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Presumed diagnosis: Otomycosis. A study of 451 patients

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Abstract: Otomycosis is a common disease. We have tried to clarify the different causative factors for otomycosis in our environment. 451 patients with a presumed diagnosis of otomycosis were included in our study. The patients were referred to us by ENT specialists and by family doctors; the diagnosis was only confirmed microbiologically in 24.43% and 16.16% respectively. *Aspergillus spp* and *Candida spp* were the most common fungal pathogens found. The high frequency of *Aspergillus niger* may be linked to the different sample extraction methods. The abundance of *Candida parapsilosis* in the samples that came from family doctors is believed to be a result of an inappropriate use of topical antibiotics, which led to fungal proliferation. We conclude that the predisposing factors for the development of otomycosis could be avoided or treated. Treatment with antifungal agents is not enough to ensure a complete recovery; treatment should be aimed at restoring the physiology of the external auditory canal.

Keywords: *Otomycosis. Diagnosis. Microbiology. Epidemiology.*

INTRODUCTION

Fungi, (a word of Latin origin), are eukaryotic organisms, spore carriers, that feed by absorption, lack chlorophyll and reproduce both sexually and asexually. Otomycosis or external otitis fungi are acute, subacute or chronic infections produced by yeasts and filamentous fungi that affect the squamous epithelium of the external auditory canal (EAC)[1].

It has been estimated that cases of otitis externa make up between 5 and 20% of all otological consultations; the etiology of the majority is bacterial, only 15-20% are attributed to fungi. Mixed infections are generally scarce as fungal flora tends to inhibit the bacterial kind[2,3].

Fungal external otitis (otomycosis) is a common pathology throughout the world. Its frequency varies according to different geographic zones, in relation to environmental factors (temperature, relative humidity) and the time of year[4]. A greater proportion of cases has been recorded in rural environments.

The fungi that produce otomycosis are generally saprophytic fungi species that abound in nature[5] and that form a part of the commensal flora of healthy EAC. These fungi are commonly *Aspergillus* and *Candida*[6]. *Aspergillus niger* is usually the predominant agent although *A. flavus, A. fumigatus, A. terreus* (filamentous fungi), *Candida albicans* and *C. parapsilosis* (yeast-like fungi) are also common.

Otomycosis is sometimes difficult to manage due to different factors related to the microorganism and to the local and general characteristics of the patient[7]. In the last few years the participation of fungi in external otitis has increased, mainly because of the use of broad-spectrum antibiotics for the treatment of bacterial otitis and to factors relating to changes in immunity.

The disease we studied is closely connected to the histology and the physiology of the EAC. This 2.5cm long, 7-9 mm wide cylindrical canal is lined with a stratified keratinized squamous epithelium that continues along the external face of the tympanic membrane. Its interior tympanic recess, medial to the isthmus, tends to accumulate remains of keratin and cerumen; it is a difficult area to clean. The skin changes its morphology as it progresses from lateral to medial, being at its thickest in the cartilaginous canal (0.5 to 1 mm) and very thin (30 to 50 micras) in the bony canal (the internal third). The external segment, besides being more adherent, is hairy and has sebaceous and ceruminous glands. The glandular secretions mix with flaked cellular epithelial elements to form an acidic ceruminous substance, impermeable to water, that protects the skin of the canal.

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Cerumen has antimycotic and bacteriostatic properties and is insect-repellent. It is composed of lipids (46 to 73%), proteins, free amino acids and mineral ions; it also contains lysozyme, immunoglobulins and fatty polyunsaturated acids. The long-chain fatty acids present in unbroken skin probably inhibit bacterial growth. Because of its hydrophobic composition, cerumen is capable of repelling water, making the surface of the canal impermeable and thus avoiding maceration and epithelial damage[8].

The flora found in the EAC is normal or commensal, made up of a series of microorganisms among which a great variety of bacteria is included: *Staphylococcus epidermidis, Corynebacterium spp, Bacillus spp,* Gram-positive cocci (*Staphylococcus aureus*. *Streptococcus spp*, non-pathogenic *micrococci*), Gram-negative bacilli (*Pseudomonas aeruginosa, Escherichia coli, Haemophilus influenzae, Moraxella cararralis* etc) and mycelial fungi of the *Aspergillus* genus or yeast-like fungi, particularly *Candida spp.* This commensal flora is not pathogenic as long as the balance between bacteria and fungi is maintained[3].

