Universität Zürich[UZH]

Institut für Medizinische Mikrobiologie

Molecular mechanisms of drug resistance in NTM

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Zürich, Switzerland

12:00-12:30 Saturday 27th April

Medical relevance of nontuberculous mycobacteria (NTM)

Examples:

M. leprae	Leprosy
M. abscessus subsp. abscessus	Lung disease
M. kansasii	Lung disease
M. avium	Lymphadenitis
M. ulcerans	Cutaneous ulcers
M. haemophilum	Joint infections
M. genavense	Disseminated infections

Tortoli. **Clinical manifestations of nontuberculous mycobacteria infections.** Clin Microbiol Infect. 2009 Oct;15(10):906-10.

Non-tuberculous mycobacteria

- Slow growers; approx.60 species
  - *M. avium*
  - *M. intracellulare*
  - *M. kansasii*
  - *M. genavense*
  - *M. leprae*
  - *M. xenopi*
  - *M. simiae*

- Fast growers; approx. 70 species
  - *M. fortuitum* group
  - *M. chelonae/M.abscessus* group
  - *M. smegmatis* group
  - *M. margeritense* / *M. wolinskyi* group

What do we detect in the diagnostic laboratory?

Sequence based identification of NTM directly in clinical specimens during a 6-months period:

16S rRNA gene sequence identification	n
M. chelonae/abscessus complex	36
M. kansasii/gastri	10
M. avium	9
M. fortuitum	7
M. xenopi	3
M. mucogenicum	2
M. asiaticum	1
M. intracellulare	1
M. malmoense	1
Non-pathogenic mycobacteria (mostly rapid growers)	52

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American Thoracic Society Documents

An Official ATS/IDSA Statement: Diagnosis, Treatment, and Prevention of Nontuberculous Mycobacterial Diseases

David E. Griffith, Timothy Aksamit, Barbara A. Brown-Elliott, Antonino Catanzaro, Charles Daley, Fred Gordin, Steven M. Holland, Robert Horsburgh, Gwen Huitt, Michael F. Iademarco, Michael Iseman, Kenneth Olivier, Stephen Ruoss, C. Fordham von Reyn, Richard J. Wallace, Jr., and Kevin Winthrop, on behalf of the ATS Mycobacterial Diseases Subcommittee

This Official Statement of the American Thoracic Society (ATS) and the Infectious Diseases Society of America (IDSA) was adopted by the ATS Board Of Directors, September 2006, and by the IDSA Board of Directors, January 2007

This document has an online supplement, which is accessible from this issue's table of contents at www.atsjournals.org

Am J Respir Crit Care Med Vol 175. pp 367–416, 2007
DOI: 10.1164/rccm.200604-571ST
Internet address: www.atsjournals.org

CLSI guidelines

Woods GL, et al. 2011.

Susceptibility testing of mycobacteria, nocardia, and other aerobic actinomycetes; approved standard.

CLSI document M24-A2.

Clinical and Laboratory Standards Institute, Wayne, PA.

Most common used antibiotics for treatment of NTM infections

Protein synthesis

- Macrolides
- Tetracyclines
- Aminoglycosides

RNA synthesis

- Rifampicin

DNA replication/gene expression

- Fluoroquinolones

Arabinogalactan/arabinomannan synthesis

- Ethambutol

Three principal mechanisms of resistance towards antibiotics:

a. Impermeability and efflux

b. Target modification

c. Drug inactivation

Resistance against antibiotics

Innate / intrinsic resistance

Innate presence of specific genes

- For example aminoglycoside modifying genes

Acquired resistance

Acquired mutations in chromosomal genes

- For example *rpoB*: rifampicin resistance

Reviews

Clinical Microbiology Reviews

Antimicrobial Susceptibility Testing, Drug Resistance Mechanisms, and Therapy of Infections with Nontuberculous Mycobacteria

Barbara A. Brown-Elliott, Kevin A. Nash and Richard J. Wallace Jr

Clin. Microbiol. Rev. 2012, 25(3):545. DOI: 10.1128/CMR.05030-11.

