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Original Article

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Survey of laboratory practices for diagnosis of fungal infection in seven Asian countries: An Asia Fungal Working Group (AFWG) initiative

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Abstract

An online survey of mycology laboratories in seven Asian countries was conducted to assess the status, competence, and services available. Country representatives from the Asia Fungal Working Group (AFWG) contacted as many laboratories performing mycology diagnosis as possible in their respective countries, requesting that the laboratory heads complete the online survey. In total, 241 laboratories responded, including 71 in China, 104 in India, 11 in Indonesia, 26 in the Philippines, four in Singapore, 18 in Taiwan, and seven in Thailand. Overall, 129/241 (53.5%) surveyed mycology laboratories operate as separate designated mycology laboratories, 75/241 (31.1%) conduct regular formal staff training, 103/241 (42.7%) are accredited, and 88/157 (56.1%) participate in external quality assurance scheme (EQAS) programs. Microscopy and culture methods are available in nearly all laboratories, although few perform DNA sequencing (37/219; 16.9%) or use matrix-assisted laser desorption/ionization time-of-flight mass spectroscopy

(MALDI-TOF MS) (27/219; 12.3%) for isolate identification. Antifungal susceptibility testing is performed in 142/241 (58.9%) laboratories, mainly for yeasts. The most commonly performed nonculture diagnostic is cryptococcal antigen testing (66 laboratories), followed by galactomannan testing (55), polymerase chain reaction (PCR) diagnosis (37), and beta-D-glucan testing (24). Therapeutic drug monitoring is conducted in 21 laboratories. There is almost no access to advanced diagnostic tests, like galactomannan, β-D-glucan, and PCR, in the surveyed laboratories in Indonesia, the Philippines, and Thailand. These results highlight the need for development of quality laboratories, accreditation and training of manpower in existing laboratories, and access to advanced non–culture-based diagnostic tests to facilitate the diagnosis of fungal infections in Asia.

Key words: fungal infections, diagnosis, laboratory, molecular diagnosis, antifungal susceptibility testing.

Introduction

Asia has the largest burden of fungal disease in the world when one considers that more than half the world's population lives in the region. The tropical environment in a large portion of the region, inadequately trained healthcare professionals, misuse or abuse of steroids and broad-spectrum antibiotics, and compromised healthcare practices due to over-capacity patient loads in public sector hospitals are major concerns and may contribute to the high burden of fungal infections in Asia.[1–5] In addition, many Asian countries lack high-quality mycology laboratories, and awareness of fungal diseases is limited. To overcome the challenges and achieve discernible change in morbidity and mortality due to fungal infections, the Asia Fungal Working Group (AFWG) was formed under the auspices of the International Society for Human and Animal Mycology (ISHAM). The aim of the AFWG is to improve patient care by advancing the diagnosis and management of fungal infections. The AFWG performed a gap analysis of mycology laboratories in seven Asian countries through an online survey conducted during the second half of 2016. This manuscript provides a comprehensive analysis of the survey results to describe the present status of mycology diagnostic services in seven Asian countries.

Method

In February 2016, the AFWG planned an online survey to assess the competencies of mycology laboratories in seven Asian countries in which the AFWG board has a country representative. A 36-item questionnaire was developed covering laboratory structure, equipment, manpower, personnel training, and diagnostic testing availability and frequency of use (See Supplement 1 for the survey questionnaire). AFWG country representatives were responsible for recruiting respondents for the survey in their respective countries. As no data were available on the total number of laboratories performing fungal diagnosis in each country, the goal was to recruit at least 100 randomly chosen laboratories in both China and India that performed diagnosis of fungal infections. For the other five countries, attempts were made to recruit laboratories from all medical teaching institutes. The AFWG country representatives tried to achieve this target through their personal contacts with heads of laboratories in their respective countries or used the Facebook pages or websites of relevant societies to send an appeal for laboratories performing fungal diagnosis to participate in the online survey.

Data analysis

A professional consulting firm programmed and hosted the online survey, and was responsible for data capture from the participating laboratories. The data sets were scrutinized for missing or discrepant data; discrepant data were excluded, and attempts were made to resolve incomplete responses by contacting the relevant laboratory. Finally, the authors analyzed the data, using the Marascuilo procedure for between-country comparisons. The Marascuilo procedure, an extension of the χ^2 test, is a parametric test used for multiple comparisons of proportions that can identify the data points responsible for rejection of the null hypothesis (i.e., all proportions considered equal) in the overall χ^2 test. The level for statistical significance was set at <0.05 for any data comparisons.

