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Bacterial and fungal endophthalmitis in Upper Egypt: related species and risk factors

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ABSTRACT

Objective: To study risk factors, contributing factors of bacterial and fungal endophthalmitis in Upper Egypt, test the isolated species sensitive to some therapeutic agents, and to investigate the air-borne bacteria and fungi in opthalmology operating rooms. **Methods:** Thirty one cases of endophthalmitis were clinically diagnosed and microbiologically studied. Indoor air-borne bacteria and fungi inside four air-conditioned operating rooms in the Ophthalmology Department at Assiut University Hospitals were also investigated. The isolated microbes from endophthalmitis cases were tested for their ability to produce some extracellular enzymes including protease, lipase, urease, phosphatase and catalase. Also the ability of 5 fungal isolates from endophthalmitis origin to produce mycotoxins and their sensitivity to some therapeutic agents were studied. **Results:** Results showed that bacteria and fungi were responsihle for infection in 10 and 6 cases of endophthalmitis, respectively and only 2 cases produced a mixture of bacteria and fungi. Trauma was the most prevalent risk factor of endophthalmitis where 58.1% of the 31 cases were due to trauma. In ophthalmology operating rooms, different bacterial and fungal species were isolated. 8 bacterial and 5 fungal isolates showed their ability to produce enzymes while only 3 fungal isolates were able to produce mycotoxins. Terbinafine showed the highest effect against most isolates *in vitro*. **Conclusions:** The ability of bacterial and fungal isolates to produce extracellular enzymes and mycotoxins may be aid in the invasion and destruction of eye tissues. Microbial contamination of operating rooms with air-borne bacteria and fungi in the present work may be a source of postoperative endophthalmitis.

1. Introduction

Endophthalmitis is an inflammatory reaction of intraocular fluids or tissues. Infectious endophthalmitis is one of the most serious complications of ophthalmic surgery[1]. Endophthalmitis can occur exogenously after ophthalmic surgery, post-traumatically or endogenously[2]. Endophthalmitis, although rare, is one of the most vision-threatening complication of cataract surgery, with a frequency varying from 0.07% to 0.13%[3]. The majority of these infections are bacterial. The occurrence of fungal endophthalmitis after cataract surgery is rare[4]. Fungal endophthalmitis is usually seen as cluster infections caused by the use of contaminated intraocular irrigating solutions, intraocular lenses, ventilation system[5], and hospital construction activity[6]. While postoperative fungal endophthalmitis may be rare in the Western world, apparently it is not as infrequent in developing countries. In recent studies from India, fungi accounted for up to 21.8% of all the culture-positive postoperative endophthalmitis cases[7]. Operating theatres in developing countries often do not adhere to standards for physical parameters. Most conventional operating theatres in hospitals of such countries are equipped with window-mounted air conditioning units, mainly installed for comfort rather than for the delivery of clean air. Fungi that can cause healthcare-associated infections include *Aspergillus* spp., members of the orders *Mucorales* and *Moniliales*. Many of these fungi have the potential to proliferate in air filtration devices and air conditioning units[8]. The lack of information about endophthalmitis in Upper Egypt and its possible reasons were the motivation of this study. This work was designed to study bacterial and fungal species associated with endophthalmitis, different risk factors that predispose patients to this infection, contributing factors, and to test the isolated species sensitive to some therapeutic agents.

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Also the contamination of air in opthalmology operating rooms by bacteria and fungi was studied.

2. Materials and methods

2.1. Clinical diagnosis and sampling of endophthalmitis specimens

A total of 31 patients clinically diagnosed to have endophthalmitis were investigated. Patients were from Upper Egypt (El-Minya, Assiut, The New Valley, Sohag, Qena, Aswan) and their informed consent for vitreous sampling was obtained. All patients were admitted and treated in the Department of Ophthalmology of Assiut University Hospitals. A history was taken of circumstances in which the eye became infected, of the predisposing factors. The patients were thoroughly examined using slit-lamp biomicroscope by an ophthalmologist. Vitreous sampling was done at the pars plana, 3 mm from the limbus with 22- to 26-Gauge needle and 2 mL disposable syringe[9]. From each endophthalmitis case, a vitreous sample was taken for direct microscopic examination and culturing. The potassium hydroxide wet mount 10% and two stains were used in examining the direct smears, lactophenol cotton blue stain and Gram stain. The vitreous samples were divided into four parts and inoculated directly into the following 4 media: two for bacteria (Blood agar and Endo agar) and two for fungi (Sabouraud dextrose agar and Czapek glucose agar). The standard microbiological methods were used for identification of bacterial and fungal genera and species[10]. Bacterial and fungal isolates were given numbers and deposited in the Assuit University Mycological center.

