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   | Amphotreacin 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  | Trichosporon beigelii   | +   | -     | ++   | +   | -   | ++     | 6   | 10 |
| 2  | Candida parapsilosis    | -   | -     | -    | +   | +   | -      | 2   | 3.33 |
| 3  | Yarrowia lipolytica     | -   | -     | -    | -   | -   | +      | 1   | 1.666 |
| 4  | Prototheca zopfii       | -   | -     | -    | -   | -   | +      | 1   | 1.666 |
| 5  | Candida lambica        | -   | -     | -    | -   | -   | +      | 1   | 1.666 |
| 6  | Aspergillus flavus      | -   | ++    | ++   | +   | -   | -      | 7   | 11.666 |
| 7  | Penicillum             | ++  | +     | -    | +   | +   | +      | 6   | 10 |
| 8  | Rhizopus              | +   | +     | ++   | -   | +   | -      | 6   | 10 |
| 9  | Aspergillus niger      | -   | -     | +    | -   | ++  | +      | 5   | 8.333 |
| 10 | Trichophyton rubrum    | +   | ++    | -    | +   | -   | -      | 5   | 8.333 |
| 11 | Mucor                  | +   | ++    | ++   | -   | -   | -      | 5   | 8.333 |
| 12 | Alternaria             | -   | -     | +    | -   | +   | ++     | 4   | 6.666 |
| 13 | Aspergillus fumigates  | +   | -     | -    | -   | -   | +      | 2   | 3.333 |
| 14 | Aspergillus terrus     | -   | -     | +    | -   | -   | -      | 2   | 3.333 |
| 15 | Epidermophyton floccosum | +   | -     | -    | -   | -   | +      | 2   | 3.333 |
| 16 | Helminthosporium       | -   | -     | +    | -   | -   | -      | 1   | 1.666 |
| 17 | Geotricum              | -   | -     | -    | -   | +   | -      | 1   | 1.666 |
| 18 | Fusarium               | -   | -     | -    | -   | +   | -      | 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  | Candida albicans    | 3                 | 6   |
| 2  | Yarrowia lipolytica | 2                 | 4   |
| 3  | Trichosporon beigelii | 1             | 2   |
| 4  | Cryptococcus albidus | 1             | 2   |
| 5  | Prototheca zopfii   | 1                 | 2   |
| 6  | Candida guilliermondii | 1             | 2   |
| 7  | Aspergillus niger   | 6                 | 12  |
| 8  | Aspergillus flavus  | 4                 | 8   |
| 9  | Aspergillus fumigates | 3             | 6   |
| 10 | Penicillum          | 5                 | 10  |
| 11 | Rhizopus            | 3                 | 6   |
| 12 | Alternaria          | 2                 | 4   |
| 13 | Fusarium            | 1                 | 2   |
| 14 | Trichophyton rubrum | 1                 | 2   |
| 15 | Mucor               | 1                 | 2   |
| 16 | Helminthosporium    | 1                 | 2   |
| 17 | Geotricum           | 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 Fusarium7.4%.

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 begali, 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 human | From animal | Nys. | Amp.B | FLC. | KT. | IT. |
|------|---------------------|------------|-------------|------|-------|------|-----|-----|
| 1    | Candida albicans    | 3          | -           | S    | S     | R    | R   | R   |
| 2    | Candida parapsilosis| -          | 2           | R    | R     | R    | R   | R   |
| 3    | Candida Guilliermondii| 1         | -           | S    | S     | S    | S   | S   |
| 4    | Candida lambica     | -          | 1           | S    | S     | R    | R   | R   |
| 5    | Cryptococcus albidus | 1          | -           | R    | R     | R    | R   | R   |
| 6    | Trichosporon begali | 1          | 6           | R    | R     | R    | R   | R   |
| 7    | Prototheca zopfii   | 1          | 1           | S    | S     | R    | R   | R   |
| 8    | Yarrowia lipolytica | 2          | 1           | S    | S     | R    | S   | R   |
| 9    | Aspergillus flavus  | 4          | 7           | R    | S     | R    | I   | I   |
| 10   | Aspergillus fumigatus| 3         | 2           | S    | S     | R    | S   | R   |
| 11   | Aspergillus niger   | 6          | 5           | R    | R     | R    | R   | R   |
| 12   | Aspergillus terreus | 2          | -           | S    | S     | R    | R   | R   |
| 13   | Alternaria          | 2          | 4           | R    | R     | R    | R   | R   |
| 14   | Epidermophyton floccosum| 2     | -           | R    | R     | R    | R   | R   |
| 15   | Fusarium            | 1          | 1           | R    | S     | R    | R   | R   |
| 16   | Geotrichum          | 1          | 1           | S    | S     | S    | S   | S   |
| 17   | Helminthosporium    | 1          | 1           | S    | S     | S    | R   | R   |
| 18   | Mucor               | 1          | 5           | S    | S     | R    | R   | R   |
| 19   | Penicillium         | 5          | 6           | S    | S     | R    | S   | R   |
| 20   | Rhizopus            | 3          | 6           | R    | R     | R    | R   | R   |
| 21   | Trichophyton rubrum | 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 14α-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 والحواظ في الإنسان والحيوانات الحقلية

الخلاصة

أجريت هذه الدراسة لعزل وتشخيص الفطريات التي تسبب جروح في الإنسان والجروح المكتسة أو جروح المبكرة في الأنسان والحيوانات الحيوانية في الجروح الحقلية. جمعت هذه الدراسة 110 مسحة قطنية. المجموع 50 مسحة من البكتيريا، 50 مسحة من الفطريات، 10 مسحة من الحيوانات الحيوانية. من حيوانات الإنسان، كل هذه العينات تم زرعها على وسط البايرن رينو (أوكسيد، إنجلند). وتم تحليل عينة من الفطريات في زرع المختبر RapID™ Yeast Plus System و تم تم الكشف عن الفطرية (Rotala, USA) في مختبر الصحة العامة. كتبت هذه الدراسة لعزل وتشخيص الفطريات التي تسبب جروح في الإنسان والحيوانات الحيوانية في الجروح الحقلية. جمعت هذه الدراسة 110 مسحة قطنية. المجموع 50 مسحة من البكتيريا، 50 مسحة من الفطريات، 10 مسحة من الحيوانات الحيوانية. من حيوانات الإنسان، كل هذه العينات تم زرعها على وسط البايرن رينو (أوكسيد، إنجلند). وتم تحليل عينة من الفطريات في زرع المختبر RapID™ Yeast Plus System و تم تم الكشف عن الفطرية (Rotala, USA) في مختبر الصحة العامة. كتبت هذه الدراسة لعزل وتشخيص الفطريات التي تسبب جروح في الإنسان والحيوانات الحيوانية في الجروح الحقلية. جمعت هذه الدراسة 110 مسحة قطنية. المجموع 50 مسحة من البكتيريا، 50 مسحة من الفطريات، 10 مسحة من الحيوانات الحيوانية. من حيوانات الإنسان، كل هذه العينات تم زرعها على وسط البايرن رينو (أوكسيد، إنجلند). وتم تحليل عينة من الفطريات في زرع المختبر RapID™ Yeast Plus System و تم تم الكشف عن الفطرية (Rotala, USA) في مختبر الصحة العامة.