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Sensitivity of Fungi Isolated from Different Pathogenic Cases against Common Bacterial Antibiotics

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Abstract
Forty two specimens were gathered from patients suffering from different fungal diseases and from different areas of the body including skin, hair, nails and mouth. Five types of fungi were isolated and diagnosed, namely *T. mentagrophytes, T. rubrum, M. canis, A.niger* and *C.albicans*. By using the comb method, drug sensitivity for the isolated fungal species was tested and the test included fifteen type of antibiotics: Ciprofloxacin,Meropenem,Azithromycin,Levofloxacin,Cefepime,Erythromycin,Linezolid,Clindamycin,Rifampicin,Streptomycin,Ceftazidime,Colistin,Nalidixic acid,Cefuroximeand Amikacin . The results showed that Erythromycin and Ciprofloxacin were the most inhibiting antagonists of *T. mentagrophytes* , whereas Erythromycin, Meropenem and Ciprofloxacin had the highest effect against *T. rubrum* . Meropenem, from another hand, showed its inhibitory activity against M. canis , while Azithromycin had the greatest frustration effect on *C.albicans* yeast and with clear inhibitory diameters. *A. niger* did not show sensitivity to any antibiotic. The lowest inhibitory concentration of Erythromycin was determined against *T. mentagrophytes* and *M. canis* with its value (0.15 and 0.31). The lowest inhibitory concentration of Nalidixic acid for *T. rubrum* was 0.15 mg / ml, whereas the minimum inhibitory concentration value for Azithromycin for *C.albicans* was 0.31 mg / ml.

Keywords
Antibiotics, *T. mentagrophytes*, *T. rubrum*,*C.albicans*, Azithromycin

Introduction

"Human pathogenic fungi", specifically "Opportunistic fungi", are regarded to be the origin of bore and bane to a wide range of people, since they cause diseases which importance are rotating from being simple in its symptoms to the skin and its accessories but do not have a serious threat to the lives of the affected. However, this kind of fungi cause skin malformations, hair loss, discoloration and cracking of the nails, which lead to aesthetic and psychological problems that accompany the sick after their recovery . In the same respect, there are serious damages that cause a direct threat to life, containing subcutaneous and systemic injuries, particularly those affecting the heart, lungs, brain, bones, liver, kidneys and other internal organs (Sullivan, 2014)

The importance of "Human pathogenic fungi" don't confine itself to the physical and psychological harm that they cause to human being, rather, they also harm the world economy when they cause economical sever loses due to the relatively high cost of the anti-fungal productions and drugs. In the United States alone, the cost for 2017 was estimated at

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11.3 billion dollars, that included only medications used against dermatophytosis, Candidiasis, and Aspergillosis (A.D.M; 2018).

Worthy to mention that the irresponsible, random and long term use of the anti-fungal drugs causes genetic mutations that make the pathogen resistant to these antibiotics. Noting that most fungal diseases require long-term treatment compared to bacterial diseases (Lewis, 2011).

Meanwhile, the use of antibiotics cause a defect in the natural balance between the number of fungi and bacteria found as a normal flora in our bodies. So, it is obvious that the use of bacterial antibiotics for long periods after surgery is accompanied by a stable increase in the number of Candida yeast in the patients' mouth and urinary and generative systems (Abdelmonem *et al.*, 2012). Around the world, most of the researchers are doing their best efforts to find out the best and cheapest ways of resisting the "Human pathogenic fungi" , and it is great to hear that there is a drug that can be used for doing two functions together like for example the "actidione" which is an insecticide that is also called "Cycloheximide" .It is used to frustrate the growth of the repaired fungi by obstructing the protein building process inside the cell and for this reason this insecticide has been regarded indispensible in the process of isolating the pathogenic fungi as it prevents infection of media with repaired fungi that may obstruct the growth of pathogenic fungi (Baliga *et al.*, 1969). Azithromycin and Meropenem have also been used to treat Covid-19, although they are antibacterial drugs .(Nestler *et al.*, 2020)

In spite of the huge differences in the shape, composition and functions of both the fungi and the bacteria, there are many studies pointing out that number of antibacterial drugs are successfully used to obstruct the growth and reduce the ability of fungi to ail whether they were molds or yeasts (Kerr & disease, 1999)