Drug Resistance Updates 15 (2012) 149–161

Contents lists available at SciVerse ScienceDirect

Drug Resistance Updates

journal homepage: www.elsevier.com/locate/drup

Resistance mechanisms and drug susceptibility testing of nontuberculous mycobacteria

Jakko van Ingen[a,*], Martin J. Boeree[b], Dick van Soolingen[a,b], Johan W. Mouton[a]

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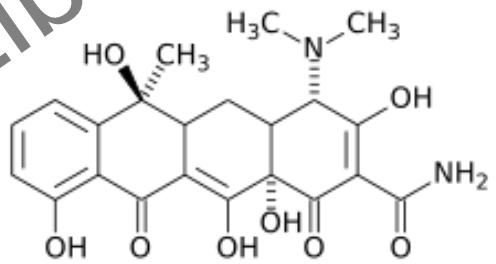
Resistance against macrolides (protein synthesis)

Resistance against clarithromycin / erythromycin

- Intrinsic resistance
  - *erm* genes
- Acquired resistance
  - 23S rRNA gene

Resistance against tetracyclines



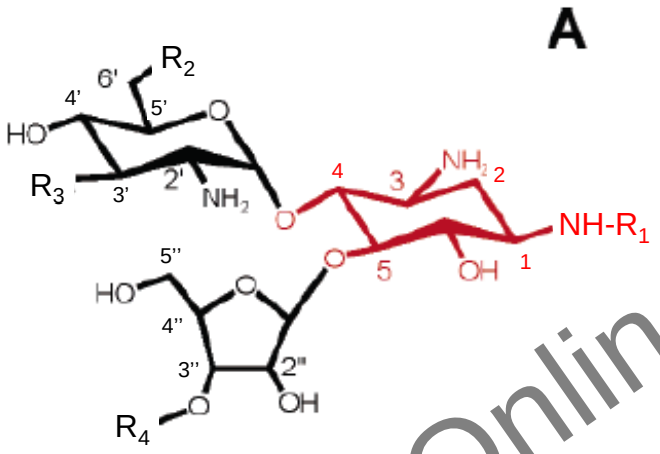
- tetracycline and doxycycline
- glycylcyclines, such as tigecycline
- binds to the 30S subunit of the ribosome and inhibit protein synthesis
- resistance to tetracyclines is associated primarily with ribosome protection and drug efflux
- ribosome protection protein genes *otr(A) and tet(M),*
- efflux pumps: homologs of *tet(K), tet(L), tet(V), and otr(B)*
- homology analysis and the inconsistent presence in mycobacteria suggest that these genes were transferred horizontally: acquired resistance
- genomic sequence data suggest that mycobacteria lack genes encoding tetracycline-inactivating enzymes

Resistance against aminoglycosides

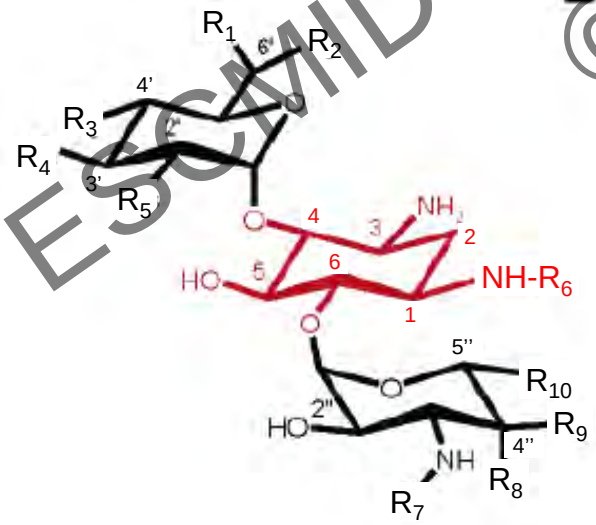
- Aminoglycosides: for example kanamycin and streptomycin
- Mode of action: inhibition of protein synthesis by binding to the ribosome
- Acquired resistance:
    - Mutations in the 16S rRNA gene
    - Mutations in rpsL gene; encoding S12 ribosomal protein)
    - Mutations do not confer resistance to all aminoglycosides, for example 1408 mutation confers resistance against amikacin and kanamycin, but NOT to streptomycin
- Intrinsic resistance: aminoglycoside modifying enzymes, such as acetyl transferases (AAC) and phospho transferases (APH).
    - Rapidly growing mycobacteria*: M.fortuitum, M. smegmatis, M. abscessus;* clinical significance?
    - Homologs also found more recently in *M. tuberculosis*

