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Original article

Fungal profile of otomycosis in a sample of Egyptian patients in Zagazig university hospitals: A prospective study

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ABSTRACT

Background: Otomycosis has been described as fungal infection of the external auditory canal with infrequent complications involving the middle ear. Otomycosis is challenging for both patients and otolaryngologist as it frequently requires long term treatment and follow up. Inspite of proper treatment and follow up, the recurrence rate remains high. The aim of current study is to detect the most common fungi in otomycosis patients and determine its risk factors. **Methods:** The study included 194 patients clinically diagnosed with otomycosis. Each swab is subjected to direct microscopic examination with 10 % KOH and culture on Sabroud's dextrose agar (SDA) and blood agar. Filamentous fungal growth is identified by macro- and micro morphological characteristics. Yeast like growth is identified by (API 20C) AUX. **Results:** The mean age ±SD of the patients was 24.18± 15.9 years. Males (n=106,54%), were more vulnerable to otomycosis than females (n=88, 45%). The most common presenting symptom was otorrhea and pruritus (n= 78 ,40%) with unilateral involvement being more common. The most common predisposing factor is self-cleaning (n=84 ,43%) followed by topical antibiotic ear drops with steroid use (n=30,15%). *Aspergillus fumigatus was* the most common fungus causing otomycosis in this study (n=70, 36%) followed by *Candida tropicalis* (n=28,16%). Positive fungal cultures were observed in 180 specimens (90%). **Conclusion:** Otomycosis was common in people with the bad habit of self -cleaning with unsterilized objects and using unnecessary steroid containing ear drops. *Aspergillus fumigatus* and *Candida tropicalis* were the most prevalent isolated fungi in otomycosis patients.

Introduction

Due to constant exposure of the ear to atmospheric biotic elements, it becomes easy to access numerous microorganisms, such as fungi, which contribute to inflammation as well as fluid production within the ear [1]. Such fungal ear infection was first identified by Andrall as well as Gaverret in 1843 in addition to Mayer in 1844 [2].

Numerous of fungi are found to be in soil or sand comprising decomposition vegetative matter. Such fungi are subjected to rapid dehydration in tropical area, whereas they were blown as small dust particles in wind. Fungal spores found in air are borne by water vapors that lead to a higher infection rate when relative humidity (RH) is elevated to 80% [2, 3].

Local lesions such as congestion found in otitis externa have a great impact on increasing both vascular permeability and temperature, which are considered as preferable circumstances to grow fungi and develop mycoses throughout external as well as middle ear [4].

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Otomycosis is a superficial fungal infection of the external ear canal that is frequently seen in otolaryngology practices. It is a worldwide disease but is common in tropical and subtropical zones and more prevalent in monsoon season [2,5].

Although rarely otomycosis may be a life-threatening condition, in immuno-compromised patients it can lead to skull base osteomyelitis along with multiple cranial nerve palsies with serious mortality and morbidity. The disease is challenging and frustrating for both patients and otolaryngologists as it frequently requires long term treatment and follow up [6].

In recent years fungal infections have gained more attention in medicine due to large number of immunocompromised patients however, these fungi may also yield infections in immunocompetent hosts. *Aspergillus niger* and *Candida* species are the most prevalent fungi in immunocompetent patients. In immunocompromised patients' other fungi can cause otomycosis like Penicillium, Mucor, Rhizopus, and Scopulariopsis [7].

An increase in otomycosis incidence has been observed because of increased dominance of comorbid settings such as diabetes mellitus, malignancies in addition to ear buds utilization, use of topical antibiotics- steroid combination and bad aural hygiene (instilling oil and water in the ear) [8].

It is important to vigorously treat otomycosis in immunosuppressed patients in order to decrease complications such as hearing loss, tympanic membrane perforations and invasive temporal bone infection. Fungal cultures are essential to confirm the diagnosis [5].

This study was conducted to detect the most common fungi in otomycosis patients and determine its risk factors.

Materials and Methods

Design of the study

This study is a prospective cross-sectional study has been carried out in Outpatient clinic of Otorhinolaryngology Department of Zagazig University Hospitals, Egypt, and Medical Microbiology and Immunology department, faculty of medicine, Zagazig university from March 2018 to August 2019. One hundred and ninety-four (194) patients clinically diagnosed to have otomycosis infection were included in this study. All patients were informed regarding the study, and prior to the ear swab, consent was granted.

