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# Isolation and Molecular Characterization of Medically Important Yeasts Isolated from Poultry Slaughterhouses and Workers

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**Authors' Contributions**

**EMD conceived and designed the study, helped in writing the article and PCR, and supervised the research. MAHI helped in collecting data poultry slaughterhouses from isolation of fungi and statistical analysis. GKK helped in collecting human data, isolation of fungi, and execution of experiments.**

**Key words**

**Yeast, PCR, RFLP, Msp I, Broiler carcasses, Slaughter worker.**

## ABSTRACT

Early identification of yeast to the genus and species level is necessary for effective antifungal therapy, and can also facilitate control of infections. A total of 50 isolates were isolated from 60 swab samples collected from two poultry processing plants represented broiler carcasses swabs and worker (hand swabs and nail scrapings). Phenotype-based methods for identifying yeast especially Candida species are often difficult, time-consuming and not enough to identify most yeast species. . Molecular biological techniques provide a useful alternative approach. Concerning with genotypic identification, polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) was applied for identification of Candida species, Cryptococcus albidus, Saccharomyces, Trichosporn, Torulopsis and Rhodotorula. Using universal primers ITS1-5.8S-ITS4 amplified ITS region. And it yielded a unique PCR size of approximately 376-930 bp. PCR amplicons were digested with enzyme MspI and the generated bans corresponded to the predicted size.

## INTRODUCTION

Yeasts have been isolated from the air and soil coming from poultry breeding and rearing houses, old litter and litter-containing water, wet feed and bird droppings. At the time of slaughtering, the feathers, feed and bodies of the birds have been found to be contaminated with yeasts (Brr and Mahmoud, 2005). Cross-contamination is a particular problem and several recommendations have been published to control pathogens throughout the chain from worker in slaughterhouses to the preparation in the home.

Yeast infections are most commonly caused by the fungus *Candida albicans*. It is a diploid fungus that grows both as yeast and filamentous cells and causes opportunistic oral, genital and skin infections in human (Ryan and Ray, 2004). Yeast infections are also infrequently caused by other *Candida* species, including *C. glabrata, C. parapsilosis, C. tropicalis* and *C. krusei.*

The incidence of skin mycosis has increased in recent years. It occurs in approximately 20-25% of the world population and constitutes a major public health problem. *Candida* species ranked second in prevalence (22.1%) were isolated from all diagnosed cases of dermatomycoses (Jautova *et al*., 2001). Marchisio *et al*. (1996) reported that yeasts were isolated from 60% of the positive cultures tested from mycotic lesions. The yeasts were mainly found on nails (60%), hands and feet (about 7.5%), the groin, anal and perianal regions (about 6.8%).

The frequency at which yeasts from the skin have been isolated was correlated with various factors such as climate, personal habits, host susceptibility, increased use of immunosuppressive therapies, diabetes, obesity, use of oral contraceptives, long-term administration of glucocorticosteroids as well as the sampling sites of the skin, and others, that render people more susceptible to opportunistic infections. A hot and humid climate can also accentuate the incidence of skin candidosis. Yeasts were more common on moist areas of skin such as the axillae, groins and toe clefts than on dry skin (Abu-Elteen, 1999; Quandt *et al*., 2005).

Therefore, the present investigation was aimed to study the incidences, isolation and identification of yeast species from poultry carcasses and poultry slaughter workers.

## MATERIALS AND METHODS

*Collection of samples*

A total of 60 swab samples (30 for each) were

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collected from two poultry processing plants represented broiler carcasses swabs and worker (hand swabs and nail scrapings) These samples were obtained and preserved in an ice box, then transferred to the laboratory under complete aseptic conditions examined as rapidly as possible.

*Yeast isolation and identification*

The swabs samples were inoculated into Sabouraud dextrose broth with chloramphenicol for 24-48 h, and then transferred to acidified Malt extract agar plates. After inoculation the plates were incubated at 37°C for 48 h (Cruickshank *et al*., 1975).

