Laboratory diagnostics
of infections caused by mycobacteria and related species

Taxonomy

- Order: Actinomycetales
  - Suborder: Corynebacterineae
    - **Corynebacteriaceae*****
    - Dietziaceae*
    - Gordoniaceae*
    - **Mycobacteriaceae*****
    - **Nocardiaceae****
    - Rhodococcaceae*
    - Tsukamurellaceae*

    CMN group, mycolic acids, acid-fastness

Infections caused by aerobic actinomycetes

- Tuberculosis
- Leprosy
- Mycobacteriosis
- Nocardiosis
- Diphtheria

System of mycobacteria according to Runyon

Group	Species	Pigment	Pathogenicity
Slow growth	***M. tbc* complex** ***M. tuberculosis, africanum, bovis, bovis* BCG** **MAI complex** ***M. avium-avium, M. avium-paratuberculosis, intracellulare*** ***M. kansasii, marinum,*** ***ulcerans,*** ***xenopi, gordonae***	N F N S,S	**TUBERCULOSIS,TBC AFRICA, LYMPHATIC NODES, VACC. STRAIN** **BIRDS,** **m. Crohn,** **lymphadenitis** **Mine water, pools, aquarium** **Skin ulcers** **Rarely pneumonia,** **Contamination**
Rapid growth	***M. fortuitum,*** ***cheloneae,*** ***smegmatis,*** ***abscessus***	N S N	Wound infections, osteomyelitis Skin infections Burns Chronic pulmonary infections, wounds
Non-culturable *in-vitro*	*M. leprae*	-	leprosy

Mycobacterium tuberculosis

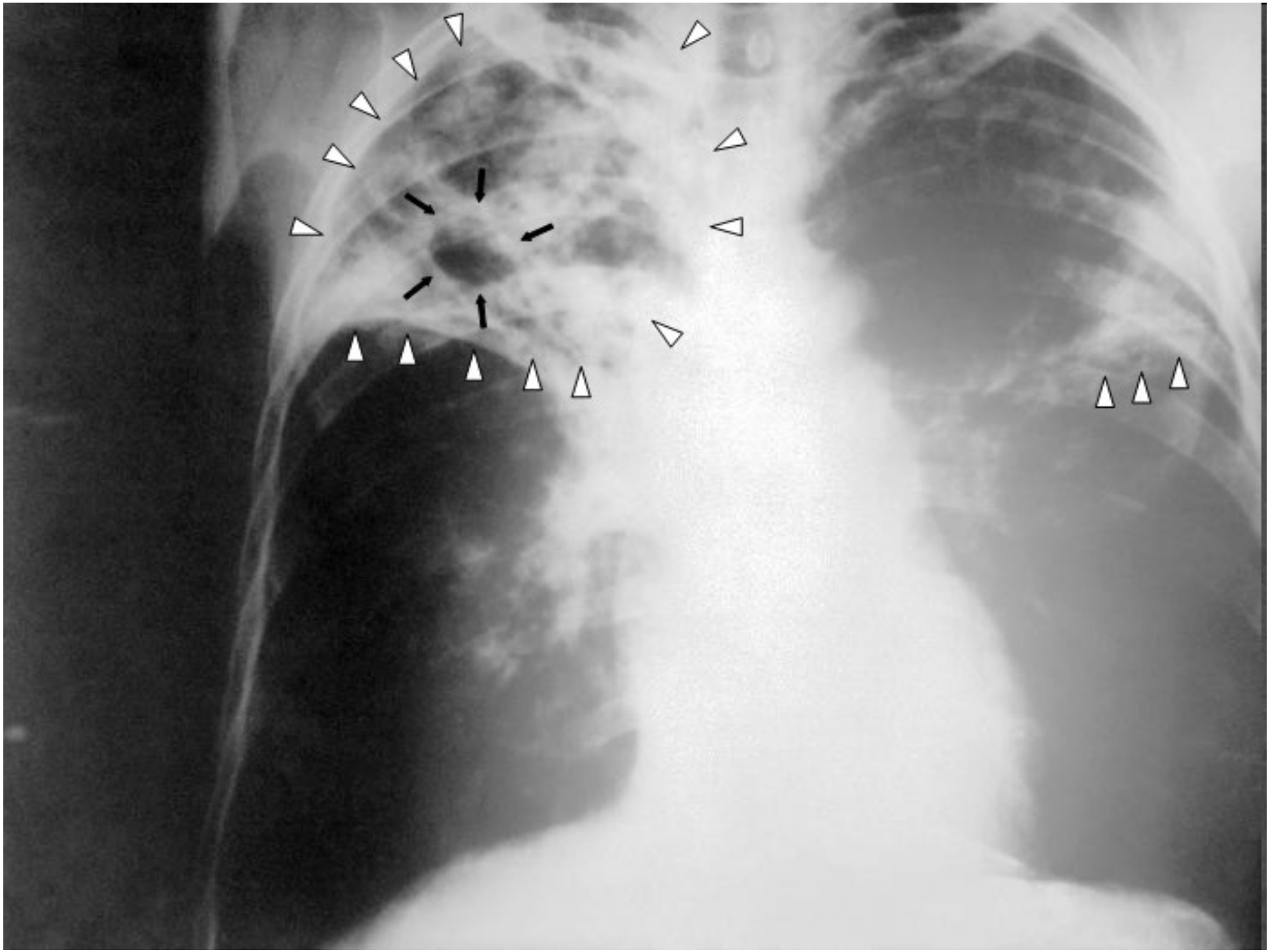
- Definition
  - **Tuberculosis** (**TBC**) – common and deadly infections caused by different mycobacteria, typically *Mycobacterium tuberculosis*
  - discovered by Robert Koch (1892) who described *M. tuberculosis*

Mycobacterium tuberculosis

- Importance
  - one of the most frequent human pathogen
  - about 1/3 of mankind is infected
  - about 10% of infected people develop symptomatic infection
  - 3,000.000 deaths attributed to TBC per year
  - Robert Koch, 1892
- *M. tuberculosis* complex
  - *M. bovis*, BCG, *M. africanum*

Mycobacterium tuberculosis

- **Pulmonary tuberculosis**
- **Extrapulmonary tuberculosis**
  - **lymphadenitis**
  - **miliary tuberculosis**
  - **neurotuberculosis – tuberculous meningitis**
  - **gastrointestinal tuberculosis**
  - **peritoneal tuberculosis**
  - **genitourinary tuberculosis**
  - **skeletal tuberculosis**
  - **Tuberculous abscess**
  - **tuberculosis and HIV/AIDS**
  - ***Mycobacterium tuberculosis* with multiply resistance to ATB (**Multi Drug Resistant TuBerculosis **= MDR-TB)**



Mycobacterium tuberculosis

- Clinical course of tuberculosis
  - Asymptomatic, latent form
  - Progressive development - phthisis
  - Disseminated infection, miliary tuberculosis
  - Granuloma
  - Reactivation, reinfection
  - Primary complex
  - Active TBC, if not treated, mortality about 50%

Mycobacterium tuberculosis

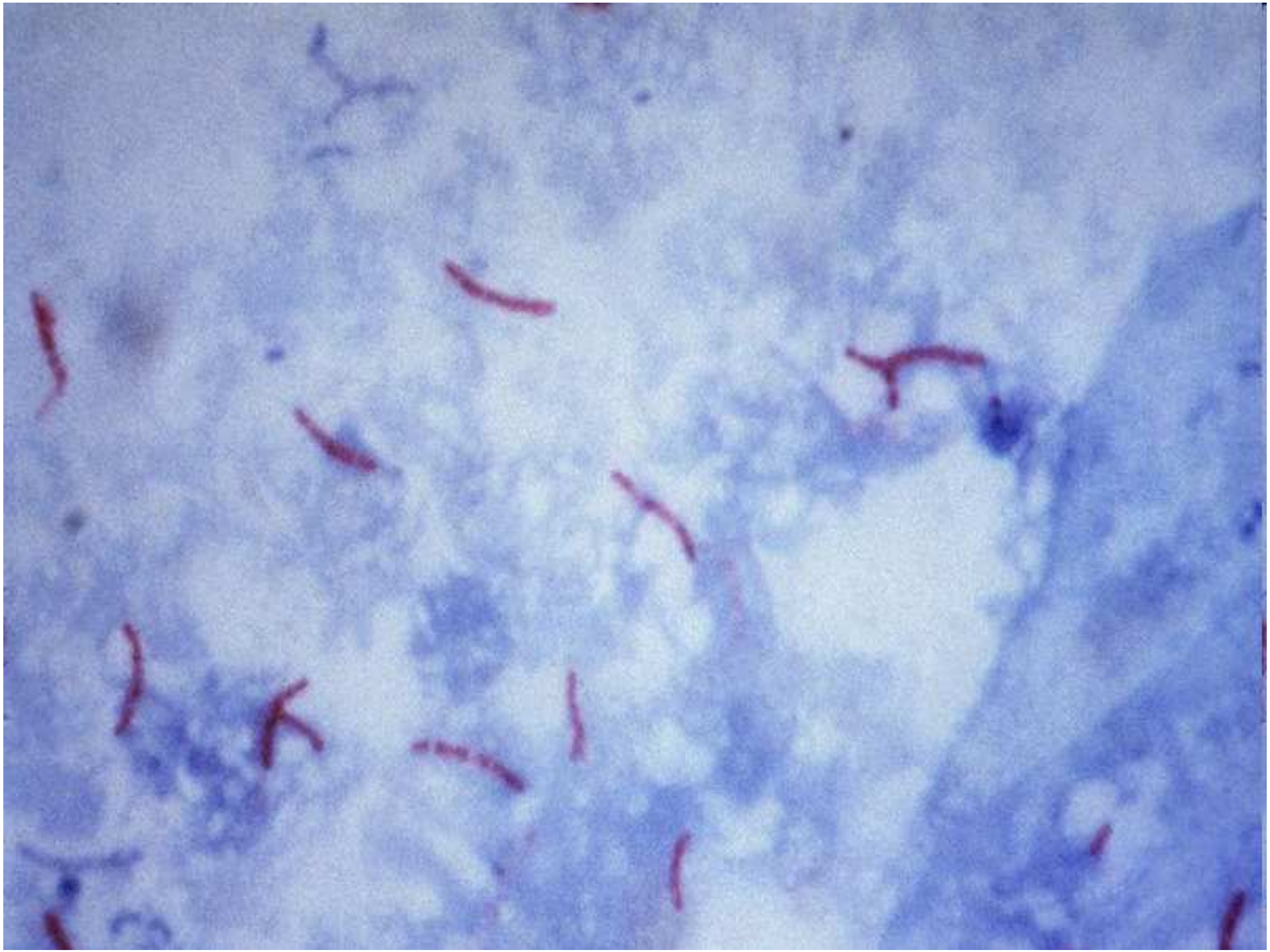
- Clinical diagnosis
  - X-ray
  - tuberculin (PPD=purified protein derivative) skin test (Mantoux)
  - Laboratory investigation of blood
  - Microscopy, culture, molecular methods
    - sputum, urine, pus, body fluids or bioptic samples

