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Molecular detection of fluoroquinolone-resistance in multi-drug resistant tuberculosis in Cambodia suggests low association with XDR phenotypes

Corinne Surcouf1†, Seiha Heng1†, Catherine Pierre-Audigier2†, Véronique Cadet-Daniel2, Amine Namouchi2, Alan Murray2, Brigitte Gicquel2*† and Bertrand Guillard1†

Abstract

Background: Drug susceptibility testing (DST) remains an important concern for implementing treatment of MDR tuberculosis patients. Implementation of molecular tests for drug resistance identification would facilitate DST particularly in developing countries where culturing is difficult to perform. We have characterized multidrug resistant strains in Cambodia using MDTDRsl tests, drug target sequencing and phenotypic tests.

Methods: A total of 65 non-MDR and 101 MDR TB isolates collected between May 2007 and June 2009 were tested for resistance to fluoroquinolones and aminoglycosides/cyclic peptides using the GenoType®MTBDRsl assay and gene sequencing. Rifampicin resistance (RMP-R) was tested using gene sequencing and genotyping was assessed by spoligotyping.

Results: A total of 95 of the 101 MDR strains were confirmed to be RMP-R by rpoB gene sequencing. Fourteen of the 101 MDR isolates (14%) carried a gyrA mutation associated with fluoroquinolone-resistance (FQ-R) (detected by the MTBDRsl assay and sequencing) compared with only 1 (1.5%) of the 65 non-MDR strains. Only 1 (1%) of the MDR isolates was found to be XDR TB. The MDR group contained a higher proportion of Beijing or Beijing like strains (58%) than the non MDR group (28%). This percentage is higher in MDR FQ-R strains (71%).

Conclusions: The new GenoType® MTBDRsl assay combined with molecular tests to detect RMP-R and isoniazid resistance (INH-R) represents a valuable tool for the detection of XDR TB. In Cambodia there is a low rate of XDR amongst MDR TB including MDR FQ-R TB. This suggests a low association between FQ-R and XDR TB. Strain spoligotyping confirms Beijing strains to be more prone to accumulate antibiotic resistance.

Background

The World Health Organization (WHO) estimated that there were 0.5 million cases of multi-drug-resistant (MDR) tuberculosis (TB) in 2007. Only 8.5% of the estimated global total of smear-positive cases of MDR-TB were notified. By the end of 2008, 55 countries and territories had reported at least one case of extensively drug-resistant TB (XDR-TB) which are defined as MDR strains that are also resistant to a fluoroquinolone (FQ) and at least one second-line injectable agent (amikacin (AM), kanamycin (KM) and/or capreomycin (CM)) [1].

The WHO underlines the absolute necessity to rapidly scale up the diagnosis and effective disease management of MDR-TB and highlights the problem of access to drug susceptibility testing (DST) for second-line drugs (SLD). Indeed, only nine of the 22 high burden countries (HBCs), who account for 80% of incident TB cases, had access to second-line DST [2].

In 2007, Cambodia with a population of 14.4 million, ranked 21st among the HBCs for TB. The incidence of all forms of TB in this country in 2007 was estimated at 495/100,000 population with a mortality rate at 89/100,000 population [1]. The directly observed therapy strategy (DOTS) has covered 100% of the TB cases for more than 10 years, with 93% treatment success and the number of sputum-smear positive MDR-TB cases (in...
2007) estimated to be 94 cases [1]. The incidence of MDR-TB appears to have remained relatively low. In 2001, a study of drug resistance carried out by the National Tuberculosis Control Program of Cambodia reported the absence of MDR among new cases and a rate of 0.4% of MDR-TB among all TB cases [3]. Nevertheless, a study undertaken between March 2003 and February 2005 found a rate of 5.1% of MDR-TB among HIV co-infected tuberculosis patients in Phnom Penh [4]. In 2007, the HIV prevalence among TB patients was 7.8% and 46% of TB relapses were MDR (data not published). It is essential that TB remains under active surveillance in Cambodia and MDR-TB diagnosis must be further developed. However, until 2011, no laboratory in Cambodia had second-line DST capability. The aim of this retrospective study was to evaluate the prevalence of XD, among MDR strains. Rifampicin-resistance (RMP-R) was confirmed using rpoB gene sequencing. The GenoType® MTBDRsl assay was used for detection of resistance to FQ, and aminoglycosides/cyclic peptides (AG/CP) using gyrA, and rrs target genes. Spoligotyping was used to determine the rate of strain transmission among the MDR population.