Results

The number of laboratories approached individually in each country included India ($n = 164$), Indonesia ($n = 182$), Singapore ($n = 6$), Taiwan ($n = 21$), and Thailand ($n = 14$). In China, an invitation was sent through the medical mycology Web net, comprising nearly 500 people; in the Philippines, an invitation was posted on the Pathology Society Facebook

Group page, with an unspecified number of members in the group. Overall, 241 laboratories from seven countries participated (in rank order): India ($n = 104$), China ($n = 71$), the Philippines ($n = 26$), Taiwan ($n = 18$), Indonesia ($n = 11$), Thailand ($n = 7$), and Singapore ($n = 4$). Responses were compiled by country and the data analyzed; results are presented in Tables 1 and 2. As the number of laboratories responding to each survey question varied, the respondent number (base) is specified for each country at each question (Tables 1 and 2). Figure 1 shows the location of the participating laboratories on a regional map.

Laboratory structure

Overall, 129/241 (53.5%) mycology diagnostic areas function as independent mycology laboratories, meaning they are stand-alone mycology laboratories with separate laboratory space and manpower (Table 1). The remaining 46.5% perform fungal diagnosis within a bacteriology/microbiology laboratory. Singapore (0/4) and the Philippines (2/26) have significantly fewer ($P < .05$) independent mycology laboratories. Regular and occasional staff training was available in 75/241 (31.1%) and 76/241 (31.5%) of all surveyed mycology laboratories, respectively (Table 1). Significantly fewer laboratories conducted staff training in Indonesia (4/11) and Thailand (0/7) ($P < .05$) compared with the other five countries. Around 11% of laboratories (26/241) indicated that there is no regular clinician/laboratory interaction. The majority (127/238, 53.4%) of the laboratories receive 0–50 mycology samples/week and only 9.7% of laboratories (23/238) receive > 500 samples every week (Table 1). Laboratories in the Philippines received the lowest ($P < .05$) number of weekly samples for fungal diagnosis. The biosafety hood is available in 192/241 (79.7%) laboratories; the availability is comparatively low in Chinese (38/71, 53.5%) and Indonesian (7/11, 63.6%) laboratories.

Accreditation

Of the surveyed laboratories, 103/241 (42.7%) were accredited by national or international agencies (Table 1). In Singapore, all four surveyed laboratories were accredited, and the lowest accreditation rate (2/11, 18.2%) was observed in Indonesia. None of the laboratories in Indonesia (0/11), but all laboratories in Singapore (4/4), participate in External Quality Assurance Scheme (EQAS) programs in the field of medical mycology (Table 1). Overall, 88/157 (56.1%) of surveyed laboratories in the seven countries participate in EQAS programs.

Table 1. Country comparison of survey responses from mycology laboratories in seven Asian countries: Laboratory characteristics.

Determinants	Overall $N = 241$	China [$N = 71$]	India [$N = 104$]	Indonesia [$N = 11$]	Philippines [$N = 26$]	Singapore [$N = 4$]	Taiwan [$N = 18$]	Thailand [$N = 7$]
Independent mycology laboratory	129/241 (53.5%)	49/71 (69.0%)	58/104 (55.8%)	5/11 (45.5%)	2/26 (7.8%)*	0/4 (0%)*	13/18 (72.2%)	2/7 (28.6%)
Number of samples processed/week:								
0–50	127/238 (53.4%)	21/71 (29.6%)	61/103 (59.2%)	9/11 (81.8%)	22/25 (88.0%)	3/4 (75.0%)	5/17 (29.4%)	6/7 (85.7%)
51–100	38/238 (16.0%)	15/71 (21.1%)	14/103 (13.6%)	1/11 (9.1%)	1/25 (4.0%)*	1/4 (25%)	6/17 (35.3%)	0
101–200	27/238 (11.3%)	11/71 (15.5%)	8/103 (7.8%)	1/11 (9.1%)	2/25 (8.0%)	0	5/17 (29.4%)	0
201–500	23/238 (9.7%)	12/71 (16.9%)	10/103 (9.7%)	0	0	0	1/17 (5.9%)	0
>500	23/238 (9.7%)	12/71 (16.9%)	10/103 (9.7%)	0	0	0	0	1/7 (14.3%)
Formal staff training:								
Regular	75/241 (31.1%)	23/71 (32.4%)	36/104 (34.6%)	1/11 (9.1%)*	3/26 (11.5%)	3/4 (75.0%)	9/18 (50.0%)	0/7 (0%)*
Occasional	76/241 (31.5%)	23/71 (32.4%)	31/104 (29.8%)	3/11 (27.3%)	9/26 (34.6%)	1/4 (25.0%)	5/18 (27.8%)	4/7 (57.1%)*
No regular laboratory-clinician interaction	26/241 (10.8%)	6/71 (8.5%)	3/104 (2.9%)*	2/11 (18.2%)	5/26 (19.2%)	0/4 (0%)*	8/18 (44.4%)	2/7 (28.6%)
Mycology laboratory is accredited	103/241 (42.7%)	26/71 (36.6%)	42/104 (40.4%)	2/11 (18.2%)	12/26 (46.2%)	4/4 (100%)	14/18 (77.8%)	3/7 (42.9%)
Participates in EQAS program	88/157 (56.1%)	11/33 (33.3%)	55/80 (68.8%)	0/5 (0%)*	6/16 (37.5%)	4/4 (100%)	10/14 (71.4%)	2/5 (40.0%)
Availability of bio-safety hood in mycology laboratory	192/241 (79.7%)	38/71 (53.5%)	98/104 (94.2%)	7/11 (63.6%)	23/26 (88.5%)	4/4 (100%)	16/18 (88.9%)	6/7 (85.7%)

EQAS, External Quality Assurance Scheme.
*Statistically significant ($P < .05$).

