ESGMYC

ESCMID STUDY GROUP FOR MYCOBACTERIAL INFECTIONS

European Society of Clinical Microbiology and Infectious Diseases

Antibiotic susceptibility of non tuberculous mycobacteria (NTM)

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Warning introduction

- Few is known about susceptibility testing and resistance of non tuberculous mycobacteria
- It is different from what is known for tuberculosis and *Mycobacterium tuberculosis* complex
- Much needs to be done!!
  - By you or others
  - One of the ESGMYC projects for the next years

Before performing antibiotic susceptibility testing (AST)

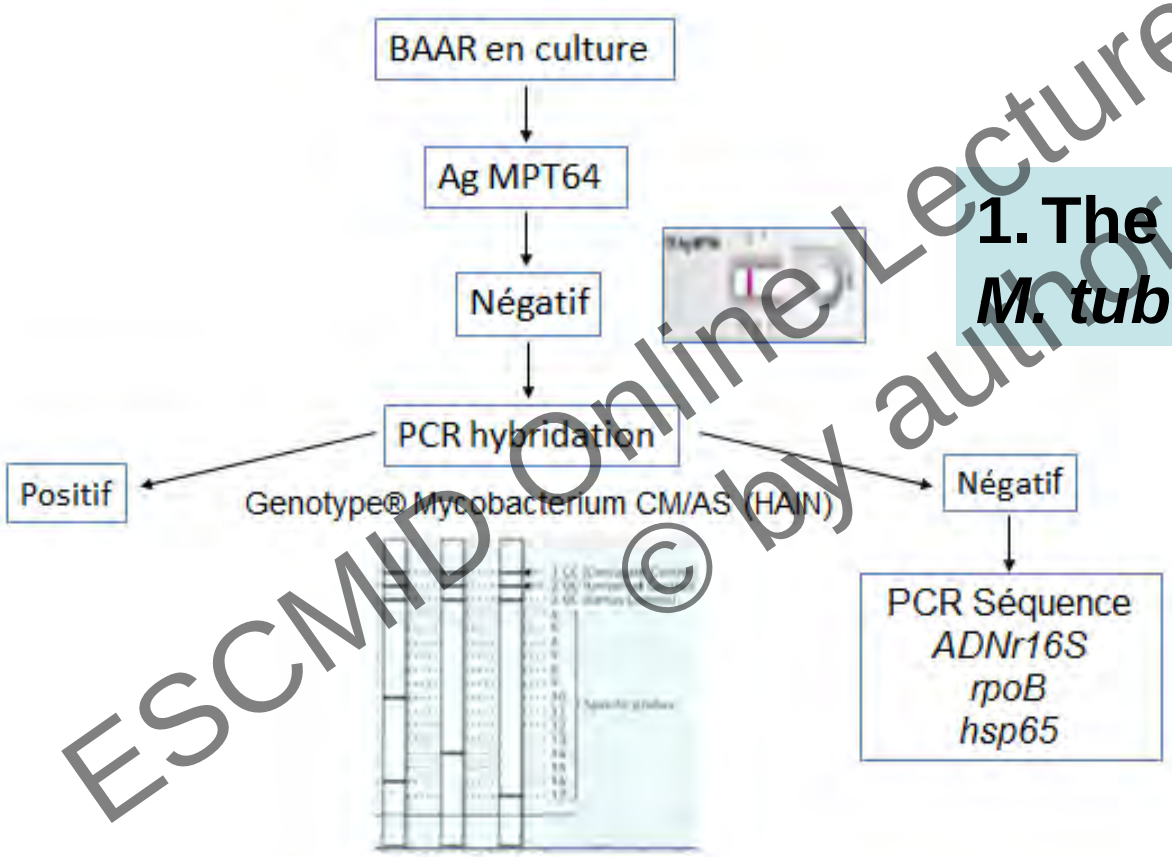
- Is there a real mycobacterial infection?
  - Clinical and radiographic signs, history of disease
- Do you know the mycobacterial species involved?
- How this species was identified?
  - At the Complex level
  - At the species level
  - At the subspecies or variant level?

⇒ If Yes, It may be worthwhile to perform antibiotic susceptibility testing, depending on the mycobacterial species

ESGMYC 2013, Pr Emmanuelle Cambau

ATS 2007, Brown-Elliott 2012

Choosing the NTM isolate



1. The isolate is not *M. tuberculosis* complex

2. Identification is done at the species level, using genetic data bases if necessary

Is this isolate involved in a NTM infection?

