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Unusual case of otomycosis caused by *Saksenaea vasiformis*

H. Trabelsi[a], S. Neji[a], I. Hadrich[a,*], M. Sellami[b], N. Khemakhem[a], H. Sellami[a], F. Makni[a], B. Hammami[b], A. Ayadi[a]

[a] Laboratory of Parasitology-Mycology, Habib Bourguiba Hospital, Sfax, Tunisia
[b] ENT Department, Habib Bourguiba Hospital, Sfax, Tunisia

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ABSTRACT

Saksenaea vasiformis is a species of the order Mucorales rarely reported as a cause of human mucormycosis. We report an unusual case of *S. vasiformis* otitis occurring in a diabetic woman after penetration of an insect in the right ear. Direct microscopic examination of the clinical sample showed hyaline and non septate hyphae belonging to the order Mucorales. Fungal identification was performed by sequencing the ITS region of the rDNA. To our knowledge, this is the first report of *S. vasiformis* infection in Tunisia.

1. Introduction

Saksenaea vasiformis is an emerging fungus which was described in 1953 by Saksena as a new zygomycete [1]. It was first isolated from a forest soil in India [1]. It has been found in soil samples, driftwood, grains and in other geographic areas [2,3]. *Saksenaea* is able to cause severe human infections in both immunocompromised and immunocompetent hosts. It is most often associated with cutaneous or subcutaneous lesions after trauma. Malignant otitis with this fungus is uncommon. In this report, we describe a case of malignant otitis externa complicated with parotid abscess and caused by *Saksenaea vasiformis* in a diabetic woman.

2. Case report

A 54-year-old diabetic woman was referred to the otolaryngology department on the first of July 2015 (Sfax-Tunisia) with complaint of ear infection and facial asymmetry. She was suffering from otalgia and purulent otorrhea of the right ear since 7 days. There was no history of fever or other systemic symptoms. A probabilistic antibiotherapy was prescribed, without any improvement. An aggravation of the facial asymmetry with parotid abscess, right ear cellulitis and fever were noted.

The patient indicated the penetration of an insect in the right ear.

Left ear examination and physical examination was normal.

The otoscopy examination showed the presence of pus in the right external auditory canal which was very inflammatory and the presence of granulations tissue and central perforation of right tympanic membrane. Neurological examination showed peripheral facial paralysis with a painful and inflammatory parotid tumefaction.

The CT scan (at day + 1) found sub-mastoidal and para-pharyngeal abscess and cerebral venous thrombosis complicating a right malignant otitis externa (Fig. 1).

Auricular swab, aspiration and samples of retro auricular collection, were practiced at day +17. The bacterial cultures were negative while the direct examination showed hyaline, broad and aseptate mycelium suggestive of Mucorales fungi. Auricular culture on Sabouraud medium without actidione allowed isolating cottony and white colonies. The microscopic examination revealed only broad aseptate hyphae without sporulation (Fig. 2). Histopathologic examination of the resected tissues showed zygomycete hyphae.

Molecular identification based on PCR amplification and sequencing of rDNA internal transcribed spacer (ITS) regions was performed to identify the Mucorales fungi. The ITS1–5.8S-ITS2 regions of rDNA were amplified with the fungal universal primer pairs ITS1/ITS4 [4]. PCR product was sequenced and the etiological agent was than identified as *Saksenaea vasiformis*. The best match was obtained (99% similarity) with sequence of *S. vasiformis* EU644757. Our sequence (TN254AU15) was submitted to GenBank under the accession number: KU314816. The above mentioned sequences were aligned using the ClustalX V2.1 program [5] as implemented in BioEdit [6], sequence alignment Editor Version. 7.0.9.0 Software (Fig. 3). Multiple alignments of our sequence with those of two *S. vasiformis* isolates (GenBank accession numbers EU644757 and AY211275) are shown in Fig. 3. Additionally,

* Corresponding author. Laboratory of Parasitology- Mycology, CHU Habib Bourguiba, Sfax, Tunisia.
E-mail addresses: thouaida@yahoo.fr (H. Trabelsi), nejisourour@yahoo.fr (S. Neji), ineshadrich@yahoo.fr (I. Hadrich), sellami_moncef@yahoo.fr (M. Sellami), Khemakhem.Nahed@yahoo.fr (N. Khemakhem), hayetsellami1@gmail.com (H. Sellami), famakni@gmail.com (F. Makni), bouthainahk@yahoo.fr (B. Hammami), ali.ayadi@rns.tn (A. Ayadi).