Morphological examination was carried by studying the macroscopic and microscopic characters of the isolates (Finegold and Martin, 1982). The colonies characteristics were described including the rate and pattern of growth, their size, consistency and surface color. Vegetative reproduction was studied on corn meal agar (Conant *et al*., 1971). Their growth was studied after transferring the isolates in Sabouraud dextrose agar slopes and incubating at 37°C for 3–5 days.

Urease test was performed according to Cruickshank *et al*. (1975) and Germ tube test was performed according to Koneman *et al*. (1992). API 20C Aux system was used for identification of yeasts (Bio Merieux, France) (Espinel-Ingroff *et al*., 1998).

As a result of morphological examination and biochemical tests performed a total of nine strains of yeasts were isolated: *C. Albicans, C. Lusituniae, C. Tropicalis, C. Parapasilosis, Saccharomyces cerevisiae, Trichosporn, Cryptococus albidus, Torulopsis glabrata* and *Rhodotorula mucilaginosa*.

*Molecular identification: PCR-RFLP*

Genomic DNA, isolated from the cultures according to the protocol of chromosomal DNA extraction kit (Gen Jet Genomic DNA purification kit K0271, Fermentas) was used for PCR amplification of internal transcribed spacers (ITS) (ITS1 and ITS2) and 5.8S rRNA gene regions. ITS1 and ITS4 primers prepared by Sigma Company were used (Mirhendi *et al*., 2006) ITS1 F-5' TCC GTA GGT GAA CCT GCGG-'3 and ITS4 R- 5' TCC TCC GCT TAT TGA TAT GC-'3.

PCR reaction was performed in a Gradient Thermal cycler (1000 S Thermal cycler Bio-RAD USA). The reaction mixture (total volume of 50 µl) was 25 µl Dream green PCR Reddy Mix (DreamTaq Green PCR Master Mix (2X) Fermentas Company, cat., No.K1080, USA.), 5 µl target DNA, 2 µl of each primers (containing 10 p mole/µl) and the mixture was completed by sterile D. W. to 50 µl. PCR amplification conditions for yeast isolates were 5 min. initial step at 94°C followed by 35 cycles at 94 °C for 1 min., 56°C for 1 min. and 72 °C for 1 min. and a final extension step at 72 °C for 7 min.

The PCR products were restricted with *MspI* (Thermo scientific), according to the manufacturer's instructions. The digests were separated on 1.5% agarose gels. The molecular sizes of the ITS digests were determined by comparison with a DNA molecular marker using Alpha View – Alpha Imager Hb Version: 3.4.0.0. (Image acquisition and analysis software).

## RESULTS AND DISCUSSION

The intensive poultry production involves slaughtering of large numbers of birds in processing plants which necessitates the implementation of effective sanitary measures against the transfer of microorganisms heavily containing the live birds in both feathers, skin and throughout the intestinal tract.

Calderone (2002) stated that *Candida albicans* is commensal and a constituent of the normal gut flora comprising microorganisms that live in the human mouth and gastrointestinal tract. *C. albicans* lives in 80% of the human population without causing harmful effects, although overgrowth of the fungus results in candidiasis (candidosis). Candidiasis is often observed in immunocompromised individuals such as HIV-infected patients. A common form of candidiasis restricted to the mucosal membranes in mouth or vagina is thrush, which is usually easily cured in people who are not immunocompromised.

In the present study sixty of swab samples collected from two poultry processing plant represented broiler carcasses swabs and worker hand swabs (skin lesion and nail scraping) were investigated for yeast species contamination and the result cleared that out of 30 broiler carcasses swab, 13 (86.7%) and 9 (60% ) broiler carcases contaminated with yeasts species, 40 yeast isolates were obtained .While, 30 worker hand swab and nail scraping , 6 (40%) and 3 (20%) cases of candidiasis, 10 yeast isolates were obtained (Table I).