Table 2. Country comparison of survey responses from mycology laboratories in seven Asian countries: Diagnostic services.

Determinants	Overall $N = 241$	China [$N = 71$]	India [$N = 104$]	Indonesia [$N = 11$]	Philippines [$N = 26$]	Singapore [$N = 4$]	Taiwan [$N = 18$]	Thailand [$N = 7$]
Culture facilities are available for isolation of fungi	215/241 (89.2%)	62/71 (87.3%)	98/104 (94.2%)	11/11 (100%)	17/26 (65.4%)*	3/4 (75.0%)	18/18 (100%)	6/7 (85.7%)
Fluorescence microscope available (for examination under calcofluor stain)	99/241 (41.1%)	24/71 (33.8%)	60/104 (57.7%)	3/11 (27.3%)	4/26 (15.4%)*	3/4 (75.0%)	3/18 (16.7%)	2/7 (28.6%)
Fungal identification methods available in laboratory:								
Noncommercial phenotypic and morphological technique	179/219 (81.7%)	47/58 (81.0%)	92/101 (91.1%)	8/11 (72.7%)	9/21 (42.9%)*	3/04 (75.0%)	15/17 (88.2%)	5/07 (71.4%)
Commercial phenotypic	98/219 (44.7%)	22/58 (37.9%)	49/101 (48.5%)	6/11 (54.6%)	8/21 (38.1%)	1/04 (25.0%)	9/17 (52.9%)	3/07 (42.9%)
PNA/FISH	2/219 (0.9%)	2/58 (3.5%)	0/101 (0%)	0/11 (0%)	0/21 (0%)	0/04 (0%)	0/17 (0%)	0/07 (0%)
MALDI	27/219 (12.3%)	6/58 (10.3%)	5/101 (5.0%)	0/11 (0%)	2/21 (9.5%)	4/04 (100%)	9/17 (52.9%)	1/07 (14.3%)
Molecular – DNA sequencing	37/219 (16.9%)	19/58 (32.8%)	10/101 (9.9%)	1/11 (9.1%)	1/21 (4.8%)	2/04 (50.0%)	3/17 (17.7%)	1/07 (14.3%)
None, send to outside laboratory	16/219 (7.3%)	9/58 (15.5%)	2/101 (2.0%)	1/11 (9.1%)	1/21 (4.8%)	0/04 (0%)	0/17 (0%)	2/07 (28.6%)
Antifungal susceptibility testing:								
Available in mycology laboratory	142/241 (58.9%)	31/71 (43.7%)	76/104 (73.1%)	5/11 (45.5%)	12/26 (46.2%)	4/4 (100%)	12/18 (66.7%)	2/7 (28.6%)
Fungal pathogens tested:								
Yeast	139/139 (100%)	31/31 (100%)	74/74 (100%)	5/5 (100%)	11/11 (100%)	4/4 (100%)	12/12 (100%)	2/2 (100%)
Mycelial fungi	38/139 (27.3%)	13/31 (41.9%)	22/74 (29.7%)	2/5 (40.0%)	1/11 (9.1%)*	0/4 (0%)*	0/12 (0%)*	0/2 (0%)*
Methods available in laboratory:								
Microbroth	52/136 (38.2%)	18/31 (58.1%)	26/73 (35.6%)	1/5 (20.0%)	3/9 (33.3%)	2/4 (50.0%)	1/12 (8.3%)	1/2 (50.0%)
Disc diffusion	62/136 (45.6%)	14/31 (45.2%)	41/73 (56.2%)	4/5 (80.0%)	3/9 (33.3%)	0/4 (0%)	0/12 (0%)	0/2 (0%)
Commercial tests	80/136 (58.8%)	16/31 (51.6%)	39/73 (53.4%)	1/5 (20.0%)	7/9 (77.8%)	4/4 (100%)	11/12 (91.7%)	2/2 (100%)
In-house tests	10/136 (7.4%)	6/31 (19.4%)	4/73 (5.5%)	0/5 (0%)	0/9 (0%)	0/4 (0%)	0/12 (0%)	0/2 (0%)
None, send to outside laboratory	5/136 (3.7%)	4/31 (12.9%)	0/73 (0%)	0/5 (0%)	1/9 (11.1%)	0/4 (0%)	0/12 (0%)	0/2 (0%)
Interpretation of antifungal susceptibility testing done by:								
CLSI	134/139 (96.4%)	27/30 (90.0%)	74/75 (98.7%)	5/5 (100%)	11/11 (100%)	3/4 (75%)	12/12 (100%)	2/2 (100%)
EUCAST	15/139 (10.8%)	5/30 (16.7%)	4/75 (5.3%)	1/5 (20%)	3/11 (27.3%)	2/4 (50%)	0 (0%)*	0 (0%)*
Not available in my hospital/laboratory	2/139 (1.4%)	1/30 (3.3%)	1/75 (1.3%)	0/5 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)

Table 2. (*Continued*).

