CENTRE D'INFECTIOLOGIE CHARLES MÉRIEUX

CICM

ACTIVITY REPORT 2014

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Introduction

The Centre d'Infectiologie Charles Mérieux (CICM) is a non-profit public research centre governed by a tripartite agreement between the Ministry of Health, the University of Antananarivo and the Fondation Mérieux. Opened in 2010, its main mission is to accompany the development of research and training on infectious diseases and to provide technical and logistic support to some clinical laboratories in public hospitals. The fastening of the CICM with the University of Antananarivo facilitates the exchanges and scientific collaborations with the Faculty of Medicine, the Faculty of Science, and the College of the Agronomic Sciences, but also with the clinical units of Teaching Hospitals in Antananarivo. These collaborations are concretized, among others, by the provision to the CICM of a biologist from the Department of Biology, Faculty of Medicine, to prepare a diploma on specialization.

In 2014, the CICM finalized the writing of its quinquennial Strategic Plan 2014-2018 and produced the first combined 2011-2013 scientific activities report. The annual Strategic Orientation Council (COS) hold in February was again an opportunity to assess its activities. The Web site of the CICM currently is available to improve its visibility regarding communication and information and to promote the exchanges with the regional laboratories, a central server was installed at the CICM this year.

In terms of research, the CICM finalized in 2014 a pre-project on endemic tropical mycosis surveillance in Madagascar (chromoblastomycosis and sporotrichosis) which enters at present in its project phase with the implication of USFR rheumatology-dermatology and an associated thesis project. This study is funded by Fondation Mérieux. In another domain, the CICM secured funding from the European & Developing Countries Clinical Trials Partnership (EDCTP) to assess the prevalence and risk factors for cryptococcosis in HIV-infected patients. This study conducted with the Department of Infectious Diseases also includes a training program in Master's degree 1 in Public health. Finally, a genotyping multicentre project on HPV associated-cervical cancers was initiated by the Institut National du Cancer (INCa) at the end 2014 with the participation of five French-speaking countries of sub-Saharan Africa including Madagascar. The CICM also provides substantial technical support to the surveillance and public health activities managed by the Ministry of Health including leprosy, paediatric bacterial meningitis or childhood bacterial diarrhoeas.

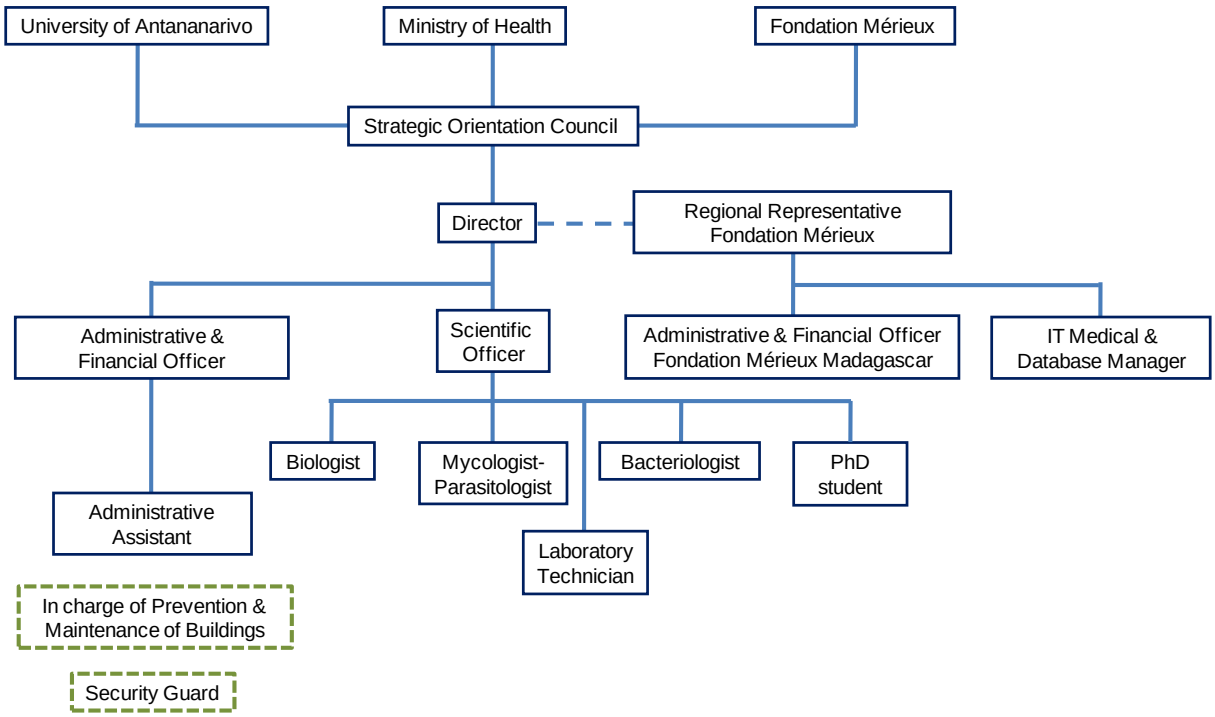
In terms of training, two training courses in Bioethics funded by the National Institute of Health (NIH) were organized in April and November, 2014 with the support of the University of North Carolina (USA) and the University of Namur (Belgium). The CICM co-organized a workshop in bacteriology with the CIRAD in February, a seminar workshop in mycology with the University of Grenoble in September and a training course in epidemiology and statistics with the University Lyon 1 in November.