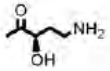
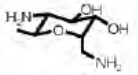
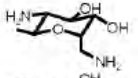
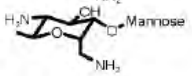
"Typical" Aminoglycosides

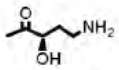
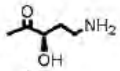
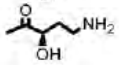
4,5-Deoxystreptamins:



4,6-Deoxystreptamins:



Antibiotic	R_1	R_2	R_3	R_4
Ribostamycin	H	NH_2	OH	H
Butirosin		NH_2	OH	H
Paromomycin	H	OH	OH	
NeomycinB	H	NH_2	OH	
Lividomycin A	H	OH	H	

Antibiotic	R_1	R_2	R_3	R_4	R_5	R_6	R_7	R_8	R_9	R_{10}
anamycin A	H	NH_2	OH	OH	OH	H	H	H	OH	CH_2OH
anamycin B	H	NH_2	OH	OH	NH_2	H	H	H	OH	CH_2OH
obramycin	H	NH_2	OH	H	NH_2	H	H	H	OH	CH_2OH
ibekacin	H	NH_2	H	H	NH_2	H	H	H	OH	CH_2OH
entamicin B	H	NH_2	OH	OH	OH	H	CH_3	OH	CH_3	H
ientmicin C1	CH_3	$NHCH_3$	H	H	NH_2	H	CH_3	OH	CH_3	H
entmicin C1A	H	NH_2	H	H	NH_2	H	CH_3	OH	CH_3	H
ientmicin C2	CH_3	NH_2	H	H	NH_2	H	CH_3	OH	CH_3	H
isomicin*	H	NH_2	H	H	NH_2	H	CH_3	OH	CH_3	H
Netilimicin*	H	NH_2	H	H	NH_2	CH_2CH_3	CH_3	OH	CH_3	H
Isepamicin	H	NH_2	OH	OH	OH		CH_3	OH	CH_3	H
Arbekacin	H	NH_2	H	H	NH_2		H	H	OH	CH_2OH
Amikacin	H	NH_2	OH	OH	OH		H	H	OH	CH_2OH

*(Δ5'>4' unsaturation)