Determinants	Overall $N = 241$	China [$N = 71$]	India [$N = 104$]	Indonesia [$N = 11$]	Philippines [$N = 26$]	Singapore [$N = 4$]	Taiwan [$N = 18$]	Thailand [$N = 7$]
Cryptococcus antigen tests used:								
Latex agglutination	43/65 (66.2%)	6/9 (66.7%)	21/36 (58.3%)	2/4 (50%)	3/4 (75%)	1/1 (100%)	9/9 (100%)	1/2 (50%)
ELISA	4/65 (6.2%)	2/9 (22.2%)	2/36 (5.5%)	0 (0%)	0/4 (0%)	0 (0%)	0 (0%)	0 (0%)
Lateral flow	19/65 (29.2%)	1/9 (11.1%)	13/36 (36.1%)	2/4 (50%)	1/4 (25.0%)	0 (0%)	0 (0%)	2/2 (100%)
Histoplasma antigen testing:								
Yes, by commercial tests	2/76 (2.6%)	1/20 (5.0%)	1/37 (2.7%)	0/3 (0%)	0/4 (0%)	0/1 (0%)	0/9 (0%)	0/2 (0%)
Yes, by in-house tests	3/76 (3.9%)	1/20 (5.0%)	0/37 (0%)	1/3 (33.3%)	0/4 (0%)	0/1 (0%)	1/9 (11.1%)	0/2 (0%)
Not available in my hospital	60/76 (78.9%)	16/20 (80.0%)	30/37 (81.1%)	2/3 (66.7%)	3/4 (75.0%)	0/1 (0%)*	7/9 (77.8%)	2/2 (100%)
Not in my hospital, send to outside laboratory	12/76 (15.8%)	3/20 (15.0%)	6/37 (16.2%)	0/3 (0%)*	1/4 (25.0%)	1/1 (100%)	1/9 (11.1%)	0/2 (0%)
Candida antigen testing:								
Yes, by commercial tests	30/203 (14.8%)	21/48 (43.8%)	6/98 (6.1%)	2/9 (22.2%)	0/25 (0%)	0/4 (0%)	0/14 (0%)	1/5 (20.0%)
Yes, by in-house tests	10/203 (4.9%)	8/48 (16.7%)	1/98 (1.0%)	0/9 (0%)	1/25 (4.0%)	0/4 (0%)	0/14 (0%)	0/5 (0%)
Yes, available (total)	37/203 (18.2%)	27/48 (56.3%)	6/98 (6.1%)	2/9 (22.2%)	1/25 (4.0%)	0/4 (0%)	0/14 (0%)*	1/5 (20.0%)
No, but I send it out	26/203 (12.8%)	4/48 (8.3%)	8/98 (8.2%)	2/9 (22.2%)	6/25 (24.0)%	1/4 (25.0%)	1/14 (7.1%)	4/5 (80.0%)
Not available	140/203 (69.0%)	17/48 (35.4%)	84/98 (85.7%)	5/9 (55.6%)	18/25 (72.0%)	3/4 (75.0%)	13/14 (92.9%)	0/5 (0%)
Available *Candida* antigen tests:								
Mannan antigen (without anti-mannan antibody)	6/35 (17.1%)	4/25 (16.0%)	2/6 (33.3%)	0/2 (0%)	0/1 (0%)	NA	NA	0/1 (0%)
Mannan antigen plus anti-mannan antibody	5/35 (14.3%)	3/25 (12.0%)	2/6 (33.3%)	0/2 (0%)	0/1 (0%)	NA	NA	0/1 (0%)
Anti-mannan antibodies	2/35 (5.7%)	1/25 (4.0%)	0/6 (0%)	1/2 (50.0%)	0/1 (0%)	NA	NA	0/1 (0%)
(1→3)-β-d-glucan (BDG)	24/35 (68.6%)	19/25 (76.0%)	4/6 (66.7%)	0/2 (0%)	0/1 (0%)	NA	NA	1/1 (100%)
Galactomannan available in laboratory	55/241 (22.8%) [32 specifying frequency]	16/71 (25.4%) [10 specifying frequency]	28/104 (26.9%) [14 specifying frequency]	1/11 (9.1%)	3/26 (11.5%)	1/4 (25.0%)	5/18 (27.8%) [2 specifying frequency]	1/7 (14.3%)
Frequency performed:								
1 day/week	6/32 (18.8%)		3/14 (21.4%)		2/3 (66.7%)		1/2 (50.0%)	
2 days/week	15/32 (46.9%)	5/10 (50.0%)	8/14 (57.1%)	1/11 (9.1%)			1/2 (50.0%)	
3 days/week	3/32 (9.4%)	2/10 (20.0%)				1/1 (100%)		
5 days/week	2/32 (3.1%)	1/10 (10.0%)			1/3 (33.3%)			

Table 2. (*Continued*).