Methods

Strains

MDR strains were collected at the Institut Pasteur of Cambodia between May 2007 and June 2009 from all patients diagnosed with MDR TB after drug susceptibility testing. Non MDR strains were selected randomly during the same period. In total, 65 non MDR and 101 MDR M. tuberculosis strains were isolated in the Mycobacteriology Laboratory at the Institut Pasteur du Cambodge (IPC, Phnom Penh). DST for first-line TB drugs was performed using the BD MGIT reading manual method before May 2009 and by the MGIT Bactec 960 method after this date. DNA was extracted from the isolates and used for sequencing, MTBDRsl assay and spoligotyping.

GenoType® MTBDRsl assay

The GenoType® MTBDRsl assay provided by BIOCENTRIC, Bandol, France, was used to determine resistance to FQ, and AM/CM. Tests were performed according to the manufacturer’s instructions.

DNA sequencing of the rpoB, gyrA, rrs genes

Genes were amplified by PCR and then sequenced using a BigDyeTerminator kit and an ABI Prism model 3100 DNA sequencer. Primer sequences are given in Table 1.

Spoligotyping

Spoligotyping was performed as previously described [5].

Results

A total of 101 MDR strains and 65 non MDR strains previously characterized by conventional DST were investigated in this study. Eighty-six MDR strains were also resistant to streptomycin (SM). Results are presented in tables 2 and 3.

RMP-R

A total of 95 out of 101 (94%) MDR strains with a RMP-R phenotype carried a mutation known to confer resistance in the core region of the rpoB genes (table 2). The codons most frequently involved were codon 516 (18/101 = 18%), codon 526 (22/101 = 22%) and codon 531 (47/101 = 47%). All of these mutations can be detected by the GenoType® MTBDRplus assay. Eighty-three strains showed a single mutation (codons involved: 516, 526, 529, 531 and 533) and 3 had a repetition of codon 514, which is associated with the mutation A532V. Ten strains displayed several mutations (or a deletion for one strain) with at least one known to be associated with RMP resistance. One strain showed a previously un-published mutation (L524W) associated with deletion of codon 526. In six strains that were phenotypically RMP-R, the sequence of the core region of rpoB was identical to the wild type. Because most RMP-R strains are known to be isoniazid resistant (INH-R), molecular INH resistance was not checked to confirm phenotypic isoniazid resistance and RMP-R strains were considered as MDR strains.

FQ-R

Mutations known to be associated to FQ-R were found in 14 out of 101 MDR strains (14%) and 1 out of the 65 non MDR strains (1.5%). The MTBDRsl assay detected all 14 FQ-R mutations that were confirmed by gyrA sequencing. The codons involved were 94 (gac D94A gcc, n = 7; gac D94G gcc, n = 5) and 90 (gcg A90V gtg, n = 2) (table 2). In 3 of these 14 strains, a superposition of nucleotides was observed suggesting that they were a mixture of wild type and mutant populations. All the strains were shown to carry a mutation at codon 21 [6] and 95 [7] of the gyrA gene. This polymorphism is not associated with resistance. The FQ-R group contained a higher proportion of Beijing strains identified by spoligotyping (10/14, 71%) than the FQ-S group (47/87, 54%).

