unesp
UNIVERSIDADE ESTADUAL PAULISTA
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FACULDADE DE CIÊNCIAS FARMACÊUTICAS DE ARARAQUARA
SOLIS DOMI SALUS

Research of New Drugs Against Tuberculosis

27-28/07/2009

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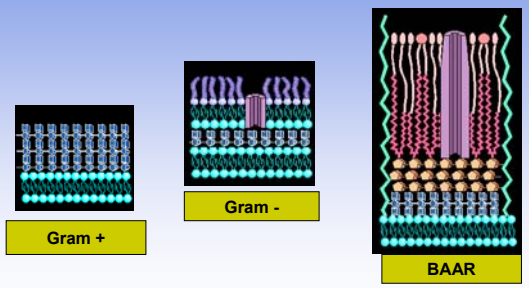
What is "Mycobacterium tuberculosis" ?

Mycobacterium tuberculosis

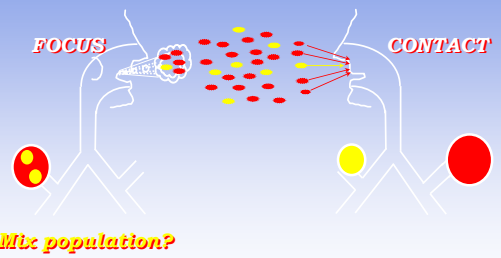
- Ancient pathogen level 3 of danger
- Acid-fast
- Very slow growth
- Complex cell wall
- Approx. 4,000 genes
- Aerobic/anaerobic
- Intracellular/extracellular
- Resistant to many common antibiotics
- No environmental reservoir
- Drying Resistance

Scanning Electron Micrograph of Mycobacterium tuberculosis

Bacteria Cell wall

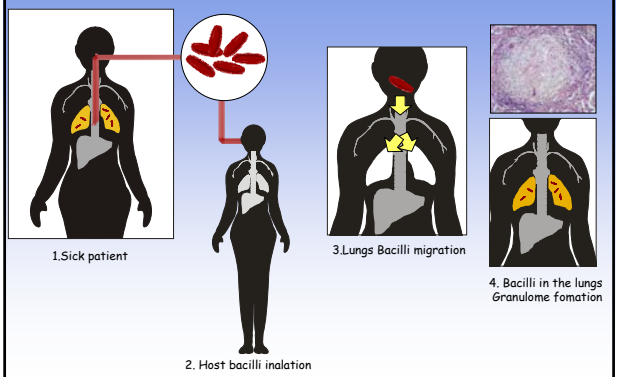


Bacilli transmission

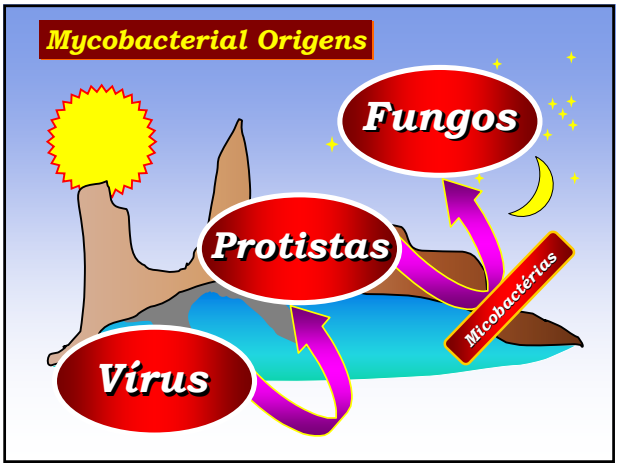


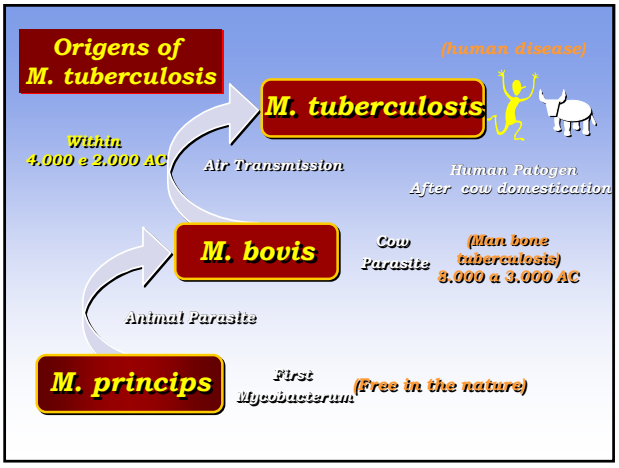
Mix population?

