QUANTITATIVE PCR DETECTION OF *ASPERGILLUS* SPP IN CLINICAL SAMPLES FROM HEZHOU PROVINCE, GUANGXI, CHINA

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Abstract. Microscopic detection of infectious fungus is frequently insensitive while traditional culture-based methods have limited sensitivity and specificity. PCR-based techniques provide rapid, sensitive and specific detection of pathogens. Quantitative (q)PCR, in comparison to direct microscopic examination and culture method, was carried out to identify *Aspergillus flavus, A. fumigatus* and *A. niger,* three of the most medically important pathogenic *Aspergillus* spp in clinical specimens from Hezhou People's Hospital in Guangxi province, PR China. Among clinical specimens (n = 161) collected between March and November 2020, qPCR-positive samples were obtained from female (n = 22) and male (n = 34) patients, with a median age of 61.0 and 62.5 years respectively (range = 10 months - 92 years old). Excellent agreement was observed between the qPCR assay and culture technique (concordance rate Κ = 0.944, p-value <0.0001), while smear method demonstrated moderate agreement (concordance rate Κ = 0.476, p-value <0.0001). *A. fumigatus, A. niger* and *A. flavus* was identified in 75, 16 and 9%, respectively of positive specimens (n = 56) obtained from patients (n = 31) >60 years of age. Of 53 qPCR *Aspergillus* spp-positive specimens, 77, 9 and 9% were from sputum, ear secretion and abscess, respectively. The superior sensitivity, specificity and rapidity of qPCR compared to microscopic and culture techniques in the detection of *Aspergillus* spp in clinical samples despite its initial hardware cost.

Keywords: *Aspergillus* spp, aspergillosis, clinical specimen, quantitative PCR

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INTRODUCTION

Aspergillus is a mold, which causes aspergillosis, a fungal infection found worldwide. There are an estimated 200 *Aspergillus* spp, of which 40, *eg*, *A. flavus, A. fumigatus* (the most common), *A. nidulans, A. niger, A. terreus,* and *A. versicolor* are pathogenic to humans, cause diseases, such as allergic *Aspergillus* sinusitis, invasive aspergillosis (IA), allergic bronchopulmonary aspergillosis (ABPA), cutaneous (skin) aspergillosis, and chronic pulmonary aspergillosis (Latgé, 1999; Geiser *et al*, 2007). Aspergillosis causes infections in several regions of the body, such as the ear canal, nail bed, sinus, and skin. *Aspergillus* spp are also the principal pathogen responsible for otomycosis, a fungal infection of the outer ear (Sood *et al*, 1967; Youssef and Abdou, 1967).

In addition to employing the culture method and microscopic examinations to identify the pathogenic organism, a diagnosis of aspergillosis or otomycosis can also be made by observing specific symptoms and thoroughly documenting the patient's medical history (Vandewoude *et al*, 2006; Samarakoon and Soubani, 2008; Agarwal and Devi, 2017). Other, more advanced technologies, including computed tomography (CT) and magnetic resonance imaging (MRI) scans, can be used to determine whether the fungus has migrated to other regions of the body.

Although microscopy and culture methods are simple and straightforward means of detecting *Aspergillus*, they lack sensitivity and specificity. In addition, immunological assays can be performed to diagnose *Aspergillus* infection as demonstrated alongside signs of chronic respiratory diseases for at least three months (Denning *et al*, 2018). PCR-based assays have now provided a rapid, specific and sensitive platform for detecting pathogens (Morton *et al*, 2017). Enzyme-linked immunosorbent assay (ELISA) have been effective in detecting galactomannan antigen (GM) in serum and bronchoalveolar lavage fluid (BAL), while DNA-based methods in detecting *Aspergillus*-specific DNA (Costa *et al*, 2002).

However, the lack of *Aspergillus*-specific IgG and insufficient training of technicians often make a diagnosis of *Aspergillus* infection problematic in a hospital setting.

The availability of validated species-specific and highly sensitive primers and probes for amplifying and detecting fungal DNA in clinical samples (Sandhu *et al*, 1995; Einsele *et al*, 1997; Morace *et al*, 1997; Loeffler *et al*, 2000), PCR-based assays have become more popular, in particular, quantitative qPCR, which takes less than a day to perform and is amenable for moderate throughput format (Goebes *et al*, 2007).

