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Detection, Identification, and Distribution of Fungi in Bronchoalveolar Lavage Specimens by Use of Multilocus PCR Coupled with Electrospray Ionization/Mass Spectrometry

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As pulmonary fungal infections continue to increase due to an increasing number of immunocompromised patients, rapid detection and accurate identification of these fungal pathogens are critical. A broad fungal assay was developed by incorporating broad-range multilocus PCR amplification and electrospray ionization/mass spectrometry (PCR/ESI-MS) to detect and identify fungal organisms directly from clinical specimens. The aims of this study were to evaluate the performance of PCR/ESI-MS for detection, identification, and determination of the distribution of fungal organisms in bronchoalveolar lavage (BAL) fluid specimens. The BAL fluid specimens submitted for fungal culture at Vanderbilt University Medical Center between May 2005 and October 2011 were included. Cultures and identification were done using standard procedures. In addition, DNA was extracted from BAL fluid specimens, and fungal DNA amplification/identification were performed by PCR/ESI-MS. The results were compared with those of the standard cultures. A total of 691 nonduplicated BAL fluid specimens with sufficient leftover volume for molecular testing were evaluated using PCR/ESI-MS. Among them, 134 specimens (19.4%) were positive for fungi by both culture and PCR/ESI-MS testing. Of the dual-positive specimens, 125 (93.3%) were positive for *Candida* and *Aspergillus* species, with concordances between culture and PCR/ESI-MS results being 84 (67.2%) at the species level and 109 (87.2%) at the genus level. In addition, 243 (35.2%) and 30 (4.3%) specimens were positive only by PCR/ESI-MS or by culture, respectively (odds ratio [OR] = 11.95, 95% confidence interval [CI] = 7.90 to 18.17, $P = 0.0000$). Codetection of fungal organisms was noted in 23 (3.3%) specimens by PCR/ESI-MS, which was significantly higher than the 4 (0.6%) in which they were noted by culture (OR = 5.91, 95% CI = 1.93 to 20.27, $P = 0.0002$). Among 53 specimens in which cultures failed because of bacterial overgrowth, at least one fungus was identified in 26 specimens (47.3%) by PCR/ESI-MS. PCR/ESI-MS provides an advanced tool for rapid and sensitive detection, identification, and determination of the distribution of fungal organisms directly from BAL fluid specimens. Moreover, it detected fungal organisms in specimens in which cultures failed because of bacterial overgrowth. The clinical relevance of the significantly higher detection rate of fungal organisms by PCR/ESI-MS merits further investigation.

As pulmonary fungal infections continue to increase due to an increasing population of immunocompromised patients, rapid detection and accurate identification of fungal pathogens are critical for optimal patient care. A delay in diagnosis may result in high mortality and morbidity rates if the fungal infection proceeds to invasive disease (1–3).

At present, culture is the "gold standard" for the diagnosis of a fungal pulmonary infection and is the standard procedure used. However, it may take days or weeks to grow fungal pathogens by culture and an additional 1 to 2 days to identify these fungi by standard biochemical and phenotypic testing methods (4–6). Moreover, considerable experience is needed at the bench to identify mold-form fungi to the species level. To overcome these limitations, several nonculture diagnostic methods have been introduced. Among them, fungal antigen testing is convenient and fast, but it lacks sensitivity and specificity, restricting its use in clinical laboratories. Molecular methods using PCR and real-time PCR techniques are useful for the diagnosis of fungal infection but can detect only one or a few specific pathogens (7, 8).

PCR with electrospray ionization/mass spectrometry (PCR/ESI-MS) is a relatively new technique based on ESI-MS combined with PCR. This method enables detection and identification of causative pathogens directly from clinical specimens or mixtures of organisms with rapid turnaround and high throughput (9, 10). This technique has been used to detect bacteria, viruses, yeasts, and other infectious agents (10–13), but there are no data on its value for the detection of fungal organisms, including yeasts and molds.

