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STUDY OF THE EFFECT OF ETHANOL ALCOHOLS ON YEASTS AND FUNGI ISOLATED FROM THE NAIL

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Abstract: The right to use alcoholic materials excessively, such as 75% ethanol concentration, which is produced locally by several companies, and the use of these materials as sterilizing agents to get rid of certain kinds of viruses or microorganisms, as happened recently due to the Corona virus outbreak, have resulted in an increase in resistance. This study, which involved bleaching a number of yeast species, including Candida albicans, Candida glabrata, Candida krusei, Candida tropicalis, Rhodotorula, and Aspergillus flavus, revealed the emergence of high resistance by those yeasts and fungi to the alcohols used EtOH, surgical, joonandjood, which was caused by certain types of microorganisms, including fungi and yeasts, and the emergence of resistant strains, particularly those that accompanied skin infections or nail injuries. This might be the result of patients' overuse of alcohol and the resistant strains that followed it.

Keywords: Alcohols, ETOH, Candida.

Introduction

Commonly, fungal infections and lesions are caused by fungi and yeasts such as Candida,

which are naturally found in the nail bed [1]. Due to their strong resistance to various medications used to treat alcohol-related conditions and antibiotics, these yeasts have the ability to become opportunistic organisms that can infect internal body parts, thereby making the treatment process more complicated [1-3]. Recurrent nail or skin infections can occur due to the continuous presence of candida and other yeast infections on medical equipment, which may not always have an effective treatment available [2]. Regular use of treatments associated with chronic diseases or sterilizers is a major cause of the development of resistance to yeasts and fungi, which can result in the growth of dense communities. Antifungal drugs and immune responses targeting specific hosts are ineffective in yeast cells and the hypothalamus [5-7].Candida biofilms, along with other fungi, exhibit a resistance to the standard treatment that is 1000 times greater. Azole-group medications have the potential to be 20 times more resistant than echinocandin therapy. The planktonic condition is known to be sensitive [9-11]. Moreover, the resistance of the multiple membranes is enhanced. Antifungal medications may be ineffective in penetrating primary cells during the initial phases of thin-film development [12], as well as in later stages due to the development of cell membrane resistance by yeasts [13]. The alterations in the DNA of drug targets may be the fundamental reason for the resistance of albicans. Alternatively Alterations arise in the production of metabolic rescue pathways, membrane sterols, or a reduction in quantity. The metabolic activity displayed by each cell is inherent to the inner biofilm cells [17]. Due to the scarcity of specific nutrients and oxygen in the environment, the effectiveness of antibiotics that depend on cell viability for delivery may be compromised. Treating infections caused by Candida species, particularly those that result in antifungal resistance, can be challenging. This is especially true for Candida species that cause nail infections in most patients with nail damage. Several drug combinations were evaluated as antimicrobials in vitro in previous studies. Strategies for playing chess on the board. The checkerboard test is employed to evaluate the utilization of multiple drugs. Assessing the resistance of Candida strains to these substances, as well as evaluating the ability of various fungi and Candida yeasts to resist a specific group of ethanol alcohols, proved to be an effective method for determining the effectiveness of the in vitro preparation [18–19].By employing the checkerboard method, numerous permutations of yeast activity were discovered, effectively demonstrating this phenomenon. Synergistic activity refers to the enhanced antibacterial action resulting from the combination of alcohol or drugs, where the combined effect is greater than the sum of their individual effects [20]. These studies also include various pharmaceutical combinations that have been researched and demonstrated to have synergistic effects, such as the combination of fluconazole with finasteride or ethyl alcohol [21–22]. Nevertheless, these studies demonstrated that certain antifungal medications exhibited adverse effects. Yeast and fungi biofilms offer compelling proof of their ability to resist a group of antibiotics and enzymes, such as fluconazole [23-24]. Furthermore, the study demonstrated that the majority of the donkeys exhibited a high level of resistance to the elevated ethanol doses [25]. Candida albicans is a common cause of bloody nail infections in various countries [26].The treatment process may be hindered by the presence of high resistance or by the spores generated by yeasts and fungi, which specifically promote the formation of fungal colonies.
Damage to the nails and fingers [1-4]. Despite undergoing treatment, Candida spp. can still cause persistent infections in various parts of the body, often resulting in recurring infections [2]. Candida biofilms have been shown to be 1000 times more resistant to alcohol than to the effects of Azole treatment [8] and to be up to 20 times more resistant to echinocandin allergies than plankton [9–11]. Additionally, there is a significant and varied increase in the resistance to treatment within the biofilms at the early stages of cell formation [12].