Systematic collection of data on clinical characteristics, disease conditions, and the spectrum of aspergillosis and otomycosis infections has not been established in Hezhou City, southern China. Therefore, this study aimed to evaluate the use of qPCR assay for identifying *Aspergillus* spp in comparison with microscopic examination and culture method of clinical specimens at Hezhou People's Hospital. The results should provide baseline information regarding the feasibility and utility of adopting the qPCR assay for routine detection of *Aspergillus* spp in clinical specimens at Hezhou People's Hospital.

MATERIALS AND METHODS

Fungal cultures

Standard fungal strains of *A. flavus* (ATCC 11492), *A. fumigatus* (ATCC 96918) and *A. niger* (ATCC 16404) were cultured for 4-14 days at 37°C on a fungal selective medium (Sabouraud dextrose agar, SDA) (Autobio, Zhengzhou, China). Slides were prepared using lactic acid/phenol/cotton blue staining solution (BASO, Zhuhai, China) of 7 to 10 days old cultures for microscopic examination.

qPCR protocols

Fungal strains were cultivated on SDA (Autobio, Zhengzhou, China) at 37°C for 7 to 10 days and DNA was extracted as described by Johnson *et al* (2012). Two loopfuls of fungal culture were added to 180 µl aliquot of ATL buffer (lysis buffer) (Qiagen, Hilden, Germany) and 20 µl aliquot of proteinase K (20 mg/ml) in a tube containing glass beads (0.3-1.0 g, 0.5 mm diameter), which was shaken for 3 minutes at 65 Hz (Tiss-24, Jingxin, Shanghai, China), then incubated at 56°C for 2 hours with occasional vertexing. DNA was extracted using QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) and stored at -20°C until used. DNA concentration was determined using Epoch™ Spectrophotometer (BioTek, Agilent Technologies, Santa Clara, CA).

The oligonucleotide primers and probes used in the qPCR assays are listed in Table 1. qPCR assays were performed in a 96-well plate

(FrameStarH, 4titude Ltd, Wotton, Surrey, UK), each well containing a qPCR mixture (20 µl) consisting of 10 µl of 2X Master Mix Kit (Ampliqon, Odense, Denmark). 0.8 µl of each primer (5M), 0.8 µl of TaqMan probe (5M), 2 µl of DNF buffer (Sangon Biotech, Shanghai, China), 2 µl of DNA template, and 3.6 µl of double distilled water. No template control (NTC) reaction was included in each PCR experiment. Thermocycling was carried out in a Thermo Fisher Scientific 7500 Real-Time PCR Instrument (Applied Biosystems, Waltham, MA) as follows: 50°C for 5 minutes, 95°C for 5 minutes, and 35 cycles of 95°C for 15 seconds and 60°C for 30 seconds. Positive controls were DNA from the standard strains of *A. flavus, A. fumigatus* and *A. niger* described above. A calibration curve was constructed using 0.002-20 ng extracted DNA/reaction to obtain a cutoff threshold cycle value ($Ct_{threshold}$) above which the sample is considered negative for *Aspergillus* spp. If the $Ct_{threshold}$ value is more than 35, the result is negative.

In order to test the performance of the PCR using each pair of the *Aspergillus* sp primers, an aliquot of the reaction mixture at the termination of the amplification cycle was separated by 2% agarose gel-electrophoresis, stained with 4S Green Plus dye and amplicon visualized under UV illumination. The pan-*Aspergillus* primers detected all test *Aspergillus* spp samples, but also cross-amplified *Penicillium* spp, while *Aspergillus* spp-specific primers generated the expected amplicons (Fig 1).

Clinical specimen collection

Specimens (*n* = 161) were collected in sterile tubes and immediately transported to the Microbiology Department, Hezhou People's Hospital. Individual specimens were divided into two portions, one portion was subjected promptly to culture and smear analysis, and the other stored at -20°C for qPCR assays.

Microscopic examination and culture of clinical specimens

The specimens were smeared on a slide for direct microscopic identification of fungi and then cultured on SDA (Autobio, Zhengzhou, China) for detection of filamentous fungi, fungal growth and macroscopic characteristics using standard mycological procedures based on a slide culture (Indian Council of Medical Research, 2019). Specimens that tested positive and negative by culture procedures were stored at -80°C for subsequent blinded qPCR assays in a separate laboratory.

