Implications of the genetic diversity within MTBC and *M. canettii* for the development of new DST assays

Dr Claudio U. Köser, Peacock lab (Department of Medicine)
Overview

- Importance of resistance mechanisms
- Systematic false-positive fluoroquinolone resistance results with Hain GenoType MTBDRsl:
  - Non-synonymous mutations
  - Synonymous mutations
- Importance of genomic surveillance for perchlozone
Disclaimer

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Drug-resistance mechanisms and tuberculosis drugs

Bedaquiline and delamanid, novel classes of anti-
tuberculosis drugs, have been recently approved for
the treatment of multidrug-resistant tuberculosis.\textsuperscript{1}
Antimicrobial resistance invariably follows the
introduction of new drugs, and appropriate drug-
susceptibility testing assays are needed to detect
resistance and tailor treatment regimens that contain
new agents.\textsuperscript{2,3} Given that phenotypic drug-susceptibility
testing is slow, technically demanding, and, in some
cases, unreliable, future assays are likely to be based
on rapid molecular techniques. To design such assays,
research to unravel the genetic basis of resistance is
urgently required (appendix).\textsuperscript{4} The question is how to
eNSure that this research occurs in a timely way, before
the emergence and spread of resistance.

A potential solution is to link the elucidation of
resistance mechanisms to the approval process for
new antibiotics, as is already the case for resistance to
antivirals.\textsuperscript{4,5} Where appropriate, this approach should also
include the resistance mechanisms of older antibiotics
that will be included in new regimens. For many bacteria
and antibiotics it is not feasible to identify resistance
before market release because of horizontal transfer of
resistance genes between bacteria. By contrast, resistance
in the Mycobacterium tuberculosis complex (MtbC)
 arises exclusively by chromosomal changes.\textsuperscript{6} Therefore,
mechanisms of resistance can be studied by multiple
methods, including the selection of drug-resistant
mutants in vitro and in vivo animal infection models, and
by examining drug-resistant mutants from clinical trials.\textsuperscript{7}

Next-generation sequencing showed that
bedaquiline resistance arises through mutations in
the ATP synthase.\textsuperscript{8} Yet it was only after regulatory
approval of bedaquiline—and more than 8 years after
the identification of the target of bedaquiline—that
it was shown that resistance can also arise through
the mutational upregulation of an efflux pump.\textsuperscript{9,10,11}
Importantly, this mechanism also confers cross-
resistance to clarithromycin.\textsuperscript{12,13} As a result, regimens that
contain both drugs might have to be reconsidered
if these mutations are found to be common and to
increase the minimum inhibitory concentrations
significantly to reduce treatment success.\textsuperscript{14,15} It is
questionable whether these regimens would have
been evaluated at all, had the bedaquiline resistance
mechanisms been elucidated comprehensively in
the early stages of drug development. Moreover, had
this genetic information been available at the time of
approval of delamanid, regulators might have required
for this cross-resistance to be formally labelled.\textsuperscript{16,31}

The early identification of resistance mechanisms
would also minimise the chance of developing
antibiotics that are not effective across the world.\textsuperscript{7}
Clinical trials only include patients infected with a
limited number of MtbC genotypes, which raises the
possibility that intrinsic antibiotic resistance could
be missed.\textsuperscript{7} By contrast, intrinsically resistant strains
could be screened for by assessing the conservation of
resistance genes in the genomes of the thousands of
phylogenetically diverse MtbC isolates that have been
sequenced to date.\textsuperscript{17} This approach has already raised
the possibility that Mycobacterium canetti, which causes
tuberculosis in the Horn of Africa and is intrinsically
resistant to pyrazinamide, might also be intrinsically
resistant to PA-824.\textsuperscript{18} Consequently, the regimen of
PA-824/pyrazinamide/moxifloxacin, which is about
to be assessed in phase 3 clinical trials, might lead to
monotherapy of patients with M canetti infection.\textsuperscript{19}

The development and periodic revision of guidelines
to determine resistance mechanisms as part of drug
development would benefit from close cooperation
between academic experts, funding agencies,
pharmaceutical companies, and regulatory authorities,
as has occurred for antivirals in the past.\textsuperscript{4,5} Such work
## Importance of resistance mechanisms