Sample collection

Derived from external auditory ear canals, debris, or exudate samples of 194 patients (2 swabs from each patient, one for microscopic examination, the other for culture) having otomycosis based on clinical diagnosis were subjected to mycological analysis. These samples were obtained from the external auditory canal controlled by aseptic settings by means of sterile cotton swab and directly sent to mycology lab for direct microscopic examination and fungal culture for confirmation of diagnosis. For diagnosis of otomycosis, detailed history (age, gender, residence, occupation, duration of the disease, laterality and aural symptoms), clinical examination, otoscopic results as well as fungus laboratory identification were measured [9].

Microscopic examination and culture

All samples were subjected to microscopic examination using 10% KOH for screening of fungal elements (hyphae and/or arthroconidia). Two to three drops of the KOH were kept on a grease-free, clean glass slide. The sample was placed in the KOH drops on the slide, and a clean cover slip was placed on the sample and pressed to prevent the formation of air bubbles [10].

The swab for culture was employed for SDA (Himedia,India) as well as blood agar (BA) inoculation for an incubation period at 25 °C for SDA plates up to 4 weeks and examined for growth every three days.Besides, BA plates lasted for (24-48hs) incubation at 37 °C. The culture was considered negative, in the absence of any growth after 4 weeks [10].

Fungal isolate identification

For suspected positive cultures of filamentous fungi **(Figure 1,2)**, the growth was examined both macroscopically (color of the surface and reverse, topography, and texture) and microscopically with Lactophenol cotton blue stain (LPCB) either using scotch tape or tease preparation [11]

For suspected yeast and yeast like growth **(Figure 3,4)**, Gram stain was done, and yeast identification was done by API 20C AUX (bioMérieux, France) which consists of 20 microtubes containing dehydrated substrates in which 19 assimilation tests are performed. The kit was utilized consistent with guidelines supplied by the manufacturer. Strips' reading was achieved following two- and three-days incubation period at 30 °C. After inoculation and incubation, the reactions are interpreted by comparison to growth controls and use of the Identification Table provided with each kit.

Statistical analysis

Data was entered for analysis with Statistical Package for Social Sciences (SPSSTM) software version 16. Descriptive statistics was presented as frequencies, percentage, mean and standard deviation (SD). Categorical variables were analyzed using Chi Square test. p-value less than 0.05 considered statistically significant.

Results

In this study, 194 patients clinically diagnosed with otomycosis were involved. 200 samples were collected (as some had bilateral affection). The mean age of included patient group 24.18± 15.9. Males accounts for (n=106,54%), and female (n=88,45%) with male to female ratio 1.3:1.

There is right ear (n=111,57%) affection more than left ear (n=77,29%), while bilateral involvement being only (n=6, 3%). **Table 1** presents a description of the predominant symptoms observed only or in combination in the sample group. Pruritis and otorrhea (40%) was the most common complaint.

By analysis of risk factors, self -cleaning was the most common risk factor 84(43%), followed by use of topical antibiotic ear drop (with steroid) 30(15%). patients with chronic suppurative otitis media (CSOM) accounts for (10%). the most common underlying morbid conditions associated with the patients is diabetes mellitus which accounts for (15%),

Regarding occupation of studied patient group, it was observed that otomycosis most commonly occurred in people whose occupation related to the field of agriculture 110 (57%) followed by engineers 10 (5%) and most affected people were rural residents 120(62%) **(Table 2)**.

Microscopic examination of the samples by 10% KOH were positive for fungal elements in 80 (40 %). Out of 200 samples included in this study fungal growth was positive in 180 (90%) **(Table 3)**.

The most common isolated fungi were *Aspergillus spp* 115(64%), followed by *Candida spp*. 60 (33%). *Aspergillus fumigatus* 70 (39%) was the most common isolated species in *Aspergillus spp.* and *Candida tropicalis* (16%) in candida spp **(Table 4) (Figure 5)**.