Clinical specimen pretreatment

Pretreatment method of a specimen depends on the type of clinical sample, namely, abscess, debrided tissue, fluid, sputum, or swab prior to DNA extraction.

(i) For debrided tissue and abscess, the tissue specimen was weighed, cut into small pieces, and suspended in 180 μl aliquot of ATL buffer and 20 μl aliquot of proteinase K (20 mg/ml); the abscess specimen was placed directly in the latter solution (Dilhari *et al*, 2017).

(ii) For fluid (bronchoalveolar lavage and puncture fluid), 200 μl aliquot of the specimen was gently mixed by vortexing with 10 μl aliquot of proteinase K (20 mg/ml), homogenized using glass beads at 65 Hz (Tiss-24, Jingxin, Shanghai, China) as described above, and incubated at 56°C until the sample was completely homogenized.

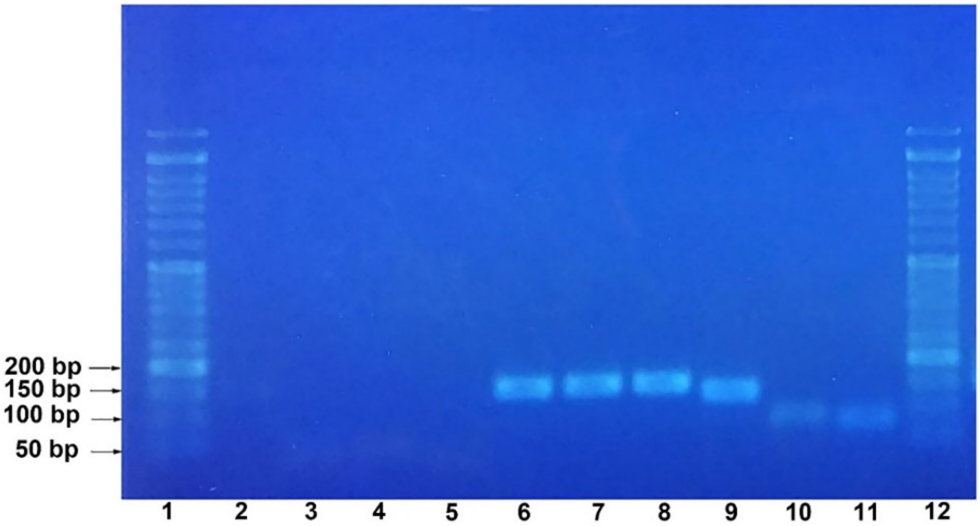

Fig 1 - Electrophoresis of amplicons using *Aspergillus* spp-specific primers

PCR was carried out as described for qPCR and amplicon analyzed by 2% agarose gel-electrophoresis and 4S Green Plus-stained gel visualized under UV illumination. Primers are listed in Table 1.

Lanes 1 and 12: 50-bp DNA markers; Lanes 2-5: no template control of each of the PCR; Lanes 6 and 9: *A. fumigatus* DNA (150 and 147 bp); Lanes 7 and 10: *A. flavus* DNA (150 and 100 bp); Lanes 8 and 11: *A. niger* DNA (150 and 100 bp).

Lanes 6, 7 and 8 using pan-Aspergillus primers; Lanes 9, 10 and 11 using *Aspergillus* spp-specific primers

bp: base pair; DNA: deoxyribonucleic acid; PCR: polymerase chain reaction; qPCR: quantitative polymerase chain reaction; UV: ultraviolet

(iii) For sputum, an aliquot was added to an equal volume of Sputolysin reagent (Calbiochem, San Diego, CA), vortexed for 30 seconds, incubated at 37°C for 15 minutes, vortexed again for 30 seconds, and then centrifuged at 13,000 g for 10 minutes. The pellet was resuspended in 200 µl aliquot of 10 mM Tris-HCl pH 8.0 (Goebes *et al*, 2007).

