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Molecular epidemiology of tuberculosis in rural Matlab, Bangladesh

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SUMMARY

OBJECTIVE: To characterise and classify clinical isolates collected from tuberculosis (TB) patients in rural Bangladesh and to investigate the mode of transmission.

DESIGN: An epidemiological study using a combination of conventional and molecular methods was performed in a rural population of Bangladesh. A total of 168 clinical isolates were collected from TB patients. Deletion analysis, used for rapid differentiation of members of the *Mycobacterium tuberculosis* complex, spoligotyping and variable number tandem repeats of mycobacterial interspersed repetitive units (VNTR-MIRU) typing were used.

RESULTS: Deletion analysis identified all isolates as *M. tuberculosis* and further divided them into 109 strains (65%) carrying the *M. tuberculosis* deletion region 1 (TbD1-intact or 'ancestral' strains) and 59 strains (35%) lacking this region (ΔTbD1 or 'modern' strains). MIRU analyses showed that 149 strains (89%) had unique patterns, whereas 19 strains (11%) clustered into eight groups. The largest cluster comprised five ΔTbD1 strains of the Beijing type. The rate of recent transmission was estimated to be 6.5%.

CONCLUSIONS: Our results suggest that TB in rural Bangladesh is caused primarily by reactivation of latent infections involving TbD1 intact strains, overlaid with the recent emergence of Beijing strain clusters that include multidrug-resistant isolates.

KEY WORDS: tuberculosis; molecular epidemiology; Bangladesh

A COMBINATION of molecular biology and conventional epidemiology, molecular epidemiology has been used to provide novel information about the spread of tubercle bacilli in mini-epidemics and outbreaks, analyse the transmission dynamics of tuberculosis (TB) and determine the risk factors for TB transmission in a community. It is also being used to track the geographic distribution and spread of clones of *Mycobacterium tuberculosis* of public health importance.

The substantial diversity of DNA patterns among the study population suggests that the chance occurrence of identical molecular characteristics among unrelated cases would be unusual. Cases of TB that are caused by strains with indistinguishable molecular characteristics are more likely to be due to recently transmitted disease, whereas cases caused by strains with unique fingerprints are typically due to reactivation of infection.

The most frequently used genotyping methods for *M. tuberculosis* are restriction fragment-length polymorphism (RFLP), which targets the insertion sequence (IS) *6110* transposable element, and spoligotyping. RFLP analysis using IS*6110* as a probe has long been considered the gold standard.[1] However, this method requires large amounts of extracted high-quality DNA from each strain and has poor discriminatory power for isolates with <6 copies of IS*6110*. In contrast, spoligotyping targets spacer sequences between repetitive elements in the so-called 'clustered regularly interspaced short palindromic repeats' (CRISPR) or 'direct repeat locus', using polymerase chain reaction (PCR) and reverse dot-blot analysis.[2,3] This method is easier, cheaper and faster, but has less discriminatory power than IS*6110*.[4] An alternative PCR-based technique targets 12 loci containing variable number tandem repeats (VNTRs) of genetic elements named mycobacterial interspersed repetitive units (MIRUs).[5,6] This method has a discriminatory power close to that of IS*6110* RFLP analysis.[7,8] Deletion analysis, another PCR-based technique, has been shown to be effective in differentiating members of the *M. tuberculosis* complex.[9] This technique is based

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on the presence or absence of 20 variable regions in the genomes of members of the *M. tuberculosis* complex, including regions of difference (RD) that differ between members of the *M. tuberculosis* complex and an *M. tuberculosis* deletion region (TbD1), which discriminates between phylogenetically 'ancestral' TbD1-intact and 'modern' ΔTbD1 strains.[9,10]

Although TB is endemic and highly prevalent in Bangladesh, molecular and epidemiological data on the mode of transmission of TB are scarce in this country. The objectives of the present study were to characterise and classify the clinical isolates prevailing in rural Bangladesh and to investigate the mode of transmission.

