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Fungal Infections in Aseer Central Hospital: A Retrospective Laboratory-based Study of 340 Cases during the Years 2011 to 2015

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Authors' contributions

This work was carried out in collaboration between all authors. Authors HMA, AMFA, NSHA, AAAA, SDHA, AMAM, HAFA, MASA, KMSA and ASAA collected data, analyzed, managed and performed the project follow up. Authors SM and MAM Isolated the organism and conducted initial identification. Author MRPJ performed identification of organisms. Author MEH designed the study, wrote the protocol and the manuscript. All authors read and approved the final manuscript.

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Case Study

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ABSTRACT

Objectives: The incidence of fungal infections is increasing due to increasing episodes of risk factors such as immune competence; broader used of antibiotics and longer hospital stays. This study aimed to analyze fungal isolates from patients admitted to Aseer Central Hospital between 2011 and 2015 and to shed light on practical recommendations based on scientific evidence for improving laboratory diagnosis.

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Methods: Retrospectively, for a period of 4 years (2011-2015), we analyzed 340 specimens submitted to the Microbiology Laboratory, at Aseer Central Hospital, Abha, Saudi Arabia. The study involved the isolation and identification of fungi using standard methods. Cultures were done on Sabouraud dextrose agar (SDA) plates and Brain Heart Infusion Agar + 5% Sheep Blood (BHIA) according to the type of the clinical specimens. Suspected mold and yeast cultures were identified on the basis of colony morphology appeared on SDA and on microscopic features as per standard criteria. Resulted were analyzed using SPSS investigating prevalence among specimens types, sex, age groups and hospital wards.

Results: Of the 340 specimens, positive fungal cultures were obtained in 105 (30.88%), no growth was seen in 218 plates (64.12%) and 17 plates (5%) had been contaminated or overgrown by bacteria. Out of the 105 positive fungal cultures, yeast represented 47 cases (44.76%) of which 23 samples (21.9%) belonged to the genus *Candida*. Dermatophytes were 18 isolates (17.14%) of which *Trichophyton tonsurans* was the dominant species 9 patients (8.57%). *Aspergillus* species were 13 cases (12.38%); Zygomycetes 9 (8.57%); *Penicillium* species, only 1 case (0.95%) and unidentified molds were 17 (16.19%). Gender showed significant differences (p=0.034) but no differences among ages groups (p = 0.187). Specimens derived from skin represented the highest percentage of fungal infections followed by the lower respiratory tract and subcutaneous tissue. Significance differences were recorded among hospital wards (p = 0.001) nonetheless male and female medical and surgical words revealed relatively higher rates of fungal infections.

Conclusion: These fungi represent a considerable hazard to patient health. What is needed in the region is to increase detection rate, by improving sample quality and expanding laboratory capacity in order to enhance patient's health.

Keywords: Fungal infections; molds; yeasts; laboratory diagnosis; Aseer Central Hospital; Saudi Arabia.

1. INTRODUCTION

The occurrence of fungal diseases is rising persistently at an alarming rate, posing a huge challenge to healthcare professionals [1]. This increase is an emerging problem and is directly related to the mounting numbers of patients suffering from immunodeficiencies from systemic disorders (e.g. diabetes, malnutrition, HIV infection), immunosuppressive treatments (cytotoxic chemotherapy, bone marrow ablation before transplantation, radiation therapy), prolonged serious illness, disease-modifying antirheumatic drugs, immunosuppressive drugs after organ transplants such as glucocorticoids [2,3]. Even though fungal infections are not often life threatening, but they can result in on a person's quality of life and may in some circumstances spread to other individuals or become invasive [4-6]. The most frequently affected body system is the skin; such infections affect millions of people globally. Dermatophilic fungi affect the superficial and subcutaneous tissues, the keratinous tissues and the mucous membranes are readily diagnosed and treatment can be dramatic [7]. However, systemic fungal infections can be fatal. Most fungi are opportunistic in nature, infecting people with risk factors or fungi sometimes are endemic to a definite geographical area. Diagnosis is at most difficult to be achieved in case of systemic infections as there are no specific symptoms [8]. The most frequently reported fungal pathogens are *Candida albicans* and *Aspergillus* spp. but other fungi such as non-albicans Candida spp. are becoming increasingly significant [1,9]. Fungal infections in health care units represented around 8% compared to bacterial infections such as E. coli (16%), *Staphylococcus* spp. (9%) and *Pseudomonas aeruginosa* (7%) [10,11].