(iv) For swab (from ear or nose), the specimen was placed in 1 ml of phosphate-buffered saline pH 7.4 (PBS), centrifuged at 13,000 g for 10 minutes and the pellet suspended in 180 µl aliquot of ATL buffer and 20 µl aliquot of proteinase K (20 mg/ml).

qPCR assay of clinical specimens

DNA extraction was carried out: DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany) was used for tissue and fluid specimens; QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) was used for swab specimens; and Roche High Purity PCR Template Preparation Kit (Roche Ltd, Welwyn Garden City, UK) was used for sputum specimens. DNA samples were stored at -20°C until used. qPCR assays were performed as described above and threshold cycle (Ct) was recorded. A Ct value $>Ct_{cutoff}$ is defined as a negative sample, *ie*, no *Aspergillus* infection.

Statistical analysis

Demographic data for quantitative variables are presented as mean ± SD and percent for qualitative variables. The sensitivity, specificity and accuracy of the qPCR assay were compared to those of the culture and smear method. Kappa test is used for statistical analysis, with a kappa value (K) = 0 indicating no difference between values observed or by chance alone, <0.4 poor, 0.4-0.75 moderate to good, >0.75 <1 excellent agreement, and 1.0 perfect agreement among all variables rated. Calculation was performed using Statistical Package for the Social Sciences (SPSS) version 26 (SPSS Inc, Chicago, IL).

Ethical consideration

The study was approved by the Ethics Committee for Human Research of Hezhou People's Hospital (COA no. 058/ 2020). All the patients under investigation signed the informed consent form with patient's approval for the sample collection.

RESULTS

Diagnostic performance of qPCR compared to culture and smear assays of *Aspergillus*

Clinical specimens (n = 161) were examined between March and November 2020 for *Aspergillus* infection by the traditional smear (microscopic) examination and culture assay, with the addition of qPCR using pan-*Aspergillus* primers and TaqMan probe (Table 1), with a Ct_{cutoff} value of 35. Using a combination of clinical evaluation and microbiological data as the reference standard (positive

Table 1

qPCR primers and probes used in the study

Primer name	Oligonucleotide sequence (5′→3′)	Reference
Pan-*Aspergillus* forward primer	GTGGAGTGATTTGTCTGCTTAATTG	Walsh *et al* (2011)
Pan-*Aspergillus* reverse primer	TCTAAGGGCATCACAGACCTGTT	Walsh *et al* (2011)
Pan-*Aspergillus* probe*	CGGCCCTTAAATAGCCCGGTCCG	Walsh *et al* (2011)
A. fumigatus forward primer	GCCCGCCGTTTCGAC	Luong *et al* (2011)
A. fumigatus reverse primer	CCGTTGTTGAAAGTTTTAACTGATTAC	Luong *et al* (2011)
A. fumigatus probe*	CCCGCCGAAGACCCCAACATG	Luong *et al* (2011)
A. flavus forward primer	CGAGTGTAGGGTTCCTAGCGA	Walsh *et al* (2011)
A. flavus reverse primer	CCGGCGGCCATGAAT	Walsh *et al* (2011)
A. flavus probe*	TCCCACCCGTGTTTACTGTACCTTAGTTGCT	Walsh *et al* (2011)
A. niger forward primer	GCCGGAGACCCCAACAC	Walsh *et al* (2011)
A. niger reverse primer	TGTTGAAAGTTTTAACTGATTGCATT	Walsh *et al* (2011)
A. niger probe*	AATCAACTCAGACTGCACGCTTTCAGACAG	Walsh *et al* (2011)

*TaqMan probe labelled with 5′6-carboxyfluorescein (FAM) and 3′tetramethyl rhodamine isocyanate (TAMRA) (Sangon Biotech, Shanghai, China) samples = 56, negative samples = 105), sensitivity and negative predictive value (NPV) of the culture method were higher than those of smear examination, but specificity and positive predictive value (PPV) were the same (100% for both parameters) (Table 2). qPCR assay performed better than the two traditional methods as regards sensitivity and negative predictive value (NPV) (100% for both parameters) but fared less well than the traditional techniques as regards specificity and PPV. Among the three methods, qPCR and culture methods had comparable accuracy (~98 %), with the smear method being the least accurate. Compared to qPCR assay, the culture and smear method demonstrated excellent and moderate agreement (Kappa value = 0.944 and 0.476 respectively) (Table 2). Culture and smear methods presented LR– values of 0.1 and 0.6 and qPCR method presented LR+ values of 50.