It is well known that there are common dietetics media, like for example "Potato Dextrose Aga" to which an antibacterial is added to prevent the medium to be infected of with bacteria, which, in its turn, obstructs the work of the researcher. However, sometimes, for the sake of isolating a specific kind of fungi, it is recommended not to add the antibacterial since the target fungus is sensitive to this antibacterial.(Joseph *et al.*, 2015)

According to what have mentioned before, the following study is going to test number of antibacterial that are widely used to frustrate the growth of pathogenic fungi and this will present double favor for the patient since ,in addition to its obstacle role to bacteria, it will prevent the emergence and exacerbation of fungi, especially the repaired and inflammatory one and those that are regarded part of the natural growth of the body. In the same respect, the study will present economic feasibility when it urges to buy bacterial antibodies only instead of spending money to buy both bacterial and fungal antibodies. The study includes the following axes: - Isolation and diagnosis of pathological fungi from various sources and Testing the effects of fifteen common antibacterial drugs against the above isolated fungi in terms of the frustration areas by using the comp method and the minimum inhibitor concentration.

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Materials and Methods

Samples collection

Forty-twospecimens were collected for patients suffering from mycoses. They consulted the dermatologist consultant ward at Al-Diwaniyah Teaching Hospital in Al-Diwaniyah Governorate for the period between September 2019 to January 2020. The samples were taken from different areas of the body, namely skin, hair, nails and mouth after being diagnosed by the specialist physicians. Then, the samples were preserved in sterile Petri dishes and transferred to the laboratory, where the processes of isolation, purification, examination and diagnosis were performed on them.

Samples Culture:

The collected samples of skin, hair and nail were cultured on a SDA medium which containchloramphenicol and cyclohexamide, nail and hair cuttings were directly cultured on the middle and incubated at 28 ° C for 7-21 days. While cotton swabs taken from the patients' mouths were planned on the surface of the food medium and incubated at 37 ° C. (Baron *et al.*, 1994)

Colonies Morphological Examination

The number of days it took for the fungus to develop the first growth were recorded and also examined the color and shape of the colonies , whether they were flat or shaped like a dome (comb) or whether they contained grooves or not and watching their weaving if they were wooly, granular, Powdery, Cottony, or Glabrous Waxy. Also, on the basis of the kind of pigments they produced, the color of the colonies were recorded from the front view and their color from the opposite side. (Kidd *et al.*, 2016)

Colonies Microscopic Examination :

In order to describe and diagnose the isolated fungi, a part of the developing colony was taken and placed on a glass slide containing a drop of Lactophenol blue stain, and then covered with the cover of the slide and left for a short period to be saturated with the dye . After being examined under a microscope, the diagnosis of the fungi were based on their microscopic properties and their phenotypic characteristics by referring to the sources.(Walsh *et al.*, 2018)

The antifungal activity test

It made for the isolated fungi *T. rubrum* , T. *mentagrophytes* , *M.canis* , *A. niger* and *C. albicans* for fifteen antibacterial drugs including Ciprofloxacin ,Meropenem ,Azithromycin ,Levofloxacin,Cefepime,Erythromycin,Linezolid,Clindamycin,Rifampicin,Streptomycin ,Ceftazidime ,Colistin ,Nalidixic acid,Cefuroxime ,and Amikacin by using the comb method in which the effectiveness and the inhibition validity of the antibacterial were measured .

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Preparation of the fungal inoculum

The fungal inoculum was prepared by transmitting a part of the growing colony onto SDA medium after diagnosing it using a sterile needle ((Loop) and placing it in a sealed tube (Vial) containing 5 ml of (Normal Saline) and shaking the solution using a (Vortex) mixture. Then the number of spores were counting by using aHemocytometer to get 10^5 spore / ml .(McGinnis, 1980)

