

# Workflow of laboratory diagnosis of *Candida auris*

PHLSB, Centre for Health Protection

Aug 2019



# Overview

- Specimen reception
- Fungal culture
- Species identification
- Susceptibility testing
- Typing



# Specimen sources

## Potential sources of *C. auris*:

1. Referral isolates from other laboratories
  - Hospital Authority (HA) hospitals
  - Private hospitals & other laboratories
2. Directly isolated from clinical specimens at PHLSB
  - GOPC
  - Sentinel surveillance
  - Dermatology/ social hygiene clinics



# Fungal culture

- Incubation at two different temperatures (29°C & 37°C) for 24 to 72h on:
  - Blood agar
  - Sabouraud agar
  - Chromogenic agar (CHROMID Candida) (37°C only)
- Enrichment broth for screening



# Appearance of *C. auris* colonies



- Pink, red or purple colonies on CHROMagar
- Cream to white colonies on SAB agar
- *C. auris* colonies can resemble those of other *Candida* spp. on CHROMagar
  - *C. haemulonii*, *C. duobushaemulonii*, *C. pseudohaemulonii*
  - *C. krusei*, *Kluyveromyces marxianus*, *Wickerhamomyces anomalus* (also pink, but may be morphologically different)



# Species identification

- Conventional phenotypic methods
  - e.g. VITEK 2 YST card
- Matrix-assisted Laser Desorption/Ionization – Time of Flight (MALDI-TOF) Mass Spectrometry
  - e.g. Bruker MALDI Biotyper
- Internal transcribed spacer (ITS) sequencing

All of the above are currently available and in use in PHLSB.



# *C. auris* species identification

Method	Advantage	Disadvantage
Conventional phenotypic identification	Traditional method	Occasional misidentification
MALDI-TOF MS	Fast, cheap, modern method of choice	Expensive hardware, needs up-to-date database
ITS sequencing	Informative, can be used even for novel species	Relatively higher cost and technical complexity



# Species identification (cont'd)

- PHLSB has capacity for routine identification of *C. auris* since Oct 2016:
  - MALDI-TOF database update on Oct 2016 included *C. auris* identification
  - Reference strain JCM15448 (as control) acquired in Oct 2017
  - Correctly identified all *C. auris* in RCPAQAP samples in Dec 2017 and Sep 2018





