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Analysis of *embCAB* Mutations Associated with Ethambutol Resistance in Multidrug-Resistant *Mycobacterium tuberculosis* Isolates from China

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Ethambutol (EMB) plays a pivotal role in the chemotherapy of drug-resistant tuberculosis (TB), including multidrug-resistant tuberculosis (MDR-TB). Resistance to EMB is considered to be caused by mutations in the *embCAB* operon (*embC*, *embA*, and *embB*). In this study, we analyzed the *embCAB* mutations among 139 MDR-TB isolates from China and found a possible association between *embCAB* operon mutation and EMB resistance. Our data indicate that 56.8% of MDR-TB isolates are resistant to EMB, and 82.2% of EMB-resistant isolates belong to the Beijing family. Overall, 110 (79.1%) MDR-TB isolates had at least one mutation in the *embCAB* operon. The majority of mutations were present in the *embB* gene and the *embA* upstream region, which also displayed significant correlations with EMB resistance. The most common mutations occurred at codon 306 in *embB* (*embB*306), followed by *embB*406, *embA*(−16), and *embB*497. Mutations at *embB*306 were associated with EMB resistance. DNA sequencing of *embB*306–497 was the best strategy for detecting EMB resistance, with 89.9% sensitivity, 58.3% specificity, and 76.3% accuracy. Additionally, *embB*306 had limited value as a candidate predictor for EMB resistance among MDR-TB infections in China.

Multidrug-resistant tuberculosis (MDR-TB) is attributed to an estimated 3.7% new cases and 20.2% previously treated cases of TB annually worldwide and is becoming a major threat to global public health (1). In China, the significantly high prevalence (5.7% new cases and 25.6% previously treated cases) of MDR-TB makes TB control especially challenging (2). Ethambutol (EMB) is an important first-line anti-TB drug routinely recommended for therapy of drug-resistant TB, including MDR-TB. Disturbingly, in some regions of China, substantial proportions (51.3% to 66.7%) of MDR-TB isolates demonstrated EMB resistance (3–5). Development of new rapid and reliable molecular methods for detecting drug resistance is essential to optimize treatment regimens, prevent treatment failure, and thus reduce the further spread of drug-resistant isolates. However, these molecular assays require precise knowledge of the genetic mutations associated with drug resistance. Prior studies indicated that the characteristics of resistance-associated mutations vary in different regions (6, 7).

EMB acts against TB by inhibiting membrane-associated arabinosyl transferases encoded by the *embCAB* operon (including *embC*, *embA*, and *embB*), which are involved in the synthesis of cell wall arabinogalactan (8, 9). Approximately 50% to 70% of EMB-resistant TB isolates harbor mutations in a relatively short fragment (codons 306–497) in *embB* genes, with mutations occurring most frequently at codon 306 in *embB* (*embB*306), *embB*406, and *embB*497 (5, 8, 10–13). Sequence analysis of this fragment has been a tool for the rapid detection of EMB resistance. However, approximately one third of EMB-resistant isolates do not carry changes in this region and therefore are not detectable by using DNA sequencing (12, 14). Although other mutations in the *embCAB* operon were suggested to confer resistance (14–16), only limited data have been available until now in China, with most studies analyzing only a short fragment of the *embB* gene encompassing codons 306, 406, and 497 (3, 4, 17). In addition, some studies showed a widespread presence of mutations at these three codons among EMB-susceptible isolates (13, 18–20). The roles of mutations within *embB*306, *embB*406, and *embB*497 were uncertain. Thus, the aim of this study was to explore the mutations within the entire *embCAB* operon (nucleotide positions in H37Rv [GenBank accession number NC_000962]: 4239863–4249810) among 139 MDR-TB clinical isolates from 8 provinces in China and to find a possible association between *embCAB* operon mutation and EMB resistance.

MATERIALS AND METHODS

Ethical approval. The study obtained approval from the ethics committee of the National Institute for Communicable Disease Control and Prevention, Chinese Center for Disease Control and Prevention. The patients with TB included in the present research were given a subject information sheet, and they all gave written informed consent to participate in the study.

