



THE EFFECT OF FUNGUS FILTRATES FOR SOME SPECIES *ASPERGILLUS* SPP. GENUS ON THE YEAST AND BACTERIA ISOLATED OF PERSONS INFECTED BY SKIN DISEASES

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Abstract

This study was conducted from October 2017 to March 2018 for isolation and diagnosis *Candida* spp. and bacteria and Competency delineation of *Aspergillus* spp. genus for isolations, 100 samples had been collected from patients with different ages & both sexes for samarra Hospital. The age of the patients ranged between more than 11 Day to 75 years, which include 53 males and 47females. Samples the highest of *candida* spp. include 57.14% males and 42.86% females. While bacteria include 53.03% males and 46.97% females. *C. albicans* it's showed the higher ratio 27.06%. while *S. epidermidis* 43.08% and *Salmonella typhi* 7.69% and it's first isolation on Iraq and second isolation in Arab World from patients and identification tests were carried out by studying morphological, microscopic characteristic and using of Vitek2 compact system. results showed *A. niger*, *A. terreus*, *A. nidulans* Filtrates showed significant differences at the probability level ($P < 0.05$) in inhibition. *A. terreus* filter was higher inhibition rate with 100, 98% include *C. albicans* from *candida* spp. and *S. typhi*, *S. epidermidis* from bacteria. *A. niger* filter showed the higher inhibition rate with rate 100,98% for *C. albicans*. While *A. nidulans* filter showed the higher inhibition rate for *C. albicans* and *S. typhi* with rate 100, 98%.

Keywords : *Aspergillus* spp., Bacteria Isolated, skin diseases, Fungus Filtrates

Introduction

Skin is one of the most vulnerable to external, environmental and visible effects to others and is associated with the rest of the body. The skin can infect large numbers of microorganisms, some of which are pathogenic and others are naturally co-infected (David, & Michael, 2011). *Candida* spp. The first of these organisms that are present on the skin is normal flora, which can become opportunistic to infect different areas of the skin when appropriate conditions are available. There are about 20 types, including *Candida albicans*, which can cause infections to the nails, skin, hair and subcutaneous tissue then *Candida glabrata* and other yeast (Satana, Dilek *et al.*, 2010; Yaper, 2014). *Candida* spp. is not limited to *Candida*. There are several types of Gram positive bacteria, including *Staphylococcus epiderimids*, as well as Gram negative bacteria that cause inflammation of wounds and burns by secretion of enzymes Which cause the injury (Abraham Pulido-Cejudo *et al.*, 2017). The use of broad-spectrum antibiotics in the form of excessive and indiscriminate adverse effects on humans has been used for more effective and economical methods. Among the proposed control methods are biological control using antibiotic organisms, which has proved effective in many experiments (Cazar *et al.*, 2005). *Aspergillus* spp is a fungus that has proven to produce a lot of inhibitory substances such as toxins, acids and enzymes, which act as antimicrobial agents, either reduce the number of pathogens, alter the metabolism of these pathogens, and inhibit other microorganisms (Maqueda, 2008; Oelschlaeger, 2010). And that the genotypes of *Aspergillus* spp can play an important role in the inhibition and control of many microorganisms by the antibiotic and can be a key to the treatment of many of the diseases, the research aims to isolate and diagnose yeasts and bacteria causing skin diseases in the city of Samarra, Types of *Aspergillus* spp against the isolates diagnosed and compared with antibiotics.

Materials and Methods

Specimens collection : The samples of the study were collected based on (Askarian *et al.*, 2009) and included references to the special form for collecting information (age, sex, place of injury).

Laboratory diagnosis of Candida yeast: Agricultural media based on (Baron & Finegold, 1990; Ellis *et al.*, 2007; Emmons *et al.*, 1974; Pfaller *et al.*, 1996), Dyes and solutions (Catalase test, Oxidase test) based on (Davise, 1995) and (Collee *et al.*, 1996; Pfaller *et al.*, 1996).

Laboratory diagnosis of bacterial isolates: Agricultural media and dye based on (Collee *et al.*, 1996) and (Koneman *et al.*, 1997; Macfaddin, 2000) and solutions based on (Macfaddin, 2000; Todar, 2004).