Characteristics of qPCR-positive specimens

The *Aspergillus* spp-positive reference specimens were obtained from female (*n* = 22) and male (*n* = 34) patients, with a median age of 61.0 and 62.5 years respectively (range = 10 months - 92 years). The majority of samples were from the intensive care unit (ICU) (*n* = 17; 30%) and the Department of Respiratory Care (*n* = 15; 27%), and mainly were sputum specimens (*n* = 43, 77%), followed by ear secretion and abscess specimens (5 (9%) cases each) (Table 3). Using qPCR assay with pan-*Aspergillus* primers and TaqMan probe on culture samples, 52/56 (93%) samples were confirmed to be *Aspergillus* infected, the same percentage being obtained from culture fungi using *Aspergillus* spp-specific primers and TaqMan probe, whereas 100% detection was achieved with qPCR assay employing *Aspergillus* spp-specific primers and TaqMan probe on DNA extracted directly from the specimens (Table 3). *A. fumigatus* infection accounted for 75% (*n* = 42) of the specimens, with *A. niger* and *A. flavus* present in 9 (16%) and 5 (9%) samples respectively. However, in ear secretion specimens, *A. niger* was predominant (4/5 samples), followed by *A. flavus* (*n* = 1). Among the abscess specimens, *A. flavus* was detected in 3/5 samples and *A. fumigatus* and *A. niger* in each of the remaining two specimens.

When stratified according to the age range of the patients, the majority of sputum samples (28/43, 65%) were from cases >60 years of age, followed by the 40-60 years of age group (9 cases, 21%) (Fig 2). The number of specimens from other clinical sources was too small to make any meaningful distinction among the age groups.

DISCUSSION

This is the first study of *Aspergillus* spp detection in clinical specimens at Hezhou People's Hospital, Guangxi Province, PR China. Three detection

Table 2

Aspergillus spp identification of clinical specimens (n = 161) using quantitative (q)PCR, culture and smear (microscopy) assays

Method	Number of test result		Sensitivity[a] (%)	Specificity[a] (%)	PPV (%)	NPV (%)	LR+	LR-	Accuracy (%)	Kappa value (p-value)
	Positive	Negative								
qPCR[b]	58	103c	100	98	96	100	50	0	99	-
Culture	52	109	93	100	100	96	-	0.1	97	0.944 (<0.0001)
Smear (microscopy)	23	138	40	100	100	86	-	0.6	79	0.476 (<0.0001)

[a]Reference standard: clinical evaluation and microbiological data; true positive (TP) (n = 56), true negative (TN) (n = 105).

[b]Using pan-*Aspergillus* primers

[c]Ct_{cutoff} = 35

Sensitivity (%) = TP/[TP + False negatives (FN)]×100

Specificity (%) = TN)/[TN+ False positives (FP)]×100

PPV (Positive predictive value) (%) = TP/(TP + FP)×100

NPV (Negative predictive value) (%) = TN/(TN + FN)×100

Likelihood ratio: LR+ = sensitivity/1-specificity; LR- = 1-sensitivity/specificity

Accuracy (%) = (Number of correct predictions/Total number of predictions) ×100

Ct_{cutoff}: cycle threshold cutoff; qPCR: quantitative polymerase chain reaction

Table 3

Numbers of clinical sample types, medical department origins of samples, positive smear examinations, positive cultures, and positive quantitative (q)PCR results of patients (n = 56) with positive *Aspergillus* diagnosis

Clinical sample type (number)	Department (number of samples)	Number of smear positive	Culture positive (number)	qPCR positive	
				Culture (number)	Clinical sample (number)
Sputum (43)	Respiratory (15)	6	*Aspergillus* spp (13)	*A. fumigatus* (11)	*A. fumigatus* (12)
				A. flavus (1)	*A. flavus* (1)
				A. niger (1)	*A. niger* (2)
	Intensive care unit (17)	8	*Aspergillus* spp (17)	*A. fumigatus* (17)	*A. fumigatus* (17)
	Hematology (2)	1	*Aspergillus* spp (2)	*A. fumigatus* (2)	*A. fumigatus* (2)
	Cardiovascular (4)	2	*Aspergillus* spp (4)	*A. fumigatus* (4)	*A. fumigatus* (4)
	Pediatric ward (1)	-	*Aspergillus* spp (1)	*A. fumigatus* (1)	*A. fumigatus* (1)
	Neurosurgery (3)	-	*Aspergillus* spp (3)	*A. fumigatus* (2)	*A. fumigatus* (2)
				A. niger (1)	*A. niger* (1)
	2nd ward of a department (1)	-	*Aspergillus* spp (1)	*A. niger* (1)	*A. niger* (1)

