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Spread of *M. chimaera* in Cardiac Surgery Facilities in Quebec: Outbreak Investigation

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Infection Controls 2017 Chicago, IL – USA.

www.inspq.qc.ca

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8 Departments – clinical diagnostic:

- Bacteriology
- Mycobacteriology
- Mycology
- Parasitology
- Virology / mol biology / Serology
- Physico-chemistry

- BSL3
- Bioforensic
- Quality assurance
- Genomics

Introduction

Mycobacterium chimaera:

- Validated in 2004 (Tortolli et al.)
- *M. avium* complex
- opportunistic human pathogens

- Resistant to antibiotics and desinfectants

- biofilm formation and hydrophobicity favouring aerosolisation

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Worldwide outbreak

Achermann *et al.* (2013): first 2 fatal cases (late endocarditis associated to *M. chimaera* following a cardiac surgery.

In 2015, Sax *et al.* (2015): *M. chimaera* outbreak occurring in Zurich (2008 and 2012) and affecting patients subjected to open-chest heart surgery.

Over 100 cases of *M. chimaera* infectionshave been notified in Switzerland, Germany, the Netherlands, UK, USA, and Australia,

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M. chimaera investigation history in Quebec

European Alert April-2015

09-2015:

Filtration protocol: 1 site investigated (6 HCU contaminated)

08-2016: 1st human case of M. chimaera detected (associated with cardiac surgery)

09-2016: 2nd human case of M. chimaera detected (associated with cardiac surgery)

10-2016: investigation in all cardiac surgery sites

(filtration, culture, Sequencing, RT-PCR for rapid detection in HCU water)

02-2017: 3rd and 4th human cases

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Table: Characteristics of patients

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M. chimaera investigation history in Quebec

Active Laboratory surveillance

10-2016: Retrospective 2010-2016

all patients (+ cultures of M. chimaera):

- 8324 isolates of MAC identified,
- 625 M. chimaera were analysed
- 25 strains were isolated from sterile sites.



hospitals

Four patients were identified as suspected to have risk associated with surgery and were included in this study.

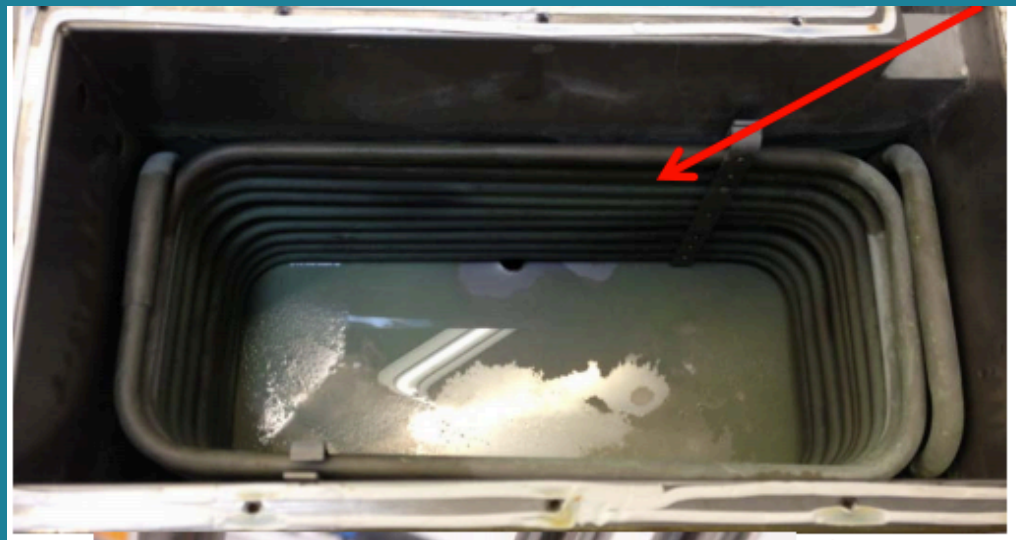
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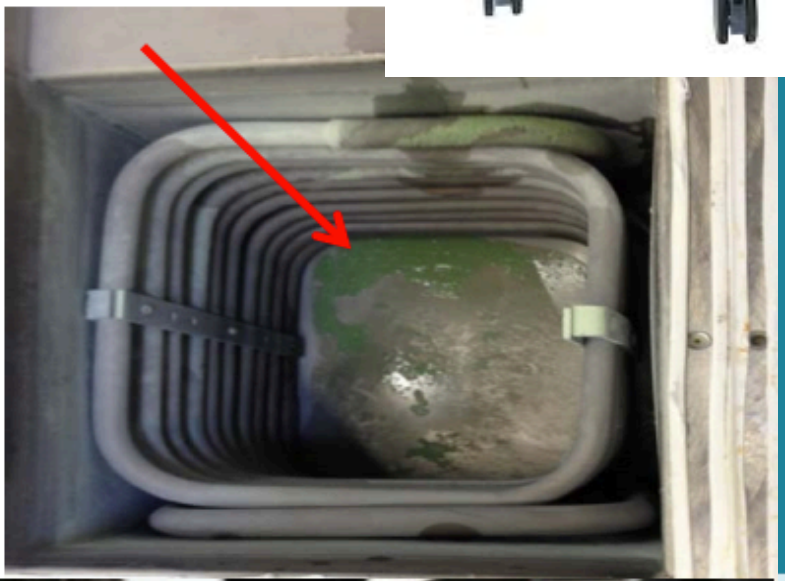
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Example of contaminated HCU