Effect of Filtrates of some species of *Aspergillus* spp In inhibition of yeast and bacteria isolated from the skin : Collect and isolate species of genus *Aspergillus* spp depending on: (Jay & Stephen, 2013; Kown–chung *et al.*, 1992; Patron, 2010).

Tests for the determination of the inhibitory effectiveness of some types of Filtrates *Aspergillus* spp and the sensitivity test of isolates of antibiotics:

- Agricultural circles depending on (Emmons *et al.*, 1974) and (Prize *et al.*, 1990) Solutions based
- Physiological Saline Solution 0.85% depending on (Prescott *et al.*, 1996).
- McFarland Standard depending on (Baron and Finegold, 1990).

Preparation of anti-fungal Filtrates

A. niger, *A. terreus* and *A. nidulans* were used as antibiotics for yeast and bacteria based on a method (Chang & Kommedahl, 1968).

Preparation Suspension of *Candida* spp and Bacteria

Preparation Suspension the *Candidia* spp. and bacteria using method 10 and (CISI, 2002; Meyer, 1974)

Antibiotics used in the study:

Antibiotic solutions: Clotrimazole (0, 2 mg; Ce); Ketoconazole (200mg; Kt) Ketoconazole; Nystatin (Ns; 100000IU / ml) Clindamycin (CA; 300 mg), Cefixime (CFM; 100 mg); Ciprofloxacin (CIP, 500 mg) and by method (Prize *et al.*, 1990).

Determination of the inhibitory effectiveness of Filtrates the *Aspergillus* spp

Taking 0.1 ml of each dilution of Yeast and Bacterium Yeast and published on the Mueller-Hinton agar medium using a cotton scanner and incubated the dishes in order to get full sowing in the middle and use it, using the drill method (Gupte, 1994). then puncture the agar with the 5mm diameter holes and then add 0.2 µg / mL of the fungus filtration with 0.2 mL of distilled water and then incubate at 37°C for 24 h. Inhibition is calculated in millimeters and according to the law below, which was statistically analyzed (Vigonolo *et al.*, 1998). Damping rate = diameter rate of growth inhibition - diameter hole rate inhibition.

Antibiotics sensitivity

The *candida* spp Sensitivity test and the bacterial species studied were performed using the drill method (Prize *et al.*, 1990) using the fungal and bacterial antifungal solutions mentioned above depending on method (Vandepitte *et al.*, 1991).

Determination of the minimum inhibitory concentration (MIC) and killer (MFC)

A series of concentrations of *Aspergillus* spp species were introduced with the addition of controlled distilled water for the purpose of determining the MIC and MFC for pathological isolates and using Agar Diffusion Method Well (Pavia *et al.*, 2007). The filtration was measured using the standard Maufferland standard 0.5 and the total number of spores Each according to its concentration as follows: 25% (1.6X²10cell / ml), 50% (5.7X²10 cells / ml), 75% (1.1X³10 cells / ml) and 100% (2.6X⁴10 cells / ml). The results recorded the lowest concentration promise where not growth

of *Candida* spp and Bacteria was the lowest inhibitory concentration (Yaper, 2014). The results were statistically analyzed using the SPSS package according to the ANOVA and the Duncan's New Multiple Range Test (1955), which is characterized by taking the differences between the different averages at the probability level (P <0.05). There were significant differences in the different inhibition ratios. The values were adopted in the form of (Rate ± standard deviation).

Results and Discussion

The results of the study showed that the number of cases infected 42 samples of *Candida* spp. and the percentage of male isolation of males 57.14% and 42.86% of females and the percentage of males from the city 83.33% and 94.44% of females and the rural, the proportion of males reached 16.67% and 5.56% (Vignesh *et al.*, 2017). As for bacterial species, the number of infected cases was 66 and the percentage of male isolation was 53.03% and 46.97% for females. The percentage of isolation from the city was 43.94 males, 42.42% females, and rural males 9.09% and 4.55% females (Xim Wang, et al 2017). The results showed that *C. albicans* were found to be 27.06%, followed by *C. glabrata* 22.35%. This was in line with (Vignesh Kanna B., et al 2017). The results showed that *S. epidermidis* was the most affected with 43.08% . *S. typhi* by 7.69% This corresponds to the findings (Mackenzie, 1963).