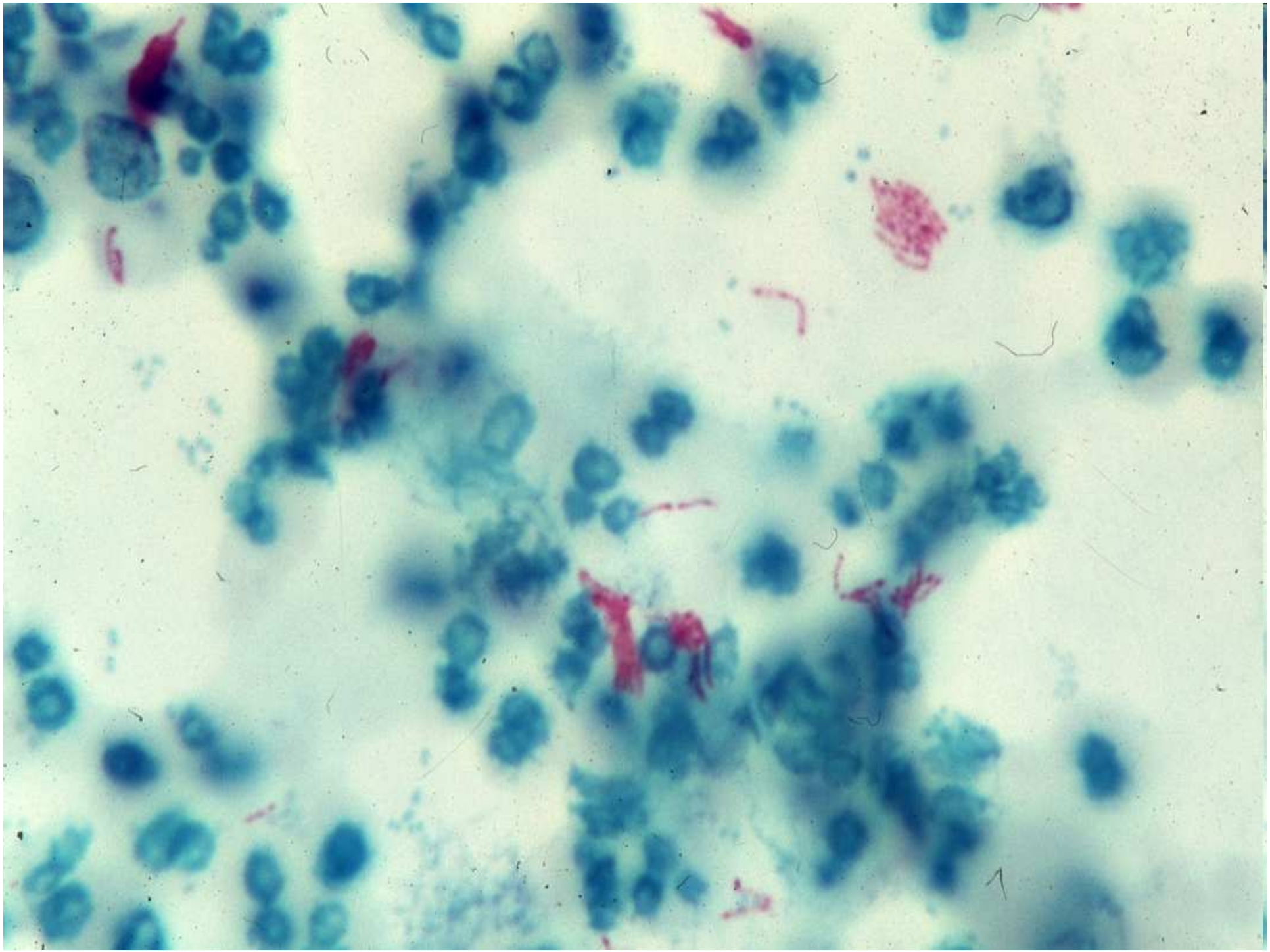
Mycobacterium tuberculosis

- Common characteristics of mycobacteria
  - Morphology (difficult to stain by Gram)
  - Culture (very slow growth on special media)
  - Pathogenicity (tuberculosis and leprosy)
    - cell-mediated immunity
  - Diagnostics: variable
  - Therapy
    - different mechanisms of action - different antibiotics
    - antituberculotic drugs

Mycobacterium tuberculosis

- Morphologie
  - slender rods, acid-fast, 0.3-0.6 x 1-4 µm, „beads“, separatively, clusters, palisades
  - cords in virulent strains
- Staining
  - Ziehl-Neelsen (carbolfuchsin, heating, decolorization of acid alcohol, counter-staining with methylene blue or malachite green)
  - Modification – Kinyoun staining (cold stain).
  - Fluorochrome staining with auramine-rhodamine stain





Mycobacterium tuberculosis

- Cell wall
  - similar to Gram-positives (peptidoglycan)
  - in addition, arabinogalactan and mycolic acids $C_{60\text{-}90}$
  - Outer lipid layer + polypeptides stimulating cell-mediated immunity (preparation of PPD extract instead of Koch´s tuberculin)

Mycobacterium tuberculosis

- Physiology
  - Strictly aerobic
  - long generation time: severAl hrs to 1 day
  - Incubation temperature: <37°C, 37°C, 45°C
    M. leprae non-culturable *in vitro*
  - Resistance to environmental factors (desiccation, chemical compounds, disinfectans)

Mycobacterium tuberculosis

- Cultivation
  - Generation time 18-24 hrs.
  - Solid Löwenstein–Jensen agar
    - growth of 3-6 weeks
    - R colonies (eugonic growth)
  - Liquid Šula medium – pellicle growth
  - Liquid media in semiautomatic devices
- Composition of media
  - Salts, asparagine, glycerine, starch, eggs, malachite green; coagulation

Mycobacterium tuberculosis

- Resistance to environmental factors
  - highly refractory to desiccation
  - survival in dust up to 10 days/ desiccated sputum up to 8 months
- Disinfectans
  - Phenoles, aldehydes. **1% lysol**, **Orthosan BF12** (3-5%), **5% formaldehyde, 2% glutaraldehyde,** 0.5% Persteril, 1% Jodonal B, 0.1-0.5%sodium hypochlorite, **Chloramine B (5%),** 0.5% Dikonit Detergents are inefficient
  - **UV irradiation is highly active**
  - **autoclaving** – standard devitalization process

Mycobacterium tuberculosis

- Pathogenesis
  - no toxins
  - trehaloso-6,6´-dimycolate, cord factor, glycolipid
  - virulence linked to survival inside of macrophages of non-immunized person
  - mycobacteria decrease pH in phagosomes and thus following fusion of them with lysosomes
  - damage to organism is derived from delayed hypersensitivity reaction to mycobacterial antigens

Mycobacterium tuberculosis

- Immunity
  - Cell-mediated immunity
  - Lymphocytes T
  - Interleukins
  - Resistance to phagocytosis
  - Granulomas
  - Immunopathogenic damage

Mycobacterium tuberculosis

- Histopathology
  - Primary complex – primary focus + lymphatic node
  - Generalization
  - Or hard tubercle (granuloma) and calcification
  - Meninges, bones, joints, kidney, skin, lungs
  - Hematogenic „miliary“ dissemination
- Reactivation
- Exogenic reinfection in immunosuppressed pts (elderly, drug abusers, alcohol, transplantation, corticoids, HIV infection)
- Tuberculoma, white cheese-like necrotic material, cavern, open TBC, hemoptysis, TNF release (cachectin, T, weight loss)

