

Article

Identification of Fungi on Mobile Phone Surfaces and Evaluation of the Effectiveness of Antibiotics Against Them

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Abstract: Mobile phones are a means by which many pathogens, including fungi, are transmitted. Therefore, this research examined the insulation and recognition of fungi isolated from mobile phones used by university professors and staff, as well as doctors, pharmacists, taxi drivers, and restaurant workers. A total of 80 samples were collected from 2024-12-1, to 2025-2-1. The study addressed the virulence factors of *Aspergillus chevalieri*, one of the fungi isolated from mobile phones, which include the capacity to adhere, the capacity to make blood disintegrate, and the synthesis of proteolytic enzymes, lipids, and proteases. Drug sensitivity of *A. chevalieri* to antifungals was tested. The study's findings demonstrated the existence of fungi upon mobile phones, as fungus *Aspergillus* spp was prevalent in all types of phones, and the species appeared in *A. fumigatus* 17 at a rate of (17%), as the highest rate was recorded, followed by the fungus *A. niger* at a rate of (13%), the fungus *A. candidus* at a rate of (12%), the fungus *A. chevalieri* and *A. flavus* at a rate of (11%), the fungus *A. terreus* eight isolates at a rate of (8%), seven isolates *Penicillium* spp at a rate of (7%), six isolates of *Mucor* spp, three isolates of *Alternaria* spp, two isolates of each fungus, *Cladosporium* spp and *C. parapsilosis*, and four isolates of each fungus *Paecilomyces* spp, *Rhizopus* spp. Molecular identification of *A. chevalieri* revealed that it contained a 500 bp DNA band. The results of the genetic tree analysis and registration on the global GenBank website also showed a similarity between our isolates and global isolates. *A. chevalieri*'s virulence factors were examined, and the findings demonstrated that spores could cling to epithelial cells by 25%. Regarding the capacity to dissolve blood, favorable outcomes were shown, as it was an alpha-type hemolysis, and it could produce the protease, as the diameter of the hemolysis area on the test medium reached about 22.16 mm, and the lipase, as the hemolysis area reached 15.18 mm, and the production of the urease was 100%. Regarding the drug sensitivity of *A. chevalieri* to antifungals utilizing the disk diffusion technique, Nystatin proved to be the most effective in inhibiting fungal growth with an average diameter of 25.3 mm, while Amphotericin B and Itraconazole gave a positive result but less inhibition than Nystatin, and their inhibition percentage was 10.5 mm and 18.1 mm, while the result of the test for Fluconazole was negative and did not provide effectiveness in inhibiting fungal growth.

Keywords: Mobile Phone, Fungi, Antibiotics, Virulence Factors.

1. Introduction

In recent years, the use of objects like mobile phones has increased. Has become widespread due to the ease of use of applications and the small size of the devices. Despite their small size, their surfaces contain many microscopic organisms that are harmful to humans. The phone is considered a means of transmitting microbes, including fungi, and

is one of the most dangerous means of transmitting microbes because it is widely used, which raises concerns that threaten human health [1].

The hand is considered an important means of transmitting microbes through touching people, surfaces, and other objects. This leads to the accumulation of microbes on the hand and then transferring them to the phone. The transmission of these germs through the nose or mouth, coming close to it, may lead to illness, and therefore, hands must be sterilized before using the phone, and the phone must also be sterilized [2].

The relationship between mobile phones and fungi: Opportunistic fungi are among the most common types on phones, including the genus *Aspergillus*, which can cause skin, respiratory, and eye infections [3], and it has also been found on mobile phones. *Candida* spp. It is not widely spread, but it has the potential to cause pathogens in people with weak immunity and children, causing oral infections [4].

Healthcare workers are considered the group most exposed to contamination of their phones because they deal with patients, place their phones on tables, and also wear gloves, so these microbes multiply in the humidity. And if they were wearing rings, a study was conducted on 40 samples, 23 samples without rings, and 17 of them with rings, at the Sapienza University of Dental Hygiene, Rome's Department of Infectious Diseases and Public Health. These samples were collected shortly after removing the gloves from brushing teeth. The most common types of fungi were *C. albicans*, *A. niger*, and *A. flavus* [5]. In light of this research carried out by [6], the number of studies related to mobile phones from which bacteria, fungi, and viruses were isolated was 54-56 studies were found on bacteria, 16 on fungi, and a minimal number of investigations focused on viruses.