It was found during the current study that the age group between 30-21 years was the highest in the incidence of *Candida* spp in males with the highest rates of infection in this category 41.67% of the city and 8.33% of the countryside. This may be due to the nature of the work carried out by the males and the length of the period of work and not to shower regularly or the use of deodorants and wear shoes for long periods, causing the incidence of infection, as indicated by the researcher (Vignesh *et al.*, 2017) and females were the age group 1 day -10 years The percentage of *Candida* spp infection in the city was 27.78%. The other groups did not have any infection rates in females, while the rate of infection among the age group between 11-20 years was 5.56% in rural areas. The other age groups did not have any male or female infection rates. This corresponds to the findings (Hee *et al.*, 2014).

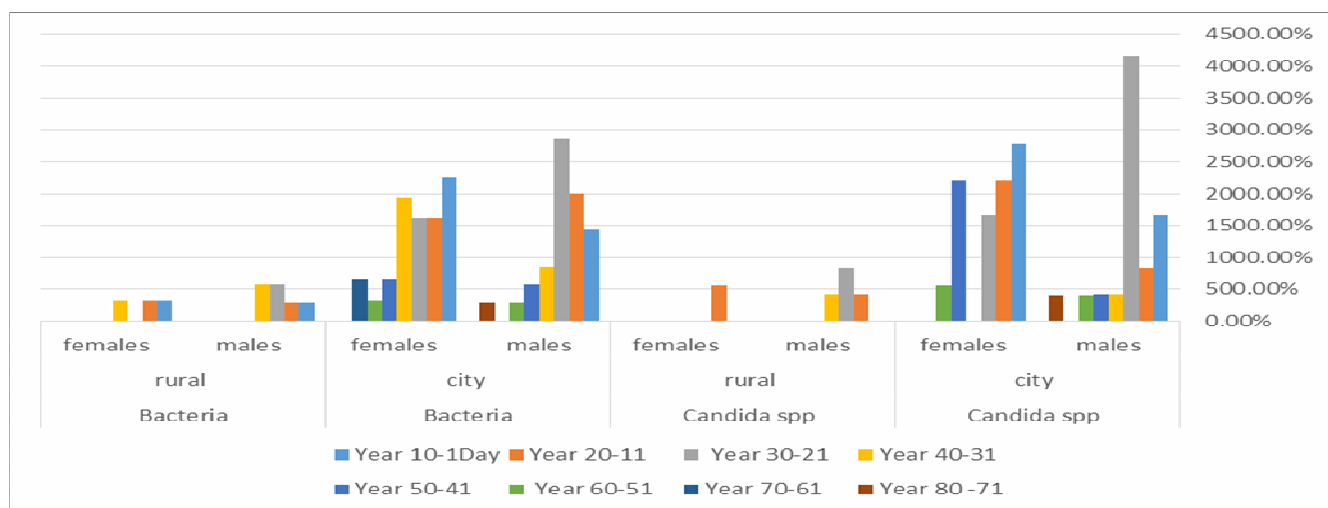


Fig. 1 : Percentage of *candida* spp and bacterial infections and their distribution by age group of males and females

As for bacterial species the age group between 21-30 years recorded the highest incidence of infection among males in 28.57% of the city. While the age groups 21-30 years and 31-40 years recorded the highest incidence rate of 5.71% in the countryside. While the female age groups 31-40 years recorded the highest incidence rate of 19.35% of the city. while In rural areas, the rural population of the age groups between 1day - 10 years, 11-20 years and 31-40 years only 3.23%. This is in line with the findings of (Horak *et al.*, 1996), this disparity is due to the fact that it reflects the environmental level of the population (urban or rural). Rural health services are low compared to the city and the distance between rural areas and health institutions is lower. Such as agriculture and livestock Is one of the carriers of the disease.

The results revealed that the incidence of *Candida* spp infection during the months reached the highest incidence of males in the city during the month of March, as it reached 50% for males and 38.89% for females, while rural areas had the highest rate of infection during the month of March 16.67% While females recorded only one infection during January of 5.56%. Which stated that the lack of fungi in some months may be caused by the use of some patients with topical treatments at random and without consultation with the specialist doctor, which may lead to the effect in the vitality of the fungus and not grow after the transplant.

