



UNIVERSITÀ
DEGLI STUDI DI BARI
ALDO MORO

MYCOLOGY

SHORT MASTER



DIPARTIMENTO
MEDICINA
VETERINARIA



I LIEVITI A LIVELLO DI SPECIE

Claudia Cafarchia, Dipartimento di Medicina Veterinaria, Università degli Studi di Bari Aldo Moro.

CARATTERE MACROSCOPICO



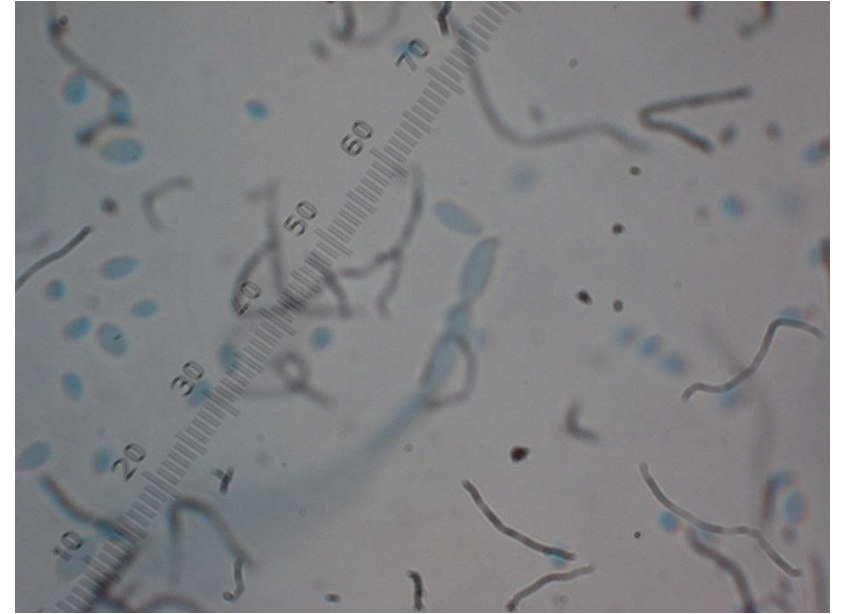
De Hoog, G.S., Guarro, J., 1999. Atlas of clinical fungi. In: de Hoog GS, Guarro J, eds. Centraal bureau voor Schimmel cultures Baarn and Delft, 2nd edn. The Netherlands.

Simplified key to the main groups of clinically relevant fungi

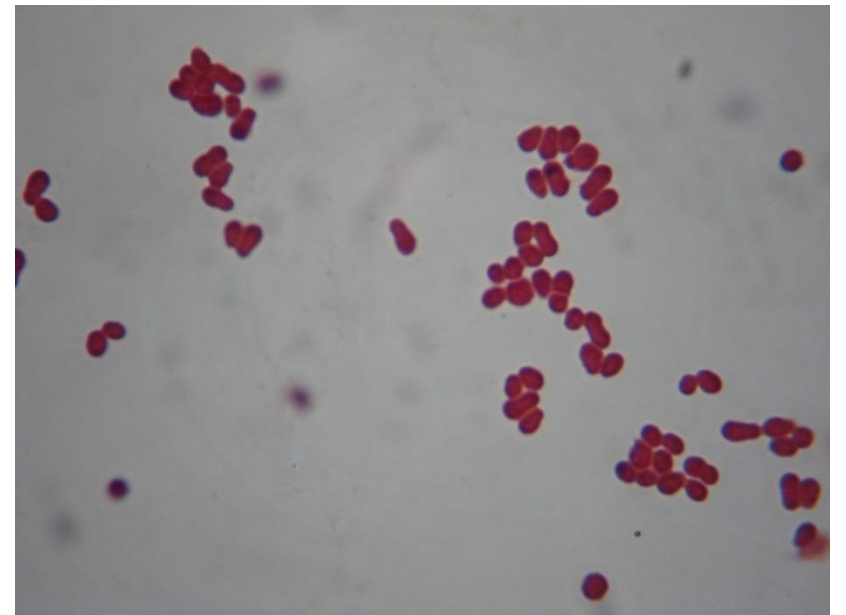
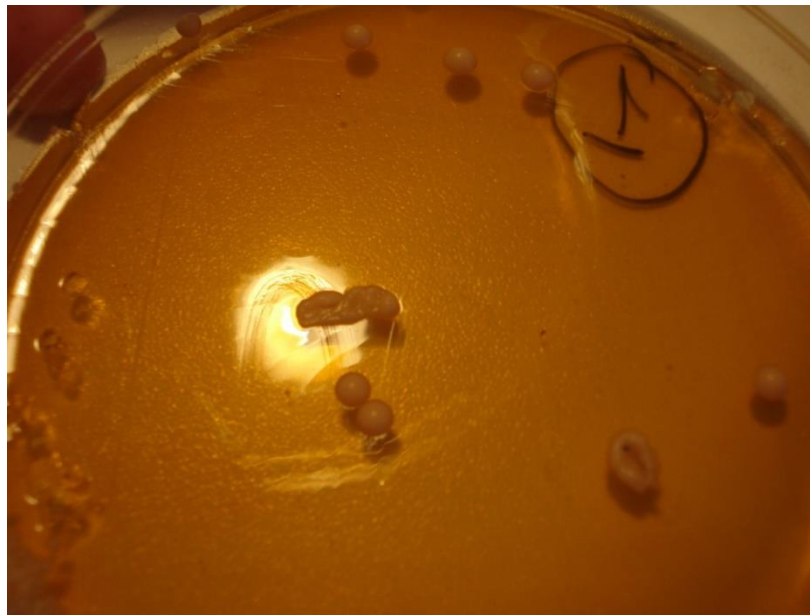
- 1a. Fungus not culturable *Mesomycetozoa, Archiascomycetes*
- 1b. Fungus culturable 2
- 2a. Loose budding cells abundant 3
- 2b. Thallus entirely consisting of filaments 4
- 3a. Colonies white or pink **Yeasts**
- 3b. Colonies black **Black yeasts and relatives**
- 4a. Mycelium regularly septate 5
- 4b. Mycelium nearly aseptate 8
- 5a. Clamp connections present, at least on
some hyphae **Filamentous basidiomycetes**
- 5b. Clamp connections absent 6
- 6a. Fruit bodies absent **Hyphomycetes**
- 6b. Fruit bodies present 7
- 7a. Fruit bodies containing spores in asci *Eusascomycetes*
- 7b. Fruit bodies containing loose conidia **Coelomycetes**
- 8a. Sporulation abundant *Zygomycota*
- 8b. Sporulation absent; zoospores formed in water cultures *Oomycota*