The faculty of a fungus to cause an ailment is based on multiple agents, such as weak immunity in the host body, the surrounding environmental conditions, and the virulence of the pathogen. Virulence is the means and mechanisms by which the fungus can cause disease. The harm and penetration of the host body include the ability to adhere, enzymes, dissolve blood, and others. The fungus that possesses these factors increases its danger to humans [7].

Azoles and polyenes are chemical compounds that kill or inhibit fungi by interfering with cell membrane metabolism, specifically with ergosterol. This causes the fungal cell membrane to deform, causing it to lose its contents and die. These antifungals have been used with opportunistic fungi, with mixed results, as sources indicate that opportunistic fungi are sensitive to these antifungals [8], [9], and other sources indicate that opportunistic fungi resist these antifungals due to mutations that occur in the fungal genome [10], [11].

The investigation aimed:

- Find and recognize fungi that live on mobile phones using conventional methods and PCR.
- Focus was placed on *A. chevalieri*, whose virulence was tested by testing its enzyme capacity, adhesion ability, and hemolysis.
- Evaluate the antifungal susceptibility of *A. chevalieri*."

2. Methodology

Sample Collection

Eighty samples were randomly collected from mobile phones and included in this study. The samples were divided into four groups: 20 samples from mobile phones of university professors and staff, 20 samples from mobile phones of doctors and pharmacists, 20 samples from mobile phones of taxi drivers, and 20 samples from mobile phones of restaurant workers. Samples were collected using sterile swabs during the period from 2024-12-1 to 2025-2-1.

Culture media

Sabouraud dextrose agar (S.D.A.) was used to grow, isolate, and identify fungi. This is a medium developed according to the manufacturer's guidelines. 65 grams of the medium in one liter of distilled water, diluted, and the media underwent a process of autoclaving for 15 minutes. It was brought down to 45°C, then 250 mg of chloramphenicol was added and poured into Petri dishes [12].

Specimens Culture

The samples have been grown in Petri dishes, which were sterilized using S.D.A. medium and inoculated by applying the Streaking technique using a Swab. The plates were then labeled based on the type of sample taken from them (University professors and staff devices, doctors and pharmacists devices, taxi drivers devices, restaurant workers devices) and incubated at 27 °C for a week, then the dishes were taken out for examination, and then the purification and diagnosis process was carried out [13].

Diagnosis of fungi

After obtaining pure colonies, diagnosis is based on color form and growth pattern within the colony as well as microscopic characteristics. Glass slides are prepared by transferring segment of the colony by use of a loop to the slide and include a small methylene blue dye, to reveal the fungal hyphae if they are split or not, as well as the conidia's shape and sporangia under the microscope. The identification was produced according to the sources listed below [14], [15], [16], [17].

Diagnosis using chrom candida different agar medium:

This test was performed by plotting Candida yeast on chrom candida different agar medium, and the plates were cultured at 37°C for two days until distinguishing between Candida species, and the examination was carried out based on the colony type, depending on the method [18].

Molecular Genetics Study:

Diagnosis Method Using the PCR Test

PCR testing was performed to detect the fungus *Aspergillus* spp. Using primers specific to the 18S rRNA gene for fungal diagnosis. The primer (18S rRNA gene ITS region) was used. Forward and reverse primers are responsible for fungal identification. The primers were designed for this study from NCBI Genbank, and the primers were prepared by IDT Integrated DNA Technologies, Canada.

Table 1. Primers; used in specific reaction for the "ITS" gene.

Primer	Sequence	Tm (°C)	GC (%)	Product size
Forward,	5'- TCCGTAGGTGAACCTGCGG -3'	60.3	50 %	550
Reverse,	5' TCCTCCGCTTATTGATATGC-3'	57.8	41 %	(base pair)

Table 2. Thermal Program.

