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The Emergence of Rare Clinical Aspergillus Species in Qatar: Molecular Characterization and Antifungal Susceptibility Profiles

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Aspergillus are ubiquitous mold species that infect immunocompetent and immunocompromised patients. The symptoms are diverse and range from allergic reactions, bronchopulmonary infection, and bronchitis, to invasive aspergillosis. The aim of this study was to characterize 70 Aspergillus isolates recovered from clinical specimens of patients with various clinical conditions presented at Hamad general hospital in Doha, Qatar, by using molecular methods and to determine their in vitro antifungal susceptibility patterns using the Clinical and Laboratory Standards Institute (CLSI) M38-A2 reference method. Fourteen Aspergillus species were identified by sequencing β-tubulin and calmodulin genes, including 10 rare and cryptic species not commonly recovered from human clinical specimens. Aspergillus welwitschiae is reported in this study for the first time in patients with fungal rhinosinusitis (\(n = 6\)) and one patient with a lower respiratory infection. Moreover, Aspergillus pseudonomius is reported in a patient with fungal rhinosinusitis which is considered as the first report ever from clinical specimens. In addition, Aspergillus sublatus is reported for the first time in a patient with cystic fibrosis. In general, our Aspergillus strains exhibited low MIC values for most of the antifungal drugs tested. One strain of Aspergillus fumigatus showed high MECs for echinocandins and low MICs for the rest of the drugs tested. Another strain of A. fumigatus exhibited high MIC for itraconazole and categorized as non-wild type. These findings require further analysis of their molecular basis of resistance. In conclusion, reliable identification of Aspergillus species is achieved by using molecular sequencing, especially for the emerging rare and cryptic species. They are mostly indistinguishable by conventional methods and might exhibit variable antifungal susceptibility profiles. Moreover, investigation of the antifungal susceptibility patterns is necessary for improved antifungal therapy against aspergillosis.

Keywords: aspergillosis, molecular identification, antifungal susceptibility, Qatar, Middle East
INTRODUCTION

Aspergillus species are common environmental fungi found in soil and decaying vegetative materials. They can infect immunocompetent (Chaturvedi et al., 2017; Emiralioglu et al., 2017; Kumar et al., 2017; Saedi et al., 2017) and immunocompromised (Taccone et al., 2015) patients. Individuals with underlying diseases or immune deficiencies can develop a variety of symptoms ranging from allergies, bronchopulmonary infections, and bronchitis, to invasive aspergillosis (IA) (Ruping et al., 2008; Guinea et al., 2010; Sugui et al., 2014). IA is associated mainly with neutropenic patients suffering from hematological malignancies (Gerson et al., 1984; Abers et al., 2016). Other risk factors include hematopoietic stem cell transplant (HSCT) (Marr et al., 2002), solid organ transplant (SOT) (Patterson et al., 2000), patients receiving prolonged high doses of corticosteroids (Palmer et al., 1991; Lewis and Kontoyiannis, 2009), human immunodeficiency virus (HIV) infection with advanced acquired immune-deficiency syndrome (AIDS) (Libanore et al., 2002) and chronic granulomatous disease (CGD) (Beaute et al., 2011). IA is associated with a high mortality rate among immunocompromised patients (Baddley et al., 2010; Kontoyiannis et al., 2010; Neofytos et al., 2013; García-Vidal et al., 2015). During the last two decades, species other than A. fumigatus, namely, Aspergillus flavus, Aspergillus terreus, Aspergillus niger, and other cryptic and rare species have increasingly been isolated from clinical specimens (Lass-Flori et al., 2005; Krishnan et al., 2009; Alastruey-Izquierdo et al., 2012). This epidemiological shift is attributed to the increasing number of immunocompromised patients, advances in the detection and identification of pathogenic fungi, and the selective pressure caused by extensive use of broad-spectrum antifungal drugs (Krishnan et al., 2009; Alastruey-Izquierdo et al., 2012). Voriconazole is the first line therapy recommended for the management of IA (Patterson et al., 2016; Ullmann et al., 2018). Other alternatives are liposomal amphotericin B and isavuconazole. In patients who exhibit refractory or progressive IA after the initiation of primary therapy, an additional antifungal agent may be added or a combination of antifungal agents from different classes (e.g., a triazole and an echinocandin) may be considered (Patterson et al., 2016; Ullmann et al., 2018). Posaconazole can be used as prophylaxis for patients at high risk for IA (Patterson et al., 2016; Ullmann et al., 2018). Triazole-resistant Aspergillus, particularly A. fumigatus, became a worldwide problem with high prevalence in Europe (Alastruey-Izquierdo et al., 2013; Abdolrasouli et al., 2018; Biel et al., 2019) and recently in the United States (Berkow et al., 2018). This poses a great challenge for clinicians in patient management. Triazole resistance in Aspergillus has also been reported from other parts of the world, such as India (Chowdhary et al., 2015), Iran (Seyedmousavi et al., 2013; Mohammadi et al., 2016; Nabili et al., 2016), and Tanzania (Chowdhary et al., 2014). In the Middle East, apart from Iran, triazole resistance for A. fumigatus has also been documented in Kuwait, a neighboring Arabian gulf country, in outdoor and hospital environments (Ahmad et al., 2014) as well as from clinical samples (Ahmad et al., 2015).

The aim of the current study was to characterize 70 Aspergillus species isolated from a variety of clinical specimens received at the microbiology laboratory of Hamad general hospital in Doha, Qatar, with emphasis on emerging rare species identified as human pathogens, and to determine their antifungal susceptibility patterns using the Clinical and Laboratory Standards Institute (CLSI) M38-A2 reference method.

MATERIALS AND METHODS

Patients and Specimens

Seventy Aspergillus species were recovered from clinical specimens of 67 patients, including immunocompromised patients \( n = 17, 25.4\% \) and immunocompetent ones with other underlying diseases \( n = 50, 74.6\% \) (Table 1), presented at Hamad general hospital in Doha, Qatar, between August 2003 and November 2014 with proven or probable infection or colonization by Aspergillus species. The patients represented 15 nationalities, including countries from Southeast Asia \( n = 23, 23.3\% \) and the Middle East \( n = 42, 62.7\% \), South Africa \( n = 1, 1.5\% \) and the United States \( n = 1, 1.5\% \). The isolates were recovered from various clinical specimens, including respiratory samples (spumum, broncho-alveolar lavage (BAL) and bronchial wash), nose and nasal sinuses, ear, wounds, pus/abscess, eye, nail, burn, pleural fluid and an unknown culture plate of clinical specimen received for identification from an external facility (Table 1).

Isolation and Identification

Aspergillus species were identified by macro and microscopy according to the laboratory standard operative protocol of the microbiology laboratory at Hamad general hospital in Qatar. Specimens were cultured on Sabouraud dextrose agar (SDA; Difco Laboratories, Detroit, MI) with and without chloramphenicol. Culture plates were incubated at 26 and 37°C and were observed daily for growth up to 10 days. Direct microscopy from clinical specimens was performed using Blankophor P fluorescent stain (Bayer AG, Germany). Cultures were preserved at −70°C using cryo-tubes (Mast Diagnostics, Bootle, Merseyside, UK) until further use.