The PCR/ESI-MS broad fungal assay (BFA; Ibis Biosciences, Carlsbad, CA) incorporates broad-range multilocus PCR amplification and ESI-MS to detect and identify fungal organisms directly from clinical specimens. The aims of this study were to evaluate the performance of PCR/ESI-MS for detection and identification of fungal organisms and to investigate the distribution of these fungi in bronchoalveolar lavage (BAL) fluid specimens.

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TABLE 1 Broad-range primers and targets for PCR/ESI-MS BFA

Target gene[a]	Primer sequence (5′ → 3′)	Locus	Amplicon size (bp)	Range of targeted fungi
LSU rRNA	TCATTTTTGGTAAGCAGAACTGGCGA TGTCTAGATGAACTAACACCTTTTGTGGT	5186	107–108	Broad
LSU rRNA	TGCCGAAGTTTCCCTCAGGATAGCA TCAAGGCCTCTAATCATTCGCTTTACCTC	5187	72–78	Broad
SSU rRNA	TGCAGGCCTATGCTCGAATACATTAGCATTGTCCCTATT AATCATTACGGCGGTCCTA	5178	99–101	Ascomycetes and basidiomycetes
LSU rRNA	TGTGAAGCGGCAAAAGCTCAAATTT TTCTCACCCTCTGTGACGGCCTGTTCC	3030	116–130	Ascomycetes and basidiomycetes
LSU rRNA	TGGAGTCTAACATCTATGCGAGTGTT TCCCACAGCTATGCTCTTACTCAAATCC	5185	137–153	Ascomycetes and some basidiomycetes
LSU rRNA	TTGTGTAGAATAGGTGGGAGCTTCGGC TCTGACAATGTCTTCAACCCGGATC	3766	148–154	Ascomycetes and basidiomycetes
mtSSU rRNA	TCCAAATTACGTGCCAGCAGTCG TCCCTACCGTCTAGGTACCCTTTAAACC	5181	90	Ascomycetes (except *Candida* spp) and zygomycetes
SSU rRNA	TCTTGGATTGACCGAAGACAAACTACTG TCTCTAGTCGGCATCGTTTGTGGTTAAG	4836	136–136	Most ascomycetes and basidiomycetes, zygomycetes
tub	TGCCGCGGTGCTCACTCTTTC TGTATCGGCCGTTGCGGAAGTC	4837	109	Most ascomycetes (except *Candida* spp.)
mt *cytB*	TCCCCGTAACAATTTTCTTATTCTTCTTAGTATTAGTGCA TTGGGTTAGCTGGAATATAATTATCAGGATG	5172	106	Zygomycetes
SSU rRNA	TCGAAGACGATTAGATACCGTCGTAGTC TCAGAGCCCA AAAACTTTACTTTCGCTAAG	5174	113–116	Zygomycetes
mtSSU rRNA	TCCAAGTACTTGACACATGCTAATCG TCCCCTTACTTTAAGGTAGCCAAATTATC	3862	102–133	Eurotiales (*Aspergillus*, *Penicillium*)
mtSSU rRNA	TGGTACAGTGGAGTATGCTGTTTAATTGGA TCTGACGACAACAATGTAACGCCTG	3865	93–131	*Saccharomycetales* (*Candida*)
mtSSU rRNA	TTCGATACCCGTGTAGTTCTAGTAGTAAACTCATTATTGC TAACGTACTCTTCAGGTGG	3867	113–127	*Saccharomycetales* (*Candida*)
mtSSU rRNA	TACCAAGCCAATGACGAGTAGGG TGCTGCACATAATCTATGCTCTGGAC	4145	87	*Tremellales* (*Cryptococcus*)
hpr	TGACGAGTTCATGAGGGCAGGC TCTGGCCTTTCAGCAAGTTTCCAAC	4437	77	Extraction control (pumpkin DNA)

[a] LSU, long subunit; SSU, short subunit; mt, mitochondrial.

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MATERIALS AND METHODS

Clinical specimens. A total of 859 BAL fluid specimens were collected for fungal culture between May 2005 and October 2011 at Vanderbilt Medical Center. These specimens were stored in −80°C freezers until testing. We excluded 168 specimens because the amount of stored material was insufficient for PCR/ESI-MS analysis or the stored material was a duplicate of an already collected specimen. As a result, 691 BAL fluid specimens were included and analyzed by PCR/ESI-MS.