- Clinical and radiological signs
  - Without tuberculosis
  - Not explained by another disease
- Isolated from
  - 2 distinct sputums
  - 1 protected or sterile specimen
  - Biopsy with histological signs
- If not: ask an expert in your country

ATS 2007, Tortoli 2009, Van ingen 2013

Is it one NTM species we know about its intrinsic pattern of antibiotic sensitivity?

- Slow growing mycobacteria
  - *M. avium, M. intracellulare (M. simiae)*
  - *M. kansasii (M. szulgai)*
  - *M. xenopi*
  - Others: *M. malmoense*, *M. ulcerans,*
- Rapid growing mycobacteria
  - *M. abscessus , M. bolletii, M. massiliense*
  - *M. fortuitum*
  - *M. chelonae*
  - *M. marinum*

Wolinski 1979, Henriques 1994, Aubry 2002, Jenkins 2003, Arendt 2004, ATS 2007

Does the patient previously been treated by antibiotics?

- For an infection due to the same mycobacterial species infection?
- Is it a relapse form of disease or a new case?
- For another infection?
  - Chronic broncho-pneumopathy (cystic fibrosis)
  - General disease: diabetes, renal failure, transplant….

If Yes, I need to perform AST and
I will search acquired
or secondary resistance to antibiotics

Which antibiotic to be tested?

- Most NTM are resistant to antituberculous antibiotics,
- except
  - *M. kansasii, M. szulgai*

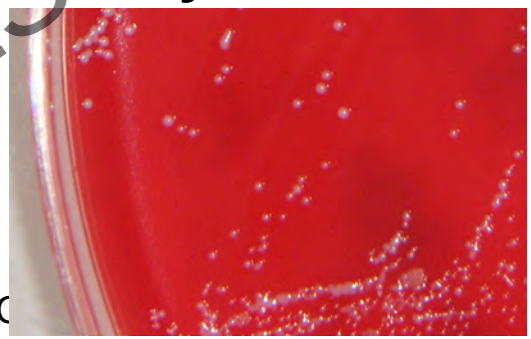
⇒Do not test NTM the way you test *M. tuberculosis* complex isolates

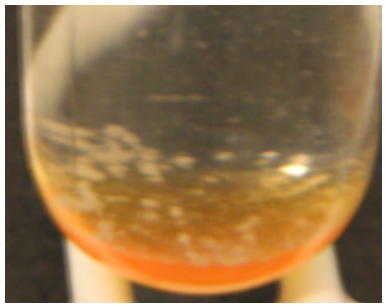
Different systems, different antibiotics, different concentrations

Shitrit 2007, Klein 2011, Brown Elliott 2012

Where does the isolate come from?

- Rarely AFP positive specimens
- Mostly culture
  - Liquid (7H9, Dubos, BHI, ..)
  - Solid (Lowenstein Jensen, Coletsos, 7H10 or 7H11 agar)
  - common media used for bacteria other than myobacteria





Which method of antibiotic susceptibility testing (AST) I will use?

- Phenotypic AST method :
  - testing the *in vitro* activity of antibiotics
  - Detecting acquired resistance
  - Standardization only for some antibiotics and some mycobacterial species
- Genotypic AST method:
  - detection of mutations conferring resistance
  - Known only for some antibiotics and some mycobacterial species

Drawbacks for AST in NTM

- Slow growth (3 days to 3 months)
  => not adapted to standardized DST (CLSI, EUCAST, …) nor automated systems
- Enriched medium = not standardized Mueller Hinton
  => problems of pH, medium/ATB interferences…
- High % GC => difficulties for PCR and sequencing
- Impermeable cell wall and multiple resistance mechanisms
  => Intrinsic resistance to many antibiotics
- Exchange of genetic materials
  => are strains different from one to another?

Good news: Biosafety Level 3 lab is not mandatory!!

