Non tuberculous mycobacterial infections

Katharina Kranzer

Outline

- Epidemiology and clinical presentation of NTM
- Phenotypic DST – challenges and opportunities
- Sequencing and what does it mean

Rare disease

Life-threatening or chronically debilitating conditions that affect not more than 5 in 10,000 people in the EU.

Rare disease

Life-threatening or **chronically debilitating** conditions that affect not more than **5 in 10,000** people in the EU.

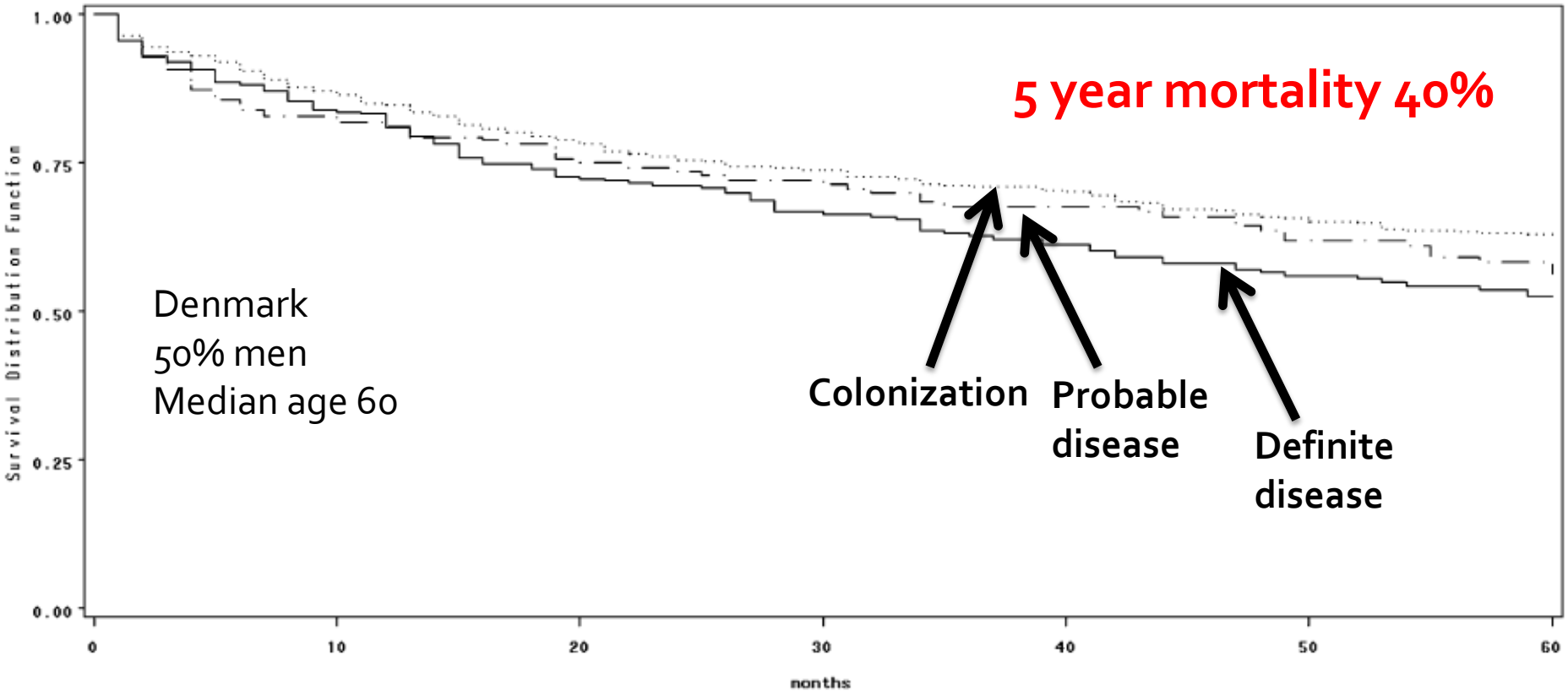
Do NTM cause rare disease?

European Medicine Agency

NTM incidence - less than 50/100,000?

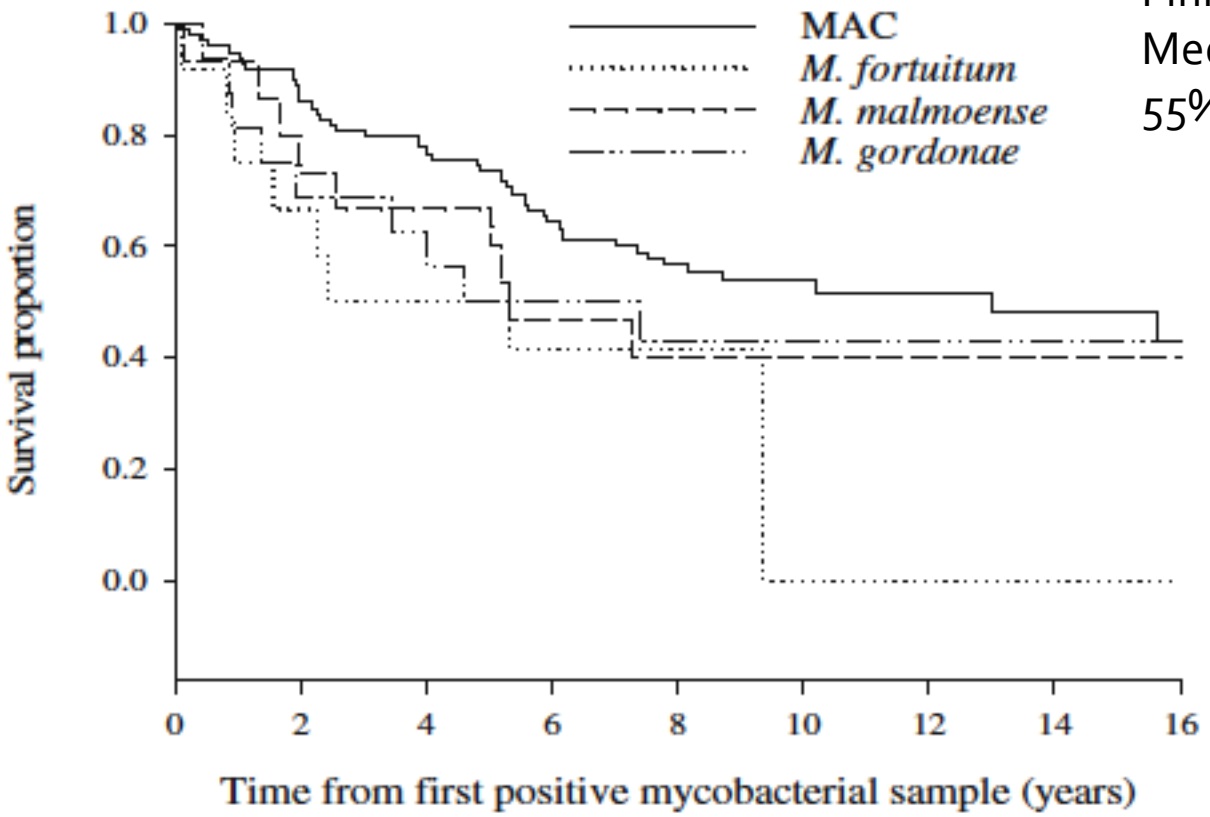
Author	Year	Country	NTM Incidence (per 100,000)	TB Incidence (per 100,000)
Moore 2010	2006	England, Wales	2.9	10
Hernández 2009	1990-2006	British Columbia, Canada	6.7	5.1
Andrejak 2010	1197-2008	Denmark	1.1	6
Shah 2016	2012	England, Wales, Northern Ireland	6.3	10
Henel 2016	2012	Oregon, USA	5.6	3.2