Mechanism of Transmission



When and how appeared "Mycobacterium tuberculosis" ?





Tuberculosis: infectious disease
Agente: "Mycobacterium tuberculosis"

Ordem:
Actinomycetales
Family:
Micobacteriaceae
Gendre:
Micobacterium

Aerobic bacillus, non mobile, no spores, with persistent forms

"Mycobacterium tuberculosis Complex"

- M. tuberculosis
- M. bovis
- M. africanum
- M. ulcerans
- M. microti

The genome (DNA) of M. tuberculosis has totally determined also its chemical characteristic and virulence. The M. microti is not pathogenic and the M.ulcerans cause skin lesion. The M. bovis is a zoonose The M. africanum" Is less pathogenic that M. tuberculosis

Current situation of "tuberculosis" ?

Current Global Status

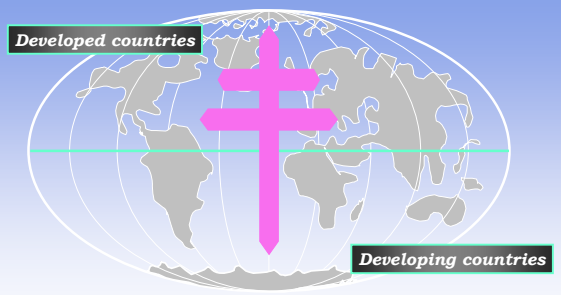
Infected: 1.86 billion (32%)
New cases/yr: 8.7 million

Deaths/yr: 1.7 million (5,000/day)
26% of avoidable deaths in developing world
Brazil : reports the second-highest TB mortality and morbidity among all countries in the Americas

Drug resistance: ubiquitous (WHO/TB)
primary: 1.4% MDR, 10.4% SDR
acquired: 13% MDR, 36% SDR

"No new drugs excepting rifabutin and rifapentine after rifampicin"

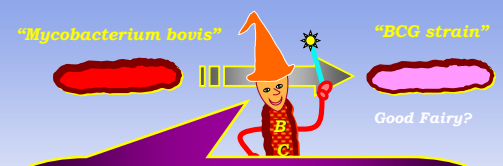
TB distribution in the world



Over 95% of TB cases in the world are in developing countries

For this reason , we who live in the developing countries, feel great responsability for the search for new anti TB drugs

BCG Vaccine



The "BCG strain" is a viable bacterium, originated of the bovine bacillus that was cultivated in glycerin potato middle with ox bile, during 13 years and 230 cultivations biweekly. The bacillus suffered a mutation becoming Non virulent, maintaining the immunogenic properties

Rosemberg J. Vacinação BCG

Current treatment for TB

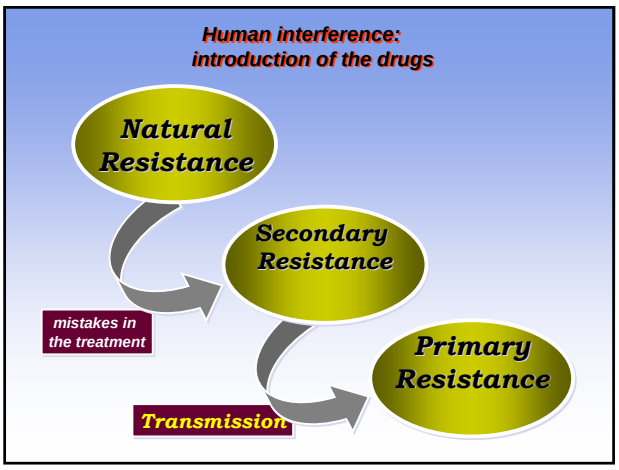
American Thoracic Society, CDC, WHO

- 2 months, daily **(intensive phase)**
  - Isoniazid (INH), 5 mg/kg po (300 mg)
  - Rifampin, 10 mg/kg po (600 mg)
  - Pyrazinamide, 15-30 mg/kg po (1-2 g)

  And (if primary resistance >4% in community)
  - Ethambutol, 15-25 mg/kg po (2-5g)

  or
  - Streptomycin, 15 mg/kg im (1g)
- 4 months daily **(continuation phase)**
  - Isoniazid (INH), 5 mg/kg po (300 mg)
  - Rifampin, 10 mg/kg po (600 mg)

How to undertand the phenomon of "M. tuberculosis" resistance?

