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Isolation and Identification of Fungi in Hospital Wastewater in Enugu Metropolis

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Abstract: **Fungi were isolated from hospital wastewater in Enugu State to investigate hospital-acquired infections. Identified fungi, including Candida spp, Pichia spp, Trichoderma spp, Cryptococcus spp, and Trichosporon spp, are known to cause human diseases were identified through standard methods. Cryptococcus spp and Trichosporon spp had high counts (4.4×10^3 cfu/ml and 3.7×10^3 cfu/ml) in samples A and B, indicating a potential health risk. Proper treatment and disposal of hospital wastewater are necessary to prevent the spread of these pathogens, protecting public health and mitigating infection risks, especially in immunocompromised individual achieving a positive result.**

Keywords: **Isolation, Identicaion, Fungi, Hospital, Waste, Water.**

1. INTRODUCTION

Hospital wastewater is distinct from domestic wastewater due to its hazardous and infectious nature. It comprises waste from operating rooms, diagnostic labs, radiology, and infectious wards (Saguti 2021).

The presence of many disease-causing microorganisms in hospital wastewater poses a potential threat to public health when discharged into water sources (Azuma 2021).

Hospital wastewater poses significant environmental risks, being both harmful and infectious, unlike other wastewater sources. It's generated from areas like surgical rooms, diagnostic labs, radiology departments, and infectious wards (Cai 2013).

Population growth, urbanization, and the expansion of healthcare facilities have led to a significant rise in hospital wastewater production (Ogwugwa 2021).

Fungi thrive in hospital settings due to factors like temperature, moisture, and nutrients, creating ideal conditions for their growth in healthcare waste. This highlights the need for proper waste management to curb their spread (Ozbek 2020).

Fungi in water can cause issues odour, trigger allergies, and even produce toxins, posing health risks, especially from toxigenic species (Hussain 2024). Studies using advanced molecular techniques have linked waterborne transmission of fungal infections, showing genetic similarities between fungi in patient samples and those in hospital water systems, highlighting a potential infection route (Hussain 2020).

Fungi thrive in hospital environments with moisture and poor sterility, like during construction or cleaning. This increases the spread of mold propagules, exposing patients to potential pathogens, especially those with weakened immune systems (Mesquita-Rocha 2014). Fungi can cause a range of illnesses, from allergies and skin infections to severe respiratory diseases and life-threatening conditions like meningitis (Srinivasan 2014).

2. MATERIALS AND METHODS

Sample Collection

Hospital wastewater samples were collected from five hospitals in Enugu State and transported to the microbiology laboratory for serial dilution and subsequent fungal isolation.

Isolation of Fungi

The fungal isolation process involved dispensing samples into conical flasks and creating serial dilutions (10^{-1} to 10^{-5}). Then, 1ml of the 10^{-5} dilution was plated on solidified Sabouraud Dextrose Agar (SDA) and incubated at 28°C for 72 hours. Pure cultures were obtained through subculturing and stored in a refrigerator at 4°C (Gupta 2018).

Fungal Identification

Fungal isolates were identified using colonial and microscopic characteristics, including colour, texture, shape, and growth patterns, referencing fungi atlas for comparison (Gupta 2018).

Germ Test Tube

The germ tube test distinguishes Candida albicans from other Candida species. It involves mixing a yeast colony with 0.5ml of serum, incubating at 37°C for 2-4 hours, and checking a drop on a slide for germ tube formation, indicating a positive result for C. albicans. (Sagar 2022).

Urea Test

Urea broth is made by dissolving 2.95g of urea powder in 150ml of water. The organism is inoculated into the broth and incubated at 37°C for 24 hours. A red-pink color indicates a positive result, while no color change indicates a negative result (Cheesbrough 2006).

3. RESULTS

Table 1: Fungi isolated from different hospital waste water.

Samples	Fungi species isolated
A	Pichia, Trichosporon, Trichoderma, Candida
B	Trichosporon, Trichoderma
C	Cryyptococcus, Trichosporon
D	Candida, Cryptococcus, Trichosporon

Table 2: Morphological and biochemical characterization of the fungi from the hospital waste water.

Isolate	Colonial morphology	Germ tube	Urease	Probable
A	White to cream Smooth colonies, spherical to sub-spherical budding blasteonidia	+	_	Candida spp
B	Special elliptical cells that reproduces asexually by multilateral budding	-	-	Pichia spp
C	White to yellow conies	-	+	Trichoderma spp
D	Mucoid or slimy in appearance with ted carotenoid pigments	-	+	Cryptococcus spp
E	White cream colonies powdery, suede-like to Farnese with radical furrows	_	+	Trichosporon spp

Table 3: Fungal counts of the hospital waste water

Sample	Fungal counts (cfu/ml)
A	$4.4x10^3$cfu/ml
B	$3.7x10^3$cfu/ml
C	$3.5x10^3$cfu/ml
D	$4.0x10^3$cfu/ml
E	$4.1x10^3$cfu/ml

4. DISCUSSION

Fungi found in hospital wastewater samples from Enugu include Candida spp, Cryptococcus spp, Trichosporon spp, Trichoderma spp, and Pichia spp, as shown in Table 1. These samples were collected from different locations.

