ESGMYC

ESCMID STUDY GROUP FOR MYCOBACTERIAL INFECTIONS

European Society of Clinical Microbiology and Infectious Diseases

Leprosy and Buruli ulcer

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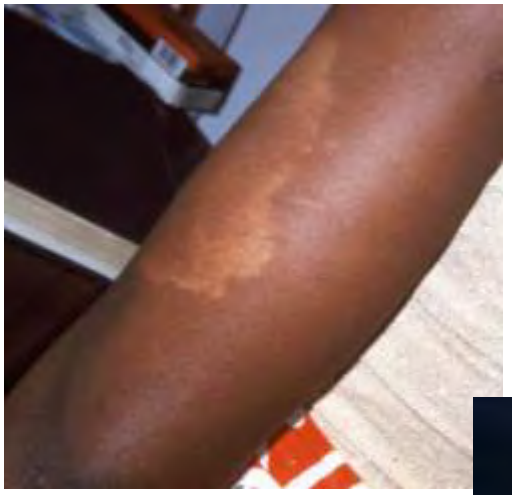
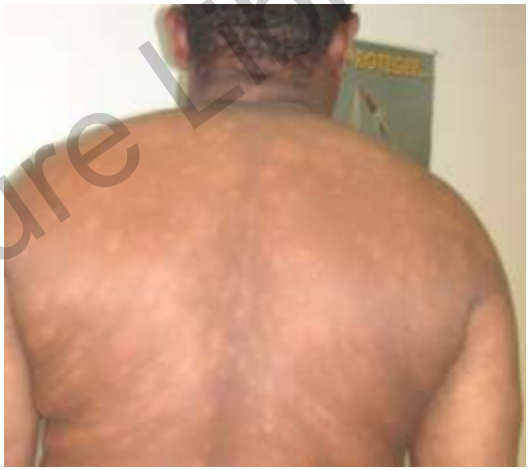
Educational Workshop - ECCMID 2014 Barcelona
Mycobacterial infections in low income countries