**Table I.- Total number of collected samples and prevalence of positive one.**

| | Chicken carcases | | Worker | |
|---|---|---|---|---|
| | Positive samples | %age | Positive samples | %age |
| Poultry processing plants I | 13 | 86.7 | 6 | 40 |
| Poultry processing plants II | 9 | 60 | 3 | 20 |

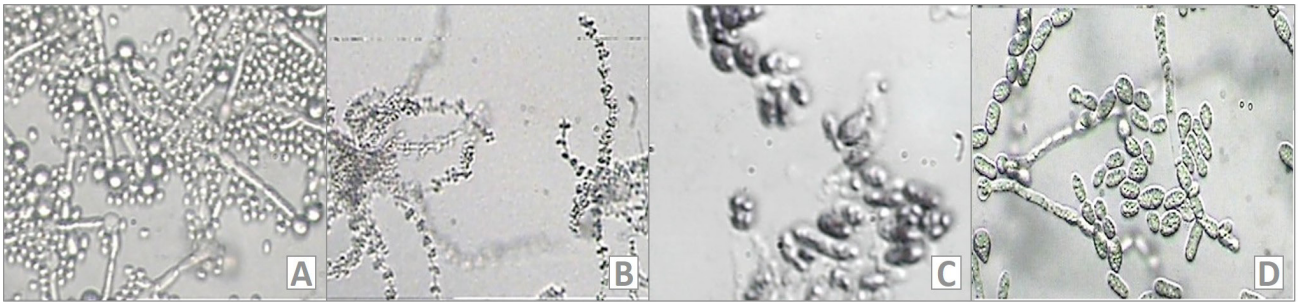


Fig. 1. Yeast isolates: A, *C. albicans* on corn meal agar; B, *C. parapasilosis* on corn meal agar; C, *Saccharomyces cerevisiae* stained with ascospore stain and D, *Trichosporn* sp. on corn meal agar.

Miliaevic *et al*. (2008) reported that skin lesions characteristic skin lesions occur in approximately 10% of patients with disseminated candidiasis and candidemia.

The lesions may be numerous or few. Lesions are generally described as erythematous, firm, nontender macronodular lesions with discrete borders. Biopsy specimens of these lesions demonstrate yeast cells, hyphae, or pseudohyphae, and cultures are positive for *Candida* species in approximately 50% of the cases.

Quandt *et al*. (2005) mentioned that 22 workers (88%) reported one or more skin ailments. Fungal infections were among the most common self-reported conditions, with 17 workers (68%) reporting foot fungus, 7 (28%) reporting nail fungus, and 6 (24%) reporting other skin fungus. Warts were reported by 4 workers (16%); 12 workers (48%) reported acne, and 10 (40%) reported dandruff. Calluses were reported by 9 workers (36%) and rashes by 7 (28%).

Table II and Figure 1 revealed that the identified yeast genera in the examined broiler carcase samples were; *C. albicans* 7 (23.3%), *C. lusitaniae* 7 (23.3%), *C. tropicalis* 5 (16.7%), *C. parapasilosis* 4 (13.3%), *Cryptococus albidus* 3 (10%), *Saccharomyces* 2 (6.7%), *Rhodotorula* spp. 8 (26.7%) and *Trichosporon* spp. 2 (6.7%). The frequencies of isolated yeast genera in examined worker samples *C. albicans* 5 (16.7%), *C. lusitaniae 2(6.7%), C. parapasilosis* 2 (6.7%) and *Trichosporon* spp. 1 (3.3%). Nearly similar results obtained by Deak et al. (2000), Deak (2001), Jautova et al. (2001), Gad (2004), Miceli et al. (2011), Shawish (2011), El-Diasty et al. (3013) and El-Saadany (2014) who mentioned that four yeast genera isolated from examined samples of Worker's hand swab were *Candida* 9 (42.86%), *Torulopsis* 7 (33.33%), *Trichosporon* 4 (19.05%) and *Cryptococcus* 1 (4.76%). While, Broiler carcass swabs examined samples showed that *Candida* (31.43%) was the most frequent yeast species isolated. From such samples followed by *Torulopsis* (25.71%), *Rhodotorula* (21.43%), *Trichosporon* (10%), *Cryptococcus* (7.14%) and *Saccharomyces* (4.29%). Also recorded that the incidence of *Candida albicans* isolated from broiler carcass and worker's hand swab samples was 4(18.18%) and 2 (22.22%), respectively.