Determinants	Overall $N = 241$	China [$N = 71$]	India [$N = 104$]	Indonesia [$N = 11$]	Philippines [$N = 26$]	Singapore [$N = 4$]	Taiwan [$N = 18$]	Thailand [$N = 7$]
6 days/week	2/32 (3.1%)	1/10 (10.0%)	1/14 (7.1%)					
Other	4/32 (12.5%)	90 samples/wk: 1	As required: 11/month: 1					25 samples/wk: 1
Beta-glucan available in laboratory	24/241 (10.0%) [20 specifying frequency]	18/71 (25.4%) [14 specifying frequency]	4/104 (3.8%)	No	1/26 (3.8%)	No	No	1/7 (14.3%)
Frequency performed:								
1 day/week	3/20 (15.0%%)	2/14 (14.3%)			1/1			
2 days/week	4/20 (20.0%)	3/14 (21.4%)	1/4 (25.0%)					
4 days/week	1/20 (5.0%)	1/14 (7.1%)						
5 days/week	5/20 (25.0%)	5/14 (35.7%)						
1 day/month	1/20 (5.0%)		1/4 (25.0%)					
Other	6/20 (30.0%)	10 samples/wk: 1 90 samples/wk: 1 120 samples/wk: 1	As required: 1 Research only: 1					0-3 samples/wk: 1
IgE is available in my/other laboratory in hospital	48/75 (64.0%)	13/20 (65.0%)	23/36 (63.9%)	2/4 (50.0%)	1/4 (25.0%)	1/1 (100%)	7/8 (87.5%)	1/2 (50.0%)
Total IgE estimation	27/48 (56.3%)	7/13 (53.8%)	14/23 (60.9%)	0/2 (0%)	1/1 (100%)	0/1 (0%)	4/7 (57.1%)	1/1 (100%)
Aspergillis-specific IgE	6/48 (12.5%)	1/13 (9.1%)	5/23 (21.7%)	0/2 (0%)	0/1 (0%)	0/1 (0%)	0/7 (0%)	0/1 (0%)
Not further specified	21/48 (43.8%)	6/13 (46.2%)	9/23 (39.1%)	2/2 (100%)	0/1 (0%)	1/1 (100%)	3/7 (42.9%)	0/1 (0%)
PCR for fungal diagnosis done using:								
Commercial tests	14/37 (37.8%)	7/16 (43.8%)	6/13 (46.2%)	0/2 (0%)	1/1 (100.0%)	0/2 (0%)	0/2 (0%)	0/1 (0%)
In-house tests	26/37 (70.3%)	11/16 (68.8%)	8/13 (61.5%)	2/2 (100%)	0/1 (0%)	2/2 (100%)	2/2 (100.0%)	1/1 (100%)
Therapeutic drug monitoring is available using:								
Bio-assay	8/21 (38.1%)	7/12 (58.3%)	1/6 (16.7%)	NA	NA	NA	0/2 (0%)	0/1 (0%)
HPLC	10/21 (47.6%)	4/12 (33.3%)	4/6 (66.7%)	NA	NA	NA	1/2 (50.0%)	1/1 (100%)
LC-MS	4/21 (19.0%)	1/12 (8.3%)	2/6 (33.3%)	NA	NA	NA	1/2 (50.0%)	0/1 (0%)

CLSI, Clinical & Laboratory Standards Institute; ELISA, enzyme-linked immunosorbent assay; EUCAST, European Committee on Antimicrobial Susceptibility Testing; HPLC, high-performance liquid chromatography; IgE, immunoglobulin E; LC-MS, liquid chromatography–mass spectrometry; MALDI, matrix-assisted laser desorption/ionization; PCR, polymerase chain reaction; PNA-FISH, fluorescence in situ hybridization using peptide nucleic acid probes.

*Statistically significant ($P < .05$).

