

How to Cite:

Khudhair, I. H., & Daeikh, A. K. (2022). Study of the effect of ethanol alcohols on yeasts and fungi isolated from the nail. *International Journal of Health Sciences*, 6(S3), 6902–6910. <https://doi.org/10.53730/ijhs.v6nS3.7590>

Study of the effect of ethanol alcohols on yeasts and fungi isolated from the nail

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Abstract---The excessive use of alcoholic such as ethanol at a 75%, which are locally by of companies, it result by using of materials as sterilizing in order to eliminate of microorganisms, as is the case result of the outbreak such Corona virus, has led to in resistance by some of microorganisms and fungi, and the resistant strains, especially skin infections or nail, as the current study, which included *C. albicans*, *C. glabrata*, *C. krusei*, *Rhodotorula*, and *A. flavus* showed the high resistance by fungi to the alcohols, surgical, joonand jood, which The reason for this may be due to the resistant fungi by use of alcohol and those excessively patients.

Keywords---alcohols , ETOH, candida.

Introduction

Fungi are a major source of infections and injuries which part of the natural nail normal flora [1]. The drug can be complicated by these yeasts into opportunistic organisms that infect the internal parts of the body due to the emergence of high resistance to many alcoholic substances or antibiotics [1-3]. Also, the infection of medical tools with candida and other fungi can occur despite treatment, resulting in infaction skin or nail [2]. Also, the use of treatment related to chronic diseases or the frequent use of sterilizers is one of the main reasons for the emergence of resistance to yeasts and fungi, which may lead to the emergence of dense communities. Hypothalamus and yeast cells are characterized by increased resistance to immune responses to certain hosts and Antifungal therapy [5-7].

Biofilms of of fungi are 1000 times resistant to the drugs in the azole group [8], or resistance may be up to 20 times than that of echinocandin Sensitivity [9-11]. The resistance within the multifactorial membranes also increases As the treatment;

In the early stages of thin-film formation [12], and during later stages, the emergence of cell membrane resistance to yeasts may prevent antifungal drugs from reaching primary cells [13]. In albicans resistance may also be due to DNA mutations in drug targets, or occur in formation membrane sterol, metabolic pathways [15-16] or reduction In the metabolic that cell exhibits and is inherent by the cells of the biofilms [17]. . Due to the limited existence of a group of Nutrients and oxygen in this environment, which result in the delivery of antibiotic dependent on survival inactive cell. Thus, related infections to yeasts that cause the resistance to antibiotics, including those caused by It is often difficult to treat Candida spp, that cause inhpition in the nail with nail injuries. In many studies a number of drug tested in vitro as antimicrobials technique.

The chessboard test is designed for testing Multidrug activity In addition to testing the resistance efficacy of Candida yeasts and a number of fungi against selected group of ethanol alcohols combined a successful approach to identify in Efficacy of the in vitro preparation, including strains of Candida resistant to substances [18-19]. The technique of chessboard show different combinations of fungi activity illustrating, which is increase in action antimicrobial caused combination of alcohol than the activity of each individual antimicrobial [20]. These tests also include some of the tested drug combinations that have shown activity with finasteride or alcohol [21-22], and others. However, these showed the Drawbacks of antimicrobial agents, resistance of fungi and yeast to of antibiotics and enzymes (fluconazole has been documented by biofilms). [23-24] Also, the concentrations of ethanol coused most of the highly resistant to it [25]. the major source of nail infections or bloody is Candida albicans [26]. The complicated can be treatment by high resistance or by produced the spores of fungi, which is cause the reproduction colonies of fungal. Finger and nail injuries [1-4]. Continuous infection of different areas of the body is also caused by Candida spp it can occur despite treatment, leading to recurrent infections in most cases [2]. Candida biofilms are 1000 times more resistant to alcohol than they are to treatments Azole treatment [8] and resistance up to 20 times greater than that of echinocandin allergy, which is in the form of plankton [9-11]. . The increase in resistance within biofilms is also multifactorial in terms of treatment; In the early stages of the formation of biofilms of cells [12].

Materials and Methods

Sterilization methods

Wet heat sterilization methods

It is used for the purpose of sterilizing solutions, dyes and food media with an oxidizing agent at 121°C and a pressure of 15 psi² for 15 minutes dissertations. [26].

