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CASE REPORT

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Extensively drug resistant tuberculosis in Mali: a case report

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Abstract

Background: Drug resistant tuberculosis presents a major public health challenge.

Case presentation: We present here the first two patients diagnosed with extensively drug resistant tuberculosis in Bamako, Mali. Genotypic findings suggest possible nosocomial transmission from the first patient to the second one, resulting in superinfection of the second patient. After being diagnosed with extensively drug resistant tuberculosis in August 2016, the patients only started receiving appropriate treatment 10 months later.

Conclusion: The identification of these patients highlights the need for improved diagnostic and treatment algorithms for better surveillance and management of drug resistance in Mali. In the interest of these as well as future patients suffering from resistant tuberculosis, all steps recommended for programmatic management of drug resistant tuberculosis must be urgently prioritized in order to strengthen the multidrug resistant tuberculosis program.

Keywords: XDR-TB, Treatment, Bamako, Mali

Background

Multidrug-resistant (MDR) tuberculosis is defined as disease caused by *Mycobacterium tuberculosis* complex strains with resistance to, at least, isoniazid and rifampicin, while extensively drug resistant tuberculosis (XDR-TB) is defined as MDR-TB plus resistance to a fluoroquinolone and to a second-line injectable agent [1]. By 2015, 117 World Health Organization (WHO) member states had reported at least one patient with XDR-TB [1]. Out of the 7579 XDR patients worldwide in 2015, 1100 (14.5%) were notified in the Africa region. Underreporting is higher in this part of the world, likely due to limited availability of drug susceptibility testing (DST), especially to second line drugs [1, 2].

Mali reported in 2015 a TB incidence of 57 per 100,000 population and was estimated to have 3.5% of primary rifampicin resistance (RR) among smear positive new patients [1]. In Mali, after the first GeneXpert machine was installed in March 2014, another eight have since been installed throughout the country. GeneXpert is used for the detection of RR in high risk patients, such as those who failed, are lost to follow up, or relapse after completion of the standardized 6 month first line therapy. At the University Clinical Research Center (UCRC) SEREFO Laboratory, DST is offered to selected patients enrolled in research protocols, since the biosafety level 3 (BSL-3) laboratory was opened in 2006 [3]. In addition, per national guidelines, all samples from patients at risk of MDR-TB are sent to UCRC for culture and 1st line DST, while confirmed MDR patients are referred to the TB unit of the University Teaching Hospital of Point-G (UTH), Bamako for further assessment, treatment and hospitalization until smear conversion [3]. Second line DST is conducted at the WHO/supra national laboratory at the Institute of Tropical Medicine (ITM), Antwerp, Belgium. We present here the first two documented

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patients with XDR-TB identified among the MDR-TB patients in Mali.

Case presentation

Description of patients

From 20 MDR-TB or RR patient's samples shipped to ITM in August 2016, two were confirmed as XDR. Both showed high-level resistance for different anti-tuberculosis drugs (Table 1). Although this was for routine surveillance per national guidelines, patients provided their informed consent.