Molecular Identification

DNA Extraction

Genomic DNA was extracted as described by Bolano et al. (2001), with minor modifications. In short, Aspergillus biomass, which was grown on oatmeal agar (OA; home-made at Westerdijk Institute) for 5 days, was bead-beaten with sterile sand, 750 µl of lysis buffer, and 750 µl of phenol-chloroform in 2 ml screw-capped tube. The mixture was centrifuged and the supernatant was transferred to 1.5 ml Eppendorf’s tube with an equal amount of ice-cold 96% ethanol. One hundred microliter of 3.0 M ice-cold sodium acetate was added, mixed gently, and stored at −20°C for 30–60 min. The mixture was then centrifuged at 4°C. The DNA pellet was air-dried and re-suspended in 100 µl Tris Ethylenediaminetetraacetic acid (TE) buffer. The solution was incubated successively at 37 and 65°C both for 10 min, and
| Specimen number | Gender/age | Origin | Specimen type | Clinical data | Histopathology/ CT | Mortality within 30 days | Treatment | Aspergillus species |
|-----------------|------------|--------|---------------|---------------|---------------------|--------------------------|-----------|-------------------|
| Q1444           | M/48       | Qatar  | Nasal swab    | Nephrotic syndrome, on immunosuppressant, fungal sinusitis | +                    | Alive                     | NA        | Aspergillus terreus |
| Q3252           | M/62       | KSA    | Burn          | Deep left knee burn | NA                  | Alive                     | NA        | Aspergillus flavus |
| Q0098           | M/49       | Sudan  | Tissue        | TBI, pulmonary aspergillosis, aspergilloma, X-ray+ abscess | +                    | Alive                     | ITC<sup>b</sup> | Aspergillus flavus |
| Q0224           | F/49       | KSA    | BAL<sup>3</sup> | Endometrial adenocarcinoma | NA                  | Alive                     | AMB<sup>f</sup>, CAS<sup>d</sup> | Aspergillus flavus |
| Q4000676        | F/29       | India  | Sinus mucosa  | Nasal polyp, diabetic | +                   | Alive                     | NA        | Aspergillus welwitschiae |
| Q0180           | M/22       | Jordan | Nasal swab    | Nasal polyp | +                   | Alive                     | NA        | Aspergillus flavus |
| Q1013           | M/27       | India  | Tissue        | Skull lesion, sphenoid sinus extending to pteryoid fascia | NA                  | Died                      | NA        | Aspergillus flavus |
| Q0878           | M/23       | Qatar  | Tissue        | Maxillary ethmoid sinus | NA                  | Alive                     | NA        | Aspergillus flavus |
| Q8000006        | M/17       | KSA    | BAL           | Cystic fibrosis | NA                  | Alive                     | NA        | Aspergillus sublatus |
| Q0078           | M/60       | Pakistan | BW<sup>i</sup> | Pneumonia | NA                  | Alive                     | CAS        | Aspergillus terreus |
| Q0139           | M/21       | India  | Nasal swab    | Allergic rhinosinusitis | NA                  | Alive                     | NA        | Aspergillus flavus |
| Q6070           | M/11       | Qatar  | Wound         | Fracture (RTA<sup>3</sup>) | NA                  | Alive                     | NA        | Aspergillus citroterreus |
| Q1129           | M/43       | Pakistan | Debris from nose | Fungal sinusitis | NA                  | Alive                     | NA        | Aspergillus flavus |
| Q0206           | M/22       | Nepal  | BAL           | Neutropenia, pancytopenia | –                   | Died                      | NA        | Aspergillus nidulans |
| Q0404           | F/33       | Pakistan | Tissue | Fungal sinusitis | +                   | Alive                     | NA        | Aspergillus welwitschiae |
| Q0782           | F/35       | India  | Tissue        | Fungal sinusitis | +                   | Alive                     | NA        | Aspergillus pseudonomus |
| Q0807           | M/6        | Pakistan | Tissue | Chronic granulomatous disease with Aspergillus brain abscess | NA                  | Alive                     | VCZ<sup>3</sup> + AMB | Aspergillus fumigatus |
| Q0657           | M/48       | Egypt  | Plate Culture | Unknown | NA                  | Alive                     | NA        | Aspergillus fumigatus |
| Q1047           | M/27       | Bangladesh | Tissue | Fungal sinusitis with intracranial extension | NA                  | Alive                     | VCZ + CAS | Aspergillus fumigatus |
| Q1072           | M/13       | Qatar  | Sputum        | Cystic fibrosis | NA                  | Alive                     | NA        | Aspergillus tubingenisis |
| Q1332           | M/30       | Nepal  | Pus aspirate (Brain) | Fungal sinusitis | +                   | Alive                     | FL<sup>1</sup>, AMB, VCZ, ITC | Aspergillus fumigatus |
| Q6746           | M/29       | Sudan  | Sphenoid sinus swab | Fungal sinusitis | +                   | Alive                     | NA        | Aspergillus terreus |
| Q0012           | F/61       | UAE    | Tissue        | Nasal polyp, breast Ca | –                   | Alive                     | NA        | Aspergillus tubingenisis |
| Q1374           | M/35       | India  | Tissue        | Nasal polyp | NA                  | Alive                     | NA        | Aspergillus flavus |
| Q0120           | M/52       | Sudan  | Sputum        | Interstitial lung disease, aspergillosis | NA                  | Alive                     | VCZ        | Aspergillus fumigatus |
| Q0140           | M/47       | Sudan  | Wound Tissue  | Brain tumor | NA                  | Died                      | CAS, FL, Miconazole, VCZ | Aspergillus nidulans |
| Q1490           | F/18       | Qatar  | Sputum        | Cystic fibrosis | NA                  | Alive                     | NA        | Aspergillus fumigatus |
| Q0338           | M/27       | India  | Tissue        | Fungal sinusitis | NA                  | Alive                     | AMB, ITC, VCZ | Aspergillus flavus |
| Q0334           | F/15       | Qatar  | Sputum        | Cystic fibrosis | NA                  | Alive                     | NA        | Aspergillus terreus |
| Q0416           | F/56       | Sudan  | Tissue        | Fungal sinusitis, CT+ | +                   | Alive                     | VZC, ITC | Aspergillus welwitschiae |
| Q0521           | F/74       | Qatar  | Tissue        | RTA | NA                  | Alive                     | AMB        | Aspergillus tamani |
| Q0609           | F/36       | South Africa | BW | Bronchopulmonary Aspergillosis | NA                  | Alive                     | VCZ        | Aspergillus fumigatus |
| Q4672           | F/18       | Qatar  | Sputum        | Cystic fibrosis | NA                  | Alive                     | NA        | Aspergillus terreus |
| Q0688           | M/52       | Qatar  | Pleural Fluid | Lung Cancer | NA                  | Died                      | VZC        | Aspergillus fumigatus |
| Q0234A          | F/37       | Sudan  | Tissue        | Fungal sinusitis | NA                  | Alive                     | VZC, ITC | Aspergillus welwitschiae |
| Q0234B          | F/37       | Sudan  | Tissue        | Fungal sinusitis | NA                  | Alive                     | VZC, ITC | Aspergillus flavus |
| Q0438           | M/80       | Tunisia | BAL | Lung cancer | NA                  | Died                      | NA        | Aspergillus terreus |