NTM disease – life-threatening or chronically debilitating ?



Andrejak 2010

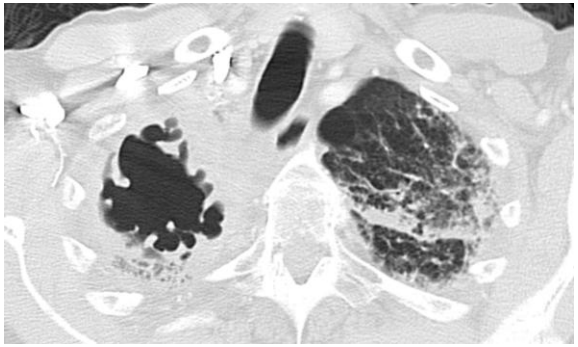
NTM disease – life-threatening or chronically debilitating ?



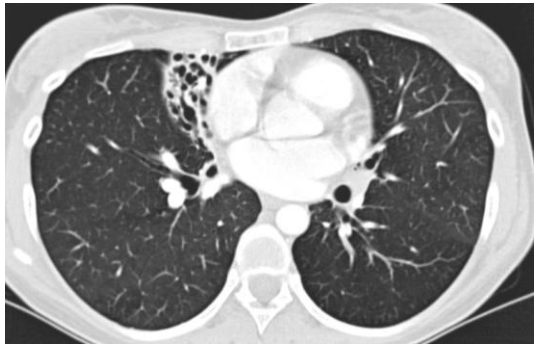
Finnland
Median age 60
55% women

Kotilainen 2015

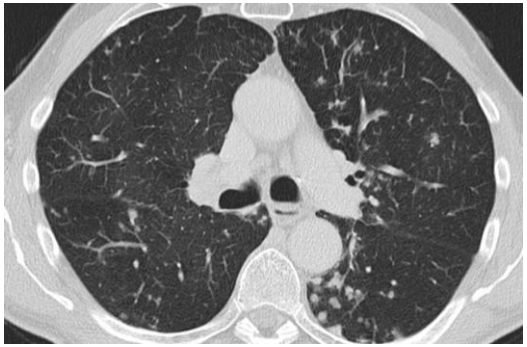
NTM disease – life-threatening or chronically debilitating ?