Patient 1

Is a 27 year old woman living in Bamako. She was first treated for TB in 2009 [category 1: rifampicin (R), isoniazid (H), pyrazinamide (Z), ethambutol (E): 2RHZE/4RH], achieving cure. In 2013 she relapsed and failed category 2 [rifampicin (R), isoniazid (H), pyrazinamide (Z), ethambutol (E), streptomycin (S): 2RHZES/1RHZE/5RH] treatment. She was diagnosed as human immunodeficiency virus (HIV) infected in 2013, when she started taking Atripla® (efavirenz/emtricitabine/tenofovir). In 2014 she was admitted in the specialized tuberculosis unit of the UTH for chronic tuberculosis and suspicion of drug resistance. At first she was started on second-line treatment empirically in February 2014 [kanamycin (K), ofloxacin (O), ethionamide (Et): 6KOEtZ/15OEtZ], with culture revealing a non-tuberculous mycobacterium (NTM) only. She was recognized as suffering from RR-TB through GeneXpert testing in May 2014, and continued to receive second-line treatment but without any clinical improvement. A sputum culture from June 2015 grew *M. tuberculosis* complex (MTBc), and the sample was sent for second-line testing at ITM in December 2015, where fluoroquinolone resistance was diagnosed. As appropriate and effective drugs for pre-XDR were not available in-country, she was re-admitted in the hospital in December 2015, and treated with a weak treatment regimen consisting of kanamycin, cycloserine, amoxicillin/clavulanic acid, and erythromycin. During the hospitalization, observation of therapeutic compliance was irregular. In August 2016, samples were collected and tested again at ITM, and revealed additional resistance to injectable agents. The patient had a close contact with MDR who had been living with her between 2012 and 2013. He died in June 2014, 18 months after starting a second-line drug regimen (6KOEtZ/15OEtZ). He had returned from Cote d'Ivoire in 2010 with a history of interrupted TB treatment.

Patient 2

Is an HIV negative 50 year old woman who was referred from Southern Mali in January 2016 for chronic tuberculosis, with GeneXpert identifying RR. She had first been diagnosed with TB in 2014 and started category 1 treatment (2RHZE/4RH) but did not complete due to stock interruptions at her health center. Her health further deteriorated in 2015 with persistent cough and weight loss, and sputum tested positive for acid fast bacilli (AFB) in July 2015, with chest X-ray showing bilateral disseminated micronodular infiltrates associated with a cavity in the right upper lung. She started MDR treatment in January 2016 (6KOEtZ/15OEtZ) after GeneXpert showed RR. Although the treatment was not directly observed during the first 6 month, there was no treatment interruption, and the patient was discharged in June 2016 after her sputum smear had converted to negative.

She had no known close contact with a MDR patient, except for patient 1 described above, with whom she shared the same hospital ward between January and June 2016. Her spoligotype pattern changed from *M. tuberculosis* (MTB) T4 family in January 2016, to MTB T1 family (same as patient 1) in May 2016 (Fig. 1b). Despite both patients having the same MTB T1 spoligotype pattern on cultures isolated in May 2016, by 24 locus mycobacterial interspersed repetitive units, variable number of tandem repeats (MIRU-VNTR) the patterns differed in six loci, while patient 2 had proof of mixed infection defined as

Table 1 Second-line drug susceptibility results for patient 1 and 2

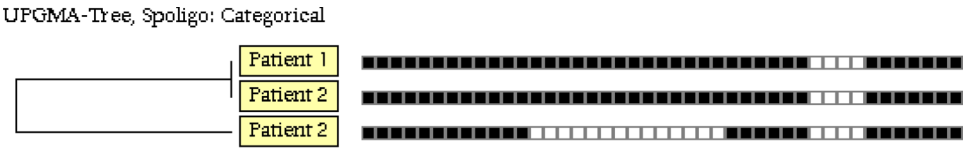
	Sample collection date	MTBDRplus rpoB	MTBDRplus katG	MTBDRplus inhA	MTBDRsld gyrA	MTBDRsld rrs	MTBDRsld eis
Patient 1	May 2016	MUT1 (D516V)	MUT1 (S315T)	MUT3B (T8A)	MUT3C (D94G)	MUT1 (A1401G)	WT
Patient 2	May 2016	MUT1 (D516V)	WT	MUT3B (T8A)	MUT3C (D94G)	MUT1 (A1401G)	WT
Patient 3	Feb 2017	MUT1 (D516V)	MUT1 (S315T)	MUT3B (T8A)	MUT3C MUT3B (D94G)	MUT1 (A1401G)	WT