(Continued)
TABLE 1 | Continued

| Specimen number | Gender/age | Origin | Specimen type | Clinical data | Histopathology/CT | Mortality within 30 days | Treatment | Aspergillus species |
|-----------------|------------|--------|---------------|---------------|-------------------|------------------------|-----------|---------------------|
| Q0490           | F/26       | Sudan  | Tissue        | Fungal sinusitis | NA                | Alive                  | ITC       | Aspergillus welwitschiae |
| Q0406           | M/4        | Qatar  | Ear Swab      | Recurrent tonsillitis, otalgia | NA                | Alive                  | Miconazole | Aspergillus terreus |
| Q0477           | M/66       | Pakistan | BAL            | Interstitial lung disease | NA                | Alive                  | NA        | Aspergillus pallidofulvus |
| Q6630           | F/49       | Pakistan | Ear Swab      | ALL¹            | NA                | Alive                  | Miconazole | Aspergillus chevalieri |
| Q1114           | F/36       | India  | BAL            | Aspergillus pneumonia | NA                | Alive                  | AMB, CAS, VCZ | Aspergillus welwitschiae |
| Q0725           | F/39       | India  | Tissue        | Fungal sinusitis | NA                | Alive                  | VCZ       | Aspergillus flavus |
| Q4260           | F/49       | Sudan  | Ear Swab      | Hearing loss     | NA                | Alive                  | NA        | Aspergillus terreus |
| Q0567           | M/72       | Palestine | Exit site swab | ESRDΤ⁶       | NA                | Alive                  | NA        | Aspergillus fumigatus |
| Q1177           | M/78       | Qatar  | BW            | Chest infiltrate | NA                | Alive                  | NA        | Aspergillus fumigatus |
| Q1169           | F/44       | Qatar  | Foot tissue   | Septic shock, diabetic, ESRD, bed sore | NA                | Alive                  | NA        | Aspergillus flavus |
| Q1165           | F/47       | UAE    | BAL            | Lung fibrosis    | NA                | Alive                  | VCZ       | Aspergillus flavus |
| Q6596           | M/63       | Qatar  | BAL            | Polyneuropathy   | NA                | Died                   | Anidulafungin | Aspergillus terreus |
| Q1301           | F/25       | Qatar  | Nail          | Onychomycosis    | NA                | Alive                  | NA        | Aspergillus quadrilineatus |
| Q6198           | M/32       | Jordan | Eye swab      | NA               | NA                | Alive                  | NA        | Aspergillus flavus |
| Q1467           | F/35       | Egypt  | Nasal swab    | Skull base meningioma | NA                | Alive                  | NA        | Aspergillus terreus |
| Q4000006        | F/55       | Qatar  | BW            | Bronchial asthma | NA                | Alive                  | Miconazole | Aspergillus terreus |
| Q0518           | M/29       | Sri Lanka | Ear          | ASOM³           | NA                | Alive                  | NA        | Aspergillus flavus |
| Q0333           | M/38       | Sri Lanka | Tissue        | Nasal polyp     | +                 | Alive                  | NA        | Aspergillus tubingenis |
| Q2266           | F/35       | Pakistan | Ethmoid sinus tissue | Fungal sinusitis | +                 | Alive                  | VCZ, ITC  | Aspergillus welwitschiae |
| Q6811           | F/8        | Qatar  | Ear           | Otomycosis      | NA                | Alive                  | NA        | Aspergillus terreus |
| Q1651           | F/18       | Qatar  | Sputum        | Cystic fibrosis  | NA                | Alive                  | NA        | Aspergillus terreus |
| Q7463           | M/25       | India  | Ear           | Ear pain        | NA                | Alive                  | NA        | Aspergillus terreus |
| Q7675           | 21/F       | Qatar  | Sputum        | Cystic fibrosis  | NA                | Alive                  | NA        | Aspergillus fumigatus |
| Q1787           | M/28       | Jordan | Ethmoid sinus tissue | Fungal sinusitis | +                 | Alive                  | ITC       | Aspergillus citrinitoreus |
| Q2779           | M/79       | Qatar  | Sputum        | COPD², lung fibrosis | NA                | Alive                  | NA        | Aspergillus terreus |
| Q0486           | M/40       | India  | BAL            | TB              | NA                | Alive                  | NA        | Aspergillus caespitosus |
| Q3118           | M/60       | Qatar  | Ear           | Chronic kidney disease | NA                | Alive                  | NA        | Aspergillus flavus |
| Q0700           | M/6        | Qatar  | BAL            | Cystic fibrosis  | NA                | Alive                  | NA        | Aspergillus fumigatus |
| Q3996           | F/74       | Syria  | Ear           | Sensorineural hearing loss since birth | NA                | Alive                  | NA        | Aspergillus terreus |
| Q4145           | M/42       | Nepal  | Sputum        | RTA             | NA                | Alive                  | NA        | Aspergillus terreus |
| Q0861           | F/39       | Sudan  | BAL            | Chronic cough   | NA                | Alive                  | ITC       | Aspergillus flavus |
| Q5260           | M/57       | Sudan  | ETT¹          | Colitis, Pleural effusion | NA                | Died                   | NA        | Aspergillus citrinitoreus |
| Q5254           | F/48       | USA    | Sputum        | URTP            | NA                | Alive                  | NA        | Aspergillus flavus |

¹ Data not Available.
² Itraconazole.
³ Amphotericin B.
⁴ Caspofungin.
⁵ Voriconazole.
⁶ Fluconazole.
⁷ Broncho-Alveolar Lavage.
⁸ Bronchial Wash.
⁹ Endotracheal Tube secretion.
¹⁰ Tuberculosis.
¹¹ Road Traffic Accident.
¹² Acute Lymphoblastic Leukemia.
¹³ End Stage Renal Disease.
¹⁴ Acute Suppurative Otitis Media.
¹⁵ Chronic Obstructive Pulmonary Disease.
¹⁶ Upper Respiratory Tract Infection.
+, Positive; –, Negative; CT, Computed Tomography.
stored at −20°C. The DNA quality was checked by 1.5% agarose gel electrophoresis.

**PCR and Sequencing**

For identification of the isolates, two loci were amplified, namely β-tubulin (BenA), and calmodulin (CaM). A segment of the β-tubulin gene was amplified using primers Bt2a (5’-GGTACCAATCGGTCGCT-3’) and Bt2b (5’-ACCTCTAGTTATGACCCCTTC-3’) (Glass and Donaldson, 1995), and a fragment of the calmodulin gene was amplified using primers cmd5 (5’-CGGTAGTACAAGGAGGCCTTC-3’) and cmd6 (5’-CGGATAGAGGTCATAACGTGG-3’) (Hong et al., 2005). The amplification of BenA and CaM loci for some of our strains resulted in poor sequence data and these strains were identified by at least one gene (BenA or CaM). Each PCR mixture (final volume 24 µl) contained 16.45 µl water, 0.75 µl (50 mM) Magnesium chloride, 2.5 µl 10 x PCR buffer, 1.95 µl dNTP mix (1 mM), 1.25 µl dimethyl sulfuroxide (DMSO), 0.5 µl of each primer (10 µM), 0.1 µl Taq polymerase (BioTaq 5 U/µL), and 1 µl of template DNA. ThePCR and sequencing reactions were performed as described previously (Visagie et al., 2014). Sequences were identified using the Basic Local Alignment Search Tool (BLAST) of The NCBI database (NCBI, 2015). A Westerdijk Institute in-house database with the latest taxonomic names and additions was also used for identification. The sequences were then deposited to the GenBank database and accession numbers are presented in Table 2.