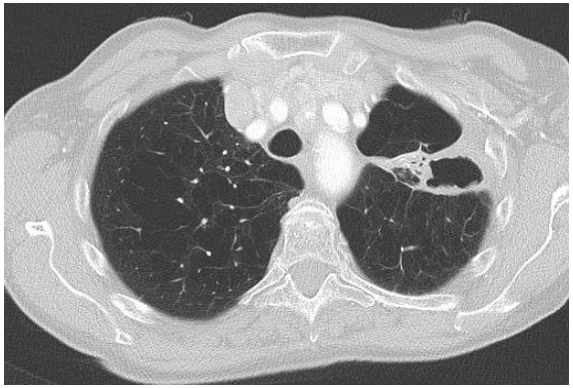
M. intracellulare complex



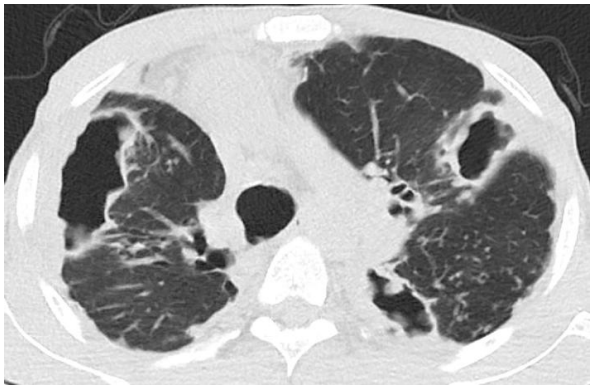
M. abscessus



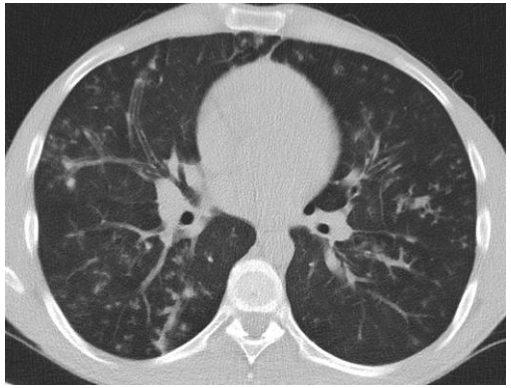
MAC



M. xenopi



M. simiae



M. abscessus

NTM disease chances for cure… relapse rates after culture conversion

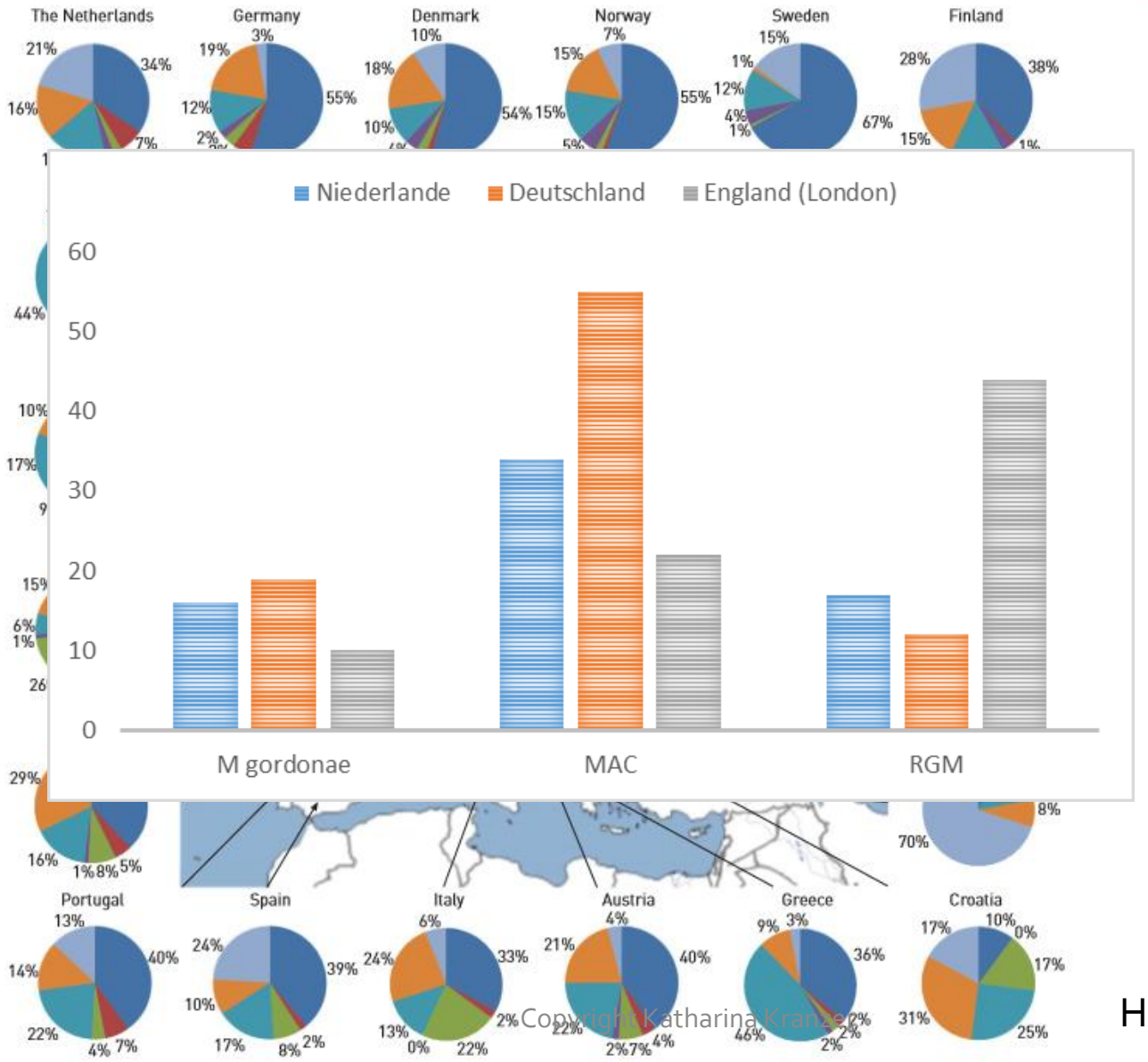
LONDON SCHOOL of HYGIENE &TROPICAL MEDICINE

Microbiological recurrence after sputum conversion

Measure	Still on Therapy[a]	After Completion of Therapy
MR after sputum conversion	25 of 180 (14)	74 of 155 (48)
Genotyping on ≥ 2 MR isolates	21 of 25 (84)	53 of 74 (72)
New infection	10 of 21 (48)	40 of 53 (75)
True relapse	11 of 21 (52)	13 of 53 (25)
Genotyping of single MAC isolates	22 of 23 (93)	37 of 45 (82)
New infection	18 of 23 (78)	28 of 37 (76)
True relapse	5 of 23 (22)	9 of 37 (24)