Methods

1- Methods of sterilization

Moist heat sterilization methods The process involves subjecting solutions, dyes, and food media to an oxidizing agent at a temperature of 121°C and a pressure of 15 psi2 for a duration of 15 minutes in order to sterilize them. The user's input is the string "[26]".

2- Collecting specimens: We collected a total of 15 nail samples from individuals who had skin ulcers and simultaneous fungal infections. The samples were obtained by extracting the outer layer of the nail and a section of the infected nail. The samples were kept in sterile plastic tubes until they were used.

Step 3: Preparation of cultivation media. Cultural media preparation involves the systematic and strategic preparation for the creation and distribution of media content that is centered around cultural themes and topics. The media used in the study was prepared following the instructions provided by the manufacturer and as described in reference [26]. The Hinton agar medium and sapiroide agar medium were prepared according to the instructions provided by the manufacturers of the culture media for the purpose of cultivating pathogenic fungi. The media were employed to cultivate samples and isolate pathogenic fungi from them.

The Candida agar, also known as the Chromium medium, is used to identify different species of Candida based on the color changes that occur on the medium [27]. The tobacco agar medium was prepared by dissolving approximately 47 grams of powdered medium in 100 milliliters of distilled water. Subsequently, the mixture was heated in a water bath until it reached its boiling point, and then it was poured into Petri dishes for future utilization. The number 28 is surrounded by square brackets.

Isolates i refer to pathogenic yeasts that were obtained and diagnosed from nail samples after being cultured on culture media. The isolates consist of Candida and Rhodoella yeasts. The fungi were identified through the application of the following diagnostic techniques:

A- Authorization of the outward appearance The phenotypic diagnosis is established by observing the color, texture, and height of colonies that develop on SDA medium [29].

B- Microscopic examination: Microscopic diagnosis was utilized to identify precise fungal species and genera, utilizing taxonomic keys specifically designed for fungal diagnosis.

Utilize Chromagar Diagnostic Medium for C- Chromagar Medium. The samples were positioned on the designated substrate and subjected to incubation at a temperature of 37°C. Observations were made on the colors of the developing colonies on the medium to distinguish between various yeast species and the standard [30].

D- Alcohol sensitivities: The experiment was carried out utilizing a particular methodology detailed in a previously published investigation [35], adhering to the Micro-dilution method as specified in the CLSI M27-A 2 guidelines. Prior to each analysis, a new set of alcohols was prepared with an XTT/menadione ratio of 12.5:1. Subsequently, 50 ml of the aforementioned alcohols were introduced into every well, including the control wells. The plates were hermetically sealed and positioned in an incubator programmed to maintain a temperature of 35°C for a period of 24 hours. To ascertain the optical density (OD), the experiments were carried out three times for each type of alcohol, and the obtained measurements were then compared to the standard diameter of the inhibition zones for each alcohol.

Results and Discussion

A total of 15 clinical samples collected between November 2021 and December 2021 yielded approximately 4 different types of Candida yeast and multiple types of dermatophytes upon isolation. The identification of these types was done through microscopic diagnosis, in addition to the phenotypic diagnosis of isolated fungi and yeasts. As indicated in Table 1

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

Species	number of isolates	Percent	Sample isolates
Candida albicans	2	25	Nail
Candida glabrata	1	12	Nail
Candida krusei	1	12	Nail
Candida tropicalis	1	12	Nail
Rhodotorula	1	12	Nail
Aspergillus flavus	2	25	Nail
Total	8		

Calculations of partial inhibitory concentrations:

A game of checkers played on a flat surface. The fractional inhibitory concentrations (FICs) of the following alcohol groups have been determined: EtOH, surgical, joon, and joon. When a single alcohol is utilized, the FIC value is divided by the MIC90 or MIC50 (Minimum Inhibitory Concentration) of that alcohol. The MIC90 represents the concentration at which the drug inhibits 10% of the metabolic activity, while the MIC50 represents the concentration at which the drug inhibits 50% of the metabolic activity. Alternatively, when used in isolation, the FIC value is divided by the MIC90 or MIC50 of the corresponding alcohol variant. The results suggest that the bleaches derived from the nails of individuals with nail injuries exhibited robust and unambiguous resistance to all alcohols examined through the diffusion method. In addition, it was observed that the fungus Rodotella displayed a distinct immunity to the particular group of alcohols used in the research, as shown 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	Candida albicans	+	+	+	+	resistance
2	Candida glabrata	+	+	+	+	resistance
3	Candida krusei	+	+	+	+	resistance
4	Candida tropicalis	+	+	+	+	resistance
5	Rhodotorula	+	+	+	+	resistance

Given the current rapid transmission of the Corona virus, it is reasonable to suggest that the observed resistance displayed by the yeasts and fungi under investigation is a result of their increased tolerance to alcohol and alcohol-based sterilizers. As a result of the pandemic, there has been an increase in alcohol consumption as a popular method to combat viral infections. Excessive consumption of alcohol has been found to cause a notable rise in the resistance of most fungi and yeasts. This leads to their transition from being coexisting parasites to becoming parasitic organisms that are resistant to both alcohol and antibiotics. As a result, these organisms led to skin infections and other harmful impacts on human health.

