

**The Project for the Establishment of a  
Research and Reference Collaborative  
System for the Diagnoses of Fungal Infections  
including Drug-Resistant Ones both in Brazil  
and Japan**

**PROJECT COMPLETION REPORT**

**February 2023**

**JAPAN INTERNATIONAL COOPERATION AGENCY (JICA)  
CHIBA UNIVERSITY**

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23-011

## Contents of the Project Completion Report

### I. Basic Information of the Project

#### 1. Country

Federative Republic of Brazil

#### 2. Title of the Project

The Project for the Establishment of a Research and Reference Collaborative System for the Diagnoses of Fungal Infections including Drug-Resistant Ones both in Brazil and Japan

#### 3. Duration of the Project (Planned and Actual)

September 15, 2017-September 14, 2022

#### 4. Background (from Record of Discussions(R/D))

Fungal infections develop in immunocompromised patients with Acquired Immunological Deficiency Syndrome (hereinafter referred to as "AIDS") or others, and the incidence is increasing worldwide. The fungi's drug resistance has strengthened along with the increased frequency in use of antifungal drugs, and the mortality rate of the patients having contracted drug-resistant fungal infections is 80%, reportedly.

Brazil has twice the number of patients with pulmonary tuberculosis out of chronic respiratory diseases as Japan does. 10% of such patients developed chronic aspergillosis with poor prognosis among fungal infections, and the 5-year survival rate is estimated at 50% or less (Nam HS, et al. Int J Infect Dis 2010: 14: e479). The mechanism how aspergillus gains drug resistance has not been clarified. For example, it could be through the exposure to pesticides containing ingredients similar to medical antifungal drugs in the fields, or in the body of a patient with chronic pulmonary aspergillosis, etc. who has undergone a treatment using azole-based drugs for a long time. Moreover, there are few public data within Brazil that shows the frequency of identification regarding aspergillus strains that cause drug resistance.

It is thus necessary to clarify the mechanism of fungi's causing drug resistance first, then, develop a new diagnostic method, establish a medical treatment system at clinical sites, collect accurate epidemiological data, and build up a new therapy.

## 5. Project Purpose (from Record of Discussions(R/D))

Project Purpose: A research and reference collaborative system for the diagnoses of fungal infections including drug-resistant ones is established both in Brazil and Japan.

## 6. Implementing Agency

(Brazilian side)

University of Campinas, Instituto Adolfo Lutz (São Paulo Central Reference Laboratory), Hospital Mário Gatti, Centro Hospitalar Ouro Verde, Centro Infantil Boldrini Pediatric Oncology Hospital, Prefeitura Municipal de Campinas

(Japanese side)

Chiba University, University of Nagasaki, National Institute of Infectious Diseases, University of Tsukuba, Obihiro University of Agriculture and Veterinary Medicine

## 7. Methodology of the Joint Review

The Joint Review was carried out by the joint review team comprising of Brazilian and Japanese members in accordance with JICA Project Evaluation Guidelines, which basically follows DAC Principles for Evaluation of Development Assistance issued by Organization for Economic Co-operation and Development – Development Assistance Committee (OECD-DAC). The PDM Ver.9 with the statement of the project purpose, outputs and activities and the PO Ver.9 were used as the basic reference documents for the evaluation. Findings and information from discussions and reports were analyzed and assessed in line with the six evaluation criteria.

The evaluation criteria used for the review survey are the following: Relevance, Coherence, Effectiveness, Efficiency, Impact, and Sustainability (Table).

Table: Evaluation Perspectives Using the Six DAC Criteria for Evaluating Development Assistance

Relevance	Validity with project implementation (development needs) Focus on "Beneficiary." Consideration for inclusiveness and equity Appropriateness of the project plan and logic of approach
Coherence	Consistency with development assistance policies of the Japanese Government and JICA

	<p>Synergistic effect/mutual relations with JICA's other projects (technical cooperation, loans, grant aid, etc.)</p> <p>Complementarity, harmonization, and coordination with other assistance/projects in Japan, other development organizations, etc.</p> <p>Consistency with global framework (international targets, initiatives, standards, etc.)</p>
Effectiveness	The degree of achievement of target level in target year of expected project outcome (differential results across the groups)
Impact	Positive and negative indirect and long-term effects (systems and norms, people's well-being, human rights, gender equality, and the environment)
Efficiency	Comparisons of planned and actual projects inputs, project period, and project cost
Sustainability	Outlook on sustainability of effects that are realized by the project for aspects of policy/political, institutional/organizational, technical, financial, social & environment, risk, and operation & maintenance

## II. Results of the Project

### 1. Results of the Project

1-1 Input by the Japanese side (Planned and Actual) (See ANNEX1.1: Results of the Project 1)

#### a) Dispatch of experts

Total number: 30 persons

Detection of fungal genes related to resistance, Development of detection methods for the resistant-related genes, epidemiology, Establishment of BioResource bank

JICA Project Coordinator

Oct. 2017-Sep. 2022

#### b) Trainings and Lectures

Training courses in Brazil: 12 courses

Total number of Brazilian researchers received in Japan: 14 persons

Analyses of fungal genes, Epidemiological surveillance, Development of research network

c) Machinery and equipment

Equipment cost for a total of 3,792,506 Japanese yen and 3.934.827,68 Brazilian real. Most of the equipment was procured in Brazil except 'Storage System for fungal strains (L-dried)', 'Loopamp Realtime Turbidimeter (LAMP)' and 'Macbook Pro 13inches'. The breakdown and procurement cost of the equipment provided is shown in Annex 1.2: Results of the Project 2. The equipment will be handed over to the Brazilian side at the end of the Project.

1-2 Input by the Brazilian side (Planned and Actual)

- (a) Services of UNICAMP's counterpart personnel and administrative personnel  
Project Director, Project Manager, Project Co-Managers, and other researchers and students (see ANNEX1.3: Results of the Project 3)
- (b) Suitable office space with necessary equipment
- (c) Research space and facilities
- (d) Supply or replacement of machinery, equipment, instruments, vehicles, tools, spare parts and any other materials necessary for the implementation of the Project other than the equipment provided by JICA
- (e) Information as well as support in obtaining medical service
- (f) Credentials or identification cards
- (g) Available data (including maps and photographs) and information related to the Project
- (h) Running expenses necessary for the implementation of the Project
- (i) Expenses necessary for transportation within Brazil of the equipment as well as for the installation, operation and maintenance thereof
- (j) Necessary facilities to the JICA experts for the remittance as well as utilization of the funds introduced into Brazil from Japan in connection with the implementation of the Project

1-3 Local Activity Budget

The Brazilian side shared a total of R\$138.845,00, and the Japanese side shared a total of R\$1.726.751,00 as local cost incurred for the Project. For further details, see ANNEX 1.4: Local activity budget.

## 1-4 Activities (Planned and Actual)

1. Prevalence of drug-resistant fungi and its mechanisms of drug-resistance are epidemiologically elucidated.	
1-1. To perform classification and identification of fungal strains by morphological and biochemical properties with clinically-isolated fungal strains collected by UNICAMP-related and other project cooperating medical facilities.	Achieved Determination of species of fungal clinical strains isolated in the attending hospitals (UNICAMP, Mario Gatti Hospital, Boldrini Children Hospital and Barretos Hospital) and environmental strains collected by Instituto Adolfo Luz and Federal University of Mato Grosso do Sul are ongoing (over 1,000 clinical strains and 500 environmental strains). Several environmental fungal strains collected in Mato Grosso do Sul State are confirmed to be antifungal resistant.
1-2. To perform more detailed identification of fungal strains including cryptic species using genetic techniques on the fungal isolates identified on the above activity.	Almost achieved Detailed species (cryptic species) identifications of fungal strains isolated from clinical and environmental specimens using several methods such as DNA sequencing, multiplex PCR and LAMP are in progress and are expected to be completed by the end of the Project.
1-3. To examine the susceptibility of the fungal species (especially for <i>Aspergillus</i> , <i>Candida</i> , <i>Cryptococcus</i> , and <i>Fusarium</i> ) including some cryptic species in accordance with internationally-approved methods (e.g., the methods provided by the	Achieved Antifungal susceptibility testing is performed on the strains isolated clinically in the attending hospitals.

Clinical and Laboratory Standards Institute (CLSI)).	
1-4. To analyze the relationship between fungal characteristics (strains, species and drug susceptibility) and patients' characteristics (source of infection, infected sites, underlying and/or complicated diseases, clinical entity, history of chemotherapy by antifungals, etc.).	Achieved The database program (REDCap®) has been operated and over 350 clinical cases of patients with various fungal infection admitted to the attending health care institutions have already enrolled.
1-5. To elucidate mechanism of drug resistance by analyzing gene regions, which are reported to be responsible for drug resistances using the fungal isolates with drug-resistant property, confirmed by the susceptibility testing.	Achieved To elucidate the effect of fungicides on mutagenicity of fungi, studies on exposure of <i>A. fumigatus</i> strains to a fungicide in a simulated field are ongoing. The CRISPR/Cas9 system are used to elucidate the effect of some mutated genes on antifungal resistance.
1-6. To analyze geographic characteristics of drug resistance genes by comparing identified drug-resistant mechanisms against antifungals.	Achieved Compared with fungal strains isolated in Japan, a different polymorphism in <i>cyp51A</i> gene was revealed in Brazilian isolates.
1-7. To elucidate mechanisms of drug resistance using advanced genetic analysis technologies such as next-generation sequencing techniques for the fungal isolates, which are suspected to have unidentified drug resistance mechanisms.	
1-7-1. To provide Brazilian researchers with the Training in Japan in the theme of genetic	Achieved See ANNEX1.1.

analysis techniques using the next-generation sequencer for elucidating drug-resistant mechanisms at the MMRC.	
1-7-2. To identify gene regions, which are suspected to be responsible for drug resistance, by whole-genomic comparison of drug-susceptible and drug resistant fungal strains isolated in Brazil, followed by the functional analyses of the region in the UNICAMP.	Almost achieved Using a next generation sequencer, gene expression analysis (transcriptome) of antifungal-susceptible/resistant strains is ongoing and will continue after the Project.
1-8. To compile research findings and outputs into scientific articles, together with that gained in the Output 2 and in Japan, to be used as evidence for revising the existing guidelines and for newly-developing it for the management of mycoses, followed by the trial-based application to the hospitals in Campinas metropolitan areas and/or other areas in Brazil.	Achieved Based on the results of this project, the existing clinical manual for aspergillosis has been revised.
<b>2. Gene amplification technologies-based drug-resistance genes detection methods with sufficient sensitivity, specificity and operability are established for the diagnoses of drug-resistant mycoses.</b>	
2-1. To develop gene amplification (LAMP, PCR, etc.)-based gene detection methods for the diagnoses of drug-resistant mycoses.	
2-1-1. To develop basic testing condition of detection methods for drug-resistant fungi particularly for <i>Aspergillus</i> including its cryptic strains, <i>Candida</i> and other fungi, which are suitable for applied gene	Achieved Detection procedures for mutations in <i>cyp51A</i> gene using PCR or LAMP method is developed in Brazil and Japan. Development of identification



amplification techniques.	procedures of cryptic species using PCR or LAMP method are in progress.
2-1-2. To provide Brazilian researchers with the Training in Japan in the theme of gene amplification-based gene detection methods for the diagnoses of drug-resistant mycoses.	Achieved See ANNEX1.1.
2-1-3. To introduce the detection methods for drug-resistant fungi, developed in the Activity 2-1-2, to the UNICAMP by the joint efforts of Japanese researchers (JICA experts) and the Brazilian researchers who participated the Training in Japan.	Achieved Introduction of new detection method for antifungal resistant fungi using Surveyor Nuclease has been started.
2-2. To evaluate the clinical performance of the detection methods for drug-resistant fungi statistically by analyzing its sensitivity and specificity using fungal strains clinically-isolated from patients with mycoses in Brazil.	Almost achieved The new detection method for antifungal resistant fungi using Surveyor Nuclease is regularly performed in Brazil.
2-3. To evaluate the operability of the drug-resistant gene detection method(s), which are developed on the basis of LAMP techniques, by introducing them into UNICAMP-related hospitals and other selected external cooperative health or testing facilities on a trial basis.	Almost achieved Detection procedure for point mutations in <i>cyp51A</i> gene using PCR or LAMP method are developed in Brazil and Japan, and their operability are evaluated now.
2-4. To initiate discussions with Brazilian authorities concerned on the practical application of the drug-resistant gene detection methods, developed by the Project, to clinical	Achieved Counterparts of this project are the official members of the Committee on Microbial Resistance of Sao Paulo State Department of Development

<p>laboratory testing (registration/accreditation of the methods as the "Standard Methods" in Brazil).</p>	<p>and Innovation.</p>
<p><b>3. A UNICAMP-centered research collaboration system among research, medical and administrative organizations is established.</b></p>	
<p>3-1. To construct a banking system of fungal strains including drug-resistant ones in the UNICAMP, which is interoperable by the external cooperative agencies for mycoses research.</p>	
<p>3-1-1. To develop bylaws for the operation of the sample banking system, which stipulates the supply/transportation of samples to the UNICAMP, preservation and shared use of the fungal strains including drug-resistant ones, by holding meeting with external cooperative agencies of the Project such as universities, hospitals and testing facilities.</p>	<p>Achieved Material Transfer Agreement (MTA) was contracted between UNICAMP and MMRC, and some notable fungal strains has already shared each other.</p>
<p>3-1-2. To provide Brazilian researchers with the Training in Japan in the theme of the operational management of fungal strains preservation facility at the MMRC.</p>	<p>Achieved See ANNEX1.1.</p>
<p>3-1-3. To develop a facility for the preservation of fungal strains in the UNICAMP.</p>	<p>Achieved Preservation of fungal strains in UNICAMP are ongoing.</p>
<p>3-1-4. To construct an information database of clinically-isolated fungal strains, compromised of fungal and patient' characteristics in the UNICAMP.</p>	<p>Achieved The database program (REDCap®) has been operated and over 350 clinical cases of patients with various fungal infection admitted to the</p>

	attending health care institutions have already enrolled.
3-2. To hold regular conferences and/or seminars on drug-resistant fungi including the reporting of project achievements, geared to project external collaborating agencies, health and medical facilities in the project areas and the administration organizations in charge of the control of fungal infections including drug-resistant ones.	Achieved The enrolled cases were selected in clinical case conferences attended by various medical professions including physicians, nurses and microbiologists of UNICAMP hospital and other health care institutions under a video-conferencing system.
3-3. To hold regular meetings with the administration organizations in charge of the control of fungal infection including drug-resistant ones for sharing of the epidemiological information such as incidence and clinical entity, gained through the research activities of the Project.	Achieved Medical professions of other institutions have attended to the regular conference and received a training to input clinical information.
3-4. To set up a network of clinical laboratory testing and diagnosis of mycoses including drug-resistant ones among the UNICAMP (as the reference laboratory) and health and testing facilities in the State of São Paulo with the support of the Institute of Adolfo Lutz (IAL).	Almost achieved As the research network activity participated by several medical institutions and research laboratories in Sao Paulo State and Mato Grosso do Sul State, collecting clinical and environmental fungal strains, the regular conference, and scientific meetings are run.
3-5. To set up a network of clinical laboratory testing and diagnosis of mycoses including drug-resistant ones among research institutes, health facilities and the MMRC as the reference laboratory also in Japan, by referencing the achievements of	Achieved The regular conference held on every week is profitable for development of young medical experts at the participating medical institutions.

project collaborative research and technical cooperation in Brazil.	
3-6. To hold national and international conferences on the research of drug-resistant mycoses geared to research, health and administrative agencies in other provinces and nations, in light of future establishment of a network of collaborative research on mycoses including drug-resistant ones as well as wide-sharing of research findings and outcomes of the Project.	Achieved International training course or international conference were already held during this project. Based on research and multicultural experiences of the Project, UNICAMP has attended to an international research project of CDC, and the molecular epidemiology and infectious disease laboratory in UNICAMP was designated as a "reference laboratory in Latin America."

## 2. Achievements of the Project

### 2-1 Outputs and indicators

(Target values and actual values achieved at completion)

#### 1. Prevalence of drug-resistant fungi and its mechanisms of drug-resistance are epidemiologically elucidated.

Indicator	Achievement
(1) By December, 2019, the proportion of drug-resistant fungal strains in the fungal isolates (the prevalence) in the target areas is estimated.	Achieved The prevalence of antifungal-resistant fungal strains among clinical isolates has been estimated, and scientific articles have been published.
(2) By March, 2020, principal mechanisms of drug resistance against antifungal agents are identified using clinical isolates gained in the Campinas metropolitan area.	Achieved Main Mechanism of antifungal resistance was identified and scientific articles have been published.
(3) By March, 2022, the revising work of the existing guidelines, and drafting	Achieved Based on the results of this project,

work of the guidelines/booklets for the management of mycoses are commenced.	the existing clinical manual for aspergillosis has been revised.
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## **2. Gene amplification technologies-based drug-resistance genes detection methods with sufficient sensitivity, specificity and operability are established for the diagnoses of drug-resistant mycoses.**

<b>Indicator</b>	<b>Achievement</b>
(1) By December, 2020, basic testing condition and procedures of gene amplification (LAMP, PCR, etc.)-based gene detection methods for the diagnoses of drug-resistant <i>Aspergillus</i> , <i>Candida</i> and other fungi are determined.	Achieved The new detection method for antifungal resistant fungi using Surveyor Nuclease was developed and reported as scientific articles.
(2) By December, 2021, evaluation works of clinical performance of the above-mentioned methods are completed.	Achieved This detection method has already been regularly performed in UNICAMP.
3) By the time of the termination of the Project, concrete discussions with the Brazilian authorities concerned regarding practical procedures for the application of the above-mentioned methods for clinical laboratory testing (registration/accreditation of the methods as the "Standard Methods" in Brazil) are commenced.	Achieved The Brazilian specialists were discussing this issue through the research network system developed in this project.

## **3. A UNICAMP-centered research collaboration system among research, medical and administrative organizations is established.**

<b>Indicator</b>	<b>Achievement</b>
(1) By the time of the termination of the Project, practical operation of the banking system of fungal strains including drug-resistant ones in the	Achieved Determination of species and antifungal susceptibilities of fungal clinical strains isolated in the

<p>UNICAMP, which is interoperable by the external cooperative agencies for mycoses research, is commenced.</p>	<p>attending hospitals (UNICAMP, Mario Gatti Hospital, Boldrini Children Hospital and Barretos Hospital) and environmental strains collected by UNICAMP and Instituto Adolfo Luz are ongoing (over 1,000 clinical strains and 500 environmental strains). After these evaluation, preservation of the fungal strains using the L-drying system (a lyophilization method) in UNICAMP are ongoing.</p>
<p>(2) By December 2020, epidemiological information on mycoses including drug-resistant ones, gained through the research activities of the Project, is shared with the Brazilian authorities concerned at a one-year interval.</p>	<p>Achieved The database program (REDCap®) has been operated and over 350 clinical cases of patients with various fungal infection admitted to the attending several health care institutions have already enrolled.</p>
<p>(3) By the time of the termination of the Project, practical operation of the network of clinical laboratory testing and diagnosis of mycoses including drug-resistant ones, of which reference laboratory is the UNICAMP, is commenced.</p>	<p>Achieved The enrolled cases were selected in regular clinical case conferences attended by various medical professions including physicians, nurses and microbiologists of UNICAMP hospital and other health care institutions. Medical professions of other institutions have also attended to the regular conference and received a training to input clinical information.</p>

## 2-2 Project Purpose and indicators

(Target values and actual values achieved at completion)

**A research and reference collaborative system for the diagnoses of fungal infections including drug-resistant ones is established both in Brazil and Japan.**

Indicator	Achievement
(1) By the time of the termination of the Project, guidelines/booklets for the management of mycoses are subjected to trial-based application in hospitals in the Campinas metropolitan area.	Achieved Based on the results of this project, the existing clinical manual for aspergillosis has been revising.
(2) By the time of the termination of the Project, concrete discussions are commenced with Brazilian administrative organizations for the application of research findings and outcomes gained by the Project to policymaking and/or countermeasures for the control of fungal infections including drug-resistant ones.	Achieved Counterparts of this project are the official members of the Committee on Microbial Resistance of Sao Paulo State Department of Development and Innovation.
(3) By the time of the termination of the Project, at least 5 research articles in the themes of project research topics such as epidemiological analyses of drug-resistant fungal infections, mechanisms of resistances of fungi to antifungals, and drug resistance-related genes detection methods are published.	Achieved Total of 32 articles were published in this project (see ANNEX 2).

### 3. History of PDM Modification

Although the PDM has not been modified, part of the equipment procurement plan has been revised.

### 4. Others

As emergency support for the COVID-19 worldwide pandemic, we conducted the following activities, which were not planned in the PDM.

4-1. [Ad-hoc Panel of MIRE on COVID-19; AMC-19]

The Chief Advisor and counterparts started regular joint clinical conferences regarding COVID-19 and its-related issues to combat the COVID-19 pandemic as a special task force of the project since May 2020. As of August 2022, the meetings were held 23 times. Discussed issues are listed in ANNEX 6: List of the discussed issues in AMC-19.

4-2. [The Partnership for Accelerating COVID-19 Testing in Brazil (PACT Brazil)] Reliability verification test of the Loopamp 2019-nCoV detection kit was started in Brazil after exchanging the memorandum among JICA, Chiba University, UNICAMP and Eiken Co. Ltd. The objective of the PACT Brazil is to support the efforts of Brazil in increasing testing of SARS-Cov-2 through the quadripartite cooperation. The Participants worked together to conduct testing of the sample of test kits, and once the certification of clinical use is obtained, the Participants will consider the possibility of further cooperation to expand its clinical use.

### III. Results of Joint Review

#### 1. Results of Review based on DAC Evaluation Criteria

<b>Relevance</b>	The relevance of the Project is high, since the Brazilian and Japanese development policies/strategies on the health sectors in Brazil has not been changed and the sector has been highly prioritized since the commencement of the Project. Antifungal resistance of fungal isolates has strengthened worldwide, and the mortality rate of the patients with fungal infections caused by resistant fungus is 80%, reportedly. Fungal resistance is a big public health problem; however, there were few public data within Brazil. It was thus necessary to collect accurate epidemiological data, to clarify the mechanism of fungi's causing drug resistance, to develop a new diagnostic method and to establish the appropriate management at clinical sites.
<b>Coherence</b>	The coherence of this project is high. The objects of this project are consistent with the Priority Policy "Building a sustainable and resilient international community through efforts to address global challenges." Moreover, this project is in



	<p>concord with Joint Statement of the Official Working Visit of Prime-Minister Shinzo Abe to Brazil and on the Establishment of the Strategic and Global Partnership between Japan and Brazil (2014). In 2015, WHO formulated a Global Action Plan regarding antimicrobial resistance (AMR), and thereafter, National Action Plans were formulated in each country, including Japan, in 2016. In 2019, the US CDC reported Antibiotic Resistant Threats, and listed drug-resistant fungi in the document.</p> <p>Some medical staff of MMRC regularly examine the patients with fungal infection at Chiba University Hospital using knowledge gained from the Project. Furthermore, periodical clinical conference regarding COVID-19 attending several medical institutions in Brazil and Japan started.</p>
<b>Effectiveness</b>	<p>The effectiveness of this project is high. As described above, the perspective of achievement of the project purpose is judged to be high since the objectively verifiable indicators have been fulfilled appropriately. Logical sequence of the causal relationships between Outputs and the Project Purpose is enough strong since (i) epidemiological elucidation of antifungal resistant fungi and (ii) practicalities of rapid diagnostic methods for the resistant fungi is indispensable to enhanced capacities for diagnosis and treatment of fungal infections between Chiba University and State University of Campinas. The Brazilian side secured additional budget for renovation of the molecular epidemiology and infectious disease laboratory in UNICAMP. The devoted leadership of Project Manager and Chief Advisor have contributed to the anticipated outcomes and enhanced research capacities of CPs at Faculty of Medical Sciences, UNICAMP.</p>
<b>Impact</b>	<p>The impact of this project is high. Next generation</p>

	<p>sequencing and genetic transformation technologies have been developed by the Project and are expected to be applied to other research areas including virus and bacteria. Based on research and multicultural experiences of the Project, UNICAMP has attended to an international research project of CDC, and the molecular epidemiology and infectious disease laboratory in UNICAMP was designated as a “reference laboratory in Latin America.”</p>
<b>Efficiency</b>	<p>The efficiency of this project is high. With regard to quality and quantity, input from the Brazilian and Japanese sides such as allocation of CPs, Japanese experts, provided equipment, training in Japan at Chiba University, office and facilities are appropriate. Delegation of JICA experts in managerial aspects, medical aspects and research technical aspects is appropriate since the Chief Advisor and Project Coordinator took roles in project management, and medical researchers took roles in sharing a lot of research technologies.</p>
<b>Sustainability</b>	<p>The prospect of sustainability is high from institutional, technical, and human resources viewpoint. The administrative and academic status of UNICAMP is sufficiently prestigious not only in Brazil but also in Latin America, that it is expected to disseminate adopted skills and technologies to other institutions and to continue to be influential to society. In fact, some experimental technologies had been already introduced to other medical institution (Centro Infantil Boldrini Pediatric Oncology Hospital). The prestigious academic authority of the university that may influence standardization of relevant regulations and procedures is expected to obtain successive political support.</p> <p>While the political and economic situation in Brazil may bring some challenges, the counterparts have</p>

	<p>agreed to take necessary measures to maintain the machinery and equipment provided. Also, adopted technologies are expected to be transferred to other institutes in Brazil as well as other countries through international training courses or international conferences in the future. Such courses and conferences were already held during this project.</p>
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## 2. Key Factors Affecting Implementation and Outcomes

(COVID-19 pandemic)

Part of the planned dispatches of JICA experts and training for Brazilian researchers in Japan could not be performed because of the COVID-19 pandemic.

