UNIVERSITY OF CAMBRIDGE

Preliminary results of survey on DST capacity in 11 EECA countries

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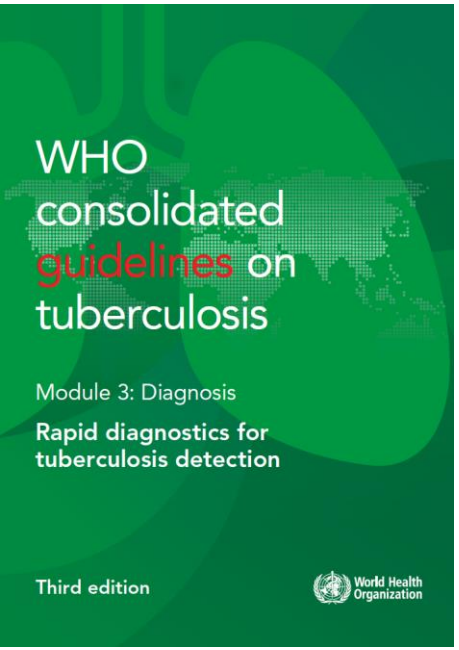
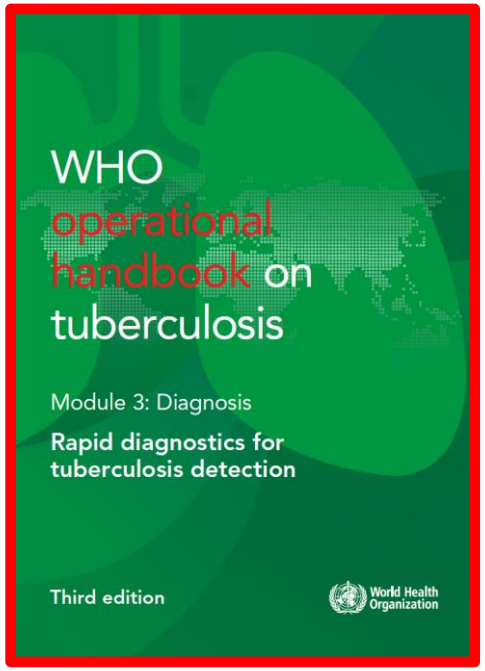
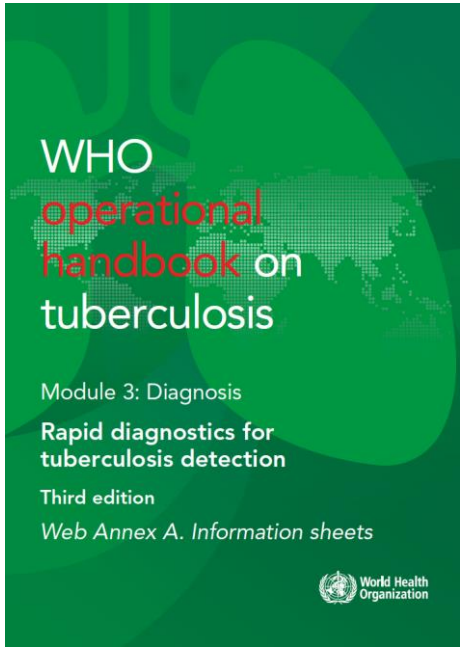
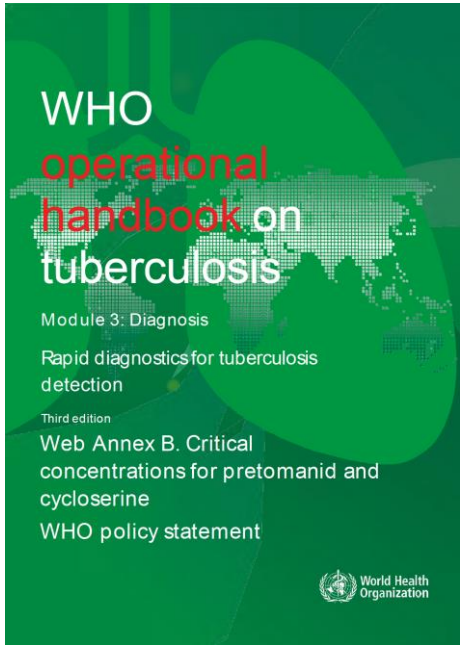
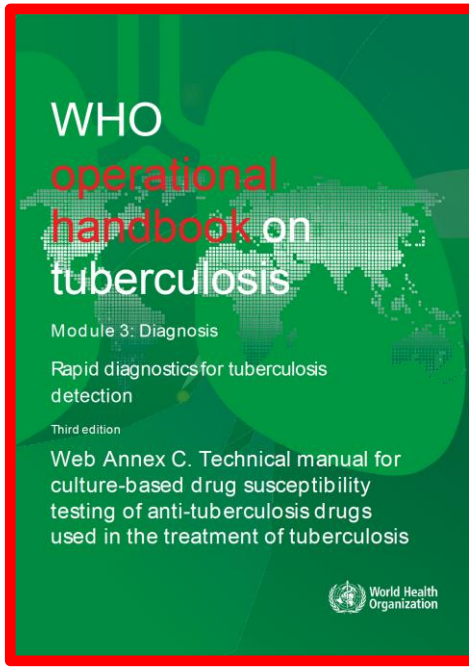
Recent WHO guideline documents related to DST