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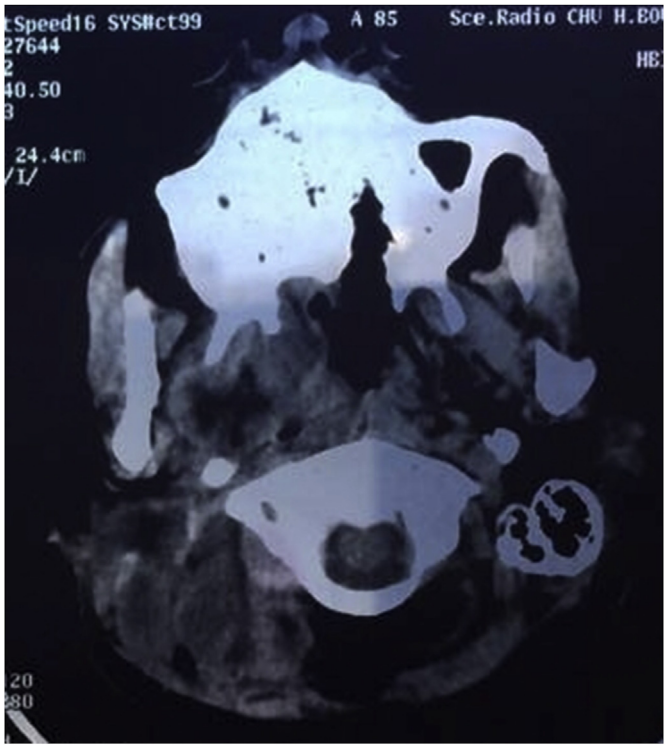

Fig. 1. CT scan: sub-mastoidal and para-pharyngeal abscess complicating a right malignant otitis externa.

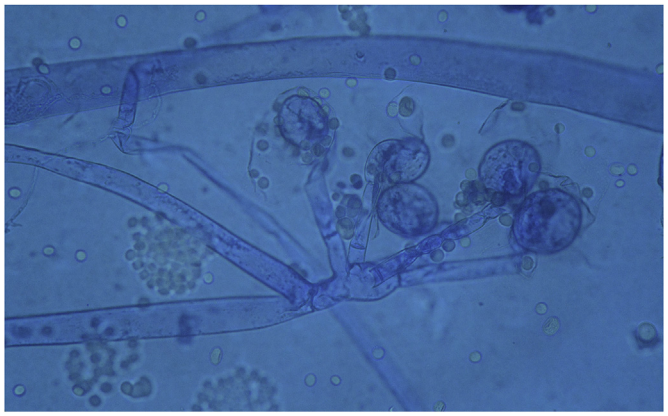

Fig. 2. Microscopic morphology in Sabouraud's dextrose agar: showed the presence of Mucorale sp.

phylogenetic tree of ITS rDNA for available sequences of *S. vasiformis* was obtained using MEGA (Molecular Evolutionary Genetic analysis program) version 6 [7]. Tree was created using UPGMA method (Fig. 4). UPGMA tree clearly showed, in one hand, clustering of our sequence with those of *S. vasiformis* and in the other hand, the closely relation with *Apophysomyces elegans* but the independence of other Mucorales (Fig. 4).

The patient was initially, treated with antibiotics (ciprofloxacin750:1 tablet x 2/day + ceftazidime: 2gx3/day) (at day 1) for 15 days and voriconazole (at day + 3) (2 bottles x 2/day then 1 bottle x 2/day) for 18 days. Then she was treated at day +20 to day +42, by amphotericin B (10mg/day J1then 50 mg/day) for 23 days, teicoplanin (800mg/24h then 400 mg/24h for 11 days), imipenem (500 mg x4/day for 20 days) and gentamicin (180mg/day: 5 days).

The control CT scan (at day 28) showed cervical collection and defect of opacification in the right sigmoid sinus and in the right internal jugular vein.