Cycle Number	Time	Temperature	PCR Steps
Cycle1	Minutes 4	94 °C	Initial denaturation
Cycle 35	Minutes 30	94 °C	Denaturation
Cycle 35	Seconds 30	56 C	Annealing
Cycle 35	Second 30	72 °C	Extension -1
Cycle1	Minutes 7	72 °C	Extension-2

Investigation of some virulence factors of *Aspergillus chevalieri*. 0.5 ml of suspended epithelial cells was mixed with 0.5 ml of suspended fungal cells in a test tube. The mixture

was incubated in a shaking incubator at 40 rpm with slow shaking at 37°C for 2 hours. Then the mixture was exposed to centrifugation at a speed of (1000 rpm) for five minutes, the supernatant was discarded to remove non-adherent fungal cells, and PBS was added to the precipitate. To see the adhering cells, one droplet of the suspended cells was collected, placed on a sterile glass slide, dried with a flame, dyed with crystal violet, and viewed via an electron microscope [19].

Test of proteolytic activity

In a beaker, the medium was made by melting 20g of agar in 900 milliliters of purified water. 100 milliliters of distilled water were employed to dissolve 10 grams of skim milk in a different beaker. After that, the two liquids were made sterile by autoclaving, each one distinct, followed by mixing and cooled to 45°C. The antibacterial Chloramphenicol 250 mg/L was added to it, then transferred to plates and allowed to set up. The medium was a pure colony, grown at 27°C with a 5 mm disk. For 72 hours. The climate was used to detect the capacity of the pathogen to produce a protease enzyme, which breaks down protein. A look at a transparent area around the colony indicates the secretion related to the protease [20].

Test of lipolytic activity

The medium was available in a beaker. 20 grams of agar were dissolved, 10 grams of hydrated calcium chloride, one gram of peptone, and one liter of distilled water containing five grams of sodium chloride. The mixture was thoroughly mixed and heated. For every 100 milliliters of the mixture, 5 milliliters of Tween 80 were added 250 mg of the antimicrobial drug chloramphenicol was added. Then, the media was poured into plates until it solidified [21], and a disc that is 5 mm from a pure population was used to inoculate the medium and kept for 72 hours at 27°C. This medium was used to detect the lipase enzyme, as the appearance of a white sediment area shows that the lipase is secreted around the colony [22].

Urealytic activity test

To prepare the urea medium, it was dissolved in 4.6 g of the medium in 100 milliliters of purified water, autoclaved for 15 minutes, and allowed to solidify. 15 ml of a previously prepared 40% urea solution was then added to the medium under sterile conditions. This media was employed. To test the ability of fungi to produce the urease enzyme that decomposes urea, and the change in the color of the medium from yellow to pink is evidence of urease production [23].

Hemolysis activity test

Forty grams were dissolved to create the medium. To one liter of purified water, sterilize it in an autoclave, then cool it to 45 °C. 50 ml, after that, sheep blood was added, which was then filled into Petri dishes and left to solidify. It was used to detect hemolysis [24].

Antifungal sensitivity test for *A. chevalieri*.

The test was performed using the antifungal-impregnated disc method. The discs were spread on SDA medium prepared for this test. The medium was inoculated with approximately 0.1 ml of fungal inoculum using a loop, so that the entire plate was covered with the fungal suspension. Then, the antifungal discs were distributed using sterile forceps, one disc for each antibody, and the dishes. In an autoclave at 27°C for 72 hours. After that, the inhibition zones were measured in mm, which represent the areas surrounding each disc, which form a halo around the antibody from growth, using a ruler [25].

3. Results and Discussion

Identification and Diagnostic

During the study period from 2024-12-1 to 2025-2-1, 80 mobile phone samples were collected from professors and university employees, from the phones of doctors and pharmacists, from the phones of taxi drivers, and from the phones of restaurant workers to isolate the fungi from them, where all the samples were given a positive result of fungal growth, where 100 fungal isolations were obtained by 100%, of which 30 isolations were distributed from the phones of doctors and pharmacists, 26 isolations from the phones of taxi drivers, 23 isolations from the phones of restaurant workers, and 21 isolations from the phones of professors and university employees according to the schedule (4).

The results of the study showed that doctors' phones are the category that is most exposed to pollution from the rest of the categories due to the nature of their work within medical environments with a high percentage of pollution such as halls and clinics and direct contact with patients, which increases the chance of microbes to being transferred to their hands and then to phones.

The most frequent presence of the genus was *Aspergillus* and was represented by species *A.fumigatus*, *A.niger*, *A.candidus*, *A.chevalieri*, *A.flavus*, *A.terreus*

Isolated fungi have shown their high ability to cause various fungal diseases, and here it is clear how dangerous these fungi are. Species belonging to the genus *Aspergillus* have known to be infected to the respiratory system, causing cases of allergies, asthma, irritation of the mucous membranes and necrosis of lung tissue [26].