CARATTERE MACROSCOPICO

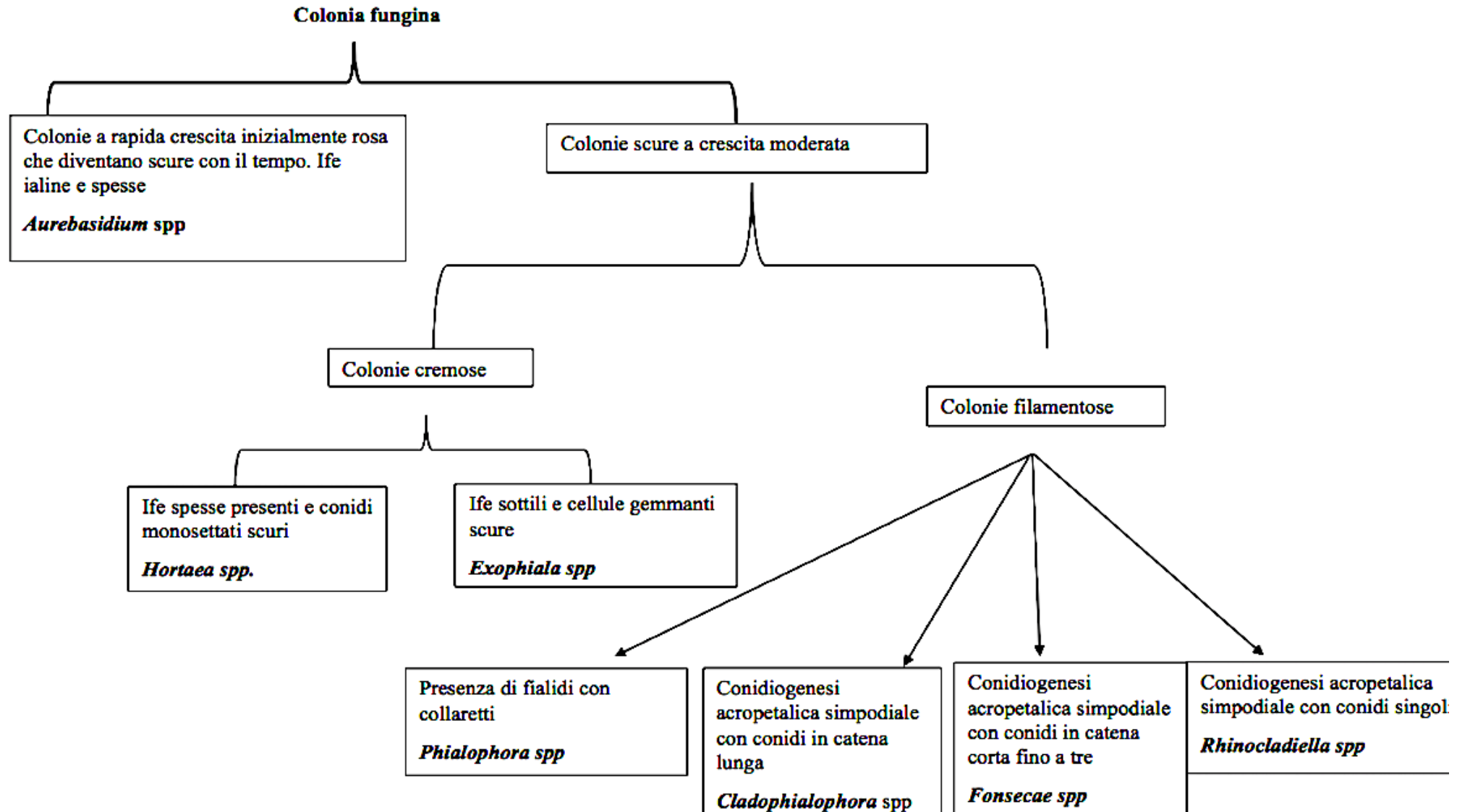
COLONIE NERE E ASPETTO
LIEVITIFORME



COLONIE BIANCHE COCCHI
GEMMANTI (ATTENTI
GEMMAZIONE)