In terms of international relationships, the Vice-president of the Union of the Comoros in charge of the health visited the CICM to look for the potential domains of cooperation with Madagascar; the team of Qualireg La Reunion also came with the aim of determining the potential areas of research in the food safety domain; finally, the representative of the University of Leeds would want to expand the fields of intervention of her establishment on infectious diseases.

Pr Luc Hervé SAMISON
Director of CICM

Summary

Organization Chart 2014-2015



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Collaboration and partnership

Scientific Partnership	
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University Claude Bernard, Lyon 1, France	Prof. Philippe Vanhems Dr Thomas Bénet
Mycobacteria and Drug Resistance National Reference Centre, Paris, France	Prof. Emmanuelle Cambau
Ecole Polytechnique Fédérale de Lausanne (EPFL), Switzerland	Prof. Stewart Cole
WHO Country Office for Madagascar	Dr Samuel H. Andrianarisoa Dr Constance Razaiarimanga
GABRIEL (*Global Approach to Biological Research, Infectious diseases and Epidemics in Low-income countries*) network	Dr Florence Pradel
Financial Partnership	
Fondation Mérieux, Lyon, France	
EDTCP (*European & Developing Countries Clinical Trials Partnership*)	
Institut National du Cancer (INCa), Paris, France	
Fogarty International Center	

Research Activities

Tropical subcutaneous mycosis

Rasamoelina T[a], Randriamampionona N[a], Raberahona M[b], Rakotomalala FA[a], Rakoto Andrianarivelo M[a], Contamin B[a], Cornet M[c], Ramarozatovo LS[d]

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Introduction

Chromoblastomycosis and sporotrichosis are endemic subcutaneous mycosis in Madagascar. The infection generally affects the subcutaneous tissue of the exposed parts (arms and legs) after injury and telluric contamination or after accidental introduction of a plant (thorn). The main responsible pathogenic agents of chromoblastomycosis are the genus *Fonsecae* and *Cladophialophora* whereas *Sporothrix* causes sporotrichosis. Because of their main distribution in developing countries by affecting essentially people living in rural and tropical areas, these diseases are qualified as neglected.

The Parasitology-Mycology Unit of the University of Grenoble has developed a PCR-based strategy to detect the pathogenic agents responsible of these subcutaneous mycoses. The assay is now operational in our laboratory in association with direct examination and culture, the classic mycological techniques. A master degree 2 of research has been presented from this subject with specialization in Healthcare and Medical Engineering (University of Grenoble).

Objectives

The main objective of this study is to identify the fungal agents responsible of chromoblastomycosis and sporotrichosis in Madagascar by means of a molecular biology. The specific objectives are to estimate their area of distribution to contribute improving the knowledge of these pathologies and to study the risk factors by associating an environmental component.

Methodology

It consists of a prospective study started on January, 2012 with recruitment of patients during field investigations and consultations provided at the dermatology department of the hospital, HUJRB. Pus, biopsies and squamous are sampled and examined microscopically with or without black Chlorazole staining. The samples were then inoculated onto Sabouraud-Chloramphénicol medium, incubated at 30°C and microscopically and macroscopically examined. Pathological examination is also performed on the biopsy material.

The PCR-based strategy was validated on the reference strains *Cladophialophora carrionii, Fonsecaea compacted, Exophiala dermatitidis* and *Sporothrix schenckii*. First, we used the universal primers NL-1/NL-4[1] and ITS5/ITS4[2] to check if the fungus belongs to this group. Secondly, their identity is

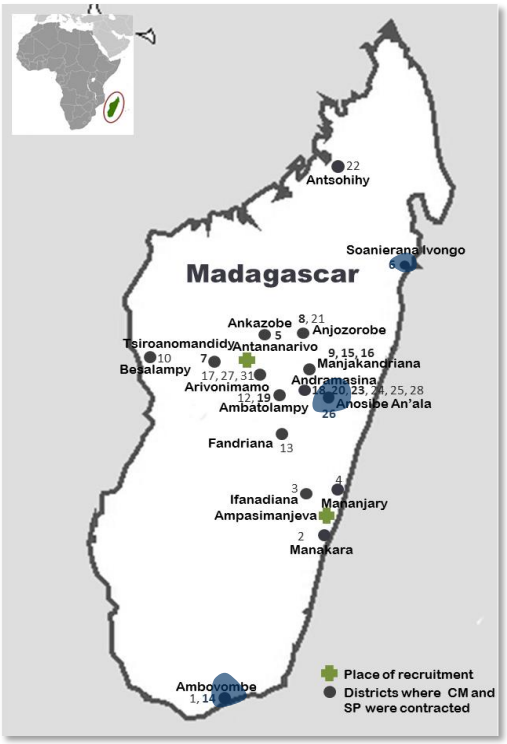
[1]Abliz P *et al*. FEMS Immunol Med Microbiol 2004;40(**1**):41-9
[2]White T *et al*. In MA Inne*set al*(ed.). Academic Press, Inc., San Diego, California, 1990, pages 315–22

specified by using specific primers that allow the amplification of *Fonsecaea* sp[3], *S. schenckii*[4], or *E. dermatitidis*. The results obtained following amplification of DNA extracts from clinical samples were compared with those of the cultures.