Preparation of the antibiotic solutions

According to what McGinnis (1980) mentioned, The stock solution of the two antibiotics Erythromycin and Azithromycin was prepared at a concentration of 10 mg / ml, by adding 25 ml of ethanol of 99% concentration in a sealed glass bottle (Vial) and put 250 mg of antibiotic powder to it, then the solution was shacked strongly. The stock solution of the Amikacin antibiotic was prepared by mixing 2 ml of the antithesis solution of original concentration (100 mg / 2 ml) in 8 ml of distilled water to obtain a storage solution of the final concentration of 10 mg / ml . The stock solution of anti-Rifampicin was prepared by dissolving 250 mg of antibiotic powder in a sealed glass vial containing 25 ml of Dimethyl Sulphoxide (DMSO) at a concentration of 100% and shake the solution strongly to obtain the stock solution at a concentration of 10 mg / ml.Stock solutions were prepared at the final concentration of 10 mg / ml for Linezolid, Clindamycin, Streptomycin, Ceftazidime, Colistin, Nalidixic acid, Cefuroxime, Ciprofloxacin, Meropenem, Levofloxacin and Cefepime by dissolving 250 mg of the antibiotic in 25 mg. of serialized water and putting the solution in a tightly sealed vial , then shake the solution strongly.

The solutions were left at normal room temperature for 30 minutes before using them, then the following steps were followed to prepare the antibiotic strip (Comb) according to the modified method(Gould & Bowie, 1952).

Strips in the shape of comb were formed , consisting of four extensions and a diameter of 6 mm from Whatman filter paper No. 3 . Each extension was marked with the required concentration of the antibiotic, prepared in a laboratory, and an abbreviation was written on the comb tape. These strips were sterilized by placing them in a vial. Closed glass and placed in the oven at a temperature of 150 ° C for 60 minutes. Four concentrations (5, 2.5, 1.25 and 0.62) mg / ml of each antibiotic were prepared from the stock solution for each antibiotic. One ml of each of the above-prepared antibiotic concentrations was added to a sterile glass vial. Each vial was labeled with the concentration and shortening of the antibiotic that was placed in it.The prepared, sterilized and labeled paper strips of each extension were immersed in bottles of glass containing the concentrations of Antibiotics, each according to its fixed concentration on the glass bottle and the extension of the paper strips for a period of five minutes, then removed and placed in an oven at a temperature of 30 C. for a period of 24 hours to dry. Then they were placed in bags and kept in the refrigerator at a temperature of 4 ° C until use.

Determination of minimal inhibitory concentration (MIC)

The minimum inhibitory concentration of some antibiotics used towards isolated fungal species was determined according to the broth dilution method described by Balouiri *et al.*,

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(2016) as follows:Two tubes were prepared after the preparation of the fungal inoculation and the stock solutions for antibiotics. Each tube contains 2 ml of SDB medium, one of which contains the medium only to ensure that it is free of contamination and the other to which the fungal inoculation was added only. Both were incubated in suitable conditions to ensure the ability of the medium to cause growth of the studied fungi.
2- 2 ml of stock solution for each of the above-mentioned antibiotics was taken and added 2 ml of SDB medium and mixed well, this lead to the obtaining of a concentration of 5 mg / ml and was given No. 1.

Three to two ml of Solution No. 1 was taken and added to 2 ml of SDB medium and mixed well, to obtain a concentration of 2.5 mg / ml and was given No. 2. Four to two ml was taken again from solution No. 2 and added to 2 ml of SDB medium and mixed well to obtain a concentration of 1.25 mg / ml and was given No. 3 .This method of dilutions was carried out until the concentration of 0.07 mg / ml was reached. Each tube was given a number that indicates dilutions of 1-7 depending on the serial dilution.Each tube was inoculated with 0.05 ml of the fungal vaccine that was prepared with the help of a micropipette, then the inoculated tubes were incubated in suitable conditions depending on the microorganisms in the test (37 ° C for 24-48 hours as for yeasts , 28 ° C for 6-12 days as for molds).
The minimal inhibitor concentration MIC was reported to be the lowest concentration of the drug that prevents the appearance of clear growth of the fungus.

