Transmission of multidrug-resistant and extensively drug-resistant tuberculosis in rural Bangladesh: lessons learnt

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We report community transmission of multidrug-resistant (MDR-) and extensively drug-resistant tuberculosis (XDR-TB) documented by fingerprinting, with secondary cases appearing over a period of 10 years. The index case failed MDR-TB treatment, with amplification to XDR-TB after refusing treatment when first diagnosed and developing pre-XDR-TB on private treatment. Some of the first MDR-TB patients were not started on appropriate treatment due to delayed diagnosis or to excessively rigid application of National TB Programme guidelines. Early presumptive MDR- and XDR-TB diagnosis and removal of barriers, such as obligatory hospitalisation, could have stopped this trend of resistance amplification and transmission.

The Damien Foundation (DF), a non-governmental organisation, covers about 20% of the Bangladeshi population for tuberculosis (TB) control. Approximately 15 000 smear-positive cases are treated annually on behalf of the National Tuberculosis Programme (NTP); however, in Rajshahi District, case detection remains far below the DF average of 51%. Drug resistance has been monitored by means of continuous systematic sampling of retreatment cases since 1996, with confirmation of all multidrug-resistant TB (MDR-TB) at a supranational reference laboratory (SRL). In 2001, MDR-TB prevalence had declined from 2.5% (1995) to 0.7%, all smear-positive cases taken together.^{1,2} Standardised MDR-TB treatment started in 1997, and has had excellent results since the introduction of a short, highly efficient gatifloxacin (GFX) based regimen ('Bangladesh regimen') in 2005.³ TB-HIV (human immunodeficiency virus) co-infection remains very rare in Bangladesh.⁴

A LOCALISED MDR- AND XDR-TB PROBLEM

Among the 765 new smear-positive cases registered from 1998 to 2011 in Charghat, a rural sub-district of Rajshahi District, 16 (2.1%) proven MDR-TB cases were detected, including two cases of primary extensively drug-resistant TB (XDR-TB). Eleven cases, including both XDR-TB cases, came from the Charghat Township Union (population 30 000).

As this was a retrospective study of transmission, and the laboratories and clinics involved had also been responsible for the routine care of these patients, ethical permission was not required. All patient data were anonymised.

The first township patient (patient C in the Table), diagnosed already in 2000, accepted free MDR-TB

treatment only in 2002 after taking various second-line drugs from private providers, with the acquisition of ofloxacin (OFX) resistance. He never converted bacteriologically, failing an OFX-based regimen following the development of secondary XDR-TB, and died in early 2004.

None of the other MDR-TB patients took second-line drugs prior to DF treatment. In 2007, the primary XDR-TB case, J, was promptly diagnosed on rapid drug susceptibility testing (DST) using slides⁵ when she remained smear-positive after 3 months of primotreatment. Despite confirmation of XDR-TB by conventional DST at the SRL, she was allowed to start the Bangladesh MDR-TB regimen only 1 year later after having failed first-line retreatment, as NTP guidelines at that time restricted the administration of second-line drugs to those patients who failed retreatment. Patient J failed MDR-TB treatment without converting, and acquired resistance to ethionamide. As slide DST also revealed pyrazinamide (PZA) resistance,⁶ she was isolated at home with DF support. Compassionate use of bedaquiline (TMC207) was granted in late 2011, with culture conversion after 3 months of individualised treatment based on this drug and linezolid.

The second primary XDR-TB (patient P) was diagnosed by slide DST in 2010 after failing primotreatment. Susceptible at 2 µg/ml GFX, he was promptly started on the unmodified Bangladesh regimen, converted at 5 months, and remains culture-negative 6 months after cure.

Five of the first diagnosed MDR-TB patients died before they could be started on specific treatment due to patient refusal, excessive SRL DST delay or the excessively strict interpretation of NTP indications once local rapid DST became available. Eight patients were cured using the Bangladesh regimen. Only one case was detected in 2010, and none during 2011.