Wallace et al Chest 2014

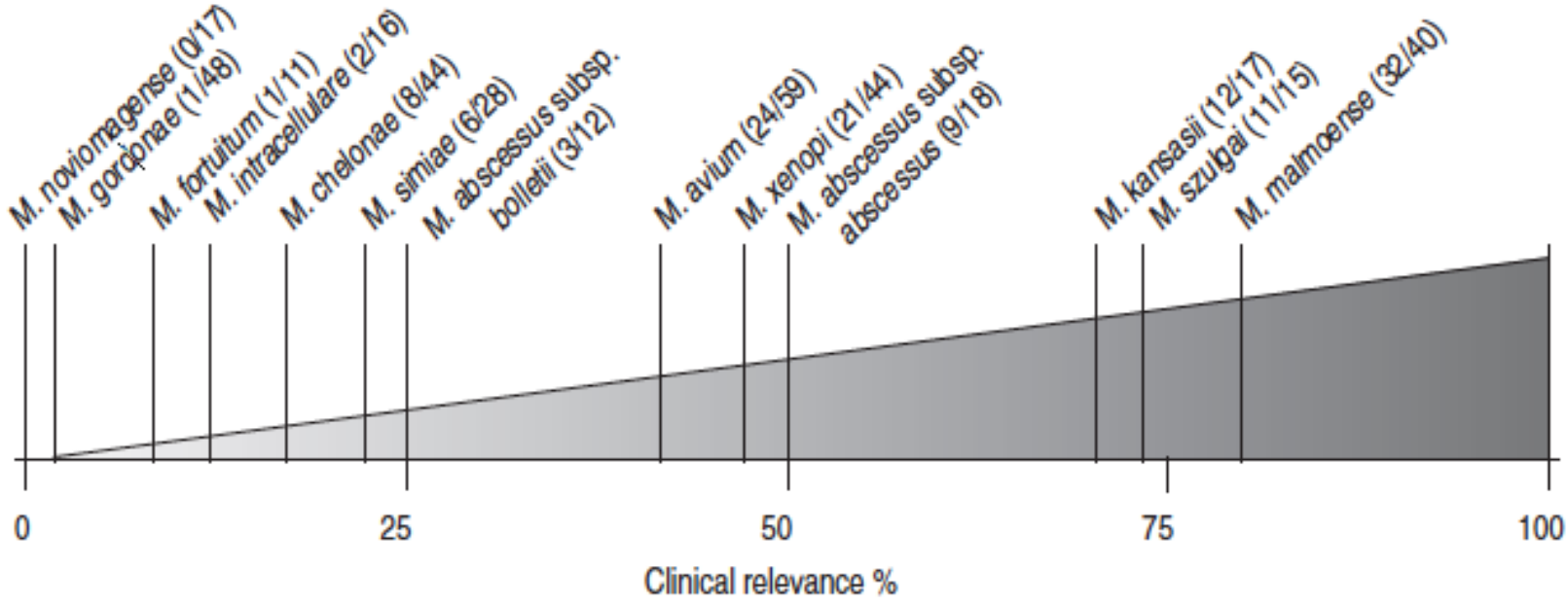
Distribution of NTMs across Europe



Hoefsloot 2014

Respiratory disease due to NTM

- Which NTMs are associated with disease?



van Ingen et al 2012

NTM disease

Disease - Risk group	NTM
Respiratory disease - chronic lung disease	MAC M. abscessus complex M. kansasii (M. xenopi) (M. malmoense)
Disseminated disease - immuno-suppression	MAC (M. genavense)
Lymphadenitis - children	MAC M. malmoense (M. scrofulaceum)
Skin, tissue disease - aquarium, chef	M. marinum M. abscessus complex (M. ulcerans)

Griffith et al AJRCCM 2007, Andréjak AJRCCM 2010; v. Ingen Chest 2009

NTM susceptibility testing

Clinical and Laboratory Standards Institute recommendations for drug susceptibility testing.

Grouping/species	First choice	Alternative(s)
M. avium complex	Broth macrodilution in 12B medium	Broth microdilution in CAMH
M. kansasii	Broth microdilution in CAMH	Macrodilution, agar proportio
other slow growers	Broth microdilution in CAMH[a]	not established
Fastidious species	no recommendation	no recommendation
M. marinum	Broth microdilution in CAMH	Macrodilution, agar dilution
Rapid growers	Broth microdilution in CAMH	not established

CAMH: cation-adjusted Mueller-Hinton broth with OADC supplement.

[a] *M. xenopi* grows poorly in this medium.

Challenges:

Media (cycloserin – pyruvat inactivates cycloserin)

Hydrolysis of imipenem

pH (clarithromycin MICs in Mueller-Hinton pH 7.3-7.4 two times lower compared to pH 6.8)

Lack of breakpoint (no EUCAST breakpoints, few CLSI breakpoints)

Van Ingen 2012

Ideal breakpoint

MIC-distribution differentiates between wildtype and mutants

Taking into account the pharmacodynamics and –kinetics

Prediction of clinical outcomes

Species/disease type	Regimen	RIF R	EMB R	STR R	CLA R	Method	Cure rate, relation
M. avium/Diss	Cla	n.a.	n.a.	n.a.	46%	MaD	n.a., Cla only
M.	R	50%, 86%, 57%	n.a.	n.a.	n.a.	MiD, AD, MaD	43%, no relation
avium/Diss	E	n.a.	50%, 92%, 50%	n.a.	n.a.	MiD, AD, MaD	75%, no relation
MAC/LD	R/Rb, E, Cla, S	ND	ND	ND	0%	MiD	82%, n.a.
MAC/LD	R, E, Cla, K	ND	ND	ND	21%	MiD	71.8%, Cla only
MAC/LD	R, E, Cla, S	ND	ND	48%	ND	MiD	71.2%, no relation
MAC/LD	R, E, Cla, S	75%	100%	90%	25%	MiD	59.6%, Cla only
MAC, *M. xenopi*, *M. malmoense*/LD	H, R, E	67%	42%	n.a.	n.a.	RRM	33%, no relation

Van Ingen 2012

Agent	System or process inhibited	Molecular target(s)[a]	Mechanism(s) of acquired resistance[b]	Mechanism(s) of intrinsic resistance[b]
β-Lactams (carbapenems and cephalosporins)	Peptidoglycan synthesis	PBP, D,L-transpeptidases	Mutation in PBP	D,L-Transpeptidases (cephalosporins), β-lactamases
Ethambutol	Arabinogalactan/arabinomannan synthesis	EmbB	Mutations in *embB*, *embR*, and other genes in the *emb* operon	Polymorphisms in *embB*, *lfrA*, efflux pump
Isoniazid	Mycolic acid synthesis	InhA	Mutations in *katG* or *inhA*	Lack of prodrug activation
Glycopeptides	Peptidoglycan synthesis	D-Alanine–D-alanine terminal amino acids	Unknown	Unknown
Aminoglycoside	Protein synthesis	Ribosome	Mutations in 16S rRNA gene *rpsL*, aminoglycoside phosphotransferase	Aminoglycoside acetyltransferases and phosphotransferases
Tetracycline	Protein synthesis	Ribosome	Mutations in 16S rRNA gene, ribosome protection [*otr*(A) and *tet*(M)], efflux [*tet*(K), *tet*(L), *tet*(V), *otr*(B), and *tap*]	Ribosome protection [*otr*(A) and *tet*(M)], efflux [*tet*(K), *tet*(L), *tet*(V), *otr*(B), and *tap*]
Glycylcycline	Protein synthesis	Ribosome	Unknown	Unknown
Macrolide-ketolide	Protein synthesis	Ribosome	23S rRNA gene mutations	*erm* genes
Oxazolidinones	Protein synthesis	Ribosome	23S rRNA gene mutations	Unknown
Fluoroquinolone	DNA replication/gene expression	DNA gyrase	Mutations in *gyrA*	*lfrA* efflux pump
Rifamycin	RNA synthesis	RNA polymerase	Mutations in *rpoB*	ADP-ribosylation
Trimethoprim	Folate metabolism	DHFR	Mutations in DHFR	Polymorphisms in DHFR
Sulfonamides	Folate metabolism	DHPS	Mutations in DHPS	Unknown