rrs gene

The rrs gene was sequenced in 95 strains to identify resistance to AM, KM and/or CM. For 6 strains, sequencing data was not interpretable because of bacteriological contamination. The MTBDRsl assay detected only one strain with a1401 g mutation in the rrs gene. No other strain had a mutation at codon 1401, 1402 or
| Table 1 Primers used for detection of antibiotic resistance |
|----------------------------------------------------------|
| **Antibiotic** | **Gene** | **Primers** | **Product size (bp)** |
|----------------|----------|-------------|-----------------------|
| Rifampicin     | rpoB     | TR1 (5'-TACGGTCGGCGAGCTGATCC-3') | 411 |
|                |          | TR2 (5'-TACGGGCTTTCGATGAAACC-3') |   |
| Fluoroquinolones | gyrA      | gyrA-F (5'-GATGACAGACAGCGTGC-3') | 398 |
|                |          | gyrA-R (5'-GGGCCTCGGTTGTAACCTCAT-3') |   |
| Kanamycin/Amikacin | rrs      | Rrs F (5'-AAACCTTTCTACCGACG-3') | 1329 |
|                |          | Rrs R (5'-GTATCCATTGATGCTGC-3') |   |

bp: base pair.

**Table 2 Results of phenotypic testing, sequencing, MTBDRs/ and spoligotyping**

| MDR strains | Number of strains | DST Phenotype | RMP | FQ | MTBDRs/ | KM/AM | Spoligotype |
|-------------|------------------|---------------|-----|----|---------|-------|-------------|
|             |                  |               |     |    |         |       |             |
| INH, RMP, SM | S531L            | A90V          | r   | a1401g | r       | EA12-NTB |
| INH, RMP, SM | D516V            | A90V mixed wt | r   | wt   | s       | Beijing |
| INH, RMP, SM | S531L            | D94A          | r   | nd   | s       | Beijing |
| INH, RMP, SM | S531L            | D94A          | r   | wt   | s       | Beijing |
| INH, RMP, SM | HS26Y            | D94A          | r   | wt   | s       | Beijing |
| INH, RMP, SM | S531L            | D94A          | r   | wt   | s       | Beijing |
| INH, RMP, SM | LS33P            | D94A mixed wt | r   | wt   | s       | Beijing |
| INH, RMP, SM | D516V            | D94G          | r   | wt   | s       | Beijing |
| INH, RMP, SM | HS26D            | D94G          | r   | wt   | s       | Beijing |
| INH, RMP, SM | S531L            | D94G          | r   | wt   | s       | EA11_SOM |
| INH, RMP, SM | S531L            | D94G          | r   | wt   | s       | U |
| INH, RMP, SM | S531L            | D94G mixed wt | r   | wt   | s       | U |
| INH, RMP, SM | S531L            | wt            | s   | wt   | s       | Beijing |
| INH, RMP, SM | S531L            | wt            | s   | wt   | s       | Beijing |
| INH, RMP, SM | S531L            | wt            | s   | wt   | s       | Beijing |
| INH, RMP, SM | S531L            | wt            | s   | wt   | s       | Beijing |
| INH, RMP, SM | S531L            | wt            | s   | wt   | s       | Beijing |
| INH, RMP, SM | S531L            | wt            | s   | wt   | s       | Beijing |
| INH, RMP, SM | S531L            | wt            | s   | wt   | s       | Beijing |
| INH, RMP, SM | S531L            | wt            | s   | wt   | s       | Beijing |
| INH, RMP, SM | S531L            | wt            | s   | wt   | s       | Beijing |
| INH, RMP, SM | S531L            | wt            | s   | wt   | s       | Beijing |
| INH, RMP, SM | S531L            | wt            | s   | wt   | s       | Beijing |
| INH, RMP, SM | S531L            | wt            | s   | wt   | s       | Beijing |
| INH, RMP, SM | S531L            | wt            | s   | wt   | s       | Beijing |