Collecting samples

About 15 samples of nails were collected from healthy people with skin ulcers, in addition to fungal infections that accompany the nail, by removing the outer part of the nail and taking part of the infected nail. The samples were kept in sterile plastic tubes until use.

Preparation of culture media

Cultural media preparation The media used in the study was prepared according to the instructions of the company producing the medium and as described in [26]. Hinton agar medium and sapiroide agar medium were prepared for the cultivation of pathogenic fungi, according to the instructions of the manufacturers mentioned on the culture media. These media were used for the purpose of culturing samples on them and isolating pathogenic fungi from them.

Chromium medium (Candida agar)

Use this medium for the purpose of detecting Candida species and according to the colors shown on the medium above [27]. Tobacco agar, this medium was prepared by dissolving the powder medium of about 47 g in 100 ml of distilled water, letting it boil in a water bath, and pouring the medium into Petri dishes until use . [28].

Define isolates

After culturing nail samples on culture media and the emergence of different types of pathogenic fungi and yeasts, pathogenic yeasts were isolated and diagnosed, which included Candida and Rhodoella yeasts. These fungi were diagnosed by the following methods:

- Approval of the outward appearance The phenotypic diagnosis is based on the colour, texture, and height of colonies developing on SDA medium [29] .
- Microscopic diagnosis: Microscopic diagnosis was adopted for the purpose of identifying fungal species and genera, according to the taxonomic keys for the diagnosis of fungi.
- Chromagar Medium Use Chromagar Diagnostic Medium The samples were planted on the mentioned medium and left in the incubator at a temperature of 37°C. The colors of the developing colonies on the medium were monitored, for the purpose of distinguishing yeast species compared to the standard [30] .

Sensitivities to alcohols

The experiment was carried out using a specific method as in a previously published study [35] using Micro-dilution method according to CLSI M27- A 2. Alcohols with a ratio of XTT/menadione (12.5:1) were newly prepared before each assay , 50 ml of Alcohols were added to each well with observation of control wells. The plates were covered and incubated at 35°C for 24 hours. To specify OD, All experiments were performed in triplicate for each type of alcohol under the result was compared with the standard diameter of the inhibition zones for each alcohol.

Result and Discussion

Approximately 4 types of Candida yeast and several types of dermatophytes were isolated from 15 clinical samples during the period from November 2021 to

December 2021. These types were identified by microscopic diagnosis in addition to the phenotypic diagnosis of isolated fungi and yeasts. As shown in Table (1)

Table 1
Percentage of yeast and fungi species identified according to the morphology

species	number of isolates	Percent	Sample isolates
<i>Candida albicans</i>	2	25	Nail
<i>Candida glabrata</i>	1	12	Nail
<i>Candida krusei</i>	1	12	Nail
<i>Candida tropicalis</i>	1	12	Nail
<i>Rhodotorula</i>	1	12	Nail
<i>Aspergillus flavus</i>	2	25	Nail
Total	8		

Calculations of partial inhibitory concentrations

Two-dimensional checkers. The partial inhibitory concentrations (FICs) were calculated for each a group of alcohols that included: EtOH .surgical, joon and joon . The FIC value has been calculated divide by MIC90 or MIC50 (Minimum Inhibitory Concentration for each drug produces 10 or 50% of the metabolic activity, respectively) from one alcohol or when used by MIC90 or MIC50 of the same type when used alone, and the results found that all alcohols that were used by diffusion method were significantly and clearly resisted by the bleaches that were isolated from the nails of a group of people with nail injuries. It was also noted that the fungus Rodotella also showed a clear resistance to the group of alcohols that were used in the study as in table 2 .