**Antifungal Susceptibility**

*In vitro* antifungal susceptibility testing was performed according to the CLSI M38-A2 microbroth dilution method for filamentous fungi (Clinical and Laboratory Standards Institute [CLSI], 2008). The antifungal agents tested were: amphotericin B (AMB), voriconazole (VRC), iraconazole (ITC), posaconazole (PCZ) (Sigma-Aldrich, St. Louis, MO, USA), isavuconazole (ISA; Basilea Pharmaceutica, Basel, Switzerland), anidulafungin (ANID; Pfizer Pharma), and micafungin (MICA; Astellas Pharma Inc.). All antifungal drugs were tested in concentrations ranging from 0.03 to 16 µg/mL. *Pichia kudriavzevii (Candida krusei)* (ATCC 6258) was used as a quality control (QC) strain as indicated in CLSI M38-A2. In addition, we tested *Aspergillus fumigatus* (ATCC 46645), a reference strain from an official culture collection with known stable MIC values. The susceptibility plates were prepared and stored at −70°C until use. Results were read after 24 and 48h of incubation at 37°C. The minimum inhibitory concentrations (MICs) for AMB and azoles were determined as the lowest concentration of the antifungal drug that prevents any discernable growth (100% inhibition) whereas the minimum effective concentrations (MECs) for echinocandins were defined as the lowest concentration of the antifungal drug that leads to rounded compact hyphal growth compared with the unchanged growth in the control well. Visual reading of the MICs/MECs was performed with the aid of an inverted mirror (Clinical and Laboratory Standards Institute [CLSI], 2008).

**TABLE 2** Aspergillus spp. isolates with Genbank accession numbers.

| Accession number | Aspergillus spp. | Genbank accession number |
|------------------|------------------|--------------------------|
| Q0098            | Aspergillus flavus | MK159746 MK038942        |
| Q0782            | Aspergillus pseudomonius | MK159747 MK038958      |
| Q0521            | Aspergillus tamarii | MK159748 MK038959       |
| Q0477            | Aspergillus palidofulvus | MK159749 MK038939      |
| Q0224            | Aspergillus flavus | MK159750 MK038951       |
| Q1013            | Aspergillus flavus | MK159751 MK038952       |
| Q1129            | Aspergillus flavus | MK159752 MK038948       |
| Q3118            | Aspergillus flavus | MK159753 MK038955       |
| Q1374            | Aspergillus flavus | MK159754 MK038943       |
| Q5254            | Aspergillus flavus | MK159755 –               |
| Q1165            | Aspergillus flavus | MK159756 MK038953       |
| Q6198            | Aspergillus flavus | MK159757 MK038956       |
| Q0234B           | Aspergillus flavus | MK159758 MK038945       |
| Q1169            | Aspergillus flavus | MK159759 MK038946       |
| Q0338            | Aspergillus flavus | MK159760 MK038957       |
| Q0139            | Aspergillus flavus | MK159761 MK038947       |
| Q0881            | Aspergillus flavus | MK159762 MK038954       |
| Q3252            | Aspergillus flavus | MK159763 MK038944       |
| Q0725            | Aspergillus flavus | MK159764 MK038949       |
| Q0012            | Aspergillus tubergensis | MK159765 MK038993      |
| Q1072            | Aspergillus tubergensis | MK159766 MK038994     |
| Q0333            | Aspergillus tubergensis | MK159767 MK038995     |
| Q0416            | Aspergillus veletrichiae | MK159768 MK038998    |
| Q0490            | Aspergillus veletrichiae | MK159769 MK039001    |
| Q1114            | Aspergillus veletrichiae | MK159770 MK039002     |
| Q2286            | Aspergillus veletrichiae | MK159771 MK039009     |
| Q4000676         | Aspergillus veletrichiae | MK159772 MK039000    |
| Q8000006         | Aspergillus sublatus | MK159773 MK039004      |
| Q1301            | Aspergillus quadlineatus | MK159774 MK039005    |
| Q0140            | Aspergillus nidulans | MK159775 MK039006     |
| Q0486            | Aspergillus caespitosus | – MK039003    |
| Q6630            | Aspergillus chevalieri | MK159776 MK039092    |
| Q0078            | Aspergillus terreus | MK159777 MK039060      |
| Q1467            | Aspergillus terreus | MK159778 MK039075      |
| Q2779            | Aspergillus terreus | MK159779 MK039063      |
| Q6596            | Aspergillus terreus | MK159780 MK039069      |
| Q4000006         | Aspergillus terreus | MK159781 MK039066      |
| Q4145            | Aspergillus terreus | MK159782 MK039068      |
| Q3996            | Aspergillus terreus | MK159783 MK039064      |
| Q6746            | Aspergillus terreus | MK159784 MK039074      |
| Q4260            | Aspergillus terreus | MK159785 MK039067      |
| Q8811            | Aspergillus terreus | MK159786 MK039072      |
| Q7406            | Aspergillus terreus | MK159787 MK039076      |
| Q4672            | Aspergillus terreus | – MK039073            |
| Q1651            | Aspergillus terreus | MK159788 MK039070      |
| Q7463            | Aspergillus terreus | MK159789 MK039065      |
| Q1444            | Aspergillus terreus | – MK039062            |

(Continued)
Aspergillus species were analyzed using the latest epidemiological cut-off values (ECVs) proposed by CLSI (Clinical and Laboratory Standards Institute [CLSI], 2018) to determine the presence of wild type (WT) and non-wild type (NWT) strains.

**RESULTS**

**Patients Groups and Aspergillosis**

Seventy *Aspergillus* strains were isolated from clinical specimens obtained from 67 patients including 40 males and 27 females. The age of female and male patients ranged from 8 to 74 (media n = 36) and 4 to 80 (media n = 36.5) years old, respectively. Eight patients were under 18 years (17, 15, 13, 11, 8, 4, and 2 patients were 6 years old) and 50% (4/8) of them suffered from cystic fibrosis (Table 1).

The majority of *Aspergillus* species were isolated from respiratory specimens (n = 28, 40%) and nasal sinuses (n = 24, 34.3%) (Table 3). Two isolates (*A. welwitschiae* and *A. flavus*) were recovered from a patient with fungal rhinosinusitis. Three strains (1 *A. fumigatus* and 2 *A. terreus*) were isolated separately from sputum samples of a patient with cystic fibrosis with 4 months interval between isolations.

Fourteen patients (20.9%) presented with IA and 55 patients with non-invasive infections. The underlying conditions of these patients were immune suppression (cancer, on immunosuppressant drugs, and diabetes), chronic pulmonary disease (tuberculosis and cystic fibrosis), pneumonia, rhinosinusitis, and onychomycosis, in addition to ear, wound, skin, and eye infections. Seventeen patients were immunocompromised (24.3%) and seven patients (10.4%) died within 30 days of diagnosis irrespective of antifungal treatment. Two of the deceased patients were infected with *Aspergillus terreus*, 2 with *Aspergillus nidulans*, and 3 patients each with *Aspergillus citrinoferreus*, *A. flavus*, and *A. fumigatus*, respectively. Patients’ demographics, clinical information and *Aspergillus* species isolated are listed in Table 1.

Fourteen *Aspergillus* species belonging to seven sections were recovered (Table 1). In addition, we detected cryptic *Aspergillus* species in 29% of our isolates (n = 20) which belong to 6 species complexes namely *A. welwitschiae* (n = 7, 10%) (section Nigri), *Aspergillus tubingensis* (n = 3, 4.3%) (section Nigri), *A. citrinoferreus* (n = 3, 4.3%) (section Terrei), *A. pseudonomius* (n = 1, 1.4%) (section Flavi), *Aspergillus chevalieri* (n = 1, 1.4%) (section Aspergillus), *A. sublatus* (1.4%) (section Nidulantes), *Aspergillus quadrilineatus* (1.4%) (section Nidulantes), *Aspergillus pallidoferreus* (1.4%) (section Circumdatai), *Aspergillus tamarri* (1.4%) (section Flavi) and *Aspergillus caesitiosus* (1.4%) (section Nidulantes).