Pruritis and otorrhea (40%) was the most common complaint in our study. It was the most common symptom in patients with self-cleaning and also in patients with topical antibiotic ear drops users. However, pruritus only was the most common presenting complaint in the patients with self-cleaning. *Aspergillus fumigatus (*n=40, 48%) and *Aspergillus niger* 20(24%) were common in self-cleaning group, *Aspergillus fumigatus* 8 (27%) and *C.tropicalis* (n=8, 27%) were common in topical antibiotic ear drop use **(Table 5)** .

Figure 1. SDA plate showing growth of *A.fumigatus.*

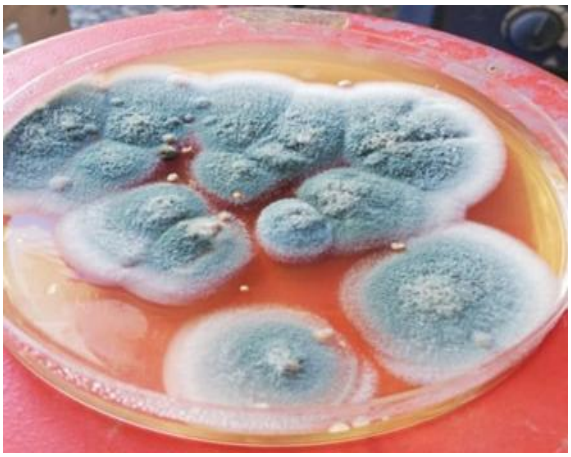

Figure 2. Microscopic appearance of *A.fumigatus.*

Figure 3. Gram stained film showing the microscopic appearance of *C. glabrata*

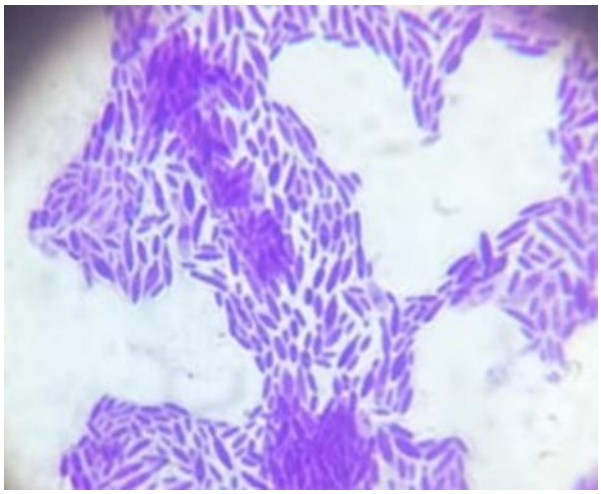

Figure 4. SDA plate showing growth of *C. albicans*

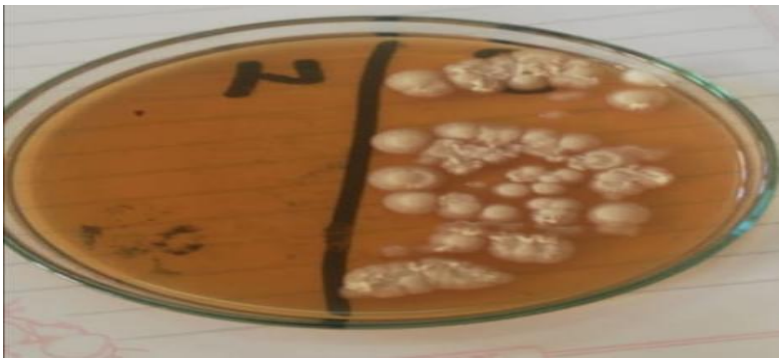

Figure 5. Frequency distribution of candida species isolated from otomycosis patients included in the study.