Elliot-Brown 2012

DST for NTM – commercial media

- Microdilution (TREK plates) for slow and rapid growing mycobacteria (weekly)
- Susceptibility testing from solid cultures
- Quality control of purity (chocolate agar) and colony count (7H10)
- Release of results for limited number of antibiotics:
  >>> M avium: clarithromycin, moxifloxacin, amikacin, linezolid
  >>> M xenopi: clarithromycin, rifampicin
  >>> M abscessus: prolonged incubation of clarithromycin susceptible strains to detect inducible resistance
- Repeat pDST and molecular testing for M avium with clarithromycin and/or amikacin resistance, repeat pDST and molecular testing for M abscessus with amikacin resistance
- Release of reports with clinical interpretations

NTM reading template

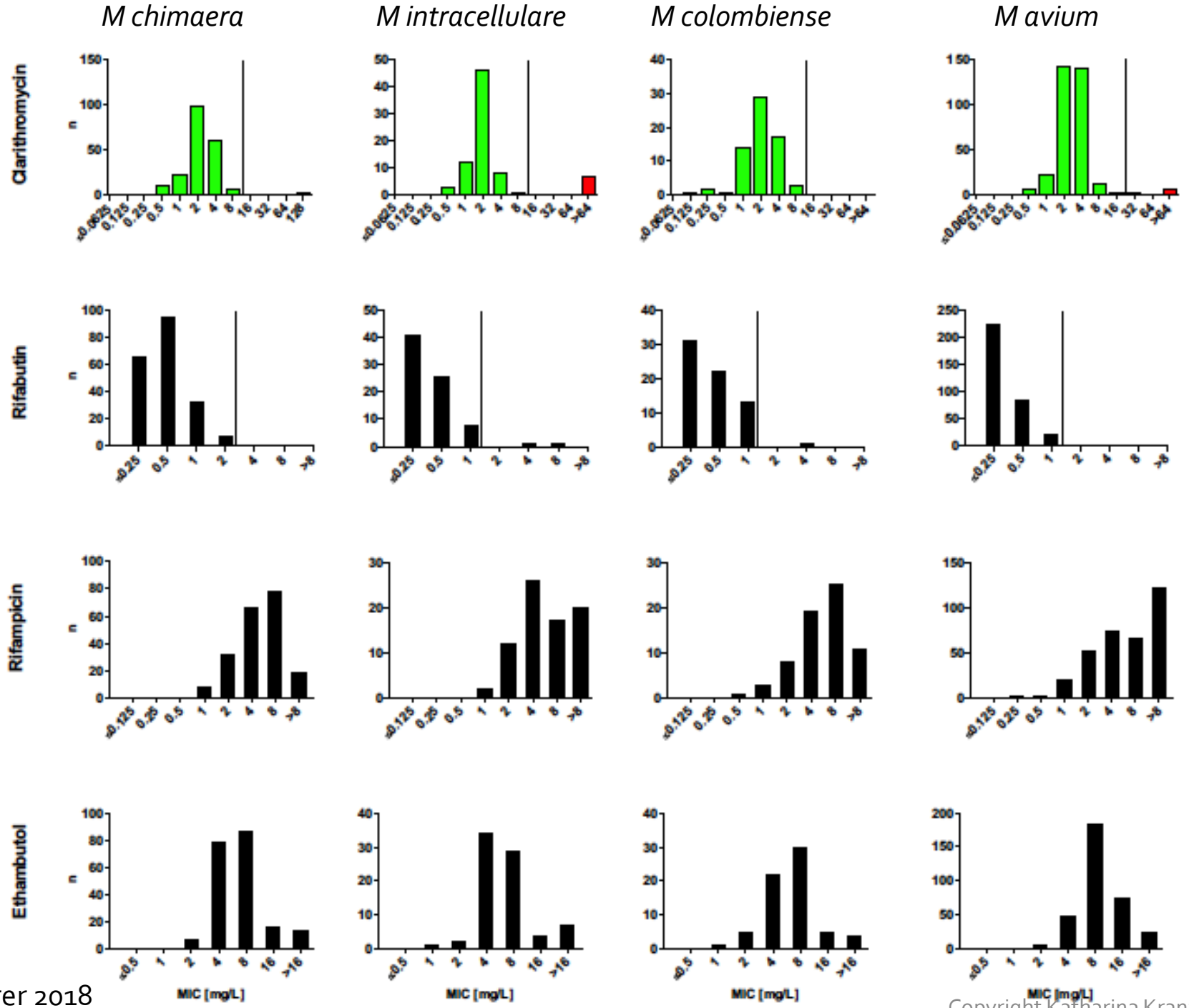
SENSITITRE langsam wachsende Mykobakterien (SLOWMYCOI)

Tgb.-Nr.		M. avium 37°C
Patienten-Name:		M. intracellulare 37°C
Angesetzt am:		
Angesetzt von:		
Abgelesen am:	Tag 7 - 14	Keimzahl (100µl) $5x10^4$ - $5x10^5$
Abgelesen von:		