*A. welwitschiae* was the most isolated cryptic species (n = 7, 35%), followed by *A. tubingensis* and *A. citrinoferreus* (each n = 3, 15%) *A. terreus* was isolated from 62.5% (5/8) of the ear specimens and 47.4% (9/19) of *A. flavus* isolates were recovered from patients presented with fungal rhinosinusitis.

**Antifungal Susceptibility**

All the MICs were within the required ranges for the QC and reference strains tested. Since there are no Clinical Break Points (CBPs) available for *Aspergillus* spp. by the CLSI, MIC data were analyzed and interpreted according to the ECVs indicated in the CLSI M59-ED2 (Clinical and Laboratory Standards Institute [CLSI], 2018). There are neither CBPs nor ECVs available for ANID and MICA.

One isolate of *A. fumigatus* showed a MIC of 2.0 µg/ml for ITC and was therefore categorized as NWT (ECV = 1). Another *A. fumigatus* strain exhibited high MEC values for ANID and MICA (4 and 16 µg/ml, respectively). One *A. flavus* showed an elevated MIC of 16 µg/ml for AMB and was considered to be NWT (ECV = 4). All *A. nidulans* isolated (n = 2) had elevated MICs of 2.0 µg/ml to AMB. Antifungal susceptibilities were not determined for *A. chevalieri* since it repeatedly failed to grow in the susceptibility test medium. The antifungal susceptibility data are presented in Table 4.

For all *Aspergillus* isolates, the range of MICs/MECs for triazoles, AMB, and echinocandins were: ITC (0.03–2.0 µg/ml), PCZ (0.03–0.5 µg/ml), VRC (0.03–1.0 µg/ml), ISA (0.03–2.0 µg/ml), AMB (0.125–16 µg/ml), ANID (0.03–4.0 µg/ml) and MICA (0.03–16 µg/ml). The overall geometric mean (GM), MIC<sub>50</sub> and MIC<sub>90</sub> are listed in Table 5.
TABLE 3 | Occurrence of Aspergillus spp. in clinical specimens.

| Respiratory | Fungal sinusitis | Wound | Ear | Brain abscess | Nail | Burn | Eye | Unknown |
|-------------|-----------------|-------|-----|--------------|------|------|-----|---------|
| A. flavus \(n = 19\) | 5 | 9 | 1 | 2 | | | | 1 |
| A. terreus \(n = 17\) | 9 | 3 | | 5 | | | | |
| A. fumigatus\(n = 12\) | 7 | 1 | 1 | 2 | | | | 1 |
| A. wallischiæ \(n = 7\) | 1 | 6 | | | | | | |
| A. citrinoètreus \(n = 3\) | 1 | 1 | 1 | | | | | |
| A. tubingenensis \(n = 3\) | 1 | 2 | | | | | | |
| A. nidulans \(n = 2\) | 1 | | | | | | | |
| A. pseudonominus \(n = 1\) | 1 | | | | | | | |
| A. chevalieri \(n = 1\) | | | | | | | 1 | |
| A. tamarin \(n = 1\) | | | | | | | | 1 |
| A. sublatus \(n = 1\) | | | | | | | | |
| A. quadrilineatus \(n = 1\) | | | | | | | | |
| A. pallidofulvus \(n = 1\) | | | | | | | | |
| A. caespitosus \(n = 1\) | | | | | | | | |
| Total \(n = 70\) | 28 | 23 | 5 | 8 | 2 | 1 | 1 | 1 |

DISCUSSION

The present study describes the molecular identification and susceptibility patterns of 70 Aspergillus strains isolated from clinical specimens of 67 patients from Hamad general hospital in Qatar, including adults (88%) and pediatric patients (12%). To our knowledge, this is considered as the first study exploring the molecular identification and antifungal susceptibility profiles of clinical aspergilli in this country.

Twenty isolates (29%) of cryptic Aspergillus spp. were recovered from patients’ samples representing 10 different species that belong to six sections. Previous studies from Spain and Brazil reported a prevalence of 14.5% (Alastruey-Izquierdo et al., 2013) and 19% (Negri et al., 2014) for cryptic Aspergillus spp., respectively, which is lower than what we found in the current study. The majority of the cryptic species isolated in this study were members of section Nigri \((n = 10/20, 50\%)\). No cryptic species were isolated from section Fumigati.

Aspergillus pseudonominus is reported in the current study for the first time ever from clinical specimens and was isolated from a patient with fungal rhinosinusitis proven by histopathology. It exhibited low MIC values for all the antifungal drugs tested (Table 4). Echinocandins were the most active drugs (MEC = 0.03 µg/ml) and ISA was the most active triazole drug against A. pseudonominus with a MIC of 0.06 µg/ml.

In addition, we report the first isolation of A. wallischiæ from six patients with fungal rhinosinusitis, three of them were proven by histopathology and one by computed tomography (CT) as invasive infections. A. wallischiæ was previously isolated from patients with respiratory infections (Pinto et al., 2018) and onychomycosis (Tsang et al., 2016). Low MICs were observed for all of the antifungal agents investigated and PCZ was the most active triazole with a MIC of 0.06 µg/ml. A. wallischiæ was also isolated in our study from a BAL of a patient with Aspergillus pneumonia.

Invasive infections caused by A. sublatus were previously reported from patients with HSCT (de Fontbrune et al., 2014; Chrenkova et al., 2018). Here we report it for the first time from BAL specimen of an adult cystic fibrosis patient with moderate obstructive pulmonary disease. However, we were unable to discern between colonization and infection caused by A. sublatus. Echinocandins MICs were relatively higher for A. sublatus compared to the other Aspergillus spp. in our set, with MIC values of 0.125 and 0.25 µg/ml for ANID and MICA, respectively. Previously reported MICs of ANID and MICA for A. sublatus were <0.0312 µg/ml (Chrenkova et al., 2018) which is lower than our findings. ITC and voriconazole were the most active triazoles with MICs of 0.125 µg/ml for both drugs (Table 4). A. sublatus belongs to Aspergillus section Nidulantes and is very closely related to A. quadrilineatus (Hubka et al., 2016). These species are indistinguishable by sequencing BenA alone (Hubka et al., 2016), whereas reliable identification can be achieved by sequencing CaM gene (Hubka et al., 2016). In our case, we identified A. sublatus by sequencing both BenA and CaM genes. AMB exhibited low MIC (0.5 µg/ml) in comparison to A. nidulans (2.0 µg/ml), which is known to be resistant to AMB (Van’t Heek et al., 1998; Kontoyiannis et al., 2002; Bowman et al., 2006). A low AMB MIC was also reported in other studies for A. sublatus (de Fontbrune et al., 2014; Chrenkova et al., 2018).

Aspergillus pallidofulvus was isolated in the present study from a BAL sample of a patient with interstitial lung disease, which is considered as the second report of this species from clinical specimens after a previous report from India where it was isolated from a BAL sample of a patient with invasive pulmonary aspergillosis (IPA) (Masih et al., 2016). The single strain isolated in the current study showed a high MIC value for AMB (2 µg/ml), which was also observed previously for this species (Masih et al., 2016). With the available data, it was not possible to recognize A. pallidofulvus as the cause of infection or colonization.

