LUMEN ET VIRTUS

Alternative protocol for antifungigram demonstrates the presence of resistant fungi in 8 samples collected in public and open spaces in the city of Tauá (Ceará)

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ABSTRACT

Conducting experiments with microorganisms is usually quite laborious, as it requires appropriate techniques and a suitable laboratory environment, particularly when looking for possible microorganisms resistant to the main antimicrobials available against bacteria and fungi. Since the nineteenth century, it has become evident that microorganisms are living beings that are impossible to be visualized without the aid of optical microscopes, since bacteria measure 1µm, viruses 1nm. Although fungi are around 100µm in diameter, the visualization of microscopic forms, yeasts require care. However, regardless of size, microorganisms are ubiquitous, found in the most diverse ecosystems. They are fundamental in the decomposition or recycling of organic matter, especially in the early stages.

Keywords: Protocol, Antifungigram, Resistance, CECITEC.

INTRODUCTION

Conducting experiments with microorganisms is usually quite laborious, as it requires appropriate techniques and a suitable laboratory environment, particularly when looking for possible microorganisms resistant to the main antimicrobials available against bacteria and fungi. Since the nineteenth century, it has become evident that microorganisms are living beings that are impossible to be visualized without the aid of optical microscopes, since bacteria measure 1µm, viruses 1nm. Although fungi are around 100µm in diameter, the visualization of microscopic forms, yeasts require care. However, regardless of size, microorganisms are ubiquitous, found in the most diverse

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ecosystems. They are fundamental in the decomposition or recycling of organic matter, especially in the early stages (MOREIRA, 2009).

Since antibiotics began to be used massively around the 40s of the twentieth century, humanity has unintentionally initiated an exacerbated process of selective pressure on microorganisms that has resulted in evolutionary changes that can be considered rapid and, increasingly, has become a problem in various sectors. such as public health, economics, medicine, evolutionary biology and, in the scope of the present work, microbiology, per se. In the years before the massive production of antibiotics, infectious diseases terrified generations. At that time, it was common for patients with untreated bacterial infections to die. From the 1960s onwards, many of these diseases were controlled and the risk of death from them decreased dramatically. However, with the production of antibiotics on an industrial scale, a new problem arose: microorganisms "developed" resistance to drugs that had been effective until then (HOGG, 2004; GUILFOILE, 2007; SUMMERS et al., 2008).

The indiscriminate use of antibiotics was described by Scheckler and Bennet, who observed in 1970 that 62% of antimicrobial prescriptions were given to patients without infection. Data reinforced by Kunin in 1973, that 50% of antimicrobial prescriptions were not indicated, and strengthened by Jogerst and Dippe, in 1981, who also classified 59% of antimicrobial prescriptions as inadequate, in agreement with MOTA et al. (2010). The excessive and inappropriate use of antibiotics has contributed to the increase in microbial resistance (FREITAS *et al.,* 2006). Antibiotic resistance is inevitable and irreversible, a natural consequence of microbial adaptation to antibiotic exposure (WANDERLEY et al., 2003; SANTOS, 2004).

The acquisition of resistance to antimicrobials is an evolutionary process, related to the modification or acquisition of genes contained in microorganisms, which encode different biochemical mechanisms that prevent the action of drugs, these mechanisms of action can be the inhibition of protein synthesis, interference in the synthesis of the cell wall, the destruction of the cell membrane structure, the reduction of permeability to the antimicrobial agent and the intervention in the synthesis of nucleic acid (TENOVER, 2006).

Due to the increase in bacterial resistance to multiple antimicrobial drugs, there has been concern and the search for new therapeutic alternatives, with the so-called medicinal plants representing an important source for obtaining these possible drugs. Research on the antimicrobial activity of plant extracts, such as essential oils and their components, has focused on the field of medicine and therapeutics, more specifically, aromatic essential oils, which have shown good effects against bacteria, yeasts, filamentous fungi and viruses (REICHLING *et al.,* 2005).