The persistence of sub-mastoidal cellulitis required additional surgical drain on day 32 of hospitalization. Histopathologic examination of the operative specimen still showed hyphae of zygomycete.

The resolution of clinical signs was, clearly, observed. However, the patient left the hospital after 42 days against medical advice.

3. Discussion

Fungi of the Mucorales order are ubiquitous in nature. They can be found in soil and decaying organic matter. They are uncommon human pathogens which can cause severe fatal disease in susceptible individuals. *S. vasiformis* is a rare fungus with worldwide distribution. The first human infection caused by *Saksenaea* was described in 1976 by Ajello et al. [2].

Up to now, approximately 54 cases of zygomycete infections, mostly cutaneous infections, have been attributed to *Saksenaea* [8,9]. Most of these cases occurred in warmer, tropical and subtropical climates and have been isolated in the United States, Australia, New Zealand, Colombia, Ecuador, French Guiana, Israel, Thailand, Spain, India, Iraq, Greece and Malaysia [9–11]. Our case is the 55th reported case of *S. vasiformis* infection and the second reported case contracted in Tunisia [12].

There are various ways by which can occur, but trauma is the most common, particularly after insect or spider bites, like our case [13]. In fact, insect or spider bites are responsible for 3% of primary cutaneous mucormycosis [12].

The other probable modes of infection in human beings are either inhalation of spores into sinuses or direct inoculation into facial wounds or sinuses by contaminated water [14].

Various predisposing factors for this fungal infection were described: skin trauma, steroids treatment, uncontrolled diabetes mellitus, and hematological malignancies [9]. However, More than 80% of *S. vasiformis* infection cases have been reported for previously healthy or non-immunocompromised individuals [9]).

The clinical spectrum of *S. vasiformis* infections range from chronic focal infections (cutaneous or subcutaneous lesions, abscess formation, osteomyelitis) to rhino-cerebral, renal and acute disseminated infections [9]. Otomycosis is uncommon mucormycosis localization. Until now, only one case of S.*vasiformis* otomycosis, which was recently published, has been reported in the literature [11].

In our case, otitis was complicated by a facial palsy and parotid abscess. In fact, Mucormycosis causing facial nerve involvement is extremely rare and was probably reflecting middle ear involvement. Only four cases were reported in the literature with the involvement of facial nerve [15–18].

The diagnosis of *S. vasiformis* may be missed as it usually does not sporulate easily in routine mycology media. So, when this infection is suspected, the fungi should be cultured in specific media to induce sporulation or it should be identified by molecular tools.

The ITS region of the nuclear rDNA has been proven to be a good phylogenetic marker in Mucorales [19]. The alignment of our sequence with *S. vasiformis* sequences deposited in GenBank and construction of phylogenetic trees allowed us to identify the fungus with confidence as the species *S. vasiformis* and demonstrate that our strain was closely related with *S. vasiformis* (EU644757) which was isolated in a French patient who developed cutaneous infection after scorpion sting in Tunisia [12]. This emphasizes the importance of molecular methods in epidemiological studies. In fact, molecular studies, based mostly on internal transcribed spacer (ITS) sequences have demonstrated high intraspecific genetic diversity for *S.vasiformis*. Currently, *Saksenaea vasiformis* is considered a complex of species that include at least two new species: *Saksenaea oblongispora* and *Saksenaea erythrospora* [19].

Treatment of *Saksenaea vasiformis* infections includes surgical procedures and polyen systemic antifungal therapy (amphotericin B, liposomal amphotericin B) [9]. Recently, some studies, reported that itraconazole and posaconazole were active against *Saksenaea vasiformis*