Results and Discussions

Antibioticsensitivity using the comb diffusion method

It has been tested for the isolated fungi especially *T. mentagrophytes , T.rubrum , M. canis , A.niger , and C. albicans*and the targeted common used antibiotics were Erythromycin(ERT) , Azithromycin(AZM) ,Amikacin(AMK),Rifampicin(RIF) , Linezolid(LNZ),Clindamycin(CLD),Streptomycin(STR),Ceftazidime(CAZ),Colistin(CLS),N alidixic acid(NAD ,Cefuroxime(CXM)Ciprofloxacin(CIP)Meropenem(MRP),, Levofloxacin(LEV), Cefepime (CEF) and by using different concentrations for each antibiotic (5 , 2.5 , 1.25 , 0.62) mg./ml. and by using the already laboratory prepared antibiotic strips (comb) . The results were determined by measuring the Zones of Inhibition
The results of this test showed that the abovementioned antibiotics for all prepared concentrations did not show any inhibitory effect against *A. niger* which showed resistance to these antibiotics.
The results showed the effect of the antibiotics used during the study in inhibition of *T. mentagrophytes*using the comb method (Table 1) . It was observed that the ERT antibiotic with a concentration of 5 mg / ml showed the largest diameter of inhibition of *T. mentagrophytes*, as the average diameter of inhibition was 19 mm, whereas the rest of the concentrations (2.5, 1.25 and 0.62)mg / ml did not show any inhibitory effect for the fungus
The antibiotic AZM at a concentration of 5 mg / ml showed a rate of inhibition of fungus of 16 mm, while the rest of the concentrations did not notice an inhibitory effect for the fungus, and the antibiotic MRP at a concentration of 5 mg / ml showed an effect Inhibition of the fungus, with a rate of 18 mm, while the rest of the concentrations had no effect to inhibit the

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growth of the fungus, .Meanwhile, the antibiotic CIP with concentrations (5, 2.5, 1.25 and 0.62 mg / ml) showed an inhibitory effect on *T. mentagrophytes*, as the rates of inhibition diameters reached (18 , 16,14 and 12)mm, respectively, showed a higher concentration of 5 mg / ml which was the highest inhibitory effect of the fungus, while the concentration was 0.62 mg / ml was the least effective. The rest of the antibiotics represented by CEF, LEV, CXM, NAD, CLS, CAZ, STR, CLD, LNZ, RIF and AMK showed no inhibitory effect for *T. mentagrophytes* and for all the prepared concentrations, and the control was treated with distilled water, which did not show any inhibitory effect for *T. mentagrophytes.* The two antibiotics, Erythromycin and Azithromycin, belong to the group of Macrolides, which are antibodies that inhibit protein synthesis in bacteria. They prevent the formation of protein by targeting the bacterial ribosome and binding to the 50S ribosomal subunit (Zin *et al.*, 2020). Meropenem is a broad-spectrum antibiotic from the Carbapenem family of-lactams that has a bactericidal effect by blocking its ability to form a cell wall. One of the anti-lactamases (Ehmann *et al.*, 2017), Ciprofloxacin is a broad-spectrum Fluoroquinolone antibiotic that has a bactericidal effect by targeting the basic enzymes gyrase and topoisomerase, thus inhibiting bacterial DNA synthesis (Conley *et al.*, 2018)

Table (1) : The effect of antibiotics in inhibiting *T.mentagrophytes* fungus using the comb method

Antibiotics	Concentration Used			
	5 mg/ml	2.5 mg/ml	1.25 mg/ml	0.62 mg/ml
Azithromycin	16Ab	0Aa	0Aa	0Aa
Erythromycin	19Bb	0Aa	0Aa	0Aa
Meropenem	18Cb	0Aa	0Aa	0Aa
Ciprofloxacin	18Cd	16Bc	14Bb	12Ba
control	0Aa	0Aa	0Aa	0Da
$LSD_{0.05}$	0.053			

The (Table 2) shows the effect of the antibiotics used during the study in inhibiting *T. rubrum* by using the comb method. The ERT antibiotic showed the largest inhibitory effect compared to the rest of the antibiotics. It showed that the following concentrations (5, 2.5, 1.25 and 0.62) mg / ml. had an inhibitory effect in which the rates of inhibition diameters were (19, 17, 14 and 13) mm. respectively. The concentration of 5 mg / ml had the greatest effect in inhibiting the growth of the fungus, while the least effect was represented by the concentration of 0.62 mg / ml, and the two antibiotics showed MRP and CIP. similar inhibitory effect for concentrations (5, 2.5, 1.25 and 0.62) mg / ml. and rates of inhibitory diameters (18, 16, 14 and 12) mm, respectively, in which the concentration of 5 mg / ml occupied the largest effect in inhibiting the growth of fungus while the concentration was 0.62 mg / Ml of least effect, and two concentrations (5 and 2.5) mg / ml of NAD antibiotic showed an inhibitory effect where the rates of inhibitory diameters were (18, 16) mm. respectively. However, the concentrations (1.25 , 0.62) mg./ml. showed no inhibitory effect. The RIF.antibiotic had shown an inhibitory effect at the concentration of 5 mg / ml, where the