The study also aimed to investigate to what extent TbD1-intact *M. tuberculosis* strains contributed to TB disease in a rural low-income community of Bangladesh with a high TB burden, and which might have been less influenced by the global emergence of certain epidemic genotypes of ΔTbD1 *M. tuberculosis* strains, as this is more often the case for highly dynamic urban areas.[11]

METHODS

Study population and data collection

This study was conducted as a part of a field study on the epidemiology and surveillance of multidrug-resistant TB (MDR-TB).[12] The study site was in rural Bangladesh in the Matlab area. The International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B) has been maintaining a Health and Demographic Surveillance System (HDSS) which consists of regular cross-sectional censuses and longitudinal registration of important events.[12] The population of the HDSS was about 220 000. A Maternal, Child Health & Family Planning Programme (MCH-FP) has been in operation, involving a population of 106 000 in the HDSS area, since 1978.

Among the 106 000 study subjects in the MCH-FP intervention area, the TB surveillance system was started in all the available population (n = 66 949) aged ⩾15 years in July 2001 and continues today. Female community health workers visited all households every month in the MCH-FP intervention area and asked if any member in the household aged ⩾15 years had had symptoms of cough for >3 weeks. Individuals with cough for >3 weeks were identified as TB suspects. The study staff interviewed all suspects and collected socio-demographic information, including history of any previous episodes of anti-tuberculosis treatment. Sputum samples from each TB suspect were collected for acid-fast bacilli (AFB) smear microscopy, which was performed at the Matlab government *Upazila* Health Complex. All AFB smear-positive samples were routinely transported to the Tuberculosis Laboratory of the ICDDR,B in Dhaka for culture and drug susceptibility testing (DST).

The study protocol was reviewed and approved by the Ethics Review Committee of the ICDDR,B.

Drug susceptibility testing

DST results were obtained from the larger surveillance study; the standard proportion method was used for DST.[12]

Genomic DNA and deletion analysis

Genomic DNA was obtained by resuspending mycobacterial colonies in 100–200 μl of distilled H_2O and incubating at 85°C for 30 min. PCR analysis was performed using the same primers and methods as described previously.[9] Sequences inside or flanking the RD9 and TbD1 regions were obtained from the following web sites: http://genolist.pasteur.fr/tuberculist/ and http://www.sanger.ac.uk/projects/m_bovis/. Primers were designed by using the primer3 web site http://www-genome.wi.mit.edu/cgi-bin/primer3-www.cgi, which would amplify ~500 bp fragments. PCR was performed on a PTC-200 amplifier (MJ Research, Inc., Watertown, MA, USA) and run on agarose gel.

Spoligotyping

Spoligotyping was performed as previously described by Kamerbeek et al.,[2] with minor modifications. Mycobacterial genomic DNA was extracted from cultured cells, as described previously.[13,14] PCR, hybridisation and detection were performed as described previously.[15]

PCR and MIRU analysis

PCR and calculation of MIRU copy number per locus were carried out as described previously.[6,7,15] All isolates were typed using 11 loci (2, 4, 10, 16, 23, 24, 26, 27, 31, 39 and 40) VNTR-MIRU typing. Most of the strains failed to give a PCR product with primers for the MIRU 20 locus, as seen in our previous study;[15] therefore, the results of MIRU 20 locus were excluded from this study. The rate of recent transmission was calculated by the formula:

$$[T(\mathrm{c}) - N(\mathrm{c})]/T(\mathrm{a}),$$

where T(c) is the total number of clustered isolates, N(c) is the number of clusters and T(a) is the total number of isolates.[16]

Epidemiological investigation

A cluster was defined as two or more isolates from different patients with identical spoligotype and MIRU patterns, whereas non-clustered patterns were referred to as unique. Clustered patients were investigated by study staff using a standardised questionnaire to further establish or strengthen potential epidemiological connections in place, time and person among cluster members. Responses from patients were rechecked by the supervisor in 10% of cases to assess their validity. Participants were considered to share an apparent

epidemiological link if they had been in the same workplace, household, village or area at overlapping times.

Analysis

Data were entered using the software package SPSS 17.0 (Statistical Package for the Social Sciences Inc, Chicago, IL, USA) and checked for errors. Pearson's χ^2 test was used to determine statistical associations between strain types and drug resistance patterns with SPSS software. $P < 0.05$ was considered as evidence of significant difference.

RESULTS

Continuous surveillance in the intervention area between June 2001 and 2007 identified a total of 227 smear-positive pulmonary TB cases. Of the 227 sputum samples, 194 were available for culture at the ICDDR,B TB laboratory. Of these 194 samples, 168 (87%) were positive on culture and 14 (7%) were culture-negative. The remaining 12 (6%) were contaminated and were unable to yield valid culture results. Results from a total of 168 isolates (74% of the total cases identified) were thus included in the current study.