Results

Since the beginning of the study, 46 cases were recruited among which 30 were from 2014. The results of 31 patients recruited between March, 2013 and May, 2014 are presented in this report (Figure). Mean age of patients was 36 years and male gender was prevalent (74.2 %). Overall, 42 % were farmers, 25 % self-employed, 23 % students and 10 % unemployed. In more than 60 % of the cases, the lesions were localized at the lower limbs including ulcerations mainly for sporotrichosis and verrucous, crusted or tumour lesions for chromoblastomycosis. The mycological analysis identified 13 sporotrichosis cases, 6 chromoblastomycosis cases, 1 case reacting favourably to sulfaméthoxazole-thrimétoprime and likely a bacterial mycetoma, whereas 11 cases were negative. The PCR identified 7 *Sporothrix*sp .cases, 1 *Cladophialophora* sp .case, 5 *Fonsecaea* sp. cases whereas the rest is under investigation. The next steps are to complete the analysis of the remaining samples and to make a thorough analysis of the specificity of primers towards *Sporothrix* sp.

These preliminary results confirm the persistence of chromoblastomycosis and sporotrichosis in Madagascar with high frequencies. The study will continue in 2015 by increasing the size of the population and by conducting an environmental study with the aim to describe the mode of distribution of fungal agents in order to avoid their contamination.



Impacts

From the point of views i) diagnostic: availability of a rapid test which will allow to confirm the aetiology of the mycosis in an early stage of the disease; ii) therapeutic: appropriate care management of patients; iii) public health: better knowledge of the epidemiology of mycosis in order to put in place appropriate prevention and control measures.

Financial support

Fondation Mérieux

[3]Abliz P *et al*. J Clin Microbiol 2003; 41(**2**):873–6
[4]Kanbe T *et al*. J DermatolSci 2005;38(**2**):99–106

Cryptococcosis and HIV

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Introduction

Cryptococcosis is a fatal systemic fungal infection that can affect both HIV-infected and uninfected patients. It is due to *Cryptococcus neoformans* complex that includes 2 species: *C. neoformans and C. gattii*. This serious opportunistic infection occurs in 80-90% of HIV-infected patients. Worldwide, the number of new cryptococcosis cases is estimated at 1 million per year, including 720,000 in sub-Saharan Africa and the deaths is attributed to 625,000. In Madagascar, no epidemiological data on HIV-related cryptococcosis is available. In 2011, 850 HIV-infected patients were monitored in the country, and nearly 250 patients are currently followed by the Department of Infectious Diseases, HUJRB, Antananarivo.

In September 2014, the Department of Infectious Diseases HUJRB, Antananarivo, in partnership with the Centre d'Infectiologie Charles Mérieux, has secured funding from EDCTP (*European and Developing Countries Clinical Trials Partnership*) in Epidemiology and Medical Statistics to drive a research project on the study of the prevalence and risk factors associated with *C. neoformans* complex in HIV-infected patients. The 2 years study period also includes a training program in master's degree 1 in Public Health at the University Paris-Sud, France.

Objectives

It consists in a prospective study with the general objective to estimate the prevalence and risk factors associated with *C. neoformans* complex in HIV-infected patients. The specific objectives are to make available to the HIV/AIDS National Programme Control, a simple, fast and reliable method for cryptococcosis diagnostic; to improve the management of patients; and to identify the circulating genotypes of cryptococcus.

Methodology

Venous blood of selected patients (CD4 count <200/mm^3, HIV-infected and consenting adults) is taken to look for cryptococcal antigen (CrAg) by an immunochromatographic assay (Immuno-Mycologics, Inc., OK, the USA). If the result is positive, the CSF is punctured in the presence of neurological and meningeal signs. A direct examination following staining with India ink and culture on Sabouraud-chloramphenicol media without cycloheximide at 30°C were done. Genetic characterization of the isolate is scheduled. Statistical analyses are applied to the collected data.

Results

From September, 2014 till February, 2015 (6 months), a total of 26 cases meeting the inclusion criteria were investigated. Mean age of the patients was 36 years, the *sex ratio* F:M 0.9 and the mean rate of CD4 87/mm^3. A case was confirmed in a 34-year-old married woman who ignored her HIV status and who was admitted for a high fever and intense headache. The CrAg titre in the CSF was > 640. Direct examination after India ink staining was positive (Figure) and the yeast was isolated on

culture. The patient died 3 days after her admission in the presence of acute meningoencephalitis. The genetic analysis of the yeast is in progress. These results confirm that cryptococcosis is really present in Madagascar and can kill. They also underline the major and priority importance to strengthen the HIV/AIDS National Control Programme in several domains: awareness campaigns, systematic screening, and effective implementation of clinical, therapeutic and biological follow-up of patients (CD4 and HIV viral load testing).