A. chevalieri fungi were chosen for the study not because it is more widespread but because of its scientific and medical importance, and it is one of the modern or few fungi studied in the field of mobile phone pollution and the lack of local studies on this type of fungus and highlights the need to study it to understand the cycle of transmission of infection in the medical environment. In addition, the presence of this fungi on the surfaces of mobile phones is a reference to its ability to adapt to non-live surfaces, and this is to make it particularly important in terms of public health, although the percentage of isolation of this fungi is lower compared to the rest of the isolations.

Table 3. Classification of Sample Types into Single and Mixed Infections".

Sample type	Single infection	Mixed infection	P value
Doctors and Pharmacists	16	4	0.007*
The devices of professors and university staff	13	7	0.180
Taxi drivers' devices	2	18	0.001*
Restaurant workers' devices	11	9	0.655

Table 4. Shows the isolated fungal species and percentages.

Fungi	The devices of university professors and employees	Devices of doctors and pharmacists	The devices of taxi drivers	The devices of restaurant workers	Total and percentage
<i>A. fumigatus</i>	6	3	5	3	(17)17%
<i>A. niger</i>	3	3	4	3	(13)13%
<i>A. candidus</i>	2	4	3	3	(12)12%
<i>A. chevalieri</i>	1	4	3	3	(11)11%
<i>A. flavus</i>	4	3	3	1	(11)11%
<i>A. terreus</i>	4	1	2	1	(8)8%

<i>Penicillium spp</i>	1	2	2	2	(7)7%
<i>Micor spp.</i>	1	2	2	1	(6)6%
<i>Alternaria spp</i>	0	2	0	1	(3)3%
<i>Cladosporium spp</i>	0	1	1	0	(2)2%
<i>C. parapsilosis</i>	1	0	1	0	(2)2%
<i>Paecilomyces ssp.</i>	1	2	0	1	(4)4%
<i>Rhizopus ssp</i>	1	0	1	2	(4) 4%
Total	21	30	26	23	100

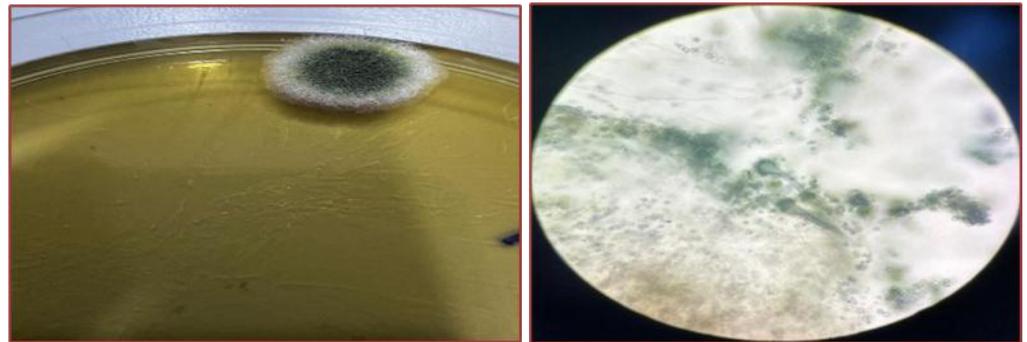


Figure 1. Shows the morphological and microscopic image of the fungus *A. chevalieri*.

Molecular PCR diagnosis of *A. chevalieri*

Traditional methods for fungal diagnosis are no longer sufficient, especially for *Aspergillus spp.*, due to the overlap of criteria between these species and other species within the genus *Aspergillus*, which number more than 300 species [27]. PCR diagnosis is considered accurate and decisive in identifying *Aspergillus* species.

The diagnosis was verified by validating the identity using the PCR method of *A. chevalieri*, as the outcomes of electrophoresis on agarose gel of the DNA sample taken out of the studied fungus appeared, with respect to the primers targeting the ITS rDNA region (ITS1-5.8S-ITS2), that the DNA bands were about 550 bp.