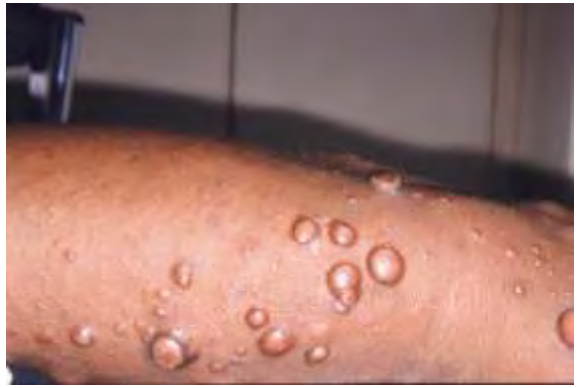
Leprosy: clinical diagnosis



Skin lesions



Nerve impairment



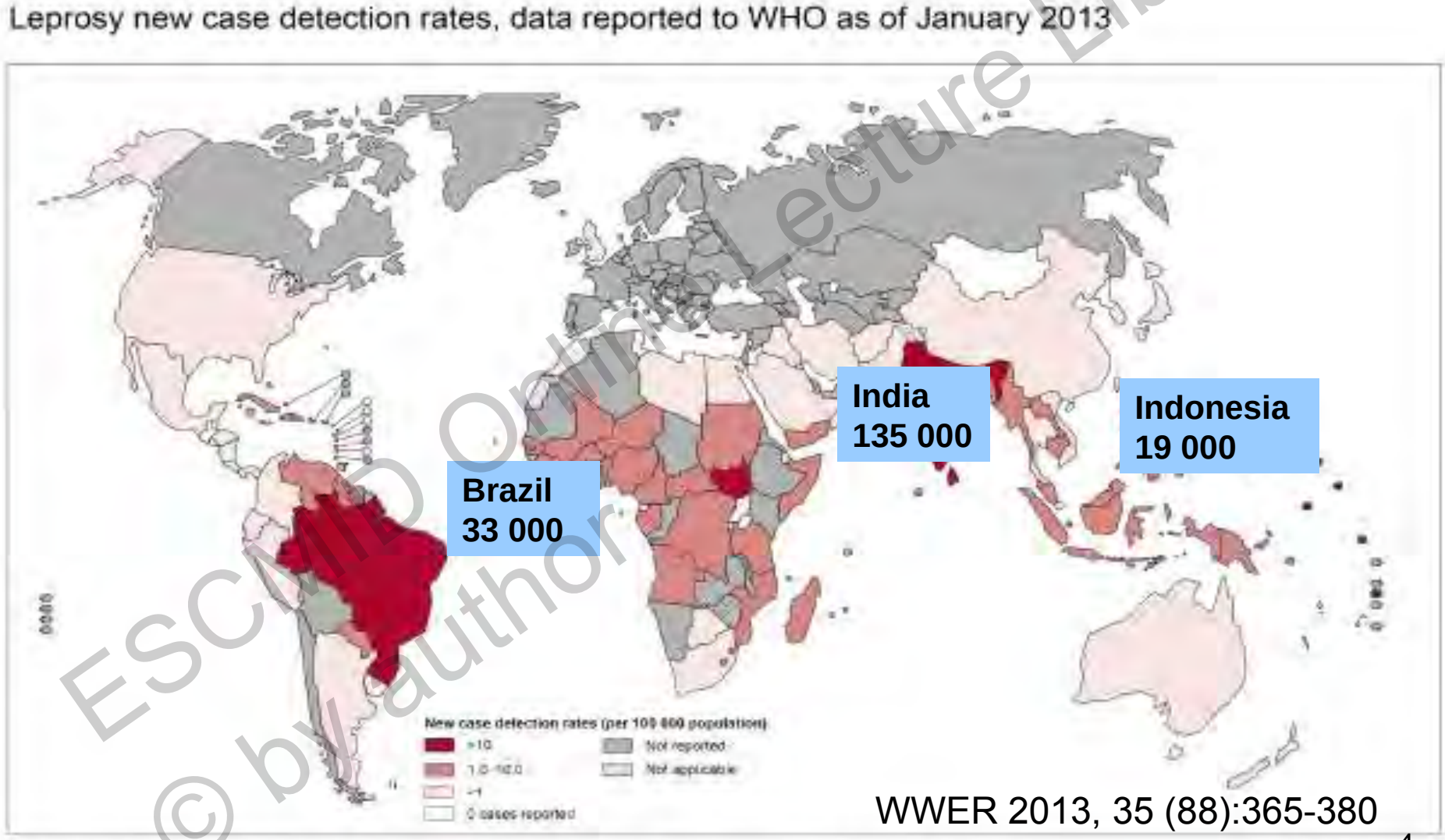
Lepromas

Diagnosis of leprosy

- Ridley and Jopling clinical forms : used in dermatology, needs histopathology
- WHO-clinical/treatment based classification:
  - One skin lesion
  - Paucibacillary leprosy ≤ 5 lesions
  - Multibacillary leprosy > 5 lesions
- Born or living in an endemic country +++

http://www.searo.who.int/entity/global_leprosy_programme/en/index.html

233 000 new cases in 2012



WWER 2013, 35 (88):365-380

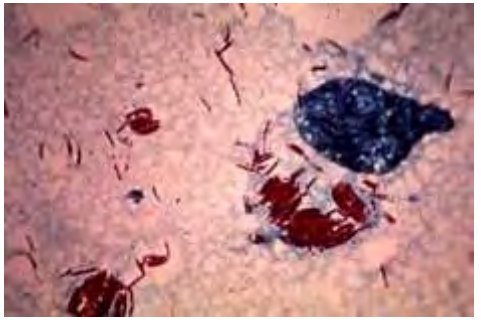
Mycobacterium leprae

- Early debate over whether leprosy was an inherited or an infectious disease
- Bacillus discovered by Armauer Hansen in Bergen (Norway) in 1872
- Slow-growing mycobacterium (14 days doubling time)
- Cannot be grown *in vitro*
- Optimal growth at $33^0/34^0$ C, in skin and nerves
- Whole genome sequencing (Cole Nature 2001)
- Related species: *M.lepraemurium, M.lepromatosis*

Polycarpou et al. 2013, Rodriguez and Lockwood LID 2011

Microbial diagnosis of leprosy

- **Skin smear or punch biopsy examination**
  - Acid fast bacilli detected (Ziehl-Neelsen staining)
  - Bacterial index : 1/100 fields (BI1+) to 1000 / field (BI 6+)



- **Paucibacillary leprosy**
  - Smear-negative = 10^{6} bacilli/body
- **Multibacillary leprosy**
  - Smear-positive
  - BI 2+ to 6+ = 10^{12} bacilli/body

Leprosy treatment : WHO Multidrug Therapy (MDT) regimens from 1982

- **Multibacillary (MB) leprosy** (> 5 lesions, 10^{12} bacilli)
  - **Rifampin**: 600 mg monthly
  - **Dapsone**: 100 mg daily
  - **Clofazimine**: 300 mg monthly and 50 mg daily

3 drugs for 12 months

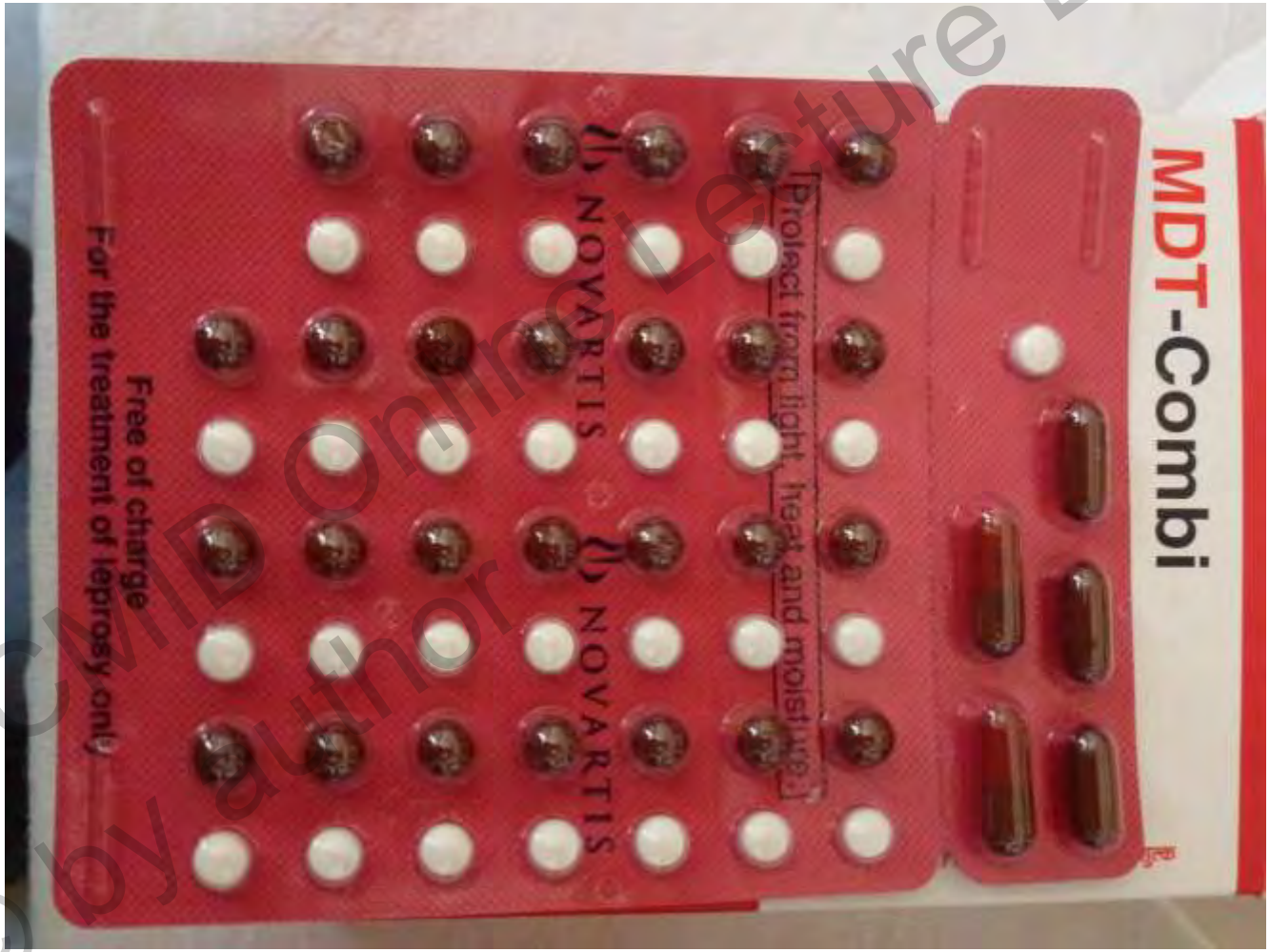
- **Paucibacillary (PB) leprosy** (< 5 lesions, 10^{6} bacilli)
  - **Rifampin**: 600 mg monthly
  - **Dapsone**: 100 mg daily