The priority in 2015 is to increase the recruitment of the patients to better estimate the prevalence and the risk factors of the disease.

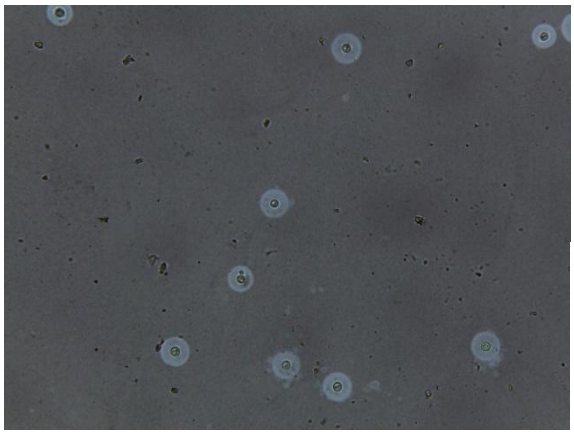

Figure. *Cryptococcus* sp. stained with India ink seen in microscope. The particles of the pigment of India ink do not penetrate into the polysaccharide capsule of the cryptococcus and form a zone cleared up in "halo" around the spherical cells of the yeast (CICM source).

Impacts

Ultimately, this study is expected to (a) make a laboratory-based diagnostic for early detection of cryptococcosis available to the programme; (b) support the implementation of an effective treatment for cryptococcosis; (c) provide a better understanding of the epidemiology of the *C. Neoformans* complex in Madagascar; and (d) serve as a model for launching similar studies to enhance the understanding of other opportunistic infections in HIV-infected patients. In addition, the study will lead to virological monitoring of HIV-infected patients, such as viral load measurement.

Financial support

European and Developing Countries Clinical Trials Partnership (EDCTP)

Human papillomavirus and cervical cancers

Rakotomalala H[a], Harioly Nirina M[b], Allegri C[a], Contamin B[b], Rakoto Andrianarivelo M[b]

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Introduction

The cervical cancer is one of the main causes of death by cancer worldwide in woman with about 266,000 deaths and 528,000 new cases in 2012[5]. In developing countries including the sub-Saharan Africa, it is the first cause of death by cancer in woman with about 231,000 deaths the same year. Cervical cancers are linked to certain types of human papillomavirus (HPV), named "high-risk"

[5]WHO.Comprehensive Cervical Cancer Control. A guide to essential practice 2nd ed. World Health Organization 2014

(essentially HPV 16 and 18), that occur at the final stage of the 10 to 20 years of evolution of the natural history of the infection. Considered rightly as an infectious disease, the cervical cancer can be prevented by use of vaccine administered before the sexual activity period of girls.

In January 2015, a conciliation meeting took place in Dakar, Senegal between the representatives of Cameroon, Côte d'Ivoire, Gabon, Madagascar and Senegal to validate a genotyping multicentre study of HPV associated-cervical cancers. The Institut National du Cancer (INCa), Paris is the promoter and the Research Unit in Biopathology of the Institut Curie is the scientific partner.

In Madagascar the project is initiated by Fondation Akbaraly, a humanitarian organization which works for the improvement of the living conditions of the population with a particular accent on the health of women and children. The virological activities are executed at the CICM.

Objectives

It consists in a prospective and retrospective study with the main objective to identify the genotypes of HPV genome integrated into the genome of invasive cancers and high-grade dysplasia of the uterine cervix. The specific objectives are i) to supply epidemiological data which can help in the decision of a vaccination drive, and ii) to favour the development of professionals' network in order to potentiate the resources and the skills and to standardize the procedures of care management of cervical cancers.

Methodology

At the first time, we shall analyze afterward 100 paraffin-embedded biopsies corresponding to invasive cancers and high-grade dysplasia of the uterine cervix. We shall implement in the course of the 2nd quarter 2015 the DNA extraction and PCR techniques allowing detecting HPV types 6, 11, 16, 18, 33, 45 and the genus HPV. In parallel, about 4,000 patients will be recruited from the health care centres of the Fondation Akbaraly to undergo a test of Papanicolaou. In this prospective study, about 300 biopsies are planned for pathological and virological testing. The study expecting to last 2 years will be submitted to the opinion of the National Ethics Committee.

Expected results

(i) Prevalence of the main genotypes of HPV associated with infiltrating cancers and high-grade dysplasia of the uterine cervix estimated; (ii) Strengthening and coordination of clinical, pathological and virological skills regarding HPV and cervical cancers established; (iii) Rationalization and homogenization of the procedures, the quality controls, the technology survey and the distribution of new indications of analysis proposed; (iv) Pooling of the results into a structured model in order to facilitate the exploitation of results and to serve as a basis for further development of an epidemiological follow-up system of tumours in French-speaking countries in Africa (register of cancers) elaborated.