**Table II.- Frequency percentages of the isolated yeast genera in the examined broiler carcass and worker samples (n=30).**

| Type of yeast | Broiler carcass swabs | | Worker samples | |
|---|---|---|---|---|
| species | No. of +ve | %age | No. of +ve | %age |
| *C. albicans* | 7 | 23.3 | 5 | 16.7 |
| *C. lusituniae* | 7 | 23.3 | 2 | 6.7 |
| *C. tropicalis* | 5 | 16.7 | 0 | 0 |
| *C. parapasilosis* | 4 | 13.3 | 2 | 6.7 |
| *Cryptococus albidus* | 3 | 10 | 0 | 0 |
| *Saccharomyces* | 2 | 6.7 | 0 | 0 |
| *Rhodotorula* | 8 | 26.7 | 0 | 0 |
| *Trichosporon* | 2 | 6.7 | 1 | 3.3 |
| *Torulopsis* | 2 | 6.7 | 0 | 0 |

N.B, percentages were calculated in relation to the total number of isolated yeast species of examined sample.

Candidiasis otherwise known as thrush is a fungal disease caused by yeasts of the genus *Candida* having nearly 200 species. Among them, six are most frequently isolated, while *C. albicans* is the most abundant and significant species *C. tropicalis, C. glabrata, C. parapsilosis, C. krusei* and *C. lusitaniae* have also been implicated as causative agents. Susceptible hosts include domestic poultry, water fowls and wild birds. Unhygienic atmosphere and secondary debilitating conditions result in both superficial and deep infections. Involvement of the digestive tract is common in young birds as compared to

older birds. Increased virulence of the fungus plays a vital role in establishing the disease (Dhama *et al*., 2013).

*Candida* nail infections occur in patients with chronic mucocutaneous candidiasis, and are caused by *C. albicans*. The organism invades the entire nail plate. *Candida* spp. may cause other syndromes, including onycholysis and paronychia. These forms occur more commonly in women than in men and often affect the middle finger, which may come into contact with *Candida* organisms that reside in the intestine or vagina. *Candida* onychomycosis can therefore be divided into three general categories. Infection beginning as a paronychia (infection of the structures surrounding the nail; also called a whitlow), the most common type of *Candida* onychomycosis, first appears as an edematous, reddened pad surrounding the nail plate. Invasion by *Candida* spp., unlike dermatophytic invasion, penetrates the nail plate only secondarily after it has attacked the soft tissue around the nail. After infection of the nail matrix occurs, transverse depressions (Beau's lines) may appear in the nail plate, which becomes convex, irregular, and rough and, ultimately, dystrophic (El-Ewski, 1998).

In the present study, PCR/RFLP system was used for the identification of nine representative yeast isolates. Using the described primers we could amplify DNA from all nine tested yeasts including *Candida*, *Saccharomyces, Trichosporon, Cryptococus, Torulopsis and Rhodotorula* representing a broad range of clinically relevant yeasts. The primer used gives an amplicon (376-930 bp) from all isolates tested. This result is in agreement with previous studies (Vazquez *et al*., 1993; Velegraki *et al*., 1999; Mirhendi *et al*., 2006; Cadez *et al*., 2010; Allam and Salem, 2012; Akhtar *et al*., 2014). They could successfully identify six Candida spp. using PCR-RFLP method and fungus-specific universal primers (ITS1 and ITS4) and then PCR amplicons were digested with *Msp* I (Table III, Fig. 2).

Analysis of RFLP derived from the DNA of *Candida* spp. in the present study has the advantage of being reliable in comparison with the phenotyping method, which is insensitive and have limited availability, the fact that also mentioned by Cirak et al. (2003).