Patient characteristics

Data on age and sex were available for 159 patients. Among these, 133 (84%) were male and 26 (16%) were female. The age range of the patients was 15–91 years, and the mean age was 47 years. Patients in the group aged 46 to 60 years had the highest proportion (30%) of isolates, followed by those aged ⩾60 years, with 26% of the isolates. Overall, there was not much difference between the proportions of clustered and unique cases among the different age groups. However, clustering was seen to be higher among the older age group (Table 1).

Deletion analysis

The presence of the RD9 region, which is strictly conserved in *M. tuberculosis,* in all 168 tested strains confirmed that the isolates were *M. tuberculosis*. Presence of the TbD1 region was observed in 109 strains (65%), indicating that these strains belonged to the 'ancestral' or TbD1-intact *M. tuberculosis* lineages, while only 59 (35%) strains had the TbD1 region deleted, thus belonging to the ΔTbD1 or 'modern' type.

Table 1 VNTR-MIRU typing clusters in different age groups

Age group, years	Isolates *n* (%)
⩽30	3 (17)
31–45	2 (11)
46–60	5 (28)
>60	8 (44)

VNTR-MIRU = variable number tandem repeats of mycobacterial interspersed repetitive units.

Spoligotyping

All 168 isolates were spoligotyped. This analysis revealed 91 different patterns, 101 (60%) of which were grouped into 24 clusters; 25% of the isolates were of East African Indian (EAI) type,[17] which corresponds to the ancestral TbD1+ *M. tuberculosis* type,[9] also named Indo-Oceanic cluster.[18] There were nine different clusters among the EAI type strains, including EAI6_BGD1, EAI, EAI3-IND, EAI5, EAI7_BGD2 and EAI-1-SOM types. The most predominant of the EAI type was the EAI6_BGD1 or 'Matlab' type, consisting of 13 isolates, which has been described previously.[19] The largest cluster comprised 26 strains belonging to the Beijing family. The remaining 14 clusters contained 39 strains, with the number of strains in each cluster ranging from 2 to 6. Other clusters included the Central Asian strain type (CAS, CAS1 or Delhi), LAM family, U-family and T-family strains (Table 2).[20]

MIRU typing

Using MIRU typing, only 19/168 (11%) strains could be grouped into eight clusters. Among the eight clusters, the largest cluster consisted of five isolates, all of which belonged to the Beijing family. The remaining seven clusters (14 isolates) consisted of two isolates. All the above 14 isolates were of 'ancestral' type; 149 (89%) exhibited unique (non-clustered) patterns. The 26 Beijing strains revealed by spoligotyping could be classified into 22 distinct types using VNTR-MIRU typing. The calculated rate of recent transmission was 6.5%, where $T(c) = 19$, $N(c) = 8$ and $T(a) = 168$. Patients with isolates that had similar MIRU patterns had medical and epidemiological data reviewed to identify any epidemiological links. No apparent epidemiological connections were found. All the cases of each clustered group were from different villages and of different occupations (Figure).

Drug resistance patterns

DST results were available for 163 isolates; 68 patients were infected with resistant isolates. Among the 68 isolates, 10 (6%) were resistant to all four drugs (i.e., MDR-TB) and overall resistance to streptomycin, isoniazid, rifampicin and ethambutol were respectively 60 (37%), 20 (12%), 16 (10%) and 18 (11%). There was no association between presence of clustering and drug resistance. However, drug resistance was significantly higher among the ΔTbD1 strains than the 'ancestral' TbD1 intact strains (Table 3). A strong association was observed between the Beijing genotype and drug resistance (Table 4). The proportion of MDR-TB was about 24% ($n = 5$) in the Beijing group, compared to only about 3% ($n = 4$) in the non-Beijing strains (Table 4).