Financial support

Institut National du Cancer (INCa), Paris, France

Support Activities

Aetiology of paediatric diarrhoeas: a comparative look on man and animal

Razafindrakoto C[a], Rahajamanana L[b], Raboba L[b], Rabenarivahiny R[c], Cardinale E[d], Robinson A[b], Contamin B[a], Rakoto Andrianarivelo M[a]

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Diarrheic diseases remain among the most important causes of childhood mortality and morbidity in developing countries especially in children under five. The main sources of contamination would be of hydric (difficulty of access to the drinking water) and of food origins. *Salmonellas* and *Campylobacters* are both bacterial genus most frequently involved in the diseases of food origin in developed countries. They are also involved in the diarrheic diseases of zoonotic origin. In developing countries including Madagascar, the data on the impact of these bacteria on the public health are poorly documented. Yet, with the exponential development of the poultry and porcine industry, accurate information would be necessary to allow the public authorities to take adequate measures to mitigate the risks. Indeed, food products derived from these sectors could represent an important risk of contamination.

The CICM and the Department of Research of the Directorate of Veterinary Services (DVS) developed together a study programme aiming: i) to determine the prevalence of *Salmonella* sp, *Campylobacter* sp and *Shigella* sp from childhood diarrhoeas in hospitalized patients; ii) to estimate the contamination of poultry products with *Salmonella* sp and to identify the species and the serotypes involved, and; iii) to estimate the antibiotic resistance of these bacteria common to man and animal.

From February till October, 2014, a total of 111 hospitalized diarrheic children were studied. The mean age of children was 25 months, the *sex ratio* F:M 0.7 and 36.9% (42) presented with signs of malnutrition. Figure 1 summarizes the observed clinical signs. The notion of previous antibiotic use shortly before admission was reported in 45.0% (50) of cases. After inoculation onto culture media, 26.1% (29) of stool specimens were positive. The use of galleries API-20E (bioMérieux, France) allowed to identify *Escherichia coli* (n=5), *Salmonella* sp. (n=2), *Shigella* sp. (n=20), *Shigella sonnei* (n=1), and *Campylobacter* sp. (n=1) [Figure 1]. *Campylobacter* sp. was identified by direct examination following Gram staining and catalase and oxydase tests.

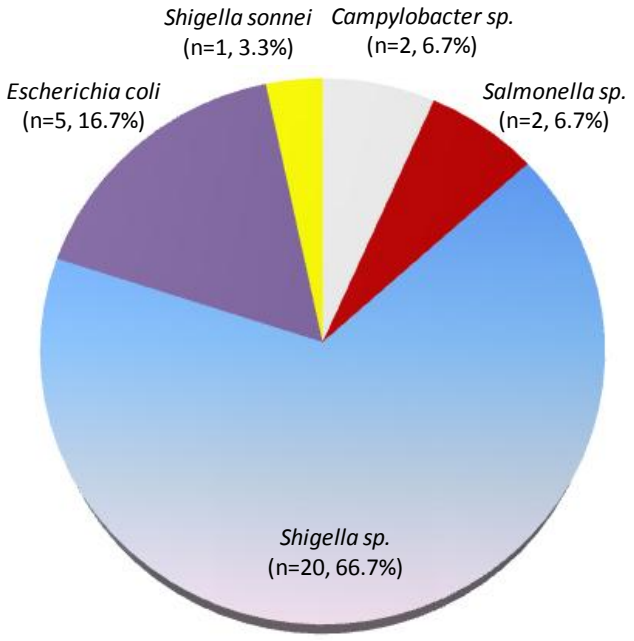

Figure 1. Bacteria isolated from diarrheic children hospitalized at CHUMET, Jan-Oct 2014

Table 1: Main clinical signs observed from diarrheic children hospitalized at CHUMET, Jan-Oct 2014.

Clinical signs	Number	%
Fever (n=109)	67	61,5
Vomiting (n=108)	63	58,3
Mucus (n=111)	51	45,9
Abdominal pain (n=108)	42	38,9
Bleeding (n=111)	20	18,0
Cough (n=108)	17	15,7

All the strains benefited from an antimicrobial susceptibility testing according to the standard antibiotic disk diffusion technique (ATB G - HAD, bioMérieux, France). For *E. coli* (n=5), their role in the occurrence of the diarrhoea is not formally proved in the absence of search of enterotoxins genes. Concerning the resistance, the most affected molecules are cotrimoxazole and tetracycline which are ineffective for 80% of strains. Forty percent (40%) of strains acquired nalidixic acid resistance but remain sensitive to fluoroquinolones; one strain produced extended-spectrum ß-lactamase (ESBL). For *Shigella* (n=21), the most affected by the antimicrobial resistance are tetracycline (95%) and cotrimoxazole (84%), whereas 66 % are high-level penicillinase producing-strains. No strain acquired resistance to third generation cephalosporin. Aminoglycoside antibiotic and fluoroquinolones remain sensitive. For *Salmonella* (n=2), they spared by the antimicrobial resistance phenomena for all classes except for one strain to tetracycline and cotrimoxazole. The *Campylobacter* strains (n=2) were tested only with five antibiotics (ampicillin, amoxicillin, gentamycin, tetracycline and ciprofloxacin) and were sensitive.

The study conducted over 10 months, and initially planned to study bacteria responsible of zoonosis, found only 13 % of *Salmonellas* or *Campylobacter*, and a majority of *Shigella* (77 %). The resistance to antibiotics is predominant for two classes: tetracycline and cotrimoxazole. A large part of strains are also resistant to ß-lactam antibiotics among which penicillinase producing-penicillin. For *E. coli* strain, resistance to the third generation cephalosporin begins to emerge but carbapenems keep an intact activity.