Table 3 (cont)

Clinical sample type (number)	Department (number of samples)	Number of smear positive	Culture positive (number)	qPCR positive	
				Culture (number)	Clinical sample (number)
Abscess (5)	Ear and nose surgery (4)	1	*Aspergillus* spp (3)	*A. fumigatus* (1)	*A. fumigatus* (1)
				A. flavus (2)	*A. flavus* (2)
					A. niger (1)
	Burn plastic surgery (1)	1	*Aspergillus* spp (1)	*A. flavus* (1)	*A. flavus* (1)
Ear secretion (5)	Ear and nose surgery (5)	4	*Aspergillus* spp (4)	*A. niger* (4)	*A. flavus* (1)
					A. niger (4)
Alveolar lavage (1)	Secretion ward (1)	-	*Aspergillus* spp (1)	*A. fumigatus* (1)	*A. fumigatus* (1)
Wound swab (1)	Bone, joint and sport (1)	-	*Aspergillus* spp (1)	*A. fumigatus* (1)	*A. fumigatus* (1)
Puncture fluid (1)	Ophthalmic ward (1)	-	*Aspergillus* spp (1)	*A. fumigatus* (1)	*A. fumigatus* (1)

qPCR: quantitative polymerase chain reaction

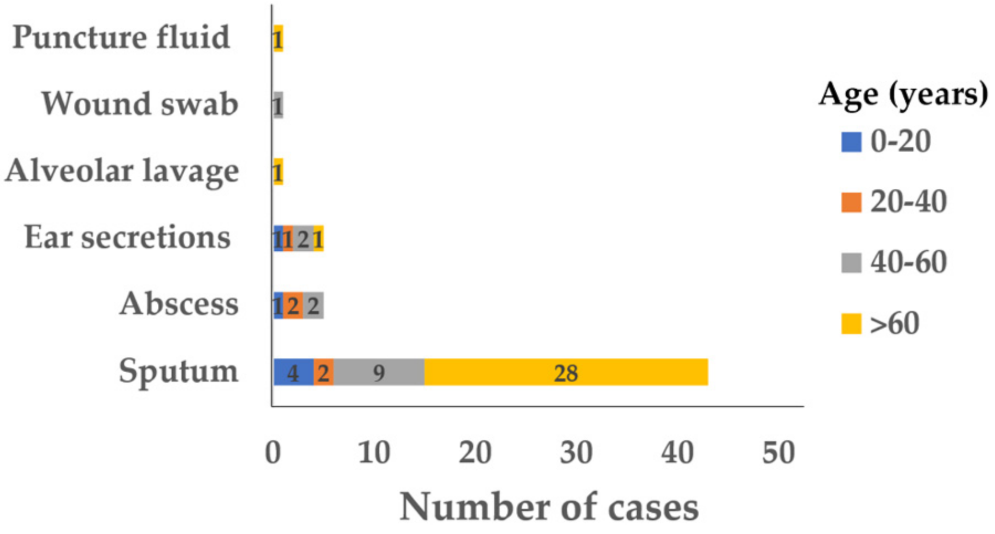

Fig 2 - *Aspergillus* spp in clinical samples stratified according to according to patients' age groups

methods were used, namely, smear (microscope), culture and qPCR (TaqMan method). The accuracy of culture and qPCR assays were comparable (~98%) and that of the smear technique, as expected, was less accurate. When the qPCR assay was tested with the reference *Aspergillus*-positive samples ($n = 56$) as determined by a combination of clinical evaluation and microbiological assays, only the qPCR assay, employing *Aspergillus* spp-specific primers and probes, used on the specimens directly was able to detected *Aspergillus* spp in 100% of these samples, compared to 98% using both pan-*Aspergillus* and species-specific qPCR assays on culture samples.