DISCUSSION

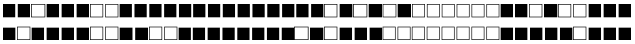
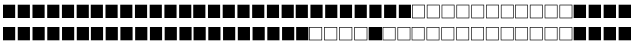
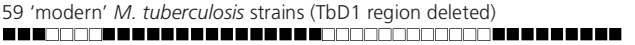
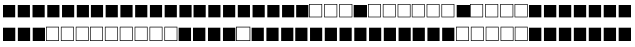
The objectives of the study were to characterise those strains that caused TB in a rural community of

Table 2 Clusters and unique *M. tuberculosis* strains determined by various typing methods and distribution of spoligotyping-defined phylogenetic clades*

n†	Spoligotype pattern of isolate(s)	Phylogenetic clade of isolate(s)‡	VNTR-MIRU type(s) associated with the spoligotype§
	109 'ancestral' *M. tuberculosis* strains (TbD1 region present)		
1			2545x7323613
1		EAI5 (236)	2543x6223314
2			2243x8223613, 2443x6221632
2		EAI6_BGD1 (591)	2445x6351533, 2446x8223523
4		EAI6_BGD1 (882)	2542x7223532, 2543x7221531, 2544x7324521, 2640x7274512
1			2543x7223522
2			2333x5221533, 2543x7213422
1			2543x3243622
1			2543x3223522
1			2743x7223531
1		EAI5 (1427)	2545x6325323
1			2543x7213521
3		EAI1_SOM (48)	1543x7213513, 15437x325513, 2544x8213313
1		EAI1_SOM (1251)	2543x8223513
1		EAI1_SOM (349)	2544x8223513
1			2543x7211313
1			2544x7222423
3		EAI5 (138)	2263x6323513, 2543x6223513, 2564x6223513,
1			2443x6223313
2		EAI6_BGD1 (1409)	2443x7332422, 2445x6322422
13		EAI6_BGD1 (292)	2333x5221523, 2333x5221624, 2343x6223225 (2 strains), 2343x6223313, 2344x6323323, 2432x6351532, 2443x6212312, 2443x6221521, 2443x6221632, 2543x3274512, 2643x2374242, 2643x7223432
1			2443x2254222
3			2443x6221533, 2443x6221534, 2443x6223533
1			2443x6233533
1			2444x7221523
1			2443x6325621
1			2453x6322642
1			2643x1324532
1			2443x6221732
1			2443x5221533
1			2653x7321624
1			2443x6212212
1		EAI (1400)	2443x5221732
1			2244x6213424
5		EAI3_IND (11)	2531x6223536, 2533x2463142, 2543x6223424, 2543x6223424, 2543x7223434
1		EAI3_IND (298)	2543x8223434
1			2443x6221332
1			2443x6221635
1			2744x6223624
2			2233x5163542, 2343x6223223
1		U (629)	2543x6223334
1		U (458)	2243x5124412
1			2543x7223532
1			2443x7333542
1			2543x7223312
1			2543x7233432
1			2543x7243532
1			2643x2374242
1			2543x8223432
1			2633x7233542
1		U (1421)	2543x1221332
1			2542x6353634
2		U (1612)	2543x6321513, 2443x7221433
2			2443x6221532, 2443x6222523
2		EAI7_BGD2 (96)	2442x2367222
4			2343x6223223, 2442x6221533, 2442x6222514, 2553x6321423

(*continued*)

Table 2 (*Continued*)

n†	Spoligotype pattern of isolate(s)	Phylogenetic clade of isolate(s)‡	VNTR-MIRU type(s) associated with the spoligotype§
	109 'ancestral' strains (*continued*)		
2			2444x6221525, 2643x6221514
1			2444x6322523
1			2443x6352534
1			2443x6221212
2		EAI (1391)	2443x6221513, 2445x2364222
1			2444x6231502
1			2543x8231511
1			2553x5321425
2			2442x6221523, 2443x6222613
1			2443x6212313
1			2443x5121534
1			2433x5221232
1			2442x6221523
1			2543x6324513
	59 'modern' *M. tuberculosis* strains (TbD1 region deleted)		
6		CASI_DELHI (26)	2234x5183525, 2244x4354146, 2254x5123513, 2254x5183423, 2264x5173432, 2276x5273544
2		CAS (1093)	2253x3454145, 2254x5173534
1		CASI_DELHI (754)	2264x5163533
1		CASI_DELHI (141)	2264x5163534
1		CAS (22)	2265x5173523
26		Beijing (1)	2213x5183523, 2223x5153522, 2223x5153532, 2223x6354241, 2232x5153533, 2233x4353141, 2233x4354241, 2233x5143532, 2233x5153422, 2233x5153532(5 strains), 2233x5153513 2233x5163232, 2233x5363242, 2234x5163613, 2243x5224434, 2243x5254333, 2244x5124523, 2244x5173533, 2244x5221523, 2244x5263642, 2244x5263643, 2343x6263223
1			2234x5162514
3		T1 (53)	2231x5153322, 2240x6152334, 2244x6262324
5		LAM9 (42)	2242x6143321, 2242x6153521, 2243x6161311, 2255x5174512, 2262x6023321
1		LAM9 (150)	2242x6153321
1		LAM3 (33)	2242x6153213
1		H1 (283)	2254x5154323
1			2233x5163335
2			2243x5153433, 2253x5264333
1		CAS (357)	2255x4167313
1		T1 (823)	2031x5252224
1			2231x6153322
1			2265x6334531
1			2242x5023332
1			2333x5131324
1			2343x5133324