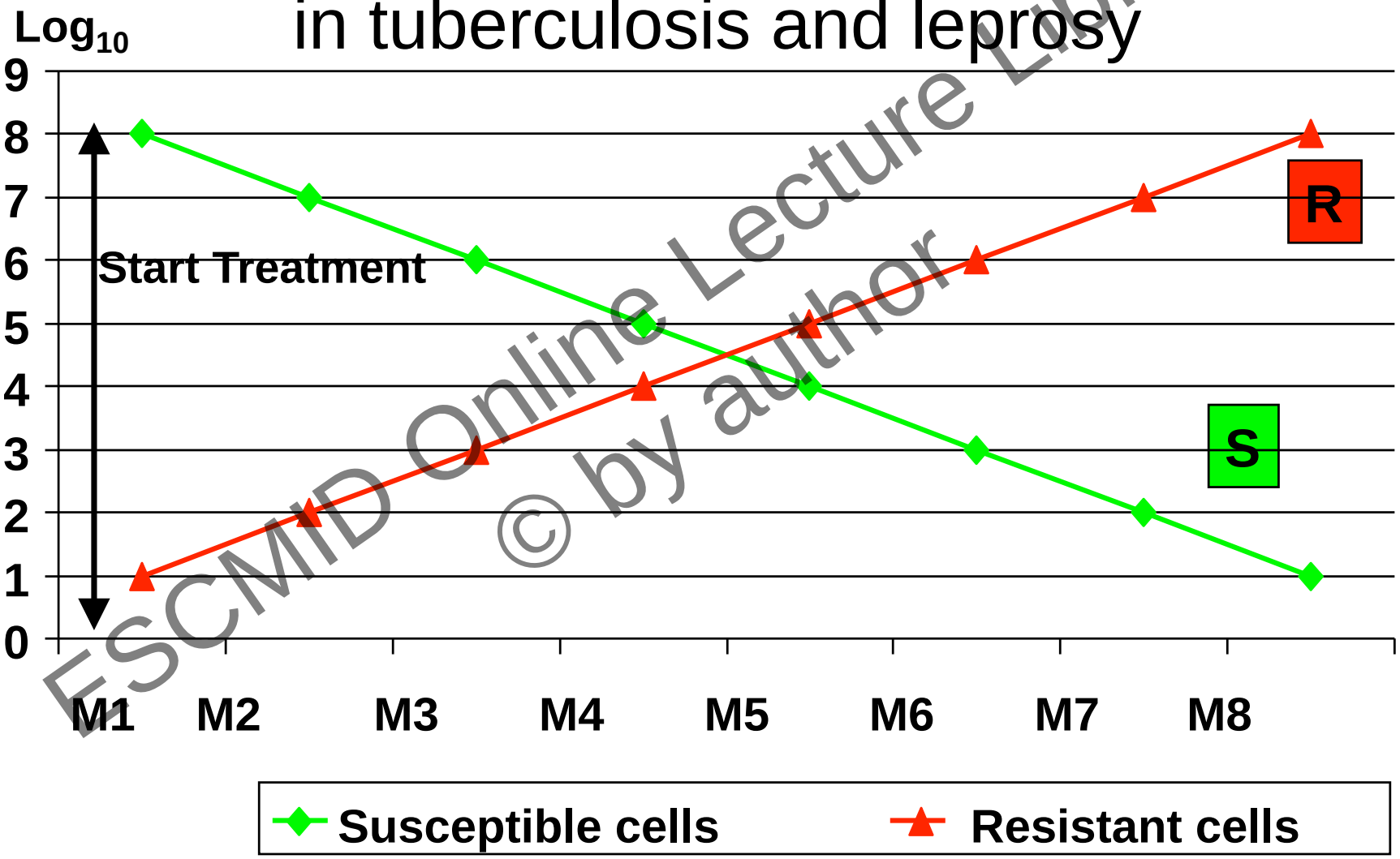
Phenotypic AST method

- Testing the *in vitro* activity of antibiotics => determination of MICs
  - Lowest concentration inhibiting 99% of growth
  - Lowest concentration inhibiting macroscopic growth
  - New ATBs or Very old ones
  - microdilution, macrodilution, dilution in solid medium, Etest
  - Duration of incubation according to slow growth

