Bernard Fourie
Dept Medical Microbiology, University of Pretoria, Pretoria, South Africa
Luke T Daum
Longhorn Vaccines and Diagnostics, San Antonio, TX , USA

Genetic analysis of Pyrazinamide Resistance in MDR/XDR *Mycobacterium tuberculosis* strains in South Africa

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Pyrazinamide Resistance among South African Multidrug-Resistant *Mycobacterium tuberculosis* Isolates[▽]

Matsie Mphahlele,[1,2] Heidi Syre,[1,5] Håvard Valvatne,[1] Ruth Stavrum,[1,5] Turid Mannsåker,[3] Tshilidzi Muthivhi,[2] Karin Weyer,[2] P. Bernard Fourie,[4] and Harleen M. S. Grewal[1,5]*

The Gade Institute, Section of Microbiology and Immunology, University of Bergen, N-5021 Bergen, Norway[1]; Medical Research Council, Pretoria, South Africa[2]; National Reference Laboratory for Mycobacteria, Norwegian Institute of Public Health, Oslo, Norway[3]; Medicine in Need, MRC Building, Pretoria, South Africa[4]; and Department of Microbiology and Immunology, Haukeland University Hospital, N-5021 Bergen, Norway[5]

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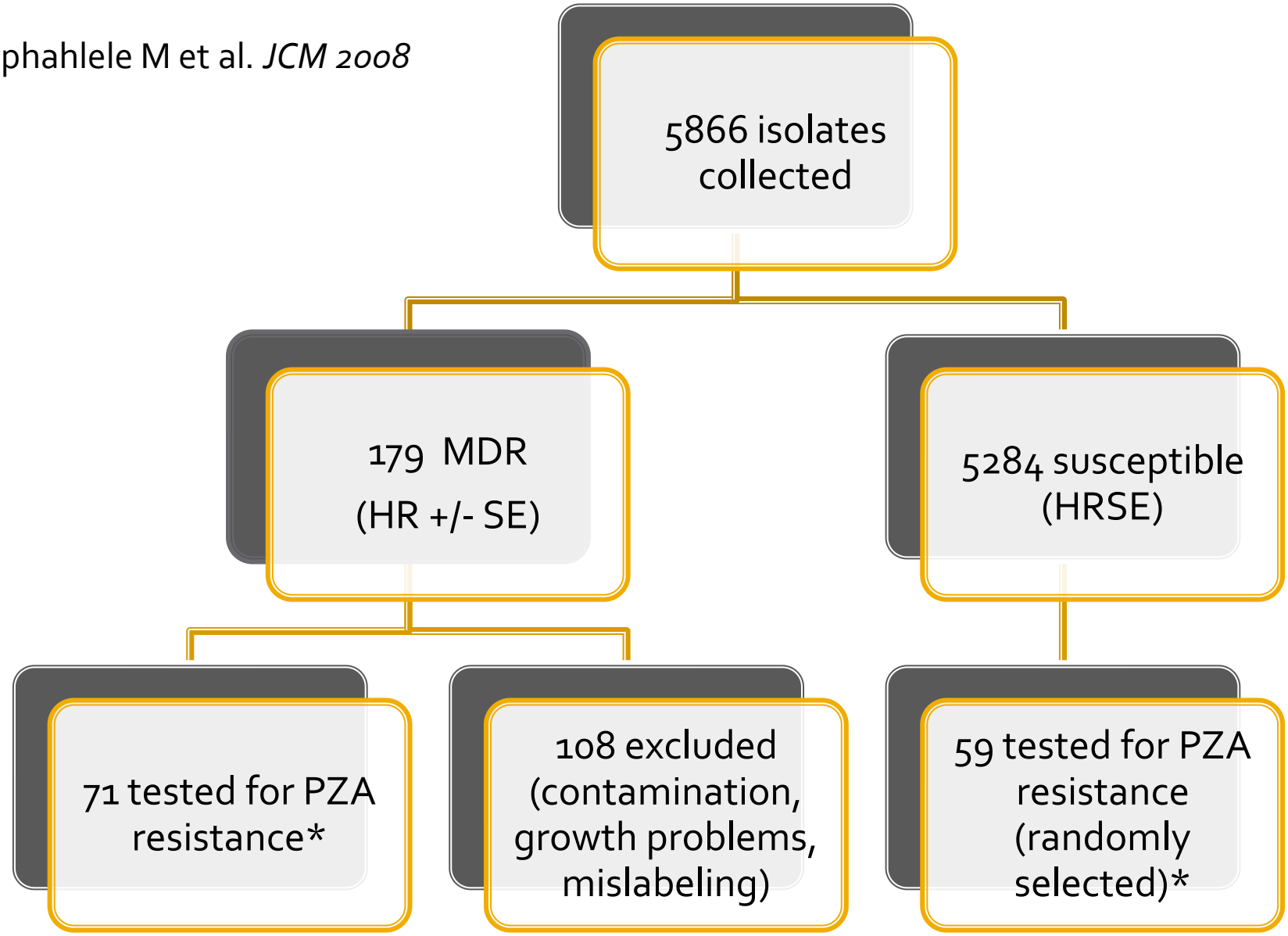
Pyrazinamide is important in tuberculosis treatment, as it is bactericidal to semidormant mycobacteria not killed by other antituberculosis drugs. Pyrazinamide is also one of the cornerstone drugs retained in the treatment of multidrug-resistant tuberculosis (MDR-TB). However, due to technical difficulties, routine drug susceptibility testing of *Mycobacterium tuberculosis* for pyrazinamide is, in many laboratories, not performed. The objective of our study was to generate information on pyrazinamide susceptibility among South African MDR and susceptible *M. tuberculosis* isolates from pulmonary tuberculosis patients. Seventy-one MDR and 59 fully susceptible *M. tuberculosis* isolates collected during the national surveillance study (2001 to 2002, by the Medical Research Council, South Africa) were examined for pyrazinamide susceptibility by the radiometric Bactec 460 TB system, pyrazinamidase activity (by Wayne's assay), and sequencing of the *pncA* gene. The frequency of pyrazinamide resistance (by the Bactec system) among the MDR *M. tuberculosis* isolates was 37 of 71 (52.1%) and 6 of 59 (10.2%) among fully sensitive isolates. A total of 25 unique mutations in the *pncA* gene were detected. The majority of these were point mutations that resulted in amino acid substitutions. Twenty-eight isolates had identical mutations in the *pncA* gene, but could be differentiated from each other by a combination of the spoligotype patterns and 12 mycobacterial interspersed repetitive-unit loci. A high proportion of South African MDR *M. tuberculosis* isolates were resistant to pyrazinamide, suggesting an evaluation of its role in patients treated previously for tuberculosis as well as its role in the treatment of MDR-TB.