Fungal cultures and identification were performed on fresh specimens by standard procedures in a clinical microbiology laboratory. Yeast-form fungi were identified according to standard clinical laboratory methods, including the germ tube test, the Phoenix Yeast ID system, and the API 20C system. Mold-form fungi were identified using colony morphology and microscopic findings. These phenotypic identifications were considered the reference method to evaluate PCR/ESI-MS.

Multilocus broad-range PCR/ESI-MS. The PCR/ESI-MS kit contains reagents to process and identify a broad range of fungal species, including *Aspergillus*, *Candida*, *Fusarium*, *Cryptococcus*, and *Mucorales*. This is a singleplex assay with 16 unique broad-range PCR primer sets, including 9 broad-range primer sets, 6 short-range primer sets, and 1 control primer set (Table 1). A synthetic DNA construct was also included within each reaction mixture (20 copies per reaction) as an internal positive amplification control as well as a quantitative calibrant. This synthetic construct (approximately 1,800 nucleotides) contained all 16 primer-binding regions; however, these regions each contained either insertions or deletions, causing a mass shift to allow product differentiation from the actual sample. By comparing the signal obtained from the synthetic construct amplicons and the target amplicons, an approximate template copy number could be determined.

DNA was extracted from 1 ml of thawed BAL fluid specimens, which was first lysed by adding 25 μl of proteinase K, 250 μl of 10% bovine serum albumin, and 142 μl of 20% SDS-extraction control mix into each bead-beating tube. After lysis of specimens with a PLEX-ID bead beater, the tubes were placed in a heating block set to 56°C for 15 min for incubation and in a centrifuge for 3 min. The specimens were then transferred to a sample plate using PLEX-ID FH, which was then ready for the PLEX-ID extractor. The PLEX-ID SP performs DNA extraction using a nonspecific capture chemistry where nucleic acids are bound to magnetic particles while cellular components and other substances are removed through a series of wash steps. Reagents are loaded in 24-well plates as follows: sample plate (lysed specimen, 1.1 ml; wash 1, 1.1 ml; magnetic particles, 160 μl), wash 1 plate (wash 1 buffer, 2 ml), wash 2 plates (wash 2 buffer, 2 ml), elution plate (elution buffer, 280 μl). DNA extracted from lysed samples was stored on an elution plate after operation of PLEX-ID SP using wash buffer 1, wash buffer 2, and elution buffer. Extracted DNAs were transferred to an assay plate by loading an elution and an assay plate for PLEX-ID FH according to the plate layout registered in the PLEX-ID software. Finally, each specimen was added to 16 wells of a 96-well plate containing primer pairs and re-

agents. After PCR amplification, total mass and base compositions were analyzed automatically by the PLEX-ID.

Molecular signature database. The manufacturer analyzed over 500 reference-cultured samples to develop the database for fungal identification. For each PCR product generated in the 16 PCRs for each reference isolate analyzed, the amplicon base composition (count of A, G, C, and T residues) was calculated from the forward and reverse strand masses determined by ESI-MS. The resulting array of base composition entries was extended by an *in silico* survey of existing fungal entries in the GenBank *nr* database, which added ca. 2,300 fungal signatures. The length of PLEX-ID signatures varies for fungal species in accordance with their taxonomy, as the 16 primers have various priming ranges; typically, reference signatures are defined across 3 (some *Mucorales* species) to 10 (*Aspergillus* spp.) loci.