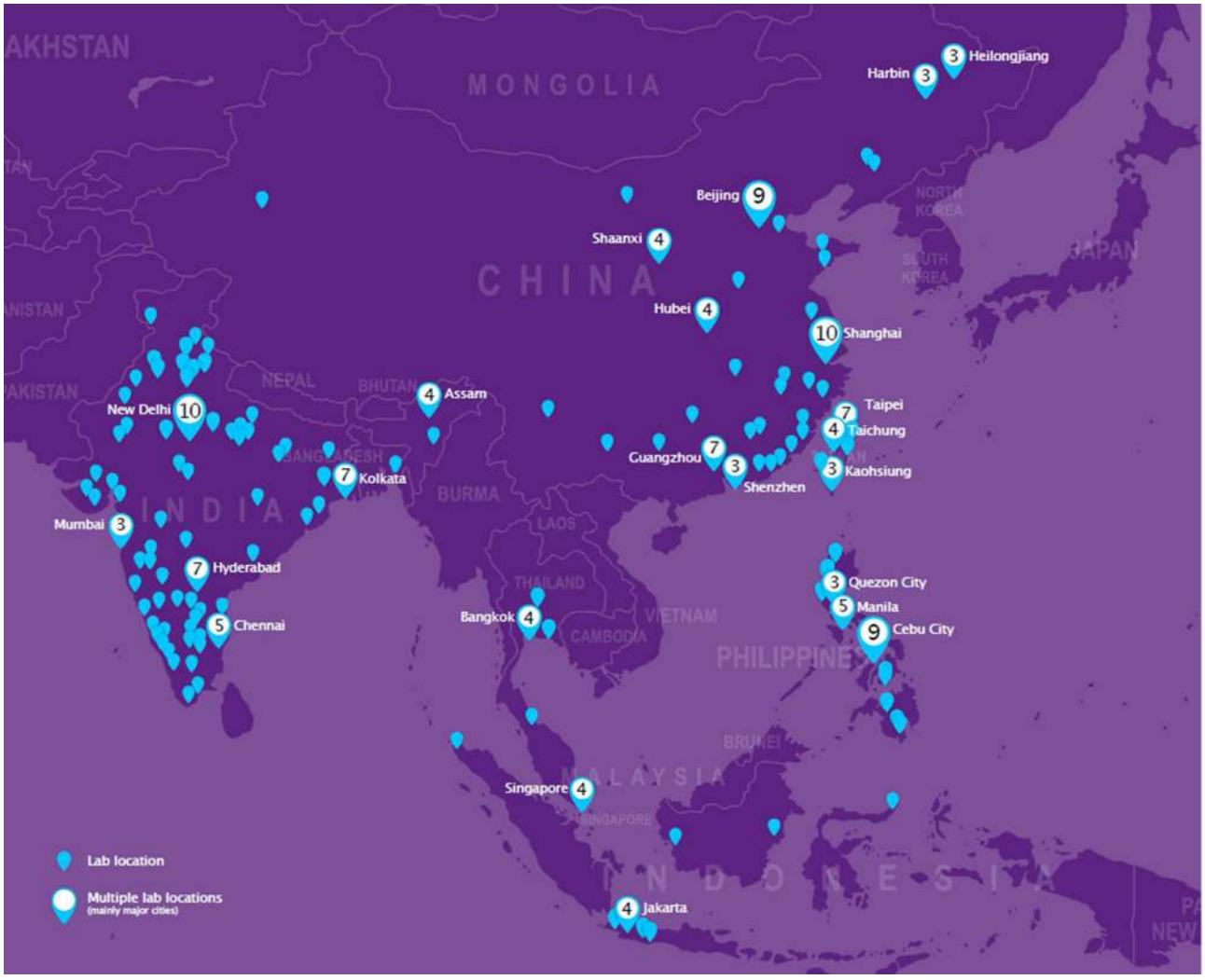

Figure 1. Locations of the laboratories that participated in the survey.

Conventional diagnosis and identification of fungi

The vast majority (230/241, 95.4%) of laboratories participating in the survey perform direct microscopy of samples, while fluorescence microscopy with the calcofluor white or fluorescent brightener technique is available in 99/241 (41.1%) laboratories (Table 2). Overall, 215/241 (89.2%) laboratories perform culture isolation of fungi. Of the 241 surveyed laboratories, 219 provided details of the fungal identification facilities or services that are available within the laboratory. For identification of fungi, although the majority (179/219, 81.7%) of all laboratories use noncommercial phenotypic and morphological methods, 44.7% (98/219) of laboratories also use commercial phenotypic methods for yeast identification; only two laboratories, both in China, use the fluorescence in situ hybridization using peptide nucleic acid probes (PNA/FISH) technique. Matrix-assisted laser desorption/ionization time-of-flight mass spectroscopy (MALDI-TOF MS) for identification of fungi is available in 27/219 (12.3%) laboratories, and nine of those laboratories are in Taiwan (9/17, 52.9%). Molecular genomic identification of fungi is available in 37/219 (16.9%) of the surveyed laboratories, and more than half of these laboratories are in China (i.e. 19/58, 32.8% of Chinese laboratories).