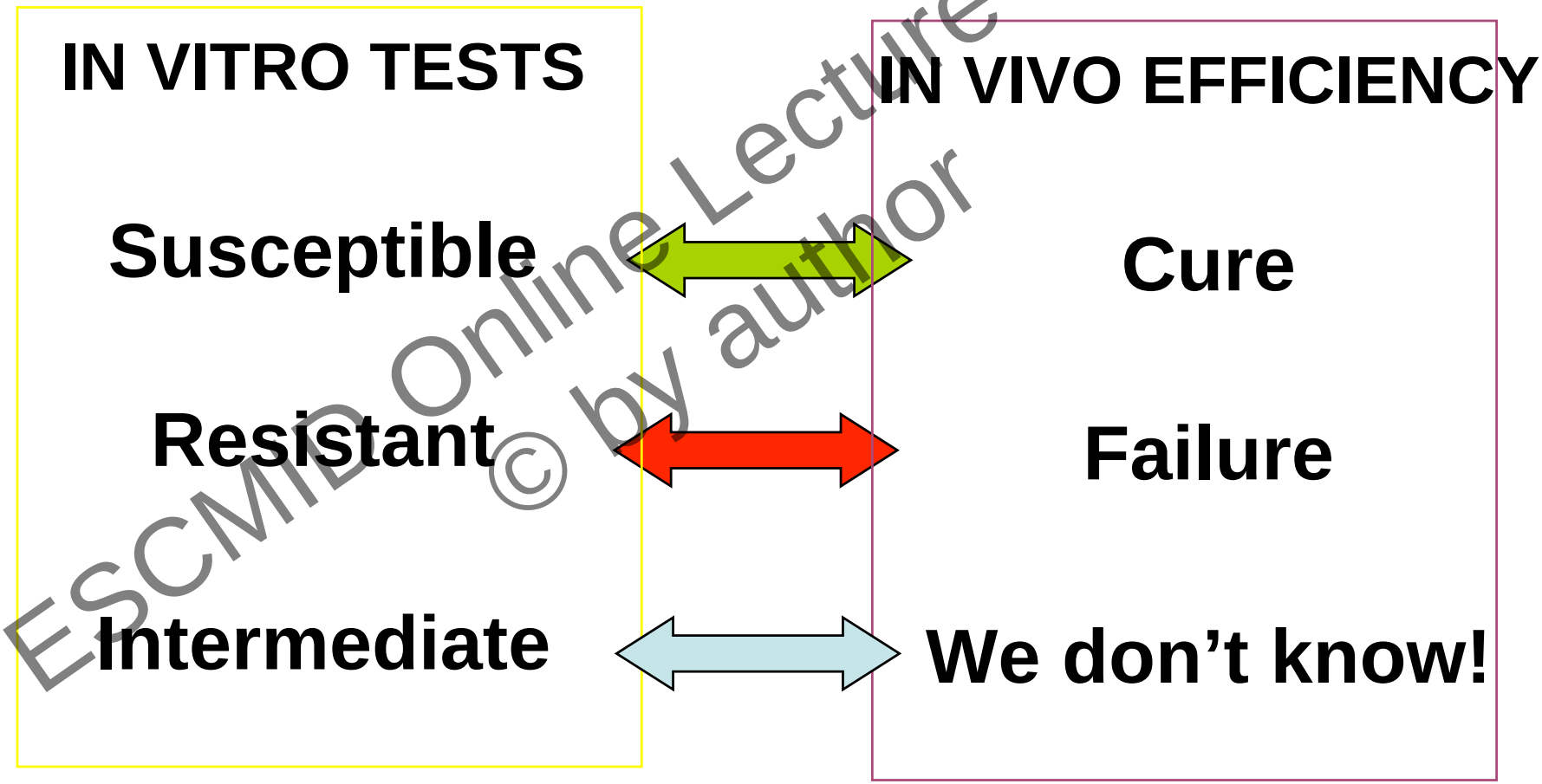
Detection of acquired resistance

Mycobacterial species	**Mandatory**	**supplement**
M. avium complex	Clarithromycin	
M. kansasii, M. szulgai	Rifampicin	
M. abscessus	Clarithromycin	Amikacin
M. bolletii	Clarithromycin	amikacin
M. massiliense	Clarithromycin	amikacin
M. chelonae	Clarithromycin	tobramycin
M. fortuitum	Ciprofloxacin	doxycycline
M. immunogenum	?	?

Scheme of selection of resistant mutant during treatment is similar to what described in tuberculosis and leprosy



What is the reference for assessing S, I, R in NTM?