This study allowed contributing to the implementation of stool culture in the CHUMET laboratory. The next step should be the comparison between human and animal strains in order to establish the relationships between *Salmonella* infection in human and carriage in the animal industry.

This study was funded by Fondation Mérieux.

Molecular diagnosis and sensitivity study of *Mycobacterium leprae*

Razafindrakoto C[a], Andrianarison A[b], Rakotomalala A[a], Contamin B[a], Rakoto Andrianarivelo M[a], Rapelanoro Rabenja F[b], Ramarozatovo LS[b]

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Leprosy is endemic in several regions of the globe with 14 countries recording more than 1,000 new cases a year since 2006[6]. Worldwide, the incidence of leprosy in 2013 is more than 215,000 new cases (3.8/100 000 inhabitants) among which about 210,000 (3.5/100.000) occurred in Africa. In Madagascar leprosy is a major public health problem despite the existence of the Leprosy National Program Control and the availability of poly-chemotherapy for approximately 20 years. For the period 2006 to 2013, between 1,500 and 1,700 new cases per year were reported[6] among which 9 % of children. With the collaboration of the Department of Dermatology, HUJRB Antananarivo, we contributed to the clinical and biological surveillance of leprosy in the Analamanga region since 2012 with the ultimate objective to extend the surveillance to the national level. The main activities in 2014 were i) to continue the dermatological consultations at the hospitals and on the field, and ii) to improve the molecular tool for diagnosis of *M. leprae*.

In 2014, we received from 14 patients juice ear samples or/and skin biopsy, a slight decrease in the number of cases compared to 2013 (16 patients). Median age of patients is 22 years (15 years 25th percentile/52 years 75th percentile) and the *sex ratio* F:M 0.6. Multibacillary (57.1 %) and tuberculoid leprosy (42.9 %) prevail. Search for acid fast bacilli resistant after Ziehl-Neelsen staining is positive on 5/14 biopsies (35.7%) and negative on the rest. For most of the juice ear samples, the excess of red blood cells made the reading of the slides difficult. Following the recommendations of the Ecole Polytechnique Fédérale de Lausanne (EPFL), Switzerland, we used an additional primers pair (REP7/REP8) targeting the repeated sequences of the genome[7], in addition to the already described primers R1/R2[8]. The results obtained with the two primers pairs were equivalent. By comparing the PCR results with those of the microscopy, the PCR allowed to detect *M. leprae* from 9 negative biopsies, whereas concordant results with PCR were obtained on 4/5 positive biopsies. We also looked for the mutations that confer resistance to rifampicin in the locus of the *rpoB* gene, to fluoroquinolones in *gyrA* gene and to dapsone in *folP1* gene, using the Genotype Leprae DR kit (Hain Lifescience, Germany)[9]. No antimicrobial resistance was described for all the isolates tested since the beginning of the study. The positive biopsies were sent to the Mycobacteria and Drug Resistance National Reference Centre, Paris (Cambau E.) for quality control. In 2014 we sent also to EPFL, Swiss an isolate (LEP06008) for further investigations. The results showed that it belongs to the genotype 1D.

In 2015 our priorities are the followings: i) to assure a better programming of the field investigations and to increase the active screening; ii) to conduct systematically an investigation around the close contact of the case; iii) to assure >95% of completeness of data with the investigation form; iv) to perform sampling(skin biopsy and juice ear) of quality and of adequate amount; v) to take part in

[6]Weekly Epidemiological Record.2014;89(**36**):389–400
[7]Monot M *et al*. Nature Genetics 2009;41(**12**):1282-9
[8]Yoon KH *et al*. J Clin Microbiol 1993;31(**4**):895-9
[9]Cambau E *et al*. PLoSNegl Trop Dis 2012;6(**7**): e1739. doi:10.1371/journal.pntd.0001739

trainings to improve the reading of slides; vii) to increase the performance of the molecular test, in particular the DNA extraction technique, and vi) to assure >95% of release of laboratory results.

In a perspective, a phylogenetic study of Malagasy isolates is planned.

This study was funded by Foundation Mérieux.

Surveillance of the paediatric bacterial meningitis

Razafindrakoto C[a], Raboba L[b], Harioly Nirina M[a], Rahajamanana L[b], Rakotomalala A[a], Rakoto Andrianarivelo M[a], Milenkov M[c], Telles JN[c], Paranhos Baccalà G[c], Contamin B[a], Robinson A[b]