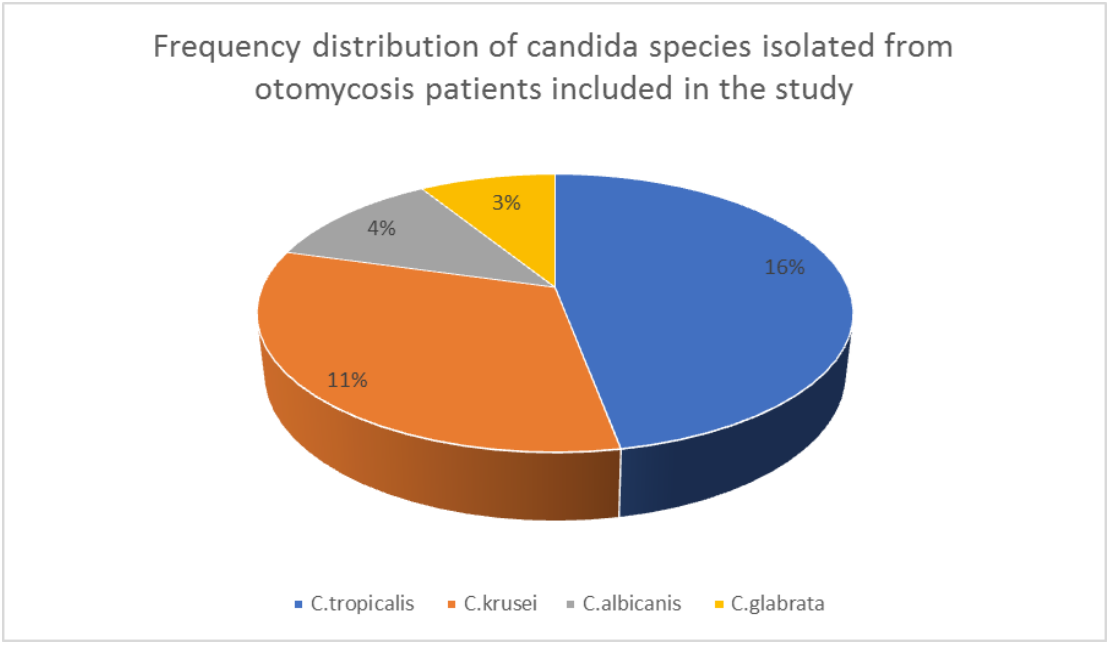

Table 1. Clinical characters of otomycosis patients included in the study (n=194).

Variable	Frequency n (%)
Presenting symptoms	
• Pruritis and otorrhea • Pruritis only • Otorrhea only • Earache • Tinnitus • Hearing loss	• 78 (40%) • 48 (25%) • 30 (15%) • 18 (9%) • 13 (7%) • 7 (4%)
Laterality distribution	
• Right ear • Left ear • Bilateral	• 111(57%) • 77(37%) • 6 (3%)

Table 2. Risk factors, occupation, residency of otomycosis patients (n=194).

Risk factor	Frequency n (%)
Self- cleaning	84(43%)
Topical antibiotic ear drops (with steroid)	30(15%)
CSOM	20(10%)
Swimming	10 (5%)
Underlying comorbid conditions • Diabetes • Chronic renal disease • Malignancy	 • 20(15%) • 18 (9 %) • 12 (6%)
Occupation • In agricultural field • Engineer • Medical field • Teacher • Others	 • 110 (57%) • 10 (5%) • 8 (4%) • 8 (4%) • 58 (30%)
Residency • Rural areas • Urban areas	 • 120(62%) • 74(38%)

Table 3. Association of fungal growth with predisposing factors (n=194).

Fungal growth	Self-cleaning	Topical antibiotic ear drops (with steroid	CSOM	Swimming	Diabetes	Malignancy	Chronic renal failure	Statistics
yes	79(94%)	24(80%)	20(100%)	10	17(85%)	9(75%)	16(89%)	X2 =5.789,
No	5(6%)	6(20%)	0(0%)	0(0%)	3(15%)	3(25%)	2(11%)	dif = 2, *p = 0.055*

Table 4. Frequency of isolated fungi from otomycosis patients positive for culture (n=180).

Isolated fungi	Frequency n (%)
Aspergillus spp. • *Aspergillus fumigatus* • *Aspergillus niger* • *Aspergillus flavus*	115 (64%) • 70 (39%) • 35 (19%) • 10 (5%)
Candida spp. • *Candida tropicalis* • *Candida krusei* • *Candida albicans* • *Candida glabarata*	60 (33%) • 28 (16%) • 20 (11%) • 7(4%) • 5(3%)
Zygomycetes	2 (1%)
Mixed growth	3 (2%)
No growth	20 (10%)

Table 5. Frequency distribution of predisposing factors with fungal growth and clinical presentations.