Genotypic AST for NTM
what is known and may be recommended

Mycobacterial species	Antibiotic	Resistance gene
M. avium complex	Clarithromycin (azithromycin)	*rrl*
M. kansasii, M. szulgai	rifampicin	*rpoB*
M. chelonae	clarithromycin	*rrl*
M. fortuitum	fluoroquinolones	*gyrA*
M. abscessus	clarithromycin	*erm41, rrl, rrs*

ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Oct. 2000, p. 2848–2852
0066-4804/00/$04.00+0

Vol. 44, No. 10

In Vitro Activities of the Ketolides Telithromycin (HMR 3647) and HMR 3004 Compared to Those of Clarithromycin against Slowly Growing Mycobacteria at pHs 6.8 and 7.4

NALIN RASTOGI,[1]* KHYE SENG GOH,[1] MYLENE BERCHEL,[1] AND ANDRÉ BRYSKIER[2,3]

TABLE 1. BACTEC MICs of the ketolides HMR 3647 (telithromycin) and HMR 3004 compared to that of clarithromycin at pHs 6.8 and 7.4 for slowly growing mycobacteria

Strain	MIC (μg/ml)					
	HMR 3647		HMR 3004		Clarithromycin	
	pH 6.8	pH 7.4	pH 6.8	pH 7.4	pH 6.8	pH 7.4
M. tuberculosis						
Type strain H37Rv	>40.0	>40.0	20.0	10.0	20.0	5.0
Clinical isolate 900145	>40.0	>40.0	40.0	10.0	20.0	5.0
Clinical isolate 900216	>40.0	>40.0	40.0	10.0	20.0	5.0
M. simiae						
Type strain ATCC 25275	40.0	20.0	20.0	5.0	20.0	10.0
AIDS isolate 91-098	>40.0	40.0	40.0	10.0	20.0	10.0
AIDS isolate 92-039	>40.0	>40.0	>40.0	10.0	10.0	5.0
AIDS isolate 94-120	>40.0	>40.0	>40.0	20.0	40.0	10.0
AIDS isolate 96-005	>40.0	40.0	40.0	10.0	20.0	5.0
AIDS isolate 96-012	>40.0	40.0	40.0	10.0	20.0	5.0
M. avium						
Type strain ATCC 25291	5.0	1.25	2.5	0.3	0.6	0.15
Clinical isolate 711	10.0	2.5	2.5	0.6	1.25	0.3
Clinical isolate 969	10.0	2.5	2.5	0.6	1.25	0.3
Clinical isolate 1110	40.0	20.0	10.0	2.5	2.5	0.6
Clinical isolate 1257	≥40.0	10.0	10.0	1.25	2.5	0.6
Clinical isolate 1295	≥40.0	20.0	10.0	2.5	2.5	1.25

CLSI 2011 recommendations for phenotypic susceptibility testing

- Microdilution
  - 0.5 Mc Farland inoculum
  - 5 105 cfu/ml
  - pH 7,3 for macrolides and others (MH+OADC)
  - Control strains
  - Not more than 5 days except macrolides
- Etest
- Proportion method for slow growers

AST in liquid medium: microdilution for rapid growers

*RAPMYCO - Mycobacteria Rapid Growers MIC Plate

	1	2	3	4	5	6	7	8	9	10	11	12
A	SXT 0.25/4.75	SXT 0.5/9.5	SXT 1/19	SXT 2/38	SXT 4/76	SXT 8/152	LZD 1	LZD 2	LZD 4	LZD 8	LZD 16	LZD 32
B	CIP 0.12	CIP 0.25	CIP 0.5	CIP 1	CIP 2	CIP 4	IMI 2	IMI 4	IMI 8	IMI 16	IMI 32	IMI 64
C	MXF 0.25	MXF 0.5	MXF 1	MXF 2	MXF 4	MXF 8	FEP 1	FEP 2	FEP 4	FEP 8	FEP 16	FEP 32
D	FOX 4	FOX 8	FOX 16	FOX 32	FOX 64	FOX 128	AUG2 2/1	AUG2 4/2	AUG2 8/4	AUG2 16/8	AUG2 32/16	AUG2 64/32
E	AMI 1	AMI 2	AMI 4	AMI 8	AMI 16	AMI 32	AXO 4	AXO 8	AXO 16	AXO 32	AXO 64	
F	DOX 0.12	DOX 0.25	DOX 0.5	DOX 1	DOX 2	DOX 4	DOX 8	DOX 16	MIN 2	MIN 4	MIN 8	
G	TGC 0.015	TGC 0.03	TGC 0.06	TGC 0.12	TGC 0.25	TGC 0.5	TGC 1	TGC 2	TGC 4	TOB 1	TOB 2	TOB 4
H	CLA 0.06	CLA 0.12	CLA 0.25	CLA 0.5	CLA 1	CLA 2	CLA 4	CLA 8	CLA 16	TOB 8	TOB 16	POS