*■ = spacer present in the DR region; □ = spacer deleted.
†Number of isolates with the same spoligotype.
‡Where shown, numbers in parentheses indicate the shared-type number (SIT) of the SpolDB4/SITVIT database; if the corresponding spoligotype was not represented in SpolDB4, the field was left empty.
§Results from MIRU-locus 20 were not included in the VNTR-MIRU type and are marked by 'x'.
VNTR-MIRU = variable number tandem repeats of mycobacterial interspersed repetitive units.

Bangladesh and to investigate its mode of transmission. A low proportion (11%) of our *M. tuberculosis* isolates were clustered, and no apparent epidemiological link was found among the clustered cases. All the clustered cases lived in different locations, their professions were different and they did not have any relationship to each other. The rate of recent transmission was 6.5%, which is low. Even among those cases with identical strains, there was no epidemiological link. Our study suggests that recent transmission in rural Bangladesh plays a lesser role in the occurrence of TB than in many other countries,[21–25] and that most TB in rural Bangladesh is due to reactivation of previous infections with strains common in that geographical area.

Population-based molecular epidemiological studies

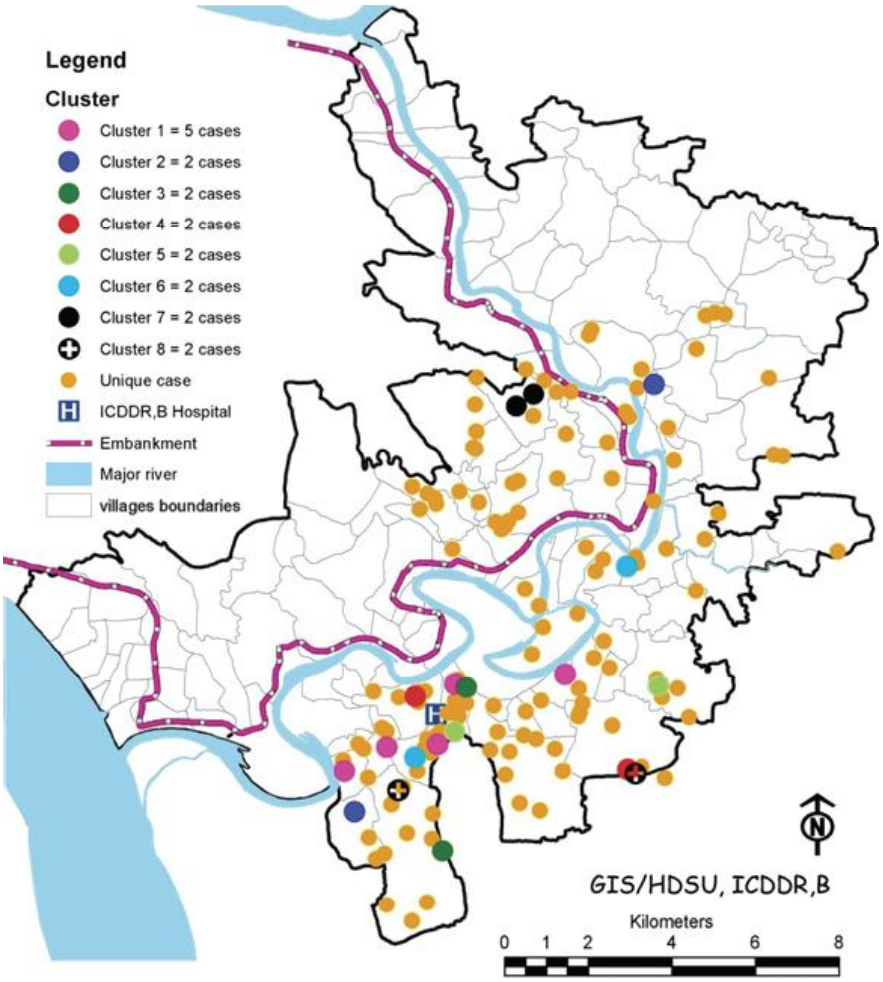


Figure Map of the Matlab area showing the location of unique and clustered strains isolated from tuberculosis cases. GIS = Geographic Information System; HDSU = Health and Demographic Surveillance Unit; ICCDR,B = International Centre for Diarrhoeal Disease Research, Bangladesh.

in San Francisco, New York and Amsterdam have demonstrated rates of recent transmission that were much higher than the estimated 10% predicted by traditional epidemiological studies.[23,25,26] In these studies, the risk factors associated with recent transmission were lower socio-economic condition, native ethnic minority and human immunodeficiency virus/acquired immune-deficiency syndrome (HIV/AIDS). This demonstrates that ongoing transmission of infection contributes to the disease burden at much higher rates than previously thought, and highlights the importance of control efforts in interrupting transmission.

Table 3 Association of antimicrobial resistance with 'ancestral' and 'modern' strains

Resistance pattern	Total (N = 156) n (%)	Ancestral (n = 104) n (%)	Modern (n = 52) n (%)	P value
Susceptible to all drugs	91 (58.3)	67 (64.4)	24 (46.2)	0.002
Total resistance to				
Streptomycin	57 (36.5)	36 (34.6)	21 (40.4)	>0.1
Isoniazid	18 (11.5)	7 (6.7)	11 (21.2)	0.008
Rifampicin	14 (9.0)	5 (4.8)	9 (17.3)	0.016*
Ethambutol	16 (10.3)	6 (5.8)	10 (19.2)	0.009