The depth of the EAC cul de sac possesses many of the requirements necessary for fungal growth: humidity, temperature, substrate (proteins and carbohydrates) and pH 5-7. The fungal colonization of the EAC is not permanent: variations are detected when patients and healthy people are studied over time, that is to say, there are temporary colonizations that are probably related to environmental or local changes.

Various factors influence the transformation of saprophytic fungi into pathogenic fungi, but they are not completely understood. All of the following factors have been considered to encourage infection: changes in the epithelial covering (dermatological diseases, microtraumas), an increase in the pH level of the EAC (bathing), qualitative and quantitative alteration of the cerumen (bathing), systemic factors (alterations in immunity, debilitating diseases, corticoids, antibiotics, cytostatics, neoplasia), environmental factors (heat, excessive humidity), a history of bacterial otomycosis, chronic secretory otitis media (CSOM), postsurgical mastoid cavities or instillation in the ear of oily substances or broad-spectrum antibiotic therapy, etc.

The objective of this study is to analyze the different epidemiological, clinical and microbiological aspects of this disease within the context of the cases that arose over a year in an urban environment with approximately 260,000 inhabitants in a city with an extreme continental climate.

MATERIAL AND METHODS

We carried out a retrospective epidemiological, clinical, and microbiological check-up of patients who had an initial diagnosis of otomycosis and who attended the West Valladolid Health Center during the year 2003. The aforementioned clinic has a catchment of approximately 260,000 people. The sample of 451 patients that were included in the study came from Health Centers (HC), where they were treated by General Practitioners (GP), and from the clinic of the ENT Department in the University Hospital of Río Hortega (UHRH), where they were treated by ENT specialists. This study was conducted with the consent of the *Comité de Ética e Investigación* (Ethics and Research Committee) of the University Hospital of Río Hortega/UHRH.

The material used in this study consists of the relevant medical histories and otic samples. The information about the otic samples was obtained from the database of the Microbiology laboratory of the UHRH. The otic samples were those collected in the West Valladolid Health Area; as was mentioned earlier, the data of 451 patients was collected. The majority of the patients live and work in an urban setting. The medical histories studied only include the ones in which the diagnosis of otomycosis was confirmed microbiologically: 102 in number.

The otic samples taken by the ENT specialists (UHRH) were obtained under microscopic analysis using a cotton wool swab (torunda) and were sent, by sterile means of transport, to the Microbiology laboratory for their bacterial and mycological study. The samples that came from health centers were sent to the same laboratory, although they were not collected under the microscope.

All the medical samples were processed as soon as possible to avoid overgrowth of the commensal saprophytic flora and the deterioration of the pathogenic flora. When immediate processing was not possible, the samples were conserved between 2ºC and 8ºC prior to being processed. When refrigeration was necessary it was never used for more than 48 hours.

The samples received in the laboratory were sown in culture media for fungi: Sabouraud- chloramphenicol agar (a selective medium that incorporates chloramphenicol to inhibit bacterial growth), blood agar and chocolate agar; and occasionally in Czapek agar, potato glucose agar, potato carrot agar, corn flour agar and malt extract agar: media in which fungal characteristics are better observed. The placa petri dish for mycological study was incubated in a heater at 30ºC (in contrast to the petri dishes used for bacterial study which were incubated in a CO_2 heater at 37ºC).

Once growth in the dish had been detected we went on to identify the fungus. The morphology of the colony enabled us to distinguish between yeast-like and filamentous fungi. The majority of white creamy, smooth or rough colonies are yeasts or, very occasionally, the yeast-like phase of dimorphic fungi.

Filamentous fungi tend to grow forming dusty, hairy, woolly, velvety or folded colonies that display a wide range of colors such as white, yellow, green, greenish blue, off-black, etc.

