

Isolation and Identification of Fungi from Wounds and Burns of Human and Farm Animals

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Summary

This study was conducted to isolate and identify the fungi that infect burn wounds in humans and acquired wounds or wounds resulted from surgical operation in farm animals. A total of 110 cotton swabs, 60 cotton swabs were collected from wounds to 6 kinds of farm animals, which include 10 swabs for each type of these animals (cattle, sheep, goats, cats, dogs, donkeys). As well as 50 swabs were collected from human burns. All these samples were cultured onto Sabouraud dextrose agar (oxoid, England) to detect fungi in wounds of humans and animals in Baghdad city. Identification of Yeasts were done by use RapID™ Yeast Plus System (remel, USA), While the Identification of moulds were done in the Central Public Health Laboratory, depending on the macroscopic appearance of the colony and microscopic examination. Growth was positive for 49 (81.66%) cases of the total 60 wounds swabs. Which detect etiological fungi that infect wounds of animals were as follows: *Trichosporon beigelii* 6 (10%), *Candida parapsilosis* 2 (3.33%), *Yarrowia lipolytica* 1 (1.66%), *Prototheca zopfii* 1 (1.66%), *Candida lambica* 1 (1.66%), *Aspergillus flavus* 7 (11.66%), *Penicillium* 6 (10%), *Rhizopus* 6 (10%), *Aspergillus niger* 5 (8.33%), *Trichophyton rubrum* 5 (8.33%), *Mucor* 5 (8.33%), *Alternaria* 4 (6.66%), *Aspergillus fumigates* 2 (3.33%), *Aspergillus terreus* 2 (3.33%), *Epidermophyton floccosum* 2 (3.33%), *Helminthosporium* 1 (1.66%), *Geotrichum* 1 (1.66%), *Fusarium* 1 (1.66%). As well as the growth was positive for 37 (74%) cases of 50 swabs taken from the burns in humans. They were found that the causative agent of burns wounds in humans were: *Candida albicans* 3 (6%), *Yarrowia lipolytica* 2 (4%), *Trichosporon beigelii* 1 (2%), *Cryptococcus albidus* 1 (2%), *Prototheca zopfii* 1 (2%), *Candida guilliermondii* 1 (2%), *Aspergillus niger* 6 (12%), *Aspergillus flavus* 4 (8%), *Aspergillus fumigates* 3 (6%), *Penicillium* 5 (10%), *Rhizopus* 3 (6%), *Alternaria* 2 (4%), *Fusarium* 1 (2%), *Trichophyton rubrum* 1 (2%), *Mucor* 1 (2%), *Helminthosporium* 1 (2%), *Geotrichum* 1 (2%). Drug susceptibility test was done by using disc diffusion method onto Muller Hinton agar, in this study use 5 types of antifungal disc were used (Amphotrecin B, Nystatin, Fluconazole, Ketoconazole, Itraconazole).

Keywords: Pathogenic fungi, Burn, Wound, Human, Farm animals.

Introduction

Wound is the disruption in the continuity of soft parts of the body structures (1 and 2). Development of wound infection depends on the interplay of many factors. The breaking of the host protective layer- the skin, and thus disturbing the protective functions of the layer, will induce many cell types into the wound to initiate host response (3) Infection of the wound is the successful invasion, and proliferation by one or more species of microorganisms anywhere within the body's sterile tissues, sometimes resulting in pus formation (4).

Wounds can be classified as accidental, pathological or post-operative. Whatever the nature of the wound, infection is the

attachment of microorganisms to host cells and they proliferate, colonize and become better placed to cause damage to the host tissues (3).

Fungi cause nosocomial infections in surgical cases as a part of polymicrobial infections or fungemia, rare causes of aggressive soft tissue infections and so-called opportunistic pathogens (5). Wound can be infected by a variety of microorganisms ranging from bacteria to fungus and parasites (5). The fungal organisms are *Candida species* and moulds *Aspergillus species* are the highest infected wounds (6). The aim of this study was to investigate the isolation and identification of fungi from wounds and burns.

Materials and Methods

Fifty burn swabs were collected from burned wounds patients' between October 2011 to May 2012. Questionnaire was made for those 50 patients including name, age, sex, residence, animal contacts, the burn reason, the burn percentage, history of infected person, recent therapy from burned patients reposed in Al- Yarmook Hospital, and Burned Hospital at Medical City in Baghdad Governorate.

Sixty wound swabs were collected between October 2011 to May 2012 from 6 types of animals include (Cow, Sheep, Goat, Cat, Dog, Donkey) in which were obtained 10 swabs from each species of animals. These suspected animals were distributed as following: the surgery section in Veterinary Medicine College of Baghdad University, and from different regions as Shialla, Ghazalia, Al-Adeel, Adan square, Al-Hurria in Baghdad Governorate. The categories of wounds infections in the study included bedsores, trauma wounds, post operation wounds and ulcers wounds resulted from complicated fractured bones.