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TABLE 1 PCR primers and conditions used for amplification and sequencing

Primers	Sequence (5′ to 3′)	Nucleotide position	PCR conditions[a]		
			D (s)	A (°C, s)	E (s)
EmbC1F	CGTCGTCGAGGACATTGGC	4239803–4241169	30	61, 40	60
EmbC1R	AGGGTCAAGGCACCGATGATG				
EmbC2F	CGGGCATGTTTCTGGCTGTCTG	4241007–4242233	30	61, 40	60
EmbC2R	GAATGCCGTTGGGTGTGAAGG				
EmbC3F	CCGGTCTAACCTACAGGCTTTGG	4242073–4243240	30	61, 40	60
EmbC3R	TGGGGCACGAGGCTCGATGGTA				
EmbA1F	AACCTAGGAACGGTGACT	4243105–4243726	30	58, 40	40
EmbA1R	CAACCTGTGGCTTCTTCT				
EmbA2F	CAACCAGGACACGGTCGTCG	4243559–4245121	40	62, 40	90
EmbA2R	TAGTTGCCGACGTAGAACCACC				
EmbA3F	CTTTGCCCGCATCGGTCTACAT	4245005–4246534	40	62, 40	90
EmbA3R	TCTGCTCGCGCACTGTGTCAT				
EmbB1F	ATCAGGGCGCTGCCATGACA	4246500–4247838	30	62, 40	60
EmbB1R	AGTGTGAATGCGGCGGTAACGA				
EmbB2F	CTCGCTGGTCACCTATGTGCTG	4247746–4248939	30	62, 40	60
EmbB2R	GGCTGGTTGGGTTTCATCACG				
EmbB3F	TGGACGGCGATTCGGGTTCT	4248813–4249826	30	62, 40	60
EmbB3R	ACTGCGGAGCCTGACGCTATG				

[a] D, length of denaturation at 94°C; A, primer annealing conditions; E, length of extension at 72°C. All PCRs were 32 cycles, were preceded by a denaturation step at 94°C for 5 min, and included a final extension step at 72°C for 7 min.

***Mycobacterium tuberculosis* isolates.** A total of 139 epidemiologically unrelated MDR-TB strains, isolated from 139 patients (98 male; age range, 16 to 83 years; median age, 43.1 years) with pulmonary tuberculosis from 2008 to 2010 were collected. These MDR-TB isolates were obtained from eight provincial tuberculosis hospitals, including Fujian (19 isolates), Guangzhou (12 isolates), Guizhou (17 isolates), Liaoning (15 isolates), Beijing (14 isolates), Shaanxi (18 isolates), Shanghai (21 isolates), and Xizang (23 isolates). H37Rv (ATCC 27294) was used as a reference.

Drug susceptibility testing. The Lowenstein-Jensen (L-J) proportion method (PM), recommended by the World Health Organization (21), was used to perform drug susceptibility testing (DST) with the following critical drug concentrations: 0.2 μg/ml isoniazid (INH), 40 μg/ml rifampin (RIF), and 2.0 μg/ml EMB. The results were read 28 days after inoculation of the media. H37Rv was used as a control with each batch of drug susceptibility testing.

DNA extraction and spoligotyping. Genomic DNA was extracted from *M. tuberculosis* isolates according to the conventional cetyltrimethylammonium bromide (CTAB) method (22). Additionally, all isolates were analyzed by the spoligotyping method as previously described by Kamerbeek et al. (23). The results in binary format were entered in an Excel spreadsheet and compared with the spoligotyping database SpolDB4 (http://www.pasteur-guadeloupe.fr:8081/SITVITDemo/index.jsp).

PCR amplification and sequencing. Expected fragments were amplified using the primers and conditions shown in Table 1. PCR products were sent for sequencing. All sequence data were aligned with the corresponding sequences of published H37Rv data (GenBank accession number NC_000962) using BioEdit software version 7.05.3. All the mutations found were compared with those contained in the TB Drug Resistance Mutation Database (www.tbdreamdb.com) (24) and previous publications (3, 5, 15, 25, 26). Novel mutations were defined as mutations not included in the database and publications. These mutations were verified by retesting PCR and sequencing.

Resolution of discrepant results. Discrepancies between phenotypic susceptibility results and genotypic data were handled with retesting the DST and DNA sequencing. If the retesting results were different from the original data, a third round of testing was completed, with the final result representing two of the three cycles of testing.

Statistical analysis. The chi-square test was used to analyze data with SPSS 16.0 software (SPSS, Inc.). Differences were considered statistically significant at a P value of <0.05.