After the morphological identification of the yeasts, a filamentation test was done; this consists of emulsifying a colony of the presumed yeast with human serum and incubating it in a heater at 37ºC for three hours. Immediately after this 3-hour period, the formation of a germinative tube or a filamentous extension of the yeast -without narrowing at the origin and characteristic of *C. albicans* - was observed under the microscope. If these structures were not correctly seen or if they were uncertain, the identification of other species of yeasts, different to *C. albicans*, was done using the automatic system (bioMérieux's Vitek 2) based on the detection of the fungal metabolism by means of a series of biochemical tests or using identification methods that study the yeast's nutritional characteristics, (fermentation and assimilation of carbon compounds), such as bioMérieux's API 20C AUX.

The identification of the filamentous fungus was done using the adhesive tape technique, starting with the colony grown in the middle of the culture. The tape was placed at one edge of the culture dish, covering the fungal colony, pressing it down firmly so that the air mycelium stuck well to the tape and then the tape was gently pulled away. This adhesive tape was then put on top of a drop of mounting fluid (blue lactophenol or lactofuchsin) on a slide. The microscopic display allows us to observe fungal structures such as conidiophores, aspergillar heads, phialides, metulas, conidial disposition, etc: characteristics of the different species of filamentous fungi.

The medical histories of the 102 patients with confirmed otomycosis were reviewed, assessing data such as sex, age, associated disease, systemic or prior topical otological medication, history, and the presence of prior otological pathology that would change the normal anatomy of the ear.

If a patient had a history of CSOM, the otological situation and the existence of nasal respiratory insufficiency were evaluated. The number of treatments required for the complete recovery from otomycosis (defined as one month without disease or microscopic findings of fungal growth) was included in the study. The treatment used was aspiration and cleaning of the EAC, with topical mercurochrome being administered in each treatment. The patients continued treatment at home with 1g per 100ml of topical ciclopirox olamine solution administered every 12 hours and with each patient's clinical situation being reviewed 5 days and 30 days after treatment. In the event of a relapse, the cycle was repeated until achieving the complete disappearance of the colonies, which was confirmed microscopically.

RESULTS

1. Distribution of positive results by origin

451 otic exudates were processed: 99 (22%) from family doctors' offices (FD) and 352 (78%) sent by the ear, nose and throat specialists (ENT). Considering them as a whole, 22.6% of samples were positive (102 in number), confirming the clinical diagnosis of otomycosis; the remaining 77.4% proved negative. 5 samples that appeared to be contaminated were excluded (Table 1).

The percentage of positive otic samples that were sent by family doctors and that confirmed the clinical diagnosis of otomycosis was 16.16%; the percentage of positive results for samples that came from the ENT clinic was 24.43%. As we mentioned above, 102 otic exudates of the 451 samples turned out to be positive.

2. Distribution of patients by age and sex

The average age of patients with a negative result was 54. The average age of patients with a positive result was 46 (18-80 years old). In regard to the sex of the patients, of the 102 that proved to be positive, 57 (55.8%) were male and 45 (44.2%), female. Of the 349 that had a negative result, 193 (55.3%) were male and 156 (44.7%), female.

3. Distribution of samples received according to the season of the year

The samples were received throughout the year with the following distribution: 21% in winter, 28% in spring, 17% in summer and 34% in the fall.

4. Distribution of cases according to previous otological alterations. Types of pathology. Frequency of chronic systemic pathologies

The patients who had positive otic cultures were assessed to see whether or not they had had previous otological pathology. Of the positive samples that came from the ENT clinic (84.3% of the positive samples), 60.56% had not had previous otological pathology. The average age of the patients without otological pathology was 42 years, the proportion between the sexes being: 47 men and 39 women.

Table 1: Distribution of positive results by origin

	HC	ENT	Laboratory
Positive	16 (15.7%)	86 (84.3%)	102 (22.6%)
Negative	83 (23.8%)	266 (76.2%)	349 (77.4%)
Total	99	352	451

39.54% of the positive samples belonged to patients who, prior to the otomycosis, had had otological alterations which were classified as follows: CSOM (64%), radical cavity (18%), atticotomy or antroatticotomy (9%), cholesteatoma (3%) and others (6%). The patients with chronic OM (64%) had central perforations of the pars tensa distributed among the following locations: subtotal (59%), posterior (32%), anterior (9%). Chronic nasal respiratory insufficiency, with or without polyposis, was listed in the medical histories of 40.9% of patients with chronic OM. 22% of these patients with nasal alterations had been operated on with different rhinological techniques.