Fungal infections in the Kingdom of Saudi Arabia have long been recognized [12]. Records have indicated that fungal infections represent about 10%; whereas Gram-positive organisms; 10%; Gram-negative organisms; 32% and the remaining 48% were polymicrobial [13,14]. *Candida* spp. associated bloodstream infections were found to cause about 5% from all other causes in health care units in Saudi Arabia. In Aseer region our recent study indicated that 2.35% revealed the presence of Candida spp. [15]. But no information is available on the prevalence of other fungal infections including molds in Aseer regions. This survey aimed to analysis the trends of fungal infection in four years periods with respect to patients, wards and specimens types. The superficial fungal infections in Riyadh region, Saudi Arabia were found more prevalent in females than males and

among children than adults and differ with climatic conditions, lifestyle, and population migration patterns [16]. *Tinea capitis* and *Tinea pedis* were most frequency encountered [16].

This study aimed to investigate fungal isolates from patients admitted to Aseer Central Hospital between 2011 and 2015 and to cast some light on practical recommendations based on scientific evidence for improving the current practice and laboratory diagnosis.

2. METHODS

2.1 Ethical Approval

The present research was approved and funded by the Deanship of Scientific Research, King Khalid University (project number: REC 2014-01-06).

2.2 Specimens

The study included the isolation and identification of fungi from patients admitted to Aseer Central Hospital between 2011 and 2015. Mycological examinations (culture and microscopy) were achieved on all patients' samples that were submitted to the laboratory (n = 340).

2.3 Isolation of Fungi

Culture was performed after a specific request was submitted. Cultures were done on Sabouraud dextrose agar (SDA) plates and Brain Heart Infusion Agar + 5% Sheep Blood (BHIA) according to the type of the clinical specimens. Inoculated plates were incubated at 30°C and examined daily for up to 10 days for the growth of molds and yeasts.

2.4 Identification of Fungi

Suspected mold usually sub cultured on SDA for improved growth and appearance of distinguished mold elements. Identification of molds was done on the basis of colony morphology appeared on SDA and on microscopic features following recommended guiding principles [17-19].

Yeasts encountered on SDA and BHIA plates were identified using conventional growth and colonial morphology criteria. [17,19] Colonies with white to cream colored, smooth, glabrous and yeast-like in appearance; with spherical to subspherical budding yeast-like cells or blastoconidia were initially identified as yeasts and considered for further identification.

2.5 Statistical Analysis

The data was collected and entered on a Microsoft Office Excel sheet. Data was then analyzed using the Statistical Package for Social Science (SPSS Inc., Chicago, IL, USA) Version 16. Confidence intervals were calculated and the analysis of variance (ANOVA) was used to evaluate the differences between group means and variations between groups. The results were demonstrated in a table and figures layout displaying comparisons and frequencies of variables. Results were considered significant when p-values equal to or less than 0.05.

3. RESULTS

The 340 specimens submitted to the laboratory and competed mycological examination revealed the following results: Positive fungal cultures were shown in 105 (30.88%), no growth was seen in 218 plates (64.12%) and 17 plates (5%) had been contaminated or overgrown by bacteria.

The distribution of the 105 positive fungal cultures is shown in Fig. 1. Yeasts represented 47 cases (44.76%) of the total 105 positive samples of which 23 samples (21.9%) identified in the genus *Candida* but 18 samples (17.14%) were have been counted as "unidentified yeasts". Dermatophytes were 18 isolates (17.14%) of which *Trichophyton tonsurans* was the dominant species 9 patient's (8.57%). *Aspergillus* species were 13 cases (12.38%); Zygomycetes were 9 (8.57%); *Penicillium* species were only 1 case (0.95%) and unidentified molds were 17 (16.19%) (Fig. 1). Variations of prevalence of these species in males and females are evident from Fig. 1.

The results of the one-way ANOVA of the fungal infections from the 340 patients at Aseer Central Hospital in relation to patient genders, ages, specimen types and hospital wards is shown in Table 1.

Regarding the gender, males and females exhibits significant differences in prevalence ($p = 0.034$) among the four epidemiological criteria (Figs. 1-4). Younger male ages (<19 years) and those above 40 years of age have higher prevalence than females (Fig. 2).

Apart from cutaneous and subcutaneous tissues, all other specimens derived from females showed higher prevalence of fungal infections (p = 0.000) including the miscellaneous ones (Fig. 3).

