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Sanitary biosecurity test against hospital acquired fungal infections: The role of hemolymph from the cochineal insect

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Abstract: Background: Fungal infections are significant risk factors for nosocomial infections. They are associated with environmental spores and they are potential colonizers in hospital infrastructure, instruments or specific vectors. Usually they are identified by means of microbiology and culture media for definitive diagnosis. The objective is to evaluate the usefulness of the application of a colorimetric assay that originates from an endemic insect in Mexico (*Dactilopius Coccus costa*); It implies a specific qualitative biochemical reaction. It is also available to be used as a quick field test in health control. Design: Prospective, transversal, descriptive, randomized sampling with control reference test. Methods: A transversal randomized sampling from surfaces, materials, solutions and organic-sanitary waste from different known risk areas in a hospital institution with a large number of patients. Samples were processed using the qualitative test, examined by colorimetric evaluations and compared with positive controls (zymosan and aspergillus spores). Results: Samples showed no evidence of fungal colonization, unlike controls, which resulted positive. The resulting sensitivity was 100%. Conclusions: First qualitative pilot test to be used in the health care field, which proved to be useful for the monitoring and timely detection of fungi of biomedical interest. The method is practical. This essay validates the potential use of a quick qualitative test for preventive control of fungal infections in hospitals.

Keywords: Carminic Acid, Dactylopius, Hemolymph, Intrahospital Infection, Fungal Infection Diagnosis

1. Introduction

To this day, hospital infections represent a risk to public health and a challenge for early detection, monitoring and decision making in health infrastructure [1, 2]. Of all the fungi species in nature, less than 200 species are known to produce infections in humans, and of those, 10% are responsible for fungal infections that have an impact on public health.

Usually opportunistic fungal species such as *Candida* and *Aspergillus* do not cause invasive illnesses in healthy people. However, these fungal varieties can cause infections, often lethal, in patients with an immunocompromised weakened immune system. Such is the case of nosocomial infections, where these two species, *Cladosporium* and *Penicillium* represent 90% of fungal infections [3-7]. To this we must add certain inputs of high-risk areas in the hospital infrastructure

that require specific monitoring [8-11].

The morbi-mortality is high, particularly when the diagnosis is delayed and the therapeutic decisions are implemented when the course of the infection is advanced.

In recent decades the incidence of fungal infections such as aspergillosis, coccidioidomycosis, cryptococcosis, in addition to the well-known prevalence of candidiasis, has increased. This is due to the increased presence of immunocompromised patients with acquired immunodeficiency syndrome (AIDS), patients undergoing chemotherapy or immunotherapy for other diseases, and patients derived from procedures such as organ transplantations. Additionally, the possibility of respiratory problems associated with fungal allergen reactivity should be taken into account, which can lead to asthma and allergic alveolitis. Other reports identified Scedosporium prolificans as a new incursion to take into account at hospital settings [12-14].

Therefore, environmental monitoring and the control of risk factors is becoming relevant due to the increasing amount of hospital-acquired infections and to the documented resistance to treatment with antibiotics and antifungals. Consequently, it is a priority to strengthen the quality control strategies that allow preventive actions and early detection in specific areas of these risks in hospital settings through environmental biosecurity strategies (EBS). The presence of opportunistic fungi in hospital environment should be understood as such: that environmental situation where there are acceptable levels of concentrated fungal spores, making it unlikely that susceptible patients acquire an infection linked by air or the hospital environment itself. [15,16] Previous studies have shown an increased presence of colony forming units (CFU) of fungi in hospital kitchens and lower concentration in operating rooms[4].

The fundamental measures recommended to maintain the level of EBS are: Proper maintenance of the air conditioning, surface cleaning, staff circulation and discipline, and proper isolation of the infrastructure under construction or being remodeled [17-23].

There is a range of evidence and studies pertaining to methods for detecting and monitoring fungal contamination in hospitals. In the emergence of a case of aspergillosis in such areas or the existence of anomalies in ventilation systems, it is recommended to perform microbiological environmental controls by sampling the air at the outlet of ventilation systems and the patient's surroundings. The sample evaluation related to the presence of aspergillus in ambient air should be <5 colony forming units (CFU) per cubic meter, to be considered a proper control.

In the case of Aspergillosis, evidence is needed to identify the fungus and make a clinical correlation with the data present in the patient. It is advisable to meet diagnostic criteria, considering definite aspergillosis; such as histological evidence in autopsy or biopsy, and destruction or tissue invasion by septated, acute-angle-branching hyphae. Either requires isolation of Aspergillus sp. (transbronchial biopsy or transthoracic needle aspiration, in patients with clinical and radiological manifestations suggestive of infection).