Nucleotide sequence accession numbers. The sequences containing novel mutations obtained in the present study were deposited in the GenBank database under the following accession numbers: KP337627, KP337626, KP337625, KP337628, and KP337629 for *embC* Val42Ala, Pro150Ser, Val417Met, Gln725Arg, and Val885Met mutants, respectively; KP337631 and KP337639, KP337633, KP337630, KP337636, KP337637, KP337638, KP337635, KP337632, KP337640, and KP337634 for G(−43)C, Gly154Ser, Thr270Pro, Ala291Thr, Ala331Thr, Gly554Asp, Thr652Lys, Pro763Ser, Pro769Thr, and Glu951Asp mutants, respectively; KP337624 and KP337617, KP337621, KP337623, KP337618, KP337619, KP337620, and KP337622 for *embB* Val50Ala, Ser412Pro, Gly603Arg, Thr642Ala, Thr643Ile, Ala679Thr, and Pro907Ser mutants, respectively.

RESULTS

EMB-resistant phenotype and spoligotype. Among 139 MDR-TB isolates, 79 isolates (56.8%) displayed EMB resistance, and the remaining 60 isolates (43.2%) were EMB susceptible. The Beijing family (114 isolates, 82.0%) was the most prevalent among all MDR-TB isolates. Isolates from the T family (11 isolates; 7.9%), H family (4 isolates; 2.9%), MANU2 family (1 isolate; 0.7%), CAS family (1 isolate; 0.7%), and orphans (8 isolates; 5.8%) were also identified in this study. Most of the EMB-resistant isolates (65/79 isolates; 82.2%) also belonged to the Beijing family. There was no association between EMB resistance and the Beijing family ($P = 0.93$).

Overall polymorphisms in *embCAB*. The entire *embCAB* operon (including the *embC* to *embA* intergenic region and entire open reading frame of *embC*, *embA*, and *embB*) of 139 MDR-TB isolates was screened. Overall, 18 sites with polymorphic synonymous substitutions presented in all isolates. Since these changes could not contribute to amino acid replacements, they are unlikely to participate in drug resistance. Thus, they were not considered further when describing the sequencing results of the *embCAB* region. (A list of 18 synonymous nucleotide changes is available by request from K. Wan)

Also, 35 codons in *embCAB* carrying nonsynonymous substitutions and 5 polymorphic nucleotide sites in the *embA* upstream region (UR) were identified (Table 2). Out of these, 19 variant

TABLE 2 Mutations in *embCAB* among 139 MDR-TB isolates

Mutations in:			No. of isolates	
embC	*embA*	*embB*	EMB resistant ($n = 79$)	EMB susceptible ($n = 60$)
Arg738Gln			0	1
	C(−16)A		0	1
	G(−43)C[a]		1	0
	Gly154Ser[a]		0	1
	Thr270Pro[a]		0	1
	Ala291Thr[a]		0	1
	Gly554Asp[a]		1	0
Val417Met[a]	Thr652Lys[a]		0	1
	Pro763Ser[a]		0	1
	C(−16)A	Val50Ala[a]/Gln497Pro	1	0
	C(−16)G	Val50Ala[a]/Gln497Lys	1	0
	C(−16)G	Met306Ile	1	0
	C(−16)T	Met306Ile	1	0
		Met306Ile/Asp354Ala	1	0
		Met306Ile/Gly406Ser	1	0
		Met306Ile/Thr643Ile[a]	1	0
		Met306Ile	14	5
	C(−16)T	Met306Leu	1	0
		Met306Leu	2	2
		Met306Val/Ile563Leu	0	1
		Met306Val/Pro907Ser[a]	0	1
		Met306Val/Asp1024Asn	1	0
	C(−12)T	Met306Val	1	0
Pro150Ser[a]	C(−12)T/Ala331Thr[a]	Met306Val	1	0
	C(−16)T	Met306Val	1	0
	Asp4Asn	Met306Val	1	0
	Pro769Thr[a]	Met306Val	1	0
Gln725Arg[a]		Met306Val	1	0
Val885Met[a]		Met306Val	0	1
		Met306Val	21	5
		Tyr319Asp	1	0
	C(−11)A	Tyr319Cys	1	0
		Tyr319Cys	1	0
		Asp328Tyr	1	0
	C(−8)T/Gly200Ser	Asp354Asn/Ala679Thr[a]	1	0
	C(−12)T	Asp354Ala	1	0
		Asp354Ala	0	1
		Asp354Asn	0	1
	G(−43)C[a]	Asn399Thr	1	0
		Gly406Ala	4	2
	Glu951Asp[a]	Gly406Asp	0	1
		Gly406Asp	2	2
	C(−16)A	Gly406Ser	1	0
		Gly406Ser	2	0
		Ser412Pro[a]	1	0
Val42Ala[a]		Gln497Arg	0	1
		Gln497Arg	1	1
		Gln497Lys	1	1
		Ile563Leu	0	1
	C(−16)G	Gly603Arg[a]	0	1
		Thr642Ala[a]/His1002Arg	1	0
		His1002Arg	1	0
		Asp1024Asn	1	0

[a] Mutation not previously reported.