Current WHO documents contain some errors that are in the process of being corrected!

Table 2.3. CCs and clinical breakpoints for medicines recommended for the treatment of MDR/RR-TB (adapted from Table 3 in Web Annex C)

Group	Medicine	Abbreviation	CCs (µg/mL) for DST by medium			
			Löwenstein–Jensen	Middlebrook 7H10	Middlebrook 7H11	BACTEC MGIT liquid culture
Group A	Levofloxacin (CC)	LFX[a]	2.0	1.0	–	1.0
	Moxifloxacin (CC)	MFX[a]	1.0	0.5	0.5	0.25
	Moxifloxacin (CB)[b]			2.0	–	1.0
	Bedaquiline	BDQ	–	–	0.25	1.0
	Linezolid	LZD	–	1.0	1.0	1.0
Group B	Clofazimine	CFZ	–	–	–	1.0
	Cycloserine/ terizidone	CS/Tad[c]	–	–	–	16
Group C	Ethambutol[d]	E	2.0	5.0	7.5	5.0
	Delamanid[e]	DLM	–	–	0.016	0.06
	Pyrazinamide[f]	PZA	–	–	–	100.0
	Imipenem-cilastatin	IMP/CLN	–	–	–	–
	Meropenem	MPM	–	–	–	–
	Amikacin	AMK	30.0	2.0	–	1.0
	(Streptomycin)[g]	(STR)	4.0	2.0	2.0	1.0
	Ethionamide	ETO	40.0	5.0	10.0	5.0
	Prothionamide	PTO	40.0	–	–	2.5
	Para-aminosalicylic acid	PAS	–	–	–	–
Other	Pretomanid	Pa				0.5 2[h]

ATU: area of technical uncertainty; CB: clinical breakpoint; CC: critical concentration; DST: drug susceptibility testing; LJ: Löwenstein–Jensen media; MDR/RR-TB: multidrug- or rifampicin-resistant tuberculosis; MGIT: Mycobacterial Growth Indicator Tube.

[a] LFX and MFX CCs for LJ established despite very limited data.

[b] CB concentration for 7H10 and MGIT apply to high-dose MFX (i.e. 800 mg daily).

[c] The CC for CS may be used as a surrogate for terizidone resistance.

[d] DST not reliable and reproducible. DST is not recommended.

[e] DLM should be stored away from light and heat, as per the manufacturer's materials safety data sheet.

[f] PZA is only counted as an effective agent when DST results confirm susceptibility in a quality-assured laboratory.