Males have shown higher prevalence in the medical and surgical wards (Fig. 4). Positive fungal cultures were recovered higher among males than females in the pediatric intensive care unit, while it is similar among neonatal ICU. In pediatric medical ward and the Coronary Care Unit (CCU) the females scored higher percentages than males. However, emergency department (ER), and pediatric surgical wards recorded similar prevalence's of fungal infections for both males and females but males recorded higher prevalence in the Outpatient Department (OPD), ICU and dermatology departments (Fig. 4).

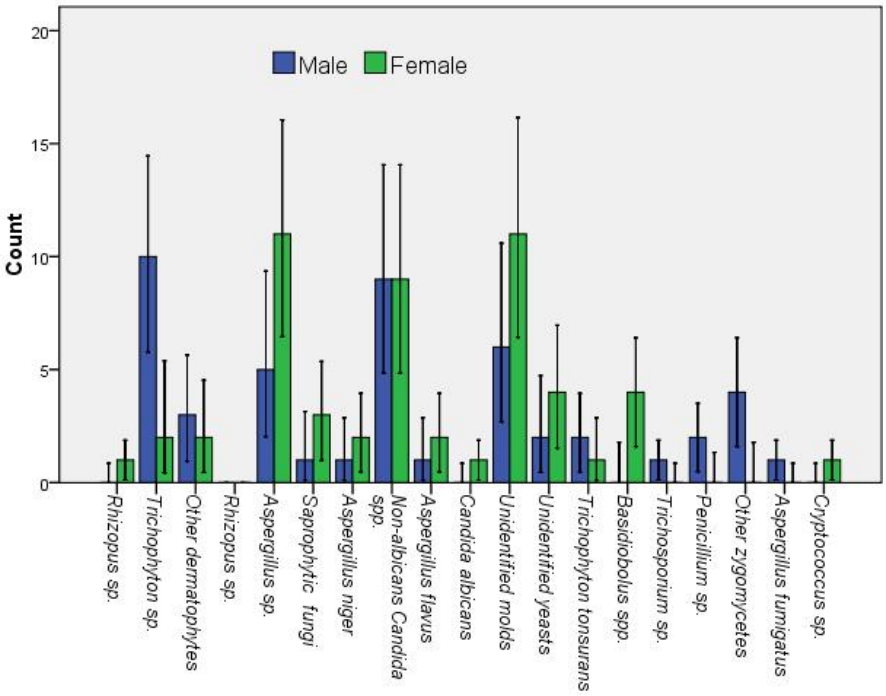

Fig. 1. Identities of the positive fungal cultures (n = 105) recovered from 340 male and female patients at Aseer Central Hospital (2011-2015)

Bars: CI 95%

Table 1. One-way ANOVA showing statistical descriptive value of fungal infections from patients at Aseer Central Hospital, Saudi Arabia in relation to specimen types, age, gender and hospital wards

		Sum of squares	df	Mean square	F	Sig.
Patient	Between Groups	1.976	2	0.988	3.418	0.034
Gender	Within Groups	89.602	310	0.289		
	Total	91.578	312			
Age	Between Groups	27.870	2	13.935	1.687	0.187
	Within Groups	2577.527	312	8.261		
	Total	2605.397	314			
Specimen	Between Groups	349.391	2	174.695	17.527	0.000
	Within Groups	3109.740	312	9.967		
	Total	3459.130	314			
Ward	Between Groups	279.619	2	139.810	6.988	0.001
	Within Groups	6242.235	312	20.007		
	Total	6521.854	314			