## 3. Evaluation on the results of the Project Risk Management

(COVID-19 pandemic)

Scientific meetings between the Chief Advisor, JICA experts and Brazilian researchers were performed periodically using a video-conferencing system and e-mail. Moreover, to keep up the efficient activities, the Japanese team frequently conducted on-line training to Brazilian researchers as far as possible. Instead of the research activities in Brazil, a part of the relevant research works was performed in Japan. Furthermore, despite the pandemic, Brazilian researchers continued the research activities (ANNEX 1.5: Other Activities in Brazil).

Through such united effort of both sides, the project was proceeded as planned.

(AMC-19)

The Chief Advisor and counterparts started regular joint clinical conferences regarding COVID-19 and its-related issues to combat the COVID-19 pandemic as a special task force of the project (Ad-hoc Panel of MIRE on COVID-19; AMC-19) since May 2020. As of August 2022, the meetings were held 23 times. Discussed issues are listed in ANNEX 6.

(PACT Brazil: The Partnership for Accelerating COVID-19 Testing in Brazil)

Reliability verification test of the Loopamp 2019-nCoV detection kit was started in Brazil after exchanging the memorandum among JICA, Chiba University, UNICAMP and Eiken Co. Ltd. The objective of PACT Brazil is to support the efforts of Brazil in increasing testing of SARS-CoV-2, through the quadripartite

cooperation. Participants worked together to conduct testing of the sample of test kits, and once the certification of clinical use is obtained, the Participants will consider the possibility of further cooperation to expand its clinical use.

#### **4. Lessons Learnt**

The Project indicates that it is indispensable for a scientific research-focused project to establish its research platform at the initial stage of project implementation including “hard” factors such as machinery, facility, office environment, and “soft” (human resource) factors such as communication, devotedness to project activities, harmonized teamwork among CPs and experts. In the Project, Brazilian and Japanese sides made possible efforts to establish and strengthen trust under the COVID-19 pandemic. It is indispensable to establish trust between Japanese and counterpart sides especially in an international co-research project with intercultural communication and cooperation.

During the Project implementation, early interventions and measures, such as holding frequent meeting to discuss the progress of the research and affirm the direction of the Project, were taken to prevent potential miscommunications caused by a multicultural communication gap between both sides. The long-term expert and the Project Manager have had a significant role in preventing potential miscommunication and alleviating the gap among the project members.

The Project successfully procured appropriate machinery that matches the needs of both the Japanese and Brazilian sides. As of this moment, a total of new 32 academic papers (See ANNEX 2: List of Products) on mycosis research have been published in peer-reviewed journals.

#### **ANNEX 1.1: Results of the Project 1**

(List of Dispatched Experts, List of Trainings)

#### **ANNEX 1.2: Results of the Project 2**

(List of Machinery and Equipment)

#### **ANNEX 1.3: Results of the Project 3**

(List of counterparts)

#### **ANNEX 1.4: Local Activity Budget**

#### **ANNEX 1.5: Other Activities in Brazil**

#### **ANNEX 2: List of Products (Research articles) Produced by the Project**

#### **ANNEX 3: PDM (All versions of PDM)**

**ANNEX 4: R/D, M/M, Minutes of JCC (copy) (\*)**

**ANNEX 5: Monitoring Sheet (copy) (\*)**

(Remarks: ANNEX 4 and 5 are for internal reference only.)

**ANNEX.6: List of discussed issues in AMC-19**

## Annex 1.1. Results of the Project

### List of Dispatched Experts

JICA expert	Affiliation	Period of visiting
Akira Watanabe (Chief Advisor)	MMRC, Chiba University	Sep. 13-26, 2017 Mar. 3-16, 2018 Sep. 22-Oct. 5, 2018 Feb.5-25, 2019 May 12-Jun. 2, 2019 Jan. 24-Feb. 16, 2020
Tetsuhiro Matsuzawa	University of Nagasaki	Sep. 13-26, 2017 Mar. 3-25, 2018 Sep. 15-Oct. 5, 2018 Feb. 9- Mar. 2, 2019 Sep. 14-Oct. 7, 2019 Jul. 7-25, 2022
Teppei Arai	MMRC, Chiba University	Sep. 13-26, 2017 Feb. 25-Mar. 25, 2018 Sep. 15-Oct. 13, 2018 Feb. 5-Mar. 18, 2019 Sep. 8-Oct.7, 2019 Jan. 24-Feb. 16, 2020 Jul. 7-25, 2022
Yasunori Muraosa	MMRC, Chiba University	Mar. 3-16, 2018 Sep. 15-Oct. 7, 2019
Hiroki Takahashi	MMRC, Chiba University	Nov. 3-16, 2019
Sayaka Ban	MMRC, Chiba University	Feb. 5-25, 2019
Yuzuru Mikami	MMRC, Chiba University	Mar. 3-16, 2018 Jun. 8-30, 2018 Jan. 24-Feb. 16, 2020
Tohru Gono	MMRC, Chiba University	Feb. 25-Mar.16, 2018 Jun. 8-30, 2018 Feb.6-16, 2020
Yutaro Hino	MMRC, Chiba University, Chiba University Hospital	May 12-Jun. 2, 2019
Yukiko Tsuchiya	MMRC, Chiba University	May 12-Jun. 2, 2019

## **JICA Project Coordinator**

**Oct. 2017-Sep. 2022**

### **List of Brazilian Counterparts**

See ANNEX1.3.

### **List of Trainings**

<in Brazil>

- Antifungal Susceptibility Tests Workshop: Mar.14-15, 2018, Dr Mikami
- Training Course of LAMP: Mar. 19-21, 2018, Dr. Matsuzawa, Dr. Arai
- Lecture "Introductory medical mycology": Jun. 22, 2018, Dr. Mikami
- Lecture "Genomic and transcriptomic analyses of human pathogenic fungi, *Aspergillus fumigatus*": Jun. 22, 2018, Dr. Gono
- Lecture and Training "Phylogenetic analysis": Feb. 8, 2019, Dr Ban
- Training on L-drying system for fungal preservation: Feb. 12-15, 2019, Dr. Ban
- Training on Antifungal Susceptibility Testings and MIC Readings: May 20-24 and 27-30, 2019, Ms. Tsuchiya and Dr. Watanabe
- Lecture "Heterogeneity of stress response in pathogenic fungus": Nov. 7, 2019, Dr. Takahashi
- Workshop on the CRISPR/cas9: Feb. 10, 2020, Dr. Arai
- International Training Course in Fungal Infection: May 4-8, 2020, Dr. Watanabe, Dr. Ban
- 1st International Symposium FUNGI: FROM THE ENVIRONMENT TO THE HOST: Nov. 23-27, Dr. watanabe, Dr. Ban
- Training on CRISPR/Cas9: Jul. 12-14, 18, 2022, Dr. Arai

<in Japan>

- Analyses of fungal genes: Aug. 5-Sep. 10, 2018, C Tararam
- Epidemiology, Development of research network: Aug. 19-Sep. 10, 2018, MR Resende
- Analyses of fungal strains, Preservation of fungal isolates, Aug. 19-Sep. 10, 2018, A Schreiber
- Methods of next generation sequencing, Development of research network: Nov. 11-Dec. 14, 2018, P Trabasso
- Analyses of fungal genes: Nov. 11-Dec. 14, 2018, L Levy

- Development of research network: Nov. 25-Dec.14, 2018, ML Moretti
- Methods of next generation sequencing: Jul. 11-Sep. 5, 2019, C Tararam
- Development of research network, Analyses of fungal genes: Oct. 6-26, 2019, ML Moretti
- Analyses of fungal strains, Development of research network: Oct. 6-Nov. 3, 2019, P Trabasso
- Epidemiological surveillance, Development of research network: Nov. 18-Dec. 5, 2019, MR Resende, T Lima, EM Psaltikidis, IS Sauer, AC martins

**Training courses provided by other institutions in Brazil and fulfilled by UNICAMP staff**

See ANNEX1.5.



## Annex 1.2 : List of Machinery and Equipment

### 1. List of Machinery and Equipment Provided on JICA Scheme

No.	Name of Equipment	Quantity	Amount
1	CO2 Incubator	1	R\$ 29,900.00
2	Chemiluminescence imager	1	R\$ 189,999.23
3	Storage System for fungal strains (L-dried)	1	¥1,654,538
4	Thermal Cycler	2	R\$ 71,596.94
5	Capillary sequencer	1	R\$ 336,693.59
6	Biosafety Cabinet -1	2	R\$ 41,800.00
7	Biosafety Cabinet -2	1	R\$ 15,200.00
8	Biosafety Cabinet -3	1	R\$ 15,700.00
9	Clean Bench	1	R\$ 11,980.00
10	Biosafety Cabinet -4	2	R\$ 100,000.00
11	Refrigerated Centrifuge	1	R\$ 126,172.42
12	Ultrapure Water System	1	R\$ 69,723.49
13	Micro Refrigerated Centrifuge	1	R\$ 38,735.51
14	Macbook Pro 13inches	1	¥247,968
15	Loopamp Realtime Turbidimeter (LAMP)	1	¥1,890,000
16	Bead Beater	1	R\$ 45,125.21
17	Deep Freezer	1	R\$ 152,175.00
18	Refrigerator (glass-fronted)	2	R\$ 30,300.00
19	Optical Microscope	1	R\$ 34,229.76
20	Dry Heat Sterilizer	1	R\$ 6,352.41
21	Microplate Washer	1	R\$ 68,137.00
22	Block Incubator	2	R\$ 34,356.21
23	Incubator Shaker	1	R\$ 17,200.00
24	Real-time PCR	1	R\$ 236,619.99
25	Incubator	1	R\$ 3,910.00
26	Vertical Autoclave	2	R\$ 24,980.00
27	Eppendorf Repeater Electronic Pipette	1	R\$ 12,807.65
28	Electrophoresis System	2	R\$ 22,022.00
29	Fluorometer	1	R\$ 15,333.67
30	Freezer	1	R\$ 2,120.31
31	Refrigerator	1	R\$ 2,549.89
32	Rotary Homogenizer	1	R\$ 4,880.00
33	Schletter	1	R\$ 3,890.00
34	Ph Meter	1	R\$ 1,850.00
35	Small Burner	2	R\$ 12,800.00
36	UPS	1	R\$ 2,280.00
37	No Break	1	R\$ 3,015.00

38	Computer	3	R\$ 18,960.00
39	Camera GPRO	1	R\$ 2,408.01
40	Mini-spinar	1	R\$ 12,202.29
41	Protective Gown	3000	R\$ 14,340.00
42	Protective Apron	3000	R\$ 25,260.00
43	Surgical Mask	3000	R\$ 2,820.10
44	Protective Gloves	3000	R\$ 17,076.00
45	PFF2 Surgical Mask	3000	R\$ 16,830.00
46	Cardioverter	10	R\$ 288,809.50
47	Electrocardiograph	10	R\$ 117,686.50
48	Multi-parameter Monitor System for ICU	20	R\$ 1,190,000.00
49	Portable Ultrasound Equipment	3	R\$ 444,000.00

Note: No.41 - 49 are ICU unit equipment has been donated for emergency assistance to combat the COVID-19.

ANNEX 1.3: Results of the Project 3 (List of counterparts)

**Project MIRE**  
List of Brazilian Counterparts

Updated August 1, 2022

	Institution	Name	Occupation	Position in the Project	Assigned to
1	Unicamp	Ivan Felizardo Contrera Toro	MD, PhD, Assistant Professor	Project Director (Dean of the FCM)	Coordination
2		Luís Carlos Zeferino	MD, PhD, Professor	Project Director (Dean of the FCM)	Coordination
3		Cláudio Saddy Rodrigues Coy	MD, PhD, Professor	Project Director (Dean of the FCM)	Coordination
4		Maria Luiza Moretti	MD, PhD, Professor	Project Manager (Vice-Rector of UNICAMP)	Coordination
5		Plínio Trabasso	MD, PhD, Associate Professor	Project Co-Manager	Molecular Biology studies
6		Angélica Zaninele Schreiber	PharmD, PhD, Assistant Professor	Project Co-Manager	Microbiology studies
7		Mariângela Ribeiro Resende	MD, PhD, Associate Professor	Project Co-Manager	Epidemiology studies
8		Lucieni de Oliveria Conterno	MD, PhD, Assistant Professor	Researcher	Epidemiology studies
9		Elisa Donalísio Teixeira Mendes	MD, PhD, Infection Disease Practitioner	Researcher	Epidemiology studies
10		Renata Fagnani	RN, PhD, Infection Control Nurse	Researcher	Epidemiology studies
11		Eliane Molina Psaltikidis	RN, PhD, Infection Control Nurse	Researcher	Epidemiology studies
12		Tiago Lima	RN, PhD, Infection Control Nurse	Researcher	Epidemiology studies
13		Cibele Aparecida Tararam	BS, PhD, Biologist at LEMDI	Researcher	Molecular Biology studies
14		Larissa Ortolam Levy	BS, Biologist at LEMDI	Researcher	Molecular Biology studies
15		Erivan Olinda de Oliveira	Technician at LEMDI	Laboratory technician	Molecular Biology studies
16		Luzia Lyra	Technician at LIF	Laboratory technician	Microbiology studies
17	Campinas Municipal Health Surveillance	Cármino Antônio de Souza	MD, PhD, Professor	Secretary of Health, Campinas Municipality	Administrative
18		Andrea von Zubem	DMV	Health Surveillance Team	Epidemiology studies
19		Marcela Torres de Lolo	MD	Health Surveillance Team	Epidemiology studies
20		Roberta Yabu-uti do Valle	RN	Health Surveillance Team	Epidemiology studies
21	Hospital Mário Gatti (Municipal General Hospital)	Marcos Eurípedes Pimenta	MD	Director of HMMG	Administrative
22		Sergio Bisogni	MD	Director of HMMG	Administrative
23		Christiane Ambrósio	RN, Infection Control Nurse	Researcher	Epidemiology studies
24		Inês Helena Leal Saraiva	MD, PhD, Infection Control Physician	Researcher	Molecular Biology studies
25		Christian Cruz Hofling	MD, PhD, Infection Control Physician	Researcher	Epidemiology studies
26		Daisy de Souza Araújo	MD, Clinical Pathology Physician	Researcher	Microbiology studies
27	Rogério Hakio Kuboyama	BS, PhD, Biologist at HMMG Laboratory	Researcher	Molecular Biology studies	
28	Centro Hospitalar Ouro Verde (Municipal General Hospital)	Roseli Aparecida de Lima	RN, Infection Control Nurse	Researcher	Epidemiology studies
29		Antônio Camargo Martins	MD, Infection Control Physician	Researcher	Molecular Biology studies
30	Centro Infantil Boldrini Pediatric Oncology Hospital	Sílvia Regina Brandalise	MD, PhD, Professor	Researcher	Molecular Biology studies
31		Hellen Caroline Alves Caldeira	RN, Infection Control Nurse	Researcher	Epidemiology studies
32		Flávio Henrique Gilli	MD, Infection Control Physician	Researcher	Epidemiology studies
33		Rosângela Aparecida Mendes	BS, Biologist at CIB Laboratory	Researcher	Microbiology studies
34	Instituto Adolfo Lutz (São Paulo Central Reference Laboratory)	Márcia M.C. Melhem	PharmD, PhD	Researcher	Environmental studies
35		Claudete Rodrigues Paula	PharmD, PhD	Researcher (USP)	Environmental studies
36		Rogério Antônio Oliveira	Laboratory Technician	Researcher	Environmental studies
37		Lucas Xavier Bonfietti	BS, PhD	Researcher	Environmental studies
38		Juliana P. Fernandes Takahashi	BS, PhD	Researcher	Environmental studies
39	Barretos' Cancer Hospital	Paulo de Tarso Oliveira e Castro	MD, PhD	Researcher	Epidemiology studies
40		José Carlos Ignácio Júnior	MD, PhD	Researcher	Epidemiology studies
41		Valéria Cristina Faustinoni	BS	Researcher	Microbiology studies
42	Federal University of Mato Grosso do Sul	James Venturini	BMedSc, PhD	Researcher	Microbiology studies
43		Marilene Rodrigues Chang	PharmD, PhD	Researcher	Microbiology studies

The following post-graduation students are also included in the Project

	Institution	Student's Name	Mentor's Name	Post-Graduation Level	Assigned to
44	Unicamp	Elisa Teixeira Mendes		Post-Doc	Environmental studies
45		Pamella Stivanelli*		Master's	Molecular Biology studies
46		Laís Sturaro*		Master's	Molecular Biology studies
47		Dayane Ruiz Costa		Trainee	Molecular Biology studies
48		Carolini Marcelli Oliveira	Maria Luiza Moretti	Trainee	Molecular Biology studies
49		Ana Paula Nascimento*		Trainee	Epidemiology studies
50		Anna Kim		Master's	Molecular Biology studies
51		Felipe W. Freire		Master's	Molecular Biology studies
52		Carlos Alberto Campos Passinho		PhD	Microbiology studies
53		Sarah Martins Craveiro		Master's	Molecular Biology studies
54		Fernanda Christina Reis		PhD	Molecular Biology studies
55		Ellen Recco		Master's	Environmental studies
56		Douglas Zamarin		Master's	Epidemiology studies
57		Dayana Fontoura	Plínio Trabasso	Master's	Epidemiology studies
58		Mariana Balduzzi		Undergraduate Research	Epidemiology studies
59		Mayara Lara Brabo		Undergraduate Research	Molecular Biology studies
60	Sarah Jarschel de Camargo		Master's	Environmental studies	
61	Franqueline Reichert Lima*		PhD	Microbiology studies	
62	Laís Pontes	Angélica Zaninele Schreiber	MsC	Microbiology studies	
63	Caio Augusto Beraquet		Master's	Microbiology studies	
64	Antônio Camargo Martins	Mariângela Ribeiro Resende	Doctorate	Molecular Biology studies	
65	Instituto Adolfo Lutz (São Paulo Central Reference Laboratory)	Lidiane de Oliveira		Master's	Environmental studies
66		Carlos Alberto Campos Passinho*		Master's	Environmental studies
67		Joana de Souza Pereira Barrel	Márcia M.C. Melhem	Master's	Environmental studies
68		Alan Antônio Kubo		PhD	Environmental studies
69		Milena Bronze Macioni		PhD	Environmental studies
70	Dálity Keffelen de Barros Rodrigues		PhD	Environmental studies	
71	Federal University of Mato Grosso do Sul (UFMS)	Suellen Raquel da Silva Ferreira	Marilene Rodrigues Chang	Master's	Environmental studies
72		Francine de Sales Dorneles	Márcia M.C. Melhem	Doctorate	Environmental studies

Obs.: \*Students that finished the post-graduation program during the Project.

#### ANNEX1.4. Local Activity Budget

Brazilian Side:

(Currency: Real)

Expenditure		FY2017 (Set-Dec)	FY2018 (Jan-Dec)	FY2019 (Jan-Dec)	FY2020 (Jan-Dec)	FY2021 (Jan-Dec)	FY2022 (Jan-Set)	Total
UNICAMP	Project Implementation Budget <sup>1)</sup>	2.150,00	8.374,00	17.930,00	7.673,00	3.215,00	1.717,00	41.059,00
	Laboratory Renovation Budget <sup>2)</sup>	1.335,00	27.420,00	56.280,00	5.290,00	4.485,00	2.976,00	97.786,00
								138.845,00

Note 1) Includes desks, chairs, meeting table, shelves, racks, and other minor furniture items, projection screen, data show equipment, microcomputers (laptop and desktops), and office supplies. Dedication of human resources to the project are not included.

2) Includes structural design project, architectural and technical projects, basic materials (e.g.: sand and cement, bricks, pipes, electricity wires, LAN wires, electrical and LAN network sockets, light bulbs, gas pipes and terminals), benches, cabinets, shelves, air conditioning equipment, cooling for the cold chamber, and miscellaneous.

Japanese Side:

(Currency: Real)

Expenditure		FY2017 (Set-Mar)	FY2018 (Apr-Mar)	FY2019 (Apr-Mar)	FY2020 (Apr-Mar)	FY2021 (Apr-Mar)	FY2022 (Apr-Set)	Total
JICA	General Expenses <sup>3)</sup>	95.569,00	360.885,00	501.225,00	137.085,00	409.570,00	222.237,00	1.726.571,00

Note 3) General expenses includes reagents, antifungal drug, consumable items for fungal preservation, travel expenses, and miscellaneous.

## ANNEX1.5. Other activities in Brazil

### Training courses provided by other institutions in Brazil and fulfilled by LEMDI staff

Title	Institution	Year	Name
Methods for Preservation of Micro-Organisms: Which is Most Efficient?	Brazilian Society of Microbiology	2017	Larissa Ortolan Levy
Logical Method for Scientific Writing	Unicamp	2018	Larissa Ortolan Levy
VI International Course on Molecular methodologies for epidemiology and diagnosis of invasive fungal infections	“Oswaldo Cruz” Foundation (Fiocruz – Rio de Janeiro)	2018	Cibele Aparecida Tararam Larissa Ortolan Levy
Fragment Sequencing and Analysis on the SeqStudio Genetic Analyzer platform	Thermo Fisher Scientific	2018	Cibele Aparecida Tararam Larissa Ortolan Levy
VIII International Course on Molecular Methodologies for Epidemiology and Diagnosis of Invasive Fungal Infections	“Oswaldo Cruz” Foundation (Fiocruz – Rio de Janeiro)	2020	Larissa Ortolan Levy
Lecture series on the cellular and molecular basis of the host-fungus interaction	Unicamp	2020	Cibele Aparecida Tararam
Environmental education and sustainability	Federal University of Santa Catarina	2020	Cibele Aparecida Tararam
Training in Environmental Waste Management	Unicamp	2021	Cibele Aparecida Tararam Larissa Ortolan Levy
Laboratory Diagnosis in Mycology	Microlab advising in Microbiology	2021	Larissa Ortolan Levy
Molecular and MALDI Identification of Molds	Association of Public Health Laboratories	2021	Larissa Ortolan Levy
Good Laboratory Practices	Unicamp	2021	Cibele Aparecida Tararam Larissa Ortolan Levy
Introductory Real-Time PCR (qPCR) online training for the QuantStudio5 platform	Thermo Fisher Scientific	2021	Cibele Aparecida Tararam Larissa Ortolan Levy

Scientific research methodology	“Oswaldo Cruz” Foundation (Fiocruz – Rio de Janeiro)	2022	Larissa Ortolan Levy
Biosafety in focus	“Oswaldo Cruz” Foundation (Fiocruz – Pernambuco)	2022	Larissa Ortolan Levy
Identification of <i>Candida auris</i>	Ministry of Health, Brazil	2022	Larissa Ortolan Levy
Update in Medical Mycology: Mycological diagnosis of opportunistic fungal infections prevalent in tertiary hospitals	Universidade Paulista Júlio de Mesquita	2022	Larissa Ortolan Levy
Invasive Fungal Infections	Rio de Janeiro Society of Infectious Diseases	2022	Cibele Aparecida Tararam Larissa Ortolan Levy
XX Bioinformatics Course “Identification, annotation and analysis of transcript expression using RNA-Seq”	Unicamp	2022	Cibele Aparecida Tararam
II International Workshop "Curation in Microbial Collections"	Unicamp	2022	Cibele Aparecida Tararam Larissa Ortolan Levy

## **Participation in Congresses with presentation of poster by Brazilian staff**

### **Cibele Aparecida Tararam**

- The gene expression profiles of susceptible and resistant strains of *Aspergillus fumigatus* after exposure to azoles appraised by RNAseq. 31st Brazilian Congress of Microbiology, ISBN: 2176-414X, October, 2021.