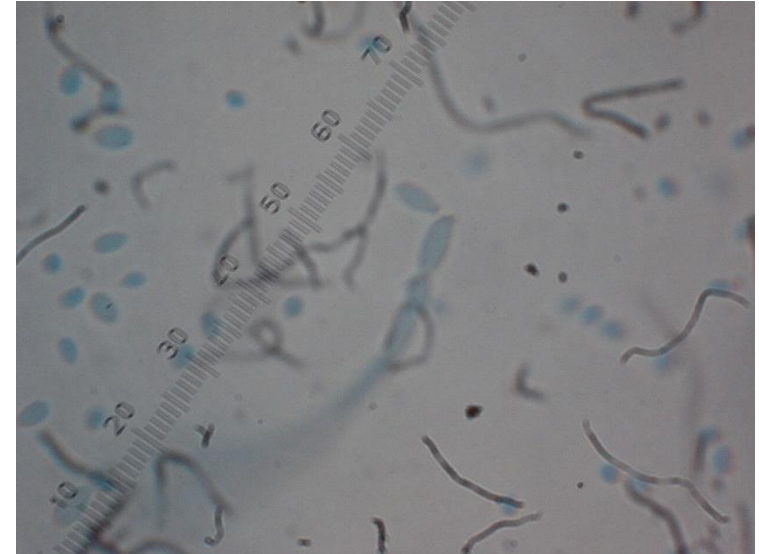


BLACK YEASTS E RELATIVES

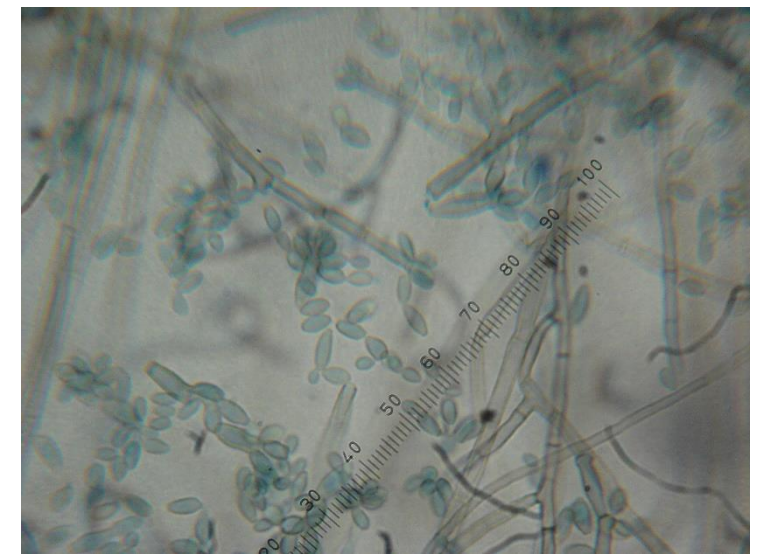


BLACK YEASTS AND RELATIVES

Exophiala spp

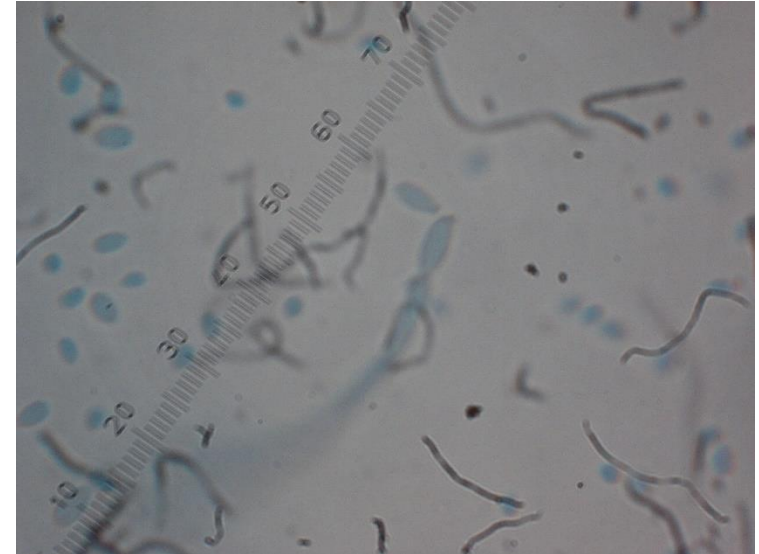


Fonsecaea pedrosoi

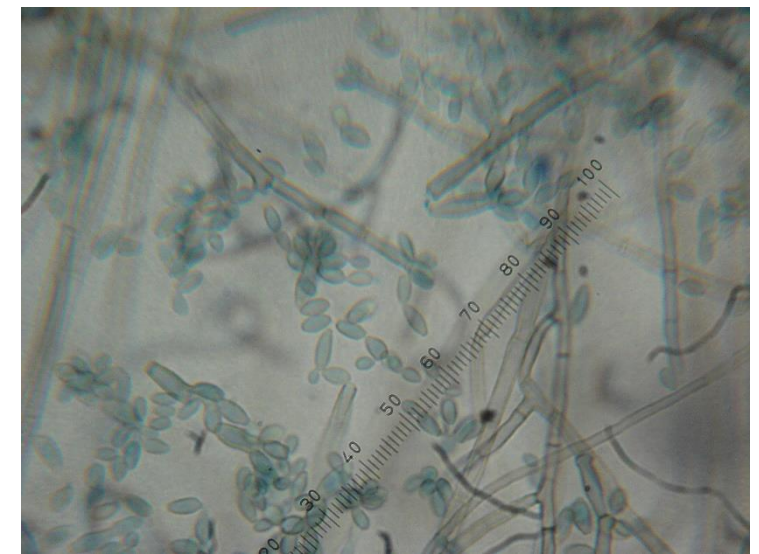


BLACK YEASTS AND RELATIVES

Exophiala spp



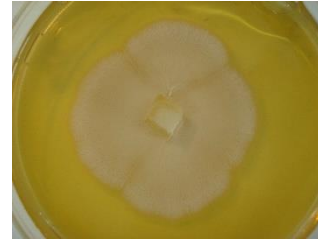
Fonsecaea pedrosoi



I LIEVITI

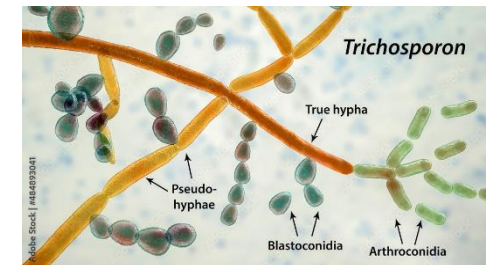
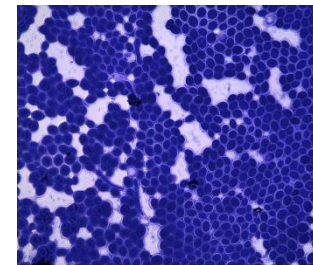
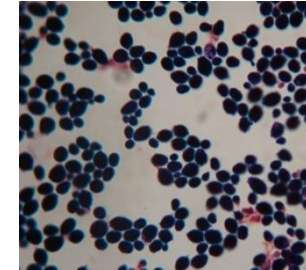
ASCOMICETI

Geotrichum
Candida



BASIDIOMICETI

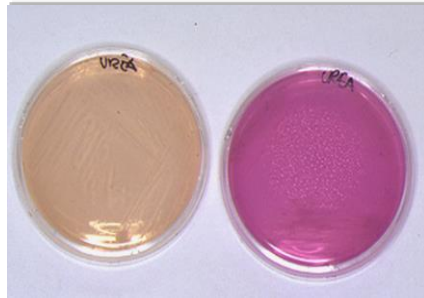
Cryptococcus
Malassezia
Rhodotorula
Trichosporon



<https://stock.adobe.com/it/images/structure-of-fungus-trichosporon-3d-illustration/484893041>

I LIEVITI

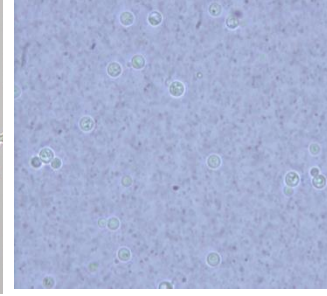
Prove morfologiche
Identificazione



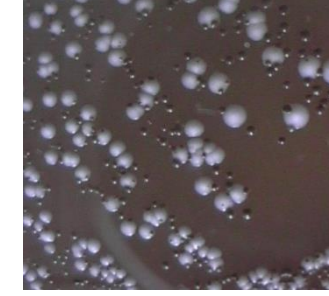