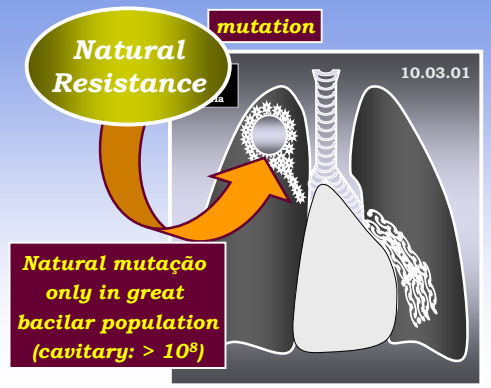


Resistência natural em cepas selvagens do "M. tuberculosis"

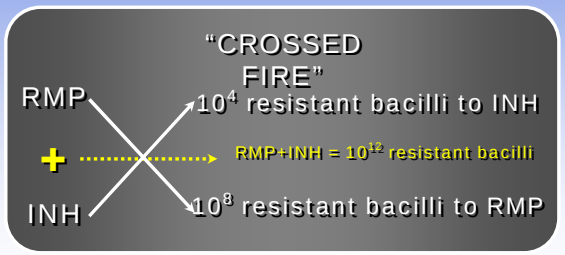
DRUGS	CONCENTRATION IN THE MEDIUM (ug/ml)	RESISTANT MUTANTS FREQUENCY
RMP	40	1 in 10^8
INH	O.2	1 in 10^4
SM	4	1 in 10^4
EMB	2	1 in 10^5
ETH	20	1 in 10^3
PZA	25	1 in 10^3

Fonte: Canetti G et al. Bull WHO, 1969

Where happens the natural resistance?

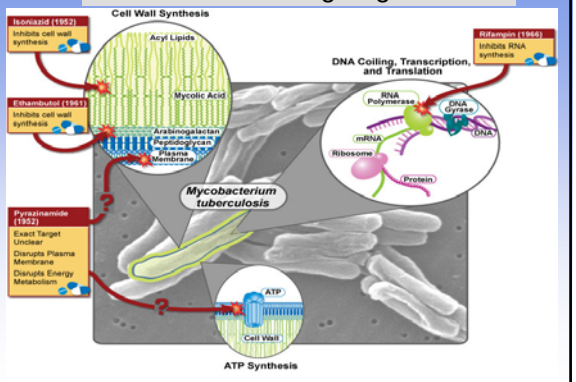


Drugs association to avoid "M. tuberculosis" resistence

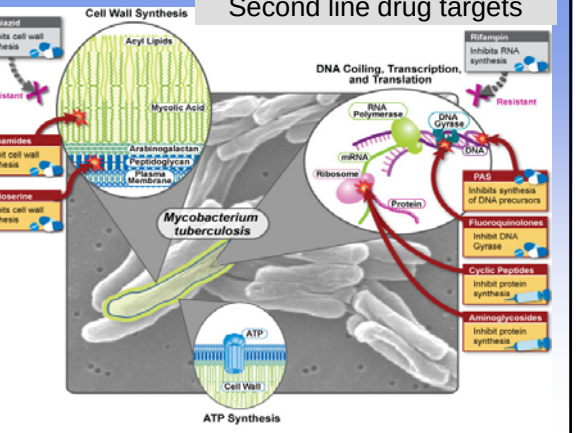


Fontes: Controle da Tuberculose, 3a.Ed., CNTC/NUTES/MS, R.J.1992
Dalcolmo MP, Tese de Doutorado, 1999.