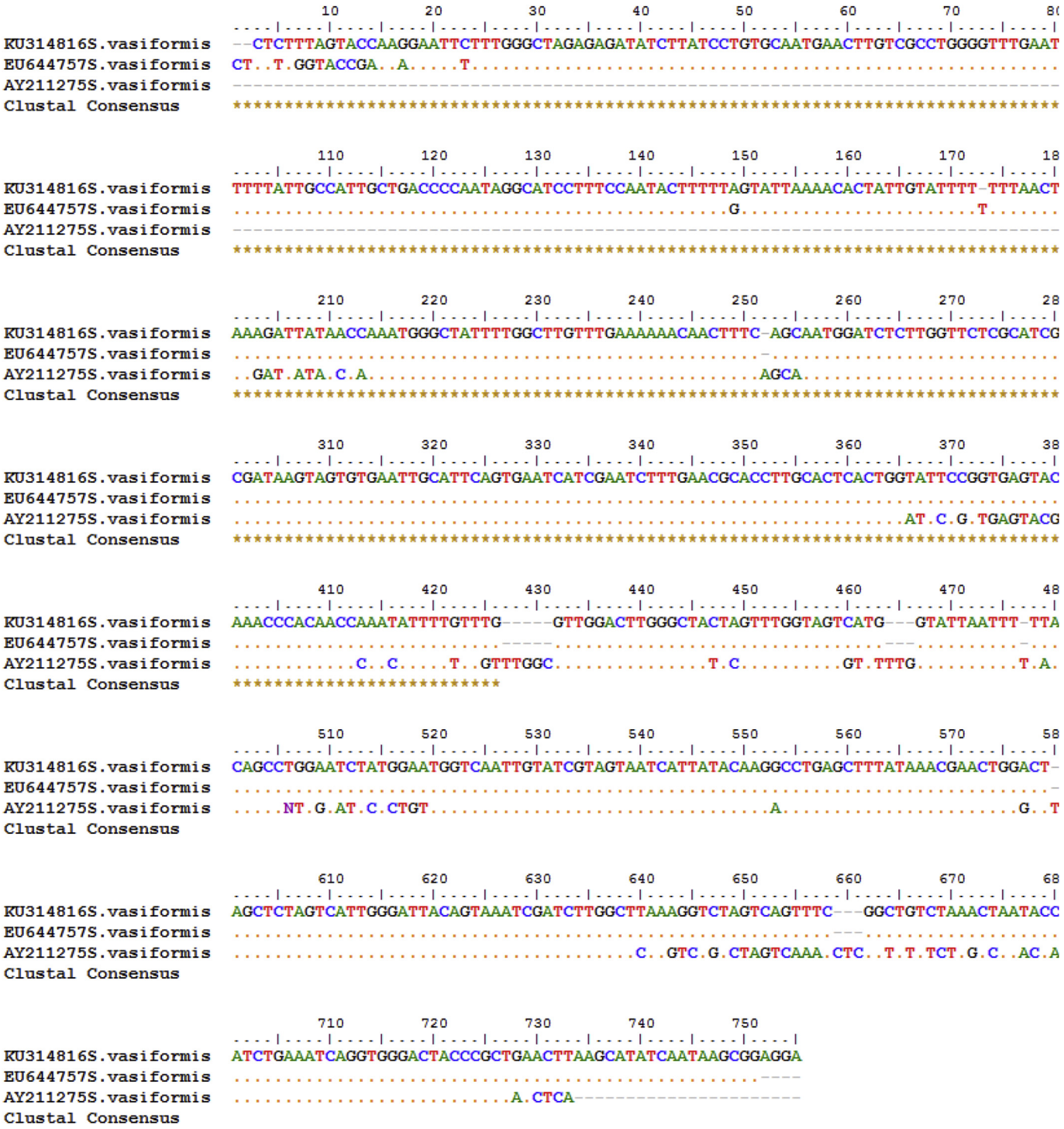

Fig. 3. Alignment of the 18 S (Partial sequence), ITS1, 5.8 S, ITS2 (Total sequence) and 28 S (Partial sequence) rDNA of *Saksenaea vasiformis* strains.

infections [19]. At present, new therapeutic approaches have been proposed, such as posaconazole associated with echinocandins, terbinafine or liposomal amphotericin B, or deferasirox associated with amphotericin B [20]. But, more studies are necessary to prove their efficacy.

Cutaneous zygomycosis generally has a good prognosis. *Saksenaea vasiformis* zygomycosis has also been reported to have a favourable outcome after treatment in most of the cases. In cutaneous infections, the mortality rate is 16% [9]. However, the prognosis of *S. vasiformis* infection can be fatal, especially, in rhino-orbito-cerebral cases (with a mortality rate of 83%), in disseminated infection (73%) and in immunocompromised patients [9].