"Atypical" Aminoglycosides

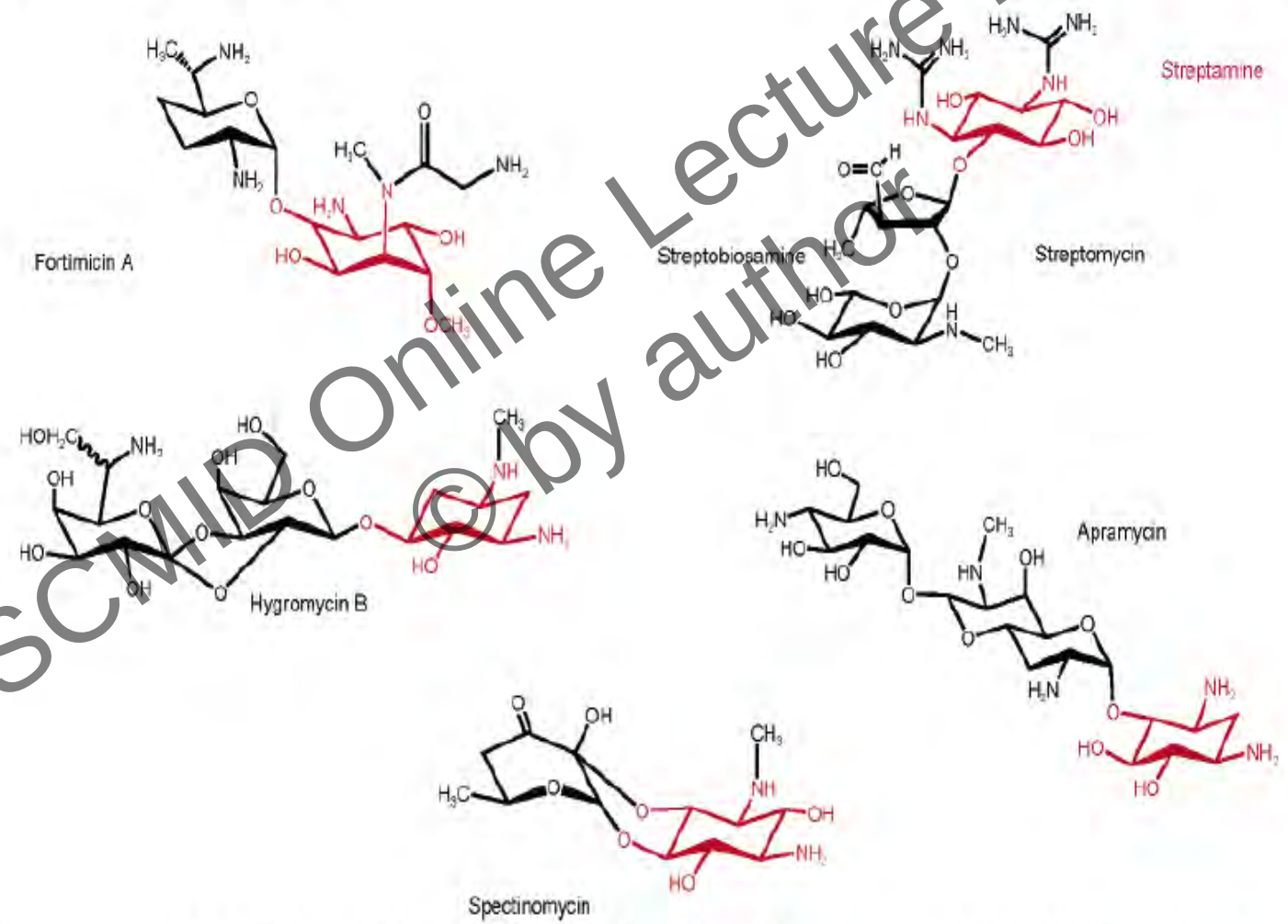
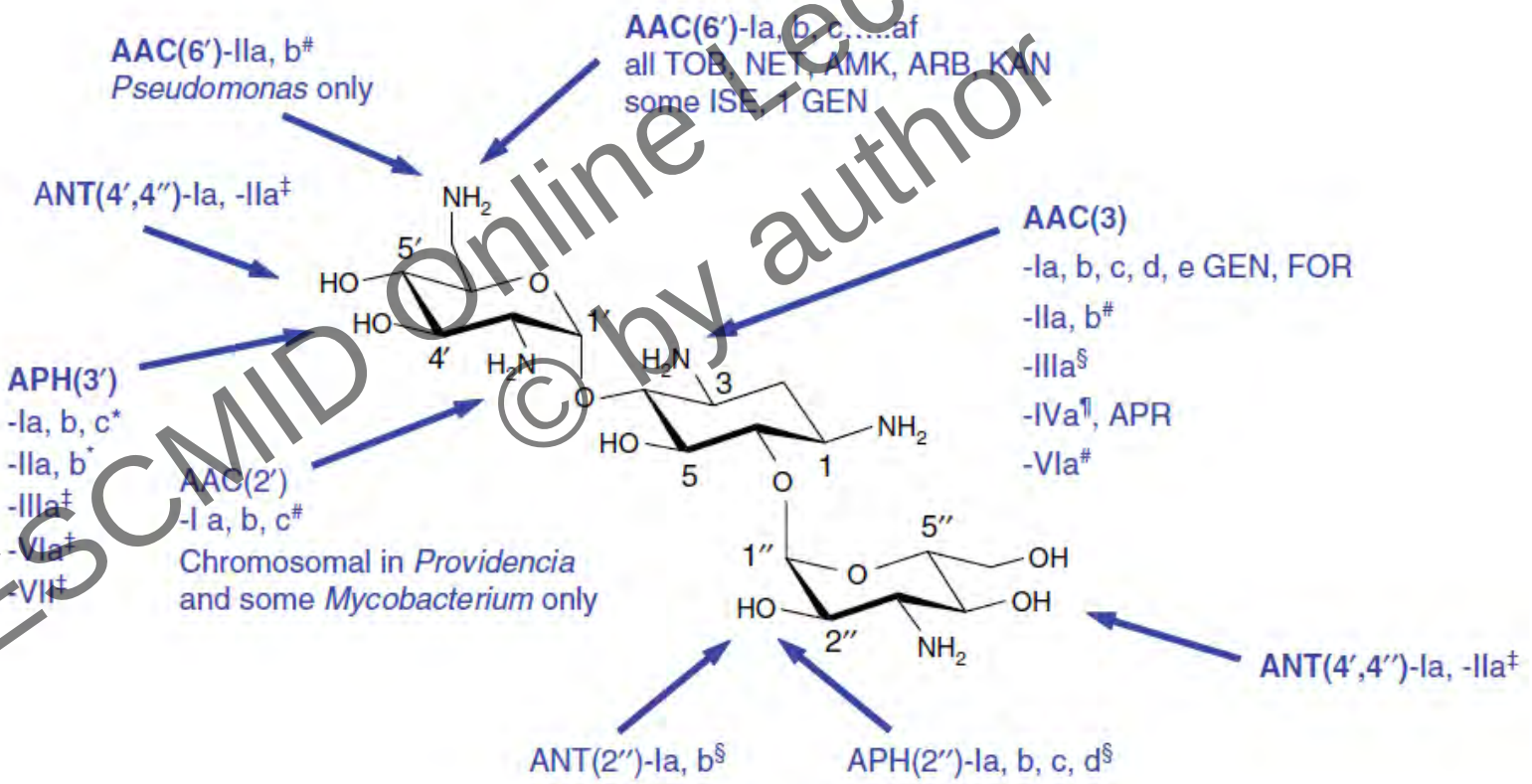