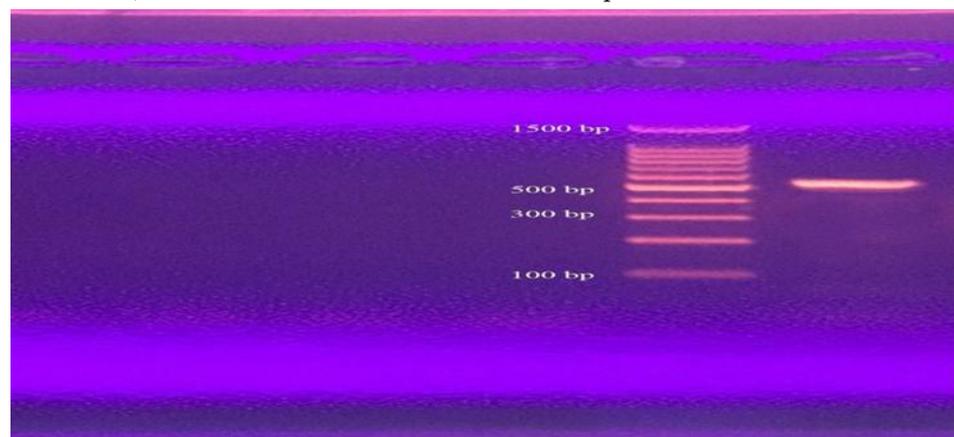


Figure 2. Indicates that the 550 bp band is present, This demonstrates that the suspected implication of fungal DNA. Species identification was confirmed later by sequencing, supporting the initial traditional diagnosis of *A. chevalieri*.

Phylogenetic tree

The sequencing technique was used to identify the isolate after completing the PCR test. Test results were shipped to Macrogen in South Korea so that an AB DNA sequencing machine could perform base pair sequencing on them. The phylogenetic tree was then created by comparing the results with the fungal sequences listed on the NCBI GenBank website using the MEGA10 tool.

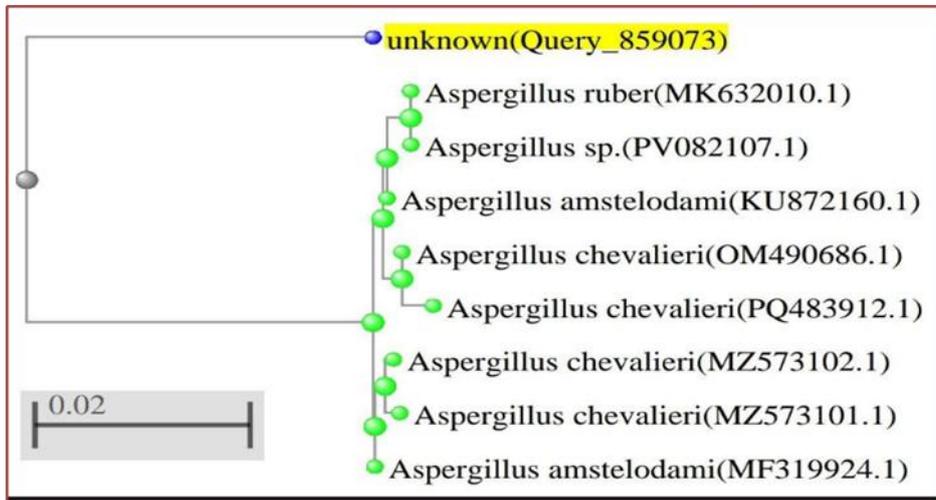


Figure 3. The results of the genetic tree analysis showed a clear similarity between the sequences of the fungi under study and the rest of the other species registered in the NCBI GenBank website and apparent in the genetic tree analysis.