2 drugs for 6 months

- **Single Skin Lesion Paucibacillary leprosy**
  - **Rifampin** (600 mg), **Ofloxacin** (400 mg) and **Minocycline** (100 mg)

Single dose

Drugs provided by Novartis fundation since 2000

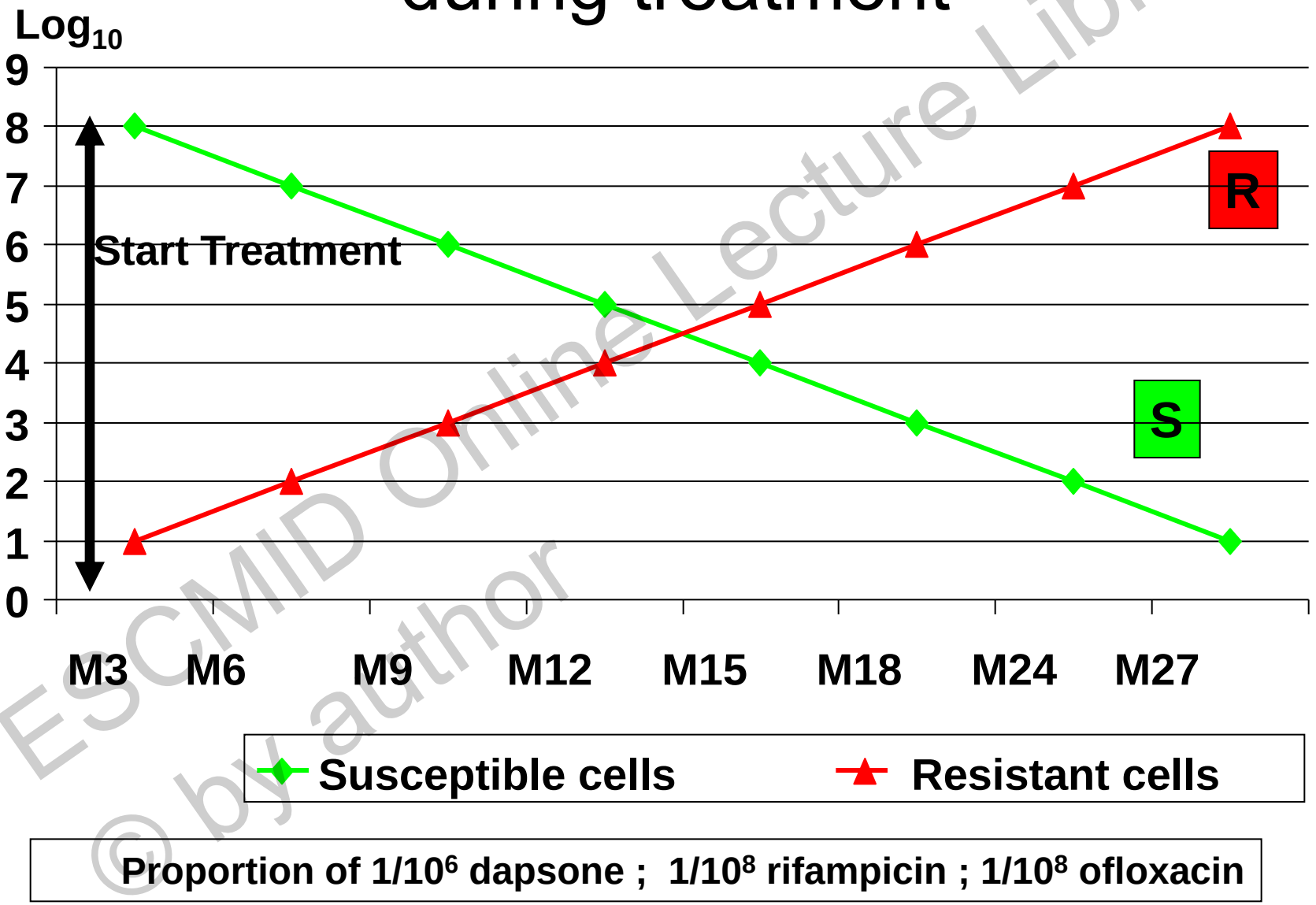


Selection of antibiotic resistance

- Dapsone monotherapy from 1950 to 1982
  => Dapsone resistance in 1964
- Rifampin available in 1968
  => Rifampin resistance in 1976
- Ofloxacin available in 1984
  => Ofloxacin resistance in 1997
- Combination therapy (MDT) in 1982: dapsone, rifampin and clofazimine
  => no resistance

Pettit 1964, Jacobson 1976, Cambau 1997 , Ji 2002

Sheme of selection of resistant mutant during treatment



Drug susceptibility testing

- *M. leprae* does not grow in vitro
- Susceptibility testing in the hind mouse footpad
  - 20 to 50 mice treated by antibiotics
  - Mice kept and fed for 1 year
  - because of slow growth of *M. leprae* (doubling generation time of ~ 10-15 days)
  - Skilled technician