The recently described A. citrinoterreus, which belongs to section Terrei, was reported mainly from patients with
TABLE 4  |  In vitro antifungal susceptibility results of Aspergillus spp.

| Aspergillus spp. isolated (n) | Antifungal | Range | GM | MIC/MEC (µg/ml) | 0.03 | 0.06 | 0.125 | 0.25 | 0.5 | 1 | 2 | 4 | 8 | 16 |
|-----------------------------|------------|-------|----|----------------|------|------|-------|------|-----|---|---|---|---|---|
| A. flavus (19)              | ITC        | 0.03–0.25 | 0.15 | 1 | 1 | 11 | 6 |
|                            | AMB        | 0.125–16 | 0.66 | 2 | 1 | 6 | 7 | 2 | 1 |
|                            | PCZ        | 0.03–0.25 | 0.06 | 10 | 4 | 4 | 1 |
|                            | VCZ        | 0.03–0.25 | 0.17 | 1 | 2 | 4 | 9 | 3 |
|                            | ISA        | 0.03–1 | 0.07 | 11 | 4 | 2 | 1 | 1 |
|                            | ANID       | 0.03 | 0.03 | 19 |
|                            | MICA       | 0.03 | 0.03 | 19 |
| A. terreus (17)             | ITC        | 0.03–0.125 | 0.07 | 3 | 7 | 7 |
|                            | AMB        | 0.25–16 | 0.92 | 2 | 8 | 3 | 2 | 1 | 1 |
|                            | PCZ        | 0.03–0.06 | 0.04 | 13 | 4 |
|                            | VCZ        | 0.06–0.25 | 0.14 | 4 | 7 | 5 | 1 |
|                            | ISA        | 0.03–0.25 | 0.04 | 15 | 1 | 1 |
|                            | ANID       | 0.03 | 0.03 | 17 |
|                            | MICA       | 0.03 | 0.03 | 17 |
| A. fumigatus (12)           | ITC        | 0.03–2 | 0.22 | 1 | 4 | 5 | 1 | 1 |
|                            | AMB        | 0.125–2 | 0.53 | 1 | 4 | 1 | 5 | 1 |
|                            | PCZ        | 0.03–0.25 | 0.06 | 5 | 3 | 3 | 1 |
|                            | VCZ        | 0.06–1 | 0.17 | 2 | 7 | 1 | 2 |
|                            | ISA        | 0.03–0.25 | 0.09 | 5 | 1 | 6 |
|                            | ANID       | 0.03–4 | 0.05 | 11 |
|                            | MICA       | 0.03–16 | 0.05 | 11 |
| A. welwitschiae (7)         | ITC        | 0.25–0.5 | 0.37 | 3 | 4 |
|                            | AMB        | 0.25 | 0.25 | 7 |
|                            | PCZ        | 0.06 | 0.08 | 7 |
|                            | VCZ        | 0.25–0.5 | 0.30 | 5 | 2 |
|                            | ISA        | 0.06–0.025 | 0.11 | 2 | 4 | 1 |
|                            | ANID       | 0.03 | 0.03 | 7 |
|                            | MICA       | 0.03 | 0.03 | 7 |
| A. tubingensis (3)          | ITC        | 1 | 1.00 | 3 |
|                            | AMB        | 0.25–0.5 | 0.40 | 1 | 2 |
|                            | PCZ        | 0.125–0.5 | 0.25 | 1 | 1 | 1 |
|                            | VCZ        | 0.5 | 0.50 | 3 |
|                            | ISA        | 0.5–2 | 1.00 | 1 | 2 |
|                            | ANID       | 0.03 | 0.03 | 3 |
|                            | MICA       | 0.03 | 0.03 | 3 |
| A. citrinoterreus (3)       | ITC        | 0.03–0.6 | 0.04 | 2 | 1 |
|                            | AMB        | 1–2 | 1.26 | 2 | 1 |
|                            | PCZ        | 0.03 | 0.03 | 3 |
|                            | VCZ        | 0.06–0.125 | 0.10 | 1 | 2 |
|                            | ISA        | 0.03 | 0.03 | 3 |
|                            | ANID       | 0.03 | 0.03 | 3 |
|                            | MICA       | 0.03 | 0.03 | 3 |
| A. nidulans (2)             | ITC        | 0.125 | 2 |
|                            | AMB        | 2 | 2 |
|                            | PCZ        | 0.03–0.125 | 1 | 1 |
|                            | VCZ        | 0.03 | 0.03 | 2 |
|                            | ISA        | 0.03 | 0.03 | 2 |
|                            | ANID       | 0.03 | 0.03 | 2 |
|                            | MICA       | 0.03 | 0.03 | 2 |

(Continued)
TABLE 4 | Continued

| Aspergillus spp. isolated (n) | Antifungal | Range | GM | MIC/MEC (µg/ml) |
|-----------------------------|------------|-------|----|----------------|
|                             |            |       |    | 0.03 | 0.06 | 0.125 | 0.25 | 0.5 | 1   | 2   | 4   | 8   | 16  |
| A. sublatus (1)             | ITC        | –     | –   | 1   |     |     |     |     |     |     |     |     |     |
|                             | AMB        | –     | –   |     |     |     |     |     |     |     |     |     |     |
|                             | PCZ        | –     | –   |     |     |     |     |     |     |     |     |     |     |
|                             | VCZ        | –     | –   |     |     |     |     |     |     |     |     |     |     |
|                             | ISA        | –     | –   |     |     |     |     |     | 1   |     |     |     |     |
|                             | ANID       | –     | –   |     |     |     |     |     |     | 1   |     |     |     |
|                             | MICA       | –     | –   |     |     |     |     |     |     |     | 1   |     |     |
| A. pseudonomius (1)         | ITC        | –     | –   | 1   |     |     |     |     |     |     |     |     |     |
|                             | AMB        | –     | –   |     |     |     |     |     |     |     |     |     |     |
|                             | PCZ        | –     | –   |     |     |     |     |     |     | 1   |     |     |     |
|                             | VCZ        | –     | –   |     |     |     |     |     |     | 1   |     |     |     |
|                             | ISA        | –     | –   |     |     |     |     |     |     |     | 1   |     |     |
|                             | ANID       | –     | –   |     |     |     |     |     |     |     |     | 1   |     |
|                             | MICA       | –     | –   |     |     |     |     |     |     |     |     |     | 1   |
| A. tamarii (1)              | ITC        | –     | –   | 1   |     |     |     |     |     |     |     |     |     |
|                             | AMB        | –     | –   |     |     |     |     |     |     |     |     |     |     |
|                             | PCZ        | –     | –   |     |     |     |     |     |     |     |     | 1   |     |
|                             | VCZ        | –     | –   |     |     |     |     |     |     |     |     |     | 1   |
|                             | ISA        | –     | –   |     |     |     |     |     |     |     |     |     | 1   |
|                             | ANID       | –     | –   |     |     |     |     |     |     |     |     |     | 1   |
|                             | MICA       | –     | –   |     |     |     |     |     |     |     |     |     | 1   |
| A. pallidofulvus (1)        | ITC        | –     | –   | 1   |     |     |     |     |     |     |     |     |     |
|                             | AMB        | –     | –   |     |     |     |     |     |     |     |     |     |     |
|                             | PCZ        | –     | –   |     |     |     |     |     |     |     |     |     | 1   |
|                             | VCZ        | –     | –   |     |     |     |     |     |     |     |     |     |     |
|                             | ISA        | –     | –   |     |     |     |     |     |     |     |     |     | 1   |
|                             | ANID       | –     | –   |     |     |     |     |     |     |     |     |     | 1   |
|                             | MICA       | –     | –   |     |     |     |     |     |     |     |     |     | 1   |
| A. quadrilineatus(1)        | ITC        | –     | –   | 1   |     |     |     |     |     |     |     |     |     |
|                             | AMB        | –     | –   |     |     |     |     |     |     |     |     |     |     |
|                             | PCZ        | –     | –   |     |     |     |     |     |     |     |     |     | 1   |
|                             | VCZ        | –     | –   |     |     |     |     |     |     |     |     |     |     |
|                             | ISA        | –     | –   |     |     |     |     |     |     |     |     |     | 1   |
|                             | ANID       | –     | –   |     |     |     |     |     |     |     |     |     | 1   |
|                             | MICA       | –     | –   |     |     |     |     |     |     |     |     |     | 1   |
| A. caespitosus(1)           | ITC        | –     | –   | 1   |     |     |     |     |     |     |     |     |     |
|                             | AMB        | –     | –   |     |     |     |     |     |     |     |     |     | 1   |
|                             | PCZ        | –     | –   |     |     |     |     |     |     |     |     |     |     |
|                             | VCZ        | –     | –   |     |     |     |     |     |     |     |     |     | 1   |
|                             | ISA        | –     | –   |     |     |     |     |     |     |     |     |     | 1   |
|                             | ANID       | –     | –   |     |     |     |     |     |     |     |     |     | 1   |
|                             | MICA       | –     | –   |     |     |     |     |     |     |     |     |     | 1   |