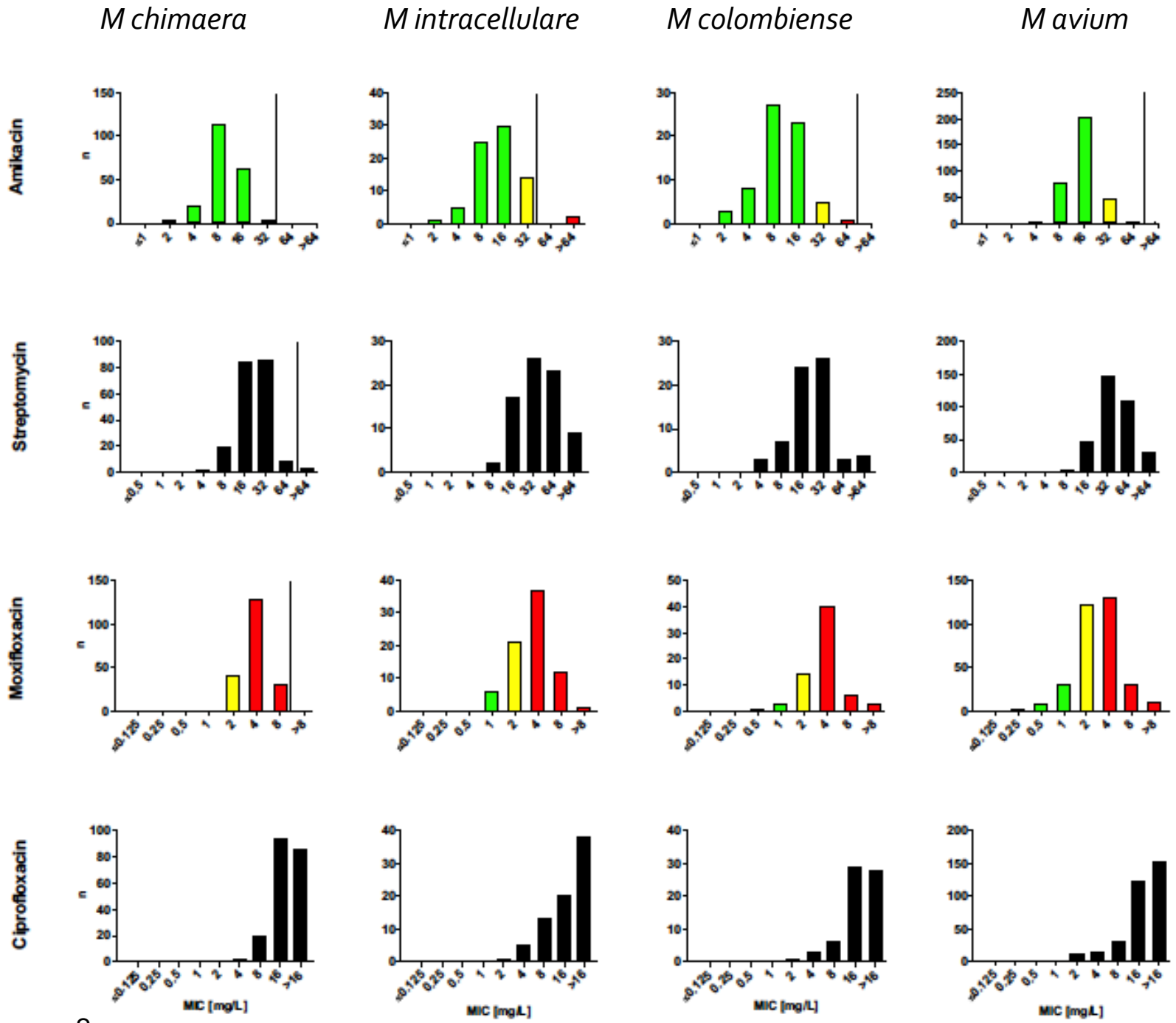
	1	2	3	4	5	6	7	8	9	10	11	12
A	CLA 0,06 -S	CLA 0,12 -S	CLA 0,25 -S	CLA 0,5 -S	CLA 1 -S	CLA 2 -S	CLA 4 -S	CLA 8 -S	CIP 16	STR 64	DOX 16	ETH 20
B	CLA 16 -I	CLA 32 -R	CLA 64 -R	MXF 8 -R	RIF 8	SXT 8/152	AMI 64 -R	LZD 64 -R	CIP 8	STR 32	DOX 8	ETH 10
C	RFB 8	EMB 16	INH8	MXF 4 -R	RIF 4	SXT 4/76	AMI 32 -R	LZD 32 -R	CIP4	STR 16	DOX 4	ETH 5
D	RFB 4	EMB 8	INH 4	MXF 2 -I	RIF 2	SXT 2/38	AMI 16 -I	LZD 16 -I	CIP 2	STR 8	DOX 2	ETH 2,5
E	RFB 2	EMB 4	INH 2	MXF 1 -S	RIF 1	SXT 1/19	AMI 8 -S	LZD 8 -S	CIP 1	STR 4	DOX 1	ETH 1,2
F	RFB 1	EMB 2	INH 1	MXF 0,5 -S	RIF 0,5	SXT 0,5/9,5	AMI 4 -S	LZD 4 -S	CIP 0,5	STR 2	DOX 0,5	ETH 0,6
G	RFB 0,5	EMB 1	INH 0,5	MXF 0,25 -S	RIF 0,25	SXT 0,25/4,75	AMI 2 -S	LZD 2 -S	CIP 0,25	STR 1	DOX 0,25	ETH 0,3
H	RFB 0,25	EMB 0,5	INH 0,25	MXF 0,12 -S	RIF 0,12	SXT 0,12/2,38	AMI 1 -S	LZD 1 -S	CIP 0,12	STR 0,5	DOX 0,12	POS