Positive culture from HCUs

06-2016 to 05-2017	Pure	Mixed / M. gordonae	/ M. intracellulare	Total	total HCU
M. chimaera	**181**	16	1	198	**34**
M. gordonae	18	0	0	18	13
M. lentiflavum	18	0	0	18	4
M. avium	2	0	0	2	2
M. intracellulare	1	0	0	1	1
Methylobacterium podarium	1	0	0	1	1

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Source of M. chimaera ?

Mycobacterium chimaera infection from:

- HCU aerolization?
- Water source in the hospital?
- Local or imported contamination?
- Related to the european outbreak ?

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Material & Methods

- Patients strains
  - 4 *M. chimaera* isolates (cardiac surgery)
  - 4 *M. chimaera* sterile sites (unknown)
  - 2 *M. chimaera* from sputum (not related)

- HCUs strains
  - 6 strains from 6 HCUs (site A)
  - 26 strains from 26 HCUs (5 sites)

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Material & Methods

- Patient strain from Zurich

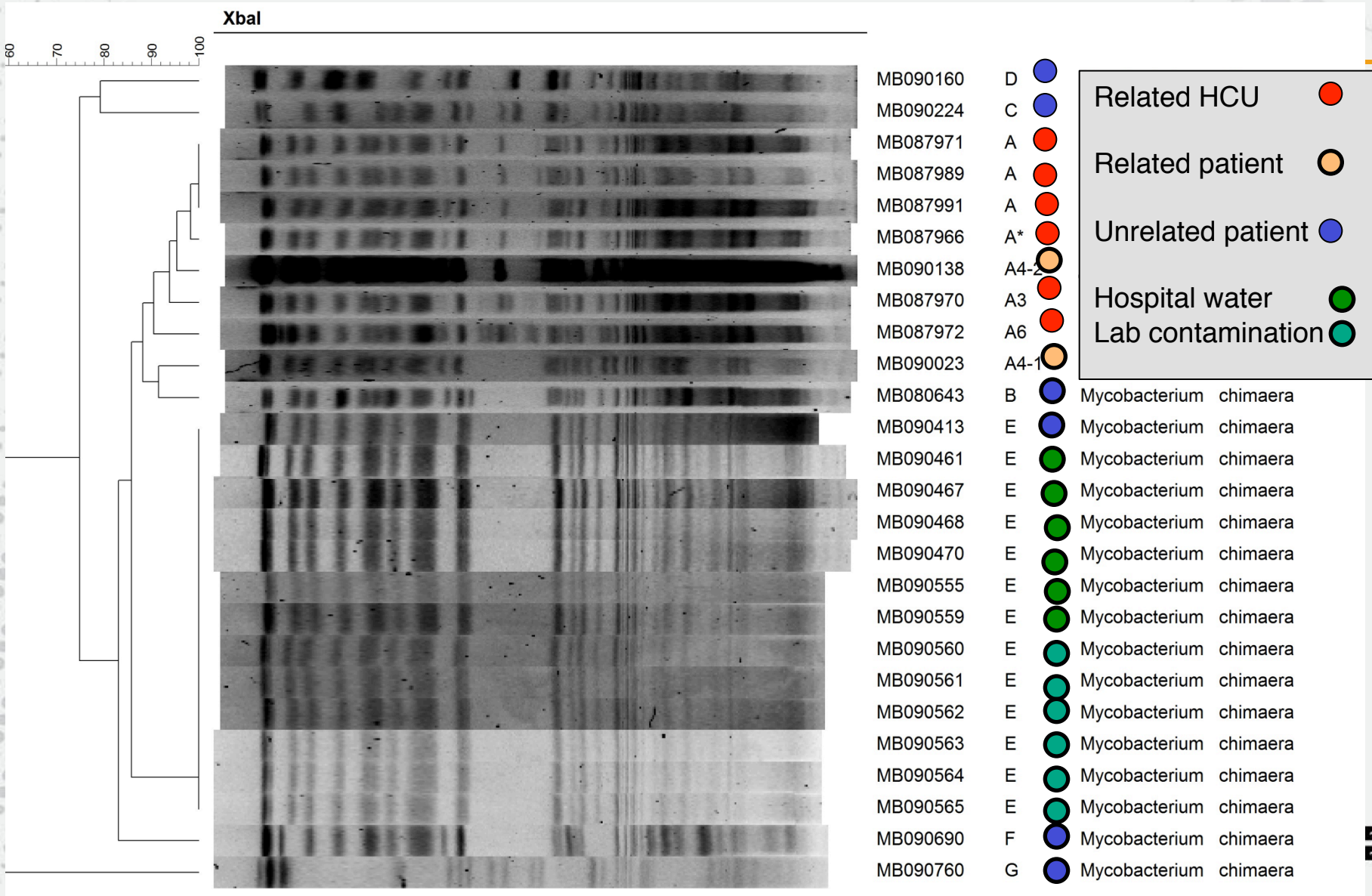
  MYCOZU1 (kindly provided by Prof E.C. Bottger and Dr P. Keller)

  PAC Bio sequenced and annotated

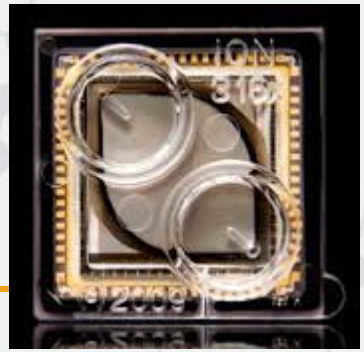
Genotyping: PFGE and WGS

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M. Chimaera genotyping = PFGE



WGS: Diagnosis and surveillance





Ion Torrent



Illumina MiSeq

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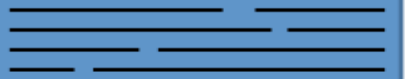
WGS Workflow

Template Preparation

Culture





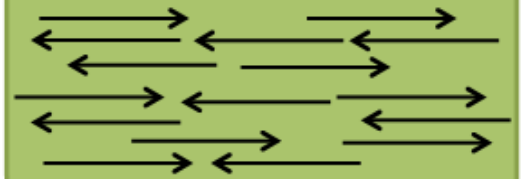


Genomic Fragments

High Throughput Sequencing



Illumina MiSeq®



Raw Reads

Data Analysis

- **Quality Control**
- **Genome Assembly**
  - *de novo*
  - Reference-guided
- **Interpretation**
  - **hqSNP Analysis**
  - wgMLST

M. Chimaera whole genome sequencing

DNA Extraction: MagAttract DNA Mini M48 Kit on the BioRobot M48 (Qiagen)

Genomic Library Construction : Nextera XT kit (Illumina, Inc.)