The swabs were cultured in Sabouraud dextrose broth (Oxoid, England) then plated onto Sabouraud dextrose agar (Oxoid, England) for isolation and identification of fungi.

The yeasts were cultured by streaking method onto the plate and incubated at 30°C according to (7) then identified by use RapID™ Yeast Plus System (Remel, USA) is a qualitative micromethod employing

conventional and chromogenic substrates for the identification of medically important yeast, yeast-like, and related organisms isolated from clinical specimens.

While the mould was cultured by stabbing method onto the plate and incubated at 25°C to mould growth, then identified by standards methods depended on macroscopic colonial morphology and microscopically finding as well (8). Then the growth was observed daily for 30 days according to (9) after which the plate show no growth were considered negative.

Antifungal susceptibility testing was done by the disc diffusion method, its solubility and diffusion rate through agar. Commercially prepared antibiotics discs were placed onto Mueller Hinton agar (LAB, England) plates those have been inoculated with the test organisms with sterile forceps.

The different antifungals agents used and their disc contents were Nystatin, Amphotrecin B, Ketoconazole, Itraconazole, Fluconazole (HiMedia). Plates were incubated for 2-3 days at 30°C for yeast and 25°C for mould after which the zone of inhibition was measured. Sensitivity of fungi was done onto Mueller Hinton agar (LAB, England) plates. In reporting the results, resistance to any antifungals was represented by R, while S represented sensitivity of the organism to the antifungals and I represented intermediate to the antifungals drugs. Their concentration in the discs and their zone of inhibition in deciding susceptibility are given in (Table, 1).

Table 1: The antifungals discs use with their remarks

No.	antifungals agent	Concentration	Diameter of zone (mm)		
			R	I	S
1	Nystatin	100 Unit /disc	15	16-17	18-20
2	Amphotrecin B	100 Unit /disc	9	-	10
3	Ketoconazole	10Mcg/disc	14	15-19	20-30
4	Itraconazole	10Mcg/disc	14	15-19	20-30
5	Fluconazole	10Mcg/disc	14	15-19	20-30

R: resist, I: intermediate, S: sensitive

Results and Discussion

Out of 60 swabs from animal cases examined, 49 (81.66%) swabs yielded growth of 58 isolates. This means that some samples

yielded more than one organism. While 11(18.33%) wounds swabs failed to yield any growth (Table, 2). In present study, 50 burn wounds swabs were examined which showed

that 37 swabs were yielded growth in percentage 74%, while 13 burn swabs were

failed to yield any growth in percentage of 26%. (Table, 3).

Table, 2: Show the types of isolates and the type of animals.

No	Type of isolate	Cow	Sheep	Goat	Dog	Cat	Donkey	No.	%
1	<i>Trichosporon beigeli</i>	+	-	++	+	-	++	6	10
2	<i>Candida parapsilosis</i>	-	-	-	+	+	-	2	3.333
3	<i>Yarrowia lipolytica</i>	-	-	-	-	-	+	1	1.666
4	<i>Prototheca zopfii</i>	-	-	-	-	-	+	1	1.666
5	<i>Candida lambica</i>	-	-	-	-	-	+	1	1.666
6	<i>Aspergillus flavus</i>	-	++	++	++	+	-	7	11.666
7	<i>Penicillium</i>	++	+	-	+	+	+	6	10
8	<i>Rhizopus</i>	+	+	++	-	+	+	6	10
9	<i>Aspergillus niger</i>	-	-	+	-	++	++	5	8.333
10	<i>Trichophyton rubrum</i>	+	++	+	-	+	-	5	8.333
11	<i>Mucor</i>	+	++	++	-	-	-	5	8.333
12	<i>Alternaria</i>	-	-	+	-	+	++	4	6.666
13	<i>Aspergillus fumigates</i>	+	-	-	-	-	+	2	3.333
14	<i>Aspergillus terreus</i>	-	-	-	++	-	-	2	3.333
15	<i>Epidermophyton floccosum</i>	+	-	-	-	-	+	2	3.333
16	<i>Helminthosporium</i>	-	-	+	-	-	-	1	1.666
17	<i>Geotricum</i>	-	-	-	-	-	+	1	1.666
18	<i>Fusarium</i>	-	-	+	-	-	-	1	1.666
Total		8	8	13	7	8	14	58	96.6

+ = yield growth (one isolate); ++ = yield growth (two isolate); - = no growth

Table, 3: Show the type and number of Human isolates.

