INTRODUCTION

Fungal infections or mycoses cause a wide range of diseases in humans. Mycoses range in extent from superficial infections involving the outer layer of the stratum corneum of the skin to disseminated infection involving the brain, heart, lungs, liver, spleen, and kidneys [1]. Superficial fungal infection is the most common fungal infections. According to the World Health Organization, the prevalence rate of superficial mycotic infection worldwide has been found to be 20–25% [2].

Dermatophytes are a group of superficial fungal infections of keratinized tissues, namely, the epidermis, hair, and nails. The causative fungi are molds belonging to three asexual genera microsporum, trichophyton, and epidermophyton [3].

Systemic fungal infection in humans can range from asymptomatic pulmonary infection difficult to treat, and life-threatening disseminated infections that involve multiple organ systems [4]. Aspergillus species are frequent causes of invasive fungal infections in immunocompromised patients; they are also associated with allergic bronchopulmonary diseases, mycotic keratitis, otomycosis, and nasal sinusitis. At least 30 Aspergillus species have been associated with human diseases, and Aspergillus fumigatus remains the most frequent cause of invasive aspergillosis [5].

Invasive candidiasis (IC) includes candidemia, disseminated candidiasis with deep organ involvement, endocarditis, and meningitis. IC has an attributable mortality of 40–50% and is increasingly reported from healthcare centers. Candida albicans and non-albicans strains are both responsible for infections [6].

METHODS

This prospective study was conducted in the Department of Microbiology at Yenepoya Medical College, Mangalore, India. A total of 274 non-duplicate clinical samples were processed from December 2016 to May 2018 after obtaining ethical clearance from the institutional ethics committee.

All clinical samples (first set) were processed according to the standard protocols, i.e., (a) skin scraping was subjected to 10% KOH, (b) nail clippings - 40% KOH, (c) hairs - 20% KOH, (d) urine - wet mount and Gram staining, and (e) blood, sputum, bronchoalveolar lavage (BAL), and swab - Gram staining. The prepared slide was examined under the microscope for the presence of hyphae and arthropores or yeast cells.

The second sets of samples were inoculated into sabouraud’s dextrose agar slope (SDA) is used, whereas the skin scraping and nail clipping were inoculated in modified SDA (SDA with chloramphenicol and cycloheximide) [7]. The inoculation was done with a sterile chromium wire spud and center of the slope. The tubes were labeled and then incubated at room temperature. The tubes were examined every day for evidence of any growth. They were discarded as negative if there was no growth after 4 weeks. In positive cases, the growth was subcultured on sabouraud’s dextrose agar slopes without antibiotics.

The growths in tubes were seen for gross morphological characteristics, and details were recorded, namely: Rate of growth, general topography, i.e., whether flat heaped up regular or irregularly folded. Characteristics of colony, i.e., yeast like powdery granular velvety or cottony, pigmentation on the surface, and on reverse [8]. Lactophenol cotton blue mount was used in all positive growth culture to check the microscopic finding. Special tests were done when necessary, namely, germ tube test, slide culture, and urease test for species identification.
RESULTS

The distribution of various types of 274 clinical samples is as follows: Skin scraping was 127, urine 37, blood culture 34, nail clipping 19, pus 16, sputum 9, tissue 9, CSF 6, BAL 5, corneal scrap 5, nasal swab 4, and hair 4. In this study, male-female ratio was found to be 6:3. Of 274 clinical samples, 125 (45.6%) showed fungal growth positive and 149 (54.4%) sample showed no growth. Of 274 samples, 172 were male and 102 were female, as shown in Fig. 1 and Table 1.

In this study, the predominant age group in blood and urine samples was 0–10 years, among skin scraping, nail clipping and hair samples were 20–30 years, among pus and tissue were 30–40 years, nasal swab and corneal scrapings were 40–50 years, sputum was 50–60 years, CSF was 0–10 years, and BAL was 60–70 years as shown in Table 2.