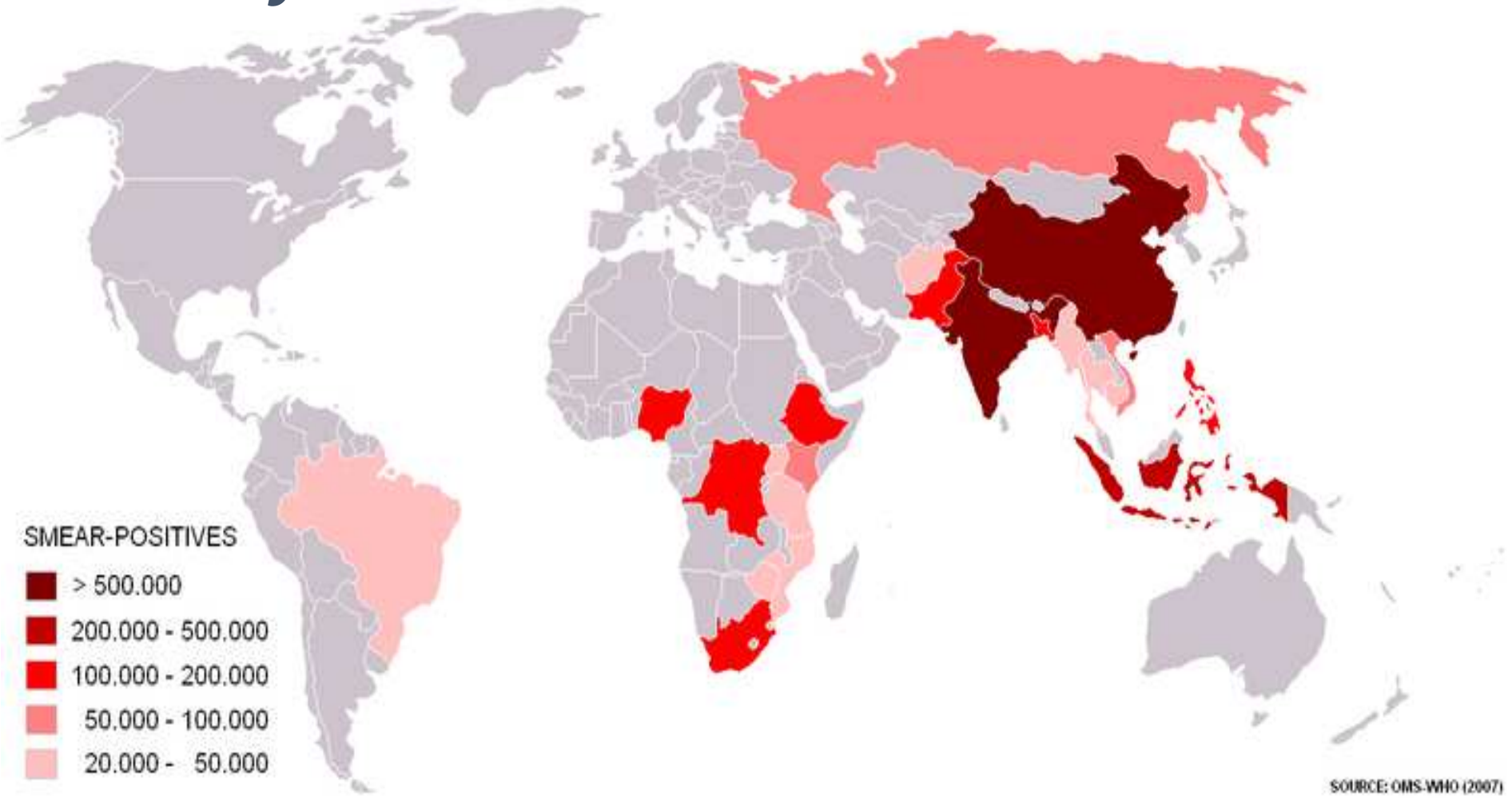
Mycobacterium tuberculosis

- Therapy
  - (surgical / climatic - history) Wolker, Kafka, Modigliani, Remarque novels
  - Antituberculotics
    - Administration of antituberculotic drugs in combination under control
      - Initial phase (2 months) – decreasing load
      - continuation phase (6-8 months) – sterilization of focus, intermittent application
    - Generics
      - INH, H: isonicotinic acid hydrazide
      - RMP, R: rifampicin
      - PZA, Z: pyrazinamide
      - EMB, E: ethambutol
      - STM, S: streptomycin
      - Second line drugs: fluoroquinolones, macrolides, aminoglycosides (AMI, ISE), rifabutin, capreomycin, clofazimin
    - Regimens
      - HRZE (HRZS) and then HR orHE
      - MDR = multidrug resistant mutants – Russian prisoners

Mycobacterium tuberculosis

- Epidemiology in Czech Republic
  - Source: human with open tbc, aerosol
  - Incidence (new cases): 14.0/100,000 in 2000
  - 10x higher incidence in homeless, addicts, prisoners and asylum seekers
  - Prevalence: 907 persons in 2001
  - Molecular typization:
    - RFLP (insertion sequences)
    - spoligotypization (ITS spacers between 16S and 23S rRNA)

Mycobacterium tuberculosis

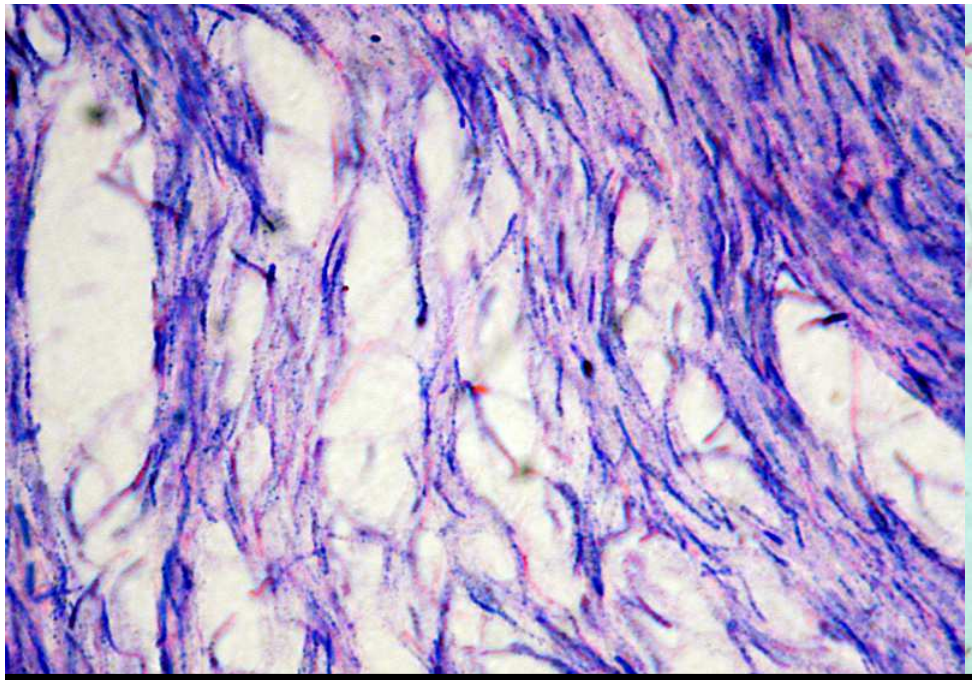


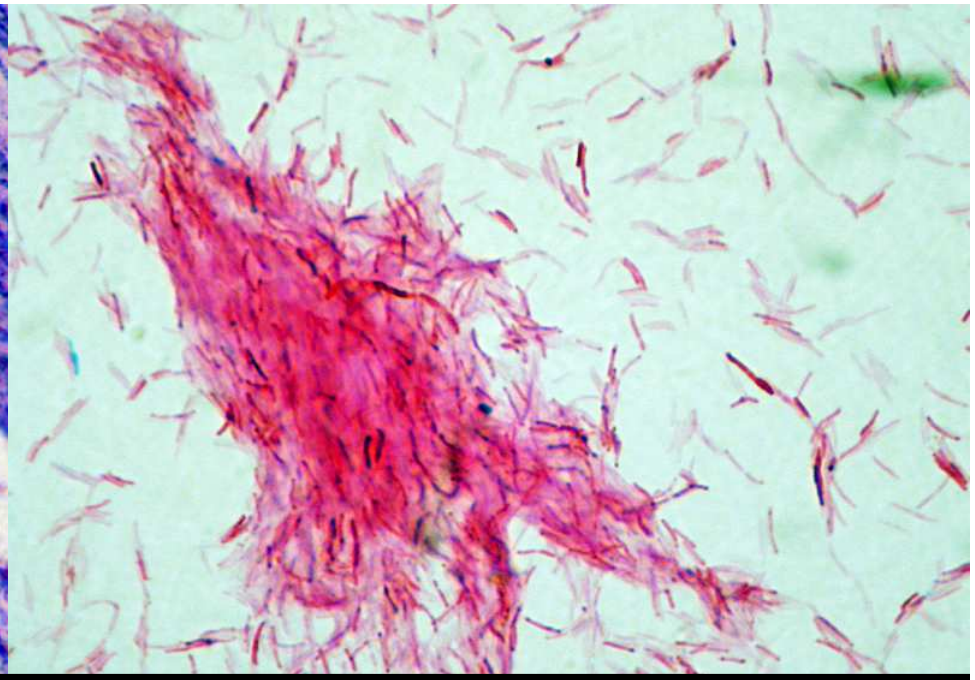
Mycobacterium tuberculosis

- Prevention
  - Screening and therapy of symptomatic TBC of people at risk of TBC (Africa)
  - Problem of overpopulation
  - Vaccination (calmetization)
    - BCG vaccine only for selected children
    - good epidemiological situation – no vaccination
      (risk of complication is higher than risk of development of TBC)

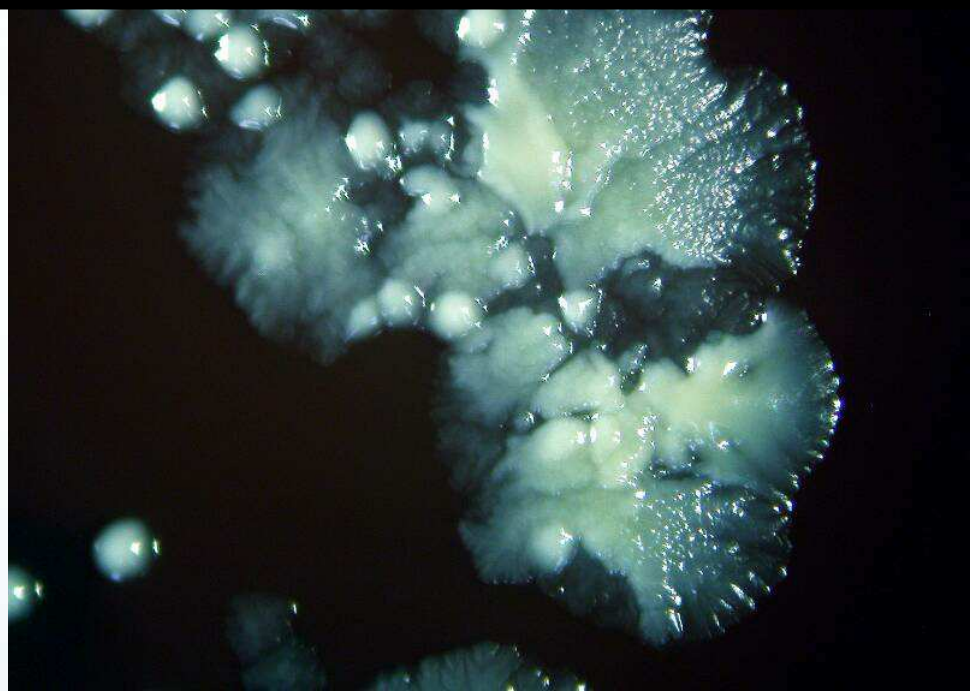
Mycobacteria Other Than Tuberculous = MOTT

- **Slowly growing species**
  - *Mycobacterium avium* complex (MAC)
  - *Mycobacterium kansasii*
  - *Mycobacterium malmoense*
  - *Mycobacterium xenopi*
  - *Mycobacterium marinum*
  - *Mycobacterium gordonae*
- **Fast growing species:**
  - *Mycobacterium abscessus*
  - *Mycobacterium cheloneae*
  - *Mycobacterium fortuitum*