Reporting. Reported fungal species names match the current taxonomic standard indicated in the NCBI taxonomy database on the basis of external references (e.g., *Candida guilliermondii* is reported as *Meyerozyma guilliermondii*). When applicable, the associated teleomorph name is reported for mitosporic species (e.g., *Bipolaris* and *Curvularia* species are reported under the *Cochliobolus* genus name). Most common pathogens with distinct PLEX-ID signatures are fully reported at the species level (e.g., *Aspergillus fumigatus*). Other organisms of lesser clinical importance, which are typically not characterized at the species level in the mycology lab, are reported at the genus level. For a specimen to be identified through matching to existing entries, at least 60% of the signature present in the PLEX-ID fungal database must be retrieved (i.e., when 3 out of 3, 4, or 5, 4 out of 6, 5 out of 7 or 8, and 6 out of 9 or 10 expected amplicon base compositions are matched). Specimens whose best database match is in the 50% to 60% range are not named specifically but prompt the instrument to issue "fungus detected—no identification can be provided" calls. No detection is reported for specimens whose best match is below the 50% signature retrieval threshold.

Statistical analysis. Statistical comparisons were performed with Epi Info software (version 6; Centers for Disease Control and Prevention, Atlanta, GA). The results of PCR/ESI-MS were compared with those of the phenotypic reference culture methods. *P* values of ≤0.05 were considered statistically significant.

RESULTS

A total of 691 nonduplicated BAL fluid specimens with sufficient leftover volume for molecular testing were included in this study. The distribution of the fungal species identified by conventional standard culture methods and PCR/ESI-MS is shown in Table 2. A total of 168 fungi could be detected by culture in 164 specimens (23.7%). No fungal organisms were detected for 53 cultured specimens (7.7%) because of bacterial overgrowth. By PCR/ESI-MS, 408 fungi were detected in 377 (54.6%) specimens; however, no specific genus or species call was made for 154 specimens (22.3%), as the instrument called fungus detected—no identification can be provided, which indicated that only partial matches to the database were retrieved. Thus, a specific genus or species could be assigned for 223 specimens (32.3%).

Among the 691 specimens, 134 (19.4%) were positive for fungi by both culture and PCR/ESI-MS, with 84 (62.7%) and 109 (81.3%) being concordant at the species and genus levels, respectively (kappa = 0.246; Table 3). Of the 134 dual-positive specimens, 125 were *Candida* and *Aspergillus* by culture, with the concordances between culture and PCR/ESI-MS results being 84 (67.2%) at the species level and 109 (87.2%) at the genus level (Table 4). In addition, 243 (35.7%) and 30 (4.3%) specimens were positive only by PCR/ESI-MS BFA and by culture, respectively (odds ratio [OR] = 11.95, 95% confidence interval [CI] = 7.90 to 18.17, $P = 0.0000$) (Table 5). A *Pneumocystis* species was detected by PCR/ESI-MS in 24 BAL fluid specimens (Tables 2, 4, and 5). No correlation between culture quantification and semiquantitative PCR/ESI-MS results was indicated (data not shown).

Codetection of fungal organisms was noted in 30 specimens (4.3%) by PCR/ESI-MS, with both detections fully identified in 22 specimens (3.2%); in contrast, codetection of fungal organisms

TABLE 2 Distribution of fungal species identified by either culture or PCR/ESI-MS

Method and organism	No. of isolates
Culture	
Candida albicans	106
Candida glabrata	24
Penicillium spp.	11
Saprophytic fungus	4
Candida tropicalis	3
Aspergillus fumigatus	2
Aspergillus niger	2
Aspergillus species, not *A. fumigatus*	2
Candida parapsilosis	2
Scopulariopsis brumptii	2
C. albicans and *C. tropicalis*	2
C. albicans and *C. glabrata*	1
C. albicans and *Penicillium* spp.	1
Candida krusei	1
Trichosporon beigelii	1
Overgrown with bacteria; unable to evaluate	53
No growth	474
Total	691
PLEX-ID BFA	
C. albicans	76
C. glabrata	22
Pneumocystis spp.	21
Cladosporium spp.	17
Cryptococcus spp. (other)	13
Candida dubliniensis	10
C. albicans and C. *glabrata*	5
C. glabrata and *C. tropicalis*	5
C. parapsilosis	5
Malassezia pachydermatis	5
C. albicans and fungus detected—no identification can be provided	4
Candida spp. (other)	4
C. tropicalis	4
A. fumigatus	3
Pichia kudriavzevii	3
Aspergillus versicolor	2
C. albicans and *Pneumocystis* species	2
C. dubliniensis and *C. glabrata*	2
C. dubliniensis and *C. tropicalis*	2
Cryptococcus neoformans and fungus detected—no identification can be provided	2
Penicillium spp.	2
Aspergillus flavus/*A. oryzae*[a]	1
A. fumigatus and *C. albicans*	1
A. versicolor and *Emericella nidulans*	1
C. albicans and *Candida* spp. (other)	1
C. albicans and *C. tropicalis*	1
C. dubliniensis and *Pneumocystis* spp.	1
C. glabrata and fungus detected—no identification can be provided	1
C. parapsilosis and *Clavispora lusitaniae*	1
Cladophialophora sp.	1
Cladosporium spp. and fungus detected—no identification can be provided	1
Cochliobolus spp.	1
Rhizomucor spp.	1
Rhizopus spp.	1
Scedosporium spp.	1
Fungus detected—no identification can be provided	154
No detection	314
Total	691