Among 126 skin scrapings, 109 samples were found to be KOH (10%) positive and 17 were negative, whereas only 87 (69%) were KOH as well as culture positive in which Trichophyton mentagrophytes was the predominant isolate. All 37 urine samples were subjected to wet mount culture, in which only 14 (37.8%) samples were positive for both, whereas C. albicans was isolated from 9 samples and C. tropicalis was isolated from 5 samples. Among 34 blood culture samples, only 2 (%) were found to be BACTEC and culture positive for C. albicans. Among 19 nail clipping samples, 6 were KOH (40%) positive and 9 (47.4%) were both KOH and culture positive; in which 3 were Trichophyton species, 3 were Fusarium species, and 3 were C. albicans. Of 16 pus samples, only 1 (6.3%) was culture positive. Of 9 sputum, 5 BAL, and 5 corneal scrapings samples; 5 (55.5%), 1 (20%), and 1 (20%) were culture positive respectively in which Candida species (including C. albicans) was the only isolate. Among 9 tissue samples only 2 (22.2%) samples were KOH and culture positive; in which one was C. albicans and one was Fonsecaea pedrosi. Among 4 nasal swabs, only 2 (50%) showed growth of Aspergillus flavus. Among 6 CSF samples, only 1 (16.7%) was Indian ink and culture positive for Cryptococcus neoformans. However, among 4 hair samples, none of them were KOH (20%) as well as culture positive as shown in Tables 1 and 3; and macroscopic and microscopic appearance as shown in Figs. 2 and 3.

DISCUSSION

In the present study, of 274 clinical samples, 125 were culture positive in which the fungal isolates obtained were dermatophytes (all 3 genera). Candida species (including C. albicans), Fusarium species, A. fumigatus, and C. neoformans.

Among superficial fungal infections, most frequent isolates belonged to the genus Trichophyton in which T. mentagrophytes was found to be predominant species followed by Microsporum gypseum, M. canis, and Fusarium species. However, in case of systemic infection, Candida species (including C. albicans) was predominant isolate followed by A. fumigatus, whereas only one C. neoformans has been grown out of 6 CSF samples. Male predominance was found among the patients being 6.3 male:female ratio. However, our study is discordance with the other studies which have reported T. rubrum is the most common isolate from the superficial infections, whereas among systemic infections, our

Statistical analysis

Descriptive statistics were used for analysis. Variables were expressed as percentages. All the data were expressed as table diagrams.

![Comparison between number of sample received and culture positive](image1)

**Fig. 1: Comparison between number of sample received and culture positive**

![Macroscopic appearance](image2)

**Fig. 2: Macroscopic appearance (a) Trichophyton mentagrophytes, (b) Trichophyton rubrum, (c) Epidermophyton floccosum, (d) Microsporum gypseum, (e) Fonsecaea pedrosi**

### Table 1: Distribution of fungus samples

| Sample                  | Male | Female | Total samples (n=274) | Culture positive (%) |
|-------------------------|------|--------|-----------------------|----------------------|
| Skin scraping           | 76   | 50     | 126                   | 87 (69)              |
| Urine                   | 19   | 18     | 37                    | 14 (37.8)            |
| Blood culture           | 22   | 12     | 34                    | 2 (5.9)              |
| Nail clipping           | 11   | 8      | 19                    | 9 (47.4)             |
| Pus                     | 12   | 4      | 16                    | 1 (6.3)              |
| Sputum                  | 7    | 2      | 9                     | 5 (55.5)             |
| Tissue                  | 8    | 1      | 9                     | 2 (22.2)             |
| CSF                     | 5    | 1      | 6                     | 1 (16.7)             |
| BAL                     | 4    | 1      | 5                     | 1 (20)               |
| Corneal scrape          | 4    | 1      | 5                     | 1 (20)               |
| Nasal swab              | 2    | 2      | 4                     | 2 (50)               |
| Hair                    | 2    | 2      | 4                     | 0 (0)                |
| Total                   | 172  | 102    | 274                   | 125                  |