10415_Mycobacterium mucogenicum



J. Scharfen

Specimen investigation *Mycobacterium* species

- **Specimen:**
  - Sputum
  - Sterile body fluids (CSF, pleural fluid etc.)
  - Skin, incl. bioptic samples
  - Pus, incl. swabs
  - Bone marrow
  - Blood
  - Gastric lavage
  - Urine
  - Stool
  - Laryngeal swab
  - Bronchoalveolary lavage fluid (BAL)
  - autopsy samples

Mycobacterium tuberculosis

- Laboratory investigation
  - **Direct methods**
  - sputum, BAL, laryngeal swab, gastric lavage, pus, CSF, biopsy, urine (40 ml)
  - stool and blood only in disseminated form
  - safety measures, professional infectios - BSL 2-3
    - Microscopy
    - Cultivation
    - PCR
  - **Indirect methods**
    - detection of memory cells in blood
    - Quantiferon

Mycobacterium tuberculosis

- Identification
  - Phenotypic (morphology, niacin production, reduction of nitrate, growth on thiofen-2-carbonic acid medium)
  - Genotypic (amplification methods)
  - Polyphasis
- Susceptibility testing
  - Proportion method by Canetti

Other mycobacteria

- *M. bovis*
- *M. bovis* BCG
- *M. leprae*
  - leprosy, Hansen bacillus, no culture *in-vitro*
  - armadillo, nude mice, paw and ear conventional mouse
  - optimal temperature 30°C, generation time 12-14 days
  - affecting peripheral nerves, granulomatous reaction, generalization, skin, subcutaneous tissue, nasal cartilage, finger bones, genetic basis of the degree of immunity
  - Patogenicity: tuberculoid leprosy, lepra lepromatosa (facies leontina)
  - Epidemiology: Norway, ponds, a special sphagnum, Middle Ages, microbiologist of Czech origin Jindřich Kazda (Germany)
  - Therapy: rifampicin, dapson, clofazimin, minocyclin, ofloxacin, clarithromycin
  - Prevention: improvement of environment, vaccination with BCG
  - DG: microscopy of skin scrapings, Z-N staining, globi.

Atypical mycobacteria

- water, soil, saprophytes, opprtunistic pathogens
- mycobacteriosis
- Clinically important species:
  - *Mycobacterium avium/ intracellulare* (MAI complex)
    - birds, poultry, pigs, MDR (multidrug resistance).
    - pulmonary processes, lymphadenitis-like TBC, AIDS – dissemination
  - *Mycobacterium kansasii*
    - Endemic, metallurgical and mining industry
    - Pulmonary/ extrapulmonary/ disseminated forms, good susceptibility
  - *M. xenopi*
    - water supply systems
  - *M. szulgai, malmoense, marinum, ulcerans,*
  - slow growing: *M. gordoneae, terrae, haemophilum, M. bohemicum* (Horák, Kaustová)
  - rapid growing: *M.fortuitum, cheloneae, abscessus, mucogenicum*
- Saprophytic: *M.phlei, M.smegmatis*
- Related: *Tropheryma whipplei*


mycobacteriosis

Host x pathogen

- Deep immunodeficiency
- Accumulation of patients with the same diagnosis
- Opportunistic pathogens, emerging infections, ubiquitous microbes
- Specific growth and chemotaxonomic properties

Classification of actinomycetes

(practical not taxonomic)

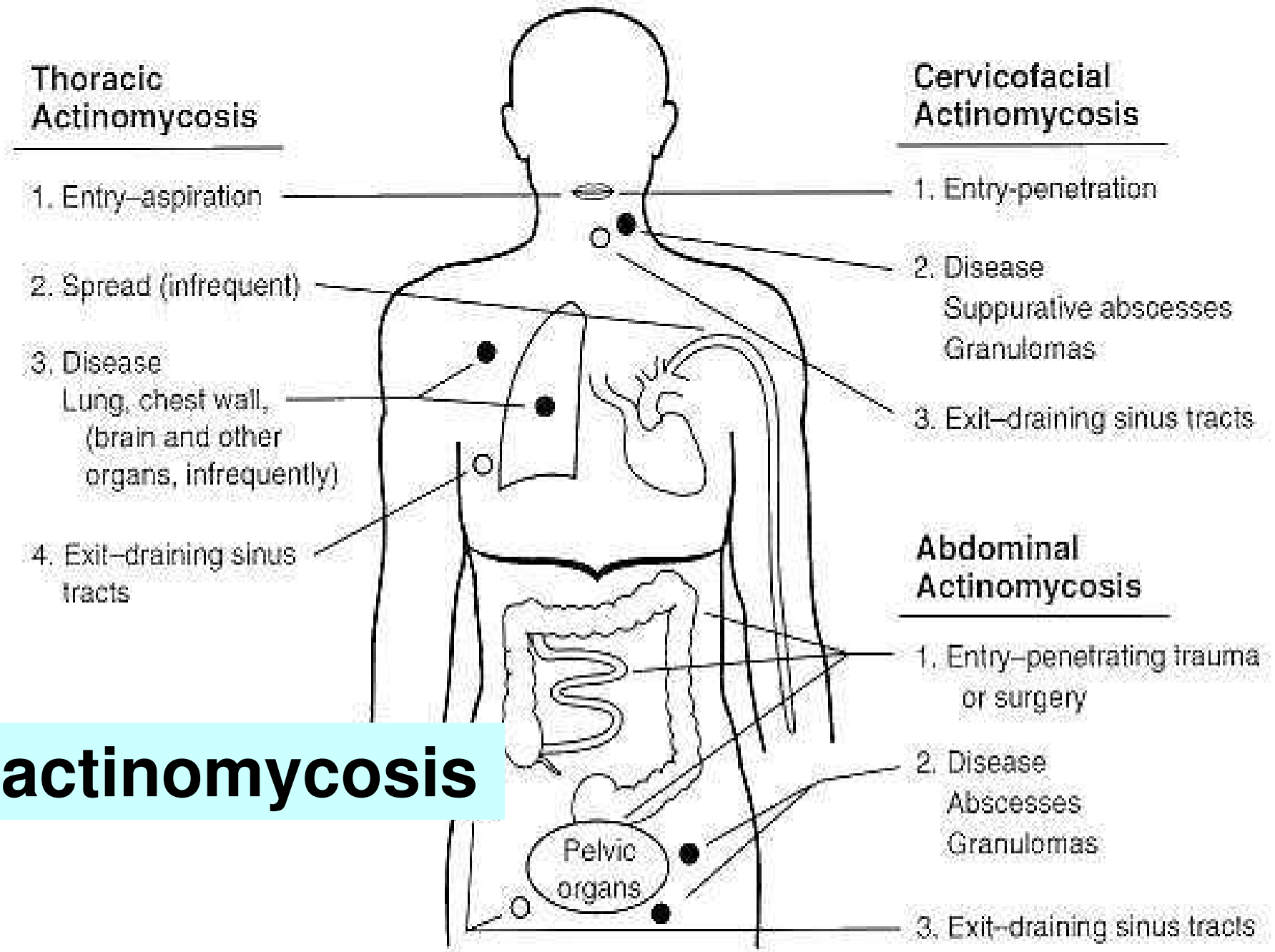
1. **Aerobic actinomycetes**
   (in narrow sense of the word)
2. **Microaerophilic actinomycetes**
3. **Other aerobic actinomycetes**

Infections

- Microaerophilic actinomycetes
  - **Actinomycosis**
- Aerobic actinomycetes
  - Tuberculosis
  - Leprosy
  - **Mycobacteriosis**
  - **Nocardiosis**
- Other aerobic actinomycetes
  - **Streptomycosis?**