The classification of a nosocomial or community case proposes difficulties, especially when certain invasive forms (eg. the surgical) can have incubation periods exceeding one year, making it difficult to define the specific moment of infection. In respiratory infections, when symptoms are present at admission or within 72 hours of the same it is considered a community-acquired infection; if the symptoms begin after that period, it is considered as a nosocomial infection. In any case it is advisable to conduct a complete epidemiological study before labeling a case as community–acquired or of nosocomial origin (whether it is definite or probable) [24-34].

In our country there are well-established criteria for defining a nosocomial infection, and what applies to the case of fungal infections, therefore it is convenient to make reference to the official Mexican norm NOM-026-SSA2-1998, for epidemiological surveillance prevention and control of nosocomial infections [35].

2. Methods

This is a prospective, transversal, descriptive, randomized sampling with control reference test. The bioethical committee and hospital infection control committee approved this study.

2.1. Colorimetric Essay

This product contains a dye and cellular components of the hemolymph of *Dactylopius coccus costa* insect. By observing the color change, in the field we can do an accurate and fast detection of the presence of contaminants such as fungi dissolved in water [36,37,38].

It has the advantage of being highly sensitive, heat stable, and at very low cost. It is made from a combination of substrates and reagents derived from hemolysates cell cultures and selected strains of *Dactylopius coccus*. Today we present the proposal for detection of fungi and spores of biomedical interest in hospital areas [39-44].

It is a product whose composition permits the evaluation of fungal contaminants in a specific environment. This product contains a dye and cellular components of the insects' hemolymph and has high affinity for specific glycans in the fungal cell wall. The reaction allows proper identification of the discoloration and enables rapid and accurate field detection in the presence of fungal contaminants from the considered risk areas. Each kit format contains several reactions allowing the use of several tests.

- Concentration of work: The contents of a vial diluted in 1 ml of water (endotoxin-free) are sufficient to evaluate and demonstrate the presence of contaminants.
- Absorption: 0.5 ml of the 1:10 dilution in water (endotoxin-free) is sufficient to determine its absorbance 450 nm-550 nm (maximum peak).
- Stability: Samples are stable in solution at a temperature range of 22 ° C - 25 ° C, they should remain in a cool place.

- Results: High reliability, the diagnosis is made in minutes.
- Restrictions: This product is for fieldwork, does not accurately quantify the concentration of fungi per sample, however, it allows preventive routine diagnosis over long periods of time.

2.2. Methods of Sampling

By random sampling under aseptic techniques and through an assay with a descriptive design, transverse cut, we proceeded to select 7 samples from different areas of a hospital with high concentration and patient flow. The biological samples obtained were inserted in a corresponding vial and left for the reactive process to take place and then were qualitatively evaluated by a colorimetric reaction.

The samples were obtained using a sterile technique directly from the source, according to the following: Sample 1) hospital masks, 2) Betadine 3) Tincture of benzoin 4) Glutaraldehyde 5) Endotracheal tube secretion from patient A from the ICU, 6) Endotracheal tube secretion from patient B from the ICU, 7) Ventilation mask.

These samples were compared with the following references as controls: Control 1) Zymosan (b, 1.3 glycans), 2) *Aspergillus sp* spores. After 5 minutes once the corresponding sample was deposited, we proceeded to identify and read the colorimetric reaction corresponding to each vial.

3. Results

We can see the basic colorimetric reaction through glycan chains, as evidence in a simple view in only five minutes. (Fig. 1).

Figure 1. *A) Basic red color of the colorimetric media without reaction, and B) the chemical reaction of Identification of glycan chains which constitute the fungal cell Wall.*

The samples conducted allowed the identification of different risk areas, which presented a negative reaction in contrast to the positive controls (Zymosan and Aspergillus sp. Spores), demonstrating the sensitivity and specificity of the test (Table 1).

In all cases the test was negative, showing the absence of biological materials derived from fungi which are determined through the colorimetric biochemical reaction, compared to the positive controls such as Zymosan and spores of Aspergillus sp, that were lysates and with formation of a precipitate proper of the reaction itself due to glycans.

Table 1. *List of the sampling and corresponding results for the qualitative tests.*

Type of Sample	Result
Surgical Facemask	- (Does not show clot formation)
Betadine	- (Does not show formation of precipitates)
Tincture of benzoin	- (Does not show formation of precipitates)
Glutaraldehyde	- (Does not show formation of precipitates)
Ventilation Mask	- (Does not show formation of precipitates)
Patient A	- (Does not show formation of precipitates)
Patient B	- (Does not show formation of precipitates)
Zymosan (b, 1.3 glycans) Positive control	+ (Does show formation of precipitates)
Aspergillus sp Spores Positive control	+ (Does show formation of precipitates)

4. Discussion

Nosocomial infections are one of the reasons for the epidemiological surveillance within the health infrastructure, which remain as a current challenge. The criteria for diagnosis are listed in various forms of management guidelines that give particular relevance to the possibility of infectious agents such as viruses, bacteria and fungi their incubation period to be classified as nosocomial acquisition. Influenced by a number of environmental conditions other than their own and intrinsic to the individual for this condition to be expressed.