[a] Single PCR/ESI-MS detection indiscriminately matching two closely related fungi.

TABLE 3 Overall concordance between culture and PCR/ESI-MS

Culture result	No. of samples with indicated PLEX-ID BFA result/total no. tested (%)		
	Positive	Negative	Total
Positive	134 (19.4)	30 (4.3)	164 (23.7)
Agreement at genus level	109/134 (81.3)		
Agreement at species level	84/134 (62.7)		
Negative	243 (35.2)	284 (41.1)	527 (76.3)
Total	377 (54.6)	314 (45.4)	691 (100)

was identified by culture in only 4 specimens (0.6%, OR = 5.91, 95% CI = 1.93 to 20.27, $P = 0.0002$). At least one fungal organism was detected and identified by PCR/ESI-MS in 26/53 specimens (47.3%) where the standard culture method failed because of bacterial overgrowth. Fifteen strains of *Candida dubliniensis* were detected only by PCR/ESI-MS, including 11 strains that were alternatively identified as *C. albicans* by culture (Tables 4 and 5).

DISCUSSION

Rapid and accurate detection and identification of pathogenic fungi can improve the diagnosis and treatment of pulmonary fun-

TABLE 4 Concordance-level distribution of specimens positive by both culture and PCR/ESI-MS

Level	Culture identification	PCR/ESI-MS BFA identification	No. of isolates
Species	*Aspergillus fumigatus*	*A. fumigatus*	1
	Candida albicans	*C. albicans*	56
	C. albicans	*C. albicans* and *A. fumigatus*	1
	C. albicans	*C. albicans* and *C. glabrata*	4
	C. albicans	*C. albicans* and fungus detected—no identification can be provided	3
	C. albicans	*C. albicans* and *Pneumocystis* sp.	2
	C. albicans and *C. tropicalis*	*C. albicans* and *C. tropicalis*	1
	C. glabrata	*C. glabrata*	10
	C. glabrata	*C. glabrata* and *C. dubliniensis*	1
	C. glabrata	*C. glabrata* and *C. tropicalis*	2
	C. glabrata	*C. glabrata* and fungus detected—no identification can be provided	1
	C. parapsilosis	*C. parapsilosis*	1
	C. tropicalis	*C. tropicalis*	1
Genus	*A. niger*	*A. versicolor*	1
	C. albicans	*C. dubliniensis*	8
	C. albicans	*C. dubliniensis* and *C. glabrata*	1
	C. albicans	*C. dubliniensis* and *Pneumocystis* sp.	1
	C. albicans	*C. glabrata*	2
	C. albicans	*C. parapsilosis*	1
	C. albicans	*C. tropicalis*	1
	C. albicans	*C. tropicalis* and *C. glabrata*	1
	C. albicans	*Pichia kudriavzevii*	1
	C. glabrata	*C. albicans*	1
	C. glabrata	*Candida* sp. (other)	1
	C. glabrata	*C. tropicalis*	1
	C. glabrata	*P. kudriavzevii*	1
	C. krusei	*C. albicans* and *Candida* sp. (other)	1
	C. tropicalis	*C. albicans*	1
	C. albicans and *C. glabrata*	*C. glabrata*	1
	C. tropicalis and *C. albicans*	*C. tropicalis* and *C. dubliniensis*	1
Fungus	*Aspergillus* sp., not *A. fumigatus*	*Cryptococcus neoformans* and fungus detected—no identification can be provided	1
	Aspergillus sp., not *A. fumigatus*	Fungus detected—no identification can be provided	1
	C. albicans	*A. flavus*/*A. oryzae*	1
	C. albicans	Fungus detected—no identification can be provided	5
	C. albicans	*Pneumocystis* spp.	2
	C. albicans and *Penicillium* sp.	Fungus detected—no identification can be provided	1
	C. glabrata	Fungus detected—no identification can be provided	3
	C. parapsilosis	Fungus detected—no identification can be provided	1
	C. tropicalis	Fungus detected—no identification can be provided	1
	Penicillium sp.	*Cladosporium* sp.	1
	Penicillium sp.	Fungus detected—no identification can be provided	2
	Saprophytic fungus	*Candida* sp. (other)	1
	Saprophytic fungus	*Cryptococcus* sp. (other)	1
	Saprophytic fungus	*Pneumocystis* sp.	1
	Scopulariopsis brumptii	*Cladosporium* sp.	1
	Scopulariopsis brumptii	Fungus detected—no identification can be provided	1
	Trichosporon beigelii	*Malassezia pachydermatis*	1