Infections due to *S. vasiforms* are probably underdiagnosed as these fungi do not easily sporulate in standard mycological media. So, the use of special cultures and the sequencing of the ITS region are recommended to facilitate the isolation and the identification of species.

Declaration of competing interest

The authors declare that they have no conflict of interest.

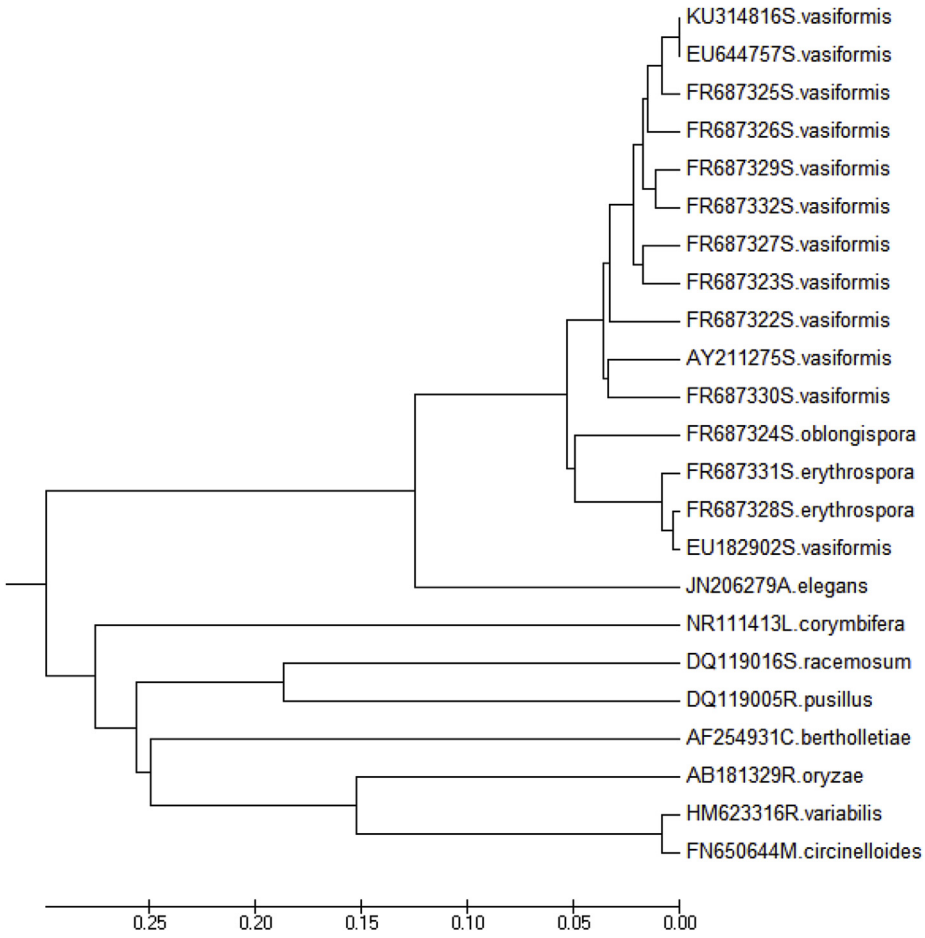

Fig. 4. Phylogenetic tree based on ITS region rDNA sequences of *Saksenaea vasiformis* complex species using UPGMA method. The percentages of bootstrap samplings, derived from 1000 samples which were supporting the interior branches, are noted.