No CLSI breakpoint for amikacin

Breakpoint according to Brown Elliot 2013

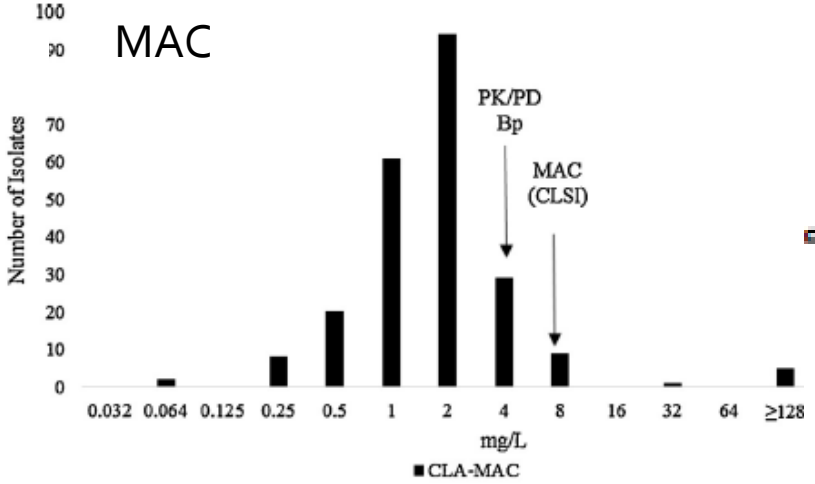


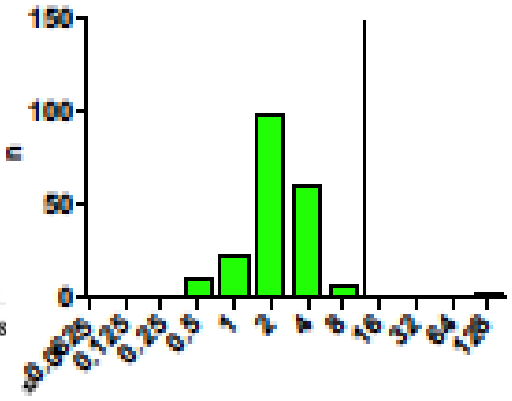
Maurer 2018

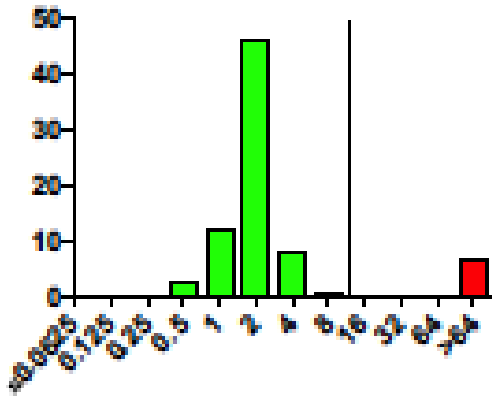


Maurer 2018

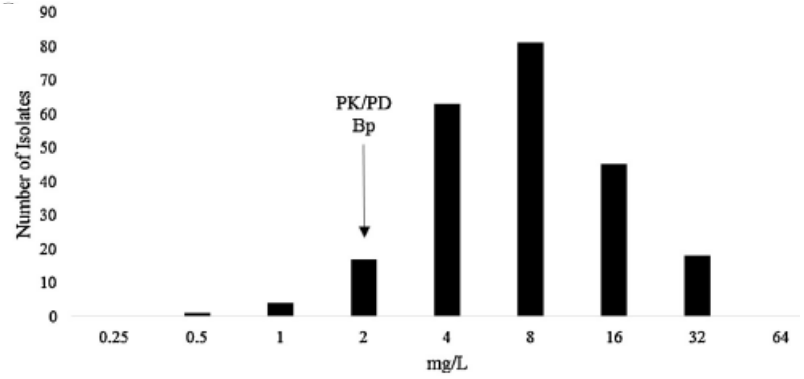
Clarithromycin

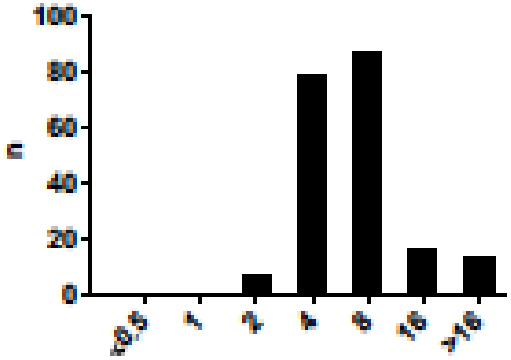


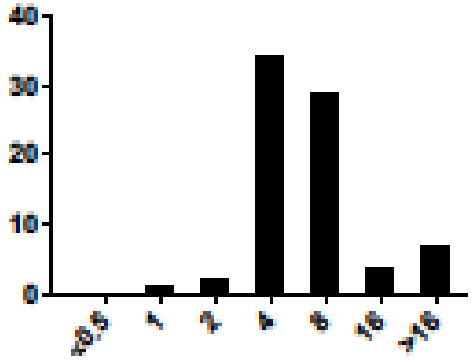




Ethambutol







Maurer 2018, Schoen 2017

J Antimicrob Chemother 2019; **74**: 1288–1294
doi:10.1093/jac/dkz027 Advance Access publication 11 February 2019

Journal of Antimicrobial Chemotherapy

Novel external quality assurance scheme for drug susceptibility testing of non-tuberculous mycobacteria: a multicentre pilot study

Vladyslav Nikolayevskyy [1]*, Florian P. Maurer [2], Yen Holicka [1], Lucy Taylor [1], Helen Liddy [1] and Katharina Kranzer [2,3]

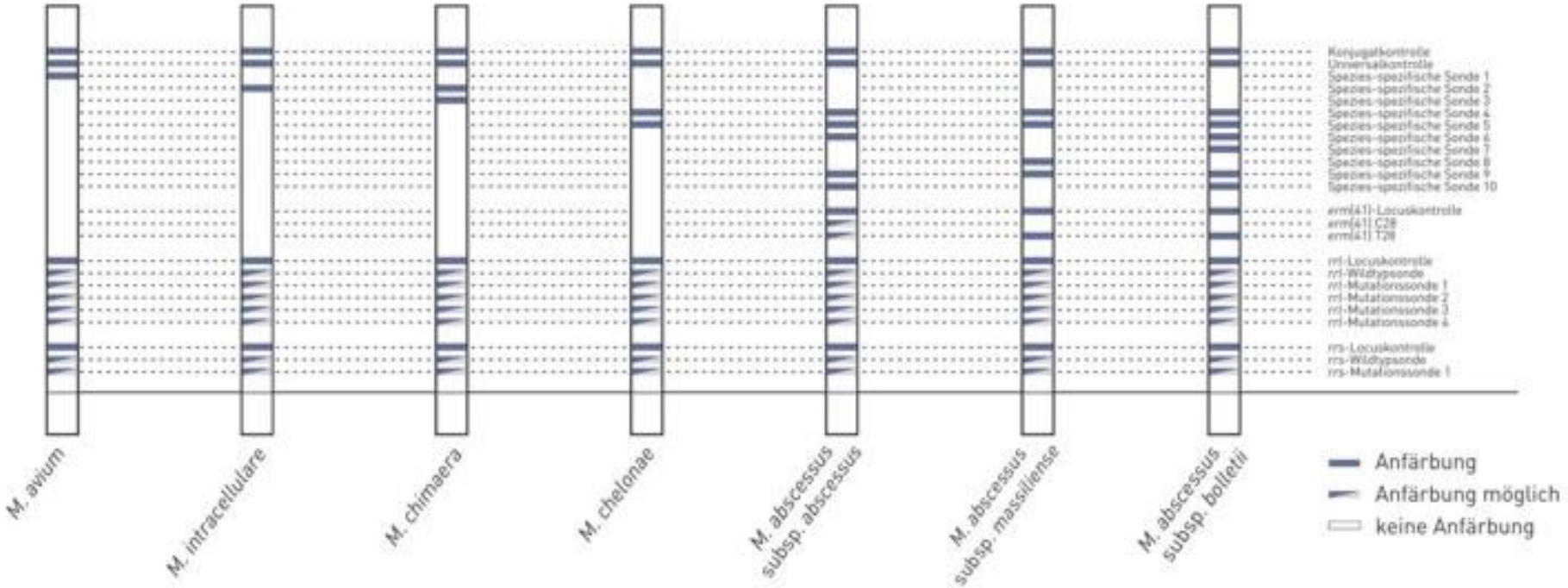
Table 1. Interlaboratory reproducibility and EA rates for phenotypic drug susceptibility testing of four MAV isolates