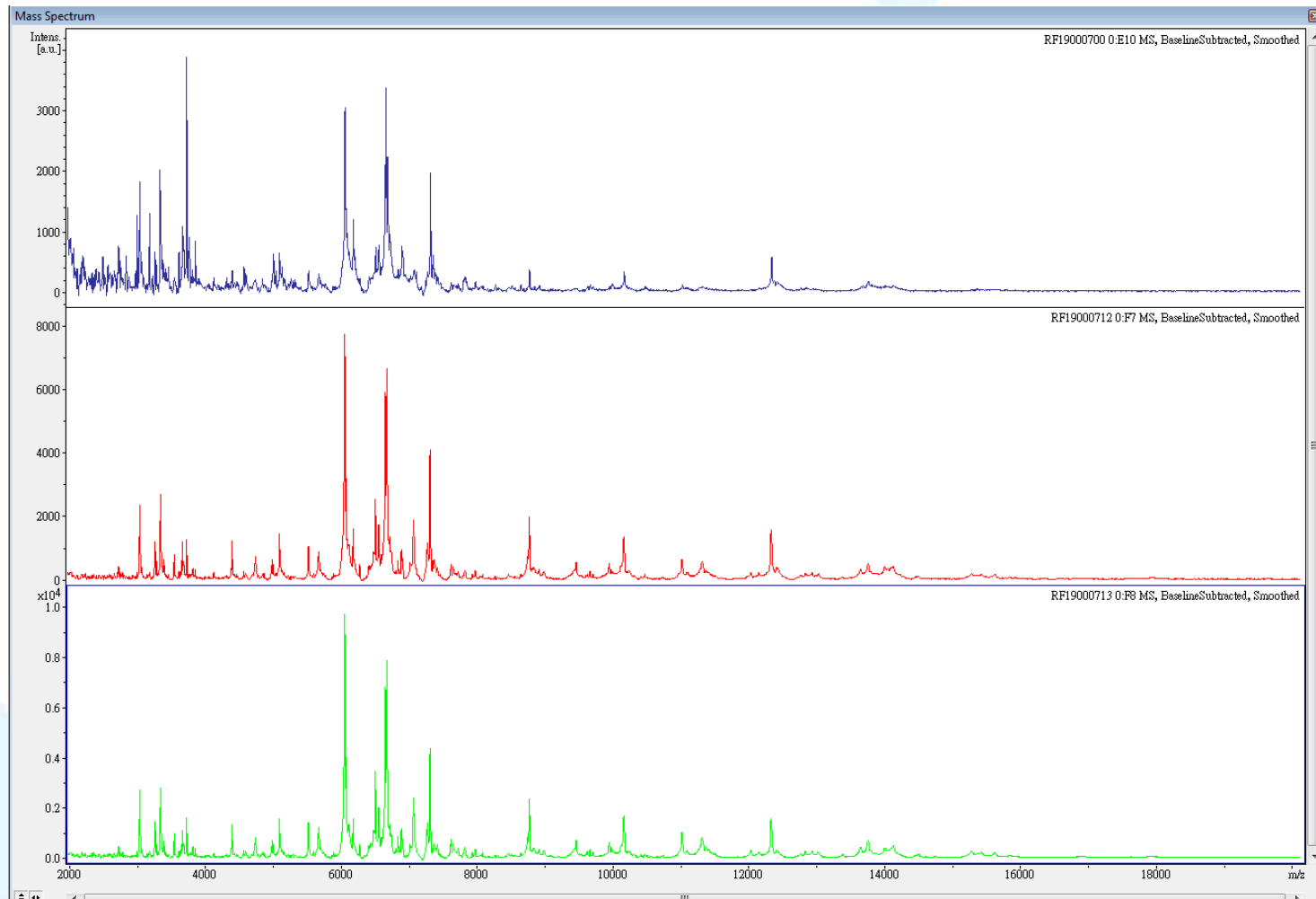
# Example of a YST result for *C. auris*

Identification Information	Card: YST	Lot Number: 2430834403	Expires: Mar 9, 2020 12:00 CST
	Completed: Jun 26, 2019 09:11 CST	Status: Final	Analysis Time: 17.83 hours
Organism Origin	VITEK 2		
Selected Organism	99% Probability <b>Candida auris</b>		Confidence: Excellent identification
	Bionumber: 4110145245321771		
SRF Organism			
Analysis Organisms and Tests to Separate:			
Analysis Messages:			
Contraindicating Typical Biopattern(s)			

Biochemical Details																	
3	LysA	-	4	IMLTa	-	5	LeuA	+	7	ARG	+	10	ERYa	-	12	GLYLa	-
13	TyrA	+	14	BNAG	-	15	ARBa	-	18	AMYa	-	19	dGALa	-	20	GENa	-
21	dGLUa	+	23	LACa	-	24	MAdGa	-	26	dCELa	-	27	GGT	-	28	dMALa	+
29	dRAFa	+	30	NAGA1	-	32	dMNEa	+	33	dMELa	-	34	dMLZa	+	38	ISBEa	-
39	IRHAa	-	40	XLTa	-	42	dSORa	+	44	SACa	+	45	URE	-	46	AGLU	+
47	dTURa	+	48	dTREa	+	49	NO3a	-	51	IARaA	(-)	52	dGATa	+	53	ESC	-
54	IGLTa	+	55	dXYLa	-	56	LATa	-	58	ACEa	+	59	CITa	+	60	GRTas	+
61	IPROa	+	62	2KGa	+	63	NAGa	+	64	dGNTa	+						