[g] AMK and STR are only to be considered in case of rescue regimens or individualized treatment, and only if DST results confirm susceptibility.

[h] No growth at 0.5 = susceptible; growth at 0.5 and no growth at 2.0 = susceptible, but with a comment on uncertainty; growth at 2.0 = resistant.

Resistant control strains for second-line drugs now available from BCCM/ITM

Annex Table 2. Recommended quality control strains (37)

Drug grouping	Drug	ATCC	BCCM/ITM	Lineage	Resistance mechanism[b]
	Susceptible H37Rv strain	27294[a]	500735	4	none
First-line	Rifampicin-R	35838[a]		4	*rpoB* S450L[c,d]
	Isoniazid low-level-R	BAA-812[a]		not known	*inhA* C-15T[e]
	Isoniazid high-level-R	35822[a]		4	complete *katG* deletion[c,d,f]
	Ethambutol-R	35837[a]		4	not known
	Pyrazinamide-R	35828[a]		4	*pncA* G132S[c]
Group A	Fluoroquinolone high-level-R		500831[g]	2	*gyrA* D94G[h]
	Bedaquiline-R		500807	4	*atpE* A63P
	Linezolid-R		501291[g]	4	*rplC* C154R
Group B	Clofazimine-R		500861[g]	1	*Rv0678* Y92Stop[i]
	Cycloserine-R		501136[g]	4	*alr* D320N[j]
Group C	Delamanid-R/		501095	2	*ddn* Q58Stop[k]
	Carbapenems-R	No DST method exists			
	Amikacin-R		501330[g]	4	*rrs* A1401G
	Streptomycin-R	35820[g]		4	*rpsL* K43R[d]
	Ethionamide/prothionamide-R	BAA-812[g]		not known	*inhA* C-15T[e]
	Para-aminosalicylic acid-R	DST not recommended			
Other	Pretomanid-R		501095	2	*ddn* Q58Stop[k]

[i] Phenotypic clofazimine DST is challenging and *Rv0678* mutants in general, including this mutant, are known not to test reliably resistant. The reasons are not understood fully (e.g. whether the critical concentration is not set optimally, the technical variability for clofazimine is inherently larger than other drugs or whether *Rv0678* confer smaller relative MIC increases for clofazimine than for bedaquiline).