TABLE 3 Summary of sequence analysis of *embCAB* and phenotypic drug susceptibility testing

Locus or codon[a]	No. of MDR-TB isolates				*P* value	Sensitivity[b] (%)	Specificity[c] (%)	Accuracy[d] (%)
	Resistant		Susceptible					
	With mutation	Without mutation	With mutation	Without mutation				
embC	2	77	4	56	0.44	2.5	93.3	41.7
embA UR	14	65	2	58	0.01[e]	17.7	96.7	51.8
embA(−16)	7	72	2	58	0.34	8.9	96.7	46.8
embA ORF	5	74	6	54	0.63	6.3	90.0	42.4
embB	74	5	27	33	0.00[f]	93.7	55.0	77.0
*embB*306–497	71	8	25	35	0.00[f]	89.9	58.3	76.3
*embB*306	50	29	15	45	0.00[f]	63.3	75.0	68.3
*embB*406	10	69	5	55	0.42	12.7	91.7	46.8
*embB*497	4	75	3	57	1.00	5.1	95.0	43.9
embB and/or *embA* UR	75	4	28	32	0.00[f]	94.9	53.3	77.0
*embB*306–497 and/or *embA* UR	72	7	26	34	0.00[f]	91.1	56.7	76.3

[a] UR, upstream region; ORF, open reading frame.
[b] Sensitivity = no. of resistant isolates with mutation/total no. of resistant isolates.
[c] Specificity = no. of susceptible isolates without mutation/total no. of susceptible isolates.
[d] Accuracy = (no. of resistant isolates with mutation + no. of susceptible isolates without mutation)/(total no. of resistant isolates + total no. of susceptible isolates).
[e] $P < 0.05$.
[f] $P < 0.01$.

codons and 4 mutated sites in the *embA* UR were uniquely present in the EMB-resistant isolates (Table 2). Overall, 110 MDR-TB isolates (79.1%) had at least one mutation in the *embCAB* operon, of which 76 (96.2%; 76/79 EMB-resistant isolates) were EMB-resistant isolates and 34 (56.7%; 34/60 EMB-susceptible isolates) were EMB-susceptible isolates. Only 3 EMB-resistant isolates did not show any mutations across the entire operon. In the following sections, the mutations observed in EMB-resistant isolates exclusively and the mutations detected in both EMB-resistant and -susceptible isolates are presented in detail (Table 2).

Mutations in *embC*. Six MDR-TB isolates had variable mutations within the *embC* gene (Table 2), including Val42Ala, Pro150Ser, Val417Met, Gln725Arg, Arg738Gln, and Val885Met. A single Arg738Gln replacement was detected in 1 EMB-susceptible isolate. The other five mutations were combined with additional mutations within *embA* and/or *embB*, of which Pro150Ser and Gln725Arg occurred in EMB-resistant isolates. Mutations within *embC* revealed no statistical correlation with EMB resistance ($P = 0.44$).

Mutations in *embA*. For *embA*, both the UR (i.e., the *embC* to *embA* intergenic region) and the entire open reading frame (ORF) were analyzed. Fourteen EMB-resistant isolates displayed nucleotide substitutions in the *embA* UR (Table 2). The most common nucleotide changes were located at position −16 bp, which was detected in 7 EMB-resistant isolates. However, this mutation was also present in 2 EMB-susceptible isolates. The other nucleotide changes found in EMB-resistant isolates occurred at positions −8, −11, −12, and −43 bp. Notably, in one EMB-resistant isolate harboring a nucleotide change at −43 bp, no other mutation in *embCAB* was detected.