The only related chronic systemic pathology that stands out does so resoundingly: 8% of the patients with a confirmed diagnosis of otomycosis suffered diabetes mellitus. 10% of the patients with otomycosis had received prior oral or parenteral antibiotic treatment for different reasons.

5. Distribution of the patients treated topically. Antibiotic administered.

98% of the patients that attended the ENT clinic had been treated on one or more occasions with topical antibiotic therapy. Out of all of them, according to the data presented in their medical histories, 49% were treated with topical ciprofloxacin and 21% with aminoglycosides. In the remaining 30%, the prescribed course of treatment was either not mentioned, or a miscellany of products figured as part of a fairly long-winded medical history.

6. Distribution of the fungal flora isolated in patients with previous otological pathology

In patients with otological pathology, *Aspergillus* was the genus most often isolated, with 57 cases representing 61% of the positive samples. The species isolated, in order of frequency, were: *Aspergillus flavus (AFL),* 26 cases (28%); *Aspergillus niger (ANI),* 16 cases (17%); *Aspergillus spp (ASP),* 9 cases (10%) and *Aspergillus fumigatus (AFU),* 6 cases (6%). The genus *Candida* was the second most common with 31 cases (33%), with the species *Candida parapsilosis (CPA)* representing 21 cases (22%) and *Candida Albicans (CAL),* 10 cases (11%). The remaining 6% were various fungi such as *Penicillium spp (PNC),* 4 cases (4%); *Paecilomyces spp (PCY)* 2 cases (2%) and there were no cases of *Candida tropicalis (CTR)* or *Candida Lusitaniae (CLU)* (Figure 1).

7. Distribution of isolated fungal flora in patients with otological pathology

a) Patients diagnosed by ENT specialists

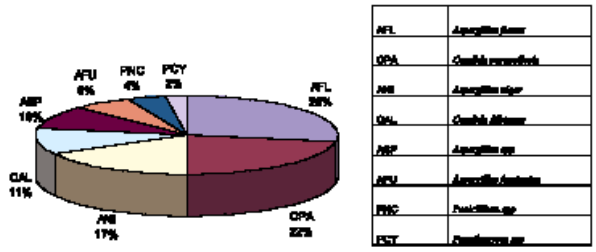

Figure 1. Distribution of the fungi isolated in patients with otologic pathology.

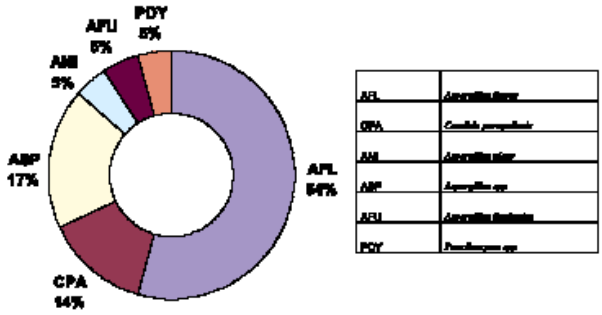

Figure 2. Fungi isolated in perforated chronic OM.

In positive samples from patients without previous otological pathology and diagnosed by ENT specialists , *CPA* was the most commonly isolated fungus species (34%). However, with respect to genus, *Aspergillus* predominated (48%) (Figure 3).

b) Patients diagnosed in Health Centers

In the positive otic samples of patients from health centers, the genera *Candida* and *Aspergillus* were isolated in equal proportions (44%) . *ANI* stands out (32%) as the species most commonly isolated (Figure 4).

8. Relationship between pathologies and duration of treatment

Two check-ups were performed on average in the ENT clinic during the follow-up of the patients without previous otological pathology. An average of one recurring otomycosis episode over the last three years was recorded.

However, in those that had had otological pathology, the average number of check-ups was 4, reflecting an average of 2 recurring episodes in the medical history of the patient in the past three years.

The relationship between the otological pathology of the patient and the duration of treatment was evaluated. The most persistent situation was with the patients who developed otomycosis and suffer from CSOM and nasal respiratory insufficiency (NRI); amongst this group, the average number of necessary annual treatments was close to 9.