Antifungal susceptibility testing

Of all surveyed laboratories, 142/241 (58.9%) perform antifungal susceptibility testing, including 76/104 (73.1%) Indian laboratories, 4/4 (100%) Singaporean laboratories, 12/18 (66.7%) Taiwanese laboratories, and 2/7 (28.6%) Thai laboratories (Table 2). Of the 142 laboratories that indicated they perform antifungal susceptibility testing, 139 specified for which pathogens the testing was performed.

All those 139 laboratories perform antifungal susceptibility testing for yeasts, but less than one in three of those laboratories (38/139, 27.3%) test mycelial fungi. At the country level, antifungal susceptibility testing of mycelial fungi is performed in some laboratories in India (22/74), China (13/31), Indonesia (2/5), and the Philippines (1/11), but none of the laboratories in Singapore, Taiwan, or Thailand. Of the 142 laboratories that perform antifungal susceptibility testing, 136 provided information on the methods they use. The microbroth dilution method of susceptibility testing is available in 52/136 (38.2%) laboratories; the majority (80/136, 58.8%) of laboratories use a commercial system (not specified) for antifungal susceptibility testing; 5/136 (3.7%) laboratories (4/31 in China and 1/9 in the Philippines) outsource the testing. Almost all (134/139, 96.4%) the laboratories performing antifungal susceptibility testing follow the Clinical and Laboratory Standards Institute (CLSI), USA, recommended protocol and 15/139 (10.8%) laboratories evaluate their results also using European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoints. Six laboratories in China (6/31, 19.4%) and four laboratories in India (4/73, 5.5%) use antifungal susceptibility testing methods that have been developed in-house (not following any commercial or standardized protocol like CLSI or EUCAST).

Serology

Of all surveyed laboratories, 77/241 (32%) indicated they perform serological/antigen testing. Of those performing *Cryptococcus* antigen detection testing ($n = 66$), 43 (66.2%) use the latex agglutination method, 4 (6.2%) use an enzyme-linked immunosorbent assay (ELISA) method, and 19 (29.2%) use a lateral flow assay (Table 2). Only 6.6% (5/76) of laboratories that conduct serological/antigen testing perform *Histoplasma* antigen assays. Some form of *Candida* antigen testing is available in 18.2% (37/203) of laboratories. (1→3)-β-D-glucan (BDG) testing is available in 24 of 35 laboratories: 18 in China, four in India and one in Thailand. Five laboratories in China perform this test 5 times a week. Galactomannan antigen testing is available in 22.8% (55/241) of surveyed laboratories; it is most commonly performed 1–2 times weekly (21/32, 65.6%); two laboratories (one each in India and China) perform the test daily (i.e., 6 times weekly). Estimation of immunoglobulin E (IgE) (total or *Aspergillus*-specific) is available to 48 of the 77 laboratories who perform serological testing, either within the laboratory or in another laboratory within the same hospital. Twenty-seven of these 48 laboratories specify that they perform total IgE estimation (7/13 in China, 14/23 in India, 4/7 in Taiwan, 1/1 in the Philippines, and 1/1 in Thailand), with six of these 27 (1/13 in China and 5/23 in India) indicating that they additionally perform *Aspergillus*-specific IgE estimations.

Molecular diagnosis and therapeutic drug monitoring for azoles

Of the 53/241 (22%) laboratories that indicated they have molecular diagnosis facilities, 37 perform polymerase chain reaction (PCR) for diagnosis of fungal infections (including 16/20 in China and 13/23 in India). Of those, 26/37 (70.3%) employ an in-house technique and 14/37 (37.8%) laboratories use commercial PCR diagnosis (manufacturer not specified) of fungal infections (Table 2). However, in our survey, respondents were not asked to specify whether PCR diagnosis was performed on a patient specimen or a fungal isolate. Twenty-one laboratories (21/241, 8.7%) perform therapeutic drug monitoring (TDM) for azoles (12/71 in China, 6/104 in India, 2/18 in Taiwan, and 1/7 in Thailand); 47.6% (10/21) of these laboratories use high performance liquid chromatography (HPLC), 38.1% (8/21) use a bio-assay, and 19.0% (4/21) use the liquid chromatography–mass spectrometry (LC-MS) technique (Table 2).

Discussion

The challenge posed by fungal infections is gaining importance worldwide,[6] especially in Asia.[1–5] The true burden of fungal infections in this region is largely unknown, and any data from developing countries are likely to be underestimates due to the absence of diagnostic mycology laboratories. The present study provides, despite certain limitations, a snapshot of the status of diagnostic mycology services in seven Asian countries. Despite our best efforts, we obtained responses from 104 and 71 laboratories in India and China, respectively, although these countries are vast in size and contain the largest populations of the world. The situation in Indonesia, the Philippines, and Thailand is no better, as we received responses from 11, 26, and 7 laboratories, respectively, in those countries.