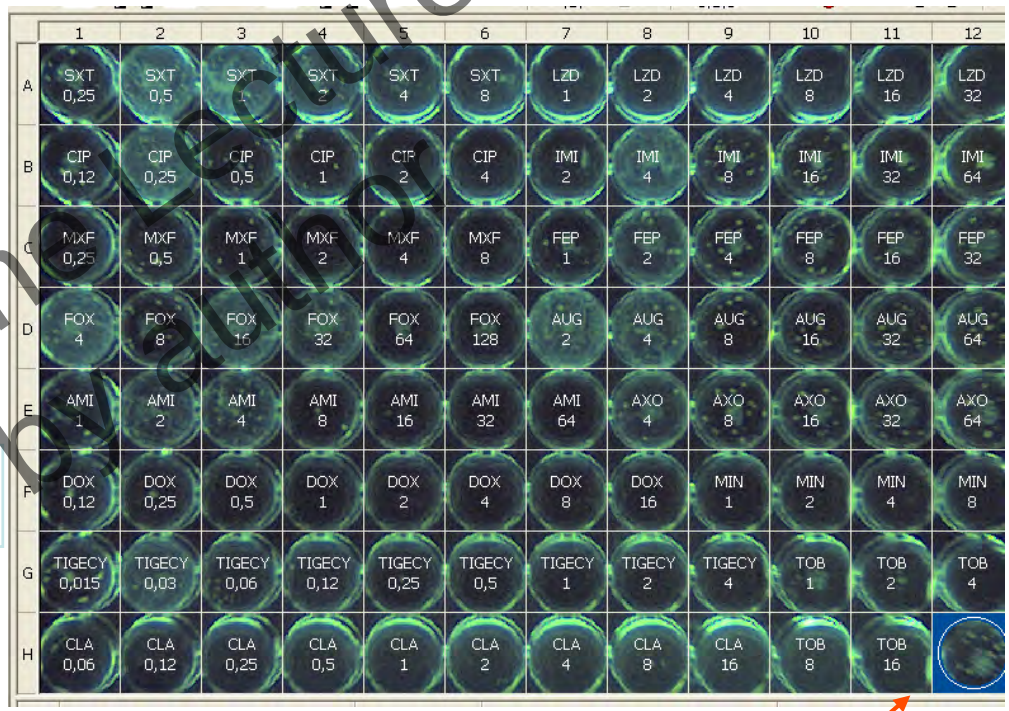
Part No. RAPMYCO**

Antimicrobics	Dilution Ranges (µg/ml)
Amikacin	1-64
Amoxicillin/ clavulanic acid	2/1-64/32
Cefepime	1-32
Cefoxitin	4-128
Ceftriaxone	4-64
Ciprofloxacin	0.12-4
Clarithromycin	0.06-16
Doxycycline	0.12-16
Imipenem	2-64
Linezolid	1-32
Minocycline	1-8
Moxifloxacin	0.25-8
Tigecycline	0.015-4
Tobramycin	1-16
Trimethoprim/ Sulfamethoxazole	0.25/4.75-8/152

ESGMYC 2013, Pr
Emmanuelle Cambau

AST in liquid medium: microdilution for rapid growers

MIC = 4 mg/L



Positive growth control

ESGMYC 2013, Pr Emmanuelle Cambau

Intrinsic susceptibility pattern for *M. chelonae*

Antibiotics	MIC50	MIC90
Amikacin	32	32
Tobramycin	2	4
Ciprofloxacin	4	4
Moxifloxacin	8	8
Clarithromycin	0,25	0,5
Linezolid	8	16
Co-trimoxazole	8/152	8/152
cefoxitine	>128	>128
imipenem	64	>64
Tigecycline	0,12	0,25
Doxycycline	>16	>16
Minocycline	>8	>8

Intrinsic susceptibility pattern for *M. chelonae*

	CMI 50	CMI90	CLSI Breakpoints (mg/L)
Amikacine	32	32	16-64
Tobramycine	2	4	2-8
Ciprofloxacine	4	4	1-4
Moxifloxacine	8	8	1-4
Clarithromycine	0,25	0,5	2-8
Linezolide	8	16	8-32
bactrim	8/152	8/152	2/38-4/76
cefoxitine	>128	>128	16-128
imipenème	64	>64	4-32
Tigécycline	0,12	0,25	-
Doxycycline	>16	>16	1-8
Minocycline	>8	>8	1-8

ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Nov. 2000, p. 3133–3136 Vol. 44, No. 11
0066-4804/00/$04.00+0