Sequencing: Illumina MiSeq platform with the MiSeq Reagent Kit v3 (600 cycles, paired ends)

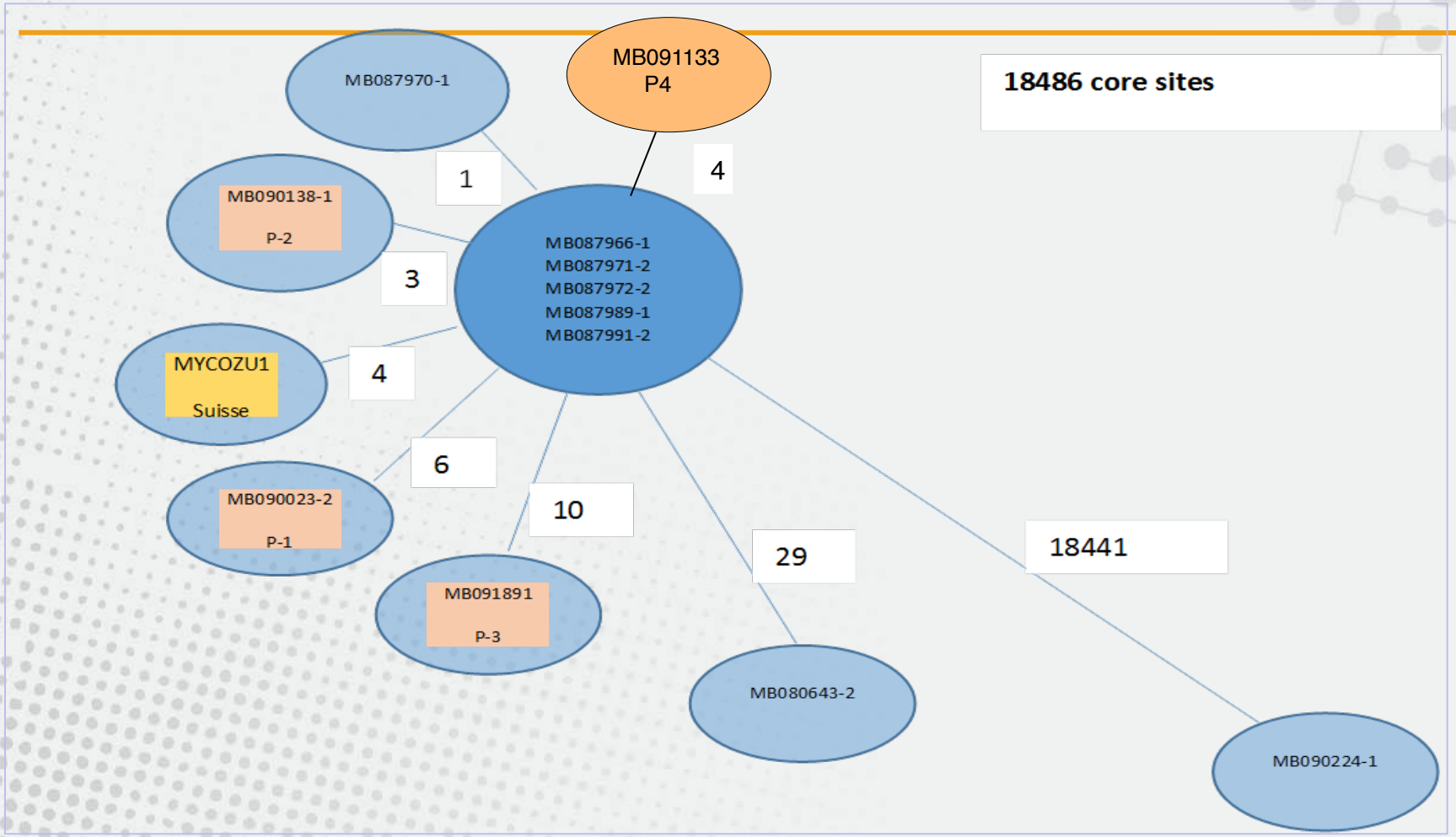
Genomic Analysis

The quality of the raw sequence reads was verified using FastQC (http://www.bioinformatics.babraham.ac.uk/projects/fastqc).