To identify the fungi, various tests were conducted, including sugar fermentation, germ tube, and urease tests. The results in Table 2 showed all isolates except E fermented glucose and sucrose, only Trichoderma spp (isolate C) fermented lactose, isolate A (Candida spp) was germ tube positive suggesting it's likely Candida albicans, and isolates C, D, and E were urease positive, breaking down urea.

The fungal colony counts from various hospitals. Sample A had the highest count of 4.4×10^3 cfu/ml, followed by sample E, D and B. Sample C had the least count of 3.5×10^1 cfu/ml as shown in Table 3

Hospital wastewater contains various fungi like Cryptococcus, Geotrichum, Trichosporon, Trichoderma, Candida, and Pichia. Some species, like T. roseum, P. viricadum, and P. rubrum, are generally non-pathogenic but can affect people with weakened immune systems, according to (Kurtman et al. 2008).

According to Chavan (2009), over 1.2 billion people worldwide suffer from fungal infections, resulting in 1.5-2 million deaths annually – higher than malaria or brucellosis death rates. Antifungal drugs target plasma membrane, sterol biosynthesis, DNA biosynthesis, and B-glucan biosynthesis to treat these infections.

Fungi in hospital water can be a source of nosocomial infections, as reported by Warris et al. (2003), who used molecular techniques to link environmental isolates to clinical isolates, suggesting waterborne transmission in hospitals. Resistant fungi in hospital wastewater, originating from clinical wards, surgical areas, and labs, can spread antifungal resistance and infections to humans and the environment, posing long-term risks, as researched by (Lovero et al. 2017).

Candida, a type of yeast, is a major cause of skin and invasive infections, leading to significant morbidity, prolonged hospital stays, high mortality rates, and increased healthcare costs, as reported by (Sharma et al. 2020).

5. CONCLUSION

In conclusion, the isolation of human-pathogenic fungi from hospital wastewater underscores a significant and often overlooked public health risk. Hospital wastewater serves as a reservoir for a wide range of opportunistic fungal pathogens, including species of Pichia spp, Candida, Cryptococcus spp, Trichosporon, and others. These microorganisms can persist through standard treatment processes and may be discharged into receiving water bodies, creating potential exposure pathways for humans and animals. The health implications are particularly concerning for immunocompromised patients, hospital staff, and communities living near wastewater discharge points, as exposure to these fungi can result in severe respiratory, skin, and systemic infections. Adequate treatment of hospital wastewater before environmental discharge is essential to prevent the spread of fungal infections and protect vulnerable populations. Conventional treatment methods, such as sedimentation, biological treatment, and chlorination, have shown limited effectiveness against many fungal species, which often exhibit resistance to standard disinfectants. Therefore, implementing advanced treatment technologies such as ultraviolet (UV) disinfection, ozonation, membrane filtration, and activated carbon adsorption is critical for achieving higher removal efficiencies. These methods not only reduce fungal loads but also help control other resistant microorganisms, contributing to overall public health safety. Beyond technological upgrades, a comprehensive approach to managing fungal contamination in hospital wastewater must include institutional controls and policy measures. Hospitals should adopt strict waste segregation and pretreatment protocols to minimize the entry of fungal spores and mycelia into wastewater streams. Regular monitoring and surveillance programs are necessary to detect and quantify fungal pathogens, evaluate treatment performance, and identify emerging threats. Training of hospital and wastewater treatment staff on biohazard risks and safe handling procedures is equally important. Future research should focus on optimizing treatment technologies for cost-effectiveness and scalability, especially in resource-limited settings. Studies should also explore the ecology and survival mechanisms of pathogenic fungi in wastewater environments, as well as their potential for antimicrobial resistance development. Developing standardized, internationally recognized guidelines for monitoring, reporting, and managing fungal pathogens in healthcare wastewater will be vital for global health security. Then in addressing the risk of pathogenic fungi in hospital wastewater requires a multidisciplinary strategy that integrates advanced treatment, institutional vigilance, policy enforcement, and ongoing research. Only through such coordinated efforts can we effectively safeguard public health, reduce the burden of fungal infections, and ensure that hospital wastewater does not become a hidden source of disease transmission.

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