⇒Very rare labs are still performing mouse footpad experiments

Shepard 1960

Mechanisms of rifampicin resistance in *M. leprae*

Region determining **rifampicin resistance** (RDRR) in the subunit B of RNA polymerase encoding gene (*rpoB*)

Insertion

432	433	436	438	441	451	456	458
Gly	Thr	Leu	Gln	Asp	His	Ser	Leu
Ser	Ile	Pro	Val	Asn Tyr	Asp Tyr	Leu Met Phe Trp	Val

*Numbering system of *M. leprae* genome strain TN
RDRR= 432 – 458 = 507-533 in *E. coli*
Position 456 in *M.leprae = 531* in *E. coli*
Position 456 = previous position 425 in M. leprae

Honore 1993, Maeda 1999, Maeda 2001, Cole 2001, Cambau 2002, Zhang 2004, Cambau 2012

Mechanisms of dapsone resistance in *M. leprae*

Region determining **dapsone resistance** (RDDR) in the Dihydropteroate synthase encoding gene (*folP1*)

53	**55**
Thr	**Pro**
Ile	**Arg**
Arg	**Leu**
Ala	**Trp**

Numbering system of *M. leprae* genome srain TN

Position 53 in *M.leprae* = 62 in *E. coli*

Position 55 in *M.leprae* = 64 in *E. coli*

Kai 1999, Gillis, 2000, Cambau 2006

Mechanisms of ofloxacin resistance in *M. leprae*

Region determining **fluoroquinolone resistance** (RDDR) in the A subunit of DNA gyrase encoding gene (*gyrA*)

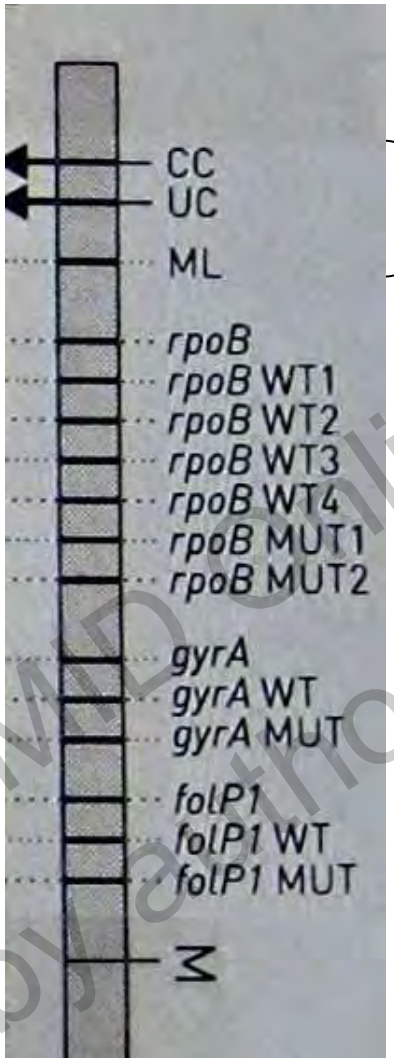
89	**91**
Gly	**Ala**
Cys	**Val**

Numbering system of *M. leprae* genome srain TN
Position 89 in *M.leprae* = 81 in *E. coli*
Position 91 in *M.leprae* = 83 in *E. coli*

Kim 2003 ; Maeda 2001, Cambau 1997, Matsuoka 2007 ; Matrat 2008, Rocha 2012

Molecular methods for resistance detection in *M. leprae*

GenoType LepraeDR DNA strip (Hain Lifesciences)