ITC, Itraconazole; AMB, Amphotericin B; PCZ, Posaconazole; VCZ, Voriconazole; ISA, Isavuconazole; ANID, Anidulafungin; MICA, Micafungin.

Respiratory infections, in addition to wound, abscess, nail and sinus infections (Guinea et al., 2015; Imbert et al., 2018; Vaezi et al., 2018). In a global study of 498 strains of A. terreus and phenotypically related species, 6 different species of section Terrei were identified and A. citrinoterreus was the second most isolated species (8.4%) (Zoran et al., 2018). In our case, among 20 strains of members of section Terrei, 3 strains (15%) were identified as A. citrinoterreus. We report the second isolation of this species from a case of fungal rhinosinusitis which was proven by histology. This patient was treated with ITC which exhibited a low in vitro MIC of 0.03 µg/ml and for AMB 1 µg/ml. However, there were no data available regarding the therapeutic outcome. The second isolate of A. citrinoterreus was from an endotracheal secretion of a patient with colitis and pleural effusion, with no other details about the underlying diseases or the immune status. Antifungal therapy information was not available for this patient who died.
few days after sample collection, and low MICs were observed (Table 4) for all the tested drugs including AMB MIC of 1 µg/ml. The third case of A. citrinoterreus was from a wound sample of a patient who had a road traffic accident. Antifungal therapy details were not available and the isolate showed low MICs in vitro except for AMB which showed an elevated MIC of 2 µg/ml. It was not possible to categorize the later 2 cases as infection or colonization.

Aspergillus tamarii is rarely encountered as a human pathogen. It was reported from few cases of cutaneous aspergillosis (Sharma et al., 2013; Kimura et al., 2018), onychomycosis (Kristensen et al., 2005), burn wound (Renner et al., 2018), keratitis (Kredics et al., 2007), respiratory (Castro et al., 2019) and sinus infections (Paludet et al., 1992). In the current study, we report the isolation of A. tamarii from wound tissue of a patient who experienced a road traffic accident. This patient received AMB with unknown treatment outcome and the in vitro MIC of AMB was 0.25 µg/ml. We could not determine whether Aspergillus tamarii was the cause of infection or a colonizer.

Aspergillus chevalieri is one of the most common species present in indoor environments (Hubka et al., 2013). Clinically, it has been recovered from a case of cutaneous aspergillosis (Naidu and Singh, 1994), a fatal cerebral aspergillosis case (Masih et al., 2016), and respiratory, corneal and sinus infections (Siqueira et al., 2018). In our study, we isolated A. chevalieri from an ear swab of a patient with acute lymphoblastic leukemia (ALL). The patient received micafungin, a topical antifungal drug.

Aspergillus tubingensis was found to be a major fungus associated with bronchial colonization in patients with lung disease (Reynaud-Gaubert et al., 2016). Previous reports of A. tubingensis were from patients with cutaneous aspergillosis (Balajee et al., 2009; Pagiotto et al., 2010), otomycosis (Szigi et al., 2012a,b), keratitis (Doczi et al., 2009), onychomycosis (Nouripour-Sisakht et al., 2015), and osteomyelitis (Hedayati et al., 2007). We recovered A. tubingensis from three patients: one with unknown underlying diseases presented with fungal rhinosinusitis and was proven by histopathology. The second patient suffered from breast cancer and presented with rhinosinusitis. It was considered as either colonization or the allergic type of Aspergillus rhinosinusitis due to the negative histology investigation. The third patient had cystic fibrosis with unknown status of invasion or colonization. The antifungal therapy data were unavailable for those patients. In general, low antifungal MICs were exhibited for A. tubingensis strains except for ISA which showed high MIC of 2.0 µg/ml for the first and the second cases. No A. niger sensu stricto was isolated in our set of strains.

Invasive aspergillosis caused by A. quadrijunicatus, which is closely related to A. nidulans, was previously reported from 2 patients who presented with fungal rhinosinusitis and had undergone bone marrow transplantation for hematological malignancy (Polacheck et al., 1992; Drakos et al., 1993), three cases of IPA in patients with CGD (Verweij et al., 2008), a patient with cerebral aspergillosis (Verweij et al., 2008) and another with onychomycosis (Gugnani et al., 2004). Our isolate was recovered from a case of onychomycosis, however, it was not possible to confirm that A. quadrijunicatus was the direct cause of infection or colonization. A lower MIC value for AMB was observed (1 µg/ml) in comparison to A. nidulans (2 µg/ml) which is in agreement with a previous report (Verweij et al., 2008).

Aspergillus caesius is a soil fungus (Raper and Thoms, 1944; Chen et al., 2016) and has not been reported previously as a human pathogen. In the current study, it was isolated from a BAL specimen of a patient suffering from tuberculosis and showed low MICs for all the antifungal drugs tested. It was unknown whether A. caesius was the cause of true infection or colonization.

Aspergillus fumigatus has been reported as the most prevalent species causing IA in different parts of the world, including the United States, Europe and Brazil (Balajee et al., 2009; Alastruey-Izquierdo et al., 2013; Negri et al., 2014). In this study, A. fumigatus was the most prevalent species (27%), which is consistent with reports from India (47%) (Xess et al., 2004), Iran (75%) (Zanganeh et al., 2018), and Tunisia (79%) (Hadrich et al., 2010). The predominance of A. fumigatus in these parts of the world is attributed to arid and semi-arid climates (Kameswaran et al., 1992; Hedayati et al., 2007). The second most prevalent species in our set was A. terreus (24%), followed by A. fumigatus (17%). This could be due to ecological preferences for environments specific to Qatar, as the highly arid deserts, which needs to be investigated thoroughly by an environmental sampling of different ecological niches. A. terreus and A. fumigatus were the most isolated species (29%) from immunocompromised patients and A. terreus was the most recovered species from respiratory samples (9/27, 33%), followed by A. fumigatus (6/27, 22%). Infections caused by A. terreus are of concern due to its reduced susceptibility to AMB in vitro and in vivo (Sutton et al., 1999; Walsh et al., 2003). This species was also found to be prevalent in Tyrol, Austria, from environmental and clinical sources (Lass-Flörl et al., 2005; Lackner et al., 2016). Moreover, a previous multicentre study from the United States reported that the incidence of A. terreus following HSCT and SOT was found to be 16 and 11.8%, respectively (Morgan et al., 2005).