UREASI



GERM TEST



INCHIOSTRO DI KINA



STAIB MEDIUM

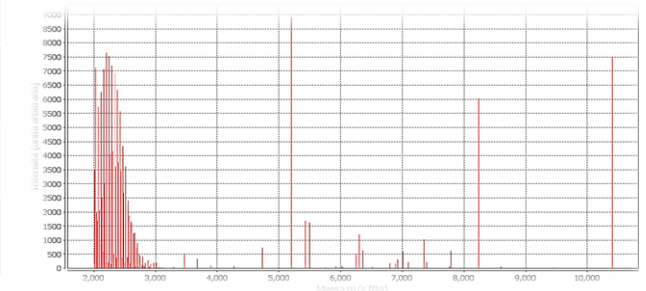
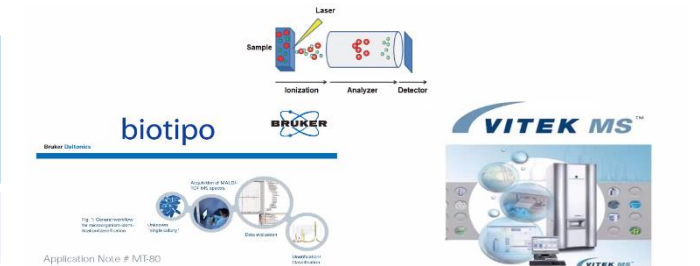


CHROMAGAR CANDIDA

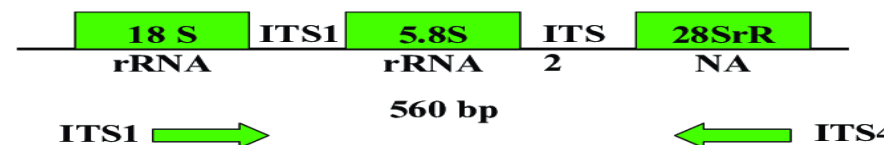
Assimilazione
zuccheri/
profilo proteico
(MALDI-TOF MS)

Prodotti (Ditte)	% di non corretta identificazione
Api Candida® (bioMérieux)	0.5 - 12.8
Api 20C AUX® (bioMérieux)	0.0 - 5.3
Auxacolor™ (Bio-Rad)	0.0 - 5.6
Candifast® (ELITech Group)	6.0 - 23.6
Fungichrom® (ELITech Group)	1.7 - 4.5
Fungifast® (ELITech Group)	1.0 - 2.6
ID 32 C® (bioMérieux)	0.0 - 23.2
RapID™ Yeast Plus (Remel)	0.7 - 16.0
Uni-Yeast-Tek® (Remel)	0.2 - 13.1

Prodotti	% Non corretta identificazione
Biolog YT Microplate™ (Biolog)	38.8 - 42.7
MicroScan® Rapid YS (Siemens)	2.8 - 8.2
Sherlock® MIS (MIDI, Inc.)	15.8 - 23.7
Vitek® YBC (bioMérieux)	1.5 - 15.1
Vitek® 2 YST (bioMérieux)	0.9 - 4.7