Control probes

Presence of *M leprae* DNA

Rifampicin resistance

rpoB wild type and mutations

Fluoroquinolone resistance

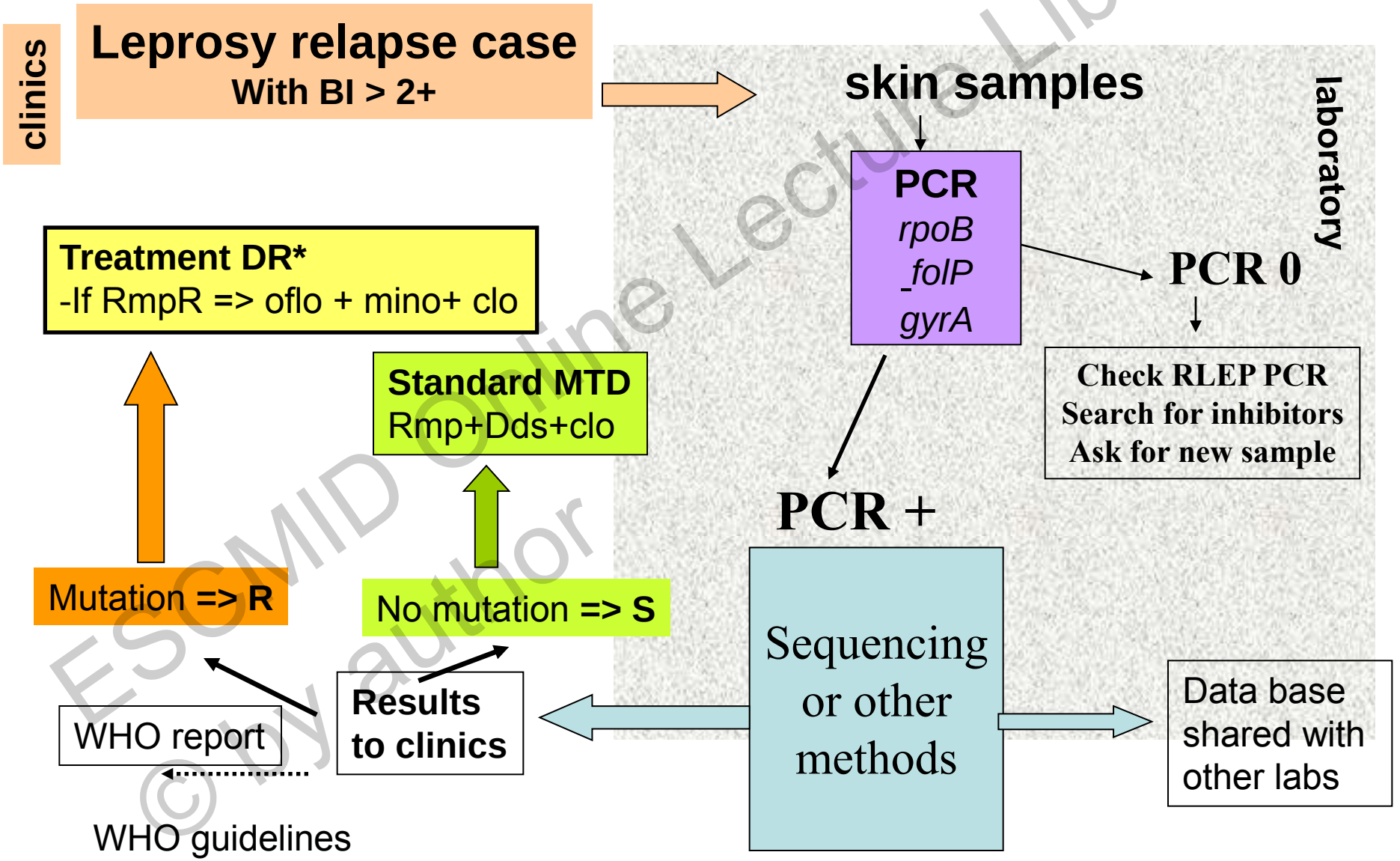
gyrA wild type and mutation

Dapsone resistance

folP1 wild type and mutation

Cambau et al. PloS NTD 2012

WHO sentinel surveillance of drug resistance in leprosy



Treatment for rifampicin-resistant cases (WHO)

- 2 year regimen
  - 6 months of
    - daily clofazimine (50mg)
    - daily Ofloxacin (400mg)
    - daily Minocycline (100 mg) or Clarithromycin (500 mg)

3 drugs daily

  - Followed by 18 months of
    - daily clofazimine
    - Daily Ofloxacin (400 mg) or Minocycline (100 mg)

2 drugs daily

- Should keep strains with ofloxacin susceptibility, minocycline susceptibility and Clarithromycin susceptibility

WHO reports for drug resistance surveillance - Rate of secondary resistance–

	2008	2009	2010	Total
N countries	**3**	**6**	**8**	**8**
N relapses	**69**	**887**	**109**	**1055**
N studied	**59**	**213**	**88**	**360 (30%)**
N PCR pos	**35**	**110**	**72**	**217 (60%)**
R DDS	**8**	**12**	**9**	**29**
R RMP	**6**	**9**	**1**	**16**
R FQs	**0**	**2**	**0**	**2**

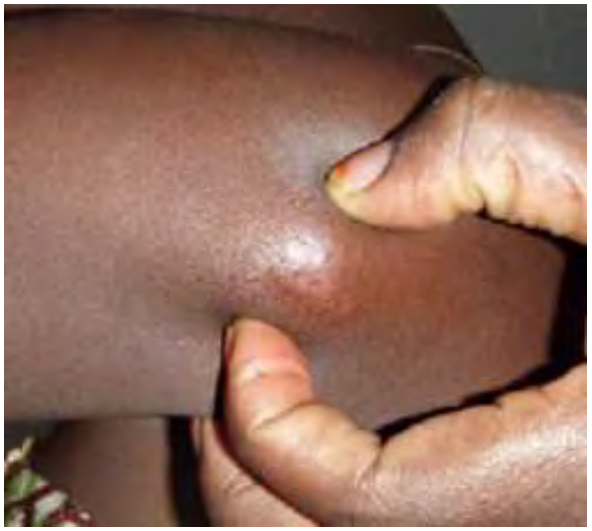
- **15% secondary resistance to dapsone**
- **7% secondary resistance to rifampicin**
- **1% secondary resistance to fluoroquinolones**

Buruli ulcer - Infection due to *M. ulcerans*

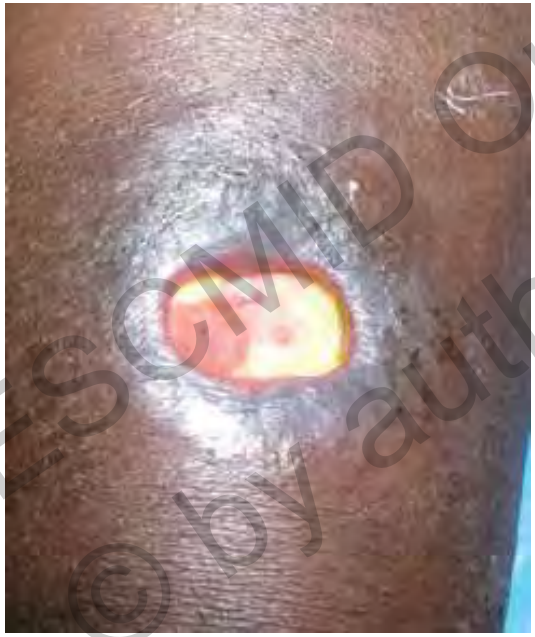
- Mycobacterium ulcerans described in 1948
- Disease was first described in Australia, then in 1960's at Buruli (Ouganda)
- WHO recognition in 1998
- Whole genome sequenced (Stinear 2002)
- Close species or Ecovar infecting non human: *M. liflandii, M. pseudoshottsii*