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|   | INH, RMP, SM | S531L | wt | s | wt | s | Beijing |
|---|--------------|-------|----|---|----|---|---------|
|   | INH, RMP, SM | S531L | wt | s | wt | s | Beijing |
| 2 | INH, RMP, SM | S531L | wt | s | wt | s | Beijing |
|   | INH, RMP, SM | S531L | wt | s | wt | s | Beijing |
|   | INH, RMP, SM | SS12N TS2ST HS26P LS27Q | wt | s | wt | s | Beijing |
| 1 | INH, RMP, SM | HS26D | wt | s | wt | s | Beijing |
| 1 | INH, RMP, SM | HS26Y | wt | s | wt | s | Beijing |
| 1 | INH, RMP, SM | HS26Y | wt | s | wt | s | Beijing |
|   | INH, RMP, SM | HS26N LS33P | wt | s | wt | s | Beijing |
| 1 | INH, RMP, SM | HS26R | wt | s | wt | s | Beijing |
| 1 | INH, RMP, SM | HS26R | wt | s | wt | s | Beijing |
| 1 | INH, RMP, SM | LS33P | wt | s | wt | s | Beijing |
|   | INH, RMP, SM | D516V mixed wt, SS31L | wt | s | wt | s | Beijing |
|   | INH, RMP, SM | insertion ttc S14 | wt | s | wt | s | Beijing |
| 2 | INH, RMP, SM | SS31L | wt | s | wt | s | Beijing |
|   | INH, RMP, SM | SS31L | wt | s | wt | s | Beijing |
| 1 | INH, RMP, SM | SS31L | wt | s | wt | s | Beijing |
| 1 | INH, RMP, SM | S531L | wt | s | wt | s | Beijing |
|   | INH, RMP, SM | L524W T525P del 526 | wt | s | wt | s | Beijing |
|   | INH, RMP, SM | wt | s | wt | s | Beijing |
| 1 | INH, RMP, SM | SS31L | wt | s | wt | s | Beijing |
| 1 | INH, RMP, SM | wt | s | wt | s | Beijing |
| 1 | INH, RMP, SM | wt | s | wt | s | Beijing |
| 1 | INH, RMP, SM | del 518 | wt | s | wt | s | EAI1_SOM |
|   | INH, RMP | SS31L | wt | s | wt | s | EAI1_SOM |
|   | INH, RMP | wt | s | nd | s | EAI1_SOM |
| 1 | INH, RMP, SM | SS31L | wt | s | wt | s | EAI1_SOM |
| 1 | INH, RMP, SM | HS26D | wt | s | wt | s | EAI1_SOM |
| 1 | INH, RMP, SM | SS31L | wt | s | nd | s | EAI1_SOM-EAI2 |
|   | INH, RMP, SM | SS31L | wt | s | wt | s | EAI4_VNM |
|   | INH, RMP, SM | SS31L | wt | s | wt | s | EAI5 |
|   | INH, RMP, SM | HS26D | wt | s | wt | s | EAI5 |
|   | INH, RMP, SM | HS26D | wt | s | wt | s | EAI5 |
| 1 | INH, RMP, SM | D516Q QS17N del 518 | wt | s | wt | s | EAI5 |
| 1 | INH, RMP, SM | SS31L | wt | s | nd | s | EAI5 |
| 1 | INH, RMP | LS33P | wt | s | wt | s | EAI5 |
| 1 | INH, RMP | LS33P | wt | s | wt | s | U |
| 1 | INH, RMP | D516V | wt | s | wt | s | U |
| 1 | INH, RMP, SM | D516V mixed wt | wt | s | wt | s | U |
| 2 | INH, RMP, SM | HS26D | wt | s | wt | s | U |
| 1 | INH, RMP, SM | SS31L | wt | s | wt | s | U |
| 1 | INH, RMP, SM | SS31L | wt | s | wt | s | U |
| 1 | INH, RMP | HS26D mixed wt | wt | s | wt | s | Unknown |
| 1 | INH, RMP | SS31L | wt | s | wt | s | Unknown |
| 1 | INH, RMP | SS31L | wt | s | wt | s | Unknown |
| 1 | INH, RMP | wt | s | wt | s | Unknown |
1484, which are all known to be associated with resistance to AM, KM and CM. Among the MDR strains, all except one of the 14 FQ-R strains were found to be AM-S. The AM-R strain was also FQ-R, thus, this single XDR strain represents 1% of the MDR population.