Mutations within the ORF of *embA* were observed in 5 EMB-resistant isolates and 6 EMB-susceptible isolates (Table 2). Among 5 EMB-resistant isolates, only 1 isolate had a single Gly554Asp mutation in *embA*. The remaining 4 isolates carried other mutations in *embC* and/or *embB*, of which 2 also harbored mutations in the UR of *embA*. Furthermore, mutations in the *embA* UR were found to be associated with EMB resistance ($P = 0.01$), whereas mutations in the *embA* ORF demonstrated no association with EMB resistance ($P = 0.63$).

Mutations in *embB*. A total of 29 *embB* mutated types were detected in 18 distinct codons among 101 (72.7%) isolates tested, including 74 (93.7%) EMB-resistant isolates and 27 (45.0%) EMB-susceptible isolates (Table 2). The majority of *embB* mutations were observed in the region between codons 306 and 497. Mutations in this region showed strong correlations with EMB resistance ($P = 0.00$).

An amino acid replacement at codon 306 was the most prevalent and occurred in 74 MDR-TB isolates (53.2%). The proportion of *embB*306 mutants in EMB-resistant isolates (50/79 isolates; 63.3%) was >2 times that in EMB-susceptible isolates (15/60 isolates; 25.0%). Statistical analysis indicated that there was a significant difference in the mutation frequency of *embB*306 between EMB-resistant and EMB-susceptible isolates ($P = 0.00$). The next most predominant mutated codons were *embB*406 and *embB*497. Mutations in *embB*406 were found in 10 of 79 EMB-resistant isolates (12.7%) and 5 of 60 EMB-susceptible isolates (8.3%). Four of 79 EMB-resistant isolates (5.1%) and 3 of 60 EMB-susceptible isolates (5.0%) harbored *embB*497 mutations. There was no statistical difference in the mutation rate of these two codons between EMB-resistant and EMB-susceptible isolates ($P_{406} = 0.42$ and $P_{497} = 1.00$).

Other mutations were also detected in codons 50, 319, 328, 354, 399, 412, 534, 563, 603, 642, 643, 679, 907, 1002, and 1024 (for a detailed summary of mutations, see Table 2). Among them, a single mutation at codon 319, 328, 412, 1002, or 1024 was found exclusively in EMB-resistant isolates. To our knowledge, codon 412 has not been described previously.

Predicting EMB resistance based on *embCAB*. With reference to the phenotypic data, the sensitivities, specificities, and accuracies for detecting EMB resistance using DNA sequencing based on analysis of different codons, regions, and genes within *embCAB* are summarized in Table 3. In our study, screens of *embB* and *embB*306–497 achieved the best prediction accuracy. Inclusion of

the *embA* UR did not increase the prediction accuracy (Table 3). Sequence analysis of *embB*306 predicted for EMB resistance with 63.3% sensitivity, 75.0% specificity, and 68.3% accuracy.

DISCUSSION

In our study, 56.8% of MDR-TB isolates were resistant to EMB, and 82.2% of EMB-resistant isolates belonged to the Beijing family, consistent with data from Henan, China (5). No correlation between EMB resistance and the Beijing family was observed ($P = 0.93$). A total of 110 (79.1%) MDR-TB isolates were found to harbor mutation(s) in the *embCAB* operon.

It was reported that the EMB resistance phenotype correlated with the mutations in the *embCAB* operon, most prevalently in the *embB* gene (15, 27). We identified 76 EMB-resistant isolates (96.2%) with mutations in *embCAB*, of which 74 isolates (93.7%) had mutations in *embB*. We also found that the overall mutated rate in the *embB* gene among all MDR-TB isolates was 72.7% (101/139 isolates), supporting the idea that *embB* mutation was associated with multidrug resistance (5, 19).

It was notable that among 74 EMB-resistant isolates with mutations in *embB*, 21 isolates harbored at least one additional mutation in *embCBA*. Some reports suggested that the isolates with mutation combinations presented higher-level EMB resistance (14, 25, 26). Nevertheless, the relevance of these mutations in EMB resistance was unclear and deserved further investigation.

The majority of *embB* mutations were concentrated in a small fragment (codons 306 to 497). Of them, *emb*B306 was the most frequent mutation, with a mutated rate of 47% to 70.6% (4, 5, 12, 13, 17, 18, 28). Mutations in *emb*B306 were also detected in EMB-susceptible isolates. In some studies, the frequencies of those mutations among EMB-susceptible isolates even approached those of EMB-resistant isolates (19, 29). Accordingly, our findings revealed that *embB*306 mutations were predominant (46.8% of all MDR-TB isolates) and occurred at a higher frequency in EMB-resistant than in EMB-susceptible isolates (63.3% versus 25.0%). The correlation between *embB*306 mutations and EMB resistance was statistically significant, suggesting that *embB*306 was associated with EMB resistance (5, 28).