Walsh et al Dermatol Clin 2011; 29: 1-8
www.who.int/buruli

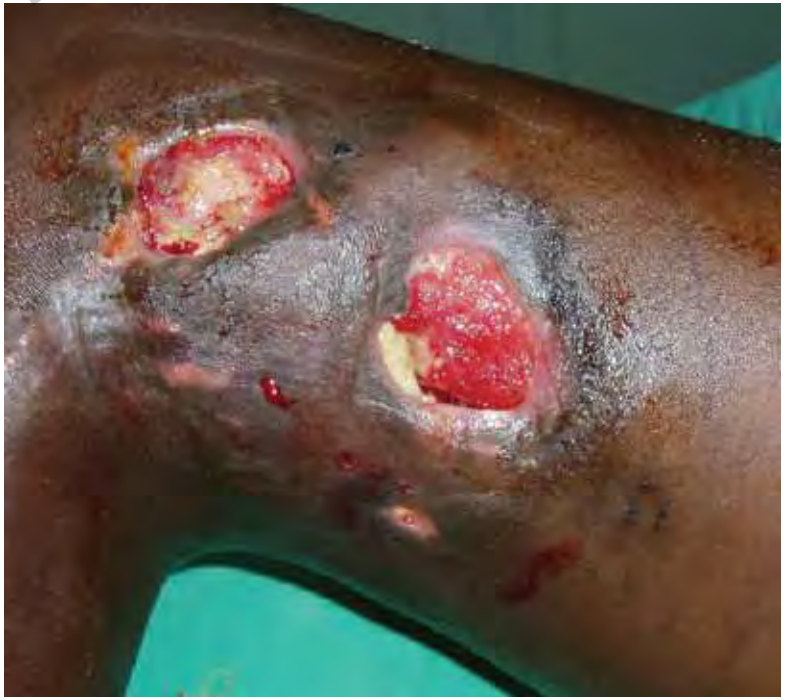
Stinear 2002, Roltgen 2012, Merritt 2010, Williamson 2014