PCR: ITS1-ITS4

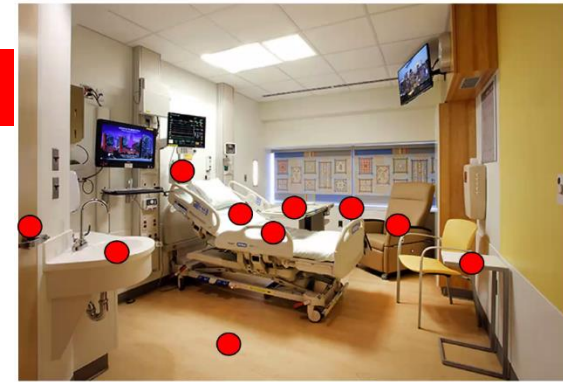
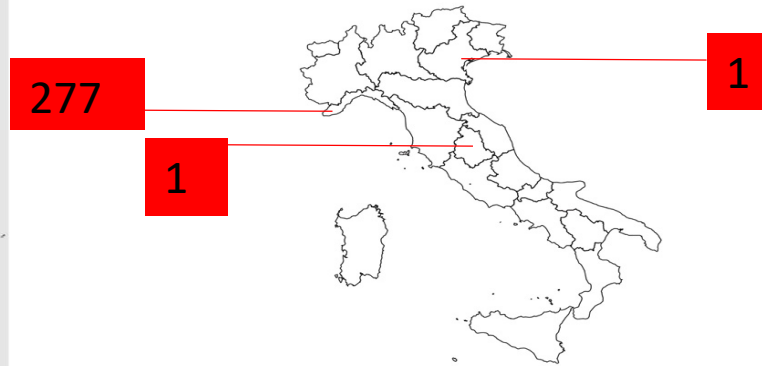
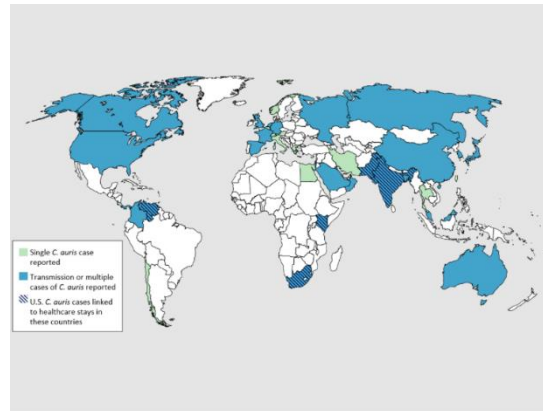


BLAST CBS:

https://wi.knaw.nl/page/Pairwise_alignment

Candida auris ???????

- Colonizzatore cute altamente diffusibile
- Aumento di casi registrati
- Frequenti i focolai epidemici
- Ceppi resistenti
- Patologia notificabile
- Misure di profilassi



(Screening dei pazienti; Precauzioni per il contatto; Screening degli ambienti e del personale a contatto con il paziente; Procedure per la decolonizzazione; Management degli ambienti; Management della comunità)

Regno Unito (PHE), Stati Uniti (CDC), Europa (ECDC) e Sudafrica (COTHI)



80%



31%



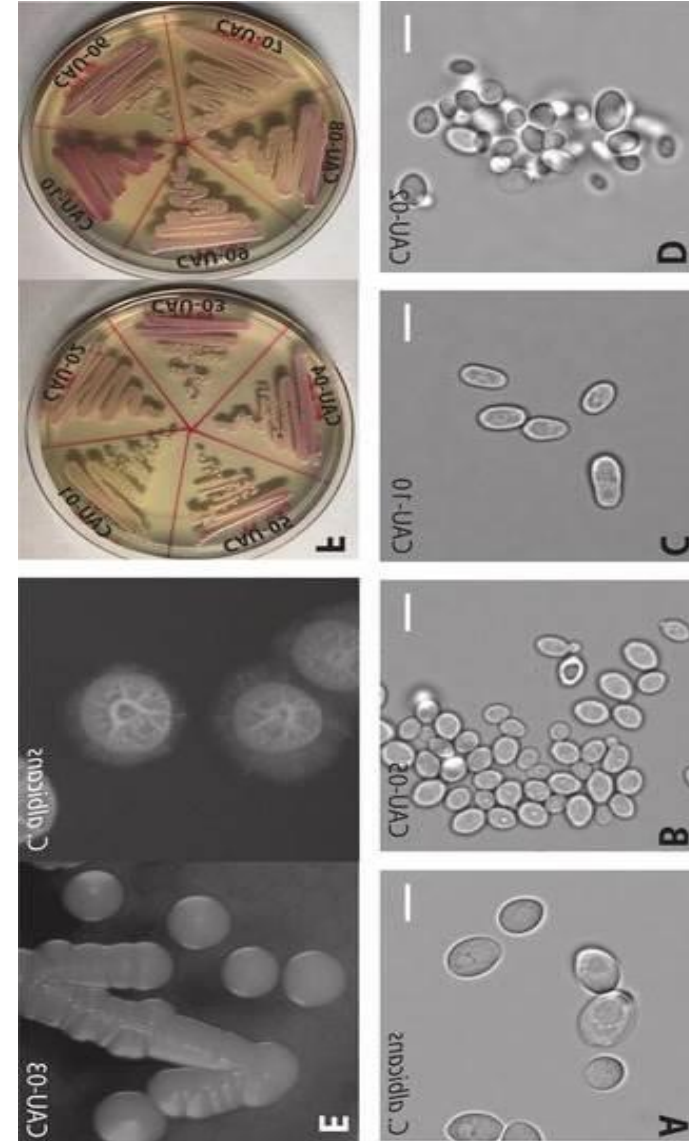
1 %

Table 1 Key points for *C. auris* prevention and control by the European Centre for Diseases Prevention and Control (ECDC) and Centers for Disease Control and Prevention (CDC)