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average diameter of inhibition was 14 mm, . The rest of the concentrations showed no inhibitory effect, while both the antibiotics AZM and CAZ at the concentration of 5 mg / ml showed a rate of inhibition diameter of 12 mm and the rest of the concentrations did not show any an inhibitory effect. The rest of the antibiotics represented by CLS, LNZ, CLD, LEV, CXM, AMK, STR and CEF showed no inhibitory effect for *T. rubrum* and for all the prepared concentrations. The control treatment was represented by distilled water, which did not show any inhibitory effect on the growth of the fungus *T. rubrum*. The two antibiotics, Erythromycin and Azithromycin, inhibit protein synthesis in bacteria, as they prevent protein synthesis by targeting the bacterial ribosome (Zin *et al.*, 2020). Meropenem antibiotic is one of the antibiotics that has the advantage of killing bacteria by blocking its ability to form cell wall (Ehmann *et al.*, 2017). Ciprofloxacin and Nalidixic Acid are broad-spectrum antibiotics that have a bactericidal effect by inhibiting bacterial DNA synthesis (Conley *et al.*, 2018). RNA polymerase of most bacterial genera, as it works to inhibit the process of RNA transcription, with effective fatal activity for bacteria (Rothstein, 2016). Ceftazidime belongs to the class of cephalosporin antagonists and it is one of the antagonists that target the cell wall by inhibiting cell wall biosynthesis Bacterial, which has a bactericidal effect (Shi *et al.*, 2018)

Table (2): The effect of antibiotics in inhibiting *T. rubrum* fungus by using the comb method

Antibiotics	Concentration Used			
	5 mg/ml	2.5 mg/ml	1.25 mg/ml	0.62 mg/ml
Azithromycin	12A	0Aa	0Aa	0Aa
Erythromycin	19Bd	17bc	14Bb	13Ba
Meropenem	18Cd	16Cc	14Bb	12Ca
Nalidixic acid	18Cc	16Cb	0Aa	0Aa
Rifampicin	14Db	0Aa	0Aa	0Aa
Ciprofloxacin	18Cd	16Cc	14Bb	12Ca
Ceftazidime	12Ab	0Aa	0Aa	0Aa
control	0Aa	0Aa	0Aa	0Ea
$LSD_{0.05}$	0.862			

In (Table 3) shows the effect of the antibiotics used during the study in inhibiting *M. canis* fungus by using the comb method. In concentration of (0.62, 1.25, 2.5, 5) and the average diameter of inhibition were (19, 17, 15 and 12 mm), respectively, the MRP antibiotics showed an inhibiting effect to the above mentioned fungus and the concentration of 5 mg / ml had the greatest effect in inhibiting the growth of the fungus, while the least effect was represented by the concentration of 0.62 mg / ml. The rest of the concentrations did not have an inhibitory effect on the fungus. The remaining antibiotics of AZM, CIP, RIF, NAD, LNZ, CAZ, CLD, STR, CLS, CXM, AMK, LEV and CEF showed no inhibitory effect for *M. canis* and for all concentrations. The preparation was treated with distilled water, which showed no growth inhibitory effect for *M. canis.*

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Table (3): The effect of antibiotics in inhibiting *M. canis* by using the comb method

Antibiotics	Concentration Used			
	5 mg/ml	2.5 mg/ml	1.25 mg/ml	0.62 mg/ml
Erythromycin	18Bb	0Aa	0Aa	0Aa
Meropenem	19Cd	17Bc	15Bb	12Ba
control	0Aa	0Aa	0Aa	0Aa
$LSD_{0.05}$	0.022			