In the case of fungi, the diagnosis concurs only through direct observation by microbiological or histopathological methods, or as a result of a specific culture. However, when this condition is positive, time can be fundamental in the deterioration and the progression of the disease in each individual case or the potential spread of infectious agents in the healthcare environment (patients, health personnel, infrastructure and inputs).

Hence the importance of having short-term alternative evidence that enables timely diagnosis within environmental biosafety criteria. This premise applies perfectly in the field of health infrastructure for fungal risk factors, which can be detected preemptively, expeditiously and timely in the context of a hospital environment.

Hence this proposal whose principle is based on a qualitative colorimetric biochemical stable reaction, controlled and with high sensitivity, which allows the identification of glycan chains that constitute the fungal cell wall, which is what gives rise to lysate and whose results are evident at a glance, regardless of spectral confirmation.

Moreover, this strategy is based on a unique link between the history of an insect *Dactylopius Coccus Costa* (Grana cochineal) endemic in Mexico, whose component in their hemocel contains a high percentage of carminic acid. This insect and its derivatives led to enormous wealth since pre color, to be used as a natural dye for dyeing textiles, artwork, ceramics, food and the same face of indigenous women in

various aesthetic expressions. In time it became one of the most important exports to Europe for the quality of staining scarlet from natural dyes. Its products were called by the natives "blood of nopal", referring to this insect as natural host to the Opuntia ficus-indica. Contemporary applications in the use of an insects' high value in ancient times, still apply for biotechnological potential uses and in this case the detection of fungi of biomedical interest. The highly sensitive nature of strict monitoring controls and favorable preliminary tests in other areas has now permitted to venture into the biomedical care. With an absorbance pattern of 495 nm identified on a curve of an average longitudinal wavelength of 500 nm, the same that modifies directly proportional to the presence of the glycan particles in the cell wall of the fungi or in proportion to the presence of spores. This fact is directly linked to highly sensitive behavior, which can detect less than 5 spores of the analyzed sample.

In the samples conducted, it allowed the identification of different risk areas, in which humid or liquid areas, surfaces of materials in contact with secretions from patients and textiles were taken into account, in addition to areas with higher risk of fungal contamination such as intensive care units.

In all cases the test was negative, showing the absence of biological materials derived from fungi which are determined through the colorimetric biochemical reaction, compared to the positive controls such as Zymosan and spores of Aspergillus sp, that were lysates and with formation of a precipitate proper of the reaction itself due to glycans. This condition confirms high sensitivity, for the purpose of this transvers test that resulted 100% (Sensitivity).

This *in situ* determination allowed for sampling with immediate results. Additionally, it permitted the verification of an existing antiseptic level in the materials studied and therefore in the sanitary management criterion of this institution.

On the other hand, it determines the status regarding environmental security risks, which may impact patients who usually are present in these areas and who are immunologically compromised by their clinical condition.

We consider that by periodic sampling of risk areas through a quick test like this, we would be able to significantly anticipate in terms of prevention. The identification and histological or microbiological cultures usually require objective evidence for therapeutic decision-making or the conformation of an epidemiological barrier to an event of this nature.

This proposal validates its incursion as a quick test in the health field, to be integrated to the quality control indicators in sanitary environment, in hospital institutions and would allow early detection of nosocomial infections, with less impact on the clinical condition of patients with intrinsic risk variables.

In turn, it promotes the development of products generated in our country, with its own patent, which also makes a particular link to biotechnological applications with the use of products from indigenous pre-Hispanic times. Today they are a legacy and have great historical value in our heritage. This strategy adds to the equity transfer of knowledge for decision-making in the prevention of fungal infections and environmental pollution, in a concrete contribution in health sciences.

5. Conclusions

This was the first qualitative pilot test that is applied in the sanitary field and whose results permitted for early identification and detection for fungi of biomedical interest. This test validates the potential utility of a quick qualitative test for preventive control of the risks of hospital fungal infections. The biotechnological use of products derived from cochineal (*Dactylopius coccus costa*) hemolymph, and an endemic insect cultivated by indigenous Mexicans since pre-Hispanic times, may represent a benefit in hospital healthcare today.

We don't have disclosure related with this project.

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