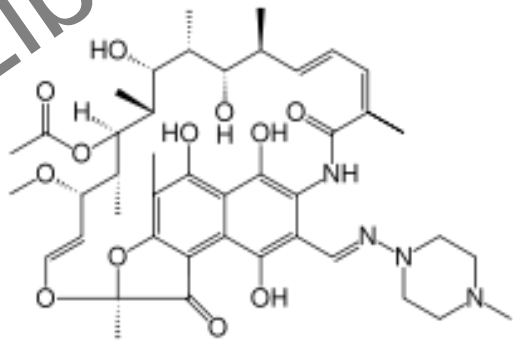
Figure 2. Structures of clinically useful atypical aminoglycosides. The aminocycitol ring is shown in red.

Enzymatic inactivation of aminoglycosides by modification



modified from Dozzo et al. New aminoglycoside antibiotics. Expert Opin. Ther. Patents 2010

Resistance against rifamycins



- Rifamycins: rifampin and rifabutin
- Inhibits RNA synthesis by binding to RNA polymerase

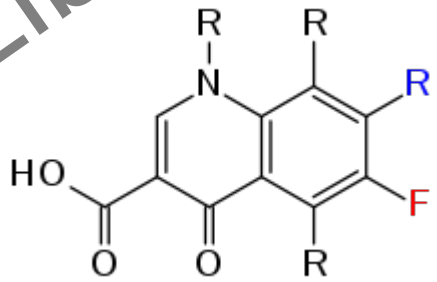
Fast growing non-tuberculous mycobacteria:

- naturally resistant; impermeability of the cell wall
- *arr* gene: ADP-ribosylation, modifying rifamycin (for example *M. smegmatis*)

Slow growing non-tuberculous mycobacteria; for example: *Mycobacterium kansasii*

- acquired resistance: mutations in *rpoB;* beta-subunit of the RNA polymerase
  - 80-bp region of the *rpoB* gene; codons 526 and 461

