**Auxarthron alboluteum** related to non-dermatophytic toenail infection in Kurdistan region, Iraq: A case report

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**ABSTRACT**

We report a rare case of non-dermatophytic onychomycosis of the big toenail caused by **Auxarthron alboluteum** in a 63-years old Iraqi female with a history of diabetes. The big toenail showed distal subungual onychomycosis with extensive yellow-white discoloration. Identification of the causative agent was confirmed by morphological and microscopical characteristics in culture and analysis of ITS-rDNA region. To the best of our knowledge, the isolated **Auxarthron alboluteum** reported here is a new etiologic species of nail infection in Iraq and this is the first case of its kind to be reported in the world.

**1. Introduction**

Fungal nail infection (tinea unguium) is a term strictly used for nails infection due to dermatophytic genera *Microsporum*, *Trichophyton*, *Epidermophyton*, *Arthroderma*, *Nannizzia*, *Lophophyton* and *Guarromyces* [1]. Onychomycosis is a chronic fungal disease and is a general term described for dermatophytic and nondermatophytic nail infections. Among the most frequently nondermatophytic fungi encountered as causal agents in nail infections are species in the genera *Acremonium*, *Alternaria*, *Aspergillus*, *Candida*, *Fusarium Geotrichum*, *Scopulariopsis* and *Scytalidium* [2]. Several studies demonstrated that the most common etiological agents among patients with nail infections were dermatophyte, followed by moulds and yeasts [1,3,4]. Our aim was to identify and report a nondermatophytic onygenalean fungus causing onychomycosis of a toenail of the 63-years old elderly women from Kurdistan region, Iraq.

**2. Case**

A 63-year-old female diabetic patient, presented in January 2017 (day 0) to the Dermatology out-patient department of Khabat Hospital with a dystrophic great left toenail, showing yellowish white to creamy discoloration (Fig. 1) of 9 months duration (-9 months). There was a history of probable onychomycosis of her left great toenails (not verified by mycological examination) three years previously (-3 years), that was completely cured after treatment with antifungal clotrimazole cream 1% (fuginid cream, SDI) used 3times/day for exactly 2 months. The associated predisposing factor for this case was diabetes mellitus, and at the time of sampling, the patient had undergone diabetic medication. No history of any underlying disease was reported.

Nail scrapings and clippings from different parts of the affected toenail were collected on day 0 after proper sterilization of the affected area with 70% alcohol and transported in folded dry-square of paper envelope to the mycology research laboratory, Biology Department at Zakho University, for direct microscopic examination and culture processing [5]. On direct KOH examination, septate filaments and arthroconidia were observed. At +3 days Portions of the nail scraping and clippings were cultured on two sets of Sabouraud's dextrose agar (SDA) medium (CM41; Oxoid, UK): two replicates of SDA plates with antibiotics and cycloheximide [Sigma-Aldrich] (500 mg/l), and one slant of SDA contained antibiotics but without cycloheximide, and incubated at 28 °C for at least 3 weeks. Mycological diagnosis was performed at +21 days. A mesh-like brown globose gymnothecia composed of network of thick-walled hyphae (reticuloperidium) related to onygenalean fungi were grew on both sets of media. No other fungi were detected on both sets of media. The onygenalean fungus was identified as a member of *Auxarthron* based on cultural and microscopic characteristics (Figs. 2 and 3) according to relevant references [6–10]. For accurate identification of the isolated causal agent, DNA sequencing, was performed.

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Fungus genomic DNA was extracted from isolated fungus using liq-
quid nitrogen and EZ-10 Spin Column Fungal Genomic DNA Mini-preps Kit, BIO BASIC INC., Canada, and the genomic DNA was analyzed by
electrophoresis on 1.5% agarose gel. The ITS region on rDNA was
amplified by using universal primers ITS1- (5'-TCCGTAGGTGAACCTG
CGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') (MWG-Biotech
AG, Germany) which yielded a PCR product size of 582bp. The PCR
reaction was done in a GenAmp9700 thermal cycler (Perkin-Elmer)
under the following conditions: an initial denaturation set up at 94 °C
for 5 min. was followed by 35 cycles of denaturation at 94 °C for 1 min.,
annealing at 58 °C for 1 min. and extension at 72 °C for 1 min., with a
final extension step of 72 °C for 10 min.

The sequencing was done at Macrogen company, Korea and the
sequence was deposited in GenBank with accession no. MK112622
(ITS). For phylogenetic analysis, sequences of amplified product was
retrieved in FASTA format from GenBank. Phylogenetic analyses using
the Neighbour-Joining (NJ) method [11] was performed with the
MEGA v. X computer program [12]. The phylogenetic tree was con-
structed using the Kimura two-parameter distance model [13] with the
‘pairwise deletion of gaps option’. The robustness of branches was as-
essed by bootstrap analysis with 1000 replicates.