	No. of results within $\log_2$ concentration								
Antibiotic	>−2	−2	−1	0	1	2	>2	total[a]	% EA
Clarithromycin	0	2	6	31	11	2	0	52	92.3%
Amikacin	2	4	9	25	7	5	0	52	78.8%
Moxifloxacin	0	5	6	30	9	0	0	50	90.0%
Linezolid	2	0	7	39	4	0	0	52	96.2%

Table 3. CA and classification errors for four MAV isolates

	Correct R	Incorrect R	Correct S	Incorrect S	Correct I	Incorrect I	Other[a]	Total	Category errors (*n*)	% CA
Clarithromycin	0	0	43	0	0	0	9	52	none	100.0%
Moxifloxacin[b]	9	10	0	9	16	0	6	50	vMEs ($n = 2$); mEs ($n = 16$)	56.8%
Linezolid[b]	32	0	0	3	4	5	8	52	vMEs ($n = 3$); mEs ($n = 9$)	81.8%

M abscessus phenotypic and molecular DST – NTM-DR



50 isolates tested
MIC (microdilution)
Sanger *rpoB* sequencing for subspecies identification
Sanger erm sequencing for macrolide resistance
NGS for discordant result

Good concordance between NTM-DR, sequencing and pDST

NTM-DR

		Reference PCR sequencing results				Genotype NTM-DR results		
Isolates	Clinical characteristics	Species	*erm (41)*	*rrl*	*rrs*	*erm(41)*	*rrl*	*rrs*
Ma1	respiratory infection	*M. avium*	–	WT + a2058c	WT	absent	WT + MUT1	WT
Ma9	CF patient	*M. avium*	–	a2059c	a1408g	absent	MUT3	MUT1
Ma10	disseminated infection in HIV patient	*M. avium*	–	a2058t	WT	absent	no WT	WT
Ma14	disseminated infection in HIV patient	*M. avium*	–	a2058t + WT + a2059c	WT	absent	MUT2 + MUT3	WT
Ma92239	respiratory infection	*M. avium*	–	a2057c	WT	absent	MUT2	WT
Mi2	respiratory infection	*M. intracellulare*	–	a2058c	WT	absent	MUT1	WT
Mi8	CF patient	*M. intracellulare*	–	WT	a1408g	absent	WT	WT + MUT1
Mi5	respiratory infection	*M. chimaera*	–	a2058c	WT	absent	MUT1 + MUT2	WT
MabC6	CF patient	*M. abscessus*	*abscessus* c28	WT + a2058g[a]	WT	*abscessus* c28	WT + MUT2	WT
MabT11	CF patient	*M. abscessus*	*abscessus* t28	a2058c	WT	*abscessus* t28	MUT1	WT
MabT13	CF patient	*M. abscessus*	*abscessus* t28	a2058g	a1408g	*abscessus* t28	MUT2	MUT1
MabT14	CF patient	*M. abscessus*	*abscessus* t28	a2058g	a1408g	*abscessus* t28	MUT2	MUT1
Mm10	CF patient	*M. abscessus*	*massiliense*	a2058g	WT + a1408g[b]	*massiliense*	MUT2	MUT1
Mcl5	respiratory infection	*M. chelonae*	–	WT + a2059g	WT	absent	WT + MUT4	WT
Mcl6	skin and soft tissue infection	*M. chelonae*	–	a2059c	WT	absent	MUT3	WT
Mcl11	skin and soft tissue infection	*M. chelonae*	–	a2059c	WT	absent	MUT3	WT

Clarithromycin resistance:
Sensitivity 79% (15/19)
Specificity 100% (83/83)

Amikacin resistance:
Sensitivity 71% (5/7)
Specificity 100% (95/95)

Mougari 2017

A challenging NTM patient

New HIV diagnoses VL > 1 million, CD4 10, started ART

Disseminated MAI, macrolide sensitive

Dec 17: rifabutin, azithro and cipro (no ethambutol because of colour blindness) >> initial clinical response (BC continuously positive)

Feb 18: deterioration ?IRIS (treated with steroids)

May 18: presentation with fevers, rigors, bone marrow suppression, cultures from blood, bone marrow and lymph node biopsy ***positive for MAI***

June 18: amikacin and ethambutol added (azithro switched to clarithro; cipro to moxi; rifabutin continued) >> no response after ~2 weeks >> meropenum and clofazamine added

July 18: sustained clinical response, asymptomatic, but BC still ***positive for MAI***, CD4 at 50, viral load suppressed, deteriorating renal function

DST isolates May 18: clarithromycin resistant, amikacin MIC >64, moxifloxacin resistant, linezolid resistant

Discusion points

Should we be stopping amikacin?

Should we be stopping macrolide?

Increase moxifloxacin dose?

What about other drugs?

Linezolid – patient is neutropenic

Bedaqualine – would the rifabutin have to be stopped

Clofazimine

Other suggestions from BHIVA and US guidelines – Ethionamide, Prothionamide, Cylecerine, thiacetazone…..

Finally, we may be able to access whole genome sequencing…..not sure how much that would take us forward???

Conclusion

- NTM disease is rare
- Treatment is challenging partly because of co-morbidities, but also because of inherent and extensive resistance mechanisms
- Meaningful breakpoints are only established for clarithromycin (and possibly amikacin)
- Breakpoints for moxifloxacin and linezolid exist, but do not fulfill the criteria of an ECOFF
- Reproducibility of phenotypic DST is suboptimal