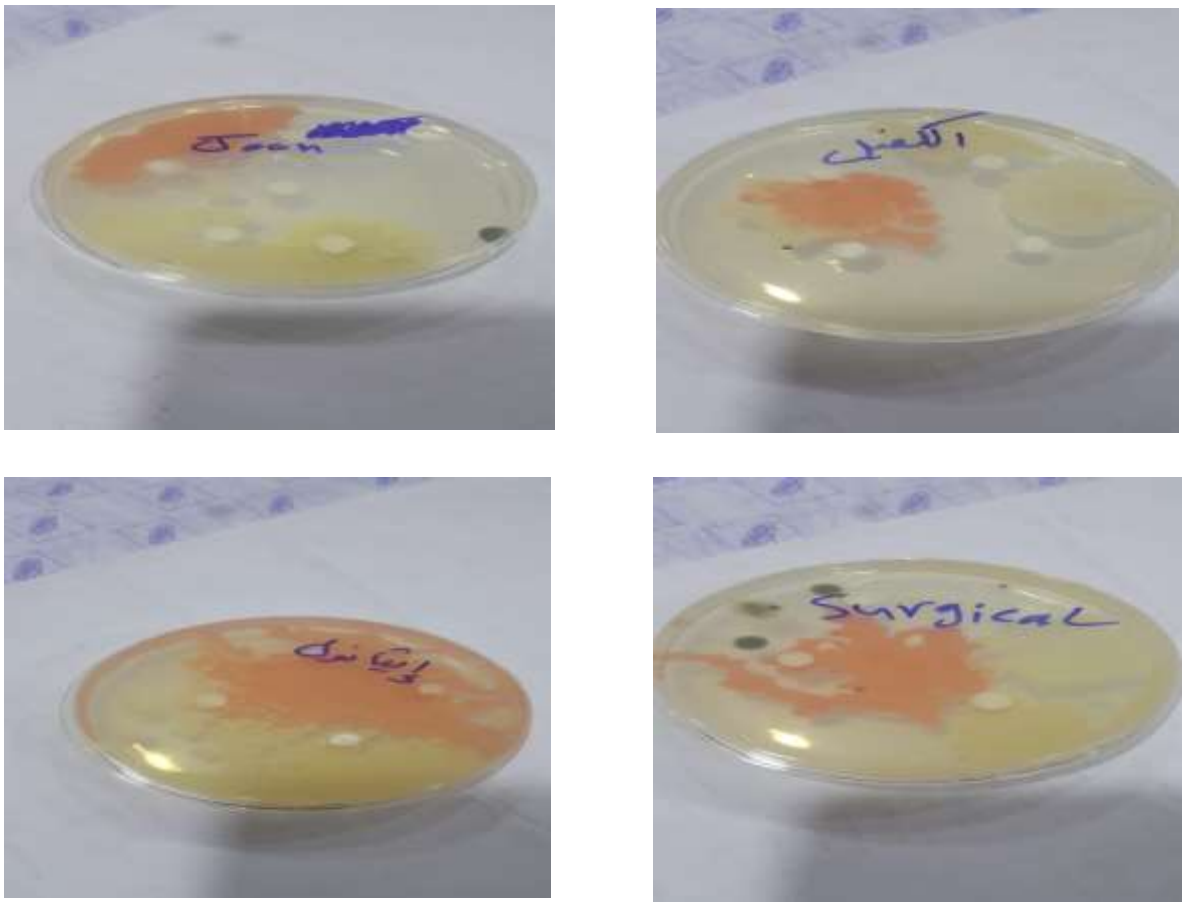
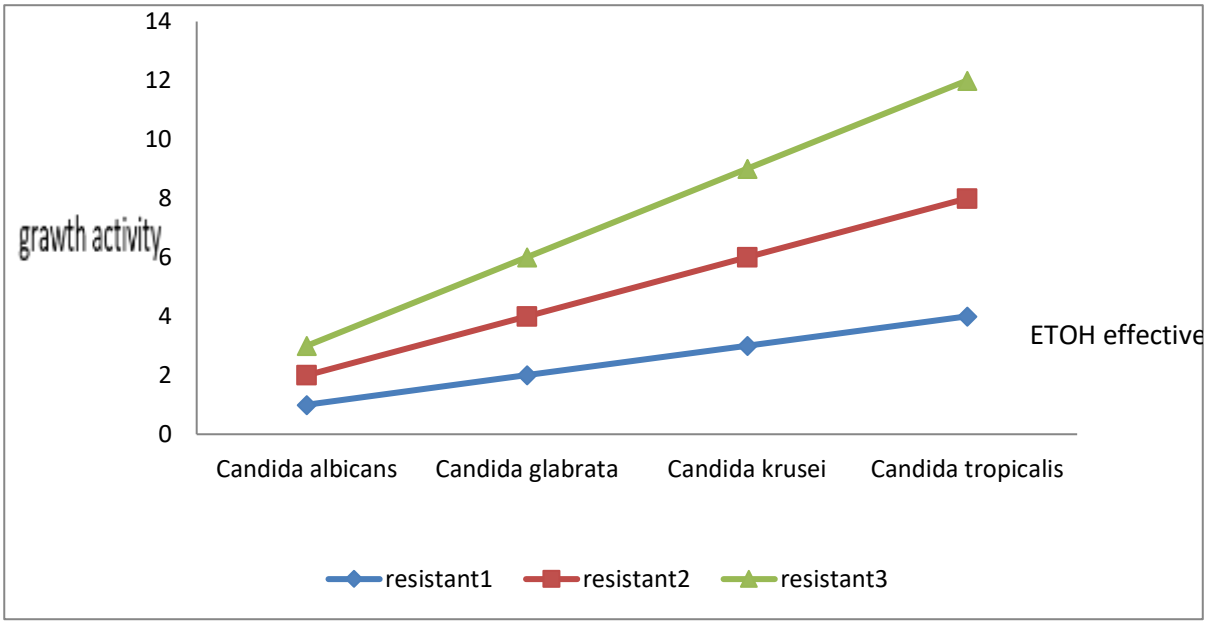