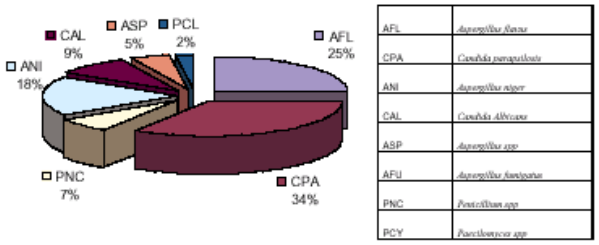

Figure 3. Fungi isolated in otomycosis diagnosed by the ENT.

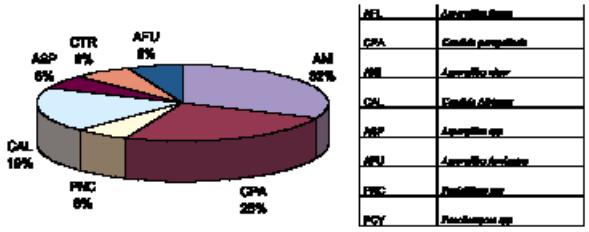

Figure 4. Fungi isolated in perforated chronic OM.

DISCUSSION

The low percentage of confirmed diagnoses of both the patients seen by family doctors as well as those treated by ENT doctors is a fact worth highlighting. Amongst the health-center patients, the family doctors only achieved 16.16% of confirmed diagnoses whereas 24.43% of the presumed diagnoses made by ENT specialists were confirmed. In the paper by Kaur et al[9], the development of fungi in the cultures was confirmed in 71 of the 95 patients diagnosed clinically. Yavo et al[10] found positive cultures for fungi in the samples of 49 out of the 115 patients (42.6%) . Pradham et al[6] obtained microbiological confirmation in 87 specimens out of the 107 ears diagnosed with presumed otomycosis (79.4%). We believe that the low number of correct diagnoses is due to the similarity that exists between the symptoms of otomycosis and other otological infections and to the tendency to support diagnoses with complementary tests. The difference found between the number of confirmed diagnoses of each group could be due to the lower availability of resources for sample taking in health centers as family doctors take samples without microscopic vision; it could also be explained by the fact that specialists – logically – have a better clinical index of suspicion, which is why the results from the ENT specialists' sample cultures confirmed the diagnosis in more cases.

Men suffer confirmed otomycosis in 55.8% of cases and women 44.2%. This significant greater frequency in men is difficult to explain as sports- or leisure-related bathing habits and exposure to climatic elements and other factors is similar for both sexes; we have not found data in the literature to confirm this finding.

The average age of patients with a negative sample was 8 years older than those with a positive sample, which indicates an earlier chronological presentation of otomycosis compared to other otopathies that resemble it.

In contrast to what could be expected according to the established criteria, which would lead us to assume that otomycosis would be more common during the summer due to bathing and climatic factors[1,11,12], a greater proportion was not found in the samples sent in summer, but in spring and especially in the fall, seasons in which salt or fresh water bathing is not so common.

The vast majority of positive samples came from the ENT clinics; a considerable percentage of these patients (39.54%) had suffered from a otological pathology prior to otomycosis. Of these otological pathologies, the one that clearly dominated was chronic otitis media (64%) with a more or less active perforation. We believe, as do the majority of authors[1,3,9,11-15], that the proliferation of fungi in these cases is due to the dampness produced by otorrhea, the scarcity of and changes in the composition of the cerumen, to a pH level that usually fluctuates between 5 and 7, to disturbances in the commensal flora that cause the balance between bacteria and fungi to deteriorate and to the fact that practically all of the cases had been treated with ciprofloxacin (49%), aminoglycosides (21%) or topical antibiotics that were not clearly specified in the patients' medical histories.

Patients with chronic otitis media are in general seen regularly by their ENT specialists, they avoid contact with the water as much as possible (which is why the probability of them developing otomycosis brought on by this factor is much lower) and, generally speaking, go to the clinic if suppuration starts. *Aspergillus flavus* is the predominant species in CSOM cases in which otomycosis is confirmed; these patients suffer symptoms that are resistant to treatment.

In patients with chronic OM and otomycosis, resistance to treatment is not influenced by the localization of the perforation but by the evolution of the otitis media, sometimes related to the state of the functionality of the tube. In our study, this type of otomycosis was difficult to treat, especially those cases associated with nasal respiratory insufficiency, as the nasal ventilatory precariousness with tube dysfunction, common in these patients, can make the chronic OM more acute, thus provoking the production of an alkaline secretion; this leads to a pH imbalance in the local medium of the canal encouraging fungal growth.