First line drug targets



Second line drug targets



Mecanismos de ação das drogas

- ***Inhibidores de síntese biossíntese de ácidos micolicos (INH, PZA ETB, PA-824, OPC-67683***
- ***Inibidores de biossíntese de arabinogalactana e peptidioglicana (ETB, D-Ciclocerina, Amoxicilina, Clofazimina***
- ***Inibidores de síntese de proteinas (SM, Claritromicina, Linezolide)***
- ***Inibidores dos processos realizados por DNA (Rifampicina, Fluoroquinolonas)***
- ***Inibidores do dehidrofolato redutase ou biossíntese de sideroforos (PAS)***

Micobacterial Mecanisms of Resistance

- *Variation of cell wall permeability*
- *Supression of Enzyms that modify the drugs (INH e PZA)*
- *Mutation of the targets of the drug*
- *Super production of targets*

Genes associated with resistency

- Gene *rpo*B – RMP
- Genes *kat*G, *inh*A, *oxy*R-*ahp*C, *kas*A – INH
- Genes *rrs* e *rps*L – SM
- Gene *pnc*A – PZA
- Genes *emb*A, *emb*B e *emb*C – ETB
- Genes *gyr*A e *gyr*B – fluoroquinolonas

Why new drugs against *Mycobacterium tuberculosis*?

Impact of New Chemotherapy

1. **Reducing Treatment Duration**
   - **Improved compliance**
2. **Successful treatment of MDR-TB**
   - **Reduce transmission of MDR-TB**
     - **Decrease cost of treatment**
3. **Cure latent TB infection**
   - **Reduce/eliminate disease reservoir**

Approaches to New TB Drugs

- **Ligand-based** whole cell screening
  - optimize non-TB antimicrobial classes
  - optimize TB drugs
  - novel synthetic
  - novel natural products
    - Ethnomedical
- **Target-based** discovery
  - target identification
  - screening (in silico, NMR, functional)
- **Random high throughput screening (HTS) of synthetic and natural products vs. *M. tuberculosis* (whole cell screening)**

Ideal properties of new anti-TB drugs

None Toxicity

- Rapid bactericidal activity on extra and Intracellular bacilli (inside macrophages)
- Long tissue half-life anti TB activity
- Good oral bioavailability and tolerability
- Low toxicity : hepato, cardio, bone marrow, nefro, neuro, and genotoxicity
- No drug-drug interations or antagonism with retro virus drugs

Reasons for delayed investigation

1. The search is expensive
2. The bacterium is hard for handle
3. The companies engaged in the development of new TB drugs perceived lack of commercial return
   - **Over 95% of TB cases in the world are in developing counties**
4. For this reason , we who live in the developing countries, feel responsible for the search for new anti TB drugs

Why synthetic metallo-organic complexes?

Why synthetic metallo-organic complexes?

- For the first time in decades, there is now a pipeline of new synthetic compounds that are being tested on TB
- Within this growing pipeline of potential new TB drugs, seven are at various stages of clinical development

Tuberculosis (TB) clinical drug development programs

Compound	Development Stage	Sponsor/Coordinator
Gatifloxacin	Phase 3	European Commission; IRD; WHO/TDR; Lupin
Moxifloxacin	Phase 2/3	Bayer; TB Alliance; CDC; University College London; Johns Hopkins University
TMC 207 (Diarylquinoline)	Phase 2	Johnson & Johnson (Tibotec)
OPC 67683 (Nitroimidazole)	Phase 1 EBA	Otsuka Pharmaceutical
PA 824 (Nitroimidazole)	Phase 1	TB Alliance
LL 3858 (Pyrrole)	Phase 1	Lupin
SQ 109 (Diamine)	Phase 1	Sequella

(Melvin K. Spigelman JID, 2007)

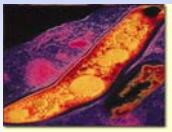
Why sinthetic metallo-organic complexes?

1. Within this group, there are no complexes between metals and organic compounds
2. Medicinal Inorganic Chemistry is an area in continuous expansion
3. Many studies have shown an increase in the pharmacological activity of pure organic compounds when complexed with metals
4. We decided to make complexes of vanadium with thiosemicarbazone, semicarbazone and hydrazone derivatives as ligands
5. For each compound made, we determined its anti-TB activity and cytotoxicity

Methods to evaluate biological activities?