2.2. Sampling for bacterial and fungal airospora in Ophthalmology operating rooms

Settle (gravity) plate method[11] was used to catch air-borne bacterial and fungal species after surgery in the atmospheres of four air-conditioned operating rooms at Ophthalmology Departement, Assiut University Hospitals. Four types of agar media were used: two for isolation of bacteria (Blood agar and Endo agar) and two for isolation of fungi (Sabouraud dextrose agar and Czapek glucose agar). Total bacterial catch for each bacterium and Total fungal catch for each fungus in 10 exposures was calculated per 160 plates, 30 minutes exposure each.

2.3. Screening for extracellular enzyme production by bacterial and fungal isolates of endophthalmitis origin

Eight bacterial and five fungal isolates of endophthalmitis origin were screened for their ability to produce extracelluar enzymes including protease, lipase, urease, phosphatase and catalase in solid media using different substrates and reagents[12]. The ability of these organisms to produce enzymes was measured by the clear zone around the colony (protease), the depth of visible precipitate (lipase), the color intensity (urease), the color zone around the colony (phosphatase) and O_2 bubbles evolution (catalse).

2.4. Determination of mycotoxins produced by some fungal isolates of keratitis and endophthalmitis origin

Mycotoxins were extracted using chloroform then the thin-layer chromatographic technique was applied for semi quantitative analysis of mycotoxins[13,14]. Five fungal isolates of endophthalmitis origin were tested for their ability to produce different mycotoxins (Aflatoxins, sterigmatocystin, zearalenone and trichothecene toxins). Thin layer of silica gel (type 60- F254) of about 0.3 mm thickness was prepared and poured on glass plates using an applicator. The samples were added to the plates as spots and for the purpose of separation of the different mycotoxins, solvent systems of reagents grade of the following composition were used:

Chloroform: methanol (97:3 v/v) and chloroform: acetone (9:1, v/v) for aflatoxins[13].

Toluene: acetone: methanol (50:30:20 v/v/v) for zearalenone and other fusarium toxins as described previously[15].

Benzene: methanol: acetic acid (90:1:15, v/v/v) for sterigmtocystin as described previously[14].

The plates were detected before and after spraying with the different reagents using shortwave (254 nm) and longwave UV light (365 nm). Mycotoxins were identified by comparison with appropriate reference standards[13,14,16-18].

2.5. Determination of antifungal activity

The disc susceptibility test[19] was employed using filter paper discs fully saturated with the antifungal agent (~10 μL). The antifungal activity of four types of antifungal therapeutic agents [amphotericin B(50 mg), cetrimide (powder), ketoconazole (200 mg) and terbinafine (250 mg)] against five fungal isolates of endophthalmitis origin were tested using three concentrations (0.1%, 0.5% and 1%). Amphotericin B at 0.005% concentration which is recommended for endophthalmitis therapy also used to find out if this concentration is effective *in-vitro* or not. Discs were placed on the surface of Sabouraud dextrose agar seeded with the test organism. Cultures were incubated at 28 ℃ for 48 h after which the zone of inhibition of fungal growth around discs was measured in mm and the data were recorded as the mean of three replicates. Dimethyl sulphoxide was used to dissolve the antifungal agents and also as a control in this test.

3. Results

Results showed that bacteria and fungi were responsihle for infection in 10 and 6 cases of endophthalmitis, respectively. Only 2 cases produced a mixture of bacteria and fungi whereas 13 were negative for microbial cultures (Table 1). *Enterobacter* species was the most common bacterial agent involved in 3 (30%) out of the 10 bacterial endophthalmitis cases followed by *Staphylococcus* species which was reported from 2 cases (20%), while *Aspergillus fumigatus* (*A. fumigatus*) (Figure 1,2) and *A. terreus* (*A. terreus*) were the dominant species associated with fungal endophthalmitis involved in 3 and 2 out of the 6 fungal cases, respectively (Table 1).

Trauma was the most prevalent risk factor of endophthalmitis where 58.1% of the 31 cases were due to trauma. Cases of trauma due to unknown factors and foreign bodies were represented by 55.6%, 44.4% of trauma cases and 32.3%, 25.8% of the total cases, respectively. Previous ocular surgery came second as a risk factor with 41.9% of the 31 cases, including cataract surgery which accounted 29.0% of