Resistance against fluoroquinolones

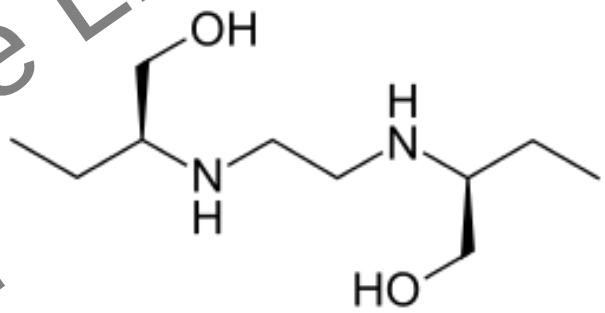


- Fluoroquinolones: synthetic antibiotics, for example ciprofloxacin and levofloxacin
- Inhbition of DNA replication and gene expression
- DNA gyrase relaxes supercoiled DNA ahead of the DNA helicase and DNA replication complex
- Inhibition of DNA gyrase by fluoroquinolones results in prevention of DNA replication
- Resistance by mutations in *gyrA* gene (acquired resistance)

Resistance against fluoroquinolones

- Second target: topoisomerase IV
- *M. abscessus, M. avium and M. tuberculosis* appear to lack a topoisomerase IV (genome sequence based)
- *M. smegmatis* and *M. vanbaalenii* do have a topoisomerase IV
- Resistance : mutations in gene encoding topoisomerase IV (acquired resistance)
- Intrinsic resistance: permeability of the cell, efflux (for example *lfrA)*

Resistance against ethambutol



- **Ethambutol**
  - Slowly growing mycobacteria
  - Limited use for rapidly growing mycobacteria

  - **Mode of action:** disruption of cell wall synthesis
  - Inhibition synthesis of arabinogalactan and lipoarabinomannan
  - Target for ethambutol: arabinosyl transferase

  - **Resistance:**
    - mutations in *embB* gene (acquired resistance); for example *M. smegmatis*
    - Possibly also in the regulatory gene *embR*
    - Polymorphisms in *embB*, *lfrA*, efflux pump (intrinsic resistance)

Clarithromycin resistance in *Mycobacterium abscessus*

J Antimicrob Chemother 2012; **67**: 2606–2611
doi:10.1093/jac/dks279 Advance Access publication 24 July 2012

Journal of Antimicrobial Chemotherapy

Acquisition of clarithromycin resistance mutations in the 23S rRNA gene of *Mycobacterium abscessus* in the presence of inducible *erm*(41)

Florian P. Maurer*, Vera Rüegger, Claudia Ritter, Guido V. Bloemberg and Erik C. Böttger

Institut für Medizinische Mikrobiologie, Nationales Zentrum für Mykobakterien, Universität Zürich, 8006 Zürich, Switzerland

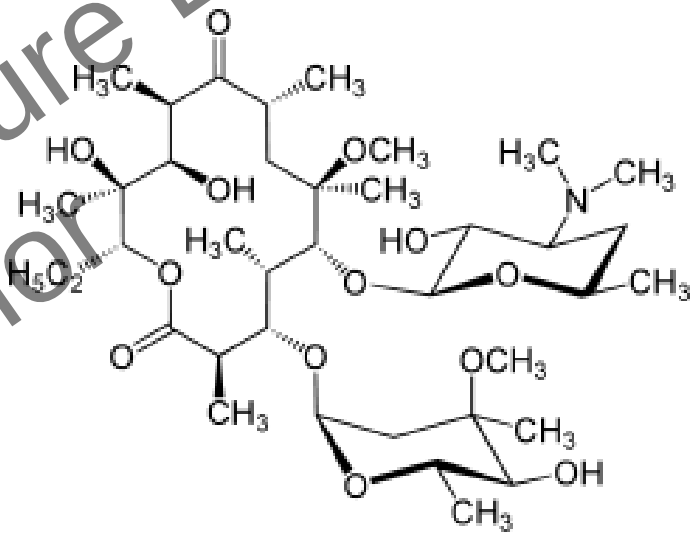
M. abscessus

General information

- Pulmonary infections; chronic infections
- Risk groups: underlying pulmonary diseases, e.g. bronchiectasis and cystic fibrosis
- a major complication following lung transplantation in CF patients
- Treatment
  - surgical resection of focal bronchiectasis and cavity
  - combination therapy including the macrolide clarithromycin
  - only drug of demonstrated efficacy that can be administered orally