2.Microaerophilic actinomycetes (MAFA) – differential diagnostics


actinomycosis

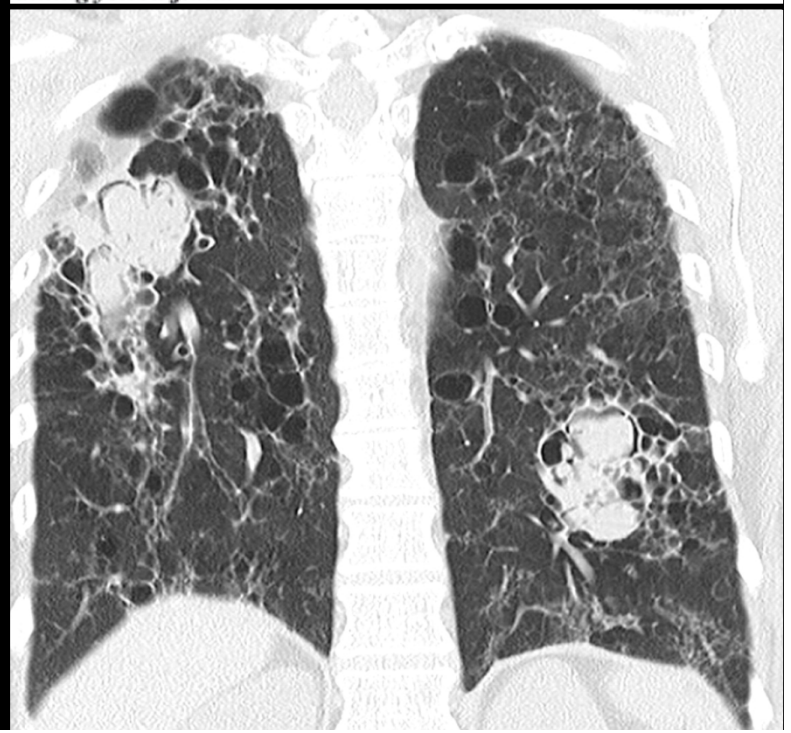


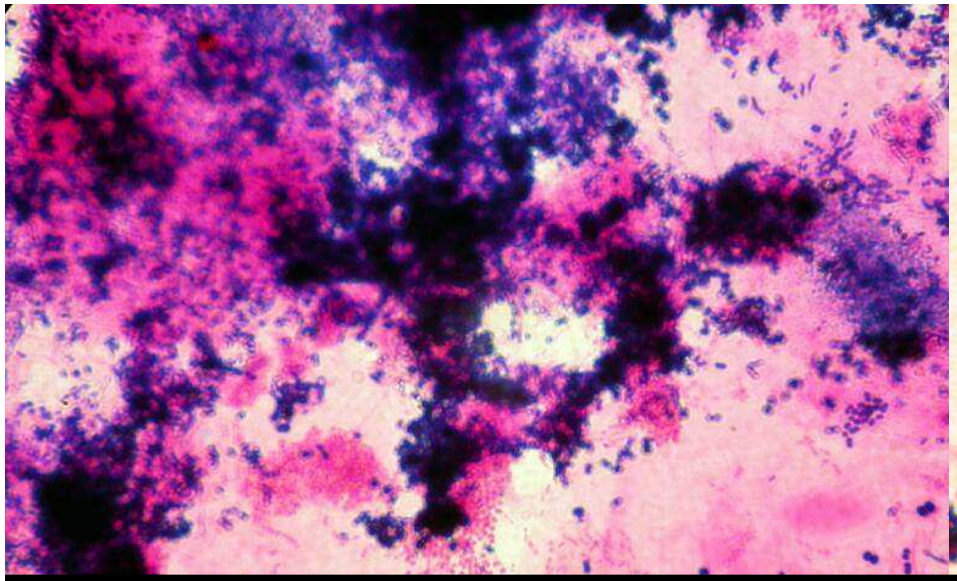
actinomycosis

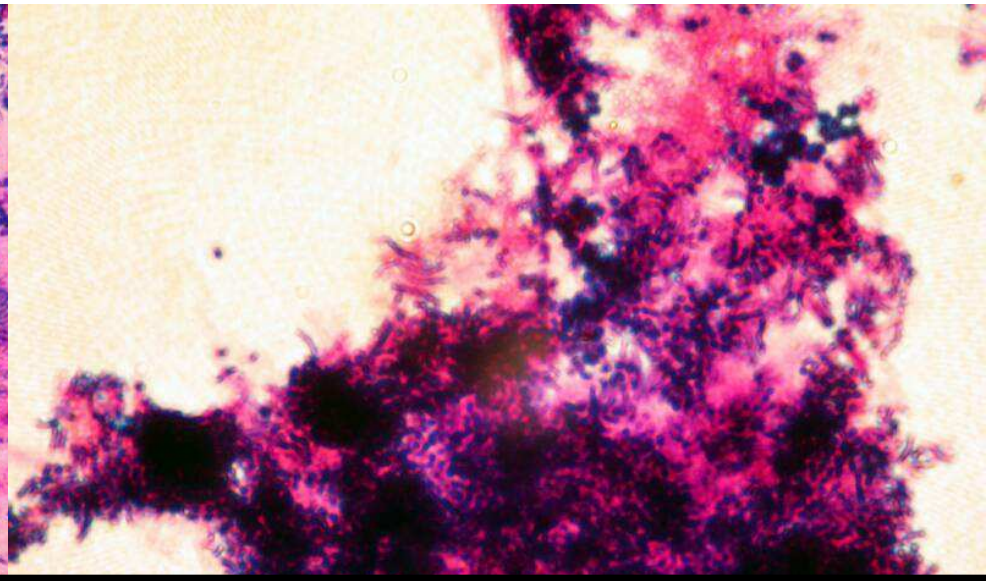
Mycetoma
Nocardia brasiliensis, Actinomadura, Nocardipsis, Streptomyces somaliensis

Figure 1 - Madura foot. Extensive involvement of the foot and leg can be seen with chronic skin abnormalities and scattered openings of sinuses draining yellow fluid.

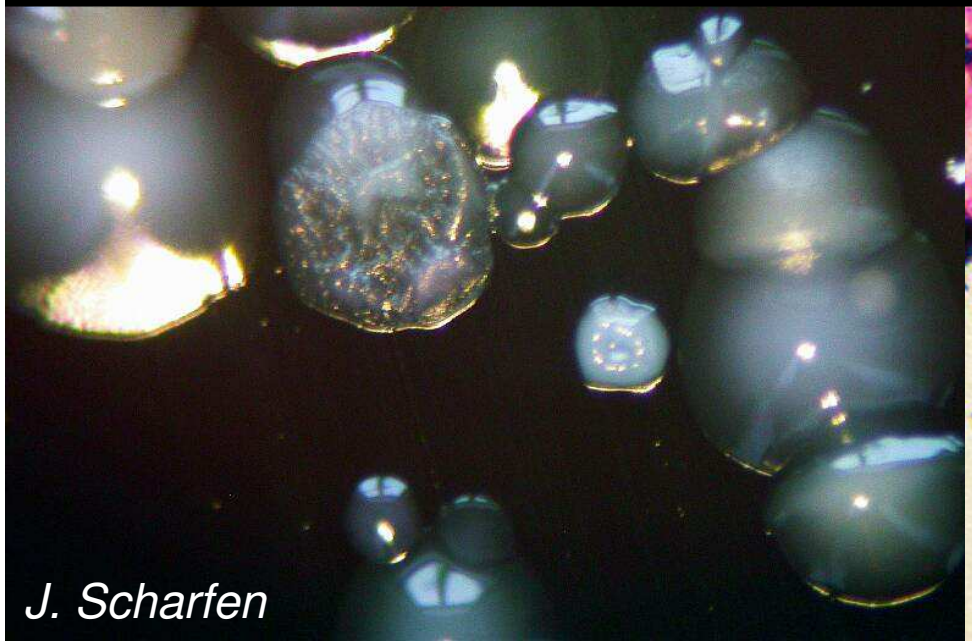
- **Chronic slowly progressing bacterial or fungal infection**
- **Usually on feet**
- **Typical granulomatous forms with the presence of fistulas, leaking pus containing druze** (aggregates of filamentous bacteria)