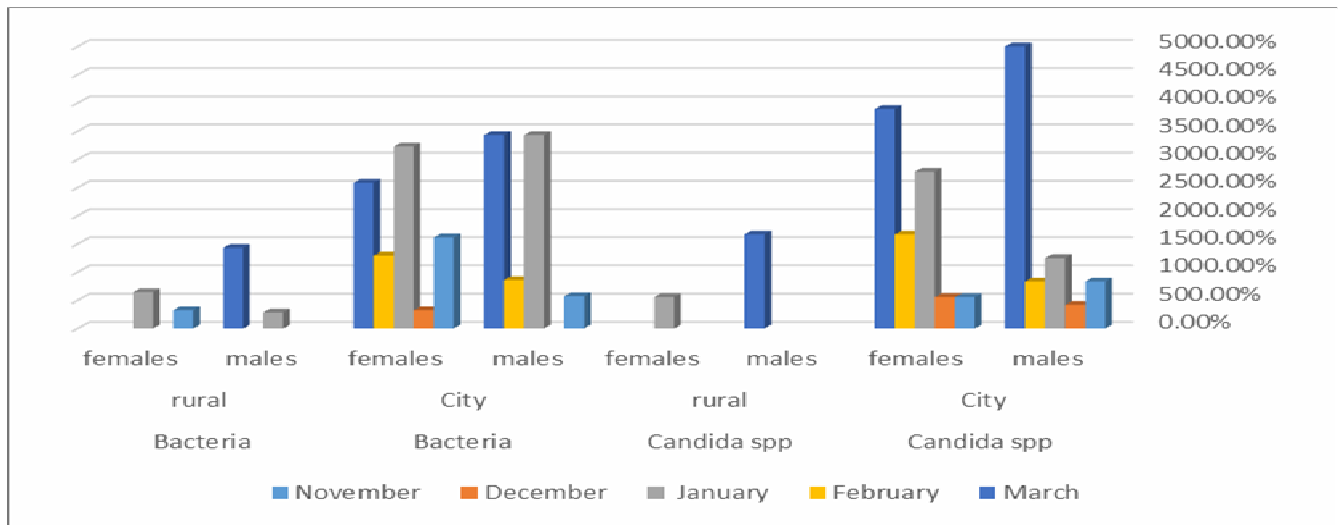


Fig. 2: Percentage of *candida* spp and bacterial infections and their distribution by months of males and females

The bacterial species had the highest infection rate during January and March at 34.29% for males and the highest infection rate for females in January was 32.26% for females. In rural areas, the highest percentage of males was infected during the month of March, 14.29%, while the highest percentage of females in January was 6.45%, while the remaining months in rural areas did not have any infection rates in males and females. Which is characterized by the engineering of mature skin layers, reduced water content in the cold months, decreased skin pH and resistance

to microflora, as indicated by researcher (Ellis *et al.*, 2007). The study included all areas of the body and the most affected areas of skin bleach in the city is ringworm, where the incidence rate of 45.83% for males and 22.22% for females and this result consistent with the findings (Kown-chung *et al.*, 1992) and occur frequently in people who wear shoes constantly, especially athletes Which is known as Athlete's foot and occurs particularly between the fifth finger and the fourth (Ingordo *et al.*, 2004).