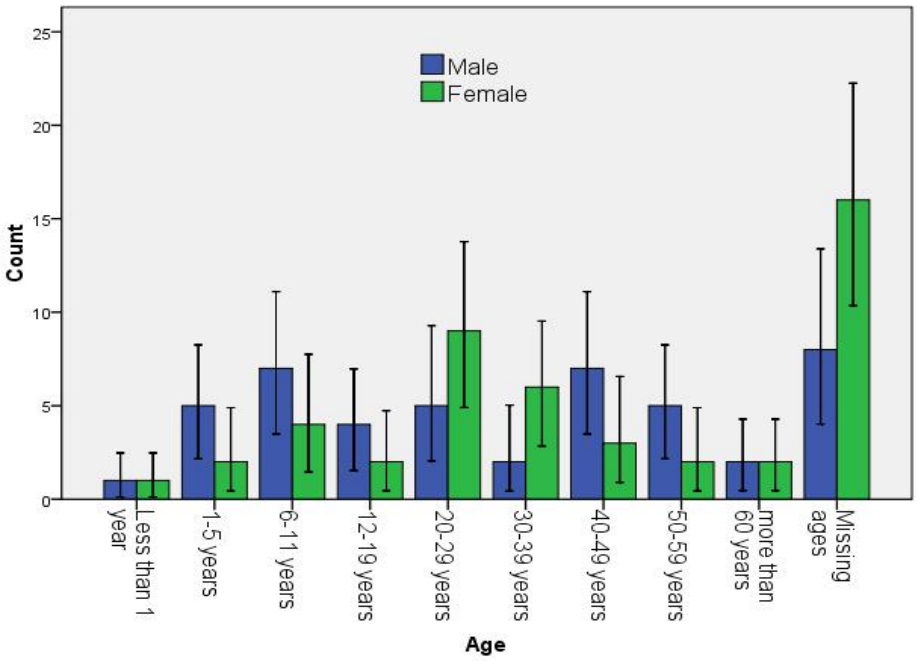

Fig. 2. Distribution of the positive fungal cultures (n = 105) among different age groups of the 340 male and female patients at Aseer Central Hospital (2011-2015)
Bars: CI 95%

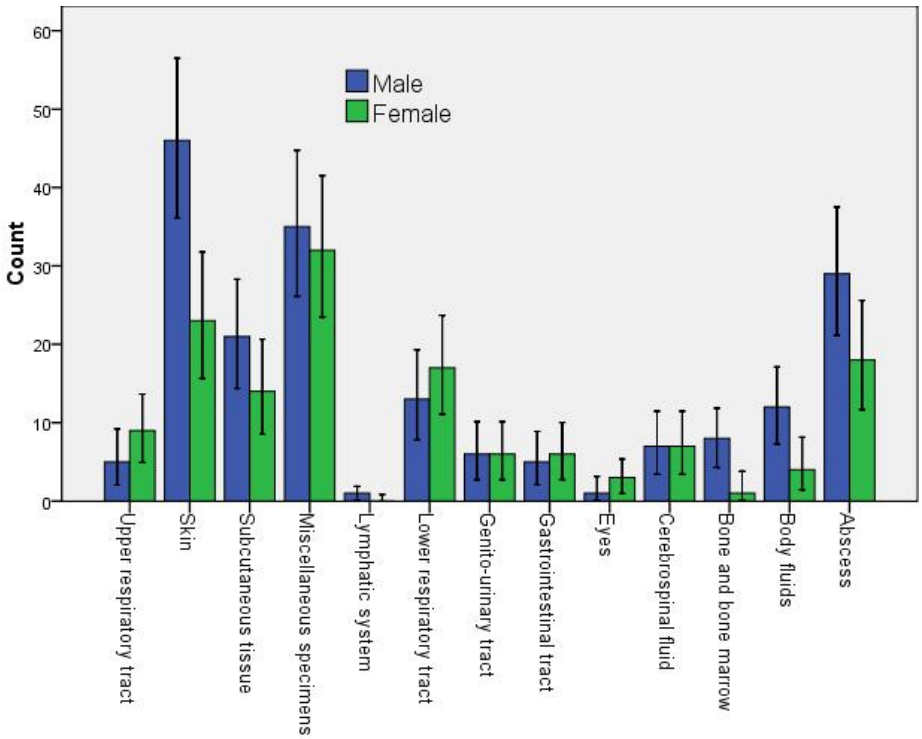

Fig. 3. Distribution of the positive fungal cultures (n = 105) recovered from different specimens types of the 340 male and female patients at Aseer Central Hospital (2011-2015)
Bars: CI 95%