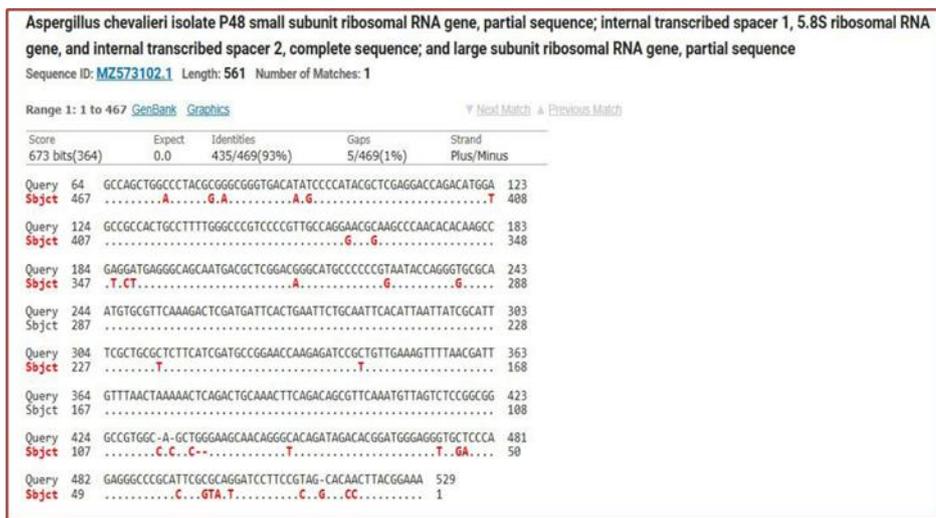


Figure 4. Multiple sequence alignment of fungal ITS sequences.

Molecular identification based on internal transcribed spacer (ITS) region sequencing is widely regarded as a reliable and accurate approach for fungal taxonomy and species-level discrimination. In the present study, PCR-amplified ITS sequences of the fungal isolate were subjected to DNA sequencing, followed by comparative analysis with reference sequences deposited in the NCBI GenBank database.

Phylogenetic tree (Figure 3) illustrates the evolutionary relationship between the studied fungal isolate and closely related fungal species retrieved from GenBank. The phylogenetic tree, constructed using the MEGA10 software, demonstrates that the isolate clusters tightly with reference sequences of the same species, forming a well-supported clade. This close clustering indicates a high degree of genetic similarity and confirms the

taxonomic identity of the isolate. The clear separation of this clade from other related species further supports the specificity and accuracy of ITS sequencing for fungal identification. Such phylogenetic consistency suggests that the isolate shares a common evolutionary origin with previously reported strains, reinforcing the reliability of molecular identification in this study.

Further support for the molecular identification is provided by the multiple sequence alignment shown in Figure 4. Alignment of the ITS sequence of the studied isolate with homologous sequences from GenBank revealed a high level of nucleotide conservation across most regions of the sequence. Only minor variations were detected, which are likely attributable to natural intraspecific genetic diversity rather than indicating species-level divergence. The presence of conserved regions confirms the suitability of the ITS region as a universal fungal barcode, while the limited variable sites contribute to distinguishing closely related taxa.

Overall, the combined results of phylogenetic analysis and multiple sequence alignment provide robust molecular evidence for the accurate identification of the fungal isolate examined in this study. The strong agreement between sequence homology, phylogenetic clustering, and alignment patterns confirms the reliability of the molecular approach employed. These findings emphasize the value of integrating ITS sequencing with bioinformatics analyses for precise fungal characterization, which is essential for advancing epidemiological investigations, understanding pathogenic potential, and supporting the development of effective control and management strategies.

Virulence Factor Test

Ability to adhesion

The test findings demonstrated that fungal spores' capacity to stick to *A. chevalieri*'s epithelial cells isolated from mobile phones yielded excellent results, with an adhesion rate of 25%. Adhesion is considered crucial to the capacity to infect, as it is the initial action in microbes attacking tissues. Having the capacity to stick to the living cell depends on the chemical composition, humidity, and physical characteristics of a cell wall, including the existence of holes or scars, among other factors. It is subject to particular systems and regulates adhesion based on the pathogenic cell's and the host cell's chemical affinity [28].

The ability to produce hydrolytic enzymes

The results showed that the fungus *Aspergillus chevalieri* has the ability to produce proteolytic and lipolytic enzymes, indicating its potential impact on human health. The results were positive for the protease enzyme, as a clear halo was formed around the colony with a mean diameter of 22.16 mm. In addition, the fungus was able to produce the lipolytic enzyme, which formed white precipitates around the colony, with a mean precipitation diameter of 15.18 mm.

These results are consistent with the findings of [29] who reported that the production of protease indicates the fungus's ability to degrade proteins, which contributes to tissue damage in the host and facilitates invasion and spread, thereby negatively affecting human health. Furthermore, the production of lipase indicates the fungus's ability to degrade lipids, which enhances its survival and adaptation in the environment, as well as its role in penetrating the cell membrane.