In (Table 4) shows the effect of the antibiotics used during the study in inhibiting *C. albicans* fungus by using the comb method. The AZM antibiotic in concentrations of (5, 2.5 and 1.25 mg / ml) showed an inhibitory effect on the growth of yeast. The rates of inhibition diameters reached (17, 11 and 9) mm, respectively, and the concentration of 5 mg / ml had the greatest effect in inhibiting the growth of yeast, while the least effect was represented by the concentration of 1.25 mg / ml, and the concentration of 0.62 mg / ml did not show any inhibitory effect on the growth of yeast . In concentrations of (5 and 2.5) mg / ml, the LNZ antibiotic showed an inhibitory effect on the growth of yeast. The rates of inhibition diameters were (15 and 10) mm, respectively, and the concentrations (1.25 and 0.62) mg / ml did not show any inhibitory effect on the growth of yeast. However, the following antibiotics ERT, MRP, CIP, RIF, NAD, CAZ, CLD, STR, CLS, CXM, AMK, LEV and CEF, haven't shown any inhibitory effect on the growth of *C. albicans*and for all the prepared concentrations, and the control was treated with distilled water, which did not show any inhibitory effect on the growth of *C. albicans* yeast. Linezolid is an oxazolidinones antibiotic that inhibits bacterial protein synthesis by targeting the bacterial ribosome and binding to the 50S ribosomal subunit (Hashemian *et al.*, 2018)

Table (4) :The effect of antibiotics in inhibiting *C. albicans* by using the comb method:

Antibiotics	Concentration Used			
	5 mg/ml	2.5 mg/ml	1.25 mg/ml	0.62 mg/ml
Azithromycin	17Ad	11Ac	9Ab	0Aa
Linezolid	15Cc	10Cb	0Ba	0Aa
control	0Aa	0Ba	0Ba	0Ba
$LSD_{0.05}$	0.027			

The study showed that *A. niger* fungus has resisted all kinds of antibiotics in all concentrations. This is due to the fact that this fungus is characterized by its high resistance to difficult conditions of dryness, salinity and extreme pH not to mention antibiotics. It was observed that this fungus has a tremendous enzymatic capacity as it can destroy and convert many pesticides and antibiotics to a food substance that benefits from it in its metabolic processes. The cell wall, also, prevents any entrance of toxic substances into the cell and can change its genetic makeup through genetic mutations (Howard & Arendrup, 2011)
The inhibitory role of antibiotics towards bacteria is known; some of them work to confuse the process of building the cell wall, others work on the cell membrane and some work on

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building protein, but their role in inhibiting fungal growth is shrouded in a lot of ambiguity as there are few researches and studies dealing with this topic . To study the effect of the Macrolides , (Karpiński, 2019) studied the effects of 74 compounds all of which belong to the Macrolides and see their effect on a number of fungi namely *A. fumigatus , C. albicans , T. rubrum , T. mentagrophytes , Neurosporacrassa , Penicilliumnotatum ,* and*Fusarium*sp. And other kinds of fungi . He found out that 29 of these compounds affect the fungi and inhibit their growth, due to their direct effect on the work of the enzymes responsible for protein metabolism by creating an increase or deficiency in minerals and salts necessary for the activity of the enzyme, which is the same reason that makes Macrolides have side effects on humans. The manufacturers attach in the leaflet accompanying the drug that it affects digestive enzymes and affects the electrocardiogram.

As for the β-lactam antibiotic, including Meropenem, their effect on fungi is by preventing the formation of proteins essential for adhesion to the host, which is the first essential step in causing disease. They also inhibit and prevent the formation of the essential membrane which is regarded a vital shelter and a vigorous factor to many diseases caused by the fungi (Sidrim *et al.*, 2015).

With regard to the anti-Ciprofloxacin, studies have shown a high efficiency in resistance to both *A. fumigatus* and *C. albicans*, especially when mixed with antifungals, as the antifungal is easily introduced into the fungal cell, while Ciprofloxacin inhibits the action of enzymes necessary for cell division(Stergiopoulou *et al.*, 2009)

(Sobieski *et al.*, 1976) pointed out that the antifungal drug Nalidixic acid inhibits the growth of fungi, and this inhibition is increased with the increase of the concentration in the food medium, and the effectiveness of the drug increases whenever the temperature is within the limits of (25-37) C. This antifungal works to deplete the nitrogen in the fungal cell. This has been observed in *C. albicans* yeast and *S. cerevisiae*, thus depriving them of the process of building nucleic acids, especially mitochondrial DNA, which makes this antifungal toxic to fungi.