**Strain diversity**

Spoligotyping results showed that the majority of MDR strains belonged to the Beijing family (57/101, 56%) or were Beijing like (2/101, 2%). This percentage is higher in MDR FQ-R strains (10/14, 71%). This confirms that Beijing strains are more prone to accumulate antibiotic resistances. The other families were: U (10/101), EA15 (6/101), EA11_SOM (6/101), EA11_SOM-EA12 (1/101), EA12-NTB (1/101), EA14-VNM (1/101), ZERO (1/101) and 16 Unknown profiles. In contrast, only 17 Beijing and 1 Beijing like out of 65 non MDR strains (28%) were identified as such. The other families were: U (3/65), EA15 (13/65), EA11_SOM (2/65), EA11_SOM-EA12 (6/65), EA12_MANILLA (2/65), H3 (1/65), and 20 Unknown profiles. MDR strain comparisons using spoligotype profiles and target gene mutations allowed us to differentiate 74/101 strains indicating that they are not epidemiologically related. The other MDR strains were grouped as follows: 27 strains were grouped into 10 pairs (6 pairs of Beijing and 4 non Beijing) and one group of three Beijing and one group of four Beijing strains.

**Discussion**

This study analyzed resistance of Cambodian MDR-TB strains to major anti-tuberculosis drugs.

Unpublished data showing that 46% of TB relapses in Cambodia are MDR is consistent with previous reports from the Central African Republic [8], Japan [9], and South Africa [10].

RMP-R was confirmed with rpoB core region sequencing in 94% of MDR strains. This agrees with other studies showing that some RMP-R strains carry a wild type rpoB core region sequence [11]. rpoB core region mutations were in the hot spot positions associated with RMP-R strains [12,13]. We observed a high number of MDR strains carrying several rpoB mutations. This may suggest a stepwise process of additive mutations (as described for FQ-R) that could generate higher levels of resistance to RMP [14-16].

To evaluate the number of XDR strains among MDR strains we identified mutations in gyrA and rrs genes which are associated with FQ resistance and AG/CM resistance respectively. Our study found that 14% of clinical MDR-TB isolates were FQ resistant. This rate is lower than that found in other studies of MDR-TB in Southern Asia: Taiwan 22.2% [17] or 42.8% using phenotypic DST; Shanghai 25.1% [18] and the Philippines 17% [19] or 51.4% [20]. However, these figures are much higher than those found on other continents (4.1% in MDR-TB in the United States and Canada [21] and 4.3% in MDR-TB in Russia [22]). Only one of the MDR FQ-R strains was AM-R. Therefore, only 1% of MDR strains are identified as XDR strains. This shows that identification of only gyrA FQ-R mutations is not a strong indication of an XDR phenotype.

Previous studies [23,24] showed a high level of concordance between MTBDRsl and phenotypic tests: I.E. 90.2% and 75.6% respectively. In this study, we had no access to phenotypic tests for FQ. MTBDRsl and sequencing data of gyrA are 100% concordant and these techniques identified 14% FQ-R in MDR strains. This FQ-R rate may have been underestimated because of the unavailability of FQ phenotypic testing in Cambodia. FQ resistance is most often associated with gyrA mutations.