TABLE 5 Distribution of fungal species identified by culture only or PCR/ESI-MS only

Method and identification	No. of isolates
Culture	
Candida albicans	16
Penicillium spp.	8
C. glabrata	3
Aspergillus fumigatus	1
A. niger	1
Saprophytic fungus	1
Total	30
PLEX-ID BFA only	
C. albicans	18
Pneumocystis spp.	18
Cladosporium spp.	15
Cryptococcus spp. (other)	12
C. glabrata	9
Malassezia pachydermatis	4
C. parapsilosis	3
A. fumigatus	2
C. dubliniensis	2
C. glabrata and *C. tropicalis*	2
Candida spp. (other)	2
Penicillium spp.	2
A. versicolor	1
A. versicolor and *Emericella nidulans*	1
C. albicans and *C. glabrata*	1
C. albicans and fungus detected—no identification can be provided	1
C. parapsilosis and *Clavispora lusitaniae*	1
C. tropicalis	1
C. tropicalis and *C. dubliniensis*	1
Cladophialophora sp.	1
Cladosporium sp. and fungus detected—no identification can be provided	1
Cochliobolus sp.	1
Cryptococcus neoformans and fungus detected—no identification can be provided	1
Pichia kudriavzevii	1
Rhizomucor sp.	1
Rhizopus sp.	1
Scedosporium sp.	1
Fungus detected—no identification can be provided	139
Total	243

gal infections. Although the conventional fungal culture method remains the gold standard, it is time-consuming and may present difficulty in identifying some mold-form fungi by morphology.

There have been major advances in molecular methods for clinical laboratory diagnosis in the past decade. Some of these techniques have been used for the detection and characterization of medically important fungi (14, 15). However, most current molecular techniques can detect only a small number of specific fungal pathogens even if multiplex PCR procedures are implemented (16, 17). To overcome this limitation, broad-range PCR and sequencing were reported for the detection and identification of unknown pathogens in cultures and various clinical specimens (18–20). However, this population-based sequencing identification often failed if two or more organisms existed in one specimen.