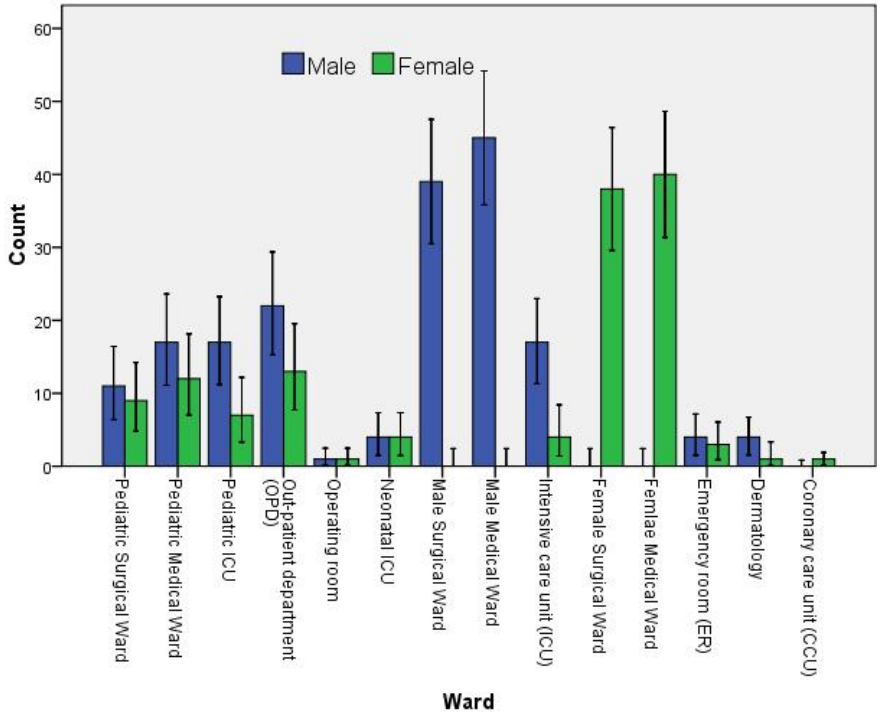

Fig. 4. Distribution of the positive fungal cultures (n = 105) derived from different wards hospitalizing 340 male and female patients at Aseer Central Hospital (2011-2015)
Bars: CI 95%

4. DISCUSSION

A considerable number of hospitals do not perform fungal cultures and other tedious mycological analyses as a routine practice because laboratory mycology is believed vastly complex to do and that the fungi are too infectious to handle [20,21].

In the present study, many pathogenic fungal have been found in association with infections which constituted a significant threat to patient health (Fig. 1). But the majority of the samples revealed no growth (63.82%). Also, 5% of the plates had been contaminated or overgrown by bacteria. These two results are serious drawback. Two aspects could help understanding these deficiencies in reaching a mycological diagnosis. The first aspect arises from the quality of the samples submitted, previous intake of antifungals or the wrong clinical diagnosis or low suspicion rates from physician. This issue has been addressed in the literature and it seems not uncommon [22].

The second aspect is a technical one related to the laboratory facilities and the technical staff. Supposing the quality of the submitted sample is good, the inefficiency of the laboratory could reveal deficiencies such as the high contamination and the low or no growth [23].

Given the rising trends of fungal infection worldwide, [24] it become necessary to improve the diagnostic capabilities of fungal infection persistently at an alarming rate, posing a huge challenge to healthcare professionals [1].

Abanmi et al. [16] found that superficial fungal infections are significantly higher in adults than children with which our findings agree. However these authors reported that females were having more infections than females which contradict with our results (Fig. 4). Children suffered commonly from tinea capitis while adults from tinea pedis [16]. Other study indicated that the median age of fungal infections was 52 years and 53% of patients were males; which agreed to some extent with our finding [25]. *Candida albicans* were the most common species (38.7%), followed by *Candida tropicalis* (18.9%), and *Candida glabrata* (16.3%). Similar results have been recently published from Aseer region [15].

Our findings indicated that dermatophytes were 17.14% and the major species was *Trichophyton tonsurans*. An earlier study revealed that of 504 positive fungal cases *Candida* species and other yeasts were responsible for 88.9% and dermatophytes for 11.1% [26]. A closely related study done between 1984 and 1988 among 4,294 clinically suspected cases, dermatomycoses were found to be 17.9% [27]. These finding were comparable with our present findings. Later, out of 372 patients with tinea capitis in Saudi Arabia, 240 were found to be positive for tinea. Tinea capitis accounted for 47.7% of all superficial mycoses, and 97% of it occurred in children below 15 years of age [28].

5. CONCLUSIONS

Molds represented 55.2% whereas yeasts represented 44.8% of the total 105 positive samples in this study. Males have shown higher prevalence in the medical and surgical wards. Such results represent a considerable hazard to patient health. What is needed in the region is to increase detection rate, by improving sample quality and expanding laboratory capacity in order to enhance patient's health and to determine accurately the fungal species associated with clinical illnesses.

CONSENT

Informed consent was not necessary for this study since the study was a laboratory-based and no direct contact with patients was undertaken. Results of this study would not affect the outcome of patient's health directly. No specific consent was taken as patient's identity or any of his/ her information or right is likely to be revealed while publishing.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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