https://iris.who.int/handle/10665/376286

Availability of novel TB drugs for DST

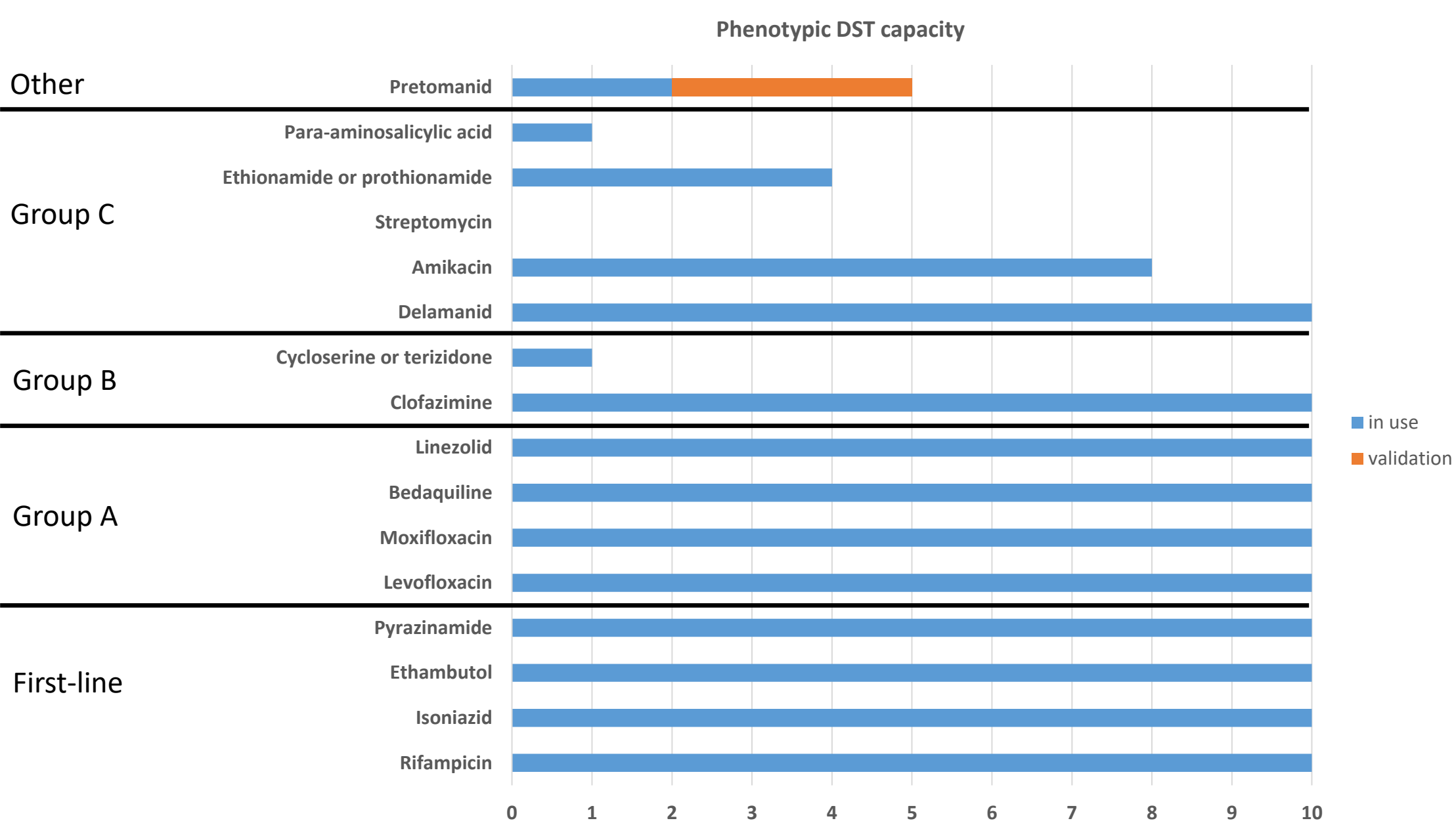
- Key drugs can now be order from BEI (www.beiresources.org):
  - Bedaquiline: ARP-12702.
  - Delamanid: NR-51636 (please remember that this drug is photosensitive!).
  - Pretomanid: NR-59591.

Survey methodology for phenotypic and genotypic DST capacity in WHO European Region