The PCR/ESI-MS technique has many attractive features compared with conventional culture methods and organism-specific PCR (9, 21, 22). It not only possesses enough sensitivity to detect pathogens directly in clinical specimens (10, 13) but also has precise discriminatory powers to simultaneously differentiate several pathogen species in a single specimen. In this study, we demonstrated the usefulness of the PCR/ESI-MS-based kit for detection and identification of fungi from BAL fluid specimens. The PLEX ID system (Ibis Biosciences, Carlsbad, CA) used for this study incorporates multilocus PCR amplification and ESI-MS detection in order to detect and characterize a variety of microorganisms, including bacteria, fungi, and viruses (21). The assay was designed to detect and identify most medically encountered fungal organisms directly from clinical specimens. This kit uses 16 primer sets of various priming ranges, including 9 for large groups of fungi; 6 narrow rangers for fine resolution of mucoraceous molds, *Aspergillus* and *Penicillium*, *Candida*, and *Cryptococcus* species; and 1 for extraction control. The PLEX-ID broad fungal assay correctly identified isolates to the species level for 154 species from 66 genera. For 12 genera, it identified organisms to the genus level when rare species beyond the 154 species were detected. With a test turnaround time from specimen to result reporting being within 6 h, final results can automatically be gained for identification without any additional analysis.

Among 134 specimens positive in both culture and PCR/ESI-MS, various fungal organisms were correctly identified from BAL fluid specimens to the species and genus levels in 62.7% and 81.3%, respectively. The concordant rates were slightly higher when only *Candida* and *Aspergillus* are included in the data analysis as 67.2% and 87.2% at the species and genus levels, respectively. In this study, *Candida dubliniensis* was identified in 15 specimens by PCR/ESI-MS. Among them, 11 were identified as *C. albicans* and 4 were negative by culture. It is well-known that it is difficult to differentiate *C. dubliniensis* from *C. albicans* by conventional commercial identification kits because the two species demonstrate a similar pattern of biochemical reactions (4). It should be noted that the *Candida* species detected in these BAL fluid specimens might represent colonizing/contamination flora. In addition, among 25 specimens that yielded only one colony or light growth by culture, PCR/ESI-MS detected one or more fungal organisms with genus-level concordance in only seven specimens. With these 25 specimens excluded to avoid potential contamination during the culture processing, PCR/ESI-MS correctly identified 77.1% of the fungi in BAL fluid specimens to the species level and 93.6% to the genus level.

In 243 specimens where fungi were detected only by PCR/ESI-MS, 139 (57.2%) were reported as fungus detected—no identification can be provided. This result was not surprising in this study, because many nonpathogenic fungi can colonize the respiratory tract. These results can be interpreted as false-positive results because the PCR/ESI-MS method can detect nonviable or contaminated fungal DNA introduced during the handling of specimens (9). It was noticed that PCR/ESI-MS also provided quantitative results for each target; whether this DNA load information facilitates the data analysis and interpretation will require further investigation.

The PCR/ESI-MS described in the study possessed overall agreements with culture of 62.7% at the species level and 81.3% at the genus level in specimens in which fungal organisms were detected by both methods. In addition, PCR/ESI-MS detected single and multiple fungal organisms at significantly higher frequencies in a given specimen than culture. While PCR/ESI-MS provides an

advanced tool for rapid and sensitive detection and identification of fungal organisms directly from BAL fluid specimens, the clinical relevance of the significantly higher rates of detection of fungal organisms by PCR/ESI-MS than by culture methods merits further investigation. Given that there are no clinical details available and that about 85% of fungal isolates can be considered contaminating flora (23), the clinical relevance of these additionally detected fungal organisms needs to be evaluated prospectively.

One obvious advantage of PCR/ESI-MS is that it can be performed on specimens with bacterial contamination. In our study, there were 53 specimens in which cultures failed because of bacterial overgrowth. In contrast, at least one fungal organism was identified in 26 of these specimens (47.3%) by PCR/ESI-MS. This speeds up the diagnostic procedure by avoiding additional specimen collection and/or decontamination.

In conclusion, the PCR/ESI-MS described in this study possessed promising concordances with culture in specimens in which fungal organisms were detected by both methods. In addition, PCR/ESI-MS detected single and multiple fungal organisms at significantly higher frequencies in a given specimen than culture. Most importantly, PCR/ESI-MS detected potential fungal organisms in specimens in which cultures failed due to bacterial overgrowth. PCR/ESI-MS provides an advanced tool for rapid and sensitive detection and identification of fungal organisms directly from BAL fluid specimens.

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