Phylogenomic links : core genome hqSNV analysis pipeline (https://github.com/apetkau/core-phylogenomics) implemented as a Galaxy (https://galaxyproject.org/)

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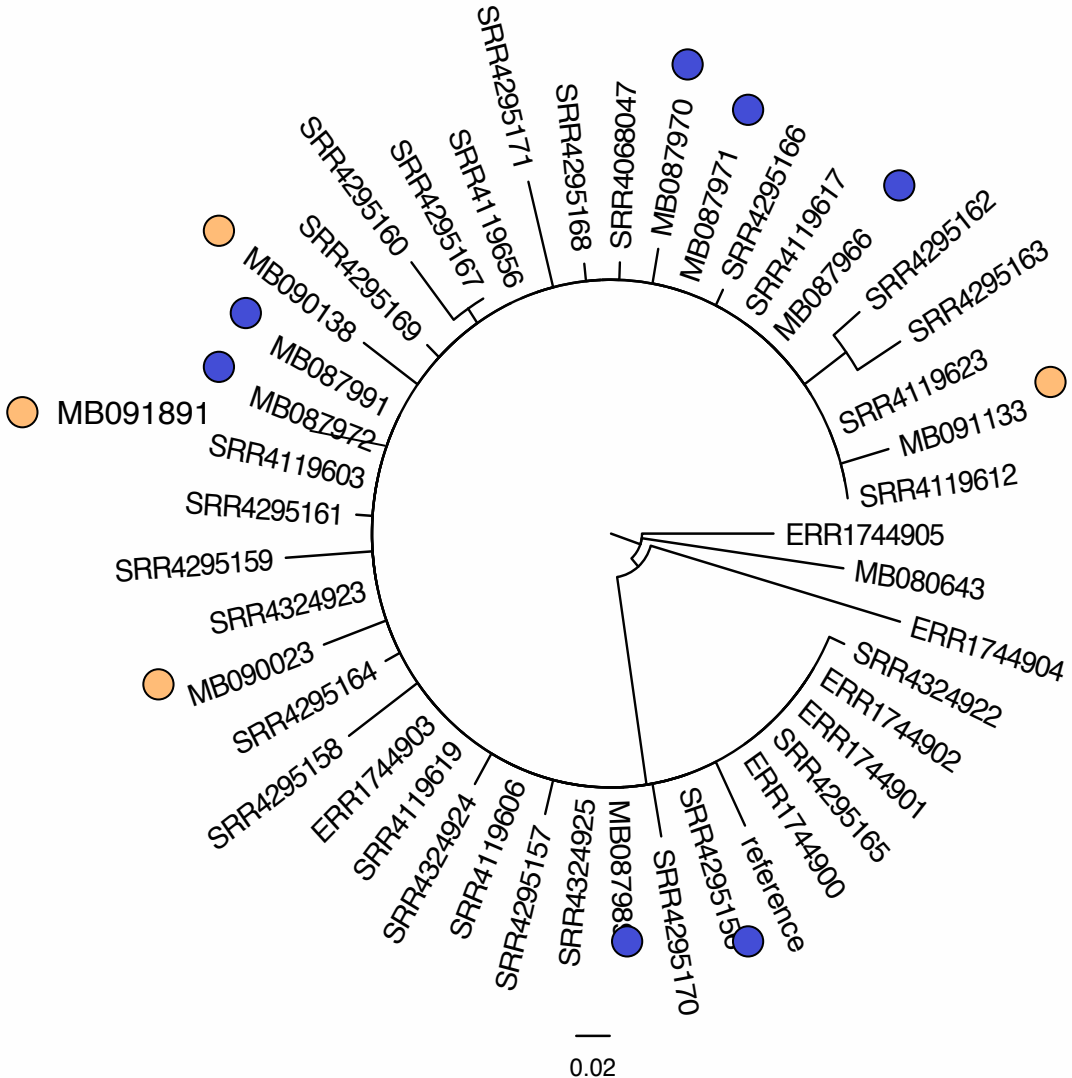
Phylogenetic tree of *M. chimaera*



4 patients related to cardiac surgery infection

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Relatedness of Montreal HCUs/ Paitens and worldwide isolates



6,2 Mbp
5 plasmids

75 % of genome analys

Conclusion 1

Mycobacterium chimaera:

- 8% of total MAC isolated in Quebec
- 4 patients associated / M. chimaera outbreak
- One site involved in patient infection
- 6 sites of cardiac surgery were contaminated
- 34 out of 51 HCUs contaminated

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9 cardiac surgery sites provided with Standardized protocoles :

Filtration (100 – 500 ml), 0.22 um resuspended in 5 ml.