Patients were admitted for initiation of MDR-TB treatment, yet none were hospitalised at the time, and there was no other evidence of nosocomial transmission. However, some were relatives, and primary XDR-TB patient J had been a neighbour of the secondary XDR-TB patient C during the last months of his life. Primary XDR-TB patient P had no history of contact with patients J or C. Clustering was verified by genotyping at the SRL, using spoligotyping and 15-locus mycobacterial interspersed repetitive units-variable number of tandem repeats (MIRU-VNTR) as per standard protocols,^{7,8} in addition to automated DNA sequencing of the *rpoB* gene.⁹ The Figure shows the fingerprinting results of strains that could be recovered, originating from 15 cases, with two clusters. The

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TABLE Patient characteristics and treatment data

Patient code	Sex/age, years	Residence Union	Treatment antecedents [§]	Drug resistance at start of MDR-TB treatment		Year of MDR-TB first diagnosis	Date of MDR-TB treatment start	Current status
				Profile first-/second-line	<i>rpoB</i> mutation			
A	Male/38	Sardah	D3/D2/D2/F2/2EHRZ	HRES	Asp516Phe	1999	March 2002	Cured
B	Female/18	Salua	2HRZ/F2	HRES	Ser531Trp	1999	Patient refused	Died
C*	Male/26	Charghat	12HR/1R/F2/6CSPthRZ/6ES/6KOPth/F2	HRESZ/OEth	Ser531Leu	2000	September 2002	Failed; died 2004
D	Male/28	Charghat	F1/D2	HRES	Failed	2001	Patient refused	Died
E	Male/33	Charghat	F2/F2	HRES/Eth	His526Leu	2004	January 2006	Cured
F	Male/44	Charghat	R1/F2	HRES/Eth	Ser531Leu	2006	May 2006	Cured
G	Male/45	Charghat	F1	HR	Ile572Phe	2006	Not started	Died
H	Male/46	Charghat	F1/F2	HRES	His526Asp	2007	September 2008	Cured
I	Female/48	Charghat	F1	HRES	Ser531Leu	2007	September 2007	Cured
J†	Female/18	Charghat	F1/F2	HRESZ/KO	Ser531Leu	2007	May 2008	Converted on XDR-TB treatment
K	Male/27	Charghat	2EHRZ/F2	HRS	Asp516Phe	2007	April 2007	Cured
L	Female/25	Nimpara	R1	HRES	Ser531Leu	2008	Not started	Died
M	Male/54	Sardah	F1/F2	HRES	Pro564Leu	2008	Not started	Died
N	Male/25	Sardah	R1/R2	HRS	His526Asp	2009	July 2011	Cured
O	Male/46	Sardah	F1/R2	HRES	His526Asp	2009	December 2010	Cured
P‡	Male/40	Charghat	F1	HRESZ/KO	Ser531Leu	2010	September 2010	Cured

*Index case.

†Primary XDR_1.

‡Primary XDR_2.

§Successive episodes are separated by slashes, interruptions are not accounted for. Pyrazinamide was not tested for all patients. Resistance profiles are composed of drug symbols for which resistance was documented. Drug symbols are preceded by the estimated number of months the drug in question was administered.

MDR-TB = multidrug-resistant tuberculosis; D3 = default from WHO Category III regimen; D2 = default from WHO Category II regimen; F2 = NTP, WHO Category II failure; E = ethambutol; H = isoniazid; R = rifampicin; Z = pyrazinamide; S = streptomycin; CS = cycloserine; Pth = prothionamide; K = kanamycin; O = ofloxacin; Eth = ethionamide; F1 = NTP, WHO Category I failure; R1 = relapse after WHO Category I; R2 = relapse after WHO Category II; WHO = World Health Organization; NTP = National TB Programme; XDR = extensively drug-resistant.

spoligotyping patterns were identical for eight patients, but the MIRU-VNTR patterns were identical for only six of these (cluster I), including all XDR-TB. All six shared the same Ser531Leu (TTG) *rpoB* mutation. Three sequential isolates from the secondary XDR-TB case C kept the same fingerprinting patterns during drug resistance amplification from MDR- to pre-XDR- and XDR-TB.

A second cluster with identical spoligotyping and MIRU profiles includes strains from patients N and O from Sardah Union, showing the same His526Asp (GAC) mutation. Patient H's strain,

with the same mutation, differed in only one allele, thus probably representing a very recent clonal variation of cluster II after MDR-TB transmission. Both clusters belonged to the Delhi/CAS family.