The majority of Aspergillus spp. were isolated from patients with non-invasive infections and a low rate of IA was observed in our study. Aspergillus rhinosinusitis was the second highest clinical presentation (23/67, 34.3%) after respiratory infections. These findings are in accordance with a report by Taj-Aldeen et al. (2015), which estimated the burden of fungal infections.
in Qatar (Taj-Aldeen et al., 2015). The study reported that Aspergillus rhinosinusitis in Qatar has a relatively high rate (2.31 cases/100,000 individuals). This is attributed to the hot and arid climate in the country and atopic young patients who develop allergic Aspergillus rhinosinusitis, in addition to the high number of residents who came from countries with elevated incidences of Aspergillus rhinosinusitis (Taj-Aldeen et al., 2003, 2004, 2015). The human population of Qatar is extremely mixed with high number of Southeast Asians, particularly Indians. In our set, the majority (13/23, 57%) of patients affected with Aspergillus rhinosinusitis originated from Southeast Asia and more than half of them (n = 7/13, 54%) were from India. Additionally, 4/13 (31%), patients were from Sudan. Previous reports showed that Aspergillus rhinosinusitis is common in these regions (Milošev et al., 1969; El Daoud et al., 1973; Chatterjee and Chakrabarti, 2010; Garg et al., 2013; Chakrabarti et al., 2015; Jain et al., 2015; Krishnan et al., 2015; Mahgoub et al., 2016). Two patients with Aspergillus rhinosinusitis, one of which infected with A. flavus and the other by A. fumigatus, died due to extension of the fungus to the brain (Table 1). Two patients were immunocompromised, one patient who was infected with A. tuberculosis suffered from breast cancer. The other patient presented with nephrotic syndrome and received immunosuppressive therapy, and was infected with A. terreus. The latter case was proven by histology (Table 1).

Taj-Aldeen et al. (2015) also showed that among respiratory aspergillosis in Qatar the rate of infection for chronic pulmonary aspergillosis post-tuberculosis (CPA-TB) was 0.75/100 000 and the other CPA was 26.8/100,000. Allergic bronchopulmonary aspergillosis (ABPA) and severe asthma with fungal sensitization (SAFS) were more common at 60.2/100 000 and 79.46/100 000, respectively (Taj-Aldeen et al., 2015). We were unable to retrieve the complete set of clinical details about the manifestation of aspergillosis. The available clinical presentations are presented in Table 1. Overall, most of the isolates showed low MIC values for the systemic antifungal agents investigated. PCZ was the most active drug with MICs ranging from 0.03 to 0.5 µg/ml (Table 5). Echinocandins are generally potent against Aspergillus spp. and are used as salvage therapy or in combination with other classes of antifungal drugs (Patterson et al., 2016). However, a recent report detected a point mutation in the fks1 gene of a strain of A. fumigatus, which caused echinocandin resistance and subsequent treatment failure (Jimenez-Ortigosa et al., 2017). We observed echinocandin resistance in one strain of A. fumigatus with high MECs for ANID and MICA i.e., 4.0 and 16 µg/ml, respectively. Another strain of A. fumigatus showed a MIC of 2.0 µg/ml for ITC and was categorized as NWT based on CLSI ECVs (Clinical and Laboratory Standards Institute [CLSI], 2018). VRC, POS, and ISA MICs for the same strain were 1, 0.25 and 0.25 µg/ml, respectively, which were categorized as WT based on CLSI ECVs (Espinel-Ingroff et al., 2010). These findings need to be investigated by analyzing the molecular mechanism(s) of resistance. One A. flavus strain showed high MIC of 16 µg/ml for AMB and therefore is considered to be non-wild type (NWT) based on CLSI ECVs. Previous studies have shown that in vitro resistance of A. flavus to AMB is correlated with treatment failure (Hadrich et al., 2012; Barchiesi et al., 2013). Most of the A. terreus strains, a species that is considered as intrinsically resistant to AMB (Lass-Florl et al., 2005), showed high MIC values for AMB (range; 0.25–8.0 µg/ml). A. chevalieri failed to grow in susceptibility medium due to its xerophilic nature, therefore, no MIC values were determined.

Patients' therapeutic outcome was not calculated in our study since the data set was incomplete. We were able to retrieve the antifungal therapeutic regimes for 26 out of 67 patients indicated in Table 1. Therapy was dependent on the site of infection and underlying disease. In short, patients with IA (n = 6): 2 received ITC, 2 ITC, and VCZ, 1 received ITC in addition to AMB, VCZ and fluconzole, and 1 was treated with VCZ and caspofungin; immunocompromised patients (n = 5): 1 was treated with AMB and caspofungin, 1 with VCZ and AMB, 1 with caspofungin, fluconzole, miconazole and VCZ, and 2 patients each with VCZ and miconazole; patients diagnosed with fungal rhinosinusitis (n = 9): 8 received either VCZ and/or ITC, and 1 treated with VCZ in addition to caspofungin. Patients with respiratory Aspergillus infection or colonization (n = 11): 4 received VCZ, 2 treated with ITC, 1 with caspofungin, 1 with caspofungin and AMB, 1 with AMB, caspofungin and VCZ, 1 with ANID, and 1 patient received miconazole probably for a superficial infection. Among 3 patients who died within 30 days of diagnosis, 1 was treated with caspofungin, miconazole and VCZ, 1 with VCZ, and 1 with ANID. For patients treated with multiple antifungal drugs, it was unknown whether the drugs were administered singly or in combinations.

The current study highlights the molecular identification and antifungal susceptibility profiles of 70 clinical Aspergillus species in Qatar. Future studies with larger sample size, including clinical and environmental samples, would provide more insight into the epidemiology of clinical aspergilli in the country.

CONCLUSION

In conclusion, we report the molecular identification and in vitro antifungal susceptibility profiles of 70 Aspergillus spp. recovered from various clinical specimens in Qatar. Rare and cryptic Aspergillus species with variable antifungal susceptibilities were detected. Triazol resistance, and recently Echinocandin resistance, is emerging in many parts of the world. Further investigation of resistance mechanism(s) is warranted for species with reduced susceptibilities to antifungal drugs. Infectious disease physicians must be aware of the emerging and resistant species to decide on accurate treatment and improved clinical outcomes.

DATA AVAILABILITY

The datasets generated for this study were deposited in Genbank. The accession numbers are listed in Table 2.

AUTHOR CONTRIBUTIONS

HS performed the technical work and wrote the manuscript draft. JH, ML, and BT provided technical assistance.
ST-A and TB assisted in designing the manuscript and writing the first draft. MA and CL-F advised on clinical aspects of the manuscript. All authors contributed to manuscript revision, editing, and approved the submitted version.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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