Cleansing and degerming the skin with a soap or detergent containing an antimicrobial agent may be useful. Drying agents, such as aluminum chloride, and keratinolytic agents, such as topical salicylate, are also helpful. Topical antimicrobial agents can be used for some infections, but systemic therapy may be necessary for patients with extensive disease. Mechanical scraping of the chalky white material on the nail plate and application of topical antifungal agents are recommended. Oral griseofulvin may be required to bring about clearing of the fingernail. For toenails with extensive involvement, oral itraconazole, fluconazole and terbinafine are effective.

**Table III.- Molecular weight of ITS1-ITS4 PCR products and restriction fragments of *Candida, Saccharomyces, Trichosporn, Cryptococus* and *Rhodotorula.***

| Yeast species | ITS1-ITS4 PCR products | Restriction fragment |
|---|---|---|
| *C. albicans* | 535 | 297, 235 |
| *C. lusituniae* | 376 | 272 |
| *C. tropicalis* | 524 | 340, 184 |
| *C. parapasilosis* | 520 | 520 |
| *Saccharomyces* | 930 | 750, 180 |
| *Trichosporn* | 515 | 333, 180 |
| *Cryptococus albidus* | 634 | 400, 240 |
| *Torulopsis* | 450 | 450 |
| *Rhodotorula* | 662 | 570, 60 |

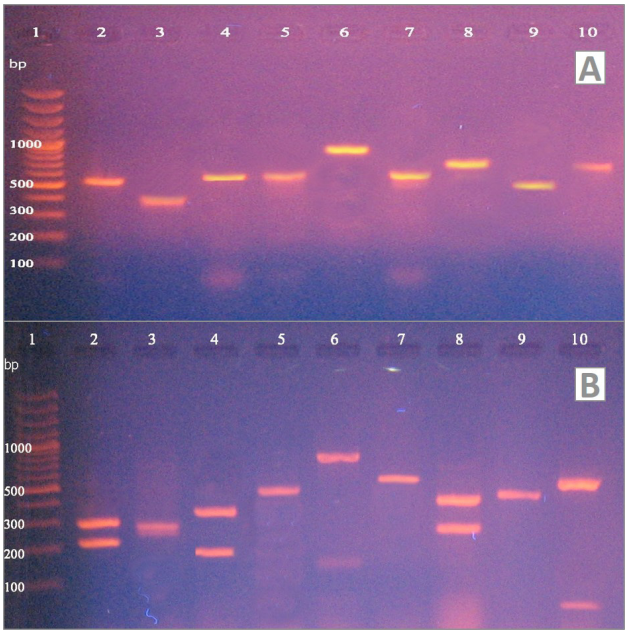


Fig. 2. Ethidium-bromide stained 1.5% agarose gel with ITS-1/4 amplicons (A) digested with *MspI*, (B) Lane 1, 100 bp DNA ladder; Lane 2, *C. albicans*; Lane 3, *C. Lusituniae;* Lane 4, *C. tropicalis*; Lane 5, *C. parapasilosis*; Lane 6, *Saccharomyces*; Lane 7, *Trichosporn*; Lane 8, *Cryptococus albidus*; Lane 9, *Torulopsis;* Lane 10, *Rhodotorulala*.

## CONCLUSIONS

In present study, *candida* was the most common genera isolated from animal and human. The most

*Candida* spp. isolated from poultry carcases and slaughter workers was *Candida albic*ans followed by *C. lusitaniae* and *C. parapasilosis.* Other *Candida* spp. as *C. tropicalis* was also isolated. Phenotypic method not enough to identify most yeast species. Genotypic method is the most accepted methods for identification of yeasts into species and varities. Also restriction digestion of the ITS amplification product with *Msp* I produced the predicted specific patterns for each species. Furthermore, the potential benefit of using the ITS-1/4 PCR-RELP products in diagnostic medical microbiology could, apart from being rapid and less costly, identify yeast and support the epidemiological studies that show an increasing number of yeasts opportunistic pathogens.

*Statement of conflict of interest*

Authors have declared no conflict of interest.

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