Mphahlele M et al. *JCM 2008*



* BACTEC 460 TB System

Findings

- **PZA resistance** in BACTEC 460 system
  - 52.1% MDR isolates (37/71)
  - 10.2% Susceptible isolates (6/59)
- In the **PZA resistant** isolates by BACTEC
  - 90.7% with mutations in *pncA* gene (39/43)
  - 9.3% lacking mutations in *pncA* gene (4/43)
- In the **PZA susceptible** isolates by BACTEC
  - 5.1% with mutations in *pncA* gene (3/59)

Key points

- In isolates with mutations in *pncA*, but PZA susceptible
  - Implication: Mutations in this region do not affect the bacterium's susceptibility to PZA
- Mutations in the *pncA* gene
  - Strongly associated with resistance to PZA by BACTEC (39/42 isolates)
  - Correlates with loss of pyrazinamidase activity (34/42 isolates,Wayne).
- CONCLUSION
  - Detection of *pncA* mutations by direct sequencing of the pncA gene by PCR could provide a rapid method for the diagnosis of pyrazinamide-resistant *M. tuberculosis*, thereby contributing to more-effective management of TB patients and potentially limiting the spread of drug-resistant *M. tuberculosis* isolates *(Louw GE et al. IJTLD 200 6; Scorpio A et al. AAC 1997)*

R&D Partnership

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