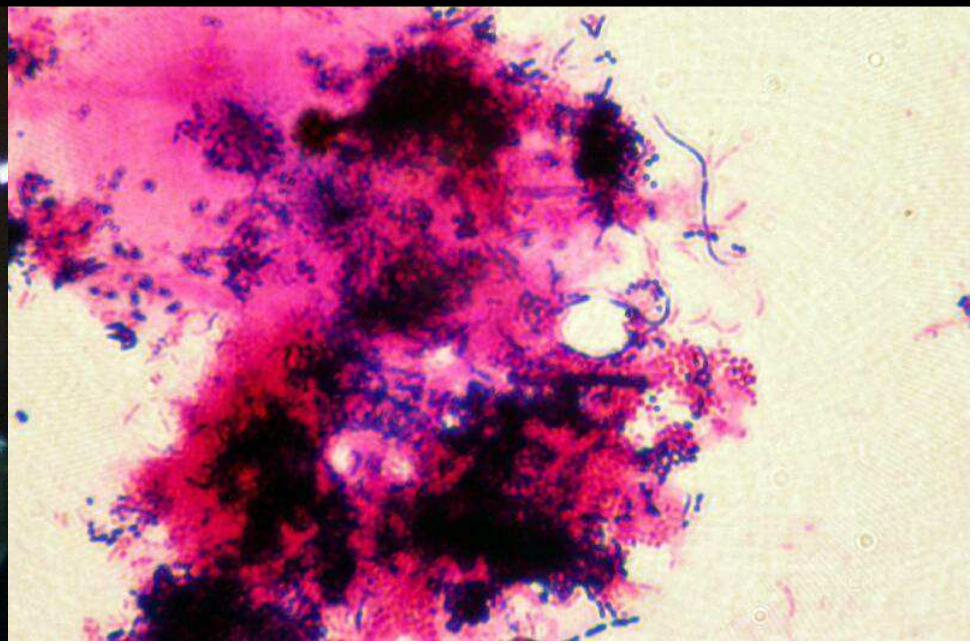


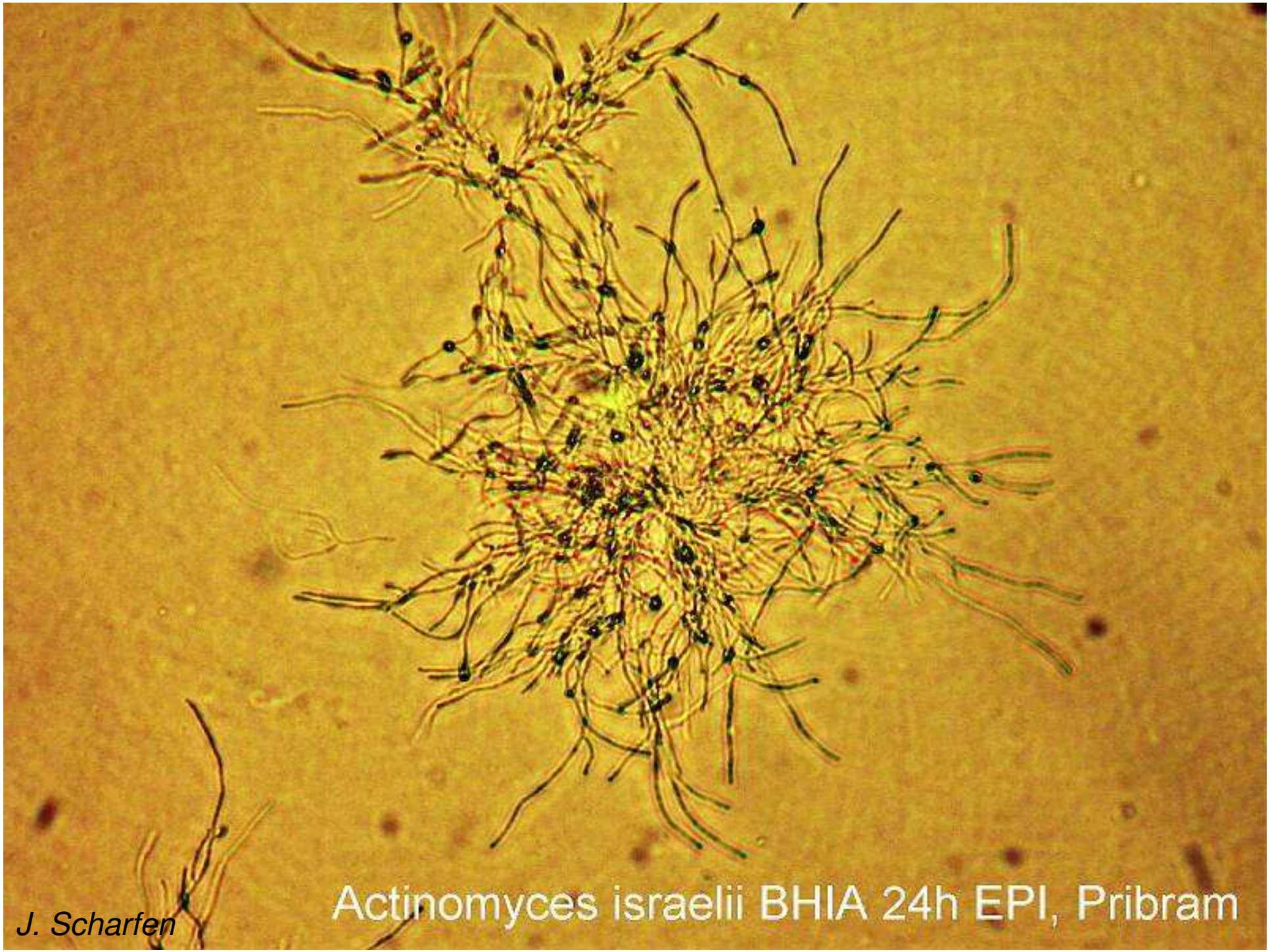




10070_CCTR957_*Actinomyces meyeri*
submandibular abscess






Actinomyces israelii BHIA 24h EPI, Pribram
J. Scharfen

Summary

- Anamnesis, imaging methods, laboratory tests
- Microscopy and choice of methods
- Anaerobic + aerobic cultivation
- phenotypic identification
- MALDI-tof MS

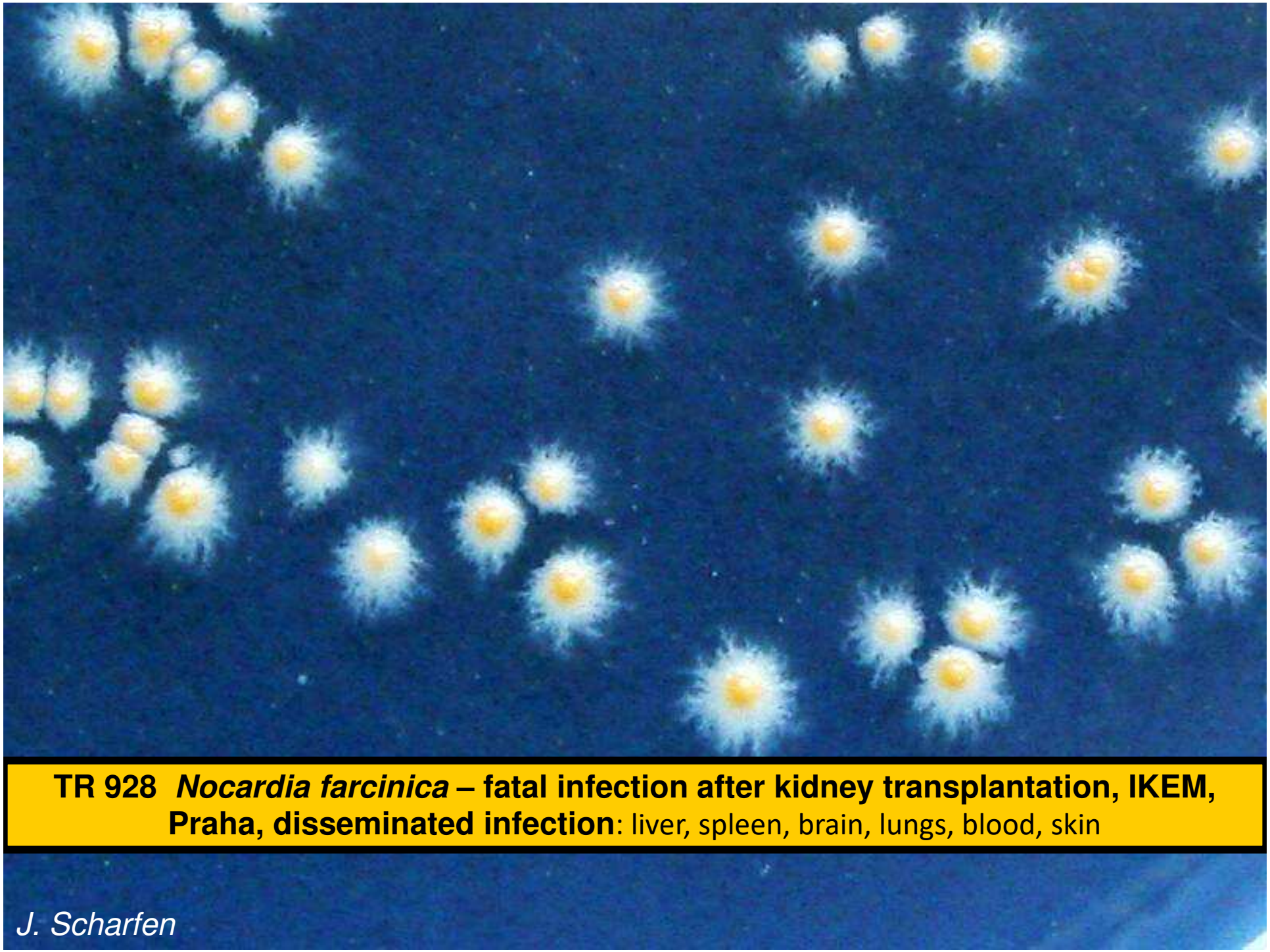
1.Aerobic actinomycetes (in narrow sense of the word)

Main etiology

- **fast growing mycobacteria**
- ***Nocardia***
- ***Gordonia***
- ***Tsukamurella***
- ***Rhodococcus***
- ***Dietzia***