Fungal organism	Self-cleaning	Topical antibiotic ear drops (with steroid	CSOM	Swimming	Diabetes	Malignancy	Chronic renal failure
Aspergillus fumigatus	40 (48%)	8 (27%)	6(30%)	5(50%)	3(15%)	3 (25%)	5(28%)
Aspergillus niger	20(24%)	3(10%)	5(25%)	2(20%)	2 (10%)	1 (8%)	2(11%)
Aspergillus flavus	2(2%)	2(7%)	2(10%)	1(5%)	0(0%)	3(15%)	2(11%)
Candida tropicalis	6(7%)	8(27%)	3(15%)	2(20%)	3 (15%)	3(25%)	3(17%)
Candida krusei	5(6%)	3(10%)	3(15%)	2(20%)	4(20%)	1(8%)	2(11%)
Candida albicans	3(4%)	0(0%)	1(5%)	(0%)	1(10%)	11(37%)	2(10%)
Candida glabarata	0(0%)	0(0%)	0(0%)	2 (20%)	1 (5%)	0(0%)	2(11%)
Zygomycetes	0(0%)	0(0%)	0(0%)	0(0%)	2(11%)	0(0%)	0(0%)
Mixed growth	3(4%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)
No growth	5(6%)	6(20%)	0(0%)	0(0%)	3(25%)	3(25%)	3(11%)
Symptoms							
Pruritis and otorrhea	20(24%)	15(50%)	15(75%)	10(100%)	9(45%)	6(50%)	3(17 %)
Pruritis only	27(32%)	10(33%)	5(25%)	0(0%)	3(15%)	2 (17%)	1(6%)
Otorrhea only	13 (15%)	5 (17%)	0(0%)	0(0%)	4(20%)	2 (17%)	6(33%)
Earache	10(12%)	0(0%)	0(0%)	0(0%)	2(10%)	2 (17 %)	4(22%)
Tinnitus	9(11%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	4(22 %)
Hearing loss	5(6%)	0(0%)	0(0%)	0(0%)	2(10%)	0(0%)	0(0%)

Table 6. Isolated fungi in different previous studies.

Author	year	*A.niger*/*A.fumigatus*/*A.flavus*	*Candida spp.*	*Zygomycetes*	*Scedosporium*	Species unidentified
Jaiswal SK [27]	**1990**	34/-/-	46	12	-	8
Jain and Agarwal SC [29]	**1992**	56.3/15.6/4.7	6.3	6.3	-	3
Kaur R et al. [26]	**2000**	36.9/41.1/1.4	13.7	4.1	-	1.4
Pradhan B et al. [24]	**2003**	25.5/6.6/37.7	10.4	-	-	-
Fasunla J et al. [20] **(Nigeria)**	**2008**	48.35/33.96/5.43	12.26	-	-	-
Pontes ZBVDS et al., [12] **(Brazil)**	**2009**	20/5/10	55	-	-	10
Aneja KR et al. [25]	**2010**	39.8/12.9/16.6	10.2	-	-	-
Barati B et al. [30] **(Iran)**	**2011**	41.6/5.5/49	7.6			0.9
Vishwanatha B et al., [3]	**2012**	56/18/-	16	-	-	-
SuShil and Kirti [1]	**2019**	15.5/20/20	22.6	2.2	2.2	-
Present study		39/15/5	23	1	-	-

Discussion

Otomycosis is defined as superficial fungal infection that affects external auditory canal, it is a common condition encountered by otorhinolaryngologists [12]. This paper aimed to study the most prevalent fungi in otomycosis patients

also studying its predisposing factors and their association.

In our study, fungal infection impact was found to be higher in males than females, since more time is spent by males outdoors resulting in high amenability to more fungal spores' exposure. The outdoor air is well recognized to be significant vector for locally widespread fungal flora. Other previous studies had shown different incidences in males as achieved by **Than and co-workers** (58%), **Kaur et al.** (60%), and **Ho et al.** (56%) [13-15].

Unilateral involvement was identified in 94% in the current study, consistent with the work achieved by **Aggarwal and Jaiswal**, showing unilateral involvement as of 92% [1]. **Ho and his colleagues** detected a bilateral involvement found among 7% of the patients, which is higher than our study where we stated that 3% of patients have bilateral otomycosis [15].