The observation that the pan-*Aspergillus* primers and probe cross-amplified *Penicillium* spp, but not *Candida* spp, was consistent with the report of Walsh *et al* (2011), owing to the close phylogenetic relationship between *Penicillium* and *Aspergillus* spp (Hope *et al*, 2005; Walsh *et al*, 2011). In the current study, two false-positive qPCR samples were due to *Penicillium* infection (data not shown), although *Penicillium* infections in humans are extremely rare (Lyratzopoulos *et al*, 2002).

The qPCR assay sensitivity, specificity, PPV, and NPV of *Aspergillus* spp in the current study were similar to those of a previous report (94.1, 98.6, 88.9, and 99.3%, respectively)

(Torelli *et al*, 2011). Grancini *et al* (2018) reported a Kappa coefficient of 0.58 in a comparison test between PCR and culture techniques. More recently, Gangneux *et al* (2020) in the diagnosis of aspergillosis in respiratory specimens from ICU patients reported concordance between qPCR and culture assay of 93.75%, with a Kappa coefficient value of 0.63, data in keeping with the current work. Other studies have demonstrated that the PCR method is more efficient than the culture technique (Klingspor *et al*, 2006; Chong *et al*, 2015; Emam and Abd El-salam, 2016) and direct microscopic examination (Torelli *et al*, 2011; Emam and El-salam, 2016; Sow *et al*, 2017).

Although there are more than 200 *Aspergillus* spp, only a small number are pathogenic, *eg, A. flavus*, *A. fumigatus* and *A. niger* (Gugnani, 2003). *A. fumigatus* is responsible for both invasive and non-invasive aspergillosis (Denning, 1998; Morgan *et al*, 2005), and is the most common pathogen in patients with invasive and chronic pulmonary aspergillosis (IPA/CPA), which is also commonly present in cancer and tuberculosis patients due to the involvement of other *Aspergillus* spp, such as *A. flavus*, *A. niger* and *A. terreus* (Walsh *et al*, 2001; Kontoyiannis *et al*, 2002; Hachem *et al*, 2004; Steinbach *et al*, 2004; Fayemiwo *et al*, 2017; Oladele *et al*, 2017).

In the current study, *A. flavus* and *A. niger* were found in ear secretion and abscess specimens in agreement with the studies of Geaney (1967), Loh *et al* (1998), Kaur *et al* (2000), Ali *et al* (2018) and Opperman and Copelyn (2020), which noted that the majority of the fungal pathogens are *Aspergillus* and *Candida* spp, with *Aspergillus* alone responsible for approximately 75% of otomycosis cases. Than *et al* (1980), Mugliston and O'Donoghue (1985), Paulose *et al* (1989), Lucente (1993), and Ali *et al* (2018) reported *A. niger* as the major etiology of otomycosis, with occasional cases due to *A. flavus* and *A. fumigatus*. However, Kiakojori *et al* (2018) observed that *A. flavus* is the most prevalent pathogen of otomycosis. It should be noted that a wide variety of fungi are known as causative pathogens of otomycosis, but as this disease is mostly present in tropical and subtropical regions, it can be expected that the unique fungal flora in a particular environment is associated with the most common fungal pathogen of otomycosis.

The current finding that the majority of fungal infections are associated with patients >60 years of age is well supported by previous studies of aspergillosis infection being commonly found in patients 40-60 years of age (Hedayati *et al*, 2015; Vázquez *et al*, 2016; Sun *et al*, 2017; Bongomin *et al*, 2020).

In conclusion, the study showed that the qPCR method employing species-specific primers and TaqMan probes accurately identified *Aspergillus* spp (*A. flavus*, *A. fumigatus* and *A. niger*) in all clinical specimens when applied using DNA directly extracted from the samples (abscess, dissected tissue, secreted fluid, sputum and swab), compared to 98% using DNA from cultured fungi. Speed, sensitivity, specificity, and convenience would recommend this method for routine diagnosis of aspergillosis in a hospital setting. It is worth noting that qPCR performed with pan-*Aspergillus* primers and TaqMan probe cross-amplified *Penicillium* spp, resulting in a false positive diagnosis. Although the cost for a diagnostic examination by the culture method is lower than that by qPCR, the more rapid identification by the latter technique allows prompt treatment of aspergillosis patients, decreased hospitalization time and, as a consequence, a lower cost of treatment.

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DISCLOSURE OF CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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