Décontamination: N-Acetyl-Cys-NaOH.

Incubation in solid and liquid (MGIT) medium

Positive cultures received at the LSPQ

Idenitifcation (16S rDNA), PFGE, WGS,

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RT-PCR *M. chimaera*

Validation ISO 15189

Species	Number of isolates tested
M. chimaera	12
M. chimaera – mixed	1
MAC other	10
M. gordonae	7
Mycobacteria other	40
Actinomycetes	2
Total	72

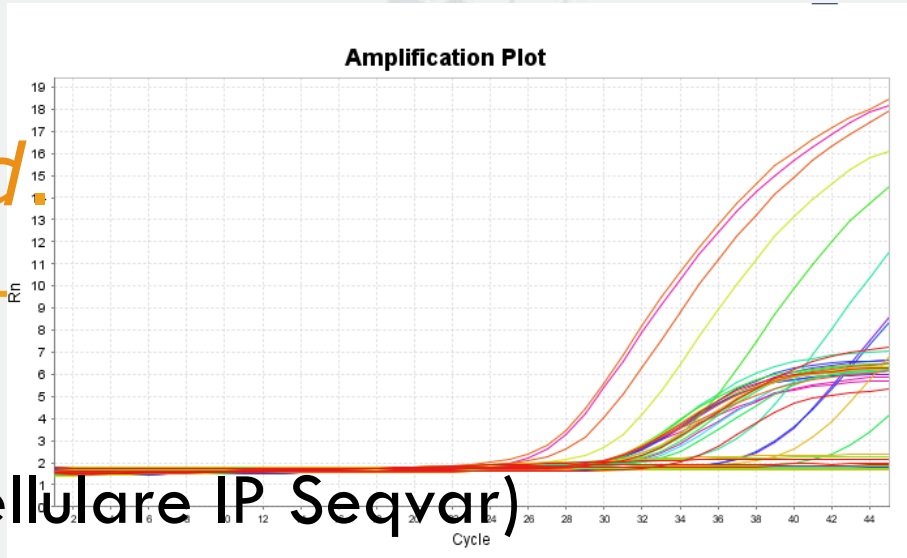
Samples	N
HCU- water	38
Spiked M.chim	3 X 26
Spiked-DNA	3 X 20
HCU-water-spiked	2 X 16
Total	208

Routine detection

01-2017 - 06-2017	*RT-PCR*
Water filtrates	349
Water	55
Total RT-PCR M chimaera	404

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Two RT-PCR evaluated



16S rDNA (mismatch M. intracellulare IP Seqvar)

Sensitivity 100% specificity 89%

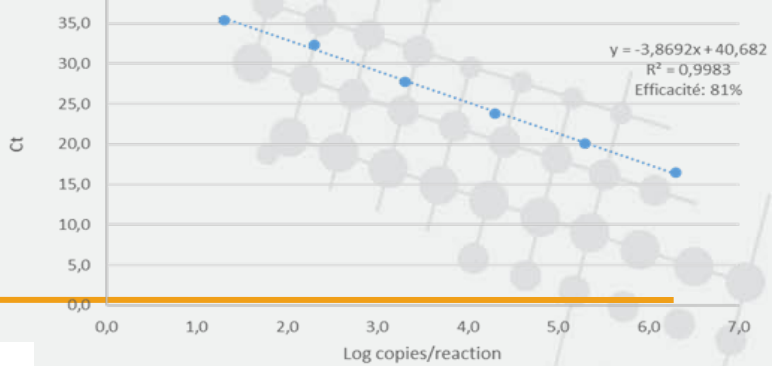
ITS (modified condition to increase specificity)