It is observed that otomycosis commonly affected farmers and people who work in the field of agriculture 110 (57%), moreover the rural residency appeared to be more common than urban residency in otomycosis patients 120 (62%), this could be attributed to the high prevalence of fungi in agricultural areas and and colonization of manmade substrata by saprophytic mold, the dust play an important rule also, as high content of suspended dust particles in the air that carry the fungal spores can be blown in the wind to be transmitted to various places specially in the rainy season[16].

As mentioned in the literature, the common symptoms of otomycosis are itching, ear discharge, ear pain, blocking sensation, decreased hearing and tinnitus [2,5]. In this study the most common presenting symptom was otorrhea and pruritis (40%), followed by pruritis only (25%). Another previous study done by **Aggarwal and Jaiswal** detected that the most common symptoms was otorrhea (34%) followed by tinnitus (22%) [1].

In our study, the most common predisposing factor was self-cleaning (34%). The routine to clean ear by matchstick, feathers, or potential contaminated fingertips is well-notorious for fungal spores' inoculation and development, particularly on the wet external auditory canal in patients using oil on the area. The germination of spores and fungal conidia are triggered by excessive cerumen in some patients featuring poor personal hygiene [8].

Chronic suppurative otitis media (CSOM) is another risk factor in our study, which is defined as a chronic inflammatory condition of the middle ear and mastoid cavity, which results in recurrent otorrhoea due to tympanic perforation. It is an important cause of preventable hearing loss in the developing world [17]. Common fungal agents causing secondary infection in CSOM are *Candida spp*., *Aspergillus spp.*, *Rhizopus spp*., and *Penicillium spp* [18,19]. In our study 10% of the patients gave history of CSOM.

Other conditions like moisture, warmth, as well as acidic pH of the external auditory canal offer typical medium of growth for fungi. Swimming was also a influencing element in current research, analogous to other previous studies done by **Fasunla et al., Ozcan et al.** and **Paulose et al.** [20-22].

Previous literatures showed that among the fungal isolates, *Aspergillus niger* as well as *Candida* were the most widespread species responsible for otomycosis globally but other causative isolated fungi species have been belonged to genera *Penicillium*, *Fusarium*, *Mucoraceae*, *Scopulariopsis*, *Alternaria*, *Malassezia* in addition to various dermatophytes [12,23].

In our study growth was seen in 90% of cases, which correlates well to study done by **Bhuwan et al.,** where the fungal growth in their study was (94%) [8]. In another study done by **Pradhan et al.** [24], they recorded positive fungal culture in (81.3%) of cases.

In the current research, we identified 64% of Aspergillus species, in correlation with the work by **Satish et al.** (54%), in addition to **Aggarwal and Jaiswal** (60%) [1,2].

In our work, the most prevalent isolated fungus was Aspergillus (64%) with predominance of *Aspergillus fumigatus* (39%). Nevertheless, in the case of the study done by **Aneja et al.** [25] the most common species was *Aspergillus niger* (43.31%).

Otomycosis correlated to *Candida* is often described by culture data due to its difficulty in diagnosis clinically as lacking of a characteristic appearance such as Aspergillus, existing as otorrhea without response to aural antimicrobials [24,26], and the discharge colour from the ear as well as laboratory identification may also assist for the sake of potential identification regarding the disease agent [27]. Generally, the creamy or white discharge may be because of *Candida* genus, and black discharge may be because of Aspergillus genus [28].

Candida spp. accounts for (33%) with more prevalent species is *C. tropicalis* (16%) in our study that is well correlated by a study done by **Aggarwal**

and Jaiswal where *C.tropicalis* accounts for (22.2%) [1].

This study showed a discrepancy to isolate fungi in comparison to different studies which can be explained by geographical distinction **(Table 6)** [1,3,12,20,23-27,29,30].

Conclusion

The study concluded that otorrhea and pruritis were the most widespread presenting symptom of otomycosis. Self-cleanings with contaminated objects and unnecessary topical antibiotic ear drops (with steroid) were the most common predisposing factors with *Aspergillus fumigatus* and *C.tropicalis* being the most prevalent isolated fungi from otomycosis.

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