates	Amplicon size
Candida krusei	20	~510 bp
Candida glabrata	7	~871 bp
Candida parapsilosis	1	~520 bp
Total	**28**	–

ITS, internal transcribed spacer; bp, base pairs.

Table 6. β-tubulin/*BenA*-based molecular identification of *Penicillium* isolates

Isolate ID	Assigned species	Identity	Coverage
PZ30560-6	*P. oxalicum*	99.0%	100%
PZ30560-7	*P. chrysogenum*	99.0%	100%
PZ30560-8	*P. chrysogenum*	99.0%	100%

Species assignment was based on BLAST comparison of β-tubulin/*BenA* sequences with reference sequences.

DISCUSSION

The present study demonstrated a fungal isolation rate of 32.26% from nasal swabs of restaurant workers, suggesting a possible association between nasal fungal colonization and occupational exposure in restaurant environments. However, because of the cross-sectional design, a causal relationship cannot be established. This finding is consistent with reports documenting occupational exposure to biological agents in restaurant settings, as well as evidence that kitchen chimneys and grease-associated microbiota may contribute to the release of bioaerosols containing fungal components [4, 12].

The highest culture positivity rate was observed among workers aged 31–40 years. This may reflect greater occupational exposure among individuals in this age group, who are often actively involved in food preparation and restaurant work. In contrast, the absence of positive cases among older workers may be related to the small number of participants in that age category rather than a true age-related difference. Although non-smokers showed a higher culture positivity rate than smokers, the association between smoking status and fungal positivity was not statistically significant. Therefore, smoking status cannot be considered a major determinant of fungal colonization in this study, and this finding should be interpreted cautiously.

Environmental conditions in restaurants may influence fungal exposure. Humidity, food handling, dust resuspension, cooking activity, and ventilation quality can all affect the distribution of fungal bioaerosols. Recent studies in public gathering places, including restaurants, support the role of activity level and ventilation quality in shaping airborne fungal distribution [13]. This may also explain the occurrence of mixed fungal growth in some samples.

The predominance of *Candida* spp., particularly non-*albicans Candida*, is clinically relevant. *Candida krusei* was the most frequent isolate, followed by *Candida glabrata*. This finding is consistent with recent epidemiological trends showing the increasing importance of non-*albicans Candida* species and the growing clinical relevance of *Pichia kudriavzevii*, formerly known as *Candida krusei*, especially because of its known intrinsic resistance to fluconazole [5, 14]. Although *Penicillium* and *Aspergillus* were isolated less frequently, their detection is consistent with their presence as common airborne fungi in indoor environments. Molecular confirmation improved the reliability of species-level identification compared with phenotypic identification alone [15].

The observed variation in ITS amplicon size, ranging from approximately 510 to 871 bp, supported molecular differentiation among *Candida* species. The ITS region is widely used for fungal identification because of its variability between species. In the present study, the distinct amplicon sizes observed for *C. krusei*, *C. glabrata*, and *C. parapsilosis* supported the phenotypic identification and strengthened the reliability of yeast identification.

A discrepancy was observed between phenotypic phospholipase activity and molecular detection of the *PLB1* gene. Phenotypic phospholipase activity was low among *Candida* isolates, whereas the *PLB1* gene was detected in a high proportion of isolates. This finding indicates that the presence of a virulence-associated gene does not necessarily confirm active enzyme expression. Gene expression may be influenced by environmental conditions, colonization site, host factors, and culture conditions [9, 16]. Therefore, molecular detection of *PLB1* should be interpreted as evidence of virulence potential rather than direct evidence of active phospholipase production.

Differences in *PLB1* detection among *Candida* species may reflect species-specific variation in virulence profiles. The *PLB1* gene was detected more frequently in *C. krusei* than in *C. glabrata*, while the single *C. parapsilosis* isolate was also positive. *Pichia kudriavzevii* / *C. krusei* has been reported to possess virulence-related traits, including the ability to produce extracellular enzymes under certain conditions [5, 17]. However, the absence of a statistically significant association between *Candida* species and *PLB1* positivity indicates that this finding should be interpreted with caution.

Sequencing of the β-tubulin gene, also referred to as *BenA*, provided reliable species-level confirmation of *Penicillium* isolates. The high similarity of *BenA* sequences to *P. chrysogenum* and *P. oxalicum* supports accurate molecular identification. The *BenA* gene is considered one of the most informative molecular markers for species-level discrimination within *Penicillium*, especially when ITS sequencing alone is insufficient [15, 18]. Minor nucleotide differences between the isolates and reference sequences do not necessarily indicate misidentification, as such variations may represent intraspecies or strain-level diversity, environmental adaptation, or database-related variation [19, 20].

Several limitations should be acknowledged. The sample size was relatively small and restricted to restaurant workers from a single geographic area, which may limit the generalizability of the findings. In addition, *PLB1* detection was based on PCR band visualization without sequencing of the amplified products. Phenotypic phospholipase activity may also be influenced by culture conditions and may not fully represent virulence expression in vivo. Future studies should include larger sample sizes from multiple regions, additional

occupational groups, sequencing of virulence-associated PCR products, and gene expression analysis under different environmental and host-related conditions.

Despite these limitations, the present study provides useful local data on nasal fungal colonization among restaurant workers in Kirkuk. The integration of culture-based methods, phenotypic characterization, molecular identification, and virulence-marker detection provides a more comprehensive understanding of fungal carriage in this occupational group. These findings provide baseline data for future studies on fungal exposure, occupational health risks, and preventive measures in food-service environments.

CONCLUSION

A nasal fungal isolation rate of 32.26% was detected among restaurant workers, suggesting possible occupational exposure to fungal bioaerosols in restaurant environments. Non-*albicans Candida* species predominated, particularly *Candida krusei*, highlighting the relevance of emerging *Candida* species in occupational and public health contexts. Molecular identification using ITS and β-tubulin/*BenA* markers supported reliable species-level identification of yeasts and molds. Frequent detection of the *PLB1* gene among *Candida* isolates indicated potential virulence capacity. However, the low phenotypic phospholipase activity compared with the high molecular detection rate suggests that the presence of a virulence-associated gene does not necessarily reflect active enzyme expression. The findings emphasize the value of combining phenotypic and molecular methods for fungal identification and virulence assessment. They also provide baseline data on nasal fungal colonization among restaurant workers in Kirkuk and support the need for broader occupational mycological surveillance in food-service settings.

ETHICAL DECLARATIONS

Ethics Approval and Consent to Participate

The study protocol was reviewed and approved by the competent scientific authorities at the College of Science, University of Kirkuk, according to College Council session No. *158S* dated *18 November 2024*, based on University Council Secretariat letter No. *4/4/S/170* dated *26 November 2024*. Official permission for sample collection was also obtained from the Kirkuk Health Directorate, Training and Human Development Center, under official letter No. *243*. Written informed consent was obtained from all participants before sample collection.

Consent for Publication

Not applicable.

Availability of Data and Material

The datasets are available from the corresponding author upon reasonable request.

Competing Interests

The authors declare that there is no conflict of interest.

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Use of Generative Artificial Intelligence

The authors declare that ChatGPT, a generative AI tool developed by OpenAI, was used solely to enhance clarity and grammatical accuracy during the final editing phase. It was not used for content generation, data analysis, or interpretation.

Authors' Contributions

All authors contributed equally to the design and conception of the study. All authors reviewed the manuscript and approved the final manuscript.

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