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The surveillance activities of paediatric bacterial meningitis and *Haemophilus influenza* (PBM-*Hib*) starting in 2012 continued in 2014. The CICM receives CSF samples taken from children <5 year-old meeting the case definition[10] and hospitalized at CHUMET Antananarivo. The surveillance sentinel site of PBM-*Hib* is based at CHUMET Antananarivo where bacterial cultures are done. The role of the CICM is to provide technical support to the national WHO surveillance programme by performing molecular diagnosis of *Streptococcus pneumoniae*, *Neisseria meningitidis* and *Haemophilus influenza* b. We carried out the method developed by LPE Lyon which also supplied reagents and advice for its implementation at the CICM.A nucleic acids extraction (QIAamp DNA blood mini kit, QIAgen) is performed prior to the identification assay with the use of an internal control (IC).The first step of the test is a quadruplex, probe-based real-time PCR(Bio-Rad CFX96), targeting the highly conserved and species specific genes *lytA* (*S. pneumoniae*), *frpA* (*N. meningitidis*),*bexA* (*Hib*), and *srtC2* (IC). Samples positive for *S. pneumoniae* were analyzed with a second assay, which comprises eleven multiplex real-time PCR reactions, targeting serotype-related genes for the identification of the 42 most prevalent *S. pneumoniae*[11].Similarly, *N. meningitidis* positive samples are submitted to analysis by two multiplex real-time PCR reactions, targeting serogroup-specific genes for the 6 meningococcal serogroups (A, B, C, W135, X, and Y) usually responsible for invasive meningococcal disease. No further investigation is carried out on samples positive for *Hib*.

In 2014, we analyzed afterward 294 CSF samples taken between September, 2012 and October, 2014. Mean age of the children was15.4 months; the median value of leukocytes in the CSF was 10 (6.5 25thpercentile / 27 75thpercentile). By PCR, 34 additional *S. pneumoniae* were detected compared to the culture. Serotypes 1 and 5 circulated up to April, 2013 then again from June, 2014 (Figure). Serotypes which are not included in the vaccine composition of PCV10 used by the EPI routine programme were identified: 2, 7C, 10A, 12F, 15B/C, 35A and 35F. "Untypable" strains were also detected and 2% of the children presented with co-infections. With the recent introduction of

[10]Axillary temperature >38°C; AND one or more of the following symptoms: seizures other than febrile seizures, neck stiffness, bulging fontanel (in children <12 months), poor sucking, altered consciousness, irritability, other meningeal signs, toxic appearance, or petechial or purpuric rash.

[11]Serotypes 1, 2, 3, 4, 5, 6A/B, 6C, 7F, 8, 9N/L, 9V, 10A, 10F, 11A, 12F, 13, 14, 15B/C, 16F, 18A/B/C /F, 19A, 19F, 21, 22F, 23A, 23B, 23F, 24F, 31, 33F, 34, 35A, 35B, 35F, 38 et 39

PCV10 vaccine in October, 2012 and although the national routine coverage was 92 % in 2013, it is too early to define a possible impact of the vaccination on the epidemiology of *S. pneumoniae*. One *Hib* meningitis case was diagnosed in July, 2013 and no *N. Meningitidis* case was detected during the study period.

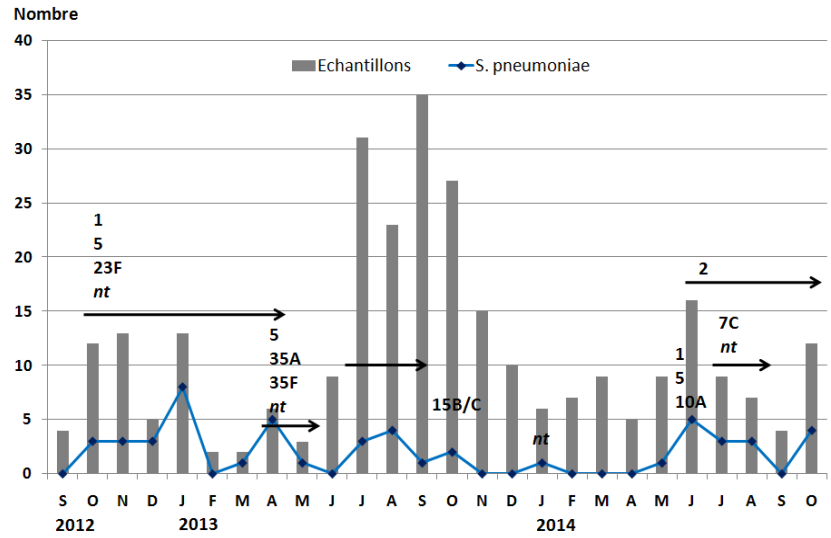

Figure. Serotypes of *S. pneumoniae* identified from hospitalized patients <5 old-year, Sep 2012 – Oct 2014 (unt = "untypable")

This study is expected to make available to the programme a fast and effective diagnostic tool for patients and during outbreaks; to improve the management of patients; to survey the circulation dynamics of strains and eventually to orient the control or the immunization strategy of these diseases.

The implementation of the molecular technique for the diagnosis of PBM-*Hib* was funded by Fondation Mérieux.

Surveillance of rotavirus diarrhoea

Rahajamanana L[a], Razafindrakoto C[b], Raboba L[a], Rakotomalala FA[a], Rakoto Andrianarivelo M[b], Contamin B[b], Robinson A[a]

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In 2014, the surveillance activities of rotavirus diarrhoea continued by analyzing stool specimens of children under five hospitalized for acute diarrhoea evolving for<7 days. Overall, 195 samples were analyzed at the CICM by an immunosorbent assay (ProSpectTM rotavirus kit, Oxoid Ltd, UK) targeting the major inner capsid protein VP6 present in group A rotaviruses. High positivity rate of 58.5% (114) was observed.