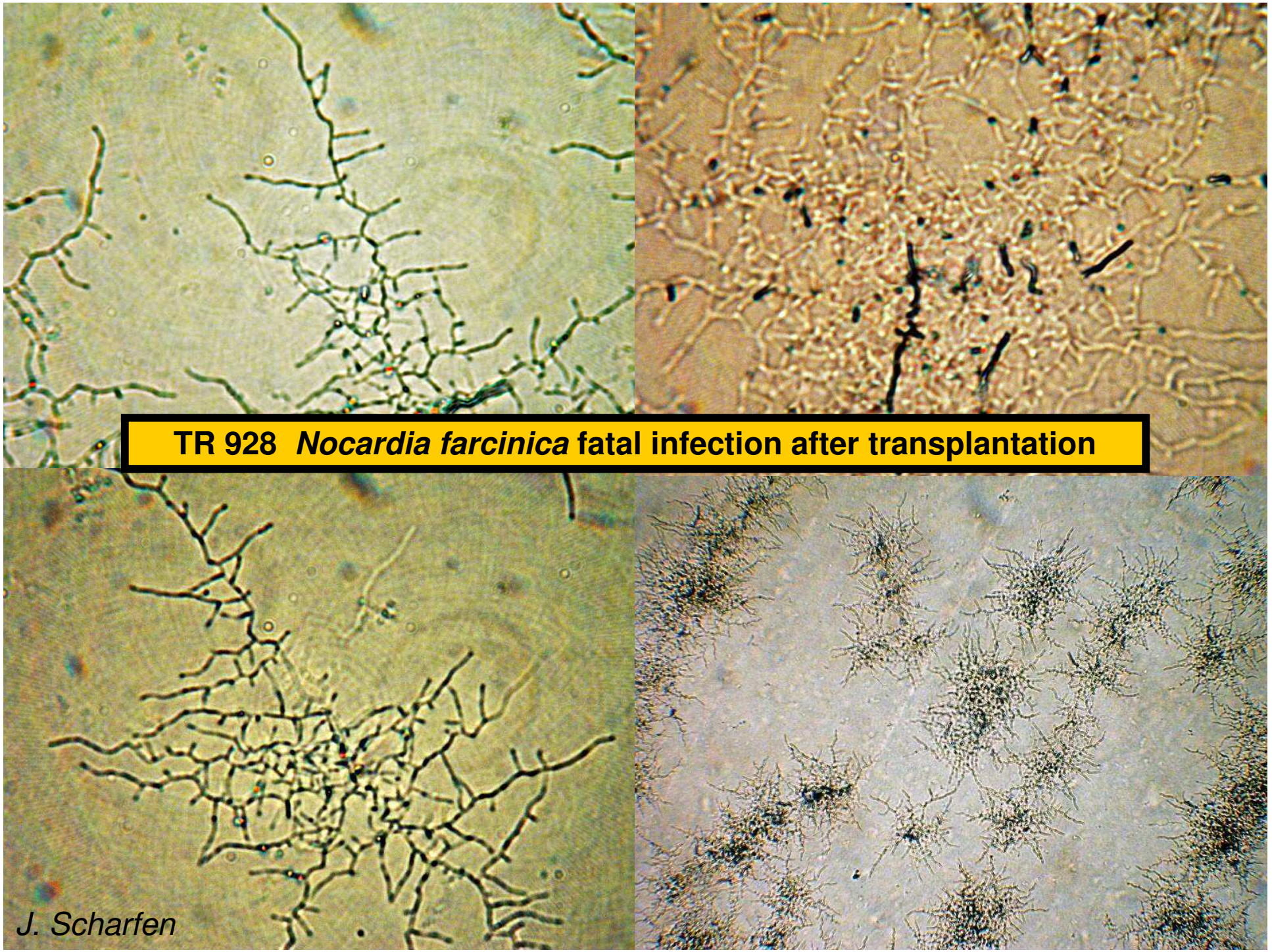
Properties

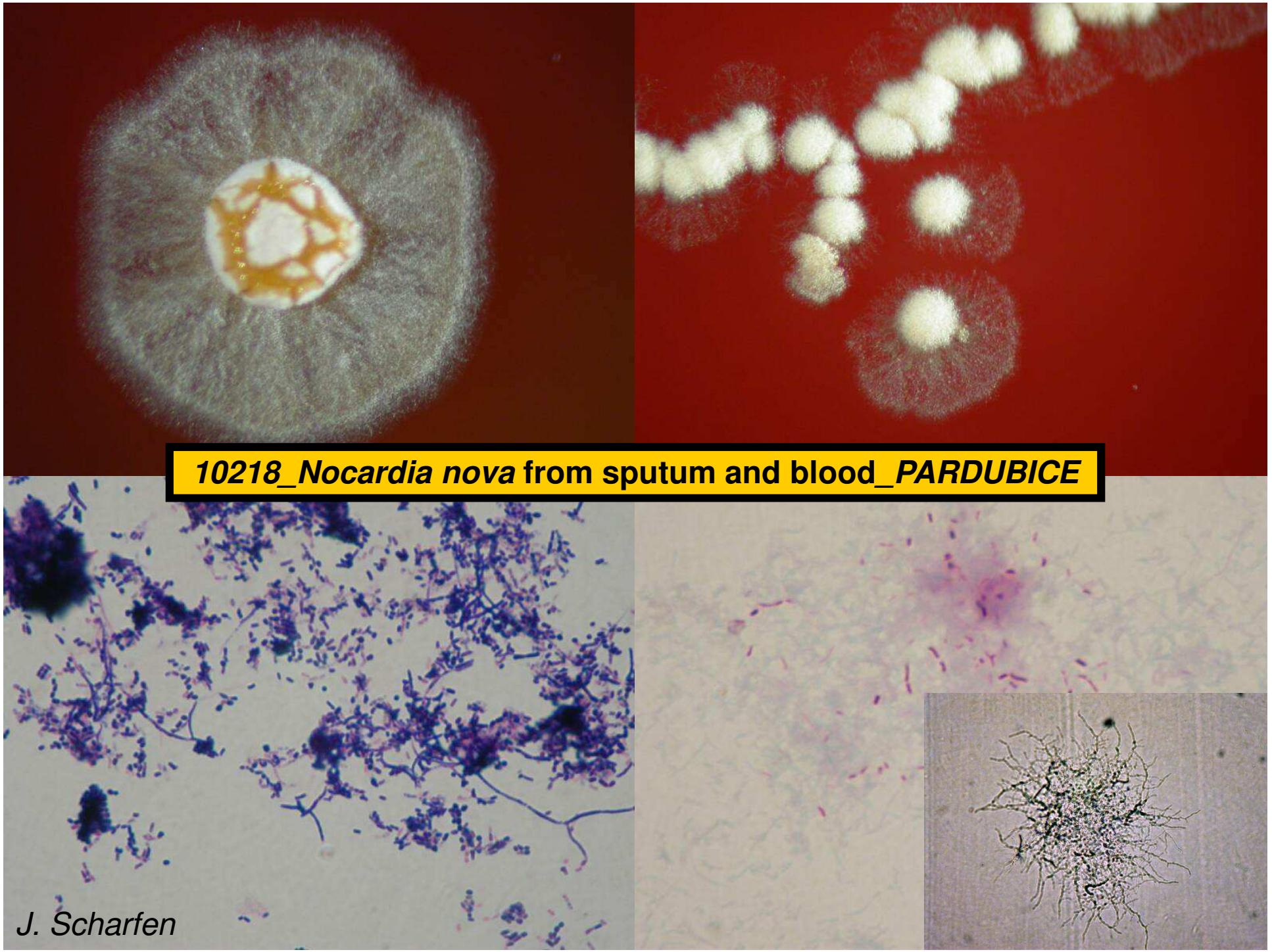
- **Exogenic infections?**
- **Immunity**
- **Phenotypic resistance**
- **Polyphasic identification**
- **Susceptibility testing**
- **Drug of choice: COT** (exceptions!)

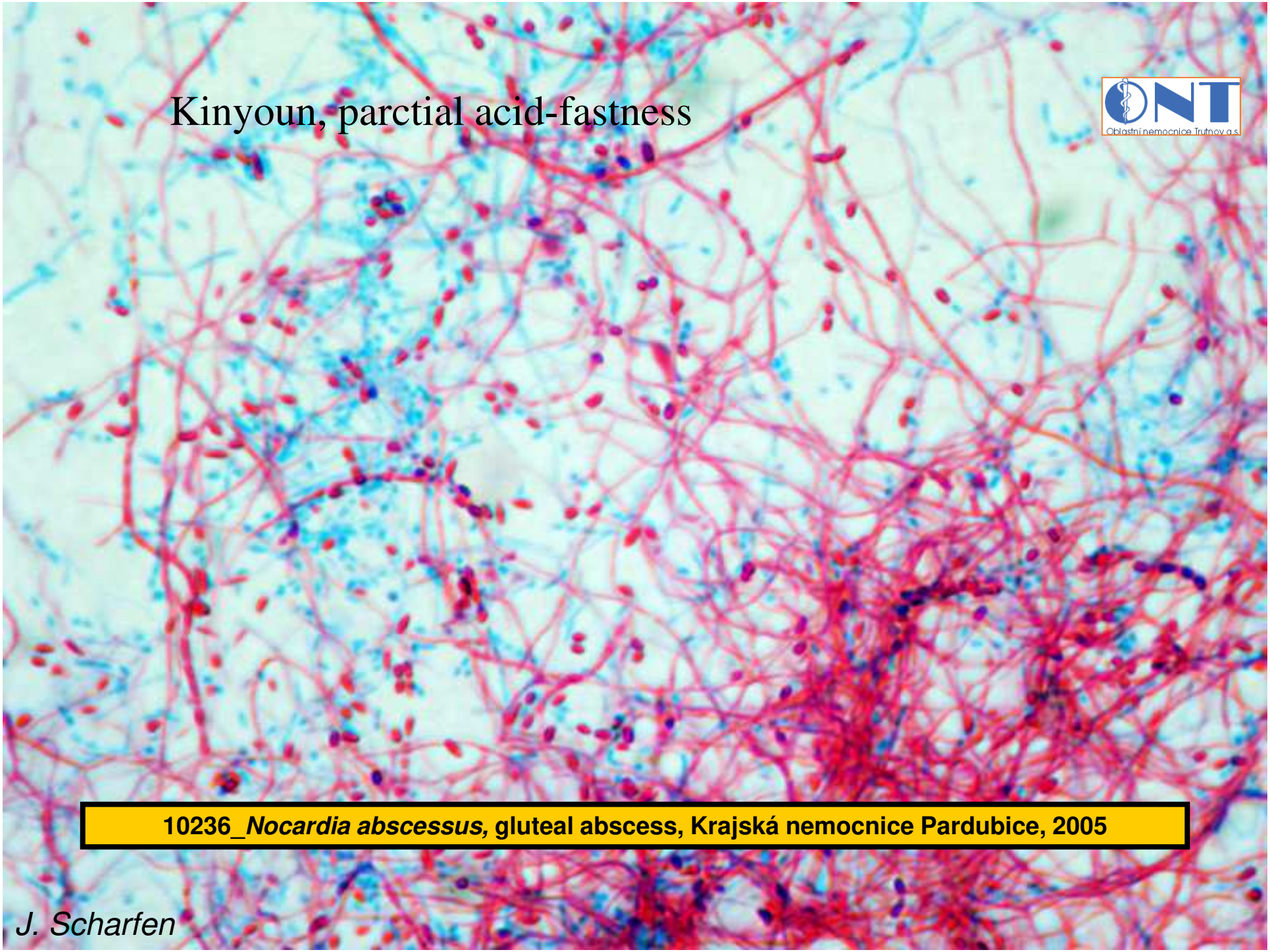


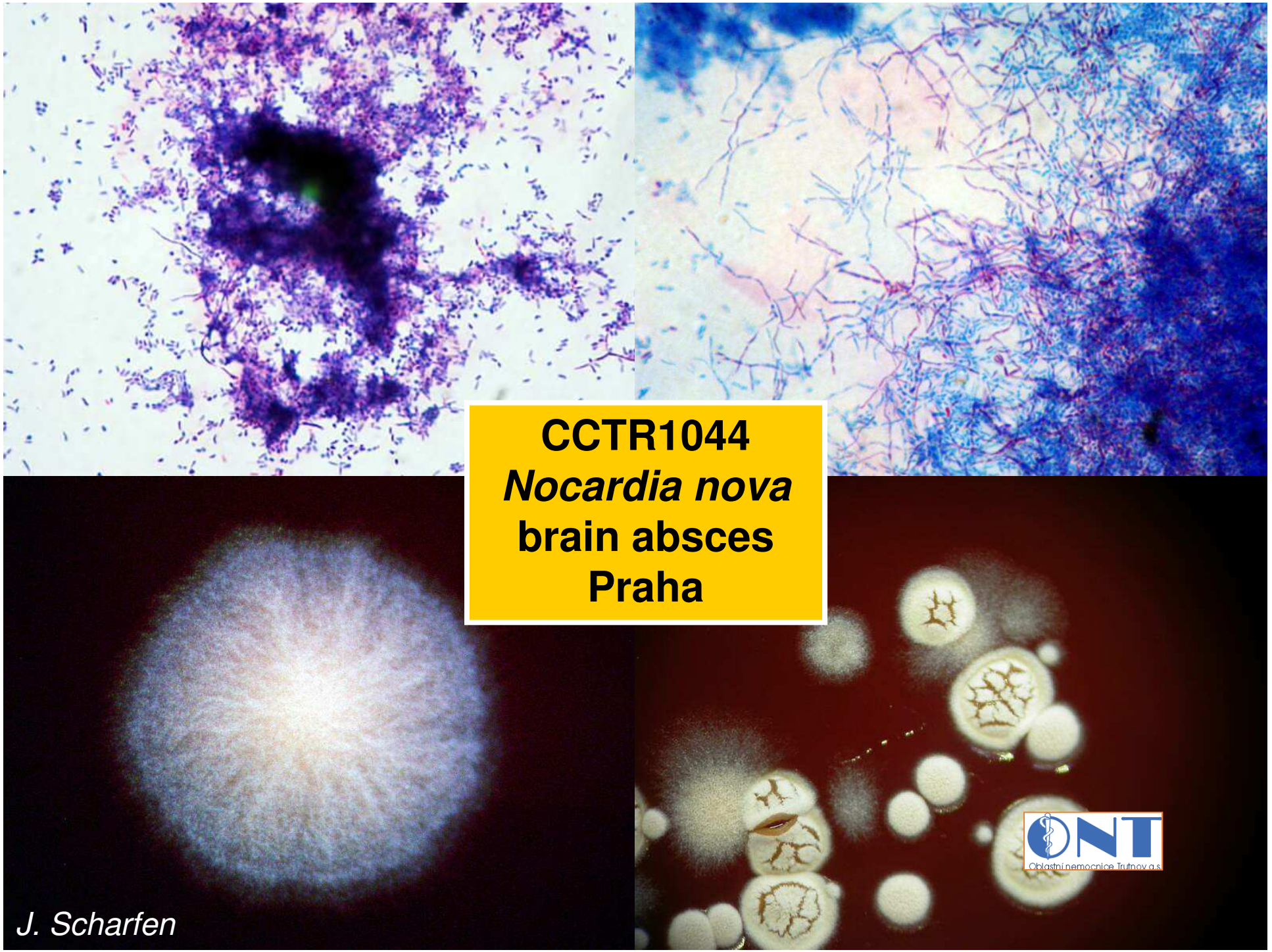
TR 928 *Nocardia farcinica* – fatal infection after kidney transplantation, IKEM, Praha, disseminated infection: liver, spleen, brain, lungs, blood, skin