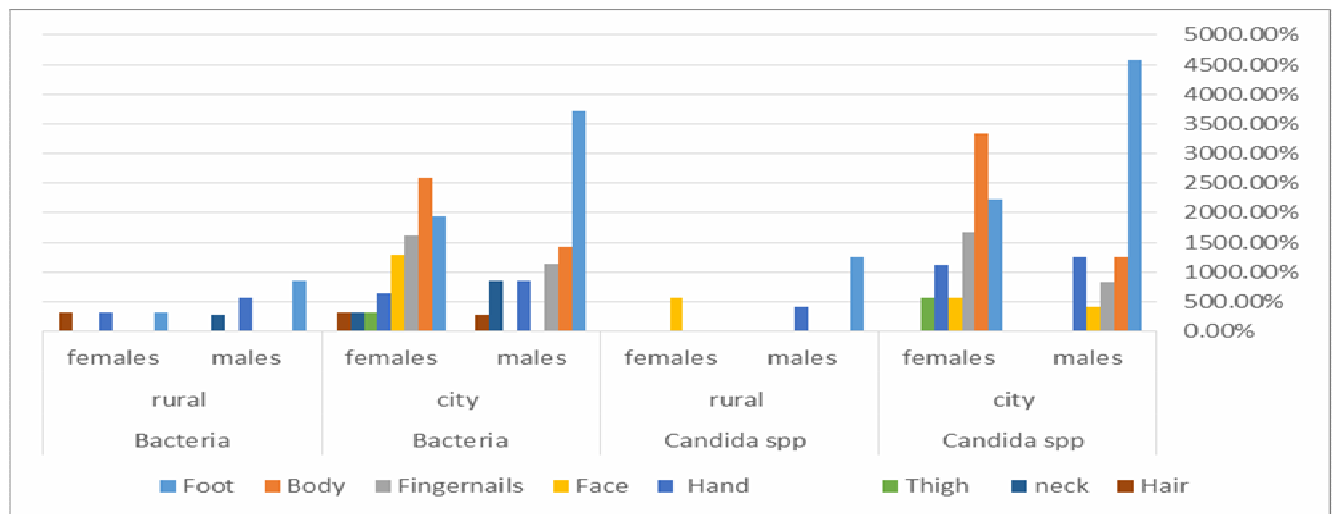


Fig. 3: Percentage of *candida* spp and bacterial infections and their distribution by areas of the body of males and females

The bacterial species have reached the highest infection in the foot area by 37.14% for males and 25.81% of females and this corresponds with the reach of (Mackenzie, 1963). The rural areas had the highest incidence of males with *Candida* spp in the 12.5 foot area and 5.56%. while the highest incidence of bacterial infection in males in the foot area reached 8.57%, while females recorded the foot, hand and hair 3.23% This may be due to the fact that most females are in contact with polluted floors when cleaning and lack of attention to personal hygiene and the use of perfumes and cleaning powders Containing chemicals without wearing gloves that may burn wounds or scratches or cause itching, which may create pimples and all these factors have helped to increase the incidence of bacteria, and this is what the researcher declared (Hang *et al.*, 2017).

Effect of Filtrates of some species of *Aspergillus* spp on *Candida* spp and bacteria species

The effect *Aspergillus* spp. of both *A. niger*, *A. terreus*, *A. nidulans* Filtrates and 75%, 50%, 25% and 100% was studied in inhibiting the growth of isolates of isolated *Candida* spp and bacteria during the study and comparing them with antibiotics. The results of inhibition of filtration types have a high ability to inhibit skin isolated even in low concentrations. The results in Figure 4 explication *A. terreus*, *A. niger*, *A. nidulans* Filtrates had a significant inhibitory effect on *C. albicans*. There were no significant differences in inhibition and their growth was completely inhibited at 100% and 98% concentrations. This finding was consistent with the findings (Hernandez-Mendoza *et al.*, 2010; Roberts, 1990).