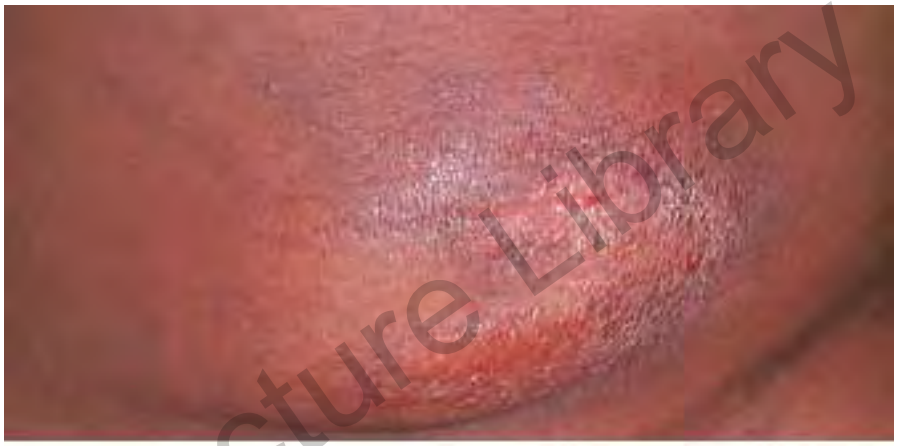


Nodules



Ulcers



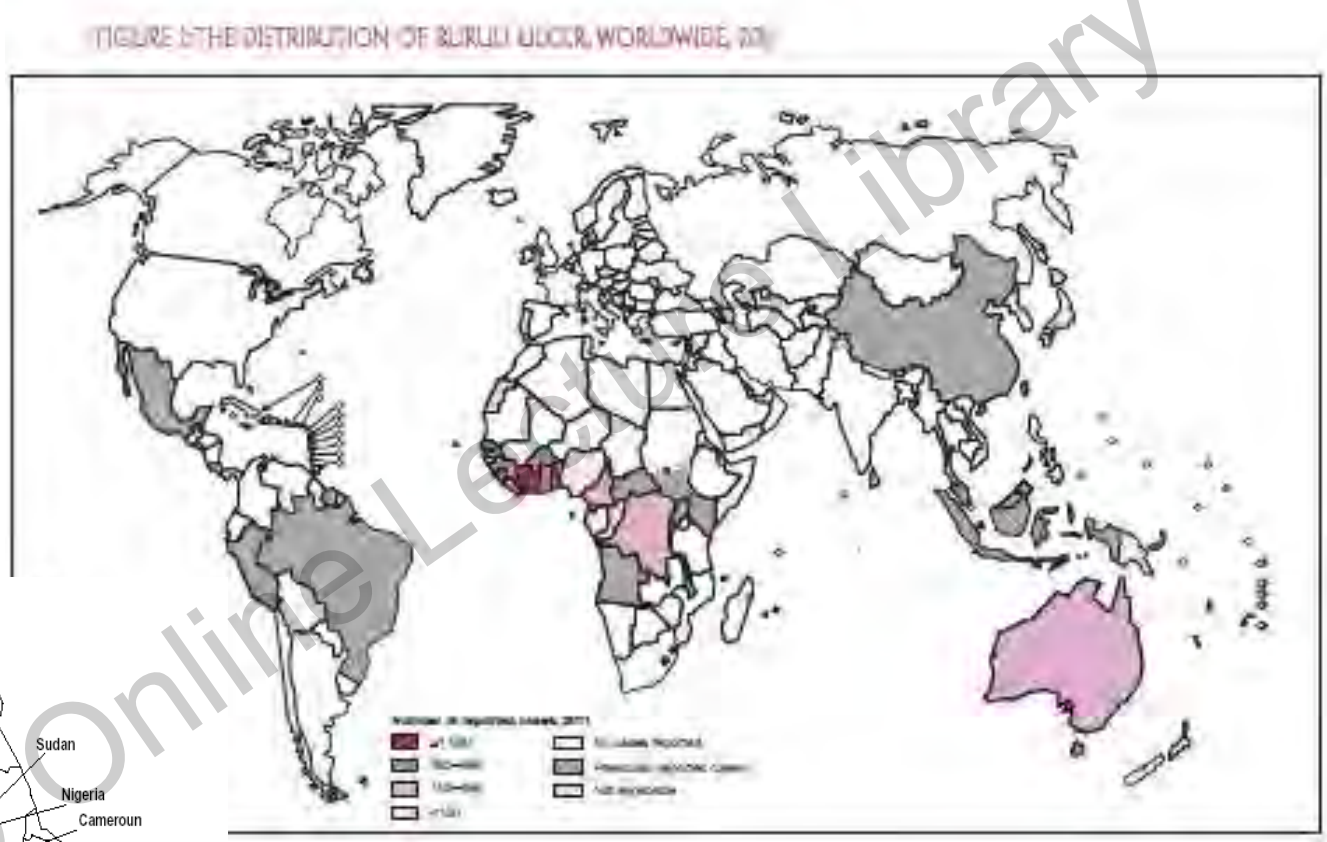


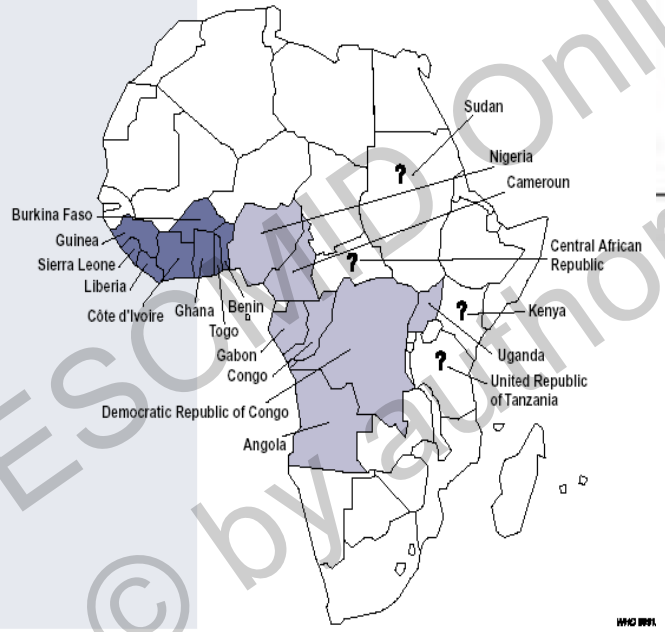
Plaques

Buruli ulcer in Africa ,

- Skin and soft tissue infection= extensive ulceration with undermed edges
- Can lead to great disability
- 50% children
- 60% lower limbs
- Painless and without fever if no staphylococcal or other bacterial surinfection
- Oedema, but no lymph node
- Ulcerative in 75% cases

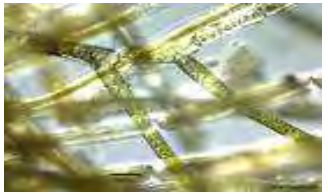
Debacker *et al* 2006; Nackers *et al* 2006; Nackers; Johnson et al 2008; Sopoh et al 2010, WHO 2012





Distribution of Buruli ulcer cases WHO - 2011

Role of water reservoir and insect bites?