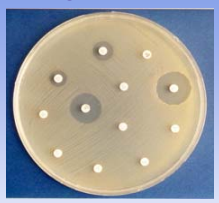
Determination of *in vitro* antimycobacterial ativity

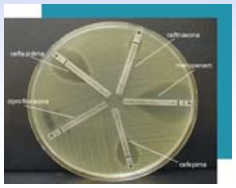
- Target Bacterium
- ***Mycobacterium tuberculosis H37Rv (Lab. nivel 3)***
- ***Mycobacterium smegmatis***



Biological assays

- Classic disk diffusion assay should be avoided because the mycobacteria grow slowly.
- Dilution methods in agar they are accomplished, for study of extracts and fractions, but the technique is difficult and slow (18 days to have resulted of MIC)





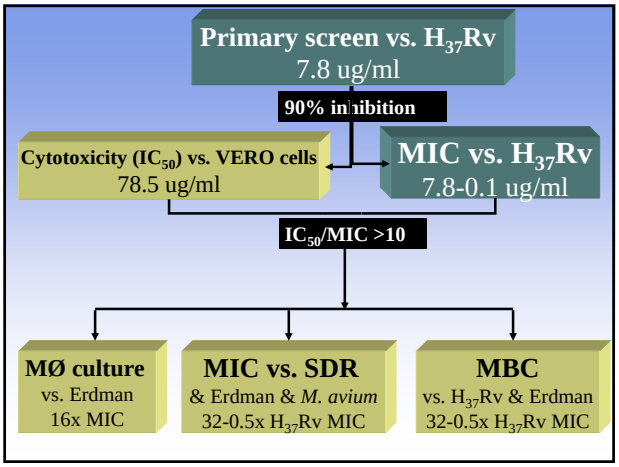
Sistema BACTEC

- Radiometric BACTEC 460 Assay : Método caro, um unico tubo de 4 ml, contendo meio radioativo, U$2.50

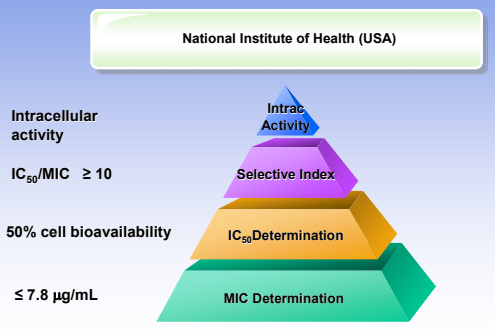
- MGIT: Non radiometric

Antimycobacterial activity in vitro Assay – REMA

- *Mycobacterium tuberculosis* H37Rv
- 96-well format, 200 ul.
- Small sample requirement
- Incubation: 6 day , 37°C
- Mycobacterial growth is determined by reduction of the blue dye (Resazurin), to the pink and fluorescent resofurin
- A change from blue to pink indicates bacterial cells growth
- The MIC is defined as the lowest concentration of drugs that inhibits 90% of cell growth
- High-throughput anti-TB assay using microplate spectrophotometer or fluorimeter



Screening of new anti-TB candidates

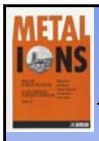


Synthesis, characterization, X-ray structure and in vitro antimycobacterial and antitumoral activities of Ru(II) phosphine/diimine complexes containing the "SpymMe2" ligand, SpymMe2 = 4,6-dimethyl-2 mercaptopyrimidine

Fábio B. do Nascimento, Gustavo Von Poelhsitz, Fernando R. Pavan , Daisy N. Sato, Clarice Q.F. Leite , Heloisa S. Selistre-de-Araújo, Javier Ellena, Eduardo E. Castellano, Victor M. Deflon, Alzir A. Batista. v. 102, 1783-1789, 2008.