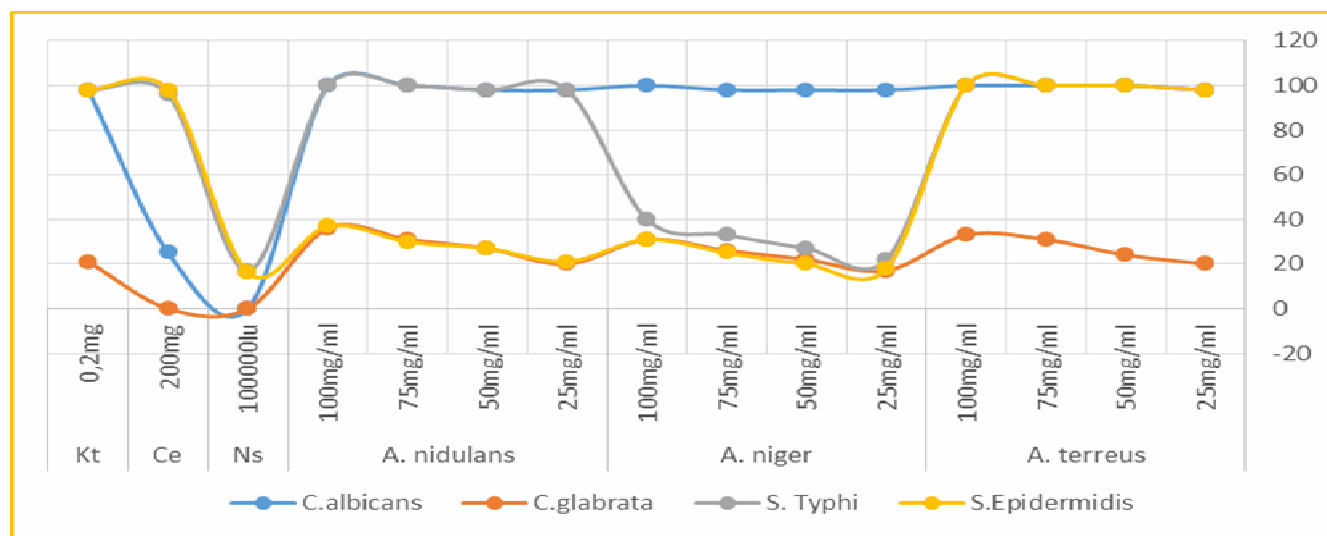


Fig. 4: Effect of Filtrates of some species of *Aspergillus* spp on *Candida* spp and bacteria species of males and females

A. terreus filter was most affected in all types of *Candida* spp and was attributed to the production of fungus Patulin toxin, which is characterized by genetic toxicity. These compounds are affected by *Candida* spp because it reacts with sulfur dioxide so antioxidants are anti-sugary agents useful in destroying of the wall (Mackenzie, 1963), while *A. niger* filter produces lactic acid, citric acid and nitropropionic acid, which has an antimicrobial effect that is sensitive to the acidic environment by dissolving the cell membrane. The acids cause scarring and a significant loss of structural regulation in the primary chitin layer, Dish B1.3glucanase and the manane layer, which reduces the complexity of manane, produces a more porous external shield, and the cell walls contain less carbohydrate than the detectable chemical detectors (Roberts, 1990).

There were significant differences at the probability level ($P < 0.05$) in the inhibitory activity of *A. terreus*, *A. niger* and *A. nidulans* Filtrates for *C. glabrata* isolation. The filtration gave an inhibitory effect of 33, 31 and 36% at 100mg / ml, respectively. *A. terreus* filter was the most effective in inhibiting the *S. typhi* and *S. epidermidis* isolates with a 100% inhibitory rate of 100, 75, 50 mg / ml and 98% at 25 mg / ml concentration. No differences were found in the inhibition ratios and this result is consistent with (Calam *et al.*, 1939; Cazar *et al.*, 2005). The susceptibility of this fungus to inhibition is attributed to the production of many toxin toxins, most notably aflatoxin, Terrirtrem A, B, C and

Cyclosporin A, which is treated as a secondary infusion that inhibits the cell membrane mechanism, which is a positive and negative anti-bacterial toxin for gram (Hernandez-Mendoza, 2010).

A. niger filter showed significant differences at the probability level ($P < 0.05$) in inhibition of *S. epidermidis* and *S. typhi* isolates with 40.31% respectively at 100mg / ml concentration This result is consistent with (Bennett and Klich, 2003). It has the potential to inhibit its production of aflatoxin and oxytoxin toxicity, which has been shown to have the above inhibitory properties and to release Citrinin, a polyketid mycotoxin that has active activity against bacteria (He, and Cox, 2016). The filtration type *A. nidulans* showed its efficiency in the inhibition ratios of *S. typhi* and inhibited its growth at concentrations of all concentration (Chang and Kommedahl, 1968).

This result is in line with the findings of aflatoxins, phosphatase, alkaline phosphatase, and phosphadystraase which break down levels of sugary and fatty substances in the walls of bacterial cells and *Candida* spp (Sebastian *et al.*, 2007). The results showed the inhibitory efficacy of *A. niger*, *A. terreus*, *A. nidulans* Filtrates were studied with a higher inhibition effect compared with the standard fungicide Ketoconazole, Nystatin and Clotrimazole The antibodies showed significant differences at the potential level ($P < 0.05$) in inhibitory activity. Nystatin did not show any inhibitory effect. The isolates exhibited resistance to this

antagonist, while Clotrimazole was effective in inhibiting *C. albicans* isolation. Which was 25% sensitive, while *C. glabrata* was resistant to antifungal due to the high level of antibiotic use, as well as the type of resistance these isolates had against most of the antibiotics used. This result was consistent with while the antifungal Ketoconazole showed the highest inhibitory rates of *C. albicans* isolates, which showed a high sensitivity of 98%, while the antibody gave 21% towards the isolation of *C. glabrata*.