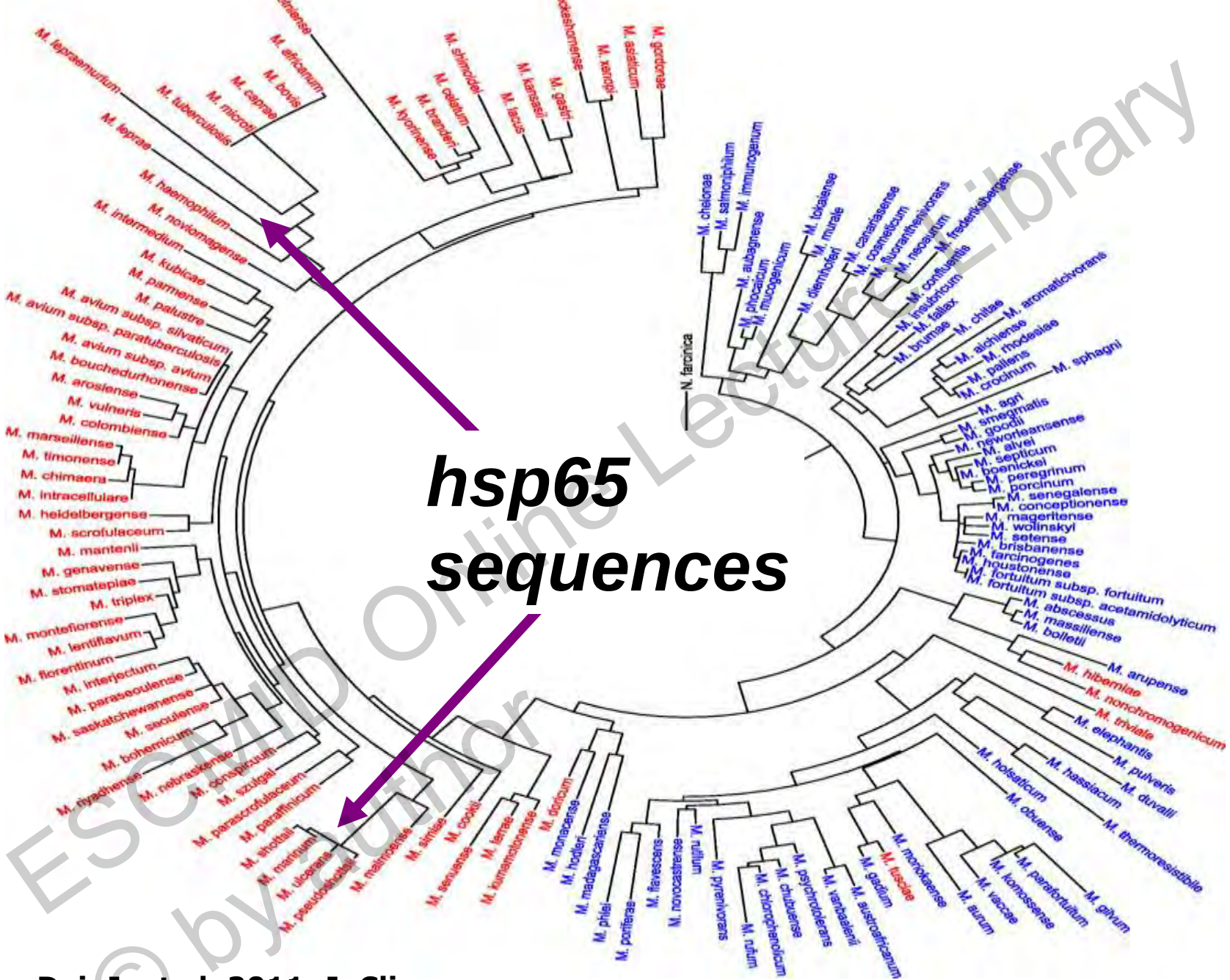
Marsollier et al, 2002, 2004

Portaels et al 2008

Laboratory diagnosis of *M. ulcerans* infection

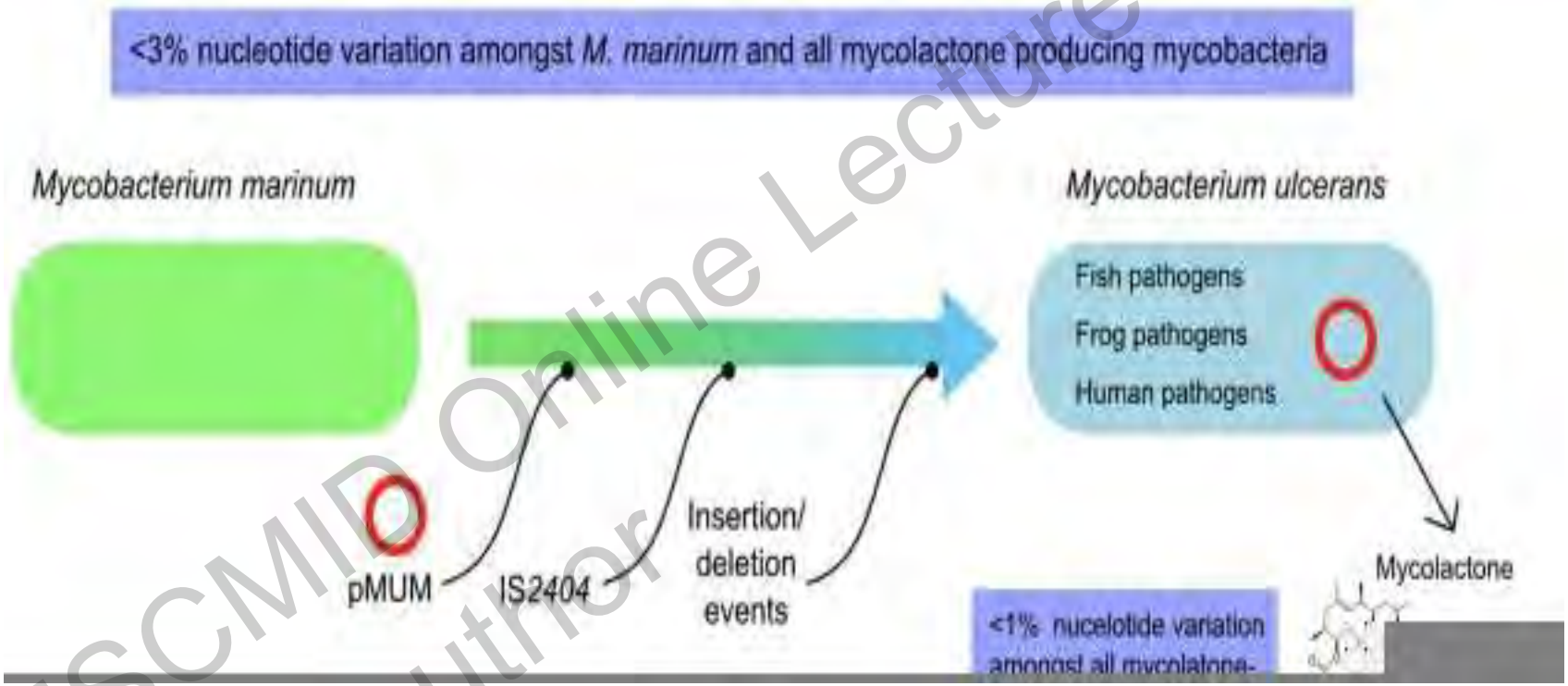
- Microscopic examination with Ziehl-Neelsen staining: positive in 50%
- Cultures on Ogawa, Coletsos solid medium
- PCR IS*2404* (200 copies per genome)
- *M. ulcerans*
  - Very slow grower (culture in 4-6 months)
  - Grows at 30°C
  - division time of 5 days
  - Produces an exotoxin called mycolactone, produced by polyketide synthases encoded by genes located on virulence plasmid pMUM001

George et al, Lancet, 1999, 283 : 854-857



Dai, J. et al. 2011. J. Clin. Microbiol. 49(6):2296-2303

Ancestor with *M. marinum*



Pidot et al. 2010

TREATMENT OF *MYCOBACTERIUM ULCERANS* DISEASE (BURULI ULCER)

- Before 2004 : Surgery until healthy tissue
- After 2004: antibiotic therapy is the first line treatment
- = Rifampicin + streptomycin or clarithromycin during 8 weeks
- Surgery after ATB : for disability prevention, wound care, skin grafting,

WHO 2012

Conclusions: two Neglected Tropical Diseases (NTD)

- Leprosy battle is not won yet, not only in low income countries
- Buruli ulcer is a new infection related to specific geographic distribution
- Early case detection is necessary to overcome these two diseases and reduce disability and stigma
- Standardized antibiotic treatments cure the patients
- Caution for acquisition of resistance
- Research in all areas is mandatory, although few money

More information

- ESCMID group on mycobacterial infections (ESGMYC): www.escmid.org/esgmyc
- ESGMYC meeting at ECCMID on Sunday 11th May 2014, 18:15-19:15, room
- Tuesday May 13th, 2014: official symposium on Leprosy and Buruli ulcer research side (Hall I, from 11:30 to 12:30)