Table 2
The types of Alcohols used and the type of resistance shown by yeasts and fungi

No	Isolates	Alcohols				resistance type
		EtOH	surgical	joon	jood	
1	<i>Candida albicans</i>	+	+	+	+	resistance
2	<i>Candida glabrata</i>	+	+	+	+	resistance
3	<i>Candida krusei</i>	+	+	+	+	resistance
4	<i>Candida tropicalis</i>	+	+	+	+	resistance
5	<i>Rhodotorula</i>	+	+	+	+	resistance

Perhaps this resistance shown by the yeasts and fungi under study may be due to the increased resistance of those yeasts to alcohol and alcohol sterilizers used only recently as a result of the increase in the spread of the Corona virus, and as a result of this pandemic, the use of alcohol has increased as a usual method for the purpose of eliminating viral infection, but excessive use Alcohols led to an increase in resistance to most fungi and yeasts, which made them turn from

coexisting parasites to parasitic or resistant to antibiotics and alcohols, causing skin infections and other injuries to humans.

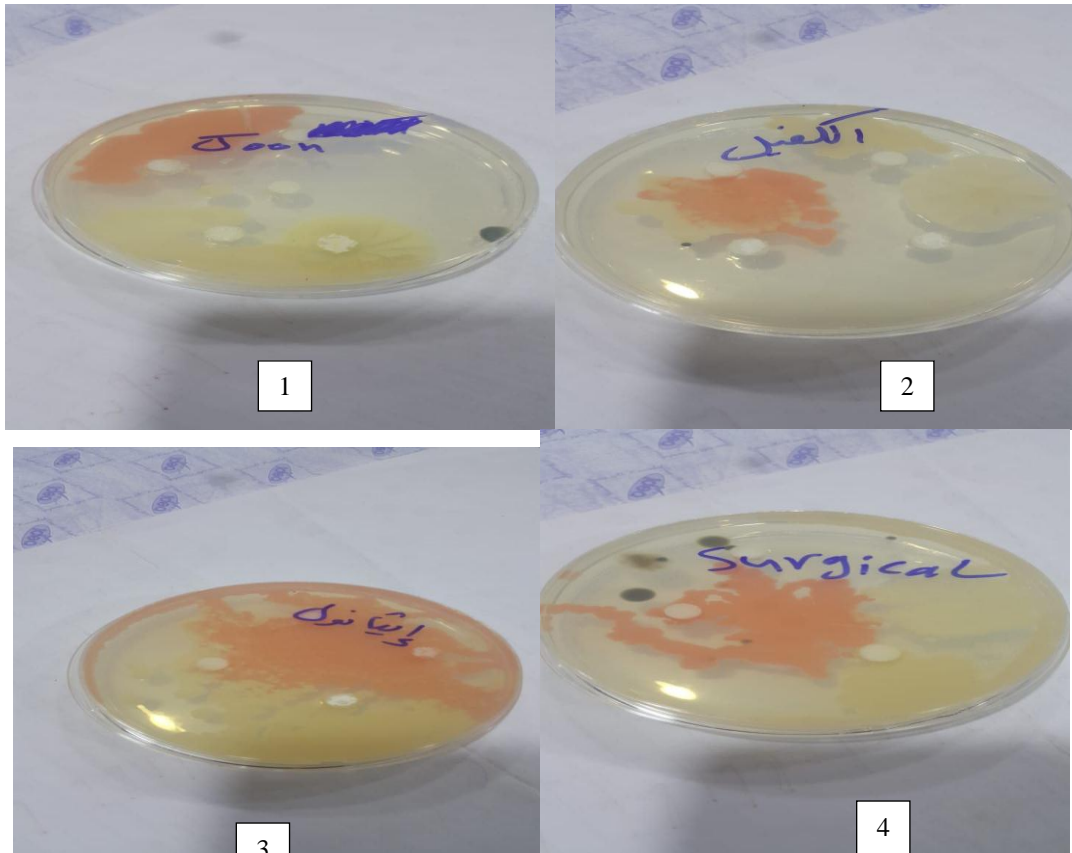


Figure 1. 1, 2, 3, 4 shows the resistance of *Candida* and *Rodotella* to the four disinfectants used in this study

Metabolic activity of improved alcohol solutions against multiple strains of *C. albicans*

The alcoholic solutions selected from the previous three-dimensional chessboard tests were additionally tested against all four alcoholic solutions, which showed high resistance to it by the *Candida* yeasts to which their species had been previously identified. sterilizers), 20% EtOH alone was the most resistant by *Candida* to it, as it was found that yeasts are able to form vital membranes for them, as well as divide and multiply in a large and vital way, which resulted in a metabolic activity of 1% for prevention and <5% of metabolic activity. 10% EtOH alone is also highly effective but has been shown to be highly resistant to *Candida* (Figur 2).