# Example of MALDI-TOF spectra for *C. auris*



# Example of a MALDI-TOF report for *C. auris*

Analyte4



Analyte Name: E10  
 Analyte Description:  
 Analyte ID: RF19000700  
 Analyte Creation Date/Time: 2019-06-24T10:37:08.969  
 Applied MSP Library(ies): PHLSB LIBRARY, PHLSB Mycology, Filamentous Fungi, BDAL  
 Applied Taxonomy Tree:

Rank (Quality)	Matched Pattern	Score Value	NCBI Identifier
1 (++)	Candida auris AR0390_CAU_10 CDC	2.083	<a href="#">5475</a>
2 (++)	Candida auris 10051297 CWZ	2.036	<a href="#">5475</a>
3 (+)	Candida auris DSM 21092T CBS	1.94	<a href="#">5475</a>
4 (+)	Candida auris JCM15448	1.905	<a href="#">129541386</a>
5 (+)	Candida auris CBS KCTC_17810 CBS	1.881	<a href="#">5475</a>



# Susceptibility testing

- *C. auris*-specific resistance breakpoints not available yet
- Susceptibility testing results interpretation “borrowed” from other *Candida* spp.
- Methods:
  - Disk diffusion
  - Etest
  - Broth microdilution (CLSI reference method)
    - Vitek 2
    - Sensititre



# Sensititre YeastOne



## List of drugs tested:

- Azoles – fluconazole, itraconazole, voriconazole, posaconazole, ketoconazole
- Polyenes – amphotericin B
- Echinocandins – caspofungin, anidulafungin, micafungin
- Others – 5-flucytosine



# Typing

## Rapid methods:

- MALDI-TOF MSP analysis
- ITS sequencing

## “Proper” typing methods:

- AFLP
- MLST
- Whole Genome Sequencing (currently “best” method)



# Clades

Figure

*C. auris* strains can be separated into 4 main geographic clades:

- South Asian
- South African
- South American
- East Asian

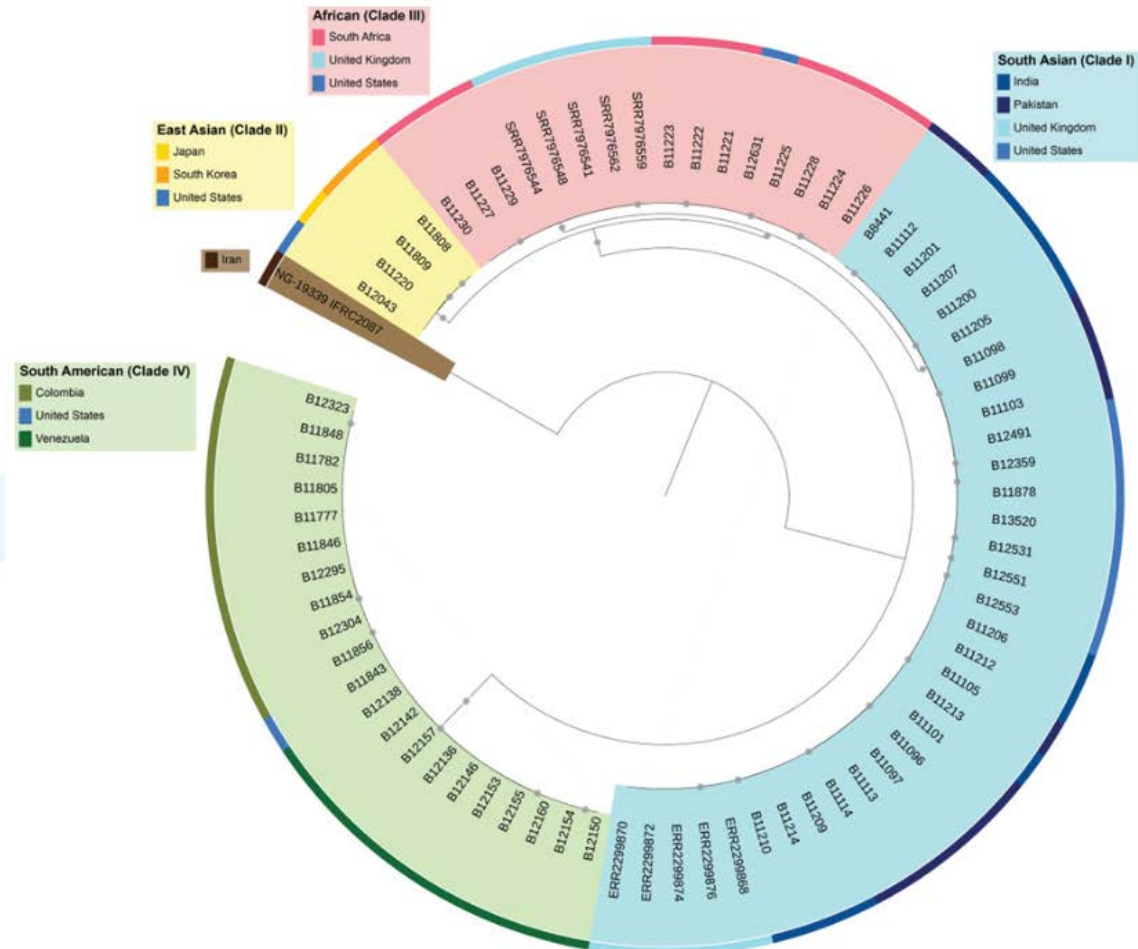


Figure. Major clades of *Candida auris*. Maximum-likelihood phylogenetic tree shows isolates from *C. auris* cases from 10 countries. Circles at nodes indicate separations with a bootstrap value  $\geq 99\%$ .

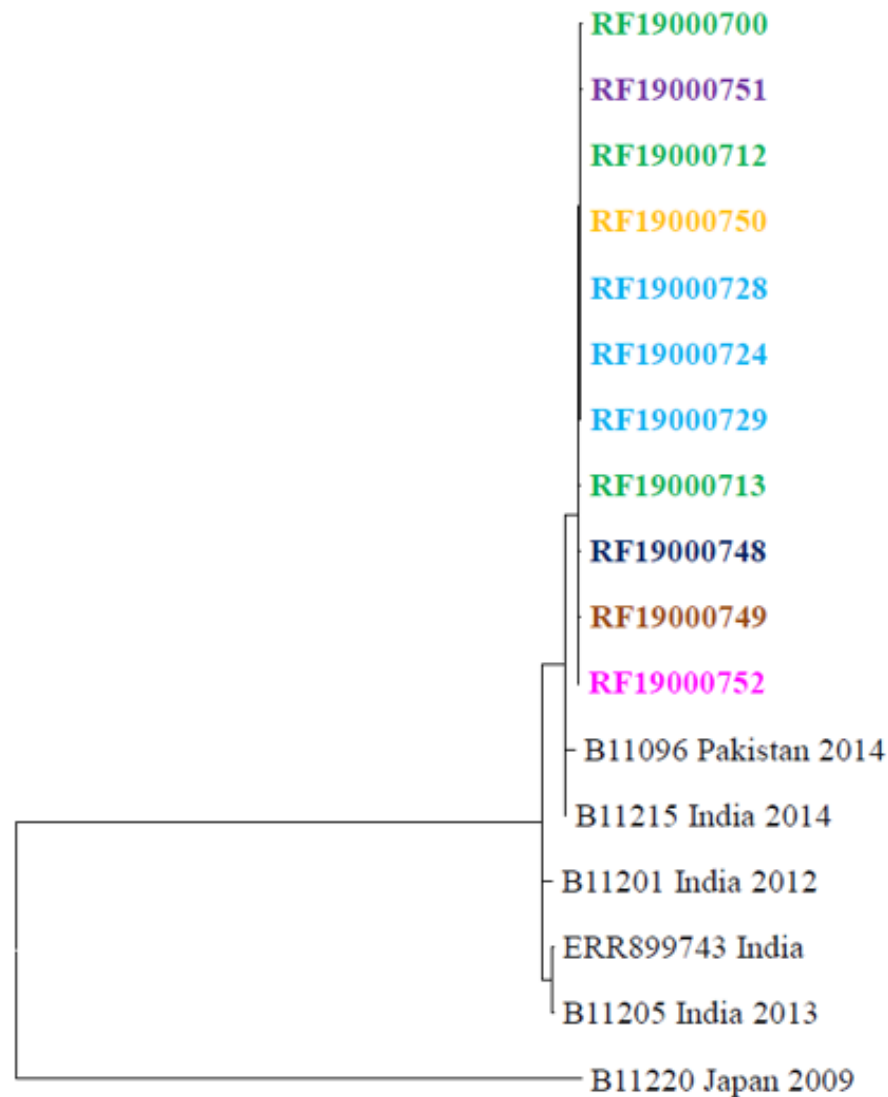
- So far, only WGS is known to be useful for reliable differentiation between and within clades