In our laboratory routine, we have tested numerous substances from native plants of the Caatinga, with the main protocol being the use of discs for antibiogram and antifungigram. However,

logistical and marketing problems caused us to look for an alternative to commercial records. Therefore, the objective of this article is to demonstrate that the use of handmade discs manufactured in our teaching laboratory of the Full Degree Course in Biological Sciences can be a cheap and effective alternative as an initial procedure in antifungigram and, possibly, in antibiograms. Additionally, a secondary objective was to demonstrate the existence of unknown fungi that present resistance to the main antifungal drugs sold in pharmacies and drugstores in the city of Tauá.

METHODOLOGY

The methodological procedure involved sample collection in the public space of Praça da Lagoa in the city of Tauá between 08:00 and 10:00. The collection was carried out by means of sterile swabs on the benches of the square. Then, the samples were taken to the Teaching Laboratory of the Full Degree Course in Biological Sciences of CECITEC in sterile microtubes. The samples were plated in two different culture media: 1) MacConkey Agar + Blood Agar Base and 2) Rappaport Vassiliadis + Potato Glucose. Later, the handmade records manufactured in the Teaching Laboratory were added. In the construction of the handmade antifungigram, 4 mm diameter discs of cardboard paper were produced in different colors (Blue, orange, yellow and pink). The discs were submitted to degermination/assepcia with 70% alcohol and then sterilized in an oven at 70º for 12 hours. In addition, both sides of the handmade discs were subjected to ultraviolet (UV) radiation for 10 minutes. Subsequently, 2uL (2ug) of commercial antifungal (Chart 1) was added to the upper surface of each disc. Over 4 weeks, measurements were performed using a caliper.

All the materials used in the preparation of the methodological collection procedures as well as the culture media (including microtubes, Petri dishes) were previously autoclaved at the Central Laboratory of the city of Tauá (LACEN/Tauá).

In the present study, a mixed control was used, i.e., it is negative and positive at the same time. Negative, because it does not have drugs; and positive because it did not present any evidence of contamination, ensuring that the protocol used in its construction works.

The subsequent procedures of the experiment were carried out under aseptic conditions in a laminar flow chamber on the premises of LACEN/Tauá.

RESULTS & DISCUSSION

The experiments carried out jointly in the Teaching Laboratory of the Full Degree Course in Biological Sciences of CECITEC and in the Central Laboratory of the city of Tauá (LACEN/Tauá) clearly demonstrate that the use of handmade discs presents a result considered satisfactory (Figure 1; Tables 1, 2 and 3). Of the 8 fungal isolates tested, it is evident that the handmade discs are stable and have not shown any sign of modification or contamination. The mixed control also presented a

satisfactory performance, especially when compared to the other disks containing the drugs tested (Chart 1). Therefore, the initial results conspicuously demonstrate that the discs manufactured (called handmade) in our Laboratory are satisfactory as a low-cost resource in methodological practices involving discs, such as antibiograms and antifungigrams. Additionally, it is observed that the various isolates present variable resistance to antifungal drugs available in pharmacies and drugstores in the city of Tauá. Although the experiment is preliminary, it implies that residents of the city of Tauá who frequent public spaces may be in contact with species of fungi with different degrees of resistance to the drugs available, including in the clinic. Although the antifungals chosen are usually used in the clinic for the treatment of mycoses and eczema, accompanied by inflammatory processes, we consider that their use may be an indication of possible occasional cases of resistant microorganisms. As well as species of fungi that are still unknown. It is notorious that oral medications can be eliminated through urine or feces. Those used in dermal treatments can be washed through baths or only parts of the body, such as the face, hands and arms. Such materials are often released *in natura*, in sewage systems, with inadequate treatment protocols for such waste. Therefore, we consider that such residual substances or not, are part of the ecosystems existing in cities. The city of Tauá, which has lotic ecosystems such as the Trici River and lentic, such as the lagoon of the City Park. Both receive materials from homes and commercial points. Noting that the Parque da Cidade lagoon was the target of an intense urban reform in 2023 that may have minimized or eliminated such a situation. Another preponderant abiotic factor is the wind. Microbial forms can be transported via wind action. The so-called winds are common in the natural landscape of the city of Tauá.