At +22 days, the patient was treated with oral itraconazole 200 mg
daily for 2 consecutive months. At +82 days, the patient visited the
dermatology out-patient department, her toenail was not completely
cured, yet, the direct microscopy and culture of nail clippings were
negative for fungal elements. At +82 days, oral itraconazole (200 mg/
day) in combination with topical fucidin cream (clotrimazole cream
1%) (SDI), twice per day were given for a further 2 months. The patient
did not visit the dermatology out-patient department of Khabat hospital
after the last therapy, for that reason the patient medical condition
cannot be confirmed.

3. Discussion

In the present study, we isolated an onygenalean fungus from the
toenail of a 63-year old elderly woman that was assigned to the genus
Auxarthron Orr&Kuehn. Auxarthron is characterized by developing
globose gymnothecium with reticuloperidium, evanescent asci, with
globose or ovoid, finely pitted to echinulate ascospores and a
Malbranchea anamorph [6,7]. Currently, the genus Auxarthron is com-
prised 16 species according to Refs. [6–10,14]. Species of Auxarthron
have been repeatedly isolated from soil [6,14], dung [6,10], river se-
diments [8], and soil enriched with keratinaceous substances [6].

We confirmed the identification of our isolate to species level as A.alboluteum (accession no. MK112622.1) by DNA sequence analysis.

A nucleotide BLAST search with 582bp showed a maximum homology of 99% with A. alboluteum of Egyptian origin (MF503899.1),
98% with Greek and Turkish A. alboluteum strains (KC253973.1 and
KF938454.1) respectively, 95% with A. alboluteum from China
(KP216931.1) and 94% accordance with several other strains from USA
and Canada.

The phylogenetic tree based on analysis of ITS rDNA sequences in Fig. 4 evidently designated that our fungal isolate (accession no. MK112622.1) clustered with the accessions (KF938454.1, KC253973.1 and MF503899.1) in a common ancestor with the supporting of 59, 99 and 100 bootstrap. The highest bootstrap of our isolate was revealed with the accession MF503899.1. The comparison between our isolate and the ITS reference sequences that were obtained from the NCBI database confirmed the identification of our isolates as A. alboluteum.

Auxarthron alboluteum has been reported in several occasions as a soil-borne fungus [7,15]. Our report is the first cause of onychomycosis with A. alboluteum as a causal agent.

Onychomycosis due to Auxarthron species was rarely encountered. There were few reports caused by Auxarthron. The first proven case was due to A. ostraviense isolated from the fingernails of 26-year old male with psoriasis [9]. Other reports demonstrated A. umbrinum as causative agent of onychomycosis on the right great toenail of 36-year old male, 54-year old man and a 29-year old woman. All cases were reported from the Czech Republic [9].

Our knowledge on incidence and causal agents of nails infection in Iraq is poorly known, perhaps may be due to a lack of research. A high incidence in tinea unguiis (34.9%) was reported in 1999 in Basrah province [16], whereas, in another study, a lower incidence (6.7%) was reported in 2002 in patients with onychomycosis from the same city [17]. From the later study, dermatophytes were responsible for 45.5% of onychomycosis, whereas, yeasts accounted for 54.5% of cases. Etiologic agents of onychomycosis due to dermatophytes were Epidermophyton floccosum, Trichophyton verrucosum and T. violaceum. Yeasts infections of nails caused by Candida albicans, C. famata, C. parapsilosis and Geotrichum candidum [17]. A case of white superficial onychomycosis was related to non-dermatophyte Cladosporium spp. in a patient from Baghdad [18]. In a recent study, 12% of onychomycosis was related to Malassezia furfur infection in patients from Baghdad [19].

In conclusion, to the best of our knowledge, we report the first case of onychomycosis of a toenail of a diabetic elderly woman caused by A. alboluteum. Identification of the causal agent was confirmed by molecular methods. Ageing and diabetic mellitus are likely to be a predisposing factor in our reported case. The study provides further information’s on etiological agents of onychomycosis in Iraq.

**Ethical Form**

Please note that this journal requires full disclosure of all sources of funding and potential conflicts of interest. The journal also requires a declaration that the author(s) have obtained written and signed consent to publish the case report from the patient or legal guardian(s).

The statements on funding, conflict of interest and consent need to be submitted via our Ethical Form that can be downloaded from the submission site www.ees.elsevier.com/mmcr. Please note that your manuscript will not be considered for publication until the signed Ethical Form has been received.

**Declaration of competing interest**

There are none.

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