### **Larissa Ortolan Levy**

- Prevalence of fungal isolates in respiratory material and blood cultures from hospitalized patients with and without COVID-19 diagnosis. Levy, L. O.; Pontes, L.; Luz, E. A. de Sá, C. F.; Watanabe, A.; Trabasso, P.; Moretti, M. L.; Schreiber A. Z. 31st Brazilian Congress of Microbiology, ISBN: 2176-414X, October, 2021.

### **Cibele Aparecida Tararam and Larissa Ortolan Levy**

- Cases of fungemia caused by *Candida haemulonii var. vulnera* resistant to Amphotericin B among children at philanthropic hospital Boldrini Child Center. Freire, F.W.; Tararam, C.A; Levy, L.O.; Silva, R.A.M.; Caldeira, H.C.A; Gilli, F.H.; Brandalise, S.R.; Watanabe, A.; Trabasso, P.; Moretti, M.L. 31st Brazilian Congress of Microbiology, ISBN: 2176-414X, October, 2021.
- Visible DNA microarray system as an adjunctive molecular test to differentiate *Candida dubliniensis* from *Candida albicans*. Levy, L. O., Tararam, C. A., Sturaro, L. L., Busso-Lopes, A. F., Lyra, L., Schreiber, A. Z., Trabasso, P., Moretti, M. L. 28th European Congress of Clinical Microbiology and Infectious Diseases (ECCMID). Madrid, Spain, 04/21 to 04/24/2018.

## **ANNEX 2 List of products**

1. Shikanai-Yasuda MA, Mendes RP, Colombo AL, Queiroz-Telles F, Kono ASG, Paniago AMM, Nathan A, Valle ACFD, Bagagli E, Benard G, Ferreira MS, Teixeira MM, Silva-Vergara ML, Pereira RM, Cavalcante RS, Hahn R, Durlacher RR, Khoury Z, Camargo ZP, Moretti ML, Martinez R. Brazilian guidelines for the clinical management of paracoccidioidomycosis. *Rev Soc Bras Med Trop.* 2017 Sep-Oct, 50(5):715-740.
2. Passos AIM, Dertkigil RP, Ramos MC, Busso-Lopes AF, Tararan C, Ribeiro EO, Schreiber AZ, Trabasso P, Resende MR, Moretti ML. Serum markers as an aid in the diagnosis of pulmonary fungal infections in AIDS patients. *Braz J Infect Dis* 2017, 21:606-612.
3. Reichert-Lima F, Lyra L, Pontes L, Moretti ML, Pham CD, Lockhart SR, Schreiber AZ. Surveillance for azoles resistance in *Aspergillus* spp. highlights a high number of amphotericin B-resistant isolates. *Mycoses.* 2018 Feb 22.
4. Sturaro LL, Gonoï T, Busso-Lopes AF, Tararam CA, Levy CE, Lyra L, Trabasso P, Schreiber AZ, Kamei K, Moretti ML. Visible DNA microarray system as an adjunctive molecular test in the identification of pathogenic fungi directly from a blood culture bottle. *J Clin Microbiol.* 2018 Mar 7, pii: JCM.01908-17.
5. Hagiwara D, Arai T, Takahashi H, Kusuya Y, Watanabe A, Kamei K, Non-cyp51A azole-resistant *Aspergillus fumigatus* isolates with mutation in HMG-CoA reductase, *Emerging Infectious Diseases*, 2018, 24(10), 1889-1897.
6. Sturaro LL, Gonoï T, Busso-Lopes AF, Tararam CA, Levy CE, Lyra L, Trabasso P, Schreiber AZ, Kamei K, Moretti ML, Visible DNA microarray system as an adjunctive molecular test in the identification of pathogenic fungi directly from a blood culture bottle, *Journal of Clinical Microbiology*, 2018, 56(5), e01908-17.
7. Watanabe A, Kamei K, international Joint Research on Antifungal Resistant Fungi: Collaborative studies with the University of Campinas, Brazil, *Journal of Disaster Research*, 2018, 13(4), 751-753.
8. Moretti ML, Busso-Lopes AF, Tararam CA, Moraes R, Muraosa Y, Mikami Y, Gonoï T, Taguchi H, Lyra L, Reichert-Lima F, Trabasso P, de Hoog GS, Al-Hatmi AMS, Schreiber AZ, Kamei K, Airborne transmission of invasive fusariosis in patients with hematologic malignancies, *PloS One* 2018, 13(4),



e0196426.

9. Reichert-Lima F, Lyra L, Pontes L, Moretti ML, Pham CD, Lockhart SR, Schreiber AZ, Surveillance for azoles resistance in *Aspergillus* spp. highlights a high number of amphotericin B-resistant isolates, *Mycoses* 2018, 61(6), 360-365.
10. Sturaro LL, Gonoï T, Busso-Lopes AF, Tararam CA, Levy CE, Lyra L, Trabasso P, Schreiber AZ, Kamei K, Moretti ML, Visible DNA Microarray system as an adjunctive molecular test in identification of pathogenic fungi directly from a blood culture bottle, *J Clin Microbiol* 2018, 56(5), e01908-17.
11. Ruas LP, Genaro LM, Justo-Junior AS, Coser LO, de Castro LF, Trabasso P, Mamoni RL, Roque-Barreira MC, Blotta MHSL, Effect of ArtinM on Human blood cells during infection with *Paracoccidioides brasiliensis*, *Front Microbiol* 2018, 9, 867.
12. Pontes L, Beraquet CAG, Arai T, Pigolli GL, Lyra L, Watanabe A, Moretti ML, Schreiber AZ, *Aspergillus fumigatus* clinical isolates carrying CYP51A TR34/L98H/S297T/F495I mutation detected after 4 years retrospective azole resistance screening test in Brazil, *Antimicrob Agents Chemother* 2019, pii: AAC.02059-19.
13. Arai T, Majima H, Watanabe A, Kamei K, A simple method to detect point mutations in *Aspergillus fumigatus* cyp51A gene using a Surveyor Nuclease assay, *Antimicrob Agents Chemother* 2019, pii: AAC.02271-19.
14. Toyotome T, Arai T, Watanabe A, Kamei K, Detection of substitutions at 98th, 121st, and 289th amino acid residues in Cyp51A using cycling probes, *Med Mycol J* 2020, doi: 10.3314/mmj.19-00014.
15. Izumikawa K, Kakeya H, Sakai F, Shibuya K, Sugita T, Takazono T, Takata T, Tashiro M, Teruya K, Nakamura S, Noguchi H, Hiruma M, Makimura K, Miyazaki T, Miyazaki Y, Yamagishi Y, Yoshida K, Watanabe A: Executive Summary of JSMM Clinical Practice Guidelines for Diagnosis and Treatment of Cryptococcosis 2019. *Med Mycol J* 2020, doi: 10.3314/mmj.20.001.
16. Khalifa HO, Arai T, Majima H, Watanabe A, Kamei K: Genetic Basis of Azole and Echinocandin Resistance in Clinical *Candida glabrata* in Japan. *Antimicrob Agents Chemother* 2020, doi: 10.1128/AAC.00783-20.
17. Hino Y, Muraosa Y, Oguchi M, Yahiro M, Yarita K, Watanabe A, Sakaida E, Yokote K, Kamei K: Drain outlets in patient rooms as sources for invasive fusariosis: an analysis of patients with haematological disorders. *J Hosp*

Infect 2020, doi: 10.1016/j.jhin.2020.04.029.

18. Takeda K, Suzuki J, Watanabe A, Matsuki M, Higa K, Inoue E, Akashi S, Shimada M, Kawashima M, Ohshima N, Fukami T, Masuda K, Yamane A, Tamura A, Nagai H, Matsui H, Tohma S, Kamei K: Species identification, antifungal susceptibility, and clinical feature association of *Aspergillus* section Nigri isolates from the lower respiratory tract. *Med Mycol* 2020, doi: 10.1093/mmy/myz072.
19. Stivanelli P, Tararam CA, Trabasso P, Levy LO, Melhem MSC, Schneider AZ, Moretti ML, Visible DNA microarray and Loop-mediated Isothermal Amplification (LAMP) for the identification of *Cryptococcus* species recovered from culture medium and cerebrospinal fluid of patients with meningitis, *Braz J Med Biol Res* 2020, doi: 10.1590/1414-431X20209056.
20. Khalifa HO, Majima H, Watanabe A, Kamei K: In Vitro Characterization of Twenty-One Antifungal Combinations against Echinocandin-Resistant and -Susceptible *Candida glabrata*. *J Fungi* 2021, doi: 10.3390/jof7020108.
21. Takeda K, Suzuki J, Watanabe A, Arai T, Koiwa T, Shinfuku K, Narumoto O, Kawashima M, Fukami T, Tamura A, Nagai H, Matsui H, Kamei K: High detection rate of azole-resistant *Aspergillus fumigatus* after treatment with azole antifungal drugs among patients with chronic pulmonary aspergillosis in a single hospital setting with low azole resistance. *Med Mycol* 2021, doi: 10.1093/mmy/myaa052.
22. Khalifa HO, Arai T, Majima H, Watanabe A, Kamei K: Evaluation of Surveyor Nuclease for rapid identification of FKS genes mutations in *Candida glabrata*. *J Infect Chemother* 2021, doi: 10.1016/j.jiac.2021.01.016.
23. Zhao S, Ge W, Watanabe A, Fortwende JR, Gibbons JG: Genome-Wide Association for Itraconazole Sensitivity in Non-Resistant Clinical Isolates of *Aspergillus fumigatus*, *Front Fungal Biol* 2021, doi: org/10.3389/ffunb.2020.617338.
24. Martins AC, Psaltikidis EM, Lima TC, Fagnani R, Schreiber AZ, Conterno LO, Kamei K, Watanabe A, Trabasso P, Resende MR, Moretti ML: COVID-19 and invasive fungal coinfections: a case series at a Brazilian referral hospital. *J Med Mycol* 2021, doi: 10.1016/j.mycmed.2021.101175.
25. Trabasso P, Matsuzawa T, Arai T, Hagiwara D, Mikami Y, Moretti ML, Watanabe A: Development and validation of LAMP primer sets for rapid identification of *Aspergillus fumigatus* carrying the cyp51A TR46 azole resistance gene. *Sci Rep* 2021, doi: 10.1038/s41598-021-96651-7.

26. Toyotome T, Onishi K, Sato M, Hagiwara D, Watanabe A, Takahashi H: Identification of novel mutations contributing to azole tolerance of *Aspergillus fumigatus* through in vitro exposure to tebuconazole. *Antimicrob Agents Chemother* 2020, doi: 10.1128/AAC.02657-20.
27. Hino Y, Watanabe A, Seki R, Tsukamoto S, Takeda Y, Sakaida E, Kamei K: A Reference Laboratory Surveillance on Fungal Isolates from Patients with Haematological Malignancy in Japan. *J Fungi* 2021, doi: org/10.3390/jof7100806.
28. Arai, T, Umeyama, T, Majima, H, Inukai, T, Watanabe, A, Miyazaki, Y, Kamei, K: Hmg1 mutations in *Aspergillus fumigatus* and their contribution to triazole susceptibility. *Med Mycol* 2021, doi: 10.1093/mmy/myab026.
29. Majima H, Arai T, Kusuya Y, Takahashi H, Watanabe A, Miyazaki Y, Kamei K: Genetic differences between Japan and other countries in cyp51A polymorphisms of *Aspergillus fumigatus*. *Mycoses* 2021, doi: 10.1111/myc.13370.
30. Khalifa HO, Watanabe A, Kamei K: Azole and Echinocandin Resistance Mechanisms and Genotyping of *Candida tropicalis* in Japan: Cross-Boundary Dissemination and Animal-Human Transmission of *C. tropicalis* Infection. *Clin Microbiol Infect* 2021, doi.org/10.1016/j.cmi.2021.10.004.
31. Takeda K, Suzuki J, Watanabe A, Sekiguchi R, Sano T, Narumoto O, Kawashima M, Fukami T, Sasaki Y, Tamura A, Nagai H, Matsui H, Kamei K: The accuracy and clinical impact of the morphological identification of *Aspergillus* species in the age of cryptic species: A single-centre study. *Mycoses* 2022, doi: 10.1111/myc.13397.
32. Takeda K, Suzuki J, Watanabe A, Narumoto O, Kawashima M, Sasaki Y, Nagai H, kamei K, Matsui H: Non-*fumigatus* *Aspergillus* infection associated with a negative *Aspergillus* precipitin test in patients with chronic pulmonary aspergillosis. *J Clin Microbiol* 2021, doi:10.1128/JCM.02018-21.

### Project Monitoring Sheet I (Revision of Project Design Matrix)

**Project Title:** The Project for the Establishment of a Research and Reference Collaborative System for the Diagnoses of Fungal Infections including Drug-Resist; **Version 1**

**Implementing Agency:** University of Campinas, Sao Paulo (UNICAMP)

**Dated 07 March, 2018**

**Target Group:** Residents of the area covered by Campinas University hospital and affiliated hospitals (Approximately 3 million people)

**Period of Project:** From September 2017 to September 2022


**Project Site:**

**Model Site:**

Narrative Summary	Objectively Verifiable Indicators	Means of Verification	Important Assumption	Achievement	Remarks
Overall Goal					
Project Purpose					
A research and reference collaborative system for the diagnoses of fungal infections including drug-resistant ones is established both in Brazil and Japan.	<p>(1) By the time of the termination of the Project, guidelines/booklets for the management of mycoses are subjected to trial-based application in hospitals in the Campinas metropolitan area.</p> <p>(2) By the time of the termination of the Project, concrete discussions are commenced with Brazilian administrative organizations for the application of research findings and outcomes gained by the Project to policymaking and/or countermeasures for the control of fungal infections including drug-resistant ones.</p> <p>(3) By the time of the termination of the Project, at least 5 research articles in the themes of project research topics such as epidemiological analyses of drug-resistant fungal infections, mechanisms of resistances of fungi to antifungals, and drug resistance-related genes detection methods are</p>	<ul style="list-style-type: none"> <li>• Reports developed by the Project</li> <li>• Meeting minutes with relevant organizations</li> <li>• Research articles published in international journals</li> </ul>		Achievement of these indicators will be verified at the end of this Project.	
Outputs					
1. Prevalence of drug-resistant fungi and its mechanisms of drug-resistance are epidemiologically elucidated.	<p>(1) By December, 2019, the proportion of drug-resistant fungal strains in the fungal isolates (the prevalence) in the target areas is estimated.</p> <p>(2) By March, 2020, principal mechanisms of drug resistance against antifungal agents are identified using clinical isolates gained in the Campinas metropolitan area.</p>	<ul style="list-style-type: none"> <li>• Reports developed by the Project</li> <li>• A draft of revised guidelines</li>   <li>• Reports developed by the Project</li> <li>• Minutes of meetings with the Brazilian authorities concerned</li> </ul>	1. The Project gains the cooperation of the Brazilian health administration organizations such as the MOH, the São Paulo State Health Office and the Campinas City Health Office in charge of the control of fungal infections including drug-resistant ones for	Among the strains isolated in UNICAMP hospital, an antifungal-resistant <i>Aspergillus fumigatus</i> strain has been found. Species identification of fungal	

	<p>(3) By March, 2022, the revising work of the existing guidelines, and drafting work of the guidelines/booklets for the management of <b>mycoses</b> are commenced.</p>	<ul style="list-style-type: none"> <li>• Reports developed by the Project</li> <li>• Documents of information sharing regarding epidemiological data with the Brazilian authorities concerned</li> </ul>	<p>implementing meetings with the Project regarding policy advocacy etc.</p> <p>2. Political superiority of the control of fungal infections including drug-resistant ones is not diminished so as to impair the achievement of the Project Purpose.</p>	<p>strains isolated from clinical and environmental specimens using a microarray method is in progress.</p>	
<p>2. Gene amplification technologies-based drug-resistance genes detection methods with sufficient sensitivity, specificity and operability are established for the diagnoses of drug-resistant mycoses.</p>	<p>(1) By December, 2020, basic testing condition and procedures of gene amplification (LAMP, PCR, etc.)-based gene detection methods for the diagnoses of drug-resistant <i>Aspergillus</i>, <i>Candida</i> and other fungi are determined.</p> <p>(2) By December, 2021, evaluation works of clinical performance of the above-mentioned methods are completed.</p> <p>(3) By the time of the termination of the Project, concrete discussions with the Brazilian authorities concerned regarding practical procedures for the application of the above-mentioned methods for clinical laboratory testing (registration/accreditation of the methods as the "Standard Methods" in Brazil) are commenced.</p>			<p>Detection experiments for mutations in antifungal-resistant genes were started using gene amplification (LAMP and PCR) methods.</p>	

<p>3. A UNICAMP-centered research collaboration system among research, medical and administrative organizations is established.</p>	<p>(1) By the time of the termination of the Project, practical operation of the banking system of fungal strains including drug-resistant ones in the UNICAMP, which is interoperable by the external cooperative agencies for mycoses research, is commenced.</p> <p>(2) By Descember 2020, epidemiological information on mycoses including drug-resistant ones, gained through the research activities of the Project, is shared with the Brazilian authorities concerned at a one-year interval.</p> <p>(3) By the time of the termination of the Project, practical operation of the network of clinical laboratory testing and diagnosis of mycoses including drug-resistant ones, of which reference laboratory is the UNICAMP, is commenced.</p>			<p>Reactivation of fungal strains stored in UNICAMP is started. L-dried system was chosen as preservation method for stored fungal strains. A room for storage of fungal strains was prepared.</p> <p>Development phase of database of clinical information using REDCap is achieved. Production phase of the software is in progress.</p> <p>Activity of building research network including workshops, meetings and visit to other institutes were started.</p>	
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Activities	Inputs		Pre-Conditions
	The Japanese Side	The Brazilian Side	
<p><b>1. Prevalence of drug-resistant fungi and its mechanisms of drug-resistance are epidemiologically elucidated.</b></p> <p>1-1. To perform classification and identification of fungal strains by morphological and biochemical properties with clinically-isolated fungal strains collected by UNICAMP-related and other project cooperating medical facilities.</p> <p>1-2. To perform more detailed identification of fungal strains including cryptic species using genetic techniques on the fungal isolates identified in the Activity 1-1.</p> <p>1-3. To examine the susceptibility of the fungal species (especially for <i>Aspergillus</i>, <i>Candida</i>, <i>Cryptococcus</i>, and <i>Fusarium</i>) including some cryptic species in accordance with internationally-approved methods (e.g. the methods provided by the Clinical and Laboratory Standards Institute (CLSI)).</p> <p>1-4. To analyze the relationship between fungal characteristics (strains, species and drug susceptibility) and patients' characteristics (source of infection, infected sites, underlying and/or complicated diseases, clinical entity, history of chemotherapy by antifungals, etc.).</p> <p>1-5. To elucidate mechanism of drug resistance by analyzing gene regions, which are reported to be responsible for drug resistances using the fungal isolates with drug-resistant property, confirmed in the Activity 1-3.</p> <p>1-6. To analyse geographical characteristic of drug resistance genes by comparing identified drug-resistant mechanisms against antifungals.</p>	<p>Experts</p> <p>(1) Chief Advisor (Short-term Expert/double as an expert for Diagnostics-related studies)</p> <p>(2) Project Coordinator/Specialist for Research Institutes Cooperation (Long-term Expert)</p> <p>(3) Diagnostics-related studies (Short-term Experts)</p> <p>(4) Molecular epidemiological studies (Short-term Experts)</p> <p>(5) Gene amplification-related studies (Short-term Experts)</p> <p>(6) Bioinformatics (Short-term Experts)</p> <p>(7) Epidemiological investigation and optimal administration-related studies (Short-term Experts)</p> <p>(8) Management of fungal strains-related study (Short-term Experts)</p> <p>(9) Other persons with necessary expertise for the project activities (Short-term Experts)</p> <p>Training in Japan</p> <p>(1) Training for gene analyses</p> <p>(2) Training for operating Next Generation Sequencer</p> <p>(3) Training for genome comparison and gene disruption</p> <p>(4) Training for management of collected fungi</p> <p>(5) Other necessary training</p> <p>Equipment and materials</p> <p>Necessary equipment and materials for research activities in the Project</p> <p>Local cost</p>	<p>Counterparts</p> <p>(1) Project Director</p> <p>(2) Project Manager</p> <p>(3) Project Co-managers (3 persons)</p> <p>(4) Researchers (Infectious Diseases Division of the Internal Medicine Department and the Clinical Pathology Department, the Faculty of Medical Sciences, the UNICAMP)</p> <p>Facilities, equipment and materials</p> <p>(1) Office space or facilities for JICA experts in the UNICAMP</p> <p>(2) Research space and facilities in the UNICAMP</p> <p>(3) Existing research instruments and equipment for research activities, etc.</p> <p>Local Costs</p> <p>- Running expenses for research activities, etc.;</p> <p>- Assignment of counterpart personnel;</p> <p>- Utility costs (electricity, water, etc.);</p> <p>- Personnel costs (to the work of counterpart) ;</p> <p>and</p> <p>- Operational costs.</p>	<p>1. Approvals are obtained by the ethical committees of each project implementing agency for the medical and/or epidemiological research, which are supposed to be performed in the Project (if necessary).</p> <p style="text-align: center;"></p> <p style="background-color: yellow; text-align: center;">&lt;Issues and countermeasures&gt;</p> <p>A capillary sequencer and other equipment for research, which were planned to be donated to UNICAMP, could not be procured in 2017FY because of budgetary limitation of Japanese side.</p> <p>The equipment is re-planned to be procured in 2018FY, and both sides try to do their best to minimize the impact of delays on activities concerned.</p>

1-7. In parallel with the Activity 1-5, to elucidate mechanisms of drug resistance using advanced genetic analysis technologies such as next-generation sequencing techniques for the fungal isolates, which are suspected to have unidentified drug resistance mechanisms.

1-7-1. To provide Brazilian researchers with the Training in Japan in the theme of genetic analysis techniques using the next-generation sequencer for elucidating drug-resistant mechanisms at the MMRC.

1-7-2. To identify gene regions, which are suspected to be responsible for drug resistance, by whole-genomic comparison of drug-susceptible and drug resistant fungal strains isolated in Brazil, followed by the functional analyses of the region in the UNICAMP.

1-8. To compile research findings and outputs into scientific articles, together with that gained in the Output 2 and in Japan, to be used as evidences for revising the existing guidelines and for newly-developing it for the management of mycoses, followed by the trial-based application to the hospitals in Campinas metropolitan area and/or other areas in Brazil.

**2. Gene amplification technologies-based drug-resistance genes detection methods with sufficient sensitivity, specificity and operability are established for the diagnoses of drug-resistant mycoses.**

2-1. To develop gene amplification (LAMP, PCR, etc.)-based gene detection methods for the diagnoses of drug-resistant mycoses.

2-1-1. To develop basic testing condition of detection methods for drug-resistant fungi particularly for *Aspergillus* including its cryptic strains, *Candida* and other fungi, which are suitable for applied gene amplification techniques.



2-1-2. To provide Brazilian researchers with the Training in Japan in the theme of gene amplification-based gene detection methods for the diagnoses of drug-resistant mycoses.

2-1-3. To introduce the detection methods for drug-resistant fungi, developed in the Activity 2-1-2, to the UNICAMP by the joint efforts of Japanese researchers (JICA experts) and the Brazilian researchers who participated the Training in Japan.

2-2. To evaluate the clinical performance of the detection methods for drug-resistant fungi statistically by analyzing its sensitivity and specificity using fungal strains clinically-isolated from patients with mycoses in Brazil.

2-3. To evaluate the operability of the drug-resistant gene detection method(s), which are developed on the basis of LAMP techniques, by introducing them into UNICAMP-related hospitals and other selected external cooperative health or testing facilities on a trial basis.

2-4. To initiate discussions with Brazilian authorities concerned on the practical application of the drug-resistant gene detection methods, developed by the Project, to clinical laboratory testing (registration/accreditation of the methods as the "Standard Methods" in Brazil).

**3. A UNICAMP-centered research collaboration system among research, medical and administrative organizations is established.**

3-1. To construct a banking system of fungal strains including drug-resistant ones in the UNICAMP, which is interoperable by the external cooperative agencies for mycoses research.