J. Scharfen


TR 928 *Nocardia farcinica* fatal infection after transplantation
J. Scharfen


10218_Nocardia nova from sputum and blood_PARDUBICE
J. Scharfen

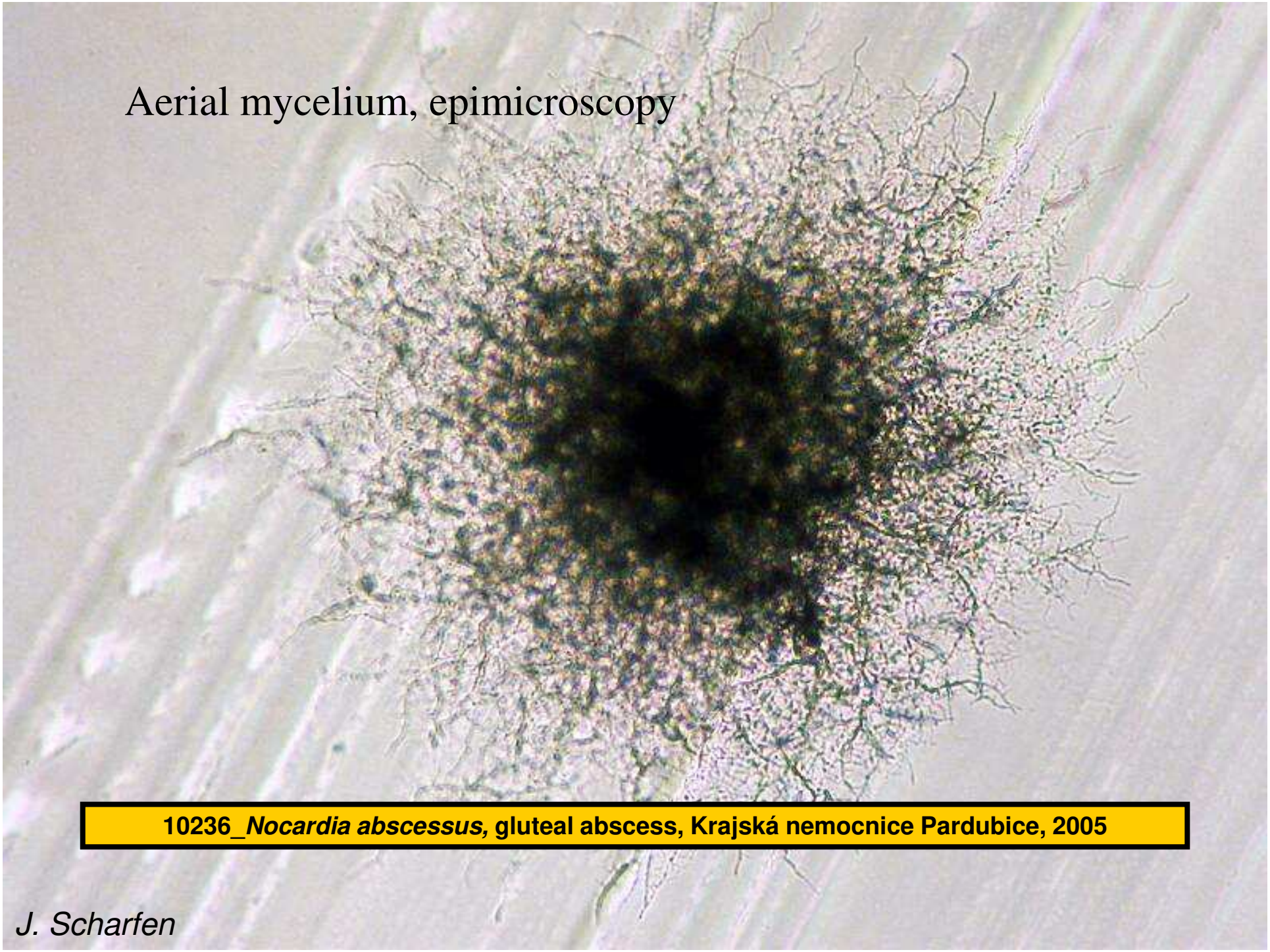

Kinyoun, parctial acid-fastness
Oblastní nemocnice Trutnov a.s.
10236_*Nocardia abscessus,* gluteal abscess, Krajská nemocnice Pardubice, 2005
J. Scharfen


CCTR1044
Nocardia nova
brain absces
Praha
J. Scharfen
ONT
Oblastní nemocnice Trutnov a.s.

Identification based on biochemical properties of bacteria

- Reaction (phenotype)
  - Biochemical properties
    (oxidation, fermentation, substrate utilization)
  - Enzymology, incl. phenotypes of ATB resistance
  - Serology
- Evaluation
  - Tables
  - Algorithms
  - Databases

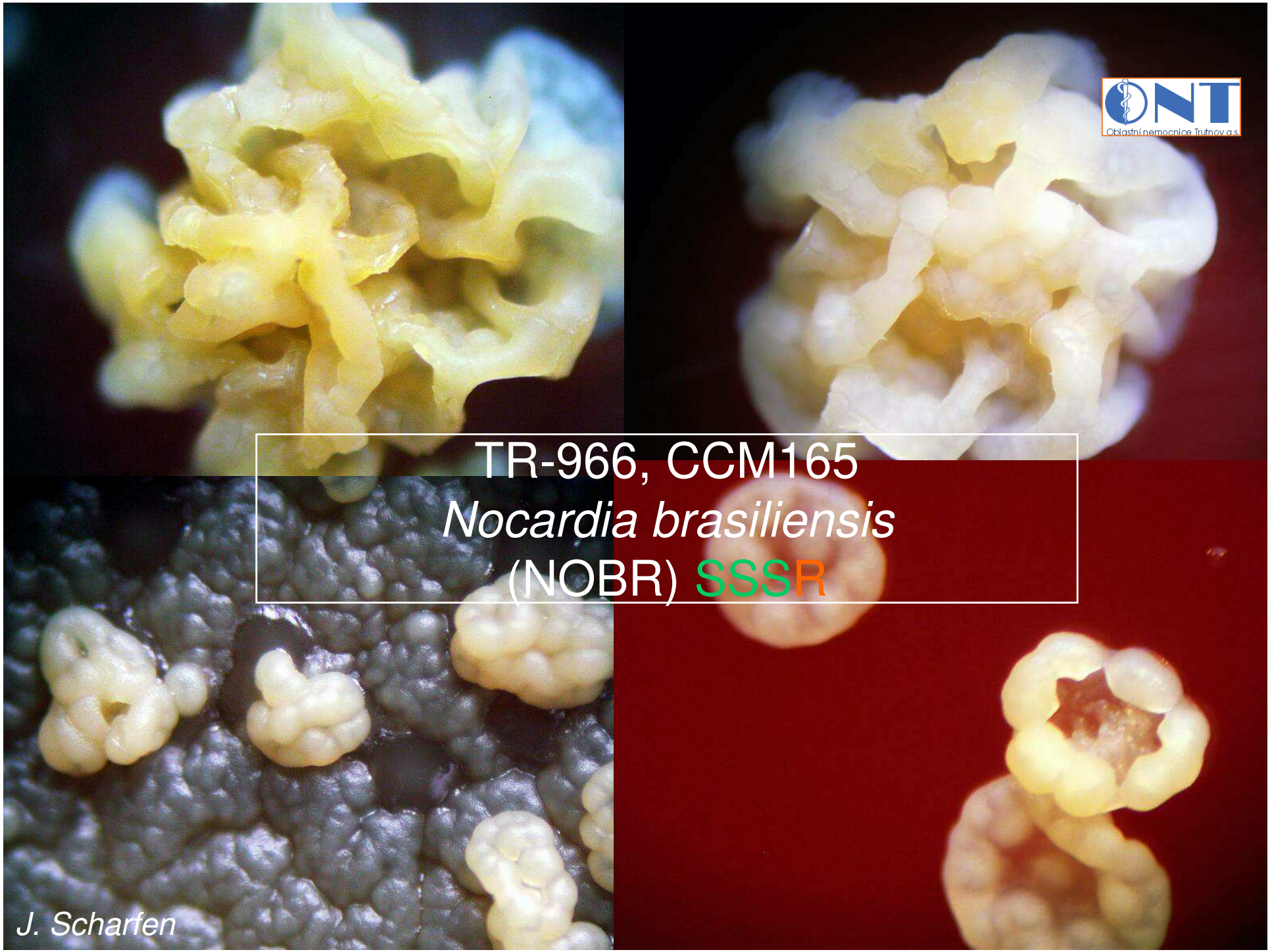
Aerial mycelium, epimicroscopy



10236_*Nocardia abscessus,* gluteal abscess, Krajská nemocnice Pardubice, 2005

J. Scharfen


TR-984, DSM 44432
Nocardia abscessus (NOAB FR I)
(NOAB) SSSR
J. Scharfen


TR-966, CCM165
Nocardia brasiliensis
(NOBR) SSSR
J. Scharfen

Diagnosis and therapy of aerobic actinomycetes
(in narrow sense of the word)

- Identification
  - Phenotypic
  - Genotypic
  - Mass spectrometry (MALDI-TOF)
- Susceptibility testing
  - Qualitative
  - Quantitative
- Consultation, proposition of therapy
- Therapy
  - drug of choice: co-trimoxazole
  - Alternative: amikacin, meropenem, imipenem, linezolid.