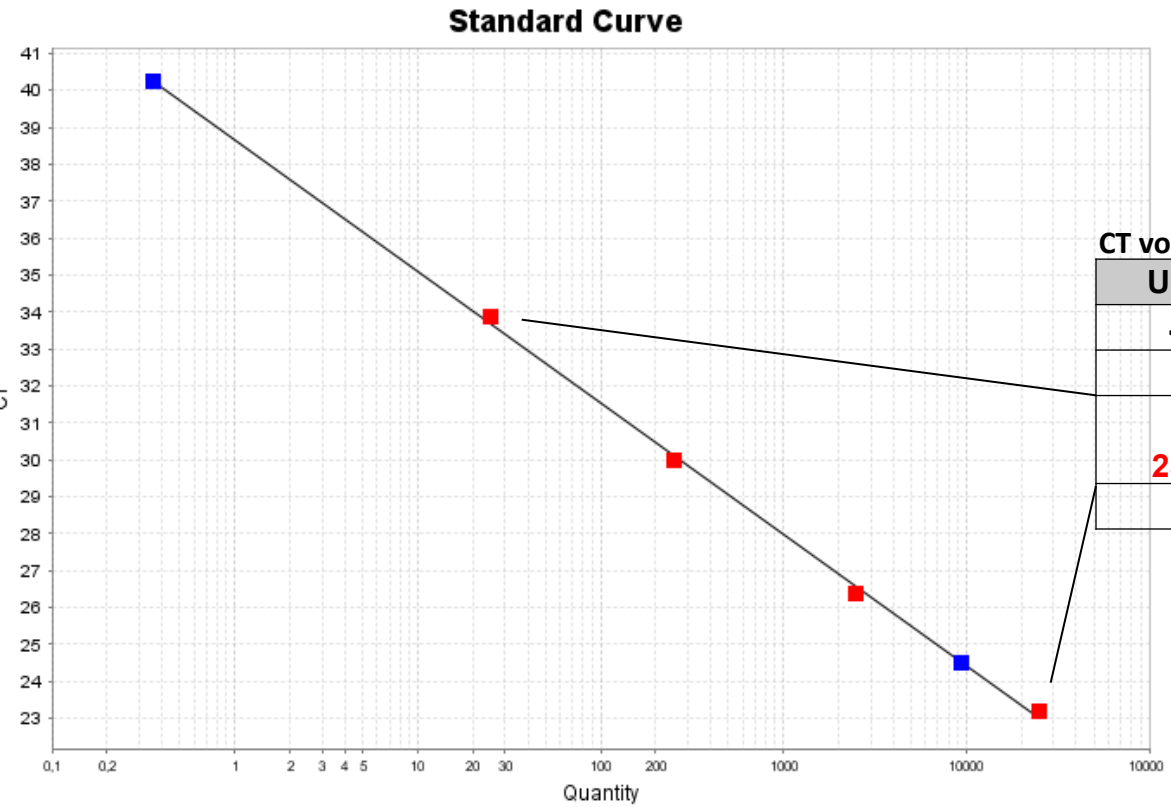
Sensitivity 100% specificity 100% (used in Routine)

TAT 48H vs 25 days

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ITS-based RT-PCR





Target: ITS-MchimP-REV Slope: -3,565 Y-Inter: 38,687 R^2: 0,998 Eff%: 90,775

LOD 90% = 10 copies/reaction

CT vonverted to UG/reaction or ml

UG/reaction	final Result
<1 ou n/a	Negative
1 to 25	Positive non quantifiable
25 to 25 000	Positive quantifiable (XXX UG/ml)*
>25 000	Positive non quantifiable