Multidrug resistance	8 (5.9)	3 (3.3)	5 (11.1)	>0.1*

*Fisher's exact test.

Table 4 Association of antimicrobial resistance with Beijing genotype

Resistance pattern	Total (N = 156) n (%)	Beijing (n = 21) n (%)	Non-Beijing (n = 135) n (%)	P value
Susceptible to all drugs	91 (58.3)	13 (61.9)	78 (57.8)	>0.1
Total resistance to				
Streptomycin	57 (36.5)	5 (23.8)	52 (38.5)	>0.1
Isoniazid	18 (11.5)	5 (23.8)	13 (9.6)	0.07*
Rifampicin	15 (9.6)	6 (28.6)	9 (6.7)	0.007*
Ethambutol	16 (10.3)	7 (33.3)	9 (6.7)	0.002*
Multidrug resistance	9 (5.8)	5 (23.8)	4 (3.0)	0.002*

*Fisher's exact test.

Population-based studies in other low-incidence countries, such as Norway and Switzerland, showed percentages of clustering that were relatively low (respectively 16% and 17.5%) compared to other studies.[27,28] This low level of recent transmission suggests that TB control was more effective in these settings. In our study, conducted in a rural population, clustering was found to be low, indicating that effective TB control measures are in place. The recently conducted national TB prevalence survey in Bangladesh revealed a prevalence of 79 per 100 000 population, substantially lower than previous estimates.[29]

The study results revealed a high prevalence of TB due to 'ancestral' or EAI strains of *M. tuberculosis* (65%). This distribution of strains is strikingly different from that of European, American and African countries, where TB is mainly caused by the 'modern', ΔTbD1 strains of *M. tuberculosis*. It is also noticeable that in this study, where isolates originate mainly from rural areas, the percentage of 'ancestral' strains was substantially higher than observed previously from samples collected in an urban area of Bangladesh,[15] suggesting that ancestral *M. tuberculosis* strains are endemic and may have a long history in this geographical region. Drug resistance was lower among 'ancestral' strains than among 'modern' strains, indicating that an effective TB control programme would be able to prevent the high incidence of TB in Bangladesh. The second most prevalent strain found in our study was the Beijing genotype, which accounted for about 16% ($n = 26$) of all isolates. In our previous study, performed in an urban hospital, the rate of Beijing strains was about 31%.[15] The strains of the largest cluster by VNTR-MIRU typing, which comprised five strains in this study, were identified as Beijing members. The lack of a direct epidemiological link between Beijing isolates with indistinguishable molecular patterns suggests that this is due to the particularly clonal population structure of Beijing strains that were brought into the study area long ago.