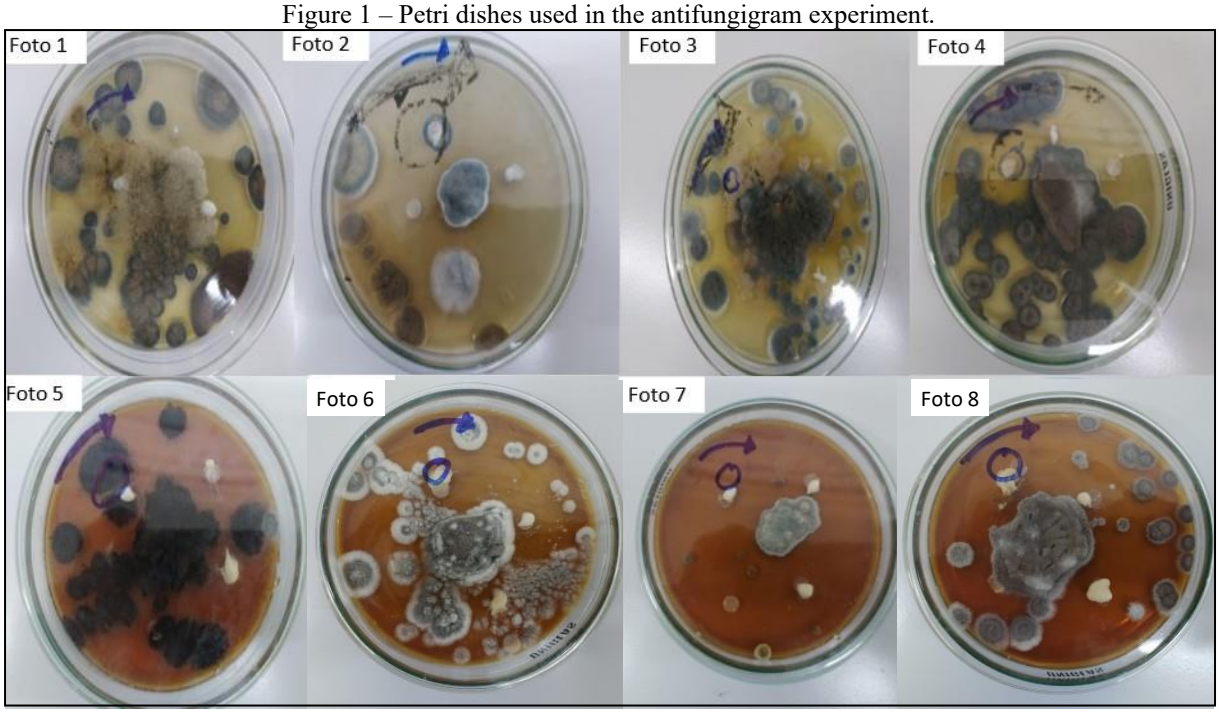
The results show that the readjustment carried out in the project in order to focus the experimental work on fungal growth, as well as the antifungigram protocol (Figure 1; Tables 1, 2 and 3) can be considered satisfactory.

TABLE 1. List of antifungals used in the antifungal.

ANTIFUNGALS	UTILITY
Cimecort	It is a drug to be applied to the skin, fights bacterial infections and mycoses, accompanied by inflammation.
Miconazole nitrate	It is an antifungal that acts by inhibiting the growth of fungi, especially of the *Candida species*, which can cause ringworm on the skin, nails, groin, or mucous membranes such as the mouth, throat or genitals, and is therefore indicated for the treatment of oral or genital candidiasis, or skin ringworm, for example.
Clotrimazole	It is indicated for the treatment of fungal infections of the skin, caused by dermatophytes, yeasts and other microorganisms, such as *Malassezia furfur*; and skin infections caused by *Corynebacterium minutissimum*. element.

Source: Prepared by the authors

Figure 1 – Petri dishes used in the antifungigram experiment.



FINAL CONSIDERATIONS

We consider that although the results are preliminary, they are promising. Handmade records are presented as a cheap and effective alternative in the initial phase of experimentation. Although they do not replace commercials. The evolutionary aspects related to microorganisms demonstrate that the evolution of this large group of living beings occurs quickly and constantly. Although only a small percentage of microorganisms are pathogenic to animals and humans, this small percentage poses a danger to animals and humans. New experimental studies should be designed and experimented, as well as the identification at the genus or species level of the 8 isolates from the benches of the Lagoa square in the city of Tauá.

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