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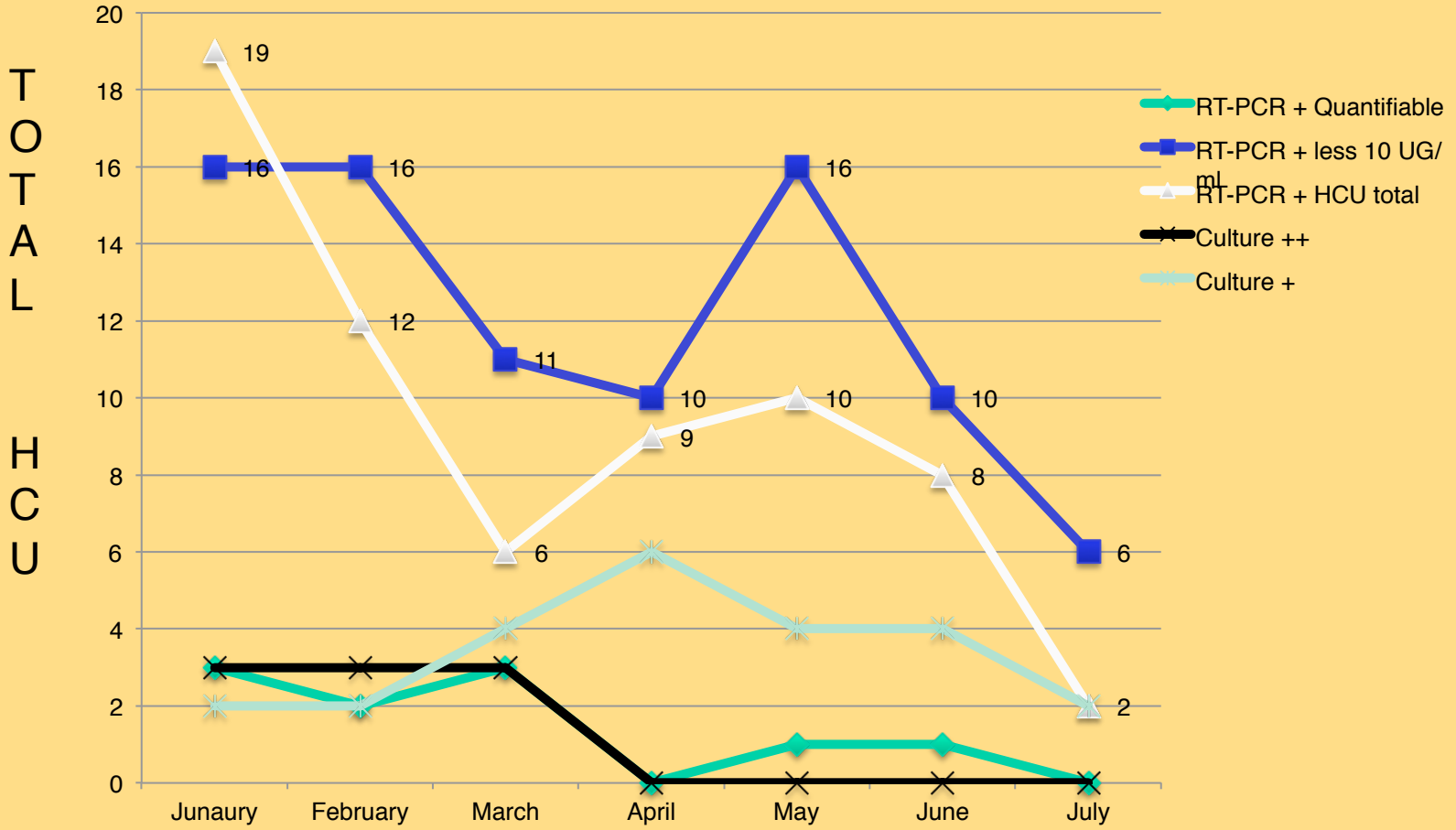
M. Chimaera contamination in the province 2016-2017.

		contaminated HCU (%)		
		Culture	RT-PCR	RT-PCR
Site (N=9)	Nbr of HCU	06-2016	05-2017	07-2017
A	11	7 (64 %)	0	1*
B	11	6 (54,5 %)	0	0
C	3	3 (100 %)	1*	1*
D	2	2 (100 %)	1*	0
E	10	10 (100 %)	*3+1	1+1*
F	6	6 (100 %)	1*	0
G	2	0	0	0
H	2	0	0	0
I	4	0	0	0
Total	51	34 (66,6 %)	7	4

* Positive culture cfu less than 5

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Evolution of HCUs contamination /



Collaborations

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Laboratoire de santé publique du Québec

- Jean Longtin
- Christine Martineau
- Huguette Faucon
- Melissa Fontaine
- Dr. M-C Domingo
- Dre Cecile Tremblay

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McGill

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- Steve Drolet

Montréal University

- Dr Richard Marchand
- Dr Louise Poirier

Zurich University

- Prof Eric Botteger
- Dr Peter Keller

NML-PHAC

- Kym antonation Ph.D
- Puilippe Mabon

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