Other major *embB* mutations were in *embB*406 and *embB*497. Despite some reports revealing that among EMB-resistant isolates, the percentage of *embB*406 mutants is significantly lower than that of *embB*497 (3, 5, 10, 15, 27, 30), other studies and our results were in contrast to this view (13, 26, 31). This difference is probably due to regional variations in the mutated frequencies at these two codons in the EMB-resistant isolates. Interestingly, mutations in *embB*406 and *embB*497 were also detected in EMB-susceptible isolates, which was similar to the prior investigations (3, 5, 10, 13, 20, 26). There was no association between mutations at codons 406 and 497 and EMB resistance.

Despite the fact that 19 EMB-resistant isolates (24.1%) carried mutations in *embC* and/or *embA*, 17 isolates had additional mutations in *embB*, implying that mutations in the first two genes are not necessary for EMB resistance. Among these 19 isolates, the majority of mutations were in the *embA* upstream region, including −43 (2 isolates), −16 (7 isolates), −12 (3 isolates), −11 (1 isolate), and −8 (1 isolate) bp. Statistical analysis indicated that *embA* UR mutations were associated with EMB resistance, consistent with the data from east China (26). Nevertheless, we observed that the proportion of isolates with a single *embA* UR mutation to isolates with all *embA* UR mutations was 7.1% (1/14 isolates), significantly lower than the earlier findings (55.6% to 71.0%) (15, 26). Considering that mutations in both the *embA* UR and *embB* were related to high-level EMB resistance (26), we determined that the number of EMB-resistant isolates with high-level resistance used in our study was relatively large.

Many novel mutations were detected among EMB-resistant isolates in this study. Since most cases were accompanied by other mutations known to confer drug resistance, it was uncertain whether these mutations were involved in EMB resistance. Interestingly, the novel mutations *embA*G(−43)C, Gly554Asp, and *embB* Ser412Pro led to EMB resistance with no additional mutations. Their actual roles in the development of EMB resistance require further exploration. There were still 3 EMB-resistant isolates with wild-type *embCAB* sequences, implying that resistance in these isolates may be attributed to mutations in other genes, such as *embR*, *iniA*, *iniB*, and *rmlD* (14).

Although 94.9% of EMB-resistant isolates among MDR-TB isolates were detected with DNA sequencing of *embB* and *embA* UR, the addition of the *embA* UR in the molecular diagnosis decreased the testing specificity from 55.0% to 53.3%. Furthermore, the *embB* gene was long and not very convenient for amplification and sequencing. Thus, the best strategy at present for molecular diagnostics was selective targeting of *embB*306–497, with 89.9% sensitivity, 58.3% specificity, and 76.3% accuracy. Mutations at *embB*306 could detect EMB resistance with 63.3% sensitivity, 75.0% specificity, and 68.3% accuracy. As the sensitivity and specificity of *embB*306 were not particularly high, its effects on identifying EMB resistance were limited.

Our research had several limitations. (i) The MIC values of all MDR-TB isolates in this study were not determined. (ii) Although a large number of MDR-TB isolates ($n = 139$) was investigated, the number of EMB-resistant isolates was relatively limited ($n = 79$). (iii) Only MDR-TB isolates were referred in our study, and other types of resistant and pan-susceptible isolates were not included. (iv) Several EMB-resistant isolates could not be explained by the mutations within *embCAB*.

To summarize, our results indicate that >50% of MDR-TB isolates in China demonstrate EMB resistance, and most EMB-resistant isolates belong to the Beijing family. Mutations in the *embB* gene and *embA* upstream region, other than in *embA* and *embC*, revealed significant correlations with EMB resistance. Compared with phenotypic data, sequence analysis of *embB*306–497 showed a satisfactory sensitivity for the detection of EMB resistance in China. The most common mutations occurred at *embB*306, *embB*406, *embA*(−16), and *embB*497. However, the *embB*306 mutation had limited value as a reliable marker for prediction of EMB resistance in China. Three novel mutations conferring EMB resistance [*embA*G(−43)C, Gly554Asp, and *embB* Ser412Pro] were identified. These findings will expand current knowledge of EMB resistance and further promote the establishment of rapid diagnostic methods to be implemented in China.

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