3-1-1. To develop bylaws for the operation of the sample banking system, which stipulates the supply/transportation of samples to the UNICAMP, preservation and shared use of the fungal strains including drug-resistant ones, by holding meeting with external cooperative agencies of the Project such as universities, hospitals and testing facilities.

3-1-2. To provide Brazilian researchers with the Training in Japan in the theme of the operational management of fungal strains preservation facility at the MMRC.

3-1-3. To develop a facility for the preservation of fungal strains in the UNICAMP.

3-1-4. To construct an information database of clinically-isolated fungal strains, compromised of fungal and patient' characteristics in the UNICAMP.

3-2. To hold regular conferences and/or seminars on drug-resistant fungi including the reporting of project achievements, geared to project external collaborating agencies, health and medical facilities in the project areas and the administration organizations in charge of the control of fungal infections including drug-resistant ones.

3-3. To hold regular meetings with the administration organizations in charge of the control of fungal infection including drug-resistant ones for sharing of the epidemiological information such as incidence and clinical entity, gained through the research activities of the Project.

3-4. To set up a network of clinical

It will take more amount of time than expected to bring out fungal strains from Brazil to Japan because of treaty ratification of Convention on Biological Diversity in May 2017.

<p>laboratory testing and diagnosis of mycoses including drug-resistant ones among the UNICAMP (as the reference laboratory) and health and testing facilities in the State of São Paulo with the support of the Institute of Adolfo Lutz (IAL).</p> <p>3-5. To set up a network of clinical laboratory testing and diagnosis of mycoses including drug-resistant ones among research institutes, health facilities and the MMRC as the reference laboratory also in Japan, by referencing the achievements of project collaborative research and technical cooperation in Brazil.</p> <p>3-6. To hold national and international conferences on the research of drug-resistant mycoses geared to research, health and administrative agencies in other provinces and nations, in light of future establishment of a network of collaborative research on mycoses including drug-resistant ones as well as wide-sharing of research findings and outcomes of the Project.</p>			
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**Project Monitoring Sheet I (Revision of Project Design Matrix)**

**Project Title: The Project for the Establishment of a Research and Reference Collaborative System for the Diagnoses of Fungal Infections including Drug-Resistant On Version 2**

**Implementing Agency: University of Campinas, Sao Paulo (UNICAMP)**

**Dated 26 September, 2018**

**Target Group: Residents of the area covered by Campinas University hospital and affiliated hospitals (Approximately 3 million people)**

**Period of Project: From September 2017 to September 2022**

**Project Site:**

**Model Site:**

Narrative Summary	Objectively Verifiable Indicators	Means of Verification	Important Assumption	Achievement	Remarks
Overall Goal					
Project Purpose	<p>(1) By the time of the termination of the Project, guidelines/booklets for the management of mycoses are subjected to trial-based application in hospitals in the Campinas metropolitan area.</p> <p>(2) By the time of the termination of the Project, concrete discussions are commenced with Brazilian administrative organizations for the application of research findings and outcomes gained by the Project to policymaking and/or countermeasures for the control of fungal infections including drug-resistant ones.</p> <p>(3) By the time of the termination of the Project, at least 5 research articles in the themes of project research topics such as epidemiological analyses of drug-resistant fungal infections, mechanisms of resistances of fungi to antifungals, and drug resistance-related genes detection methods are published.</p>	<ul style="list-style-type: none"> <li>• Reports developed by the Project</li> <li>• Meeting minutes with relevant organizations</li> <li>• Research articles published in international journals</li> </ul>		Achievement of these indicators will be verified at the end of this Project.	
Outputs	<p>(1) By December, 2019, the proportion of drug-resistant fungal strains in the fungal isolates (the prevalence) in the target areas is estimated.</p> <p>(2) By March, 2020, principal mechanisms of drug resistance against antifungal agents are identified using clinical isolates gained in the Campinas metropolitan area.</p> <p>(3) By March, 2022, the revising work of the existing guidelines, and drafting work of the guidelines/booklets for the management of mycoses are commenced.</p>	<ul style="list-style-type: none"> <li>• Reports developed by the Project</li> <li>• A draft of revised guidelines</li> <li>• Reports developed by the Project</li> <li>• Minutes of meetings with the Brazilian authorities concerned</li> <li>• Reports developed by the Project</li> <li>• Documents of information sharing regarding epidemiological data with the Brazilian authorities concerned</li> </ul>	<p>1. The Project gains the cooperation of the Brazilian health administration organizations such as the MOH, the São Paulo State Health Office and the Campinas City Health Office in charge of the control of fungal infections including drug-resistant ones for implementing meetings with the Project regarding policy advocacy etc.</p> <p>2. Political superiority of the control of fungal infections including the ones caused by drug-resistant strains is not diminished so as to impair the achievement of the Project Purpose.</p>	<p>Among the strains isolated in UNICAMP, antifungal-resistant <i>Aspergillus fumigatus</i> strains have been found both clinically and environmentally. These strains are now analyzed genetically to find the mechanism for antifungal-resistance. Training for whole-genome analyses using a next generation sequencer was performed at MMRC in August to September, 2018. Species identifications of fungal strains isolated from clinical and environmental specimens using several methods such as multiplex PCR, microarray and LAMP are in progress. Relationship between environmental strain isolated in hospital and clinical strain isolated from patients have been analyzed using DNA sequencing, susceptibility testing and DNA fingerprinting.</p>	

<p>2. Gene amplification technologies-based drug-resistance genes detection methods with sufficient sensitivity, specificity and operability are established for the diagnoses of drug-resistant mycoses.</p>	<p>(1) By December, 2020, basic testing condition and procedures of gene amplification (LAMP, PCR, etc.)-based gene detection methods for the diagnoses of drug-resistant <i>Aspergillus</i>, <i>Candida</i> and other fungi are determined.                  (2) By December, 2021, evaluation works of clinical performance of the above-mentioned methods are completed.                  (3) By the time of the termination of the Project, concrete discussions with the Brazilian authorities concerned regarding practical procedures for the application of the above-mentioned methods for clinical laboratory testing (registration/accreditation of the methods as the "Standard Methods" in Brazil)</p>			<p>Evaluation of automatic identification methods for differentiation between <i>Candida albicans</i> and <i>C.dubliniensis</i> and antifungal susceptibility testing of these strains are in progress. Training for gene analyses was performed at MMRC in August to September, 2018. Detection procedures for mutations in antifungal-resistant genes are developing using some genetic analytical methods such as mismatch-specific nucleases (Surveyor Nuclease) and LAMP.</p>	
<p>3. A UNICAMP-centered research collaboration system among research, medical and administrative organizations is established.</p>	<p>(1) By the time of the termination of the Project, practical operation of the banking system of fungal strains including drug-resistant ones in the UNICAMP, which is interoperable by the external cooperative agencies for mycoses research, is commenced.                  (2) By December 2020, epidemiological information on mycoses including drug-resistant ones, gained through the research activities of the Project, is shared with the Brazilian authorities concerned at a one-year interval.                  (3) By the time of the termination of the Project, practical operation of the network of clinical laboratory testing and diagnosis of mycoses including drug-resistant ones, of which reference laboratory is the UNICAMP, is commenced</p>	<p>A L-drying system was selected as the fungal preservation method. For the epidemiological study the REDCap software was chosen and the database system based on it has been developed. REDCap (Research Electronic Data Capture) is a secure and open web application for building and managing online surveys and databases.</p>		<p>Mycological laboratory in UNICAMP (LEMDI) has been expanded the space and upgraded. Two workshops for microbiology and a training course for LAMP were held in UNICAMP. Reactivation of fungal strains stored in UNICAMP is ongoing. Training for fungal preservation procedure using a L-drying system was performed at MMRC in August to September, 2018. Brazilian researchers attended training courses on REDCap in São Paulo in April and June, 2018. The database program has been developed and is now tested in UNICAMP by entering the information of real clinical cases of patients hospitalized in the Hospital de Clinicas of Unicamp with different fungal infection. The cases were selected by an assistance physician and three hospital epidemiology nurses are responsible to input the clinical data. Brazilian researchers were given lectures on NESID (the National Epidemiological Surveillance of Infectious Diseases) and JANIS (Japan Nosocomial Infections Surveillance) in Japan in August to September, 2018. Activity of building research network including workshops, meetings and visit to other institutes were in progress.</p>	

Activities	Inputs		Pre-Conditions
	The Japanese Side	The Brazilian Side	
<p><b>1. Prevalence of drug-resistant fungi and its mechanisms of drug-resistance are epidemiologically elucidated.</b></p> <p>1-1. To perform classification and identification of fungal strains by morphological and biochemical properties with clinically-isolated fungal strains collected by UNICAMP-related and other project cooperating medical facilities.</p> <p>1-2. To perform more detailed identification of fungal strains including cryptic species using genetic techniques on the fungal isolates identified in the Activity 1-1.</p> <p>1-3. To examine the susceptibility of the fungal species (especially for <i>Aspergillus</i>, <i>Candida</i>, <i>Cryptococcus</i>, and <i>Fusarium</i>) including some cryptic species in accordance with internationally-approved methods (e.g. the methods provided by the Clinical and Laboratory Standards Institute (CLSI)).</p> <p>1-4. To analyze the relationship between fungal characteristics (strains, species and drug susceptibility) and patients' characteristics (source of infection, infected sites, underlying and/or complicated diseases, clinical entity, history of chemotherapy by antifungals, etc.).</p> <p>1-5. To elucidate mechanism of drug resistance by analyzing gene regions, which are reported to be responsible for drug resistances using the fungal isolates with drug-resistant property, confirmed in the Activity 1-3.</p> <p>1-6. To analyse geographical characteristic of drug resistance genes by comparing identified drug-resistant mechanisms against antifungals.</p> <p>1-7. In parallel with the Activity 1-5, to elucidate mechanisms of drug resistance using advanced genetic analysis technologies such as next-generation sequencing techniques for the fungal isolates, which are suspected to have unidentified drug resistance mechanisms.</p>	<p>Experts</p> <p>(1) Chief Advisor (Short-term Expert/double as an expert for Diagnostics-related studies)</p> <p>(2) Project Coordinator/Specialist for Research Institutes Cooperation (Long-term Expert)</p> <p>(3) Diagnostics-related studies (Short-term Experts)</p> <p>(4) Molecular epidemiological studies (Short-term Experts)</p> <p>(5) Gene amplification-related studies (Short-term Experts)</p> <p>(6) Bioinformatics (Short-term Experts)</p> <p>(7)</p> <p>Epidemiological investigation and optimal administration-related studies (Short-term Experts)</p> <p>(8) Management of fungal strains-related study (Short-term Experts)</p> <p>(9) Other persons with necessary expertise for the project activities (Short-term Experts)</p> <p>Training in Japan</p> <p>(1) Training for gene analyses</p> <p>(2) Training for operating Next Generation Sequencer</p> <p>(3) Training for genome comparison and gene disruption</p> <p>(4) Training for management of collected fungi</p> <p>(5) Other necessary training</p> <p>Equipment and materials</p> <p>Necessary equipment and materials for research activities in the Project</p> <p>Local cost</p>	<p>Counterparts</p> <p>(1) Project Director</p> <p>(2) Project Manager</p> <p>(3) Project Co-managers (3 persons)</p> <p>(4) Researchers (Infectious Diseases Division of the Internal Medicine Department and the Clinical Pathology Department, the Faculty of Medical Sciences, the UNICAMP)</p> <p>Facilities, equipment and materials</p> <p>(1) Office space or facilities for JICA experts in the UNICAMP</p> <p>(2) Research space and facilities in the UNICAMP</p> <p>(3) Existing research instruments and equipment for research activities, etc.</p> <p>Local Costs</p> <ul style="list-style-type: none"> <li>- Running expenses for research activities, etc.;</li> <li>- Assignment of counterpart personnel;</li> <li>- Utility costs (electricity, water, etc.);</li> <li>- Personnel costs (to the work of counterpart) ;</li> </ul> <p>and</p> <ul style="list-style-type: none"> <li>- Operational costs.</li> </ul>	<p>1. Approvals are obtained by the ethical committees of each project implementing agency for the medical and/or epidemiological research, which are supposed to be performed in the Project (if necessary).</p> <p style="text-align: center;">▶</p> <p style="background-color: yellow; text-align: center;">&lt;Issues and countermeasures&gt;</p>

1-7-1. To provide Brazilian researchers with the Training in Japan in the theme of genetic analysis techniques using the next-generation sequencer for elucidating drug-resistant mechanisms at the MMRC.

1-7-2. To identify gene regions, which are suspected to be responsible for drug resistance, by whole-genomic comparison of drug-susceptible and drug resistant fungal strains isolated in Brazil, followed by the functional analyses of the region in the UNICAMP.

1-8. To compile research findings and outputs into scientific articles, together with that gained in the Output 2 and in Japan, to be used as evidences for revising the existing guidelines and for newly-developing it for the management of mycoses, followed by the trial-based application to the hospitals in Campinas metropolitan area and/or other areas in Brazil.

**2. Gene amplification technologies-based drug-resistance genes detection methods with sufficient sensitivity, specificity and operability are established for the diagnoses of drug-resistant mycoses.**

2-1. To develop gene amplification (LAMP, PCR, etc.)-based gene detection methods for the diagnoses of drug-resistant mycoses.

2-1-1. To develop basic testing condition of detection methods for drug-resistant fungi particularly for *Aspergillus* including its cryptic strains, *Candida* and other fungi, which are suitable for applied gene amplification techniques.

2-1-2. To provide Brazilian researchers with the Training in Japan in the theme of gene amplification-based gene detection methods for the diagnoses of drug-resistant mycoses.

2-1-3. To introduce the detection methods for drug-resistant fungi, developed in the Activity 2-1-2, to the UNICAMP by the joint efforts of Japanese researchers (JICA experts) and the Brazilian researchers who participated the Training in Japan.

2-2. To evaluate the clinical performance of

the detection methods for drug-resistant fungi statistically by analyzing its sensitivity and specificity using fungal strains clinically-isolated from patients with mycoses in Brazil.

2-3. To evaluate the operability of the drug-resistant gene detection method(s), which are developed on the basis of LAMP techniques, by introducing them into UNICAMP-related hospitals and other selected external cooperative health or testing facilities on a trial basis.

2-4. To initiate discussions with Brazilian authorities concerned on the practical application of the drug-resistant gene detection methods, developed by the Project, to clinical laboratory testing (registration/accreditation of the methods as the "Standard Methods" in Brazil).

**3. A UNICAMP-centered research collaboration system among research, medical and administrative organizations is established.**

3-1. To construct a banking system of fungal strains including drug-resistant ones in the UNICAMP, which is interoperable by the external cooperative agencies for mycoses research.

3-1-1. To develop bylaws for the operation of the sample banking system, which stipulates the supply/transportation of samples to the UNICAMP, preservation and shared use of the fungal strains including drug-resistant ones, by holding meeting with external cooperative agencies of the Project such as universities, hospitals and testing facilities.

3-1-2. To provide Brazilian researchers with the Training in Japan in the theme of the operational management of fungal strains preservation facility at the MMRC.

3-1-3. To develop a facility for the preservation of fungal strains in the UNICAMP.

3-1-4. To construct an information database of clinically-isolated fungal strains, comprised of fungal and patient' characteristics in the UNICAMP.

3-2. To hold regular conferences and/or

It will take more amount of time than expected to bring out fungal strains from Brazil to Japan because of treaty ratification of Convention on Biological Diversity in May 2017.



<p>seminars on drug-resistant fungi including the reporting of project achievements, geared to project external collaborating agencies, health and medical facilities in the project areas and the administration organizations in charge of the control of fungal infections including drug-resistant ones.</p> <p>3-3. To hold regular meetings with the administration organizations in charge of the control of fungal infection including drug-resistant ones for sharing of the epidemiological information such as incidence and clinical entity, gained through the research activities of the Project.</p> <p>3-4. To set up a network of clinical laboratory testing and diagnosis of mycoses including drug-resistant ones among the UNICAMP (as the reference laboratory) and health and testing facilities in the State of São Paulo with the support of the Institute of Adolfo Lutz (IAL).</p> <p>3-5. To set up a network of clinical laboratory testing and diagnosis of mycoses including drug-resistant ones among research institutes, health facilities and the MMRC as the reference laboratory also in Japan, by referencing the achievements of project collaborative research and technical cooperation in Brazil.</p> <p>3-6. To hold national and international conferences on the research of drug-resistant mycoses geared to research, health and administrative agencies in other provinces and nations, in light of future establishment of a network of collaborative research on mycoses including drug-resistant ones as well as wide-sharing of research findings and outcomes of the Project.</p>			
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## Project Monitoring Sheet I (Revision of Project Design Matrix)

Project Title: The Project for the Establishment of a Research and Reference Collaborative System for the Diagnoses of Fungal Infections including Drug-Resistant Ones both in Brazil and Japan

Version 3

Implementing Agency: University of Campinas, Sao Paulo (UNICAMP)

Dated 20 February, 2019

Target Group: Residents of the area covered by Campinas University hospital and affiliated hospitals (Approximately 3 million people)

Period of Project: From September 2017 to September 2022

Project Site:


Model Site:

Narrative Summary	Objectively Verifiable Indicators	Means of Verification	Important Assumption	Achievement	Remarks
Overall Goal					
Project Purpose  A research and reference collaborative system for the diagnoses of fungal infections including drug-resistant ones is established both in Brazil and Japan.	(1) By the time of the termination of the Project, guidelines/booklets for the management of mycoses are subjected to trial-based application in hospitals in the Campinas metropolitan area. (2) By the time of the termination of the Project, concrete discussions are commenced with Brazilian administrative organizations for the application of research findings and outcomes gained by the Project to policymaking and/or countermeasures for the control of fungal infections including drug-resistant ones. (3) By the time of the termination of the Project, at least 5 research articles in the themes of project research topics such as epidemiological analyses of drug-resistant fungal infections, mechanisms of resistances of fungi to antifungals, and drug resistance-related genes detection methods are published.	<ul style="list-style-type: none"> <li>• Reports developed by the Project</li> <li>• Meeting minutes with relevant organizations</li> <li>• Research articles published in international journals</li> </ul>		Achievement of these indicators will be verified at the end of this Project.	
Outputs 1. Prevalence of drug-resistant fungi and its mechanisms of drug-resistance are epidemiologically elucidated.	(1) By December, 2019, the proportion of drug-resistant fungal strains in the fungal isolates (the prevalence) in the target areas is estimated.	<ul style="list-style-type: none"> <li>• Reports (including some articles or abstracts of academic meeting) developed by the Project</li> <li>• A draft of revised guidelines</li> </ul>	<p>1. The Project gains the cooperation of the Brazilian health administration organizations such as the MOH, the São Paulo State Health Office and the Campinas City Health Office in charge of the control of fungal infections including drug-resistant ones for implementing meetings with the Project regarding policy advocacy etc.</p> <p>2. Political superiority of the control of fungal infections including the ones caused by drug-resistant strains is not diminished so as to impair the achievement of the Project Purpose.</p>	Classification and identification of fungal strains collected in UNICAMP are ongoing (over 500 strains). Lecture course on molecular epidemiology in National Institute of Infectious Diseases, Japan (NIID) were held on December, 2018.	

	<p>(2) By March, 2020, principal mechanisms of drug resistance against antifungal agents are identified using clinical isolates gained in the Campinas metropolitan area.</p> <p>(3) By March, 2022, the revising work of the existing guidelines, and drafting work of the guidelines/booklets for the management of mycoses are commenced.</p>	<ul style="list-style-type: none"> <li>• Reports developed by the Project</li> <li>• Minutes of meetings with the Brazilian authorities concerned</li>   <li>• Reports developed by the Project</li> <li>• Documents of information sharing regarding epidemiological data with the Brazilian authorities concerned</li> </ul>
<p>2. Gene amplification technologies-based drug-resistance genes detection methods with sufficient sensitivity, specificity and operability are established for the diagnoses of drug-resistant mycoses.</p>	<p>(1) By December, 2020, basic testing condition and procedures of gene amplification (LAMP, PCR, etc.)-based gene detection methods for the diagnoses of drug-resistant <i>Aspergillus</i>, <i>Candida</i> and other fungi are determined.</p> <p>(2) By December, 2021, evaluation works of clinical performance of the above-mentioned methods are completed.</p> <p>(3) By the time of the termination of the Project, concrete discussions with the Brazilian authorities concerned regarding practical procedures for the application of the above-mentioned methods for clinical laboratory testing (registration/accreditation of the methods as the "Standard Methods" in Brazil) are commenced.</p>	

<p>Lecture on and practice for fungal phylogenetic analysis were held in UNICAMP in February 2019.</p> <p>Detailed species identifications of fungal strains isolated from clinical and environmental specimens using several methods such as multiplex PCR, microarray and LAMP are in progress.</p> <p>Among the strains isolated in UNICAMP, antifungal-resistant <i>Aspergillus fumigatus</i> strains have been found both clinically and environmentally. This environmental resistant strain possesses a unique mutation in <i>cyp51A</i> gene.</p> <p>To elucidate the effect of fungicides on mutagenicity of fungi, studies on exposure of environmental fungal strains to some fungicides are on going in Instituto Adolfo Lutz.</p> <p>Introduction of whole-genome analyses using a next generation sequencer was performed at MMRC in November to December, 2018.</p>	
<p>Training for gene analyses was performed at MMRC in November to December, 2018.</p> <p>Lecture on the advanced LAMP method was held in UNICAMP in February 2019.</p> <p>Detection method for mutations in antifungal-resistant genes <i>cyp51A</i> using mismatch-specific nucleases (Surveyor Nuclease) is validated. Development of a detection procedure for point mutations in <i>cyp51A</i> gene using ARMS-LAMP method is in progress.</p>	

<p>3. A UNICAMP-centered research collaboration system among research, medical and administrative organizations is established.</p>	<p>(1) By the time of the termination of the Project, practical operation of the banking system of fungal strains including drug-resistant ones in the UNICAMP, which is interoperable by the external cooperative agencies for mycoses research, is commenced.</p> <p>(2) By December 2020, epidemiological information on mycoses including drug-resistant ones, gained through the research activities of the Project, is shared with the Brazilian authorities concerned at a one-year interval.</p> <p>(3) By the time of the termination of the Project, practical operation of the network of clinical laboratory testing and diagnosis of mycoses including drug-resistant ones, of which reference laboratory is the UNICAMP, is commenced.</p>			<p>Workshops for phylogenetic analysis and for LAMP were held in UNICAMP.</p> <p>L-drying system for fungal preservation was installed in UNICAMP. The training course for fungal preservation using this system were held in UNICAMP in February 2019.</p> <p>The database program (RedCap) has been operated and over 30 clinical cases of patients with various fungal infection admitted to the Hospital de Clinicas of Unicamp have already enrolled. These cases were selected in clinical case conferences attended by various medical professions including physicians, nurses and microbiologists. This conference held every week in the hospital, and is profitable for development of young medical experts.</p> <p>Activity of building research network including workshops, meetings and visit to other institutes were in progress.</p>	
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Activities	Inputs		Pre-Conditions
	The Japanese Side	The Brazilian Side	
<p><b>1. Prevalence of drug-resistant fungi and its mechanisms of drug-resistance are epidemiologically elucidated.</b></p> <p>1-1. To perform classification and identification of fungal strains by morphological and biochemical properties with clinically-isolated fungal strains collected by UNICAMP-related and other project cooperating medical facilities.</p> <p>1-2. To perform more detailed identification of fungal strains including cryptic species using genetic techniques on the fungal isolates identified in the Activity 1-1.</p> <p>1-3. To examine the susceptibility of the fungal species (especially for <i>Aspergillus</i>, <i>Candida</i>, <i>Cryptococcus</i>, and <i>Fusarium</i>) including some cryptic species in accordance with internationally-approved methods (e.g. the methods provided by the Clinical and Laboratory Standards Institute (CLSI)).</p> <p>1-4. To analyze the relationship between fungal characteristics (strains, species and drug susceptibility) and patients' characteristics (source of infection, infected sites, underlying and/or complicated diseases, clinical entity, history of chemotherapy by antifungals, etc.).</p> <p>1-5. To elucidate mechanism of drug resistance by analyzing gene regions, which are reported to be responsible for drug resistances using the fungal isolates with drug-resistant property, confirmed in the Activity 1-3.</p> <p>1-6. To analyse geographical characteristic of drug resistance genes by comparing identified drug-resistant mechanisms against antifungals.</p> <p>1-7. In parallel with the Activity 1-5, to elucidate mechanisms of drug resistance using advanced genetic analysis technologies such as next-generation sequencing techniques for the fungal isolates, which are suspected to have unidentified drug resistance mechanisms.</p> <p>1-7-1. To provide Brazilian researchers with the Training in Japan in the theme of genetic analysis techniques using the next-generation sequencer for elucidating drug-resistant mechanisms at the MMRC.</p> <p>1-7-2. To identify gene regions, which are suspected</p>	<p>Experts                      (1) Chief Advisor (Short-term Expert/double as an expert for Diagnostics-related studies)                      (2) Project Coordinator/Specialist for Research Institutes Cooperation (Long-term Expert)                      (3) Diagnostics-related studies (Short-term Experts)                      (4) Molecular epidemiological studies (Short-term Experts)                      (5) Gene amplification-related studies (Short-term Experts)                      (6) Bioinformatics (Short-term Experts)                      (7) Epidemiological investigation and optimal administration-related studies (Short-term Experts)                      (8) Management of fungal strains-related study (Short-term Experts)                      (9) Other persons with necessary expertise for the project activities (Short-term Experts)</p> <p>Training in Japan                      (1) Training for gene analyses                      (2) Training for operating Next Generation Sequencer                      (3) Training for genome comparison and gene disruption                      (4) Training for management of collected fungi                      (5) Other necessary training</p> <p>Equipment and materials                      Necessary equipment and materials for research activities in the Project</p> <p>Local cost</p>	<p>Counterparts                      (1) Project Director                      (2) Project Manager                      (3) Project Co-managers (3 persons)                      (4) Researchers (Infectious Diseases Division of the Internal Medicine Department and the Clinical Pathology Department, the Faculty of Medical Sciences, the UNICAMP)</p> <p>Facilities, equipment and materials                      (1) Office space or facilities for JICA experts in the UNICAMP                      (2) Research space and facilities in the UNICAMP                      (3) Existing research instruments and equipment for research activities, etc.</p> <p>Local Costs                      - Running expenses for research activities, etc.;                      - Assignment of counterpart personnel;                      - Utility costs (electricity, water, etc.);                      - Personnel costs (to the work of counterpart) ;                      and                      - Operational costs.</p>	<p>1. Approvals are obtained by the ethical committees of each project implementing agency for the medical and/or epidemiological research, which are supposed to be performed in the Project (if necessary).</p> <p style="text-align: center;"></p> <p style="text-align: center; background-color: yellow;">&lt;Issues and countermeasures&gt;</p>

to be responsible for drug resistance, by whole-genomic comparison of drug-susceptible and drug-resistant fungal strains isolated in Brazil, followed by the functional analyses of the region in the UNICAMP.