**Table 2 Results of phenotypic testing, sequencing, MTBDRsl and spoligotyping (Continued)**

|   | INH, RMP |  |  | INH, RMP, SM |  |  | INH, RMP, SM |  |
|---|---------|---|---|-------------|---|---|-------------|---|
| 1 | INH, RMP | wt | wt | s | wt | s | Unknown |
| 1 | INH, RMP, SM | HS26Y | wt | s | wt | s | Unknown |
| 1 | INH, RMP, SM | HS26D RS29Q | wt | s | wt | s | Unknown |
| 2 | INH, RMP, SM | SS31L | wt | s | wt | s | Unknown |
| 1 | INH, RMP, SM | SS31L | wt | s | wt | s | Unknown |
| 1 | INH, RMP, SM | HS26Y | wt | s | wt | s | Unknown |
| 1 | INH, RMP, SM | DS16V | wt | s | wt | s | Unknown |
| 1 | INH, RMP, SM | DS16V | wt | s | nd | s | Unknown |
| 1 | INH, RMP, SM | SS31L | wt | s | wt | s | Unknown |
| 1 | INH, RMP, SM | SS31L | wt | s | wt | s | Unknown |
| 1 | INH, RMP, SM | DS16G | wt | s | nd | s | ZERO |

INH isoniazid, RMP rifampicin, SM streptomycin.
AM amikacin, FQ fluoroquinolones, KM kanamycin.
wt wild type, s susceptible, r resistant, nd not determined.
and more particularly mutations at codons 94 and 90. This was the case in our study. The percentage of FQ-R M. tuberculosis clinical isolates with gyrase A mutations have been reported to be between 70% and 90% [23]. Because we have no access to FQ-R phenotypic tests, our analysis may have missed a number of cases. However, as reported before [23,24], in the absence of phenotypic tests, molecular tests will detect more than 75% of FQ-R cases. Mutations other than those affecting gyrA and other mechanisms could result in FQ-R, including: decreased cell-wall permeability to drug, efflux pumps, drug sequestration or perhaps even drug inactivation [12,26]. In a small number of cases, FQ-R could be associated with gyrB mutations and a probable efflux mechanism [27,28].

FQ has become an essential part of treatment regimens for MDR tuberculosis [25,26]. Due to their efficacy and safety, the new generation of FQ's is even being evaluated as a first-line medication for tuberculosis [27-29]. Unfortunately, the extensive use of FQ has increased spontaneous acquisition of mutations associated with FQ-R. It has been suggested that routine FQ-R testing in locations where resistant strains are endemic may be clinically useful by showing a significant correlation between development of FQ-R and first-line M. tuberculosis drug resistance [30]. However, extensive use of FQ would likely increase MDR tuberculosis treatment failure. As in other developing countries, problems arise from the uncontrolled use of antibiotics. However, it is interesting to note that only 1 out of 14 MDR FQ-R strains is XDR, thus showing that detection of FQ-R strains cannot be used as a single marker for the detection of XDR cases. Additional tests are required to identify XDR cases, among them molecular tests like MTBDRsl that include the detection of mutations in gyrA associated to FQ-R and rrs associated with AM, KM and CM resistance.

Work by other researchers has demonstrated that the MTBDRsl assay detected 86.7% (39/45) and 100% (5/5) of phenotypically AM and CM resistant TB strains [23,24]. The mutation a1401 g was the most prevalent. The XDR isolate in our study was confirmed to carry a1401 g mutation in the rrs gene. This result suggests a low rate of XDR amongst MDR TB in Cambodia (1%) as compared to countries like India and Taiwan that have reported rates of 8-15% and 10% respectively [31,32]. This favorable situation could be linked to the implementation of the DOTS strategy in 1980 by the national TB control program in Cambodia [3].

Spoligotyping confirmed previous observations that the Beijing family is prevalent in Asia. The higher proportion of Beijing spoligotypes in the MDR group (58%) compared with the non MDR group (28%) is consistent with previous studies demonstrating that strains of the Beijing genotype more readily acquire resistance mutations than