In all related studies, *Aspergillus* and *Candida* are the most common causes of otomycosis[9,16-18]. Following the analysis of the results of our microbiological study, we

observed that the distribution of species varied according to the diagnostic source. Thus, when the sample came from ENT clinics, *Aspergillus flavus* and *Candida parapsilosis* were the predominant species, while if the sample came from health centers, *Aspergillus niger* was the fungus most commonly isolated. In comparison, it was significant that *Aspergillus niger* occupied third place in the ENT samples. The explanation for the differences must lie in the conditions in which the sample was obtained; these are more rigorous in ENT clinics than in health centers: in ENT clinics a combination of otoscopy and microscopy is used for the extractions while in the health centers only otoscopy is used.

Irrespective of the species, in our study the genus *Aspergillus spp* turned out to be predominant over the genus *Candida spp*. This conclusion matches the results of Martin et al[19] and differs somewhat to those obtained by Bernat Gili et al[20,] who reported *Aspergillus* and *Candida* in equal quantities.

Another fact worth highlighting is the high percentage of yeast-like fungi isolated, namely *Condida parapsilosis,* in patients that had gone to a health center and then been referred to an ENT specialist and in those patients whose samples were taken in the UHRH. These patients had in common the fact that they had been diagnosed with otitis which was resistant to topical treatment with ciprofloxacin. We must therefore assume that a mistaken diagnosis of bacterial otitis and the treatment with quinolone ear drops encouraged the proliferation of the fungus.

Some authors believe that otomycosis is not a nosological entity as such, but the manifestation of some dermatological diseases (psoriasis, seborrheic dermatitis, contact dermatitis, infectious eczema), of mechanic lesions, of excessive accumulation of cerumen or of its scarcity, of prior bacterial infections etc, in which the saprophytic and commensal fungus becomes a secondary pathogen. However, we believe that, from a conceptual point of view, otomycosis is an entity in itself as a variety of otitis, despite one or another prior pathologies exerting an influence over its pathogenia.

The existence of prior otological pathology is a fundamental factor in the prognosis, as not only does it predispose the patient to suffering from otomycosis, but also makes the disease clearly more resistant to treatment and significantly increases the likelihood of recurrences. That is to say, while the factors that facilitate the onset of fungal disease can usually be eliminated in ears that were previously healthy, in those ears with chronic OM, even when the fungal infection has been cured, the otitis media that gave rise to it remains. This problem is sometimes difficult to resolve and often perpetuated by nasal and tubaric alterations.

All of this means that the exclusive use of antifungal agents is not sufficient to completely cure the process and especially that these agents are not effective enough to avoid frequent relapses. It is important that the treatment, besides being based on cures and the use of topical antimycotic drugs, be focused on restoring the physiology of the canal; that is to say, avoiding sudden maneuvers in the EAC, taking care to avoid excessive moisture by not going in the water, receiving appropriate medical or surgical treatment for otitis media, avoiding any situation that changes the local homeostasis etc are all essential in order to bring about the definitive resolution of the disease.

CONCLUSIONS

1. In our series, the presumed diagnosis of otomycosis was confirmed by microbiology in at least a quarter of all cases.
2. In our study, the disease was not more frequent in summer: fall was the season in which it most often appeared.
3. The overinfection from fungi in chronic OM is a common occurrence. The predominant causal fungus was *Aspergillus flavus.*
4. The main species found in the samples that came from ENT clinics were *Aspergillus flavus* and *Candida parapsilosis;* however, the most common fungus isolated in the samples from health centers was *Aspergillus niger*.
5. The predisposition to suffering from otomycosis and subsequent relapses is clearly connected to individual predisposing factors localized in the EAC, along with having previously suffered certain otological pathologies and with the indiscriminate use of topical antibiotics; these circumstances, which are ideal for otomycosis, can be avoided or treated in many cases.
6. The use of antimycotic agents is generally not sufficient to provoke a complete recovery from the disease. Treatment must also be directed towards restoring the physiology of the EAC.

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