1-8. To compile research findings and outputs into scientific articles, together with that gained in the Output 2 and in Japan, to be used as evidences for revising the existing guidelines and for newly-developing it for the management of mycoses, followed by the trial-based application to the hospitals in Campinas metropolitan areas and/or other areas in Brazil.

**2. Gene amplification technologies-based drug-resistance genes detection methods with sufficient sensitivity, specificity and operability are established for the diagnoses of drug-resistant mycoses.**

2-1. To develop gene amplification (LAMP, PCR, etc.)-based gene detection methods for the diagnoses of drug-resistant mycoses.

2-1-1. To develop basic testing condition of detection methods for drug-resistant fungi particularly for *Aspergillus* including its cryptic strains, *Candida* and other fungi, which are suitable for applied gene amplification techniques.

2-1-2. To provide Brazilian researchers with the Training in Japan in the theme of gene amplification-based gene detection methods for the diagnoses of drug-resistant mycoses.

2-1-3. To introduce the detection methods for drug-resistant fungi, developed in the Activity 2-1-2, to the UNICAMP by the joint efforts of Japanese researchers (JICA experts) and the Brazilian researchers who participated the Training in Japan.

2-2. To evaluate the clinical performance of the detection methods for drug-resistant fungi statistically by analyzing its sensitivity and specificity using fungal strains clinically-isolated from patients with mycoses in Brazil.

2-3. To evaluate the operability of the drug-resistant gene detection method(s), which are developed on the basis of LAMP techniques, by introducing them into UNICAMP-related hospitals and other selected external cooperative health or testing facilities on a trial basis.

2-4. To initiate discussions with Brazilian authorities concerned on the practical application of the drug-resistant gene detection methods, developed by the Project, to clinical laboratory testing (registration/accreditation of the methods as the "Standard Methods" in Brazil).

**3. A UNICAMP-centered research collaboration system among research, medical and administrative organizations is established.**

3-1. To construct a banking system of fungal strains including drug-resistant ones in the UNICAMP, which is interoperable by the external cooperative agencies for mycoses research.

3-1-1. To develop bylaws for the operation of the sample banking system, which stipulates the supply/transportation of samples to the UNICAMP, preservation and shared use of the fungal strains including drug-resistant ones, by holding meeting with external cooperative agencies of the Project such as universities, hospitals and testing facilities.

3-1-2. To provide Brazilian researchers with the Training in Japan in the theme of the operational management of fungal strains preservation facility at the MMRC.

3-1-3. To develop a facility for the preservation of fungal strains in the UNICAMP.

3-1-4. To construct an information database of clinically-isolated fungal strains, comprised of fungal and patient' characteristics in the UNICAMP.

3-2. To hold regular conferences and/or seminars on drug-resistant fungi including the reporting of project achievements, geared to project external collaborating agencies, health and medical facilities in the project areas and the administration organizations in charge of the control of fungal infections including drug-resistant ones.

3-3. To hold regular meetings with the administration organizations in charge of the control of fungal infection including drug-resistant ones for sharing of the epidemiological information such as incidence and clinical entity, gained through the research activities of the Project.

3-4. To set up a network of clinical laboratory testing and diagnosis of mycoses including drug-resistant ones among the UNICAMP (as the reference laboratory) and health and testing facilities in the State of São Paulo with the support of the Institute of Adolfo Lutz (IAL).

3-5. To set up a network of clinical laboratory testing and diagnosis of mycoses including drug-resistant ones among research institutes, health facilities and the MMRC as the reference laboratory also in Japan, by referencing the achievements of project collaborative research and technical cooperation in Brazil.

3-6. To hold national and international conferences on the research of drug-resistant mycoses geared to research, health and administrative agencies in other provinces and nations, in light of future establishment of a network of collaborative research on mycoses including drug-resistant ones as well as wide-sharing of research findings and outcomes of the Project.



**Project Monitoring Sheet I (Revision of Project Design Matrix)**

**Project Title:** The Project for the Establishment of a Research and Reference Collaborative System for the Diagnoses of Fungal Infections including Drug-Resistant Ones both in Brazil and Japan

**Version 4**

**Implementing Agency:** University of Campinas, Sao Paulo (UNICAMP)

**Dated 20 September, 2019**

**Target Group:** Residents of the area covered by Campinas University hospital and affiliated hospitals (Approximately 3 million people)

**Period of Project:** From September 2017 to September 2022

**Project Site:**


**Model Site:**

Narrative Summary	Objectively Verifiable Indicators	Means of Verification	Important Assumption	Achievement	Remarks
Overall Goal					
Project Purpose  A research and reference collaborative system for the diagnoses of fungal infections including drug-resistant ones is established both in Brazil and Japan.	(1) By the time of the termination of the Project, guidelines/booklets for the management of mycoses are subjected to trial-based application in hospitals in the Campinas metropolitan area. (2) By the time of the termination of the Project, concrete discussions are commenced with Brazilian administrative organizations for the application of research findings and outcomes gained by the Project to policymaking and/or countermeasures for the control of fungal infections including drug-resistant ones. (3) By the time of the termination of the Project, at least 5 research articles in the themes of project research topics such as epidemiological analyses of drug-resistant fungal infections, mechanisms of resistances of fungi to antifungals, and drug resistance-related genes detection methods are published.	<ul style="list-style-type: none"> <li>• Reports developed by the Project</li> <li>• Meeting minutes with relevant organizations</li> <li>• Research articles published in international journals</li> </ul>		Achievement of these indicators will be verified at the end of this Project.	
Outputs 1. Prevalence of drug-resistant fungi and its mechanisms of drug-resistance are epidemiologically elucidated.	(1) By December, 2019, the proportion of drug-resistant fungal strains in the fungal isolates (the prevalence) in the target areas is estimated.	<ul style="list-style-type: none"> <li>• Reports (including some articles or abstracts of academic meeting) developed by the Project</li> <li>• A draft of revised guidelines</li> </ul>	1. The Project gains the cooperation of the Brazilian health administration organizations such as the MOH, the São Paulo State Health Office and the Campinas City Health Office in charge of the control of fungal infections including drug-resistant ones for implementing meetings with the Project regarding policy advocacy etc.  2. Political superiority of the control of fungal infections including the ones caused by drug-resistant strains is not diminished so as to impair the achievement of the Project Purpose.	Determination of species and antifungal susceptibilities of fungal clinical strains isolated in the attending hospitals and environmental strains collected by Instituto Adolfo Luz are ongoing (over 1,000 clinical strains and 500 environmental strains). Lecture and training course on antifungal	

	<p>(2) By March, 2020, principal mechanisms of drug resistance against antifungal agents are identified using clinical isolates gained in the Campinas metropolitan area.</p> <p>(3) By March, 2022, the revising work of the existing guidelines, and drafting work of the guidelines/booklets for the management of mycoses are commenced.</p>	<ul style="list-style-type: none"> <li>• Reports developed by the Project</li> <li>• Minutes of meetings with the Brazilian authorities concerned</li>   <li>• Reports developed by the Project</li> <li>• Documents of information sharing regarding epidemiological data with the Brazilian authorities concerned</li> </ul>
<p>2. Gene amplification technologies-based drug-resistance genes detection methods with sufficient sensitivity, specificity and operability are established for the diagnoses of drug-resistant mycoses.</p>	<p>(1) By December, 2020, basic testing condition and procedures of gene amplification (LAMP, PCR, etc.)-based gene detection methods for the diagnoses of drug-resistant <i>Aspergillus</i>, <i>Candida</i> and other fungi are determined.</p> <p>(2) By December, 2021, evaluation works of clinical performance of the above-mentioned methods are completed.</p> <p>(3) By the time of the termination of the Project, concrete discussions with the Brazilian authorities concerned regarding practical procedures for the application of the above-mentioned methods for clinical laboratory testing (registration/accreditation of the methods as the "Standard Methods" in Brazil) are commenced.</p>	

<p>susceptibility testing according to CLSI methods to researchers of UNICAMP and other attending institutions were held on May, 2019 in UNICAMP.</p> <p>Detailed species (cryptic species) identifications of fungal strains isolated from clinical and environmental specimens using several methods such as DNA sequencing, multiplex PCR and LAMP are in progress. Among the strains isolated in UNICAMP, another antifungal-resistant <i>Aspergillus fumigatus</i> clinical strains have been found. Compared with fungal strains isolated in Japan, a different polymorphism in <i>cyp51A</i> gene was revealed in Brazilian isolates. To elucidate the effect of fungicides on mutagenicity of fungi, studies on exposure of <i>A. fumigatus</i> strains to some fungicides are on going in Instituto Adolfo Lutz. Using a next generation sequencer, gene expression analysis (transcriptome) of antifungal-susceptible/resistant strains was started from July 2019.</p>	
<p>Validation of detection method for mutations in antifungal-resistant genes <i>cyp51A</i> by mismatch-specific nucleases (Surveyor Nuclease) using antifungal-susceptible/resistant strains were finished. Development of a detection procedure for point mutations in <i>cyp51A</i> gene using ARMS-LAMP method is in progress. Microbiome analysis using clinical samples (bronchoalveolar lavage fluid) was started from May 2019. The CRISPR/Cas9 system, a new method for transgenesis, was started to perform to clarify the function of mutated gene.</p>	

<p>3. A UNICAMP-centered research collaboration system among research, medical and administrative organizations is established.</p>	<p>(1) By the time of the termination of the Project, practical operation of the banking system of fungal strains including drug-resistant ones in the UNICAMP, which is interoperable by the external cooperative agencies for mycoses research, is commenced.</p> <p>(2) By December 2020, epidemiological information on mycoses including drug-resistant ones, gained through the research activities of the Project, is shared with the Brazilian authorities concerned at a one-year interval.</p> <p>(3) By the time of the termination of the Project, practical operation of the network of clinical laboratory testing and diagnosis of mycoses including drug-resistant ones, of which reference laboratory is the UNICAMP, is commenced.</p>			<p>The database program (RedCap) has been operated and over 110 clinical cases of patients with various fungal infection admitted to the attending health care institutions have already enrolled. These cases were selected in clinical case conferences attended by various medical professions including physicians, nurses and microbiologists of UNICAMP hospital and other health care institutions under a video-conference system. This conference held every week, and is profitable for development of young medical experts.</p> <p>As the research network activity participated by several health care and research institutes in Sao Paulo State, collecting clinical and environmental fungal strains, the regular conference, and scientific meetings are run.</p>	
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Activities	Inputs		Pre-Conditions
	The Japanese Side	The Brazilian Side	
<p><b>1. Prevalence of drug-resistant fungi and its mechanisms of drug-resistance are epidemiologically elucidated.</b></p> <p>1-1. To perform classification and identification of fungal strains by morphological and biochemical properties with clinically-isolated fungal strains collected by UNICAMP-related and other project cooperating medical facilities.</p> <p>1-2. To perform more detailed identification of fungal strains including cryptic species using genetic techniques on the fungal isolates identified in the Activity 1-1.</p> <p>1-3. To examine the susceptibility of the fungal species (especially for <i>Aspergillus</i>, <i>Candida</i>, <i>Cryptococcus</i>, and <i>Fusarium</i>) including some cryptic species in accordance with internationally-approved methods (e.g. the methods provided by the Clinical and Laboratory Standards Institute (CLSI)).</p> <p>1-4. To analyze the relationship between fungal characteristics (strains, species and drug susceptibility) and patients' characteristics (source of infection, infected sites, underlying and/or complicated diseases, clinical entity, history of chemotherapy by antifungals, etc.).</p> <p>1-5. To elucidate mechanism of drug resistance by analyzing gene regions, which are reported to be responsible for drug resistances using the fungal isolates with drug-resistant property, confirmed in the Activity 1-3.</p> <p>1-6. To analyse geographical characteristic of drug resistance genes by comparing identified drug-resistant mechanisms against antifungals.</p> <p>1-7. In parallel with the Activity 1-5, to elucidate mechanisms of drug resistance using advanced genetic analysis technologies such as next-generation sequencing techniques for the fungal isolates, which are suspected to have unidentified drug resistance mechanisms.</p> <p>1-7-1. To provide Brazilian researchers with the Training in Japan in the theme of genetic analysis techniques using the next-generation sequencer for elucidating drug-resistant mechanisms at the MMRC.</p> <p>1-7-2. To identify gene regions, which are suspected</p>	<p>Experts                      (1) Chief Advisor (Short-term Expert/double as an expert for Diagnostics-related studies)                      (2) Project Coordinator/Specialist for Research Institutes Cooperation (Long-term Expert)                      (3) Diagnostics-related studies (Short-term Experts)                      (4) Molecular epidemiological studies (Short-term Experts)                      (5) Gene amplification-related studies (Short-term Experts)                      (6) Bioinformatics (Short-term Experts)                      (7) Epidemiological investigation and optimal administration-related studies (Short-term Experts)                      (8) Management of fungal strains-related study (Short-term Experts)                      (9) Other persons with necessary expertise for the project activities (Short-term Experts)</p> <p>Training in Japan                      (1) Training for gene analyses                      (2) Training for operating Next Generation Sequencer                      (3) Training for genome comparison and gene disruption                      (4) Training for management of collected fungi                      (5) Other necessary training</p> <p>Equipment and materials                      Necessary equipment and materials for research activities in the Project</p> <p>Local cost</p>	<p>Counterparts                      (1) Project Director                      (2) Project Manager                      (3) Project Co-managers (3 persons)                      (4) Researchers (Infectious Diseases Division of the Internal Medicine Department and the Clinical Pathology Department, the Faculty of Medical Sciences, the UNICAMP)</p> <p>Facilities, equipment and materials                      (1) Office space or facilities for JICA experts in the UNICAMP                      (2) Research space and facilities in the UNICAMP                      (3) Existing research instruments and equipment for research activities, etc.</p> <p>Local Costs                      - Running expenses for research activities, etc.;                      - Assignment of counterpart personnel;                      - Utility costs (electricity, water, etc.);                      - Personnel costs (to the work of counterpart) ;                      and                      - Operational costs.</p>	<p>1. Approvals are obtained by the ethical committees of each project implementing agency for the medical and/or epidemiological research, which are supposed to be performed in the Project (if necessary).</p> <p style="text-align: center;"></p> <p style="text-align: center; background-color: yellow;">&lt;Issues and countermeasures&gt;</p>

to be responsible for drug resistance, by whole-genomic comparison of drug-susceptible and drug-resistant fungal strains isolated in Brazil, followed by the functional analyses of the region in the UNICAMP.

1-8. To compile research findings and outputs into scientific articles, together with that gained in the Output 2 and in Japan, to be used as evidences for revising the existing guidelines and for newly-developing it for the management of mycoses, followed by the trial-based application to the hospitals in Campinas metropolitan areas and/or other areas in Brazil.

**2. Gene amplification technologies-based drug-resistance genes detection methods with sufficient sensitivity, specificity and operability are established for the diagnoses of drug-resistant mycoses.**

2-1. To develop gene amplification (LAMP, PCR, etc.)-based gene detection methods for the diagnoses of drug-resistant mycoses.

2-1-1. To develop basic testing condition of detection methods for drug-resistant fungi particularly for *Aspergillus* including its cryptic strains, *Candida* and other fungi, which are suitable for applied gene amplification techniques.

2-1-2. To provide Brazilian researchers with the Training in Japan in the theme of gene amplification-based gene detection methods for the diagnoses of drug-resistant mycoses.

2-1-3. To introduce the detection methods for drug-resistant fungi, developed in the Activity 2-1-2, to the UNICAMP by the joint efforts of Japanese researchers (JICA experts) and the Brazilian researchers who participated the Training in Japan.

2-2. To evaluate the clinical performance of the detection methods for drug-resistant fungi statistically by analyzing its sensitivity and specificity using fungal strains clinically-isolated from patients with mycoses in Brazil.

2-3. To evaluate the operability of the drug-resistant gene detection method(s), which are developed on the basis of LAMP techniques, by introducing them into UNICAMP-related hospitals and other selected external cooperative health or testing facilities on a trial basis.

2-4. To initiate discussions with Brazilian authorities concerned on the practical application of the drug-resistant gene detection methods, developed by the Project, to clinical laboratory testing (registration/accreditation of the methods as the "Standard Methods" in Brazil).

**3. A UNICAMP-centered research collaboration system among research, medical and administrative organizations is established.**

3-1. To construct a banking system of fungal strains including drug-resistant ones in the UNICAMP, which is interoperable by the external cooperative agencies for mycoses research.

3-1-1. To develop bylaws for the operation of the sample banking system, which stipulates the supply/transportation of samples to the UNICAMP, preservation and shared use of the fungal strains including drug-resistant ones, by holding meeting with external cooperative agencies of the Project such as universities, hospitals and testing facilities.

3-1-2. To provide Brazilian researchers with the Training in Japan in the theme of the operational management of fungal strains preservation facility at the MMRC.

3-1-3. To develop a facility for the preservation of fungal strains in the UNICAMP.

3-1-4. To construct an information database of clinically-isolated fungal strains, comprised of fungal and patient' characteristics in the UNICAMP.

3-2. To hold regular conferences and/or seminars on drug-resistant fungi including the reporting of project achievements, geared to project external collaborating agencies, health and medical facilities in the project areas and the administration organizations in charge of the control of fungal infections including drug-resistant ones.

3-3. To hold regular meetings with the administration organizations in charge of the control of fungal infection including drug-resistant ones for sharing of the epidemiological information such as incidence and clinical entity, gained through the research activities of the Project.

3-4. To set up a network of clinical laboratory testing and diagnosis of mycoses including drug-resistant ones among the UNICAMP (as the reference laboratory) and health and testing facilities in the State of São Paulo with the support of the Institute of Adolfo Lutz (IAL).

3-5. To set up a network of clinical laboratory testing and diagnosis of mycoses including drug-resistant ones among research institutes, health facilities and the MMRC as the reference laboratory also in Japan, by referencing the achievements of project collaborative research and technical cooperation in Brazil.

3-6. To hold national and international conferences on the research of drug-resistant mycoses geared to research, health and administrative agencies in other provinces and nations, in light of future establishment of a network of collaborative research on mycoses including drug-resistant ones as well as wide-sharing of research findings and outcomes of the Project.

**Project Monitoring Sheet I (Revision of Project Design Matrix)**

**Project Title: The Project for the Establishment of a Research and Reference Collaborative System for the Diagnoses of Fungal Infections including Drug-Resistant Ones both in Brazil and Japan Version 5**

**Implementing Agency: University of Campinas, Sao Paulo (UNICAMP)**

**Dated 30 January, 2020**

**Target Group: Residents of the area covered by Campinas University hospital and affiliated hospitals (Approximately 3 million people)**

**Period of Project: From September 2017 to September 2022**

**Project Site:**

**Model Site:**

Narrative Summary	Objectively Verifiable Indicators	Means of Verification	Important Assumption	Achievement	Remarks
Overall Goal					
Project Purpose  A research and reference collaborative system for the diagnoses of fungal infections including drug-resistant ones is established both in Brazil and Japan.	(1) By the time of the termination of the Project, guidelines/booklets for the management of mycoses are subjected to trial-based application in hospitals in the Campinas metropolitan area. (2) By the time of the termination of the Project, concrete discussions are commenced with Brazilian administrative organizations for the application of research findings and outcomes gained by the Project to policymaking and/or countermeasures for the control of fungal infections including drug-resistant ones. (3) By the time of the termination of the Project, at least 5 research articles in the themes of project research topics such as epidemiological analyses of drug-resistant fungal infections, mechanisms of resistances of fungi to antifungals, and drug resistance-related genes detection methods are published.	<ul style="list-style-type: none"> <li>• Reports developed by the Project</li> <li>• Meeting minutes with relevant organizations</li> <li>• Research articles published in international journals</li> </ul>		Achievement of these indicators will be verified at the end of this Project.	
Outputs 1. Prevalence of drug-resistant fungi and its mechanisms of drug-resistance are epidemiologically elucidated.	(1) By December, 2019, the proportion of drug-resistant fungal strains in the fungal isolates (the prevalence) in the target areas is estimated.  (2) By March, 2020, principal mechanisms of drug resistance against antifungal agents are identified using clinical isolates gained in the Campinas metropolitan area.	<ul style="list-style-type: none"> <li>• Reports (including some articles or abstracts of academic meeting) developed by the Project</li> <li>• A draft of revised guidelines</li> <li>• Reports developed by the Project</li> <li>• Minutes of meetings with the Brazilian authorities concerned</li> </ul>	<p>1. The Project gains the cooperation of the Brazilian health administration organizations such as the MOH, the São Paulo State Health Office and the Campinas City Health Office in charge of the control of fungal infections including drug-resistant ones for implementing meetings with the Project regarding policy advocacy etc.</p> <p>2. Political superiority of the control of fungal infections including the ones caused by drug-resistant strains is not diminished so as to impair the achievement of the Project Purpose.</p>	<p>The prevalence of antifungal-resistant fungal strains among clinical isolates has been estimated.</p> <p>Determination of species and antifungal susceptibilities of fungal clinical strains isolated in the attending hospitals (UNICAMP, Mario Gatti Hospital, Boldrini Children Hospital and Ouro Verde Hospital) and environmental strains collected by UNICAMP and Instituto Adolfo Luz are ongoing (over 1,000 clinical strains and 500 environmental strains).</p> <p>Detailed species (cryptic species) identifications of fungal strains isolated from clinical and environmental specimens using several methods such as DNA sequencing, multiplex PCR and LAMP are in progress.</p> <p>A paper regarding antifungal-resistant <i>A. fumigatus</i> clinical strains has been accepted for publication.</p> <p>To elucidate the effect of fungicides on</p>	