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References

[1] S.B. Saksena, A new genus of the Mucorales, Mycologia 45 (1953) 426–436.
[2] L. Ajello, D.F. Dean, R.S. Irwin, The zygomycete *Saksenaea vasiformis* as a pathogen of humans with a critical review of the etiology of zygomycosis, Mycologia 68 (1976) 52–62.
[3] C.M. Pillai, RAhmed, *Saksenaea vasiformis*, an ecologically important fungus on rice from India, Curr. Sci. 65 (1993) 291.
[4] T.J. White, T. Bruns, S. Lee, J. Taylor, Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics, in: D.H. Gelfand, J.J. Sninsky, T.J. White (Eds.), PCR Protocols: a Guide to Methods and Applications (Innis MA, Academic Press, San Diego, 1990, pp. 315–322.
[5] J.D. Thompson, D.G. Higgins, T.J. Gibson, W. Clustal, Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice, Nucleic Acids Res. 22 (1994) 4673–4680.
[6] T. Hall, BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT, Nucleic Acids Symposium, 41 1999, pp. 95–98.
[7] K. Tamura, G. Stecher, D. Peterson, et al., MEGA6: molecular evolutionary genetics analysis version 6.0, Mol. Biol. Evol. 30 (2013) 2725–2729.
[8] A.J. Stewardson, N.E. Holmes, D.H. Ellis, B.P. Howden, Cutaneous zygomycosis caused by *Saksenaea vasiformis* following water-related wound in a 24-year-old immunocompetent woman, Mycoses 52 (2009) 547–549.
[9] M.Z.R. Gomes, R.E. Lewis, D.P. Kontoyiannis, Mucormycosis caused by unusual mucormycetes, non-Rhizopus, -Mucor, and -Lichtheimia species, Clin. Microbiol. Rev. 24 (2011) 411–445.
[10] I.D. Gkegkes, I. Kotrogiannis, F. Konstantara, A. Karetsou, S. Tsiplakou, E. Fotiou, S. Stamopoulou, C. Papazacharias, I.A. Paraskevopoulos, Cutaneous mucormycosis by Saksenaea vasiformis: an unusual case report and review of literature, Mycopathologia 30 (2018).
[11] E. Marechal, F. Barry, F. Dalle, L. Basmaciyan, S. Valot, M. Sautour, C. Duvillard, P. Chavanet, L. Piroth, M. Blot, Fatal invasive otitis with skull base osteomyelitis caused by Saksenaea vasiformis, QJM 1 111 (7) (2018) 499–500.
[12] P. Lechevalier, D.G. Hermoso, A. Carol, S. Bonacorsi, L. Ferkdadji, F. Fitoussi, O. Lortholary, A. Bourrillon, A. Faye, other authors, Molecular diagnosis of Saksenaea vasiformis cutaneous infection after scorpion sting in an immunocompetent adolescent, J. Clin. Microbiol. 46 (2008) 3169–3172.
[13] Anna Skiada, Dimitris Rigopoulos, Larios George, Petrikkos George, Andreas Katsambas, Global epidemiology of cutaneous zygomycosis, Clin. Dermatol. 30 (2012) 628–632.
[14] G. Gonis, M. Starr, Fatal rhinoorbital mucormycosis caused by Saksenaea vasiformis in an immunocompromised child, Pediatr. Infect. Dis. J. 16 (1997) 714–716.
[15] M.W. Yun, C.C. Lui, W.J. Chen, Facial paralysis secondary to tympanic mucormycosis: case report, Am. J. Otol. 15 (1994) 413–414.
[16] I. Olalla, M. Ortín, G. Hermida, M.A. Cortés, C. Richard, A. Iriondo, et al., Autologous peripheral blood stem cell transplantation in a patient with previous invasive middle ear mucormycosis, Bone Marrow Transplant. 18 (1996) 1183–1184.
[17] A. Tuzcu, M. Bahceci, M.K. Celen, N. Kilinc, S. Ozmen, Necrotizing (malignant) otitis externa: an unusual localization of mucormycosis, Indian J. Med. Microbiol. 24 (2006) 289–291.
[18] H. Ganapathy Vaishali Shah, Gopalakrishnan Ram, N. Geetha, A rare case of facial palsy due to mucormycosis, Int. J. Otolaryngol. Head Neck Surg. 4 (2015) 224–228.
[19] E. Alvarez, D. Garcia-Hermoso, D.A. Sutton, J.F. Cano, A.M. Stchigel, D. Hoinard, et al., Molecular phylogeny and proposal of two new species of the emerging pathogenic fungus Saksenaea, J. Clin. Microbiol. 48 (2010) 4410–4416 re we and where should we go from here? J. Clin. Microbiol. 47:877–884.
[20] B. Spellberg, A. Ibrahim, E. Roilides, et al., Combination therapy for mucormycosis: why, what, and how? Clin. Infect. Dis. 54 (Suppl 1) (2012) S73–S78.