Figure (1 1, 2, 3, 4) shows the resistance of Candida and Rodotella to the four disinfectants used in this study.

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

The alcoholic solutions that the Candida yeasts, previously identified as highly resistant, were tested against the selected alcoholic solutions from the earlier three-dimensional checkerboard testing. Among the sterilizers tested, Candida showed the greatest resistance to 20% EtOH alone. This is due to the yeast's ability to synthesize vital membranes and undergo rapid and extensive proliferation. The final outcome yielded a metabolic activity of less than 5% for metabolic activity and 1% for prevention. While Candida has demonstrated resistance, it is worth noting that 10% ethanol alone is also highly effective (Figure 2).



FIGUR (2) resistance shown by *candida* spp.

This study highlights the importance of minimizing the use of alcohols and chemicals in sterilization practices to prevent or mitigate the current pandemic, as illustrated by the rapid transmission of COVID-19, a previously potent pathogen. This study has shown that the excessive use of alcohols as a protective material against viral infections significantly raises the likelihood of yeasts and other fungal species developing resistance. Additionally, it hinders the formation of mature biofilms and prevents the bleaching effect when 70% EtOH is utilized. Each type of alcohol was selected with consideration for the local market. The identification of yeasts and fungi exhibiting a significant degree of resistance to these alcohols suggests the emergence of novel strains of these microorganisms that have developed the ability to withstand alcohol as a protective mechanism against viral infections. This phenomenon aligns with Subsequent studies have confirmed that clinical isolates with a significant resistance to alcohols are also highly susceptible to alcohol, as previously documented by researchers [31]. The study utilized AfLT (Active fungal load testing) and tested different concentrations of alcohols and antigens to investigate their effects on various fungal species. The results showed that these fungal species displayed significant resistance to the alcohols that were tested. Furthermore, the researcher noted that the fungus displayed significant resistance to the alcohol ethanol, as demonstrated in an in vitro lock model at a concentration of 5 Î1⁄4g/ml. According to the researcher [32], fungi have developed a substantial degree of resistance to numerous alcohol antifungals commonly used to treat the Candida group. Although the potent fungicides showed MICA action, especially when combined with EtOH, this does not imply that a higher dosage is required. The concentrations of ethanol (EtOH) and doxorubicin (DOX) were determined in 3D checkerboard assays by monitoring the activity of a single stable drug against Clostridium albicans. This is consistent with the conclusions of the researchers [32] and the outcomes of the study on a two-dimensional chessboard, where the yeasts exhibited a significant degree of alcohol resistance. The researchers postulated that the increase in resistance is attributed to the emergence of novel strains of Candida yeasts that exhibit resistance to alcohols, sterilizers, and specific anti-fungal agents.

Conclusion

In this study concluded that certain types of microorganisms, including fungi and yeasts, and the emergence of resistant strains, particularly those that accompanied skin infections or nail injuries. This might be the result of patients' overuse of alcohol and the resistant strains that followed it.

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