Clarithromycin

- Macrolide
- Binds to the 23 rRNA
- Inhibits translation
- Acid-stable, orally taken
- High concentration in phagocytes



Resistance against macrolides (protein synthesis)

Resistance against clarithromycin / erythromycin

- Intrinsic resistance
  - *erm* genes
- Acquired resistance
  - 23S rRNA gene

Clarithromycin resistance in *M. abscessus*

Two main resistance mechanisms:

- Mutations in the drug-binding pocket, in particular at nucleotide positions 2058 and 2059, of the bacterial 23S rRNA gene confering high level Cla resistance (MIC = 256 mg/L)
- Inducible *erm* gene
  - *erm* gene encodes: ribosomal methylase Erm (41)
  - mono- or di-methylate the adenine at position 2058 of the 23S rRNA
  - *Erm* genes have been described in many species of rapidly growing mycobacteria
  - Functionality dependent on the nucleotide at position 28
  - WT 28T inducible resistance; mutant 28C loss of functionality

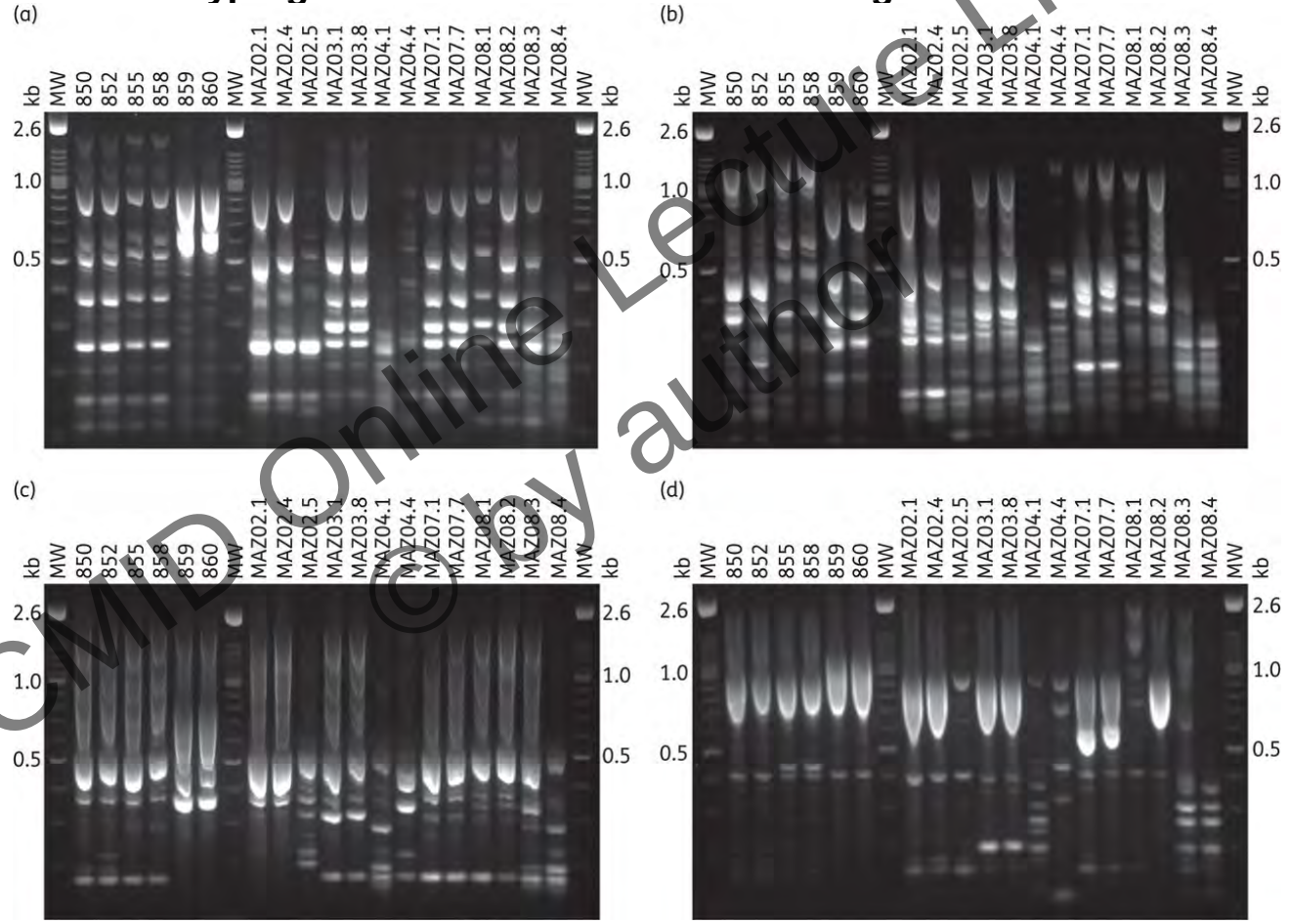
Does *M. abscessus* acquire resistance mutations in the *rrl* gene in addition to the presence of an inducible Erm(41) methylase ?