Antibiotic Susceptibility Pattern of *Mycobacterium marinum*

ALEXANDRA AUBRY,[1] VINCENT JARLIER,[1] SYLVIE ESCOLANO,[2]
CHANTAL TRUFFOT-PERNOT,[1] AND EMMANUELLE CAMBAU[1]*

TABLE 1. MICs (μg/ml) of 17 antibiotics for 54 strains of *M. marinum*, determined by the agar dilution method

Antibiotic	MIC_{50}	MIC_{90}	Modal MIC	Geometric mean MIC	Geometric standard deviation	Range
Rifampin	0.25	0.5	0.25	0.24	1.7	0.125–4
Rifabutin	0.06	0.06	0.06	0.06	1.8	0.015–1
Isoniazid	4	8	4	5.6	1.5	4–16
Ethambutol	2	4	2	1.7	1.6	1–4
Amikacin	2	4	4	1	1.7	1–8
Doxycycline	8	16	8	5.7	2	0.5–16
Minocycline	2	4	2	2.9	1.7	0.5–8
Clarithromycin	1	4	2	1.2	2.3	0.5–32
Azithromycin	32	128	32	NA[a]	NA	8–>128
Ofloxacin	4	16	4	6.1	1.7	2–32
Ciprofloxacin	4	8	4	3.8	1.8	1–16
Levofloxacin	4	8	4	4.5	1.7	2–32
Sparfloxacin	1	2	1	1	1.8	0.5–4
Moxifloxacin	0.5	1	0.5	0.6	1.7	0.25–4
Sulfamethoxazole	8	128	8	NA	NA	4–>128
Trimethoprim	64	128	128	67.4	2.3	16–512
Imipenem	2	8	2	2.6	2.6	0.5–16

[a] NA, not applicable (upper MICs were above the highest concentration tested).

TABLE 2. Reproducibility of results with the Etest as evaluated by the determination of MICs by two different operators for 39 clinical strains of *Mycobacterium marinum*

Antibiotic	No. of results within $\log_2$ concentration difference of:							% Agreement[a]
	>−2	−2	−1	0	1	2	>2	
Rifampin	1	3	4	7	6	1	17	44 ± 7.8
Minocycline	1	4	8	16	8	1	1	82 ± 5.9
Clarithromycin	5	3	4	12	3	8	4	46 ± 7.9
Sparfloxacin	4	3	1	3	4	11	13	21 ± 6.4

[a] Percentage of isolates within duplicate MICs ± $\log_2$ dilution (± standard error).

TABLE 4. Distribution of category discrepancies by a comparison of Etest results to agar dilution results for 54 strains of *M. marinum*

Antibiotic (breakpoints [μg/ml])[a]	No. of category discrepancies			% Agreement[b]
	Very major	Major	Minor	
Rifampin (1–4)	0	2	3	91 ± 3.9
Minocycline (4–16)	0	1	5	89 ± 4.3
Clarithromycin (2–8)	0	1	5	89 ± 4.3
Sparfloxacin (1–4)	0	8	15	57 ± 6.7

[a] MIC interpretive breakpoints as defined by the NCCLS.
[b] Percentage of isolates within the accuracy limits of the test ± standard error.

Intrinsic susceptibility pattern for *M. massiliense*

Antibiotiques	CMI $_{50}$ (mg/l)	Sensibilité
Clarithromycine	1	S
Cefoxitine	32	I
Imipenem	16	I
Ciprofloxacine	8	R
Moxifloxacine	16	R
Amikacine	16	S
Linezolide	32	R
Tigecycline	0.5	S

Nessar et al., JAC 2012

Prevalence of acquired resistance in *M. avium* complex in France (Groupe Azay-mycobactéries 1995 – 1997)

		N. cases	% Clari^{R}
1995	New case	261	0%
	Previously treated	100	72%
1996	New case	157	0%
	Previously treated	20	65%
1997	New case	38	1 case*
	Previously treated	19	63%
Total		606	18%

* received macrolides for other infection

Maugein J et al. Eur Respir J 2001

Antibiotic / species couples for AST

Mycobacterial species	Mandatory	supplement
M. avium complex	Clarithromycin	
M. kansasii, M. szulgai	Rifampicin	Clarithromycin, moxifloxacine
M. marinum	No ATB	
M. abscessus	Clarithromycin 14 days	Amikacin, cefoxitin
M. bolletii	Clarithromycin 14 days	amikacin
M. massiliense	Clarithromycin 14 days	amikacin
M. chelonae	Clarithromycin	tobramycin
M. fortuitum	Ciprofloxacin	doxycycline
M. immunogenum		

erm(41) polymorphism and relation with clarithromycin susceptibility

Mycobacteria l species	**MIC90 at D14 (mg/L)**	***erm(41) gene***
M. massiliense	1	-276pb deletion
M. bolletii	>256	sequevar T28
M. abscessus	> 256	*sequevar* T28
	2	*sequevar* C28