	<p>(3) By March, 2022, the revising work of the existing guidelines, and drafting work of the guidelines/booklets for the management of mycoses are commenced.</p>	<ul style="list-style-type: none"> <li>• Reports developed by the Project</li> <li>• Documents of information sharing regarding epidemiological data with the Brazilian authorities concerned</li> </ul>		<p>mutagenicity of fungi, studies on exposure of <i>A. fumigatus</i> strains to a fungicide in a simulated field are on going.</p> <p>Training on next-generation sequencer was performed from July to September, 2019 in MMRC.</p> <p>Training on antifungal susceptibility test according to CLSI methods was performed on November, 2019 in MMRC.</p> <p>Using a next generation sequencer, gene expression analysis (transcriptome) of antifungal-susceptible/resistant strains are ongoing.</p> <p>Using a next generation sequencer, metagenomic analysis of clinical specimens are ongoing.</p>	
<p>2. Gene amplification technologies-based drug-resistance genes detection methods with sufficient sensitivity, specificity and operability are established for the diagnoses of drug-resistant mycoses.</p>	<p>(1) By December, 2020, basic testing condition and procedures of gene amplification (LAMP, PCR, etc.)-based gene detection methods for the diagnoses of drug-resistant <i>Aspergillus</i>, <i>Candida</i> and other fungi are determined.</p> <p>(2) By December, 2021, evaluation works of clinical performance of the above-mentioned methods are completed.</p> <p>(3) By the time of the termination of the Project, concrete discussions with the Brazilian authorities concerned regarding practical procedures for the application of the above-mentioned methods for clinical laboratory testing (registration/accreditation of the methods as the "Standard Methods" in Brazil) are commenced.</p>			<p>A paper regarding a simple method for detecting gene mutations of <i>A. fumigatus</i> using Surveyor Nuclease assay has been accepted for publication.</p> <p>Introduction of new detection method for antifungal resistant fungi using Surveyor Nuclease has been started.</p> <p>Development of a detection procedure for point mutations in <i>cyp51A</i> gene using PCR or LAMP method is in progress.</p> <p>Developments of identification procedures of cryptic species using PCR or LAMP method are in progress.</p> <p>Training on gene amplification methods including LAMP was performed from October to November, 2019 in MMRC.</p> <p>The CRISPR/Cas9 system are used to elucidate the effect of some mutated genes on antifungal resistance.</p>	
<p>3. A UNICAMP-centered research collaboration system among research, medical and administrative organizations is established.</p>	<p>(1) By the time of the termination of the Project, practical operation of the banking system of fungal strains including drug-resistant ones in the UNICAMP, which is interoperable by the external cooperative agencies for mycoses research, is commenced.</p> <p>(2) By December 2020, epidemiological information on mycoses including drug-resistant ones, gained through the research activities of the Project, is shared with the Brazilian authorities concerned at a one-year interval.</p> <p>(3) By the time of the termination of the Project, practical operation of the network of clinical laboratory testing and diagnosis of mycoses including drug-resistant ones, of which reference laboratory is the UNICAMP, is commenced.</p>			<p>The database program (RedCap) has been operated and over 160 clinical cases of patients with various fungal infection admitted to the attending health care institutions have already enrolled.</p> <p>As the research network activity participated by several medical institutions and a research laboratory in Sao Paulo State, collecting clinical and environmental fungal strains, the regular conference, and scientific meetings are run.</p> <p>Training on the preservation method of fungal strains was performed from October to November, 2019 in MMRC.</p> <p>Preparation of the freeze-dried ampouls for preservation of fungal strains in UNICAMP are on going.</p> <p>Training on epidemiological and multi-center collaborative studies to Brazilian researchers in UNICAMP and other medical institutions was performed from November to December, 2019 by several Japanese researchers in Tokyo and Chiba.</p>	

Activities	Inputs		Pre-Conditions
	The Japanese Side	The Brazilian Side	
<p><b>1. Prevalence of drug-resistant fungi and its mechanisms of drug-resistance are epidemiologically elucidated.</b></p> <p>1-1. To perform classification and identification of fungal strains by morphological and biochemical properties with clinically-isolated fungal strains collected by UNICAMP-related and other project cooperating medical facilities.</p> <p>1-2. To perform more detailed identification of fungal strains including cryptic species using genetic techniques on the fungal isolates identified in the Activity 1-1.</p> <p>1-3. To examine the susceptibility of the fungal species (especially for <i>Aspergillus</i>, <i>Candida</i>, <i>Cryptococcus</i>, and <i>Fusarium</i>) including some cryptic species in accordance with internationally-approved methods (e.g. the methods provided by the Clinical and Laboratory Standards Institute (CLSI)).</p> <p>1-4. To analyze the relationship between fungal characteristics (strains, species and drug susceptibility) and patients' characteristics (source of infection, infected sites, underlying and/or complicated diseases, clinical entity, history of chemotherapy by antifungals, etc.).</p> <p>1-5. To elucidate mechanism of drug resistance by analyzing gene regions, which are reported to be responsible for drug resistances using the fungal isolates with drug-resistant property, confirmed in the Activity 1-3.</p> <p>1-6. To analyse geographical characteristics of drug resistance genes by comparing identified drug-resistant mechanisms against antifungals.</p> <p>1-7. In parallel with the Activity 1-5, to elucidate mechanisms of drug resistance using advanced genetic analysis technologies such as next-generation sequencing techniques for the fungal isolates, which are suspected to have unidentified drug resistance mechanisms.</p> <p>1-7-1. To provide Brazilian researchers with the Training in Japan in the theme of genetic analysis techniques using the next-generation sequencer for elucidating drug-resistant mechanisms at the MMRC.</p>	<p>Experts</p> <p>(1) Chief Advisor (Short-term Expert/double as an expert for Diagnostics-related studies)</p> <p>(2) Project Coordinator/Specialist for Research Institutes Cooperation (Long-term Expert)</p> <p>(3) Diagnostics-related studies (Short-term Experts)</p> <p>(4) Molecular epidemiological studies (Short-term Experts)</p> <p>(5) Gene amplification-related studies (Short-term Experts)</p> <p>(6) Bioinformatics (Short-term Experts)</p> <p>(7) Epidemiological investigation and optimal administration-related studies (Short-term Experts)</p> <p>(8) Management of fungal strains-related study (Short-term Experts)</p> <p>(9) Other persons with necessary expertise for the project activities (Short-term Experts)</p> <p>Training in Japan</p> <p>(1) Training for gene analyses</p> <p>(2) Training for operating Next Generation Sequencer</p> <p>(3) Training for genome comparison and gene disruption</p> <p>(4) Training for management of collected fungi</p> <p>(5) Other necessary training</p> <p>Equipment and materials</p> <p>Necessary equipment and materials for research activities in the Project</p> <p>Local cost</p>	<p>Counterparts</p> <p>(1) Project Director</p> <p>(2) Project Manager</p> <p>(3) Project Co-managers (3 persons)</p> <p>(4) Researchers (Infectious Diseases Division of the Internal Medicine Department and the Clinical Pathology Department, the Faculty of Medical Sciences, the UNICAMP)</p> <p>Facilities, equipment and materials</p> <p>(1) Office space or facilities for JICA experts in the UNICAMP</p> <p>(2) Research space and facilities in the UNICAMP</p> <p>(3) Existing research instruments and equipment for research activities, etc.</p> <p>Local Costs</p> <p>- Running expenses for research activities, etc.;</p> <p>- Assignment of counterpart personnel;</p> <p>- Utility costs (electricity, water, etc.);</p> <p>- Personnel costs (to the work of counterpart) ; and</p> <p>- Operational costs.</p>	<p>1. Approvals are obtained by the ethical committees of each project implementing agency for the medical and/or epidemiological research, which are supposed to be performed in the Project (if necessary).</p> <p style="text-align: center;">■</p> <p style="text-align: center; background-color: yellow;">&lt;Issues and countermeasures&gt;</p>

1-7-2. To identify gene regions, which are suspected to be responsible for drug resistance, by whole-genomic comparison of drug-susceptible and drug resistant fungal strains isolated in Brazil, followed by the functional analyses of the region in the UNICAMP.

1-8. To compile research findings and outputs into scientific articles, together with that gained in the Output 2 and in Japan, to be used as evidences for revising the existing guidelines and for newly-developing it for the management of mycoses, followed by the trial-based application to the hospitals in Campinas metropolitan areas and/or other areas in Brazil.

**2. Gene amplification technologies-based drug-resistance genes detection methods with sufficient sensitivity, specificity and operability are established for the diagnoses of drug-resistant mycoses.**

2-1. To develop gene amplification (LAMP, PCR, etc.)-based gene detection methods for the diagnoses of drug-resistant mycoses.

2-1-1. To develop basic testing condition of detection methods for drug-resistant fungi particularly for *Aspergillus* including its cryptic strains, *Candida* and other fungi, which are suitable for applied gene amplification techniques.

2-1-2. To provide Brazilian researchers with the Training in Japan in the theme of gene amplification-based gene detection methods for the diagnoses of drug-resistant mycoses.

2-1-3. To introduce the detection methods for drug-resistant fungi, developed in the Activity 2-1-2, to the UNICAMP by the joint efforts of Japanese researchers (JICA experts) and the Brazilian researchers who participated the Training in Japan.

2-2. To evaluate the clinical performance of the detection methods for drug-resistant fungi statistically by analyzing its sensitivity and specificity using fungal strains clinically-isolated from patients with mycoses in Brazil.

2-3. To evaluate the operability of the drug-resistant gene detection method(s), which are developed on the basis of LAMP techniques, by introducing them into UNICAMP-related hospitals and other selected external cooperative health or testing facilities on a trial basis.

2-4. To initiate discussions with Brazilian authorities

2-4. To initiate discussions with Brazilian authorities concerned on the practical application of the drug-resistant gene detection methods, developed by the Project, to clinical laboratory testing (registration/accreditation of the methods as the "Standard Methods" in Brazil).

**3. A UNICAMP-centered research collaboration system among research, medical and administrative organizations is established.**

3-1. To construct a banking system of fungal strains including drug-resistant ones in the UNICAMP, which is interoperable by the external cooperative agencies for mycoses research.

3-1-1. To develop bylaws for the operation of the sample banking system, which stipulates the supply/transportation of samples to the UNICAMP, preservation and shared use of the fungal strains including drug-resistant ones, by holding meeting with external cooperative agencies of the Project such as universities, hospitals and testing facilities.

3-1-2. To provide Brazilian researchers with the Training in Japan in the theme of the operational management of fungal strains preservation facility at the MMRC.

3-1-3. To develop a facility for the preservation of fungal strains in the UNICAMP.

3-1-4. To construct an information database of clinically-isolated fungal strains, comprised of fungal and patient' characteristics in the UNICAMP.

3-2. To hold regular conferences and/or seminars on drug-resistant fungi including the reporting of project achievements, geared to project external collaborating agencies, health and medical facilities in the project areas and the administration organizations in charge of the control of fungal infections including drug-resistant ones.

3-3. To hold regular meetings with the administration organizations in charge of the control of fungal infection including drug-resistant ones for sharing of the epidemiological information such as incidence and clinical entity, gained through the research activities of the Project.

<p>3-4. To set up a network of clinical laboratory testing and diagnosis of mycoses including drug-resistant ones among the UNICAMP (as the reference laboratory) and health and testing facilities in the State of São Paulo with the support of the Institute of Adolfo Lutz (IAL).</p> <p>3-5. To set up a network of clinical laboratory testing and diagnosis of mycoses including drug-resistant ones among research institutes, health facilities and the MMRC as the reference laboratory also in Japan, by referencing the achievements of project collaborative research and technical cooperation in Brazil.</p> <p>3-6. To hold national and international conferences on the research of drug-resistant mycoses geared to research, health and administrative agencies in other provinces and nations, in light of future establishment of a network of collaborative research on mycoses including drug-resistant ones as well as wide-sharing of research findings and outcomes of the Project.</p>			
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**Project Monitoring Sheet I (Revision of Project Design Matrix)**

**Project Title: The Project for the Establishment of a Research and Reference Collaborative System for the Diagnoses of Fungal Infections including Drug-Resistant Ones both in Brazil and Japan Version 6**

**Implementing Agency: University of Campinas, Sao Paulo (UNICAMP)**

**Dated 30 September, 2020**

**Target Group: Residents of the area covered by Campinas University hospital and affiliated hospitals (Approximately 3 million people)**

**Period of Project: From September 2017 to September 2022**

**Project Site:**


**Model Site:**

Narrative Summary	Objectively Verifiable Indicators	Means of Verification	Important Assumption	Achievement	Remarks
Overall Goal					
Project Purpose  A research and reference collaborative system for the diagnoses of fungal infections including drug-resistant ones is established both in Brazil and Japan.	(1) By the time of the termination of the Project, guidelines/booklets for the management of mycoses are subjected to trial-based application in hospitals in the Campinas metropolitan area. (2) By the time of the termination of the Project, concrete discussions are commenced with Brazilian administrative organizations for the application of research findings and outcomes gained by the Project to policymaking and/or countermeasures for the control of fungal infections including drug-resistant ones. (3) By the time of the termination of the Project, at least 5 research articles in the themes of project research topics such as epidemiological analyses of drug-resistant fungal infections, mechanisms of resistances of fungi to antifungals, and drug resistance-related genes detection methods are published.	<ul style="list-style-type: none"> <li>• Reports developed by the Project</li> <li>• Meeting minutes with relevant organizations</li> <li>• Research articles published in international journals</li> </ul>		Achievement of these indicators will be verified at the end of this Project.	
Outputs 1. Prevalence of drug-resistant fungi and its mechanisms of drug-resistance are epidemiologically elucidated.	(1) By December, 2019, the proportion of drug-resistant fungal strains in the fungal isolates (the prevalence) in the target areas is estimated.	<ul style="list-style-type: none"> <li>• Reports (including some articles or abstracts of academic meeting) developed by the Project</li> <li>• A draft of revised guidelines</li> </ul>	1. The Project gains the cooperation of the Brazilian health administration organizations such as the MOH, the São Paulo State Health Office and the Campinas City Health Office in charge of the control of fungal infections including drug-resistant ones for implementing meetings with the Project regarding policy advocacy etc.  2. Political superiority of the control of fungal infections including the ones caused by drug-resistant strains is not diminished so as to impair the achievement of the Project Purpose.	The prevalence of antifungal-resistant fungal strains among clinical isolates has been estimated. Determination of species and antifungal susceptibilities of fungal clinical strains isolated in the attending hospitals (UNICAMP, Mario Gatti Hospital, Boldrini Children Hospital and Ouro Verde Hospital) and environmental strains collected by UNICAMP and Instituto Adolfo Luz are	

	<p>(2) By March, 2020, principal mechanisms of drug resistance against antifungal agents are identified using clinical isolates gained in the Campinas metropolitan area.</p> <p>(3) By March, 2022, the revising work of the existing guidelines, and drafting work of the guidelines/booklets for the management of mycoses are commenced.</p>	<ul style="list-style-type: none"> <li>• Reports developed by the Project</li> <li>• Minutes of meetings with the Brazilian authorities concerned</li>   <li>• Reports developed by the Project</li> <li>• Documents of information sharing regarding epidemiological data with the Brazilian authorities concerned</li> </ul>		<p>ongoing (over 1,000 clinical strains and 500 environmental strains).                  Detailed species (cryptic species) identifications of fungal strains isolated from clinical and environmental specimens using several methods such as DNA sequencing, multiplex PCR and LAMP are in progress. Discussion on clinical handbook/manual for aspergillosis was started on the Web. To elucidate the effect of fungicides on mutagenicity of fungi, studies on exposure of <i>A. fumigatus</i> strains to a fungicide in a simulated field are on going. Using a next generation sequencer, gene expression analysis (transcriptome) of antifungal-susceptible/resistant strains are ongoing. Using a next generation sequencer, metagenomic analysis of clinical specimens are ongoing.                  A paper regarding genetic basis of antifungal resistance in clinical <i>Candida glabrata</i> was published.                  A paper regarding azole-resistant <i>A. fumigatus</i> isolated from plant bulbs was published.</p>	
<p>2. Gene amplification technologies-based drug-resistance genes detection methods with sufficient sensitivity, specificity and operability are established for the diagnoses of drug-resistant mycoses.</p>	<p>(1) By December, 2020, basic testing condition and procedures of gene amplification (LAMP, PCR, etc.)-based gene detection methods for the diagnoses of drug-resistant <i>Aspergillus</i>, <i>Candida</i> and other fungi are determined.                  (2) By December, 2021, evaluation works of clinical performance of the above-mentioned methods are completed.                  (3) By the time of the termination of the Project, concrete discussions with the Brazilian authorities concerned regarding practical procedures for the application of the above-mentioned methods for clinical laboratory testing (registration/accreditation of the methods as the "Standard Methods" in Brazil) are commenced.</p>			<p>Introduction of new detection method for antifungal resistant fungi using Surveyor Nuclease has been started.                  Development of a detection procedure for point mutations in <i>cyp51A</i> gene using PCR or LAMP method is on going in Brazil and Japan.                  Developments of identification procedures of cryptic species using PCR or LAMP method are in progress.                  The CRISPR/Cas9 system are used to elucidate the effect of some mutated genes on antifungal resistance.</p>	

<p>3. A UNICAMP-centered research collaboration system among research, medical and administrative organizations is established.</p>	<p>(1) By the time of the termination of the Project, practical operation of the banking system of fungal strains including drug-resistant ones in the UNICAMP, which is interoperable by the external cooperative agencies for mycoses research, is commenced.                  (2) By December 2020, epidemiological information on mycoses including drug-resistant ones, gained through the research activities of the Project, is shared with the Brazilian authorities concerned at a one-year interval.                  (3) By the time of the termination of the Project, practical operation of the network of clinical laboratory testing and diagnosis of mycoses including drug-resistant ones, of which reference laboratory is the UNICAMP, is commenced.</p>			<p>The database program (RedCap) has been operated and over 200 clinical cases of patients with various fungal infection admitted to the attending health care institutions have already enrolled.                  As the research network activity participated by several medical institutions and a research laboratory in Sao Paulo State, collecting clinical and environmental fungal strains, the regular conference, and scientific meetings are run.                  One medical institution has newly joined to the research network.                  Preparation of the freeze-dried ampouls for preservation of fungal strains in UNCAMP are on going.                  A paper regarding a potential cause of invasive fusariosis in hospitals or residences was published.</p>	
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Activities	Inputs		Pre-Conditions
	The Japanese Side	The Brazilian Side	
<p><b>1. Prevalence of drug-resistant fungi and its mechanisms of drug-resistance are epidemiologically elucidated.</b></p> <p>1-1. To perform classification and identification of fungal strains by morphological and biochemical properties with clinically-isolated fungal strains collected by UNICAMP-related and other project cooperating medical facilities.</p> <p>1-2. To perform more detailed identification of fungal strains including cryptic species using genetic techniques on the fungal isolates identified in the Activity 1-1.</p> <p>1-3. To examine the susceptibility of the fungal species (especially for <i>Aspergillus</i>, <i>Candida</i>, <i>Cryptococcus</i>, and <i>Fusarium</i>) including some cryptic species in accordance with internationally-approved methods (e.g. the methods provided by the Clinical and Laboratory Standards Institute (CLSI)).</p> <p>1-4. To analyze the relationship between fungal characteristics (strains, species and drug susceptibility) and patients' characteristics (source of infection, infected sites, underlying and/or complicated diseases, clinical entity, history of chemotherapy by antifungals, etc.).</p> <p>1-5. To elucidate mechanism of drug resistance by analyzing gene regions, which are reported to be responsible for drug resistances using the fungal isolates with drug-resistant property, confirmed in the Activity 1-3.</p> <p>1-6. To analyse geographical characteristics of drug resistance genes by comparing identified drug-resistant mechanisms against antifungals.</p> <p>1-7. In parallel with the Activity 1-5, to elucidate mechanisms of drug resistance using advanced genetic analysis technologies such as next-generation sequencing techniques for the fungal isolates, which are suspected to have unidentified drug resistance mechanisms.</p> <p>1-7-1. To provide Brazilian researchers with the Training in Japan in the theme of genetic analysis techniques using the next-generation sequencer for elucidating drug-resistant mechanisms at the MMRC.</p>	<p>Experts</p> <p>(1) Chief Advisor (Short-term Expert/double as an expert for Diagnostics-related studies)</p> <p>(2) Project Coordinator/Specialist for Research Institutes Cooperation (Long-term Expert)</p> <p>(3) Diagnostics-related studies (Short-term Experts)</p> <p>(4) Molecular epidemiological studies (Short-term Experts)</p> <p>(5) Gene amplification-related studies (Short-term Experts)</p> <p>(6) Bioinformatics (Short-term Experts)</p> <p>(7) Epidemiological investigation and optimal administration-related studies (Short-term Experts)</p> <p>(8) Management of fungal strains-related study (Short-term Experts)</p> <p>(9) Other persons with necessary expertise for the project activities (Short-term Experts)</p> <p>Training in Japan</p> <p>(1) Training for gene analyses</p> <p>(2) Training for operating Next Generation Sequencer</p> <p>(3) Training for genome comparison and gene disruption</p> <p>(4) Training for management of collected fungi</p> <p>(5) Other necessary training</p> <p>Equipment and materials</p> <p>Necessary equipment and materials for research activities in the Project</p> <p>Local cost</p>	<p>Counterparts</p> <p>(1) Project Director</p> <p>(2) Project Manager</p> <p>(3) Project Co-managers (3 persons)</p> <p>(4) Researchers (Infectious Diseases Division of the Internal Medicine Department and the Clinical Pathology Department, the Faculty of Medical Sciences, the UNICAMP)</p> <p>Facilities, equipment and materials</p> <p>(1) Office space or facilities for JICA experts in the UNICAMP</p> <p>(2) Research space and facilities in the UNICAMP</p> <p>(3) Existing research instruments and equipment for research activities, etc.</p> <p>Local Costs</p> <p>- Running expenses for research activities, etc.;</p> <p>- Assignment of counterpart personnel;</p> <p>- Utility costs (electricity, water, etc.);</p> <p>- Personnel costs (to the work of counterpart) ; and</p> <p>- Operational costs.</p>	<p>1. Approvals are obtained by the ethical committees of each project implementing agency for the medical and/or epidemiological research, which are supposed to be performed in the Project (if necessary).</p> <p style="text-align: center;"></p> <p style="text-align: center; background-color: yellow;"><b>&lt;Issues and countermeasures&gt;</b></p> <p>(Issues)</p> <p>Due to the pandemic of COVID-19 worldwide, Japanese researchers' visiting to Brazil and receiving Brazilian trainees in Japan were deferred.</p> <p>(Countermeasures)</p> <p>Research meetings between the chief advisor/Japanese experts and Brazilian researchers were performed periodically using a video-conferencing system.</p>

1-7-2. To identify gene regions, which are suspected to be responsible for drug resistance, by whole-genomic comparison of drug-susceptible and drug resistant fungal strains isolated in Brazil, followed by the functional analyses of the region in the UNICAMP.

1-8. To compile research findings and outputs into scientific articles, together with that gained in the Output 2 and in Japan, to be used as evidences for revising the existing guidelines and for newly-developing it for the management of mycoses, followed by the trial-based application to the hospitals in Campinas metropolitan areas and/or other areas in Brazil.

**2. Gene amplification technologies-based drug-resistance genes detection methods with sufficient sensitivity, specificity and operability are established for the diagnoses of drug-resistant mycoses.**

2-1. To develop gene amplification (LAMP, PCR, etc.)-based gene detection methods for the diagnoses of drug-resistant mycoses.

2-1-1. To develop basic testing condition of detection methods for drug-resistant fungi particularly for *Aspergillus* including its cryptic strains, *Candida* and other fungi, which are suitable for applied gene amplification techniques.

2-1-2. To provide Brazilian researchers with the Training in Japan in the theme of gene amplification-based gene detection methods for the diagnoses of drug-resistant mycoses.

2-1-3. To introduce the detection methods for drug-resistant fungi, developed in the Activity 2-1-2, to the UNICAMP by the joint efforts of Japanese researchers (JICA experts) and the Brazilian researchers who participated the Training in Japan.

2-2. To evaluate the clinical performance of the detection methods for drug-resistant fungi statistically by analyzing its sensitivity and specificity using fungal strains clinically-isolated from patients with mycoses in Brazil.

2-3. To evaluate the operability of the drug-resistant gene detection method(s), which are developed on the basis of LAMP techniques, by introducing them into UNICAMP-related hospitals and other selected external cooperative health or testing facilities on a trial basis.

2-4. To initiate discussions with Brazilian authorities

2-4. To initiate discussions with Brazilian authorities concerned on the practical application of the drug-resistant gene detection methods, developed by the Project, to clinical laboratory testing (registration/accreditation of the methods as the "Standard Methods" in Brazil).