**Table 1.** Characteristics of the first seven cases of *Candida auris* received by PHLC.

Patient	Lab no.	Specimen Type	Date collected
1	RF19000700	Endotracheal aspirate	14/6/2019
	RF19000712	Pooled swab, nasal, axilla and groin	24/6/2019
	RF19000713	Rectal swab	22/6/2019
2	RF19000724	Pooled swab, nasal, axilla and groin	25/6/2019
	RF19000728	Pooled swab, axilla and groin	28/6/2019
	RF19000729	Nasal swab	29/6/2019
3	RF19000748	Skin swab (axilla and groin)	4/7/2019
4	RF19000749	Skin swab (axilla and groin)	4/7/2019
5	RF19000750	Skin swab (axilla and groin)	4/7/2019
6	RF19000751	Skin swab (axilla and groin)	5/7/2019
7	RF19000752	Skin swab (axilla and groin)	4/7/2019







H  
50 SNPs

**Fig 1b.** Phylogenetic tree showing the genetic relationships among isolates within the South Asia (India/Pakistan) clade. The isolates from the seven patients were indicated by different colors.



# Referral for confirmation / characterization

## Appendix 1

Microbiology Division, Public Health Laboratory Services Branch (PHLSB)  
Centre for Health Protection, Department of Health, HKSAR

### Referral Form for Identification / Characterization of Culture Isolates

Date: \_\_\_\_\_

Referring hospital: \_\_\_\_\_

Requesting doctor: \_\_\_\_\_

Contact phone no.: \_\_\_\_\_ Your lab. no: \_\_\_\_\_

Patient identifier: (OR Gum label)

Name \_\_\_\_\_

Sex / age \_\_\_\_\_

HKID no. \_\_\_\_\_

Clinical information:

Clinical diagnosis: \_\_\_\_\_ Onset date of illness: \_\_\_\_\_

Details of request:

Specimen site: \_\_\_\_\_ Date of collection: \_\_\_\_\_

Test requested\*: Identification / Others: \_\_\_\_\_

Medium sent: Name: \_\_\_\_\_ Incubated for: \_\_\_\_\_ hours

Incubation\*: Aerobic / MICROaerophilic / ANaerobic \_\_\_\_\_

Preliminary laboratory findings:

Gram stain morphology: \_\_\_\_\_

MALDI-TOF ID: \_\_\_\_\_ Log score / Confidence: \_\_\_\_\_ \*BioTyper / VITEK MS

Presumptive identification of isolate: \_\_\_\_\_

Other relevant information: \_\_\_\_\_

\*Please circle



Thank you