ECDC	CDC
<p>Correct identification (MALDI-TOF; DNA sequencing of the D1/D2 domain); Clinicians and microbiologists alertness; Notification and retrospective case-finding</p> <p>Good standard infection control measures (including environmental cleaning, reprocessing of medical devices and patient isolation) and prompt notification</p>	<p>Correct identification (MALDI-TOF; molecular methods) Confirmed isolates of <i>C. auris</i> should be reported to local and state public health officials and to CDC</p> <p>Infection control measures:</p> <ul style="list-style-type: none"> • Placing the patient with <i>C. auris</i> in a single-patient room and using contact precautions • Emphasizing adherence to hand hygiene • Cleaning and disinfecting the patient care environment (daily and terminal cleaning) with recommended products • Screening contacts of newly identified case patients to identify <i>C. auris</i> colonization
<p>Early identification of carriers by using active surveillance cultures (sites considered for sampling include nose/throat, axilla, groin, rectum, insertion sites of venous catheters; clinical samples such as urine, feces, wound drain fluid, and respiratory specimens)</p>	<p>Screening should be performed to identify colonization among potentially epidemiologically linked patients, including:</p> <ul style="list-style-type: none"> • Current roommates • Roommates at the current or other facilities in the prior month (even if they have been discharged from the facility) <p>Screening for <i>C. auris</i> should be done using a composite swab of the patient's axilla and groin (sites of consistent colonization). Patients have also been found to be colonized with <i>C. auris</i> in nose, external ear canals, oropharynx, urine, wounds, and rectum.</p>
<p>Establish the source of the outbreak (epidemiological investigation, cross-sectional patient screening and environmental sampling); prevention of inter-hospital and cross-border transmission</p>	<p>All laboratories, especially laboratories serving healthcare facilities where cases of <i>C. auris</i> have been detected, should:</p> <ul style="list-style-type: none"> • Review past microbiology records to identify cases of confirmed or suspected <i>C. auris</i> • Conduct prospective surveillance to identify <i>C. auris</i> cases in the future • Consider screening close contacts of patients with <i>C. auris</i> for presence of colonization
<p>Enhanced control measures to contain outbreaks (such as contact precautions, single room isolation or patient cohorting, and dedicated nursing staff for colonized or infected patients)</p>	<p>Education of all healthcare personnel, including staff working with environmental cleaning services about <i>C. auris</i> and need for appropriate precautions; Monitor adherence to infection control practices</p>
<p>Education and practice audits (for healthcare workers and contacts)</p>	<p>Antibiotic and antifungal stewardship</p>

Candida auris DIAGNOSI

- Colonie bianche o crema (SDA)
- Rosa o beige (CHROMagar)
- Cellule ovali e gemmanti prive di pseudoife
- Crescita a 37 o 42° C
- Assimila N- acetili glucosammina , succinati e gluconati



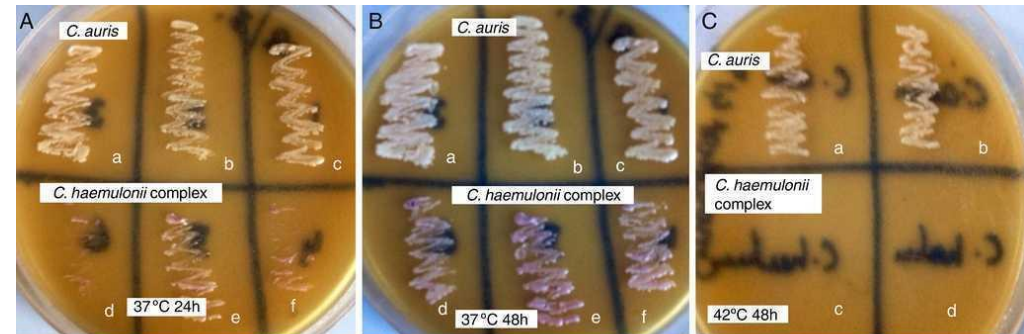
Candida auris DIAGNOSI

PROVE MORFOLOGICHE IDENTIFICAZIONE

CHROMAGAR CANDIDA



CHROMAGAR CANDIDA + PAL'S AGAR (uguale volume dei due terreni mescolati)



Rev Iberoam Micol. 2017;34:109-11

- *C. auris*: colonie lisce e bianche 37° C e 42° C senza pseudoife dopo 24 e 48h.
- *C. haemulonii complex*: colonie lisce e rosa a 37° C con pseudoife dopo 48 h.
- Assenza di crescita a 42° C.

Candida auris DIAGNOSI

ASSIMILAZIONE ZUCCHERI

PROFILO PROTEICO (MALDI-TOF MS)