In the same respect, the anti-Rifampicin affects fungi, including *Aspergillus*, *H.capsulatum* and yeasts, and its effect increases when mixed with fluconazole as it helps to penetrate the cell wall, while it works on nucleic acids by preventing the process of replication ((Medoff, 1983)

Ceftazidime, from another hand, is one of the antibiotics that targets the cell wall by inhibiting the biosynthesis of the bacterial cell wall, but in fungi it affects by inhibiting the proteins that work on the fungal cell attachment to the host as well as preventing the formation of biofilms (Sidrim *et al.*, 2015)

As for the anti-Linezolid, it is known that its action is made by blocking the building of protein in the cells, but it must first penetrate the outer wall of the fungal cell. This antibiotic succeeded in inhibiting the growth of both *Candida albicans* and the fungus *Pythium* after being mixed with Amphotericin B and also inhibited biofilm formation (Lu *et al.*, 2019)

Generally speaking, the wall of the fungal cell is the first obstacle that prevents the antibiotic from reaching the inside of the cell and therefore most of the fungi, as is evident from the above table, were not affected by the antibiotics. In addition , the physiological nature of the fungal cell as it is an uninuclear that contain a complex genome that can adapt and make

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mutations that enable the fungi to overcome by pesticides, antibiotics and other anti-fungal substances.

The Determination of the minimum inhibitory concentration of the antibiotic used against the isolated fungi under study

The results in (Table 5) indicate the determination of the Minimum Inhibitory Concentrations (MIC) values of some antibiotics used against the isolated fungal under study. We note that the isolated fungal showed varying sensitivity towards antibiotics. The tested isolates, as the effect of Azithromycin was clear in the fungal isolates *T. mentagrophytes, T. rubrum* and *C.albicans*, with the minimum inhibitory concentration value of 0.31 mg / ml, while the Erythromycin antibiotic showed its clear effect on *T. mentagrophytes* and *T. rubrum* isolates. And *M. canis* where the value of the minimum inhibitory concentration was 0.15 mg / ml for *T. mentagrophytes*, while the value of the minimum inhibitory concentration was 0.31 mg / ml for *T. rubrum* and *M. canis*. The Nalidixic acid antibiotic showed a clear effect on *T. rubrum*. The value was The lowest inhibitory concentration was 0.15 mg / ml, whereas the Ciprofloxacin antibiotic was effective on *T. mentagrophytes* and *T. rubrum*. The minimum inhibitory concentration was 1.25 mg / ml.

Table (5) The values of the minimum inhibitory concentration of some antibiotics towards the isolated fungi under study (mg / ml)

Antibiotics	Species of Fungi			
	T.mentagrophytes	***T. rubrum***	***M. canis***	***C. albicans***
Azithromycin	0.31	0.31		0.31
Erythromycin	0.15	0.31	0.31	
Nalidixic acid		0.15		
Ciprofloxacin	1.25	1.25		

The determining of the lowest inhibitory concentration method is regarded to be one of the best approved methods for evaluating the effectiveness of antibiotics, as well as plant extracts against microorganisms. It also has a great economic importance since it guides the specialists to the best and lowest concentration at which the growth of fungi stops and then the picture becomes clear to the specialists who will no more use meaningless and useless high concentrations . On the contrary, it costs them additional sums and this is essential in the pharmaceutical industry. Not only that, but the use of high concentrations causes the emergence of antibiotic-resistant isolates and puts the pharmaceutical industry in a permanent dilemma as they have to develop medicines in line as the resistance emerges(Rangseekaew & Pathom-aree, 2019)

One of the things that must be taken into consideration is that each drug has side effects such as headache, diarrhea, fever, high blood pressure, etc.Worthy to mention that whenever the drug concentration becomes higher in the body, its effects become more dangerous. Thus, it is vital to determine the minimal concentration that can be safely used and that gives perfect inhibit to the pathogen (Ribeiro da Cunha *et al.*, 2019)

The results of our study are promising results and open the way for this type of studies, especially after the laboratories are looking for multifunctional drugs . Both Azithromycin and Erythromycin were distinguished in inhibiting fungi. This may be due to its ability to penetrate the fungal cell wall. And when enter inside the cell, this kind of drug will affects the process of protein synthesis inside the cell(Karpinski, 2019)

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