BAL: Bronchoalveolar lavage

### Table 2: Age wise distribution of sample

| Age group (years) | BC | BAL | SPU | PUS | NS | SS | Hair | NC | Urine | Tissue | CS | CSF |
|-------------------|----|-----|-----|-----|----|----|------|----|-------|--------|----|-----|
| 0-10              | 29 | 01  | -   | -   | -  | 03 | -    | -  | 30    | -      | -  | 03  |
| 10-20             | -  | 01  | -   | 01  | -  | -  | 29   | 01 | 02    | 03     | -  | 01  |
| 20-30             | -  | -   | -   | 02  | 01 | 40 | 02   | 09 | 01    | -      | -  | -   |
| 30-40             | 01 | 01  | 01  | 04  | -  | 06 | -    | -  | -     | 06     | -  | 01  |
| 40-50             | 02 | -   | 02  | 03  | 03 | 20 | -    | 04 | -     | -      | 03 | -   |
| 50-60             | -  | 01  | 04  | 03  | -  | 23 | 01   | 02 | 01    | 02     | 01 | 01  |
| 60-70             | 01 | 02  | 02  | 02  | -  | 05 | -    | -  | 01    | -      | 01 | -   |
| 70-80             | -  | -   | -   | -   | 01 | -  | -    | -  | 02    | 01     | -  | -   |
| Total             | 34 | 05  | 09  | 16  | 04 | 126| 04   | 19 | 37    | 09     | 05 | 06  |

BC: Blood culture, BAL: Bronchoalveolar lavage, SPU: Sputum, NS: Nasal swab, SS: Skin scraping, NC: Nail clipping, CS: Corneal scraping, CSF: Cerebrospinal fluid
study is in accordance with the other studies in which *Candida* species being the most common pathogen.

In this study, among children (0–10 years), the most common samples received were blood and urine, whereas among adults (20–30 years), the most common sample received was skin scrapings.

In accordance with our study, a study was conducted by Malik et al. [9] also showed the higher incidence of fungal infection mainly dermatophytes in males than in females has been reported in India. Among dermatophytoses, total 86 isolates were obtained from the all skin scrapings and nail clippings. The dermatophytes isolated were *T. mentagrophytes*, *T. rubrum*, *M. gypseum*, *M. canis*, and *Epidermophyton floccosum*. *Candida* spp. were isolated from 30 cases, of which 26 (86.6%) were *C. albicans* and 6 (4.8%) were *C. tropicalis*. However, according to Kannan et al. [10] showed the higher prevalence of *T. rubrum* among cases of dermatophytoses, whereas *Candida* species being the predominant isolate from all non-dermatophytic fungal infections. Kuruvilla and Dias [11] and Jandial and Shaveta [12] reported that *Fusarium* species from dermatophytoses samples as similar to our study, whereas Malik et al. reported higher prevalence as compared to the present study. However, in the same study, by Malik et al., *A. fumigatus* was 15.6% which is much higher than the present study (1.6%). In the present study, *Fonsecaea pedrosoi* was detected from one out of two tissue sample. A similar result was also reported in case report done by Khan et al. from Kochi [13].

### CONCLUSION

The frequency of superficial and systemic mycoses has increased significantly. This increase infection is mostly because of underdiagnosis of the causative pathogen and is directly related to the increasing numbers of patients who are at risk for the development of serious fungal infections. Therefore, all the fungal infections should be dealt with due care. The development of new approaches to diagnosis and treatment of invasive fungal infections is the subject of intensive research.

### AUTHOR’S CONTRIBUTION

Jitendra Chandra Devrari and Varsha Saxena have contributed to the study conception, design, sample collection, analysis, and interpretation of the data and manuscript preparation. Vidya Pai has contributed to the conception and critical revision of the manuscript.

### CONFLICT OF INTEREST

None.

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