Table 3 Results of phenotypic testing, sequencing and spoligotyping

| non MDR strains | Number of strains | DST Phenotype | Sequencing | Spoligotype |
|----------------|------------------|---------------|------------|-------------|
| rpoB            | gyrase A          |               |            |             |
| 1 INH, SM       | wt wt Beijing     |               |            |             |
| 3 SM            | wt wt Beijing     |               |            |             |
| 13 no resistance| wt wt Beijing     |               |            |             |
| 1 no resistance | wt wt Beijing-like|               |            |             |
| 1 SM            | wt wt EAI1_SOM    |               |            |             |
| 1 no resistance | wt wt V55V EAI1_SOM|               |            |             |
| 4 no resistance | wt wt EAI1_SOM    |               |            |             |
| 1 no resistance | wt wt EAI1_SOM    |               |            |             |
| 1 no resistance | wt wt EAI2_MANILLA|               |            |             |
| 1 no resistance | wt wt EAI2_MANILLA|               |            |             |
| 1 INH            | Q513K wt EAIS     |               |            |             |
| 1 INH            | wt wt EAIS        |               |            |             |
| 1 no resistance  | wt wt EAIS        |               |            |             |
| 2 no resistance  | wt wt EAIS        |               |            |             |
| 4 no resistance  | wt wt EAIS        |               |            |             |
| 1 no resistance  | wt wt EAIS        |               |            |             |
| 1 no resistance  | wt wt EAIS        |               |            |             |
| 1 no resistance  | wt wt EAIS        |               |            |             |
| 1 no resistance  | wt wt EAIS        |               |            |             |
| 1 no resistance  | wt wt H3          |               |            |             |
| 1 INH, SM       | wt wt U           |               |            |             |
| 2 no resistance  | wt wt U           |               |            |             |
| 1 INH, SM       | wt wt D94A Unknown|               |            |             |
| 1 INH            | wt wt Unknown     |               |            |             |
| 1 RMP            | H526Y wt Unknown  |               |            |             |
| 1 RMP            | wt wt Unknown     |               |            |             |
| 1 no resistance  | wt wt Unknown     |               |            |             |
| 1 no resistance  | wt wt Unknown     |               |            |             |
| 1 no resistance  | wt wt Unknown     |               |            |             |
| 1 no resistance  | wt wt Unknown     |               |            |             |
| 1 no resistance  | wt wt Unknown     |               |            |             |
| 1 no resistance  | wt wt Unknown     |               |            |             |
| 1 no resistance  | wt wt Unknown     |               |            |             |
| 1 no resistance  | wt wt Unknown     |               |            |             |
| 1 no resistance  | wt wt Unknown     |               |            |             |
| 1 no resistance  | wt wt Unknown     |               |            |             |
| 1 no resistance  | wt wt Unknown     |               |            |             |
| 1 no resistance  | wt wt Unknown     |               |            |             |
| 1 no resistance  | wt wt Unknown     |               |            |             |

INH isoniazid, RMP rifampicin, SM streptomycin, wt wild type.
non-Beijing strains [33-35]. This percentage is increased in the MDR FQ-R group (71%). Although most of the spoligotypes had the Beijing profile, no major MDR outbreak by this family has been reported in Cambodia. Further studies with new markers are warranted to provide a more accurate picture of the epidemiology of the Beijing strains in Cambodia.

Conclusions

A molecular study of 101 MDR M. tuberculosis strains in Cambodia identified only 1 (1%) XDR strain although a high number of the MDR cases showed FQ-R (14%). The new GenoType® MTBDRsl assay represents a reliable tool for the detection of FQ and AG/CAP resistance and in combination with molecular tests for the detection of RMP-R and INH-R, XDR TB can potentially be identified within 1 or 2 days. These tests could provide valuable information to facilitate the management of patient therapy and the prevention of transmission.

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Authors’ contributions

CI participated in the planning of the study, acquisition of samples and demographic data, SH participated in culture and isolation of mycobacteria, molecular assays, CPA participated in data analysis and drafting of the manuscript, VCD, AM and AN participated in molecular assays, data analysis and drafting of manuscript, AM participated in general supervision of the study and critical revision of the manuscript, BG and BG the conception of the study, general supervision of the study, critical revision of manuscript. All authors read and approved the final manuscript.

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