There have been few attempts worldwide at conducting this type of survey to provide a status report on medical mycology services.[7–10] Our survey is the maiden attempt in Asia. Aside from a few laboratories in our survey, most laboratories lack continuing education and training in medical mycology. A special effort may be required in Indonesia as significantly fewer laboratories conducted formal training of laboratory staff than in other countries. The use of

regular external mycology proficiency testing also reflects the competence of the laboratory.[11] A little over half (56.1%) of the surveyed Asian laboratories participate in an EQAS program; none of these is in Indonesia. The AFWG is taking action to start EQAS programs for mycology laboratories in each country. Laboratory accreditation is another method for improving laboratory proficiency, but more than half (57.3%) of the surveyed Asian laboratories are not accredited. The biosafety hood, an essential equipment of biosafety, is not available in 20.3% surveyed laboratories.

Accurate identification of fungal species is important for selection of suitable antifungal therapy as the available medications differ in their spectrum of activity. The recent worldwide threat posed by the identification of multidrug-resistant *Candida auris*[12] is looming large in Asian hospitals. Commercial phenotypic methods used by the majority of laboratories in Asia would fail to identify this superbug,[13] which would require a MALDI-TOF MS database or molecular identification by DNA sequencing. However, our survey suggests that MALDI-TOF MS and DNA sequencing facilities are not widely available in surveyed Asian countries.

With the rise of antifungal resistance, especially in yeasts in Asian countries,[14–16] routine use of antifungal susceptibility testing is essential for all laboratories associated with tertiary care facilities. However, 41.1% of surveyed laboratories do not conduct antifungal susceptibility testing, and the standard microbroth dilution technique is used by less than half (38.2%) of those laboratories performing antifungal susceptibility testing.

Serology for fungal diagnosis is performed in very few laboratories (32%, $n = 77$), and cryptococcal antigen testing is the most commonly used. Histoplasmosis is prevalent in Asian countries,[5] but *Histoplasma* antigen testing is performed by only five of the surveyed laboratories. Most of the laboratories perform conventional techniques, including direct microscopy and culture, for diagnosis of histoplasmosis. Galactomannan antigen detection, an important test for diagnosis of invasive aspergillosis, is performed in 55 laboratories, and most (62.5%) of these laboratories perform it 1–2 times per week. This would suggest a long turnaround time for a test that is essential for diagnosing the life-threatening disease aspergillosis. The β-D-glucan test, another important pan-fungal diagnostic test, is performed in 24 laboratories, covering China, India, and Thailand (only one laboratory in Thailand). The test is not available in the surveyed laboratories from the other four Asian countries. Similarly, advanced tests like PCR for fungal diagnosis and TDM for azoles are available in few laboratories in the surveyed countries. Though allergic bronchopulmonary aspergillosis[17] and allergic fungal sinusitis[18] are highly prevalent diseases in India, and possibly other Asian countries, total IgE and *Aspergillus*-specific IgE estimations (essential tests for diagnosing these diseases) are performed in 23 and seven laboratories, respectively.

The present survey identifies an urgent need to increase investment in mycology laboratories, especially to fund the incorporation of MALDI-TOF MS and non–culture-based biomarker tests like galactomannan, β-D-glucan and fungal PCR. The capital investment in MALDI-TOF MS may be high, but overall the procedure is cost-effective due to early identification, and reduced reagent and labor costs.[19] The situation appears worse in Indonesia, the Philippines, and Thailand, where those biomarker tests are nearly non-existent. The lack of facilities hinders epidemiological and outbreak investigations and compromises the management of patients with fungal infections in Asian countries. Biosafety in the laboratory is also an important issue. All laboratories performing fungal diagnosis should be equipped with biosafety hood.

The study has certain limitations. We do not know the exact number of laboratories performing mycology diagnostic services in each country and, thus, what percentage of those laboratories participated in the survey from each country. There is an absence or lack of readily available support from government or non-government agencies on fungal diseases in all Asian countries. Reviewing the map of the countries and laboratories surveyed, it appears the study is well represented in India, the Philippines, and Singapore, but skewed in China, Indonesia, and Thailand. In addition, it is possible that there were some instances where survey respondents might have misunderstood the meaning of a question. To avoid including misleading data in the analysis set, we censored some clear outlier responses from the database, for example, responses to the bed count of the catchment area of the laboratory. Still, the present study is the first serious attempt to develop a status report of diagnostic mycology services in Asian countries.

An urgent concerted effort from government, academia, and other stakeholders is required to support the development of new quality mycology laboratories and the improvement of existing laboratories with regular staff training, accreditation, and inclusion of essential advanced rapid biomarker tests and equipment. Only then we will have the tools and skills in place to curb the morbidity and mortality of invasive fungal diseases in the Asian population.

Supplementary material

Supplementary data are available at *MMYCOL* online.

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Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and the writing of the paper.

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