No	Type of isolate	No. Human isolate	%
1	<i>Candida albicans</i>	3	6
2	<i>Yarrowia lipolytica</i>	2	4
3	<i>Trichosporon beigeli</i>	1	2
4	<i>Cryptococcus albidus</i>	1	2
5	<i>Prototheca zopfii</i>	1	2
6	<i>Candida guilliermondii</i>	1	2
7	<i>Aspergillus niger</i>	6	12
8	<i>Aspergillus flavus</i>	4	8
9	<i>Aspergillus fumigates</i>	3	6
10	<i>Penicillium</i>	5	10
11	<i>Rhizopus</i>	3	6
12	<i>Alternaria</i>	2	4
13	<i>Fusarium</i>	1	2
14	<i>Trichophyton rubrum</i>	1	2
15	<i>Mucor</i>	1	2
16	<i>Helminthosporium</i>	1	2
17	<i>Geotricum</i>	1	2
Total		37	74

This result was agreed with (6, 10 and 11) whom showed that the fungal organisms are yeasts *Candida species* and moulds (*Aspergillus species*) were the highest infected wounds. Followed by *Rhizopus*, *Penicillium*, *Alternaria*.

In Iraq the present study was in agreement with (12) who showed the *Aspergillus spp.* and *Candida spp.* were the higher fungal isolation rate of infection which were more common in patients treated with open dressing (25.5%) than occlusive dressing (16.0%) in Basrah governorate.

In Jordan (13) the microbiological analysis of burn wound infection showed that fungal infection was being responsible for 50.74% of the infections *Candida* 11.3%, *Aspergillus* and *Fusarium* 7.4%.

Human and animals fungal isolates were almost similar with distinct sensitivity and resistant to antifungals. Only, the number of isolates varied (Table, 4). In general, *Candida albicans*, *Candida lambica*, *Prototheca zopfii*, *Aspergillus terreus* and *Mucor* were sensitive to polyenes and resistant to azoles. This result

agreed with (14 - 17). On the other hand, *Yarrowia lipolytica*, *Aspergillus fumigatus* and *Penicillium* were sensitive to Polyenes and Ketoconazole and resistant to other azoles. This result coincide with (18 and 19). Moreover, the *Helminthosporium* and *Trichophyton rubrum* were sensitive to polyenes and Fluconazole and resistant to other azoles. This result agreed with (20).

Our record showed that *Candida Guilliermondii* and *Geotrichum* were sensitive to all antifungal agents. This result agreed with (21 and 22). The rest, including *Candida parapsilosis*, *Cryptococcus albidus*, *Trichosporon begalii*, *Aspergillus niger*, *Alternaria*, *Epidermophyton floccosum* and *Rhizopus* were resistant for polyenes and azoles. This result was supported by (14, 16, 23 and 24). *Fusarium* isolates were resistant for all antifungal agents except Amphotrcin B. this result agreed with (25), in Texas. Finally, *Aspergillus flavus* was sensitive for amphotrcin B, intermediate for ketoconazole and itraconazole, resistant for nystatin and fluconazole. This result coincided with (26).

Table, 4: Show the results of antifungal disc.

List	Isolates	from		Antifungal disc				
		human	animal	Nys.	Amp.B	FLC.	KT.	IT.
1	<i>Candida albicans</i>	3	-	S	S	R	R	R
2	<i>Candida parapsilosis</i>	-	2	R	R	R	R	R
3	<i>Candida Guilliermondii</i>	1	-	S	S	S	S	S
4	<i>Candida lambica</i>	-	1	S	S	R	R	R
5	<i>Cryptococcus albidus</i>	1	-	R	R	R	R	R
6	<i>Trichosporon begalii</i>	1	6	R	R	R	R	R
7	<i>Prototheca zopfii</i>	1	1	S	S	R	R	R
8	<i>Yarrowia lipolytica</i>	2	1	S	S	R	S	R
9	<i>Aspergillus flavus</i>	4	7	R	S	R	I	I
10	<i>Aspergillus fumigatus</i>	3	2	S	S	R	S	R
11	<i>Aspergillus niger</i>	6	5	R	R	R	R	R
12	<i>Aspergillus terreus</i>	2	-	S	S	R	R	R
13	<i>Alternaria</i>	2	4	R	R	R	R	R
14	<i>Epidermophyton floccosum</i>	2	-	R	R	R	R	R
15	<i>Fusarium</i>	1	1	R	S	R	R	R
16	<i>Geotrichum</i>	1	1	S	S	S	S	S
17	<i>Helminthosporium</i>	1	1	S	S	S	R	R
18	<i>Mucor</i>	1	5	S	S	R	R	R
19	<i>Penicillium</i>	5	6	S	S	R	S	R
20	<i>Rhizopus</i>	3	6	R	R	R	R	R
21	<i>Trichophyton rubrum</i>	1	5	S	S	S	R	R

(Nys.)=nystatin, (Amp.B)= Amphotrcin B, (Flc.)=Fluconazol, (KT.)=Keteconazol, (IT)=Itraconazol.