<table>
<thead>
<tr>
<th>Class of resistance mechanisms</th>
<th>Examples</th>
<th>Likelihood of elucidating genetic basis of resistance comprehensively</th>
<th>Likelihood that assay that targets few loci will have high sensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1) Always essential</td>
<td><em>rpoB, inhA, rpsL, rrs</em></td>
<td>High</td>
<td>High</td>
</tr>
<tr>
<td>2) Non-essential <em>in vitro</em>, but “essential” <em>in vivo</em>¹</td>
<td><em>katG</em></td>
<td>Dominant mutations only</td>
<td>Medium</td>
</tr>
<tr>
<td>3) Always non-essential</td>
<td><em>pncA, gidB</em></td>
<td>Low²</td>
<td>Low</td>
</tr>
</tbody>
</table>

¹Certain mutations have higher fitness and are consequently dominant.
²e.g. *pncA* has 185 aa (excluding start codon). So, 185*23=4,255 single codon changes possible (excluding nonsense mutations).
1) Always essential
2) Non-essential in vitro, but “essential” in vivo
3) Always non-essential
# Importance of resistance mechanisms

<table>
<thead>
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<th>Class of resistance mechanisms</th>
<th>Examples</th>
<th>Likelihood of intrinsic antibiotic resistance in significant proportion of population</th>
</tr>
</thead>
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<td>1) Always essential</td>
<td>rpoB, inhA, rpsL, rrs</td>
<td>Low</td>
</tr>
<tr>
<td>2) Non-essential <em>in vitro</em>, but “essential” <em>in vivo</em>&lt;sup&gt;1&lt;/sup&gt;</td>
<td>katG</td>
<td>Medium</td>
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<td>3) Always non-essential</td>
<td>pncA, gidB</td>
<td>High</td>
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</table>

<sup>1</sup>Certain mutations have higher fitness and are consequently dominant.

some, if not all PZA & PA-824 R

Ancestral *M. tuberculosis* (PGG 1, TbD1 intact, Lineage 1, e.g. EAI)

PGG 1, Lineage 2,3, e.g. Beijing, CAS

PGG 2, Lineage 4, e.g. LAM

PGG 3, Lineage 4, e.g. H37Rv

*M. africanum* subtype 1(b) (West-African 1 lineage, Lineage 5)

sublineage 1

sublineage 2

sublineage 3

*M. africanum* subtype 1(a) (West-African 2 lineage, Lineage 6)

Chimpanzee bacillus

*M. mungi* (banded mongoose bacillus)

Dassie bacillus

*M. suricattae* (meerkat bacillus)

*M. oryxis* (oryx bacillus)

*M. microti* (vole bacillus)

*M. pinnipedii* (seal bacillus)

*M. caprae* (goat bacillus)

*M. bovis* (bovine bacillus)

Animal-adapted species

PZA R
cycloserine R
INH/ETH R

*M. bovis BCG* (older strains)

*M. bovis BCG* (newer strains)
**Systematic errors with Hain GenoType MTBDRs/**

- Only known systematic error involves Acc/Gcc T80A gCg/gGg A90G, which does not cause resistance to any FQs, but prevents binding of WT2

- Unclear whether these double mutations were mono-, para- or polyphyletic and how widespread they were, except for some circumstantial evidence:
  - Only 8/211 studies in my database featured these mutations
  - T80A known marker for Uganda genotype
  - Two studies from Democratic Republic of Congo showed highest frequency (7 and 60% of MDR TB, respectively)
Maximum likelihood phylogeny of 87 MTBC Uganda genotype strains and two related outgroup isolates. Phylogenetic variants in a fluoroquinolone resistance mediating region of the gyrA gene are color coded.
Systematic errors with Hain GenoType MTBDRs/ 

<table>
<thead>
<tr>
<th>WT1</th>
<th>WT2</th>
<th>WT3</th>
</tr>
</thead>
<tbody>
<tr>
<td>85</td>
<td>86</td>
<td>87</td>
</tr>
<tr>
<td>88</td>
<td>89</td>
<td>90</td>
</tr>
<tr>
<td>91</td>
<td>92</td>
<td>93</td>
</tr>
<tr>
<td>94</td>
<td>95</td>
<td>96</td>
</tr>
</tbody>
</table>

- MUT1 gCg/gTg A90V
- MUT2 Tcg/Ccg S91P
- MUT3A gAc/gCc D94A
- MUT3B Gac/Aac or Tac D94N or Y
- MUT3C gAc/gGc D94G
- MUT3D Gac/Cac D94H