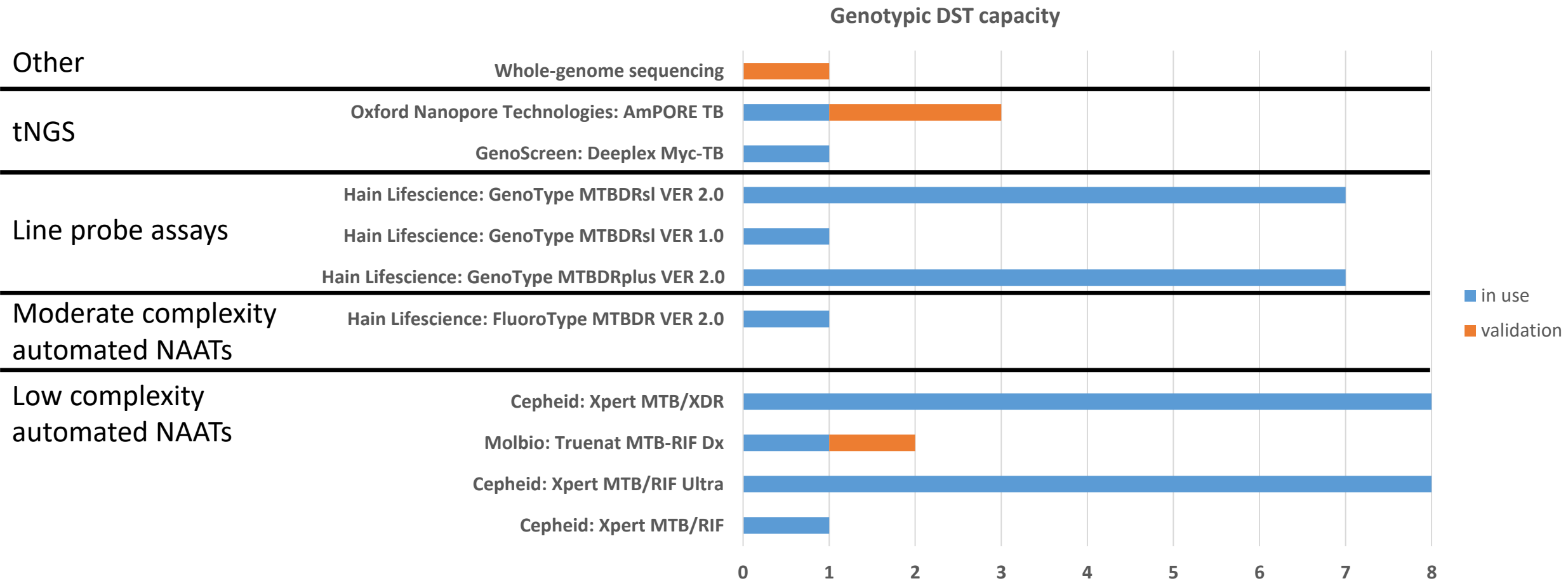
- Online questionnaire sent to officially selected representatives of all countries/regions, WHO Supranational Reference Laboratories, and National Centres of Excellence (i.e. sometimes more than one response per country).
- Main focus on BPaL(M) drugs, but all other drugs were included, except for carbapenems, for which phenotypic DST is currently impossible.
- All commercial genotypic DST assays were included, even if not WHO-recommended, to get a comprehensive view of the current capacity.
- We asked whether at least one laboratory in the country tested a particular drug/used an assay for patient care or was in the process of validating it (i.e. this excluded assays used for research only and means that some assays are only used at the reference laboratory level).
- Limitations:
  - Incomplete results for countries with decentralised testing in private laboratories (mostly in Western European countries).
  - We did not ask to which standard a test/assay was ‘validated’ or to what extent internal/external quality assessment is carried out.

Preliminary results to be discussed in this presentation

- 11 EECA countries that are supported by the Global Fund:
  - Armenia
  - Azerbaijan
  - Belarus
  - Georgia
  - Kazakhstan
  - Kyrgyzstan
  - Republic of Moldova
  - Tajikistan
  - Turkmenistan
  - Ukraine
  - Uzbekistan
- All results from one country and genotypic DST results from two countries were excluded pending clarifications.



- All countries have phenotypic DST capacity to detect MDR-, pre-XDR, and XDR-TB.
- Confusion about interpretation of pretomanid results reported due to lack and/or contradictory guidance by EUCAST, EMA, and WHO.


Genotypic DST capacity
Other
Whole-genome sequencing
tNGS
Oxford Nanopore Technologies: AmPORE TB
GenoScreen: Deeplex Myc-TB
Line probe assays
Hain Lifescience: GenoType MTBDRsl VER 2.0
Hain Lifescience: GenoType MTBDRsl VER 1.0
Hain Lifescience: GenoType MTBDRplus VER 2.0
Moderate complexity automated NAATs
Hain Lifescience: FluoroType MTBDR VER 2.0
Low complexity automated NAATs
Cepheid: Xpert MTB/XDR
Molbio: Truenat MTB-RIF Dx
Cepheid: Xpert MTB/RIF Ultra
Cepheid: Xpert MTB/RIF
0
1
2
3
4
5
6
7
8
in use
validation

Thank you for your attention

- For any questions that cannot be answered by those on this call, please email cuk21@cam.ac.uk.