"Point mutations in: the rpoB gene confer resistance to rifampicin; katG gene and in the inhA gene promoter confer resistance to isoniazid; gyrA and gyrB confer resistance to fluoroquinolones; and rrs confers resistance to kanamycin"WT = wild type (no resistance). MUT = mutation in position 1, position 3B, or position 3C of the gene, with specific mutation indicated

Patients	Sample date	0154	0424	0577	0580	0802	0960	1644	1955	2059	2163b	2165	2347	2401	2461	2531	2687	2996	3007	3171	3192	3690	4052	4156	4348
Patient 2	07/01/2016	2	2	4	2	1	3	3	3	1	5	4	4	2	2	5	1	5	3	3	3	3	5	2	2
Patient 1	18/05/2016	2	2	3	2	7	3	3	4	2	2	3	4	4	2	5	1	4	3	2	3	3	6	1	3
Patient 2	18/05/2016	2	2	3+4	2	1	3	3	3	1	2+5	3+4	4	2	2	5	1	5	3	3	3	3	5	2	2

original MIRU pattern of patient 2's isolate
MIRU pattern of patient 1's isolate
Loci with identical copy numbers in isolates from patient 2 (original) **and** patient 1 (non-discriminatory)
Loci with **evidence for superinfection** of patient 2 by patient 1's isolate based on double peaks that match both patient 2 (original) and patient 1 copy numbers

a



b

Fig. 1 **a** 24-locus mycobacterial interspersed repetitive units, variable number of tandem repeats patterns of both extensively drug resistant tuberculosis patients. Three loci show double peaks for the second sample of patient 2, with the same alleles as patient 1's MIRU pattern, suggestive of *possible* nosocomial superinfection of patient 2 from patient 1. In addition each copy number in patient 2's second isolate matches either the original isolate (red = original patient 2, and green = both original patient 2 and patient 1), or is a double pattern with copy numbers matching both original patient 2 AND patient 1 copy numbers. **b** Spoligotyping results of the extensively drug resistant tuberculosis patients: [patient 2, sample 1 (Jan 16) is at the bottom and sample 2 (May 16) is in the middle] showing the superimposed pattern of patient 1 obscuring the missing spacers 13–26 in the first isolate. All isolates belong to the EuroAmerican lineage 4

double peaks in three of 24 loci (Fig. 1a), with the additional peaks matching the MIRU pattern of patient 1. This suggests that patient 2 had chronic TB with a different strain, but was superinfected by patient 1 through nosocomial transmission. The different resistance profiles [*katG* mutation for patient 1, and wild type (WT) for patient 2] suggest that the Line Probe Assay missed the superinfecting strain in patient 2 (Fig. 1, Table 1). In addition each copy number in patient 2's second isolate matches either the original isolate (red = original patient 2, and green = both original patient 2 and patient 1), or is a double pattern with copy numbers matching both original patient 2 AND patient 1 copy numbers.

From diagnosis to treatment initiation

After diagnosis of these patients as XDR in August 2016, in November 2016 the Mali national tuberculosis program (NTP) placed an order for assistance with the Global Drug Facility (GDF) Stop TB Partnership to initiate a treatment regimen with new drugs. Despite two additional requests through The TB Union and also WHO, appropriate treatment was not made available until finally the Global Fund through its representative program in Mali bought the drugs, bedaquiline (Bdq), clofazimine (Cfz), delamanid (Dlm), linezolid (Lzd), para acid salicylic (PAS): 6 (Bdq-Cfz-Dlm-Lzd-Z-H-PAS)/14 (Bdq-Cfz-Lzd-Z) and patients started treatment on May 9th, 2017. Sadly, during this 10 month delay, patients were hospitalized in isolation rooms without receiving TB specific therapy, only nutritional support.