• The following synonymous mutations cause false-positive results:
  - gcG/gcA A90A (WT2)
  - atC/atT I92I (WT2 & WT3)
  - gaC/gaT D94D (WT3)
  - **Ctg/Ttg L96L** (WT3)

• But not these:
  - caC/caT H85H
  - **ctG/ctA L96L**

• More extensive evaluation of synonymous mutations using plasmids ongoing to explore more complex patterns (e.g. if binding of MUT bands is prevented)

NB: These results are preliminary.
Perchlozone

- Developed by JSC Pharmasyntez and approved for the treatment of MDR TB in Russia in 2012
- Added to ‘List of Vital and Essential Medicines’ in Russia in 2014
- Registration in African countries and the Commonwealth of Independent States is being considered
- Phase IV trials ongoing
- Official Russian national guidelines do not feature perchlozone, but the ones by the Russian Association of Pulmonologists do (for empiric treatment)

http://en.perchlozone.info/ (accessed 29.11.2015)
http://www.pipelinereport.org/2013/tb-treatment
<table>
<thead>
<tr>
<th>Drug</th>
<th>Resistance genes</th>
<th>High confidence gene</th>
</tr>
</thead>
<tbody>
<tr>
<td>Perchlozone</td>
<td><em>hadA (Rv0635)</em>&lt;br&gt;<em>ethA (aka, etaA, Rv3854c)</em></td>
<td></td>
</tr>
<tr>
<td>Ethionamide/prothionamide</td>
<td><em>mshA (Rv0486)</em>&lt;br&gt;<em>mmaA3 (Rv0643c)</em>&lt;br&gt;<em>fabG1 (mabA, Rv1483)</em>&lt;br&gt;<em>inhA (Rv1484)</em>&lt;br&gt;<em>ndh (Rv1854c)</em>&lt;br&gt;<em>mshC (cysS2, Rv2130)</em>&lt;br&gt;<em>nudC (Rv3199c)</em>&lt;br&gt;<em>ethA (aka, etaA, Rv3854c)</em>&lt;br&gt;<em>ethR (aka, etaR, Rv3855)</em></td>
<td>✓</td>
</tr>
<tr>
<td>Thioacetazone</td>
<td><em>hadA (Rv0635)</em>&lt;br&gt;<em>hadB (Rv0636)</em>&lt;br&gt;<em>hadC (Rv0637)</em>&lt;br&gt;<em>mmaA4 (Rv0642c)</em>&lt;br&gt;<em>mmaA2 (Rv0644c)</em>&lt;br&gt;<em>ethA (aka, etaA, Rv3854c)</em>&lt;br&gt;<em>ethR (aka, etaR, Rv3855)</em></td>
<td>✓</td>
</tr>
</tbody>
</table>

Perchlozone

- According to the guidelines by the Russian Association of Pulmonologists perchlozone could be prescribed in combination with ethionamide/prothionamide

- JSC Pharmasyntez recommends the following regimen on its website:
  - Cycloserine
  - Capreomycin
  - Perchlozone
  - Prothionamide
  - Para-aminosalicylic acid
  - Pyrazinamide

http://en.perchlozone.info/ (accessed 29.11.2015)
Genomic surveillance for perchlozone

428 MDR strains from Samara

277 MDR strains from Nukus
Maximum likelihood tree of 705 MDR strains:

- **Clade A - Samara**: ethA -7 t/c (also homoplastic)
- **Clade C - Nukus/Samara**: ethA 111_del (homoplastic)
- **Clade B - Nukus/Samara**: ethA T314I

**Table: ethA category in Beijing strains**

<table>
<thead>
<tr>
<th>ethA category (for 575/591 strains with valid DST results)</th>
<th>Percentage of Beijing strains</th>
</tr>
</thead>
<tbody>
<tr>
<td>wild-type</td>
<td>26% (155)</td>
</tr>
<tr>
<td>any mutation</td>
<td>74% (436)</td>
</tr>
<tr>
<td>indels or nonsense</td>
<td>20% (117)</td>
</tr>
<tr>
<td>111_del</td>
<td>8% (48)</td>
</tr>
<tr>
<td>-7 t/c</td>
<td>30% (177)</td>
</tr>
<tr>
<td>T314I</td>
<td>16% (97)</td>
</tr>
</tbody>
</table>

**Graph: Thioamide resistance**

- wild-type
- any mutation
- indels or nonsense
- 111_del
- -7 t/c
- T314I

*homoplastic*
Thank you very much for your attention