Identification Method	Database/Software, if applicable	<i>C. auris</i> is confirmed if initial identification is <i>C. auris</i> .	<i>C. auris</i> is possible if the following initial identifications are given. Further work-up is needed to determine if the isolate is <i>C. auris</i> .
Bruker Biotyper MALDI-TOF	RUO libraries (Versions 2014 [5627] and more recent)	<i>C. auris</i>	n/a
	CA System library (Version Claim 4)	<i>C. auris</i>	n/a
bioMérieux VITEK MS MALDI-TOF	RUO library (with Saramis Version 4.14 database and Saccharomycetaceae update)	<i>C. auris</i>	n/a
	IVD library (v3.2)	<i>C. auris</i>	n/a
	Older IVD libraries	n/a	<i>C. haemulonii</i> <i>C. lusitaniae</i> No identification
VITEK 2 YST	Software version 8.01*	<i>C. auris</i>	<i>C. haemulonii</i> <i>C. duobushaemulonii</i> <i>Candida</i> spp. not identified
	Older versions	n/a	<i>C. haemulonii</i> <i>C. duobushaemulonii</i> <i>Candida</i> spp. not identified
API 20C		n/a	<i>Rhodotorula glutinis</i> (without characteristic red color) <i>C. sake</i> <i>Candida</i> spp. not identified
API ID 32C		n/a	<i>C. intermedia</i> <i>C. sake</i> <i>Saccharomyces kluyveri</i>
BD Phoenix		n/a	<i>C. catenulata</i> <i>C. haemulonii</i> <i>Candida</i> spp. not identified
MicroScan		n/a	<i>C. lusitaniae</i> ** <i>C. guilliermondii</i> ** <i>C. parapsilosis</i> ** <i>C. famata</i> <i>Candida</i> spp. not identified
RapID Yeast Plus		n/a	<i>C. parapsilosis</i> ** <i>Candida</i> spp. not identified

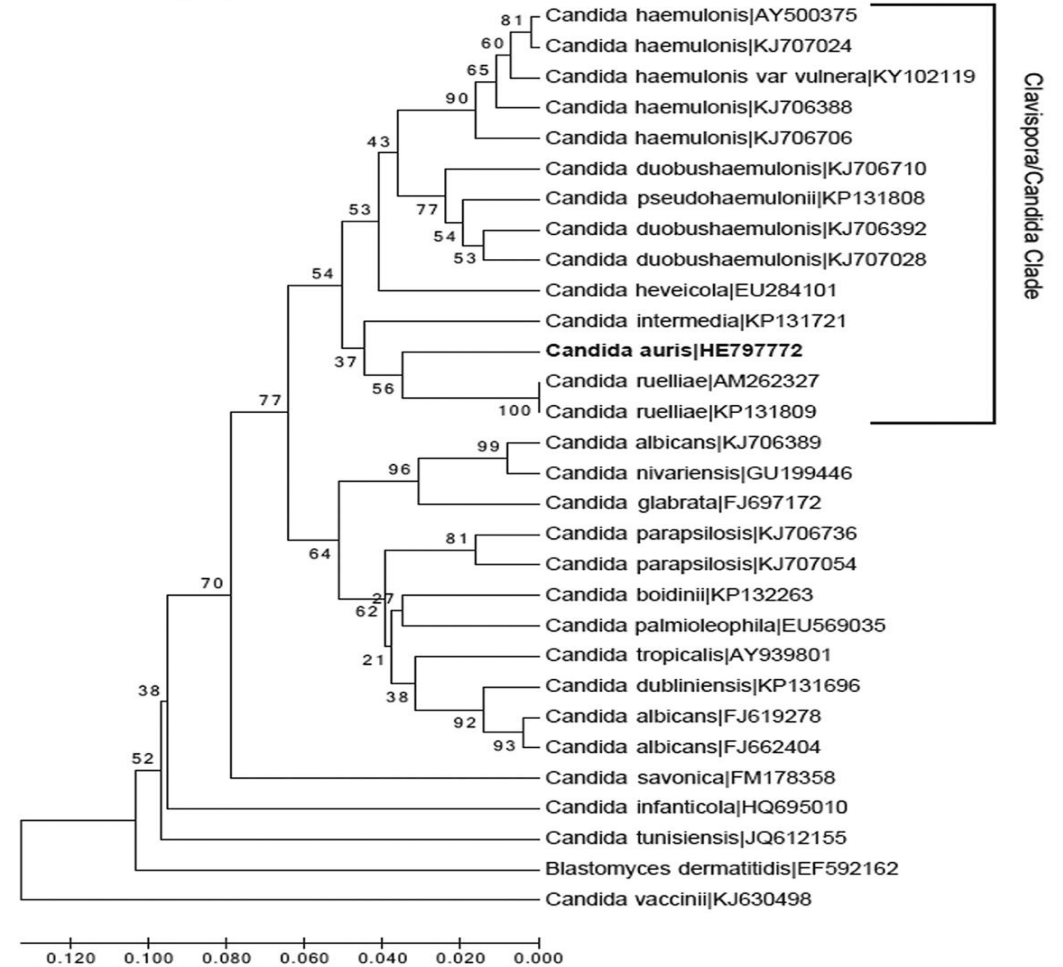
Candida auris DIAGNOSI

IDENTIFICAZIONE MOLECOLARE

ITS1 (5'-TCCGTAGGTGAACCTGCGG-3')

ITS4 (5'-TCCTCCGCTTATTGATATGC-3')