While all of the bacterial isolates were sensitive to Cefixime and Ciprofloxacin with a 98-96% inhibition ratio for all bacterial isolates and showed no differences in inhibitory efficacy. Clindamycin showed significant

differences at the probability level ($P < 0.05$) in the inhibition rate, this is consistent with his findings.

The minimum inhibitory concentration (MIC) and the minimal killer concentration (MFC) for the filtration of certain species of *Aspergillus* spp on *Candida* spp and bacterial species

The results showed that the value of MIC and MFC varies depending on the type of leachate. Table 4. *Aspergillus terreus* MIC, *Aspergillus niger*, *Aspergillus nidulans* Filtrates (25 mg / mL), and MFC (100 mg/ml) and this is identical to what mentioned (Cazar *et al.*, 2005; Chang and Kommedahl, T. 1968; Hernandez-Mendoza *et al.*, 2010; Park *et al.*, 2013).

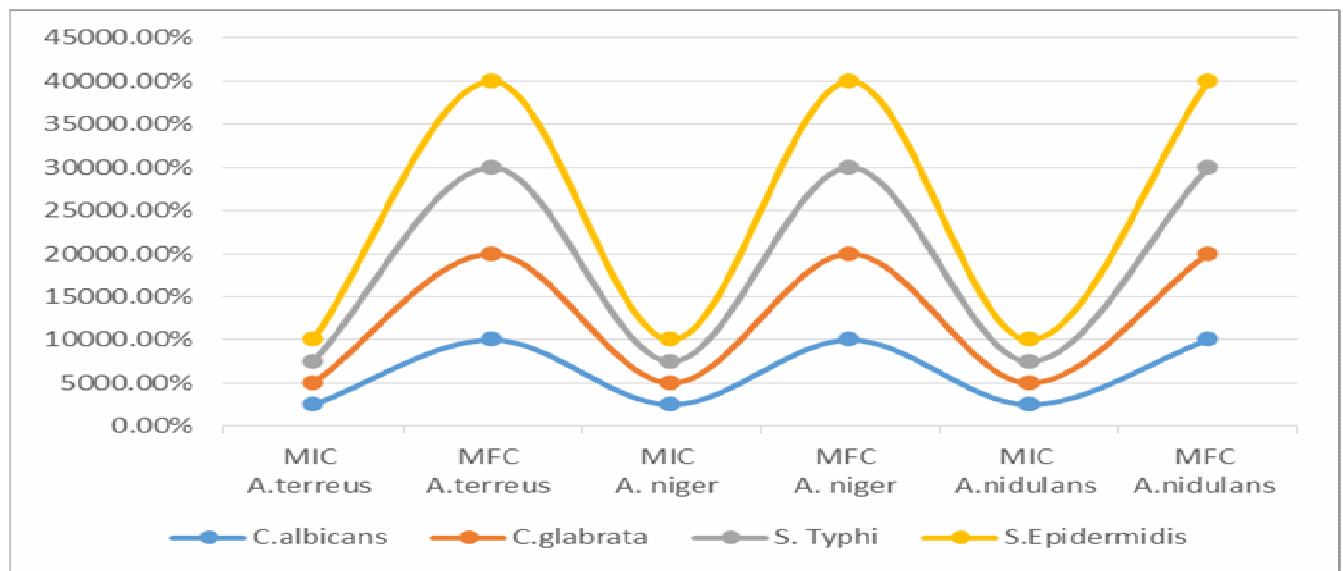


Fig. 5: MIC and MFC concentration for the filtration *Aspergillus* spp on *Candida* spp and bacteria

Conclusions

- The results of the study showed that the incidence of bacteria is higher than candida yeast.
- 2 *Candida* yeast for the isolation of *C. Albicans* is the highest cause of inflammation of the skin and bacteria are the isolation of *Staph. epidermidis*.
- The presence of isolates disease together cause the development and complexity of skin infection in many people if it was found that the proportion of male infection is higher than females for both *Candida* and bacteria.
- *Aspergillus terreus* was more efficient in inhibiting all isolates than the other studied filtrate species

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