Bennett, (27), noted the resistance in *C. albicans* occurs by way of mutations in the ERG11 gene, which codes for 14 α -demethylase. These mutations prevent the azole drug from binding, while still allowing binding of the enzyme's natural substrate, lanosterol. Development of resistance to one azole in this way will confer resistance to all drugs in the class. Another resistance mechanism employed by both *C. albicans* is increasing the rate of efflux of the azole drug from the cell, by both ATP-binding cassette and major facilitator superfamily transporters. Other gene mutations are also known to contribute to development of resistance. On other hand (26) some *Aspergillus spp.* resistant for azoles due to mutations in the cyp51A-gene, the target for antifungal azoles lead to failure treatment of cases infected with *Aspergillus spp.*

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عزل وتشخيص الفطريات من الجروح و الحروق في الانسان و الحيوانات الحقلية

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الخلاصة

أجريت هذه الدراسة لعزل وتشخيص الفطريات التي تصيب جروح الحروق في الانسان و الجروح المكتسبة او جروح العمليات الجراحية في الحيوانات الحقلية. جمعت لهذه الدراسة 110 مسحة قطنية، 60 مسحة قطنية جمعت من جروح 6 انواع من الحيوانات الحقلية و التي تشمل 10 مسحات لكل نوع من هذه الحيوانات (أبقار، أغنام، ماعز، قطط، كلاب، حمير). كذلك تم جمع 50 مسحة من حروق الانسان، كل هذه العينات تم زرعها على وسط السابروييد دكستروز اكار (Oxoid, England) للكشف عن الفطريات في العينات المأخوذة من الانسان و الحيوانات في مدينة بغداد. تشخيص الخمائر أجري باستخدام RapID™ Yeast Plus System (remel, USA)، و قد تم تأكيد تشخيص الاعفان في مختبر الصحة العامة المركزي بالاعتماد على الشكل الخارجي للمستعمرة و الفحص المجهرى. النمو كان موجبا لـ 49 (81.66%) حالة من مجموع 60 مسحة جرح، تم من خلالها الكشف عن المسببات الفطرية التي تصيب جروح الحيوانات وهي كما يلي: *Trichosporon beigelii* 6(10%), *Candida parapsilosis* 2(3.33%), *Yarrowia lipolytica* 1(1.66%), *Prototheca zopfii* 1(1.66%), *Candida lambica* 1 (1.66%), *Aspergillus flavus* 7 (11.66%), *Penicillium* 6 (10%), *Rhizopus* 6(10%), *Aspergillus niger* 5 (8.33%), *Trichophyton rubrum* 5(8.33%), *Mucor* 5(8.33%), *Alternaria* 4(6.66%), *Aspergillus fumigates* 2 (3.33%), *Aspergillus terreus* 2 (3.33%), *Epidermophyton floccosum* 2 (3.33%), *Helminthosporium* 1(1.66%), *Geotrichum* 1(1.66%), *Fusarium* 1(1.66%).
مأخوذة من الحروق في الانسان، حيث وجد ان المسببات الفطرية للجروح الناتجة عن الحروق في الانسان هي: *Candida albicans* 3(6%), *Yarrowia lipolytica* 2 (4%), *Trichosporon beigelii* 1 (2%), *Cryptococcus albidus* 1 (2%), *Prototheca zopfii* 1 (2%), *Candida guilliermondii* 1 (2%), *Aspergillus niger* 6 (12%), *Aspergillus flavus* 4 (8%), *Aspergillus fumigates* 3 (6%), *Penicillium* 5 (10%), *Rhizopus* 3 (6%), *Alternaria* 2 (4%), *Fusarium* 1 (2%), *Trichophyton rubrum* 1(2%), *Mucor* 1(2%), *Helminthosporium* 1(2%), *Geotrichum* 1(2%).
أختبار الحساسية الدوائية تم باستخدام طريقة القرص المنتشر، حيث ان له قابلية الذوبان و الانتشار خلال المادة المتخنة (الكار)، أستخدم في هذه الدراسة 5 انواع من المضادات الفطرية و التي تشمل (النستاتين، الامفوترسين بي، الفلوكونازول، كيتوكونازول، اتركونازول) (HiMedia).

الكلمات المفتاحية: الفطريات المرضية، الجروح، الحروق، الانسان، الحيوانات الحقلية.