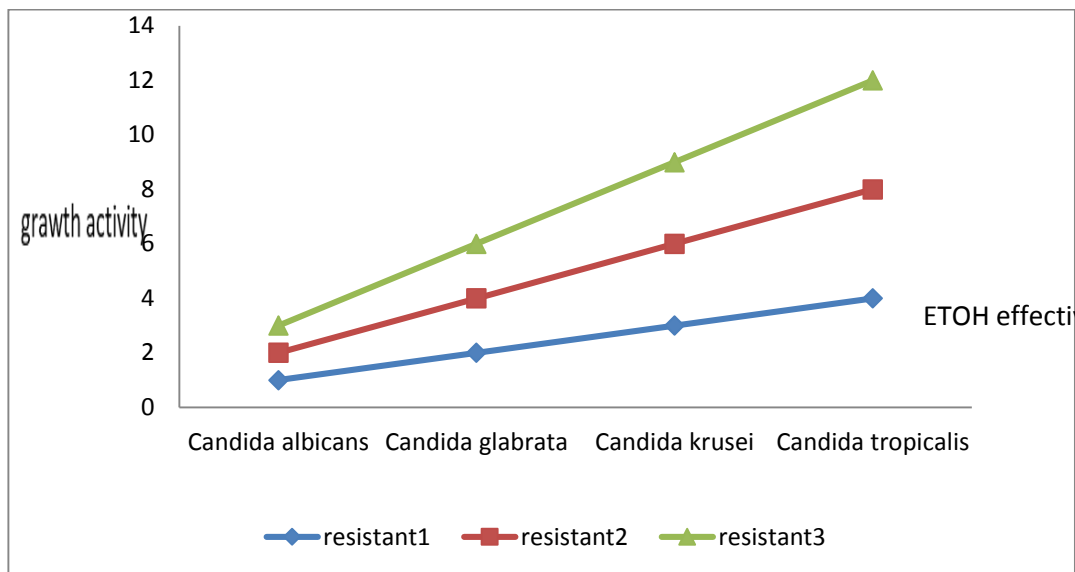


figure 2. Resistance shown by *candida* spp

Discussion

In this study, we show the extent of the importance of reducing and excessive use of chemicals and alcohols that are used in an excessive manner in sterilization in order to save or prevent the pandemic that has spread widely in recent times, which was represented by Covid 19, which was characterized in the past by being a very effective compound Against microorganisms, including viruses, which prevents bleach white and mature biofilms are formed especially when using 70% EtOH, as this study showed that the excessive use of alcohols as a protective material against infection with viruses poses a great risk in showing resistance by yeasts and some other fungal species Each type of alcohol was selected according to the type most commonly used in the local market. It was found that yeasts and fungi have shown a high resistance to these types of alcohols, which may be due to the emergence of new strains of these types showing resistance to alcohol as a result of excessive use of alcohol as a type of prevention from Virus infection, which corresponds to Previous studies in which measure the susceptibility of clinical isolates that showed high resistance to alcohols, a number of other studies have reported a high resistance to alcohol, as indicated by researchers [31].

Which used AfLT and with different concentrations of alcohols and antigens for the purpose of knowing their effect on the different fungi under study, and those fungal species showed high resistance to the alcohols used. The researcher also indicated [40] that there is a high resistance shown by the fungus to alcohol ethanol, which was used in a concentration, it showed 5 $\mu\text{g/ml}$ in an in vitro lock model. The researcher [32] also indicated the emergence of high resistance by fungi to different types of alcohols antifungals against *Candida* group. However, the powerful fungicides showed MICA activity, especially when used with EtOH, does not guarantee a larger dose. EtOH and DOX concentrations were determined for 3D checkerboard assays based on the activity of one stable drug against *Clostridium albicans* and this is in agreement with what the researchers said [42]

and the two-dimensional chess board that was used in which the yeasts showed high resistance to alcohol, in which they indicated that the reason for the emergence of this resistance is due to the presence of new strains of *Candida* yeasts that showed high resistance to alcohols and sterilizers, as well as some types of anti-fungals.

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