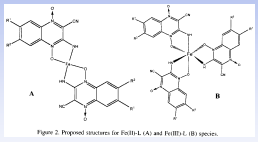
Compounds	Structures	MIC μg/mL	MIC μM
Free Phosphines and Diimines Ligands			
dppb		>50	>117,20
Bipy		25	160,10
Me-Bipy		25	135,70
HspymMe2		25	178,30

Compounds	Structures	MIC μg/mL	MIC μM
Ru(II) phosphine/diimine complexes			
$[Ru(SpymMe_2)(dppb)(bipy)]PF_6$		0,78	0,80
$[Ru(SpymMe_2)(dppb)(Me\text{-}bipy)]PF_6$		0,78	0,78
cis-$[RuCl_2(dppb)(bipy)]$		3,90	5,17
cis-$[RuCl_2(dppb)(Me\text{-}bipy)]$		6,25	7,99



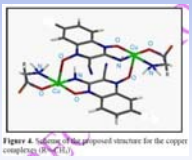
Novel Iron Complexes with quinoxaline N^1, N^4 – dioxide derivatives: synthesis, characterization and Antimycobacterial Activity

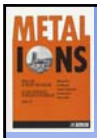
M. Belén Tarallo, Carolina Urquiola, Antonio Monge, Fernando R. Pavan, Clarice Q.F. Leite, María H. Torre, Dinorah Gambino. John Libbey Eurotext, Paris; v. 10, 867-872, 2008.

Compounds	Structures	REMA IC50 MIC (μg/mL)	IS
Iron Complexes with quinoxaline N^1, N^4 – dioxide derivatives			
Fe(II) – L1		3.9 125	32
Fe(III) – L1		7.8 156	20
Fe(III) – L2		3.9 156	40
Ligand			

Research of new mixed-chelate copper complexe with quinoxaline N1,N4-dioxide derivatives and alanine as ligands, potential antimycobacterial agents

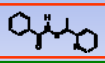
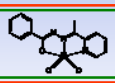
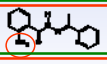
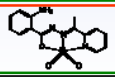
M. Belén Tarallo, Antonio J. Costa-Filho, Ernanni D. Vieira, Antonio Monge, Clarice Q.F. Leite, Fernando R. Pavan, Graciela Borthagaray, Dinorah Gambino, María H. Torre. "in press", 2009.

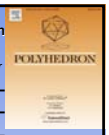
Compounds	Structures	REMA MIC (μg/mL)
mixed-chelate copper complexe with quinoxaline N1,N4-dioxide derivatives and alanine as ligands		
CuL1ala		7.8
CuL2ala		7.8
CuL3ala		7.8
Ligand		



Synthesis and *anti-Mycobacterium tuberculosis* activity of Vanadium complexes with N,N,O-donor ligands

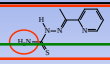
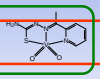
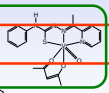
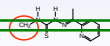
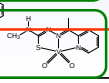
Pedro I. da S. Maia, Victor M. Deflon, Fernando R. Pavan, Clarice Q.F. Leite, Claudia C. Gatto, Sebastião S. Lemos, Alzir A. Batista. John Libbey Eurotext, Paris; v. 10, 197-203, 2008.

Compounds	Structures	REMA (MIC) (μg/mL)	IC_{50}
Hidrazones, Semicarbazones and Vanadium Complexes			
Hapbh		1.9	1.9
$[VO_2(apbh)]$		0.97	1.9
Hapah		15,6	1.9
$[VO_2(apah)]$		7.8	1.9



Vanadium complexes with thiosemicarbazones: Synthesis, characterization crystal structures and *anti-Mycobacterium tuberculosis* activity

Pedro I. da S. Maia, Fernando R. Pavan, Clarice Q.F. Leite, Sebastião S. Lemos, Gerimário F. de Sousa, Alzir A. Batista, Otaciro R. Nascimento, Javier Ellena, Eduardo E. Castellano, Elke Niquet, Victor M. Deflon.v. 28, 398-406, 2009.

Compounds	Structures		REMA(MIC)	IC_{50}
	Ligands	Complexes	μg/mL	
Thiosemicarbazones and Vanadium Complexes				
Haptsc			31.3	156
$[VO_2(aptsc)]$			31.3	19.5
Happtsc			15.6	3,9
VO(acac)(apptsc)]			1.6	3.9
Hapmtsc			7.8	1.9
$[VO_2(apmtsc)]$			3.9	1.9

Collaborators

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Ph.D. Sergio Roberto de Andrade Leite
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Marcelo Miyata
José Rodrigo Pandolfi

Master Students
Natália Mendes

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