**3. A UNICAMP-centered research collaboration system among research, medical and administrative organizations is established.**

3-1. To construct a banking system of fungal strains including drug-resistant ones in the UNICAMP, which is interoperable by the external cooperative agencies for mycoses research.

3-1-1. To develop bylaws for the operation of the sample banking system, which stipulates the supply/transportation of samples to the UNICAMP, preservation and shared use of the fungal strains including drug-resistant ones, by holding meeting with external cooperative agencies of the Project such as universities, hospitals and testing facilities.

3-1-2. To provide Brazilian researchers with the Training in Japan in the theme of the operational management of fungal strains preservation facility at the MMRC.

3-1-3. To develop a facility for the preservation of fungal strains in the UNICAMP.

3-1-4. To construct an information database of clinically-isolated fungal strains, comprised of fungal and patient' characteristics in the UNICAMP.

3-2. To hold regular conferences and/or seminars on drug-resistant fungi including the reporting of project achievements, geared to project external collaborating agencies, health and medical facilities in the project areas and the administration organizations in charge of the control of fungal infections including drug-resistant ones.

3-3. To hold regular meetings with the administration organizations in charge of the control of fungal infection including drug-resistant ones for sharing of the epidemiological information such as incidence and clinical entity, gained through the research activities of the Project.

3-4. To set up a network of clinical laboratory testing and diagnosis of mycoses including drug-resistant ones among the UNICAMP (as the reference laboratory) and health and testing facilities in the State of São Paulo with the support of the Institute of Adolfo Lutz (IAL).

3-5. To set up a network of clinical laboratory testing and diagnosis of mycoses including drug-resistant ones among research institutes, health facilities and the MMRC as the reference laboratory also in Japan, by referencing the achievements of project collaborative research and technical cooperation in Brazil.

3-6. To hold national and international conferences on the research of drug-resistant mycoses geared to research, health and administrative agencies in other provinces and nations, in light of future establishment of a network of collaborative research on mycoses including drug-resistant ones as well as wide-sharing of research findings and outcomes of the Project.

### Project Monitoring Sheet I (Revision of Project Design Matrix)

**Project Title:** The Project for the Establishment of a Research and Reference Collaborative System for the Diagnoses of Fungal Infections including Drug-Resistant Ones both in Brazil and Japan **Version 7**

**Implementing Agency:** University of Campinas, Sao Paulo (UNICAMP)

**Dated 31 March, 2021**

**Target Group:** Residents of the area covered by Campinas University hospital and affiliated hospitals (Approximately 3 million people)

**Period of Project:** From September 2017 to September 2022

**Project Site:**

**Model Site:**

Narrative Summary	Objectively Verifiable Indicators	Means of Verification	Important Assumption	Achievement	Remarks
Overall Goal					
Project Purpose  A research and reference collaborative system for the diagnoses of fungal infections including drug-resistant ones is established both in Brazil and Japan.	(1) by the time of the termination of the Project, guidelines/booklets for the management of mycoses are subjected to trial-based application in hospitals in the Campinas metropolitan area. (2) By the time of the termination of the Project, concrete discussions are commenced with Brazilian administrative organizations for the application of research findings and outcomes gained by the Project to policymaking and/or countermeasures for the control of fungal infections including drug-resistant ones. (3) By the time of the termination of the Project, at least 5 research articles in the themes of project research topics such as epidemiological analyses of drug-resistant fungal infections, mechanisms of resistances of fungi to antifungals, and drug resistance-related genes detection methods are published.	<ul style="list-style-type: none"> <li>• Reports developed by the Project</li> <li>• Meeting minutes with relevant organizations</li> <li>• Research articles published in international journals</li> </ul>		Achievement of these indicators will be verified at the end of this Project.	
Outputs 1. Prevalence of drug-resistant fungi and its mechanisms of drug-resistance are epidemiologically elucidated.	(1) By December, 2019, the proportion of drug-resistant fungal strains in the fungal isolates (the prevalence) in the target areas is estimated.	<ul style="list-style-type: none"> <li>• Reports (including some articles or abstracts of academic meeting) developed by the Project</li> <li>• A draft of revised guidelines</li> </ul>	<p>1. The Project gains the cooperation of the Brazilian health administration organizations such as the MOH, the São Paulo State Health Office and the Campinas City Health Office in charge of the control of fungal infections including drug-resistant ones for implementing meetings with the Project regarding policy advocacy etc.</p> <p>2. Political superiority of the control of fungal infections including the ones caused by drug-resistant strains is not diminished so as to impair the achievement of the Project Purpose.</p>	The prevalence of antifungal-resistant fungal strains among clinical isolates has been estimated. Determination of species and antifungal susceptibilities of fungal clinical strains isolated in the attending hospitals (UNICAMP, Mario Gatti Hospital, Boldrini Children Hospital and Barretos Hospital) and environmental strains collected by UNICAMP and Instituto Adolfo Luz are ongoing (over 1,000 clinical strains and 500	

	<p>(2) By March, 2020, principal mechanisms of drug resistance against antifungal agents are identified using clinical isolates gained in the Campinas metropolitan area.</p> <p>(3) By March, 2022, the revising work of the existing guidelines, and drafting work of the guidelines/booklets for the management of mycoses are commenced.</p>	<ul style="list-style-type: none"> <li>• Reports developed by the Project</li> <li>• Minutes of meetings with the Brazilian authorities concerned</li> </ul> <ul style="list-style-type: none"> <li>• Reports developed by the Project</li> <li>• Documents of information sharing regarding epidemiological data with the Brazilian authorities concerned</li> </ul>
<p>2. Gene amplification technologies-based drug-resistance genes detection methods with sufficient sensitivity, specificity and operability are established for the diagnoses of drug-resistant mycoses.</p>	<p>(1) By December, 2020, basic testing condition and procedures of gene amplification (LAMP, PCR, etc.)-based gene detection methods for the diagnoses of drug-resistant <i>Aspergillus</i>, <i>Candida</i> and other fungi are determined.</p> <p>(2) By December, 2021, evaluation works of clinical performance of the above-mentioned methods are completed.</p> <p>(3) By the time of the termination of the Project, concrete discussions with the Brazilian authorities concerned regarding practical procedures for the application of the above-mentioned methods for clinical laboratory testing (registration/accreditation of the methods as the "Standard Methods" in Brazil) are commenced.</p>	

<p>environmental strains). Detailed species (cryptic species) identifications of fungal strains isolated from clinical and environmental specimens using several methods such as DNA sequencing, multiplex PCR and LAMP are in progress. <b>Discussion on clinical handbook/manual for aspergillosis was continued using a video-conference system.</b> To elucidate the effect of fungicides on mutagenicity of fungi, studies on exposure of <i>A. fumigatus</i> strains to a fungicide in a simulated field are on going. Using a next generation sequencer, gene expression analysis (transcriptome) of antifungal-susceptible/resistant strains are ongoing. Using a next generation sequencer, metagenomic analysis of clinical specimens are ongoing. <b>A paper regarding a rapid identification of FKS genes mutations in <i>Candida glabrata</i> was published.</b> <b>A paper regarding transcription of secondary metabolism-related genes was published.</b></p>	
<p>Introduction of new detection method for antifungal resistant fungi using Surveyor Nuclease has been started. <b>Development of a detection procedure for point mutations in <i>cyp51A</i> gene using PCR or LAMP method is on going in Brazil and Japan.</b> Developments of identification procedures of cryptic species using PCR or LAMP method are in progress. The CRISPR/Cas9 system are used to elucidate the effect of some mutated genes on antifungal resistance.</p>	

<p>3. A UNICAMP-centered research collaboration system among research, medical and administrative organizations is established.</p>	<p>(1) By the time of the termination of the Project, practical operation of the banking system of fungal strains including drug-resistant ones in the UNICAMP, which is interoperable by the external cooperative agencies for mycoses research, is commenced.                  (2) By December 2020, epidemiological information on mycoses including drug-resistant ones, gained through the research activities of the Project, is shared with the Brazilian authorities concerned at a one-year interval.                  (3) By the time of the termination of the Project, practical operation of the network of clinical laboratory testing and diagnosis of mycoses including drug-resistant ones, of which reference laboratory is the UNICAMP, is commenced.</p>			<p>The database program (RedCap) has been operated and over 250 clinical cases of patients with various fungal infection admitted to the attending health care institutions have already enrolled.                  Several medical and research insrtitution not only in Sao Paulo State but also in Mato Grosso do Sul State are presently participating the research network. As the research network activity, collecting clinical and environmental fungal strains, the regular conference, and scientific meetings are run.                  Preparation of the freeze-dried ampouls for preservation of fungal strains in UNICAMP are on going.</p>	
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Activities	Inputs	Pre-Conditions	
<p><b>1. Prevalence of drug-resistant fungi and its mechanisms of drug-resistance are epidemiologically elucidated.</b></p> <p>1-1. To perform classification and identification of fungal strains by morphological and biochemical properties with clinically-isolated fungal strains collected by UNICAMP-related and other project cooperating medical facilities.</p> <p>1-2. To perform more detailed identification of fungal strains including cryptic species using genetic techniques on the fungal isolates identified in the Activity 1-1.</p> <p>1-3. To examine the susceptibility of the fungal species (especially for <i>Aspergillus</i>, <i>Candida</i>, <i>Cryptococcus</i>, and <i>Fusarium</i>) including some cryptic species in accordance with internationally-approved methods (e.g. the methods provided by the Clinical and Laboratory Standards Institute (CLSI)).</p> <p>1-4. To analyze the relationship between fungal characteristics (strains, species and drug susceptibility) and patients' characteristics (source of infection, infected sites, underlying and/or complicated diseases, clinical entity, history of chemotherapy by antifungals, etc.).</p> <p>1-5. To elucidate mechanism of drug resistance by analyzing gene regions, which are reported to be responsible for drug resistances using the fungal isolates with drug-resistant property, confirmed in the Activity 1-3.</p> <p>1-6. To analyse geographical characteristics of drug resistance genes by comparing identified drug-resistant mechanisms against antifungals.</p> <p>1-7. In parallel with the Activity 1-5, to elucidate mechanisms of drug resistance using advanced genetic analysis technologies such as next-generation sequencing techniques for the fungal isolates, which are suspected to have unidentified drug resistance mechanisms.</p> <p>1-7-1. To provide Brazilian researchers with the Training in Japan in the theme of genetic analysis techniques using the next-generation sequencer for elucidating drug-resistant mechanisms at the MMRC.</p>	<p>The Japanese Side</p> <p>Experts            (1) Chief Advisor (Short-term Expert/double as an expert for Diagnostics-related studies)            (2) Project Coordinator/Specialist for Research Institutes Cooperation (Long-term Expert)            (3) Diagnostics-related studies (Short-term Experts)            (4) Molecular epidemiological studies (Short-term Experts)            (5) Gene amplification-related studies (Short-term Experts)            (6) Bioinformatics (Short-term Experts)            (7) Epidemiological investigation and optimal administration-related studies (Short-term Experts)            (8) Management of fungal strains-related study (Short-term Experts)            (9) Other persons with necessary expertise for the project activities (Short-term Experts)</p> <p>Training in Japan            (1) Training for gene analyses            (2) Training for operating Next Generation Sequencer            (3) Training for genome comparison and gene disruption            (4) Training for management of collected fungi            (5) Other necessary training</p> <p>Equipment and materials            Necessary equipment and materials for research activities in the Project</p> <p>Local cost</p>	<p>The Brazilian Side</p> <p>Counterparts            (1) Project Director            (2) Project Manager            (3) Project Co-managers (3 persons)            (4) Researchers (Infectious Diseases Division of the Internal Medicine Department and the Clinical Pathology Department, the Faculty of Medical Sciences, the UNICAMP)</p> <p>Facilities, equipment and materials            (1) Office space or facilities for JICA experts in the UNICAMP            (2) Research space and facilities in the UNICAMP            (3) Existing research instruments and equipment for research activities, etc.</p> <p>Local Costs            - Running expenses for research activities, etc.;            - Assignment of counterpart personnel;            - Utility costs (electricity, water, etc.);            - Personnel costs (to the work of counterpart);            and            - Operational costs.</p>	<p>1. Approvals are obtained by the ethical committees of each project implementing agency for the medical and/or epidemiological research, which are supposed to be performed in the Project (if necessary).</p> <p style="text-align: center;"><b>&lt;Issues and countermeasures&gt;</b></p> <p>(Issues)            Due to the pandemic of COVID-19 worldwide, Japanese researchers' visiting to Brazil and receiving Brazilian trainees in Japan were deferred.</p> <p>(Countermeasures)            Research meetings between the chief advisor/Japanese experts and Brazilian researchers were performed periodically using a video-conferencing system.</p>



1-7-2. To identify gene regions, which are suspected to be responsible for drug resistance, by whole-genomic comparison of drug-susceptible and drug resistant fungal strains isolated in Brazil, followed by the functional analyses of the region in the UNICAMP.

1-8. To compile research findings and outputs into scientific articles, together with that gained in the Output 2 and in Japan, to be used as evidences for revising the existing guidelines and for newly-developing it for the management of mycoses, followed by the trial-based application to the hospitals in Campinas metropolitan areas and/or other areas in Brazil.

**2. Gene amplification technologies-based drug-resistance genes detection methods with sufficient sensitivity, specificity and operability are established for the diagnoses of drug-resistant mycoses.**

2-1. To develop gene amplification (LAMP, PCR, etc.)-based gene detection methods for the diagnoses of drug-resistant mycoses.

2-1-1. To develop basic testing condition of detection methods for drug-resistant fungi particularly for *Aspergillus* including its cryptic strains, *Candida* and other fungi, which are suitable for applied gene amplification techniques.

2-1-2. To provide Brazilian researchers with the Training in Japan in the theme of gene amplification-based gene detection methods for the diagnoses of drug-resistant mycoses.

2-1-3. To introduce the detection methods for drug-resistant fungi, developed in the Activity 2-1-2, to the UNICAMP by the joint efforts of Japanese researchers (JICA experts) and the Brazilian researchers who participated the Training in Japan.

2-2. To evaluate the clinical performance of the detection methods for drug-resistant fungi statistically by analyzing its sensitivity and specificity using fungal strains clinically-isolated from patients with mycoses in Brazil.

2-3. To evaluate the operability of the drug-resistant gene detection method(s), which are developed on the basis of LAMP techniques, by introducing them into UNICAMP-related hospitals and other selected external cooperative health or testing facilities on a trial basis.

2-4. To initiate discussions with Brazilian authorities

2-4. To initiate discussions with Brazilian authorities concerned on the practical application of the drug-resistant gene detection methods, developed by the Project, to clinical laboratory testing (registration/accreditation of the methods as the "Standard Methods" in Brazil).

**3. A UNICAMP-centered research collaboration system among research, medical and administrative organizations is established.**

3-1. To construct a banking system of fungal strains including drug-resistant ones in the UNICAMP, which is interoperable by the external cooperative agencies for mycoses research.

3-1-1. To develop bylaws for the operation of the sample banking system, which stipulates the supply/transportation of samples to the UNICAMP, preservation and shared use of the fungal strains including drug-resistant ones, by holding meeting with external cooperative agencies of the Project such as universities, hospitals and testing facilities.

3-1-2. To provide Brazilian researchers with the Training in Japan in the theme of the operational management of fungal strains preservation facility at the MMRC.

3-1-3. To develop a facility for the preservation of fungal strains in the UNICAMP.

3-1-4. To construct an information database of clinically-isolated fungal strains, comprised of fungal and patient' characteristics in the UNICAMP.

3-2. To hold regular conferences and/or seminars on drug-resistant fungi including the reporting of project achievements, geared to project external collaborating agencies, health and medical facilities in the project areas and the administration organizations in charge of the control of fungal infections including drug-resistant ones.

3-3. To hold regular meetings with the administration organizations in charge of the control of fungal infection including drug-resistant ones for sharing of the epidemiological information such as incidence and clinical entity, gained through the research activities of the Project.

3-4. To set up a network of clinical laboratory testing and diagnosis of mycoses including drug-resistant ones among the UNICAMP (as the reference laboratory) and health and testing facilities in the State of São Paulo with the support of the Institute of Adolfo Lutz (IAL).

3-5. To set up a network of clinical laboratory testing and diagnosis of mycoses including drug-resistant ones among research institutes, health facilities and the MMRC as the reference laboratory also in Japan, by referencing the achievements of project collaborative research and technical cooperation in Brazil.

3-6. To hold national and international conferences on the research of drug-resistant mycoses geared to research, health and administrative agencies in other provinces and nations, in light of future establishment of a network of collaborative research on mycoses including drug-resistant ones as well as wide-sharing of research findings and outcomes of the Project.

### Project Monitoring Sheet I (Revision of Project Design Matrix)

**Project Title:** The Project for the Establishment of a Research and Reference Collaborative System for the Diagnoses of Fungal Infections including Drug-Resistant Ones both in Brazil and Japan **Version 8**

**Implementing Agency:** University of Campinas, Sao Paulo (UNICAMP)

**Dated 30 September, 2021**

**Target Group:** Residents of the area covered by Campinas University hospital and affiliated hospitals (Approximately 3 million people)

**Period of Project:** From September 2017 to September 2022

**Project Site:**

**Model Site:**

Narrative Summary	Objectively Verifiable Indicators	Means of Verification	Important Assumption	Achievement	Remarks
Overall Goal					
Project Purpose	<p>(1) By the time of the termination of the Project, guidelines/booklets for the management of mycoses are subjected to trial-based application in hospitals in the Campinas metropolitan area.</p> <p>(2) By the time of the termination of the Project, concrete discussions are commenced with Brazilian administrative organizations for the application of research findings and outcomes gained by the Project to policymaking and/or countermeasures for the control of fungal infections including drug-resistant ones.</p> <p>(3) By the time of the termination of the Project, at least 5 research articles in the themes of project research topics such as epidemiological analyses of drug-resistant fungal infections, mechanisms of resistances of fungi to antifungals, and drug resistance-related genes detection methods are published.</p>	<ul style="list-style-type: none"> <li>• Reports developed by the Project</li> <li>• Meeting minutes with relevant organizations</li> <li>• Research articles published in international journals</li> </ul>		Achievement of these indicators will be verified at the end of this Project.	
<p><b>Outputs</b></p> <p>1. Prevalence of drug-resistant fungi and its mechanisms of drug-resistance are epidemiologically elucidated.</p>	<p>(1) By December, 2019, the proportion of drug-resistant fungal strains in the fungal isolates (the prevalence) in the target areas is estimated.</p>	<ul style="list-style-type: none"> <li>• Reports (including some articles or abstracts of academic meeting) developed by the Project</li> <li>• A draft of revised guidelines</li> </ul>	<p>1. The Project gains the cooperation of the Brazilian health administration organizations such as the MOH, the São Paulo State Health Office and the Campinas City Health Office in charge of the control of fungal infections including drug-resistant ones for implementing meetings with the Project regarding policy advocacy etc.</p> <p>2. Political superiority of the control of fungal infections including the ones caused by drug-resistant strains is not diminished so as to impair the achievement of the Project Purpose.</p>	<p>The prevalence of antifungal-resistant fungal strains among clinical isolates has been estimated.</p> <p>Determination of species and antifungal susceptibilities of fungal clinical strains isolated in the attending hospitals (UNICAMP, Mario Gatti Hospital, Boldrini Children Hospital and Barretos Hospital) and environmental strains collected by UNICAMP and Instituto Adolfo Luz are</p>	

	<p>(2) By March, 2020, principal mechanisms of drug resistance against antifungal agents are identified using clinical isolates gained in the Campinas metropolitan area.</p> <p>(3) By March, 2022, the revising work of the existing guidelines, and drafting work of the guidelines/booklets for the management of mycoses are commenced.</p>	<ul style="list-style-type: none"> <li>• Reports developed by the Project</li> <li>• Minutes of meetings with the Brazilian authorities concerned</li> </ul> <ul style="list-style-type: none"> <li>• Reports developed by the Project</li> <li>• Documents of information sharing regarding epidemiological data with the Brazilian authorities concerned</li> </ul>	<p>ongoing (over 1,000 clinical strains and 500 environmental strains).  <b>Environmental fungal strains collected in Mato Grosso do Sul State are analyzing.</b>                  Detailed species (cryptic species) identifications of fungal strains isolated from clinical and environmental specimens using several methods such as DNA sequencing, multiplex PCR and LAMP are in progress. Discussion on clinical handbook/manual for aspergillosis was continued using a video-conference system.                  To elucidate the effect of fungicides on mutagenicity of fungi, studies on exposure of <i>A. fumigatus</i> strains to a fungicide in a simulated field are on going.                  Using a next generation sequencer, gene expression analysis (transcriptome) of antifungal-susceptible/resistant strains are ongoing.                  Using a next generation sequencer, metagenomic analysis of clinical specimens are ongoing.  <b>A paper regarding novel mutations caused by the exposure to a fungicide (tebuconazole) was published.</b></p>	
<p>2. Gene amplification technologies-based drug-resistance genes detection methods with sufficient sensitivity, specificity and operability are established for the diagnoses of drug-resistant mycoses.</p>	<p>(1) By December, 2020, basic testing condition and procedures of gene amplification (LAMP, PCR, etc.)-based gene detection methods for the diagnoses of drug-resistant <i>Aspergillus</i>, <i>Candida</i> and other fungi are determined.                  (2) By December, 2021, evaluation works of clinical performance of the above-mentioned methods are completed.                  (3) By the time of the termination of the Project, concrete discussions with the Brazilian authorities concerned regarding practical procedures for the application of the above-mentioned methods for clinical laboratory testing (registration/accreditation of the methods as the "Standard Methods" in Brazil) are commenced.</p>		<p>Introduction of new detection method for antifungal resistant fungi using Surveyor Nuclease has been started.                  Development of a detection procedure for point mutations in <i>cyp51A</i> gene using PCR or LAMP method is on going in Brazil and Japan.  <b>A paper regarding a detection test for point mutation in <i>cyp51A</i> gene using LAMP method has been published.</b>                  Developments of identification procedures of cryptic species using PCR or LAMP method are in progress.                  The CRISPR/Cas9 system are used to elucidate the effect of some mutated genes on antifungal resistance.</p>	

<p>3. A UNICAMP-centered research collaboration system among research, medical and administrative organizations is established.</p>	<p>(1) By the time of the termination of the Project, practical operation of the banking system of fungal strains including drug-resistant ones in the UNICAMP, which is interoperable by the external cooperative agencies for mycoses research, is commenced.</p> <p>(2) By December 2020, epidemiological information on mycoses including drug-resistant ones, gained through the research activities of the Project, is shared with the Brazilian authorities concerned at a one-year interval.</p> <p>(3) By the time of the termination of the Project, practical operation of the network of clinical laboratory testing and diagnosis of mycoses including drug-resistant ones, of which reference laboratory is the UNICAMP, is commenced.</p>			<p>The database program (RedCap) has been operated and over 300 clinical cases of patients with various fungal infection admitted to the attending health care institutions have already enrolled. Several medical and research institution not only in Sao Paulo State but also in Mato Grosso do Sul State are presently participating the research network. As the research network activity, collecting clinical and environmental fungal strains, the regular conference, and scientific meetings are run. Preparation of the freeze-dried ampouls for preservation of fungal strains in UNICAMP are on going.</p> <p>A paper regarding a case series of the COVID-19 associated fungal infections at UNICAMP was published.</p>	
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Activities	Inputs	Pre-Conditions	
<p><b>1. Prevalence of drug-resistant fungi and its mechanisms of drug-resistance are epidemiologically elucidated.</b></p> <p>1-1. To perform classification and identification of fungal strains by morphological and biochemical properties with clinically-isolated fungal strains collected by UNICAMP-related and other project cooperating medical facilities.</p> <p>1-2. To perform more detailed identification of fungal strains including cryptic species using genetic techniques on the fungal isolates identified in the Activity 1-1.</p> <p>1-3. To examine the susceptibility of the fungal species (especially for <i>Aspergillus</i>, <i>Candida</i>, <i>Cryptococcus</i>, and <i>Fusarium</i>) including some cryptic species in accordance with internationally-approved methods (e.g. the methods provided by the Clinical and Laboratory Standards Institute (CLSI)).</p> <p>1-4. To analyze the relationship between fungal characteristics (strains, species and drug susceptibility) and patients' characteristics (source of infection, infected sites, underlying and/or complicated diseases, clinical entity, history of chemotherapy by antifungals, etc.).</p> <p>1-5. To elucidate mechanism of drug resistance by analyzing gene regions, which are reported to be responsible for drug resistances using the fungal isolates with drug-resistant property, confirmed in the Activity 1-3.</p> <p>1-6. To analyse geographical characteristics of drug resistance genes by comparing identified drug-resistant mechanisms against antifungals.</p> <p>1-7. In parallel with the Activity 1-5, to elucidate mechanisms of drug resistance using advanced genetic analysis technologies such as next-generation sequencing techniques for the fungal isolates, which are suspected to have unidentified drug resistance mechanisms.</p> <p>1-7-1. To provide Brazilian researchers with the Training in Japan in the theme of genetic analysis techniques using the next-generation sequencer for elucidating drug-resistant mechanisms at the MMRC.</p>	<p>The Japanese Side</p> <p>Experts            (1) Chief Advisor (Short-term Expert/double as an expert for Diagnostics-related studies)            (2) Project Coordinator/Specialist for Research Institutes Cooperation (Long-term Expert)            (3) Diagnostics-related studies (Short-term Experts)            (4) Molecular epidemiological studies (Short-term Experts)            (5) Gene amplification-related studies (Short-term Experts)            (6) Bioinformatics (Short-term Experts)            (7) Epidemiological investigation and optimal administration-related studies (Short-term Experts)            (8) Management of fungal strains-related study (Short-term Experts)            (9) Other persons with necessary expertise for the project activities (Short-term Experts)</p> <p>Training in Japan            (1) Training for gene analyses            (2) Training for operating Next Generation Sequencer            (3) Training for genome comparison and gene disruption            (4) Training for management of collected fungi            (5) Other necessary training</p> <p>Equipment and materials            Necessary equipment and materials for research activities in the Project</p> <p>Local cost</p>	<p>The Brazilian Side</p> <p>Counterparts            (1) Project Director            (2) Project Manager            (3) Project Co-managers (3 persons)            (4) Researchers (Infectious Diseases Division of the Internal Medicine Department and the Clinical Pathology Department, the Faculty of Medical Sciences, the UNICAMP)</p> <p>Facilities, equipment and materials            (1) Office space or facilities for JICA experts in the UNICAMP            (2) Research space and facilities in the UNICAMP            (3) Existing research instruments and equipment for research activities, etc.</p> <p>Local Costs            - Running expenses for research activities, etc.;            - Assignment of counterpart personnel;            - Utility costs (electricity, water, etc.);            - Personnel costs (to the work of counterpart);            and            - Operational costs.</p>	<p>1. Approvals are obtained by the ethical committees of each project implementing agency for the medical and/or epidemiological research, which are supposed to be performed in the Project (if necessary).</p> <p style="text-align: center;"><b>&lt;Issues and countermeasures&gt;</b></p> <p>(Issues)            Due to the pandemic of COVID-19 worldwide, Japanese researchers' visiting to Brazil and receiving Brazilian trainees in Japan were deferred.</p> <p>(Countermeasures)            Research meetings between the chief advisor/Japanese experts and Brazilian researchers were performed periodically using a video-conferencing system.  <b>The experts perform the preliminary experiments in Japan as a replacement for inputs to Brazil.</b></p>

1-7-2. To identify gene regions, which are suspected to be responsible for drug resistance, by whole-genomic comparison of drug-susceptible and drug resistant fungal strains isolated in Brazil, followed by the functional analyses of the region in the UNICAMP.