Since mid-September 2014, the diagnostic activities were relocated with the acquisition of a new ELISA reader by the sentinel site.

Training activities

Bioethics

Strengthening capacity in bioethics for chronic diseases and HIV infection. Training in bioethics of research with the aim to implement an Institutional Ethics Committee at the Faculty of Medicine. Partners: University of North Carolina (USA), University of Namur (Belgium), Fogarty International Center (USA).

Epidemiology and Statistical course

A short training in Epidemiology and Statistics for researchers and specialists in public hospitals was organized from 18 till 20 November 2014 with the support of the University Claude Bernard, Lyon 1 (Dr Thomas Bénet).

Workshop

Workshop in bacteriology (03-07/02/2014, Dr Isabelle Henry, CIRAD) and mycology (22-26/09/2014, Prof. Muriel Cornet, University of Grenoble) intended for doctors and laboratory technicians of public hospitals were organized with the partnership of the Ministry of Health and the Department of Biology, Faculty of Medicine, Antananarivo.

Management Information System

Training associated with the implementation of a laboratory Management Information System was realized in a clinical lab of Teaching Hospital and 8 regional laboratory hospitals.

In May, 2014 a server (Dell power edge) was settled in the premises of the CICM to ensure the storage of databases.

Diploma

Diplôme d'Etudes de Formations Spécialisées (DEFS or University Degree in Specialized Training) delivered by the Department of Medical Biology, Faculty of Medicine, University of Antananarivo:

- Bacterial diarrhoeas in hospitalized children at the Mother & Child Teaching Hospital Tsaralalàna– CHUMET (Dr Catherine RAZAFINDRAKOTO, 2013-2014, in progress)
- Bacterial urinary Infections at the Teaching Hospital Befelatanana – HUJRB (Dr Saïda RASOANANDRASANA, 2013-2014, in progress)

Master' Degree 2 with specialization in Healthcare and Medical Engineering TIMC-TheRex laboratory, University of Grenoble:

- The Use of a PCR-based Strategy for the Diagnosis and the Identification of the Causative Agents of Chromoblastomycosis and Sporotrichosis in Madagascar: a study of 31 cases (Mr. Tahinamandranto RASAMOELINA, 2013-2014)

Master' Degree 1 in Public Health, University Paris-Sud (Dr Rivonirina Andry RAKOTOARIVELO, 2014-2015)

Master' 1 Degree in Infectiology: microbiology, virology, immunology, University Paris Diderot Paris VII, internship at the laboratory of Prof. Emmanuelle CAMBAU, Lariboisière hospital, Paris VII (Dr Saïda RASOANANDRASANA, 2014-2015)

Scientific productions

Publications

1. Randriamahazo TR, Raherinaivo AA, Rakotoarivelo ZH, **Contamin B**, Rakoto Alson OA, Andrianapanalinarivo HR, Rasamindrakotroka A. Prevalence of hepatitis B virus serologic markers in pregnant patients in Antananarivo, Madagascar. *Med Mal Infect*. 2014 Oct 30. pii: S0399-077X(14)00310-2. doi: 10.1016/j.medmal.2014.10.008
2. Picot VS, Bénet T, Messaoudi M, Telles JN, Chou M, Eap T, Wang J, Shen K, MD, Pape JW, Rouzier V, Awasthi S, Pandey N, Bavdekar A, Sanghvi S, Robinson A, **Contamin B**, Hoffmann J, Sylla M, Diallo S, Nymadawa P, Dash-Yandag B, Russomando G, Basualdo W, Komurian-Pradel F, Vernet G, Endtz H, Siqueira MM, Barreto P, Vanhems P, Paranhos-Baccalà G, GABRIEL pneumonia Network. Multicenter case-control study protocol of severe pneumonia etiology in children: Global approach to biological research infectious diseases and epidemics in low income countries (GABRIEL network). *BMC Infectious Diseases2014, 14:635. http://www.biomedcentral.com/1471-2334/14/635*
3. Cardinale E, Abat C, **Contamin B**, Porphyre V, Rakotoharinome M, Maeder M. Salmonella and Campylobacter Contamination of Ready-to-Eat Street-Vended Pork Meat Dishes in Antananarivo, Madagascar: A Risk for the Consumers? *Foodborne Pathog Dis*. 2015 Mar;12(3):197-202.

Oral communications

1. Rasamoelina T. Utilisation d'une Stratégie basée sur la PCR pour le Diagnostic et l'Identification des Agents Causaux des Chromoblastomycoses et des Sporotrichoses à Madagascar: une Etude sur 31 cas. Congrès SOMADER, Akademia Nasionaly Malagasy, 10-12 novembre 2014
2. Rakoto-Andrianarivelo M. Detection and typing of Neisseria Meningitis by molecular assay in Madagascar. 7thAnnual GABRIEL Meeting, 14-17 December 2014, Les Pensières, Annecy, France.
3. Rasamoelina T. A PCR-based strategy for the diagnosis of chromoblastomycosis and sporotrichosis in Madagascar: a study of 31 cases. 7thAnnual GABRIEL Meeting, 14-17 December 2014, Les Pensières, Annecy, France.