DISCUSSION

Our data show that intense transmission of MDR- and XDR-TB is also possible in a rural environment, without HIV, overcrowding or particularly virulent strains. Spoligotyping was not adequately

FIGURE Genotyping results of available strains: dendrogram, MIRU-VNTR and spoligotyping patterns. From right to left are shown the spoligotyping pattern, the MIRU-VNTR pattern and the strain relationship tree. Patients to whom the isolates belong are coded A to P, with year of isolation, phenotypic resistance profile classification as MDR, pre-XDR or XDR-TB, and *rpoB* mutation. Three isolates with identical fingerprints from index case C are shown, evolving from MDR- to XDR-TB with completely preserved fingerprinting patterns. Two closely related clusters with respectively 2–3 (N, O and, possibly H) and 6 patients (C, F, I, J, L and P) can be identified. K and E seem to belong to the larger cluster by spoligotyping pattern, but differ considerably by MIRU-VNTR. MIRU = mycobacterial interspersed repetitive units; VNTR = variable number of tandem repeats; MDR = multidrug-resistant; XDR = extensively drug-resistant; TB = tuberculosis.

discriminating for this investigation, with MIRU-VNTR correcting one quarter as not belonging to spoligotype cluster I. Secondary cases were detected 5–10 years after the index case was diagnosed. This patient refused MDR-TB treatment, presumably because at the time this meant obligatory hospitalisation. After the case had progressed to pre-XDR-TB on private treatment, our OFX regimen proved too weak to prevent the amplification of resistance to XDR-TB. Other MDR-TB patients had died on first-line retreatment by the time the conventional DST result was reported by the SRL. Rapid presumptive diagnosis of MDR-TB, XDR-TB and other mycobacteria by decentralised slide DST facilitated prompt treatment from 2008 onwards. However, an excessively rigid implementation of guidelines at the government TB hospital resulted in proven MDR-TB cases dying before fulfilling the NTP criteria for second-line treatment. Even a patient with known primary XDR-TB had to fail the first-line retreatment regimen before being eligible. Both primary XDR-TB cases were OFX-resistant, but were still susceptible to GFX 2 µg/ml. The standard high-dose GFX Bangladesh regimen may have cured the second case immediately after he failed primo-treatment, but it failed for the first case, possibly due to development of higher level isoniazid and PZA resistance during first-line retreatment.

CONCLUSION

A documented outbreak of MDR- and XDR-TB in rural Bangladesh led to secondary cases appearing over a period of at least 10 years, without any obvious risk factor. It may now be controlled after correction of the obstacles to early and efficient treatment, associated with poor outcome and ongoing transmission: 1) DST at the SRL was replaced by less accurate but rapid, local DST; 2) obligatory hospitalisation during the intensive phase was abolished;

and 3) the high-dose GFX MDR-TB regimen overcame most fluoroquinolone resistance.

Less than 10% of MDR-TB cases worldwide receive appropriate treatment, resulting in ongoing transmission of their strains and increasing incidence of MDR-TB.¹⁰ With the expanded availability of rapid molecular diagnostics for rifampicin resistance, prompt and highly effective second-line treatment has become more crucial than ever before to reverse this trend.

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Nous décrivons la transmission dans la collectivité de la tuberculose à germes multirésistants (TB-MDR) et ultrarésistants (TB-XDR) documentées par les empreintes digitales, ainsi que les cas secondaires apparaissant au cours d'une période de 10 ans. Dans le cas index, on a vu échouer le traitement de la TB-MDR et le passage à une TB-XDR après refus du traitement lors du premier diagnostic et l'apparition d'une pré-TB-XDR au cours d'un traitement dans le secteur privé.

Certains des premiers patients TB-MDR n'ont pas été mis sous traitement approprié en raison des délais du diagnostic ou d'une application trop rigide des directives du Programme national de la tuberculose. Un diagnostic précoce de la suspicion de la TB-MDR ou de la TB-XDR et l'élimination des barrières comme l'hospitalisation obligatoire auraient pu arrêter cette tendance vers l'amplification et la transmission de la résistance.

Se comunica la presencia de transmisión de tuberculosis multidrogo-resistente (TB-MDR) y extremadamente drogoresistente (TB-XDR), confirmada mediante las huellas genéticas, con aparición de casos secundarios en un período de 10 años. El caso inicial de TB-MDR presentó un fracaso terapéutico con progresión hacia una forma TB-XDR, tras haber rechazado el tratamiento en el momento del primer diagnóstico y recibido luego un tratamiento en el sector privado durante el cual la enfermedad evolucionó hacia una TB-pre-XDR. Algunos de los

pacientes que contrajeron la TB-MDR no iniciaron un tratamiento adecuado debido a los retrasos en el diagnóstico o a una aplicación demasiado estricta de las normas del Programa Nacional contra la Tuberculosis. Habría sido posible interrumpir esta tendencia de amplificación de la resistencia y transmisión continua si se hubiese establecido un diagnóstico presuntivo precoz de TB-MDR o -XDR y eliminado los obstáculos como la hospitalización obligatoria.