1-8. To compile research findings and outputs into scientific articles, together with that gained in the Output 2 and in Japan, to be used as evidences for revising the existing guidelines and for newly-developing it for the management of mycoses, followed by the trial-based application to the hospitals in Campinas metropolitan areas and/or other areas in Brazil.

**2. Gene amplification technologies-based drug-resistance genes detection methods with sufficient sensitivity, specificity and operability are established for the diagnoses of drug-resistant mycoses.**

2-1. To develop gene amplification (LAMP, PCR, etc.)-based gene detection methods for the diagnoses of drug-resistant mycoses.

2-1-1. To develop basic testing condition of detection methods for drug-resistant fungi particularly for *Aspergillus* including its cryptic strains, *Candida* and other fungi, which are suitable for applied gene amplification techniques.

2-1-2. To provide Brazilian researchers with the Training in Japan in the theme of gene amplification-based gene detection methods for the diagnoses of drug-resistant mycoses.

2-1-3. To introduce the detection methods for drug-resistant fungi, developed in the Activity 2-1-2, to the UNICAMP by the joint efforts of Japanese researchers (JICA experts) and the Brazilian researchers who participated the Training in Japan.

2-2. To evaluate the clinical performance of the detection methods for drug-resistant fungi statistically by analyzing its sensitivity and specificity using fungal strains clinically-isolated from patients with mycoses in Brazil.

2-3. To evaluate the operability of the drug-resistant gene detection method(s), which are developed on the basis of LAMP techniques, by introducing them into UNICAMP-related hospitals and other selected external cooperative health or testing facilities on a trial basis.

2-4. To initiate discussions with Brazilian authorities



2-4. To initiate discussions with Brazilian authorities concerned on the practical application of the drug-resistant gene detection methods, developed by the Project, to clinical laboratory testing (registration/accreditation of the methods as the "Standard Methods" in Brazil).

**3. A UNICAMP-centered research collaboration system among research, medical and administrative organizations is established.**

3-1. To construct a banking system of fungal strains including drug-resistant ones in the UNICAMP, which is interoperable by the external cooperative agencies for mycoses research.

3-1-1. To develop bylaws for the operation of the sample banking system, which stipulates the supply/transportation of samples to the UNICAMP, preservation and shared use of the fungal strains including drug-resistant ones, by holding meeting with external cooperative agencies of the Project such as universities, hospitals and testing facilities.

3-1-2. To provide Brazilian researchers with the Training in Japan in the theme of the operational management of fungal strains preservation facility at the MMRC.

3-1-3. To develop a facility for the preservation of fungal strains in the UNICAMP.

3-1-4. To construct an information database of clinically-isolated fungal strains, comprised of fungal and patient' characteristics in the UNICAMP.

3-2. To hold regular conferences and/or seminars on drug-resistant fungi including the reporting of project achievements, geared to project external collaborating agencies, health and medical facilities in the project areas and the administration organizations in charge of the control of fungal infections including drug-resistant ones.

3-3. To hold regular meetings with the administration organizations in charge of the control of fungal infection including drug-resistant ones for sharing of the epidemiological information such as incidence and clinical entity, gained through the research activities of the Project.

3-4. To set up a network of clinical laboratory testing and diagnosis of mycoses including drug-resistant ones among the UNICAMP (as the reference laboratory) and health and testing facilities in the State of São Paulo with the support of the Institute of Adolfo Lutz (IAL).

3-5. To set up a network of clinical laboratory testing and diagnosis of mycoses including drug-resistant ones among research institutes, health facilities and the MMRC as the reference laboratory also in Japan, by referencing the achievements of project collaborative research and technical cooperation in Brazil.

3-6. To hold national and international conferences on the research of drug-resistant mycoses geared to research, health and administrative agencies in other provinces and nations, in light of future establishment of a network of collaborative research on mycoses including drug-resistant ones as well as wide-sharing of research findings and outcomes of the Project.

**Project Monitoring Sheet I (Revision of Project Design Matrix)**

**Project Title: The Project for the Establishment of a Research and Reference Collaborative System for the Diagnoses of Fungal Infections including Drug-Resistant Ones both in Brazil and Japan** **Version 9**

**Implementing Agency: University of Campinas, Sao Paulo (UNICAMP)**

**Dated 31 March, 2022**

**Target Group: Residents of the area covered by Campinas University hospital and affiliated hospitals (Approximately 3 million people)**

**Period of Project: From September 2017 to September 2022**

**Project Site:**

**Model Site:**

Narrative Summary	Objectively Verifiable Indicators	Means of Verification	Important Assumption	Achievement	Remarks
Overall Goal					
Project Purpose	<p>(1) By the time of the termination of the Project, guidelines/booklets for the management of mycoses are subjected to trial-based application in hospitals in the Campinas metropolitan area.</p> <p>(2) By the time of the termination of the Project, concrete discussions are commenced with Brazilian administrative organizations for the application of research findings and outcomes gained by the Project to policymaking and/or countermeasures for the control of fungal infections including drug-resistant ones.</p> <p>(3) By the time of the termination of the Project, at least 5 research articles in the themes of project research topics such as epidemiological analyses of drug-resistant fungal infections, mechanisms of resistances of fungi to antifungals, and drug resistance-related genes detection methods are published.</p>	<ul style="list-style-type: none"> <li>• Reports developed by the Project</li> <li>• Meeting minutes with relevant organizations</li> <li>• Research articles published in international journals</li> </ul>		Achievement of these indicators will be verified at the end of this Project.	
<p><b>Outputs</b></p> <p>1. Prevalence of drug-resistant fungi and its mechanisms of drug-resistance are epidemiologically elucidated.</p>	<p>(1) By December, 2019, the proportion of drug-resistant fungal strains in the fungal isolates (the prevalence) in the target areas is estimated.</p>	<ul style="list-style-type: none"> <li>• Reports (including some articles or abstracts of academic meeting) developed by the Project</li> <li>• A draft of revised guidelines</li> </ul>	<p>1. The Project gains the cooperation of the Brazilian health administration organizations such as the MOH, the São Paulo State Health Office and the Campinas City Health Office in charge of the control of fungal infections including drug-resistant ones for implementing meetings with the Project regarding policy advocacy etc.</p> <p>2. Political superiority of the control of fungal infections including the ones caused by drug-resistant strains is not diminished so as to impair the achievement of the Project Purpose.</p>	<p>The prevalence of antifungal-resistant fungal strains among clinical isolates has been estimated.</p> <p>Determination of species and antifungal susceptibilities of fungal clinical strains isolated in the attending hospitals (UNICAMP, Mario Gatti Hospital, Boldrini Children Hospital and Barretos Hospital) and environmental strains collected by UNICAMP and Instituto Adolfo Luz are ongoing (over 1,000 clinical strains and 500 environmental strains).</p> <p>Several environmental fungal strains</p>	

	<p>(2) By March, 2020, principal mechanisms of drug resistance against antifungal agents are identified using clinical isolates gained in the Campinas metropolitan area.</p> <p>(3) By March, 2022, the revising work of the existing guidelines, and drafting work of the guidelines/booklets for the management of mycoses are commenced.</p>	<ul style="list-style-type: none"> <li>• Reports developed by the Project</li> <li>• Minutes of meetings with the Brazilian authorities concerned</li>   <li>• Reports developed by the Project</li> <li>• Documents of information sharing regarding epidemiological data with the Brazilian authorities concerned</li> </ul>	<p>collected in Mato Grosso do Sul State are confirmed to be antifungal resistant strains. Detailed species (cryptic species) identifications of fungal strains isolated from clinical and environmental specimens using several methods such as DNA sequencing, multiplex PCR and LAMP are in progress. Discussion on clinical handbook/manual for aspergillosis was continued using a video-conference system.</p> <p>To elucidate the effect of fungicides on mutagenicity of fungi, studies on exposure of <i>A. fumigatus</i> strains to a fungicide in a simulated field are on going.</p> <p>Using a next generation sequencer, gene expression analysis (transcriptome) of antifungal-susceptible/resistant strains are ongoing.</p> <p>Using a next generation sequencer, metagenomic analysis of clinical specimens are ongoing.</p> <p>A paper regarding the antifungal resistance of <i>Candida tropicalis</i> was published.</p> <p>A paper regarding genetic differences of <i>Aspergillus fumigatus</i> among several countries was published.</p> <p>A paper regarding detailed mechanism of Hmg1 mutations in <i>A. fumigatus</i> was published.</p>	
<p>2. Gene amplification technologies-based drug-resistance genes detection methods with sufficient sensitivity, specificity and operability are established for the diagnoses of drug-resistant mycoses.</p>	<p>(1) By December, 2020, basic testing condition and procedures of gene amplification (LAMP, PCR, etc.)-based gene detection methods for the diagnoses of drug-resistant <i>Aspergillus</i>, <i>Candida</i> and other fungi are determined.</p> <p>(2) By December, 2021, evaluation works of clinical performance of the above-mentioned methods are completed.</p> <p>(3) By the time of the termination of the Project, concrete discussions with the Brazilian authorities concerned regarding practical procedures for the application of the above-mentioned methods for clinical laboratory testing (registration/accreditation of the methods as the "Standard Methods" in Brazil) are commenced.</p>		<p style="text-align: center;">ca</p> <p>The new detection method for antifungal resistant fungi using Surveyor Nuclease is regularly performed.</p> <p>Development of a detection procedure for point mutations in <i>cyp51A</i> gene using PCR or LAMP method is on going in Brazil and Japan.</p> <p>Developments of identification procedures of cryptic species using PCR or LAMP method are in progress.</p> <p>The CRISPR/Cas9 system are used to elucidate the effect of some mutated genes on antifungal resistance.</p>	

<p>3. A UNICAMP-centered research collaboration system among research, medical and administrative organizations is established.</p>	<p>(1) By the time of the termination of the Project, practical operation of the banking system of fungal strains including drug-resistant ones in the UNICAMP, which is interoperable by the external cooperative agencies for mycoses research, is commenced.                  (2) By December 2020, epidemiological information on mycoses including drug-resistant ones, gained through the research activities of the Project, is shared with the Brazilian authorities concerned at a one-year interval.                  (3) By the time of the termination of the Project, practical operation of the network of clinical laboratory testing and diagnosis of mycoses including drug-resistant ones, of which reference laboratory is the UNICAMP, is commenced.</p>			<p>The database program (RedCap) has been operated and over 350 clinical cases of patients with various fungal infection admitted to the attending health care institutions have already enrolled. Several medical and research institution not only in Sao Paulo State but also in Mato Grosso do Sul State are presently participating the research network. As the research network activity, collecting clinical and environmental fungal strains, the regular conference, and scientific meetings are run. Preparation of the freeze-dried ampouls for preservation of fungal strains in UNICAMP are on going.</p>	
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Activities	Inputs		Pre-Conditions
	The Japanese Side	The Brazilian Side	
<p><b>1. Prevalence of drug-resistant fungi and its mechanisms of drug-resistance are epidemiologically elucidated.</b></p> <p>1-1. To perform classification and identification of fungal strains by morphological and biochemical properties with clinically-isolated fungal strains collected by UNICAMP-related and other project cooperating medical facilities.</p> <p>1-2. To perform more detailed identification of fungal strains including cryptic species using genetic techniques on the fungal isolates identified in the Activity 1-1.</p> <p>1-3. To examine the susceptibility of the fungal species (especially for <i>Aspergillus</i>, <i>Candida</i>, <i>Cryptococcus</i>, and <i>Fusarium</i>) including some cryptic species in accordance with internationally-approved methods (e.g. the methods provided by the Clinical and Laboratory Standards Institute (CLSI)).</p> <p>1-4. To analyze the relationship between fungal characteristics (strains, species and drug susceptibility) and patients' characteristics (source of infection, infected sites, underlying and/or complicated diseases, clinical entity, history of chemotherapy by antifungals, etc.).</p> <p>1-5. To elucidate mechanism of drug resistance by analyzing gene regions, which are reported to be responsible for drug resistances using the fungal isolates with drug-resistant property, confirmed in the Activity 1-3.</p> <p>1-6. To analyse geographical characteristics of drug resistance genes by comparing identified drug-resistant mechanisms against antifungals.</p> <p>1-7. In parallel with the Activity 1-5, to elucidate mechanisms of drug resistance using advanced genetic analysis technologies such as next-generation sequencing techniques for the fungal isolates, which are suspected to have unidentified drug resistance mechanisms.</p>	<p>Experts (1) Chief Advisor (Short-term Expert/double as an expert for Diagnostics-related studies) (2) Project Coordinator/Specialist for Research Institutes Cooperation (Long-term Expert) (3) Diagnostics-related studies (Short-term Experts) (4) Molecular epidemiological studies (Short-term Experts) (5) Gene amplification-related studies (Short-term Experts) (6) Bioinformatics (Short-term Experts) (7) Epidemiological investigation and optimal administration-related studies (Short-term Experts) (8) Management of fungal strains-related study (Short-term Experts) (9) Other persons with necessary expertise for the project activities (Short-term Experts)</p> <p>Training in Japan (1) Training for gene analyses (2) Training for operating Next Generation Sequencer (3) Training for genome comparison and gene disruption (4) Training for management of collected fungi (5) Other necessary training</p> <p>Equipment and materials Necessary equipment and materials for research activities in the Project</p> <p>Local cost</p>	<p>Counterparts (1) Project Director (2) Project Manager (3) Project Co-managers (3 persons) (4) Researchers (Infectious Diseases Division of the Internal Medicine Department and the Clinical Pathology Department, the Faculty of Medical Sciences, the UNICAMP)</p> <p>Facilities, equipment and materials (1) Office space or facilities for JICA experts in the UNICAMP (2) Research space and facilities in the UNICAMP (3) Existing research instruments and equipment for research activities, etc.</p> <p>Local Costs - Running expenses for research activities, etc.; - Assignment of counterpart personnel; - Utility costs (electricity, water, etc.); - Personnel costs (to the work of counterpart); and - Operational costs.</p>	<p>1. Approvals are obtained by the ethical committees of each project implementing agency for the medical and/or epidemiological research, which are supposed to be performed in the Project (if necessary).</p> <p style="text-align: center;"><b>&lt;Issues and countermeasures&gt;</b></p> <p>(Issues) Due to the pandemic of COVID-19 worldwide, Japanese researchers' visiting to Brazil and receiving Brazilian trainees in Japan were deferred.</p> <p>(Countermeasures) Research meetings between the chief advisor/Japanese experts and Brazilian researchers were performed periodically using a video-conferencing system. The experts perform the preliminary experiments in Japan as a replacement for inputs to Brazil.</p>

1-7-1. To provide Brazilian researchers with the Training in Japan in the theme of genetic analysis techniques using the next-generation sequencer for elucidating drug-resistant mechanisms at the MMRC.

1-7-2. To identify gene regions, which are suspected to be responsible for drug resistance, by whole-genomic comparison of drug-susceptible and drug resistant fungal strains isolated in Brazil, followed by the functional analyses of the region in the UNICAMP.

1-8. To compile research findings and outputs into scientific articles, together with that gained in the Output 2 and in Japan, to be used as evidences for revising the existing guidelines and for newly-developing it for the management of mycoses, followed by the trial-based application to the hospitals in Campinas metropolitan area and/or other areas in Brazil.

**2. Gene amplification technologies-based drug-resistance genes detection methods with sufficient sensitivity, specificity and operability are established for the diagnoses of drug-resistant mycoses.**

2-1. To develop gene amplification (LAMP, PCR, etc.)-based gene detection methods for the diagnoses of drug-resistant mycoses.

2-1-1. To develop basic testing condition of detection methods for drug-resistant fungi particularly for *Aspergillus* including its cryptic strains, *Candida* and other fungi, which are suitable for applied gene amplification techniques.

2-1-2. To provide Brazilian researchers with the Training in Japan in the theme of gene amplification-based gene detection methods for the diagnoses of drug-resistant mycoses.

2-1-3. To introduce the detection methods for drug-resistant fungi, developed in the Activity 2-1-2, to the UNICAMP by the joint efforts of Japanese researchers (JICA experts) and the Brazilian researchers who participated the Training in Japan.

2-2. To evaluate the clinical performance of the detection methods for drug-resistant fungi statistically by analyzing its sensitivity and specificity using fungal strains clinically-isolated from patients with mycoses in Brazil.

2-3. To evaluate the operability of the drug-resistant gene detection method(s), which are developed on the basis of LAMP techniques, by introducing them into UNICAMP-related hospitals and other selected external cooperative health or testing facilities on a trial basis.

2-4. To initiate discussions with Brazilian authorities concerned on the practical application of the drug-resistant gene detection methods, developed by the Project, to clinical laboratory testing (registration/accreditation of the methods as the "Standard Methods" in Brazil).

**3. A UNICAMP-centered research collaboration system among research, medical and administrative organizations is established.**

3-1. To construct a banking system of fungal strains including drug-resistant ones in the UNICAMP, which is interoperable by the external cooperative agencies for mycoses research.

3-1-1. To develop bylaws for the operation of the sample banking system, which stipulates the supply/transportation of samples to the UNICAMP, preservation and shared use of the fungal strains including drug-resistant ones, by holding meeting with external cooperative agencies of the Project such as universities, hospitals and testing facilities.

3-1-2. To provide Brazilian researchers with the Training in Japan in the theme of the operational management of fungal strains preservation facility at the MMRC.

3-1-3. To develop a facility for the preservation of fungal strains in the UNICAMP.

3-1-4. To construct an information database of clinically-isolated fungal strains, comprised of fungal and patient' characteristics in the UNICAMP.

3-2. To hold regular conferences and/or seminars on drug-resistant fungi including the reporting of project achievements, geared to project external collaborating agencies, health and medical facilities in the project areas and the administration organizations in charge of the control of fungal infections including drug-resistant ones.



3-3. To hold regular meetings with the administration organizations in charge of the control of fungal infection including drug-resistant ones for sharing of the epidemiological information such as incidence and clinical entity, gained through the research activities of the Project.

3-4. To set up a network of clinical laboratory testing and diagnosis of mycoses including drug-resistant ones among the UNICAMP (as the reference laboratory) and health and testing facilities in the State of São Paulo with the support of the Institute of Adolfo Lutz (IAL).

3-5. To set up a network of clinical laboratory testing and diagnosis of mycoses including drug-resistant ones among research institutes, health facilities and the MMRC as the reference laboratory also in Japan, by referencing the achievements of project collaborative research and technical cooperation in Brazil.

3-6. To hold national and international conferences on the research of drug-resistant mycoses geared to research, health and administrative agencies in other provinces and nations, in light of future establishment of a network of collaborative research on mycoses including drug-resistant ones as well as wide-sharing of research findings and outcomes of the Project.

## **ANNEX 6 List of the discussed issues in AMC-19**

**1st AMC-19 (June 3, 2020): Clinical cases of COVID-19 in Japan**

**2nd AMC-19 (June 17, 2020): Clinical cases of COVID-19 in Japan and Brazil**

**3rd AMC-19 (July 1, 2020): Comprehensive health care (incl. mental health care) for medical staff on COVID-19**

**4th AMC-19 (July 22, 2020): Clinical practice on COVID-19 at HC/UNICAMP – focusing on PBPM-Protocol-Based Pharmacotherapy Management –**

**5th AMC-19 (August 12, 2020): Clinical Practice on COVID-19 at Chiba University Hospital – focusing on Antiviral and anticoagulant therapy –**

**6th AMC-19 (September 2, 2020): Comprehensive health care (incl. mental health care) for medical staff on COVID-19 at UNICAMP**

**7th AMC-19 (September 23, 2020): Persistent symptoms after acute COVID-19 (Japan)**

**8th AMC-19 (October 14, 2020): Persistent symptoms after acute COVID-19 (Brazil)**

**9th AMC-19 (November 4, 2020): COVID-19 disease in Children – Japan**

**10th AMC-19 (November 25, 2020): COVID-19 disease in Children – Brazil**

**11th AMC-19 (December 16, 2020): Extrapulmonary manifestations and impact of COVID-19**

**12th AMC-19 (February 24, 2021): Management of non-ICU COVID-19 patients in Chiba University Hospital**

**13th AMC-19 (March 17, 2021): Current Status of a new SARS-CoV-2 lineage,**

**named P.1 (20J/501Y.V3) and Epidemiological Surveillance in Brazil**

**14th AMC-19 (April 28, 2021): COVID-19 - Epidemiological situation in Japan**

**15th AMC-19 (May 26, 2021): Clinical features of COVID-19 variants including P.1 lineage**

**16th AMC-19 (June 30, 2021): The organization policy of higher education facilities in the COVID-19 pandemic**

**17th AMC-19 (July 21, 2021): Campinas State University: pioneering the fight against Covid-19**

**18th AMC-19 (September 8, 2021): The infodemic about COVID-19 vaccines**

**19th AMC-19 (September 29, 2021): The infodemic; Why? How? And so what?**

**20th AMC-19 (October 12, 2021): Campinas and COVID-19: Current Overview of Cases, Vaccines and Variants**

**21st AMC-19 (November 9, 2021): Two cases of rare adverse event after COVID-19 vaccination**

**22nd AMC-19 (December 15, 2021): After-Effect of COVID-19; Psychiatric Disorders and Post Traumatic Growth**

**23rd AMC-19 (March 15, 2022): Epidemiological Aspects of VOC Omicron in São Paulo State and the City of Campinas**

**24th AMC-19 (May 10, 2022): Treatment strategies for mild to moderate COVID-19 in Japan, 2022 May**