Bastian at al. *AAC* 2010

Further studies on secondary resistance

Mycobacterial species	Antibiotic	N resistant / N treated	Reference
M. fortuitum	**Ciprofloxacin**		Wallace
M. chelonae	**clarithromycin**		Bottger
M. abscessus	**clarithromycin**		Nash
M. kansasii	rifampicin		Wallace
Wallace 1993; Bottger 1996, Nash …, Wallace , Bastian 2010			

Conclusions

- Non tuberculous mycobacteria are intrinsically resistant to most antituberculous drugs and to many antibiotics
- We know little about some mycobacterial species and antibiotics. We do not know about the others
- We still need to search for effective drugs and correlation between cure and in vitro susceptibility testing
- Effective antibiotics can select for acquired/ secondary resistance as in tuberculosis and leprosy

More

- ESCMID group on mycobacterial infections (ESGMYC): www.escmid.org/esgmyc
- Monday 29th April 2013: official symposium on NTM respiratory infections (room, from 16:00 to 18:00)
- ESGMYC meeting at ECCMID on Sunday 28th April 2013, room 13/14.

TABLE 1 Antimicrobials

Rapidly growing species	Antimicrobials[j]
M. abscessus subsp. *abscessus*	Linezolid (~50%), moxifloxacin (~15%), ciprofloxacin, levofloxacin (<5%), doxycycline (<5%), clarithromycin-azithromycin (~20%)[a] (oral); amikacin, tigecycline, cefoxitin (70%), imipenem (~50%),[b] linezolid (50%) (parenteral)
M. abscessus subsp. *bolletii* (formerly *M. massiliense*)	Clarithromycin-azithromycin,[c] linezolid (~50%), moxifloxacin (~15%), ciprofloxacin (<5%), doxycycline (<5%) (oral); amikacin, tigecycline, cefoxitin (~70%), imipenem (~50%), linezolid (~50%) (parenteral)
M. chelonae	Clarithromycin-azithromycin, linezolid, moxifloxacin (~25%), ciprofloxacin (~20%), doxycycline (~20%) (oral); tobramycin, linezolid, amikacin (~50%), imipenem (~60%),[b] tigecycline (parenteral)
M. fortuitum	Ciprofloxacin, levofloxacin, moxifloxacin, trimethoprim-sulfamethoxazole, linezolid, doxycycline (~50%), clarithromycin-azithromycin (~20%)[a] (oral); imipenem, tigecycline, linezolid, amikacin, cefoxitin (~50%) (parenteral)
M. neoaurum-M. bacteremicum	Ciprofloxacin, levofloxacin, moxifloxacin, doxycycline, linezolid, trimethoprim-sulfamethoxazole, clarithromycin-azithromycin[d] (oral); amikacin, tobramycin, linezolid, imipenem, tigecycline, cefoxitin (parenteral)
M. avium complex	Clarithromycin-azithromycin,[e] rifampin-rifabutin, ethambutol, moxifloxacin (<50%), ciprofloxacin (<25%) (oral); amikacin, streptomycin, linezolid (<50%) (parenteral)
M. kansasii	Clarithromycin-azithromycin, rifampin-rifabutin,[f] trimethoprim-sulfamethoxazole, ethambutol, isoniazid, moxifloxacin, ciprofloxacin, linezolid (oral); amikacin, linezolid (parenteral)
M. marinum[g]	Clarithromycin-azithromycin, rifampin-rifabutin, ethambutol, ciprofloxacin (~50%), trimethoprim-sulfamethoxazole, moxifloxacin, linezolid, doxycycline (~50%) (oral); amikacin, linezolid (parenteral)
M. simiae[h]	Clarithromycin-azithromycin, moxifloxacin (~60%), trimethoprim-sulfamethoxazole (oral); amikacin (parenteral)
M. xenopi[i]	Clarithromycin-azithromycin, rifampin-rifabutin, ethambutol, moxifloxacin (oral); amikacin, streptomycin (parenteral)

Brown Elliott CMR2012