A. ITS 1/2 Phylogenetic Tree



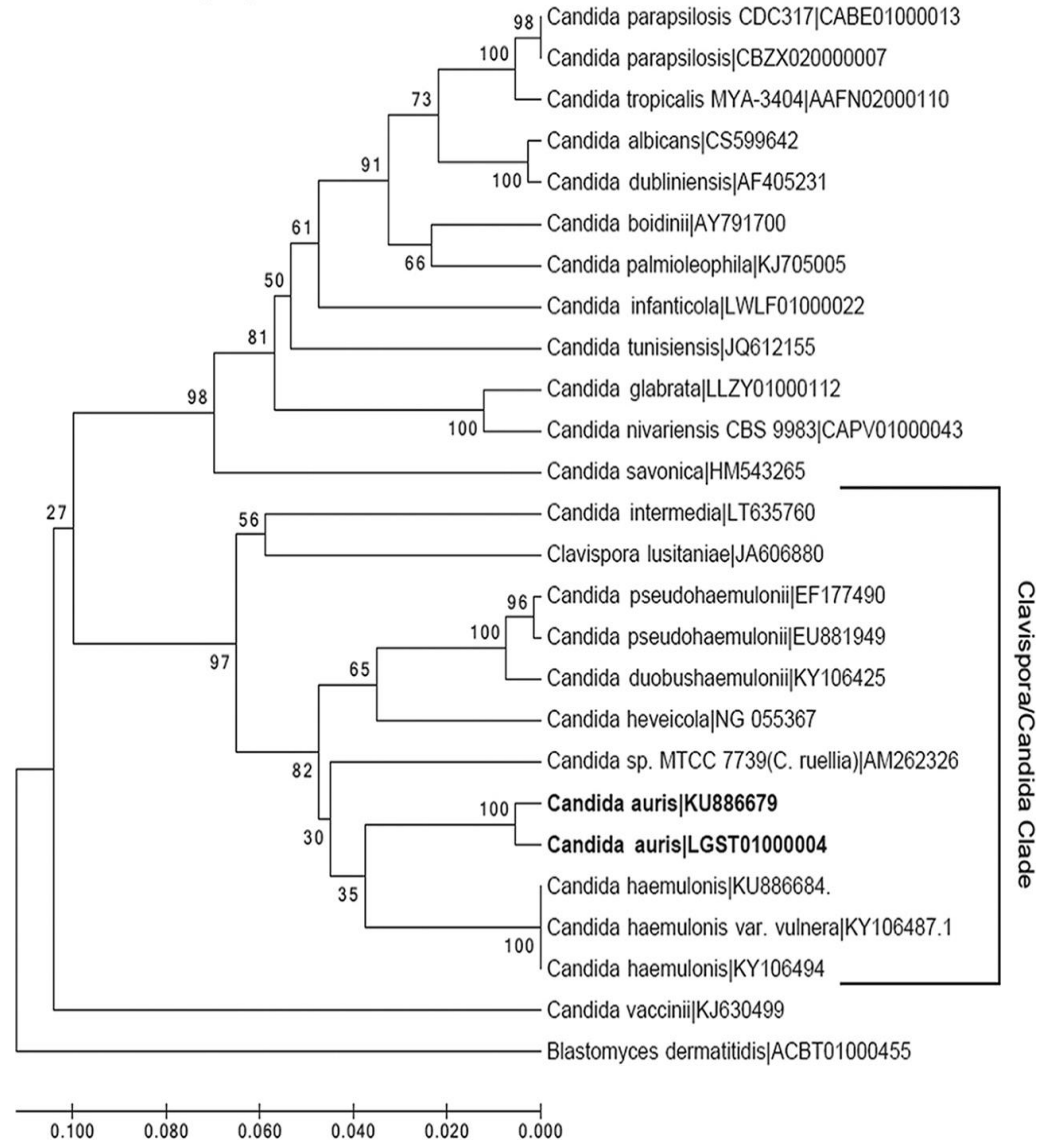
Candida auris DIAGNOSI

IDENTIFICAZIONE MOLECOLARE

NL-1 (5=-GCATATCAATAAGCG GAGGAAAAG-3=)

NL-4 (5= -GGTCCGTG TTTCAAGACGG-3=)

B. D1/D2 Phylogenetic Tree



Candida auris DIAGNOSI

IDENTIFICAZIONE MOLECOLARE

- Quattro raggruppamenti di *C. auris*. (South Asian, South African, South American, and East Asian)
- Tra i diversi clade: differenze nucleotidiche <1% (D1-D2 del 28S DNAr)
- Tra le altre specie >18% (D1-D2 del 28S DNAr)

LSU rDNA

Organism	% identity
<i>Candida auris</i> (South Asian clade)	100
<i>Candida auris</i> (South African clade)	99
<i>Candida auris</i> (East Asian clade)	99
<i>Candida lusitanae</i>	82
<i>Candida haemulonii</i>	82
<i>Candida guilliermondii</i>	80
<i>Candida ciferrii</i>	80
<i>Candida pseudohaemulonii</i>	79
<i>Candida duobushaemulonii</i>	79
<i>Candida tropicalis</i>	79
<i>Candida kefyr</i>	79
<i>Candida pelliculosa</i>	78
<i>Saccharomyces cerevisiae</i>	77
<i>Candida utilis</i>	76
<i>Candida famata</i>	75
<i>Candida parapsilosis</i>	70
<i>Candida magnoliae</i>	46
<i>Candida albicans</i>	43
<i>Candida krusei</i>	43
<i>Candida glabrata</i>	42
<i>Candida inconspicua</i>	47

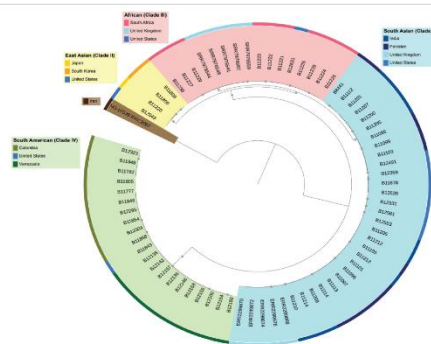


Figure. Major clades of *Candida auris*. Maximum-likelihood phylogenetic tree shows isolates from *C. auris* cases from 10 countries. Circles at nodes indicate separations with a bootstrap value $\geq 99\%$. A color version of this figure is available online (<http://wwwnc.cdc.gov/EID/article/25/9/19-0686-F1.htm>).

Candida auris DIAGNOSI

PROTOCOLLO RAPIDO DI IDENTIFICAZIONE