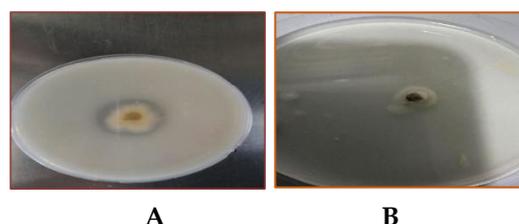


Figure 5. Testing the capacity of *A. chevalieri* for creating enzymes (A) protease (B) lipase.

Hemolysis ability

Test results showed positive results for the hemolysin enzyme secretion of the fungus *A. chevalieri*. The fungus was cultivated using fresh sheep blood as the medium. A transparent halo appeared around the colony, indicating the capacity of the enzyme to break down red blood cells. The degrading type of this fungus was alpha. The conclusions of the inquiry agree with those of [30] that the enzyme hemolysin decomposes red blood cells by making holes in the cell membranes. This leads to the release of iron, which promotes fungal growth [31]. *Aspergillus* fungi, especially *A. chevalieri*, produce the enzyme asp-hemolysin, which leads to an increase in the occurrence of pathogenesis [31].

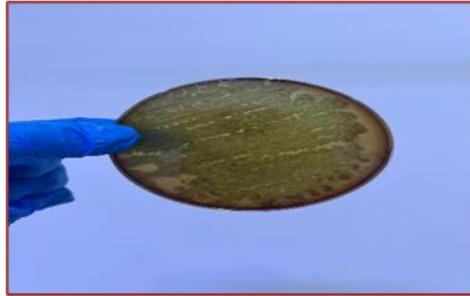


Figure 6. Testing the ability of *A.chevalieri* to generate the enzyme hemolysin.

Urease enzyme production

Test findings indicated that the ability of *A. chevalieri* to produce urease, as the isolate produced 100% urease. The findings of this investigation are in line with those of [33]. This study showed that most *Aspergillus* species are capable of producing urease. Many pathogenic microorganisms can use urea as a source of nitrogen by secreting Urease is an enzyme that breaks down urea into ammonia and carbamic acid, which then decomposes spontaneously to generate ammonia [34].



Figure 7. Testing the ability of *A. chevalieri* to produce the urease enzyme.

Table 5. Shows some virulence factor tests for the fungus.

Virulence factor	Value region Sedimentation	Virulence factor	Ratio Centenary %
Protease enzyme production	22.16	Ability to stick	25%
Lipase enzyme production	15.18	Urease enzyme production	100%
Enzyme production Hemolysin	Alpha type		

antifungal sensitivity of *A. chevalieri*

The drug sensitivity test's findings demonstrated that Nystatin was the most effective inhibitor of *A. chevalieri*, with an average diameter of the inhibition zone of 25.3 mm. Amphotericin B and Itraconazole produced positive results but less inhibition than Nystatin, and their inhibition rate was 10.5 mm and 18.1 mm, respectively. The antifungal Itraconazole is characterized by its effect on the process of building the cell membrane, which makes it completely permeable, and this causes the cell contents to exit the cell and leads to its death. While the result of the antifungal test, Fluconazole, was negative, it did not show any effectiveness in inhibiting the growth of fungi. Fungi can resist some antibiotics. After all, their walls are thick, which are similar to the walls of human cells, and also to the capacity for causing modifications in genes, specifically since they have a very high genetic stock because they are considered eukaryotic [35].

Table 6. Antifungal sensitivity of *A.chevalieri*.

No	Antifungal type	Anti-concentration	Inhibition zone in mm
1	Amphotercin B	20ug/disk	10.5
2	Itraconazole	50ug/disk	18.1
3	Nystatine	100ug/disk	25.3
4	Fluconazole	25ug/disk	0



Figure 8. Drug sensitivity of *A.chevalieri* to antifungals.

4. Conclusion

Mobile phones are one of the means through which many types of fungi can be transmitted, which can infect humans and cause diseases. Doctors' and pharmacists' devices are considered the most contaminated. The fungus *A. chevalieri* can secrete hydrolytic enzymes and hemolytic enzymes capable of causing infection. The best antifungal for control of *A. chevalieri* is Itraconazole. Fungus was diagnosed correctly by PCR, with findings indicating a genetic match with the documented strains, demonstrating the efficacy of the molecular diagnostic.

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