The results of this study suggest that the majority of the TB cases in rural Bangladesh are caused by reactivation of previous TB infection. Drug-susceptible 'ancestral' strains are more prevalent in these areas. With the widely introduced DOTS strategy, transmission of TB is likely to be reduced, as the cure rate is high and the relapse rate is low in this area. To control TB effectively, efforts should be sustained and supplemented by intensive and early case detection of TB in the context of an increasing rate of TB-HIV coinfection globally. However, the emergence of the drug-resistant Beijing genotype of *M. tuberculosis* remains a threat to effective TB control programmes.

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RÉSUMÉ

OBJECTIFS : Caractériser et classifier des isolats cliniques provenant de patients tuberculeux au Bangladesh rural ainsi que l'investigation de leur mode de transmission.

SCHÉMA : On a mené dans une population rurale du Bangladesh une étude épidémiologique utilisant une combinaison des méthodes conventionnelles et moléculaires. Au total, on a recueilli 168 isolats cliniques provenant de patients tuberculeux. On a utilisé l'analyse des délétions pour la différentiation rapide des membres du complexe *Mycobacterium tuberculosis*, le spoligotypage et la répétition en tandem de nombreux variables des unités répétitives mycobactériennes entremélées (VNTR-MIRU).

RÉSULTATS : L'analyse des délétions a identifié tous les isolats comme *M. tuberculosis* et les a répartis ensuite en 109 souches (65%) où la région 1 de délétion de *M. tuberculosis* (TbD1, appelées souches « ancestrales ») était intacte, ainsi que 59 souches (35%) où cette région faisait défaut (souches ΔTbD1, appelées souches « modernes »). Les analyses MIRU ont démontré que les types de 149 souches (89%) étaient uniques, alors que 19 souches (11%) étaient regroupées en huit grappes. La grappe la plus importante comportait cinq souches ΔTbD1 du type Beijing. On a estimé que le taux de transmission récente était de 6,5%.

CONCLUSIONS : Nos résultats suggèrent qu'au Bangladesh rural la tuberculose est principalement causée par la réactivation d'une infection latente impliquant des souches TbD1 intactes auxquelles se superposaient des grappes de souche Beijing récemment apparues et comportant des isolats multirésistants.

RESUMEN

OBJETIVOS: Caracterizar y clasificar los aislados clínicos provenientes de pacientes con tuberculosis (TB) en una zona rural de Bangladesh e investigar el modo de transmisión de la enfermedad.

MÉTODO: Se llevó a cabo un estudio epidemiológico mediante una combinación de métodos convencionales y moleculares en una población rural de Bangladesh. Se recogieron 168 aislados clínicos de pacientes con TB. A fin de diferenciar en forma rápida las cepas del complejo *Mycobacterium tuberculosis*, se utilizó el análisis de las deleciones y luego se practicaron el espoligotipado y la genotipificación con marcadores para locus múltiples de las secuencias repetitivas en tándem (VNTR-MIRU).

RESULTADOS: Se identificaron todos los aislados de *M. tuberculosis* mediante el análisis de las deleciones; estos aislados se dividieron en 109 cepas (65%) portadoras de la región de deleción 1 de *M. tuberculosis* (cepas con TbD1 intacta o 'ancestral') y 59 cepas (35%) en las cuales faltaba esta región (cepas ΔTbD1 o cepas 'modernas'). La genotipificación con MIRU puso en evidencia que 149 cepas (89%) presentaban patrones únicos y 19 cepas (11%) formaban parte de ocho conglomerados. El conglomerado más grande agrupaba cinco cepas ΔTbD1 de la familia Beijing. La tasa de transmisión reciente se calculó en 6,5%.

CONCLUSIÓN: Estos resultados indican que la TB que se observa en la zona rural de Bangladesh proviene sobre todo de la reactivación de infecciones latentes, causadas por cepas portadoras de una región TbD1 intacta, al lado de la aparición reciente de conglomerados de cepas de tipo Beijing, que incluyen aislados multidrogorresistentes.