- Monitoring 5 patients over 2-4 years
- 29 *M. abscessus* clinical isolates
- Genetic and phenotypic characterisation
- In three out of five patients acquisition of resistance mutations in the *rrl* gene in addition to the presence of an inducible Erm methylase

Phenotypic and genotypic monitoring

			Date of isolation		Clarithromycin MIC (mg/L) by method				Genotype		
					microdilution			Etest			
Patient	Isolate	Source	month/year	months since first isolate	day 3	day 7	day 14	day 7	*erm*(41)[a]	23S peptidyltransferase region	*rpoB*
Control	MAZ01.1	left forearm	08/08	0	0.5	1	1	0.064	C28	wild-type	*M. abscessus* subsp. *abscessus*
MAZ02	MAZ02.1	respiratory tract	06/08	0	1	>128	>128	0.5	T28	wild-type	*M. abscessus* subsp.
	MAZ02.2	respiratory tract	10/08	4	2	>128	>128	0.125	T28	wild-type	*abscessus*
MAZ07	MAZ07.1	respiratory tract	06/08	0	0.5	16	>128	0.19	T28	wild-type	*M. abscessus* subsp.
	MAZ07.2	respiratory tract	02/09	8	0.5	>128	>128	0.125–256	T28	wild-type/2058A→G[b]	*abscessus*
	MAZ07.3	respiratory tract	04/09	10	1–128	>128	>128	1–256	T28	wild-type/2058A→C/2058A→G[b]	
	MAZ07.4	respiratory tract	09/09	15	2–128	>128	>128	1–256	T28	wild-type/2058A→C/2058A→G[b]	
	MAZ07.5	respiratory tract	12/09	18	>128	>128	>128	>256	T28	2058A→C/2058A→G[b]	
	MAZ07.6	respiratory tract	12/10	30	>128	>128	>128	>256	T28	2058A→C/2058A→G[b]	
	MAZ07.7	respiratory tract	01/11	31	>128	>128	>128	>256	T28	2058A→C/2058A→G[b]	

Genotyping of M. abscessus isolates using RAPD-PCR.



Maurer F P et al. J. Antimicrob. Chemother. 2012;67:2606-2611

Journal of Antimicrobial Chemotherapy

Conclusion

Clarithromycin resistance mutations in the 23S rRNA peptidyltransferase region provide an additional selective advantage independent of a functional *erm*(41) gene.

Species dependent clarithromycin resistance

Interspecies differences in e*rm* genes:

The Erm methylase in *M. abscessus* subsp. *massiliense* is dysfunctional due to:

- 2 bp deletion of nucleotides 64–65
- 274 bp deletion of nucleotides 159–432

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