Actions taken to limit transmission

After diagnosis, in addition to the isolation of the two patients from the other MDR patients, all the household contacts for both patients were screened for TB, including drug resistance, by Xpert MTB/Rif. This screening allowed identifying another MDR-TB patient from patient 2's family. Unfortunately, at the end of March 2017, we identified a third XDR-TB patient also from the same hospital ward, and molecular Hain test suggest nosocomial transmission from the first XDR patient (Table 1). We think that the most logical explanation is that patient 3 has pre-XDR with a *gyrA* MUT3B, and was superinfected by patient 1, who had XDR with *gyrA* MUT3C. In total three XDR and one MDR patient were identified from this outbreak.

Culture, identification, first line drug susceptibility testing at SEREFO/UCRC and shipping of isolates to ITM and molecular second-line DST at ITM, Antwerp Belgium

In the UCRC laboratory, primary isolation is done in both liquid [manual reading of *Mycobacterium* growth incubator tubes (BBL™ MGIT™ Becton–Dickinson, Sparks MD, USA)], and solid (Middlebrook 7H11 agar and selective 7H11 agar) media, following standard protocols. Indirect first line DST is performed on confirmed *M. tuberculosis* complex (MTBc) isolates using MGIT AST/SIRE System

(Becton Dickinson, Sparks, MD, USA). Samples and cultured isolates were first heat inactivated before shipping to ITM, Antwerp, where the Hain Second Line Probe Assay, (GenoType MTBDRsl) was performed on each sample as per manufacturer's instructions [4].

Discussion and conclusions

Data on XDR-TB are scarce in Africa, especially in West African countries [2, 3]. We describe here the first documented XDR patients in Mali. Extensively drug resistant TB patients were identified in neighbouring countries Burkina Faso in 2010 and Cote d'Ivoire in 2015 [5, 6]. As culture and DST were not performed during the first episodes of TB infection in the patients presented here, we cannot exclude primary pre-XDR resistance. Also in the XDR patients in Burkina Faso and Cote d'Ivoire [5, 6] baseline resistance tests were missing, whereas in South Africa primary resistance was well documented in XDR patients [7]. The NTM isolated from the first culture of patient 1 may have been a 'colonizer' that obscured ongoing TB disease, or may have contributed to chronic pulmonary infection in this HIV co-infected patient [8]. These patients also highlight the need for new diagnostic tools that could simultaneously detect the MTBc and NTM. In addition, both patients experienced treatment interruptions, and patient 1 was inappropriately treated with a weak regimen based on kanamycin for more than 6 months after diagnosis of high level fluoroquinolone resistance [9], which likely caused the additional resistance to injectables, resulting in XDR-TB. Lastly, the ineffective treatment and poor hospital infection control likely permitted the possible nosocomial superinfection from patient 1 to patient 2 and 3. Urgent treatment initiation limits morbidity, mortality, and ongoing transmission [10, 11]. Here, early initiation of appropriate treatment could have stopped the possible nosocomial transmission and may have prevented the third XDR-TB patient. Despite the resource limited condition with the Mali NTP, it is high time that all recommended steps for programmatic management of drug-resistant tuberculosis (PMDT) implementation for strengthening the MDR TB program are taken to serve patients with rifampicin resistance in Mali.

In order to address the deficiencies presented, firstly the laboratory network in Mali should be upgraded to allow local access to second-line DST, which will considerably reduce the time to diagnosis, especially given the complexities of transportation of samples from Bamako to Antwerp. Both the Hain SL LPA and phenotypic second line DST are currently being implemented not only at the NRL, but also at UCRC. Secondly, patient 2 probably was superinfected by patient 1, and clinicians should be trained for proper management of MDR patients, including appropriate infection control measures, physiotherapy and psychosocial support for patients for retention into care. Most importantly, rapid diagnosis is futile without appropriate treatment regimens being available. As for 'simple' MDR patients, the 20 + month MDR regimen has unacceptably high drop-out and failure rates, and the 9M MDR needs to urgently be adopted in Mali. For these and additional XDR-TB patients, appropriate drugs need to be stocked.

In summary, the identification of XDR in Mali is not unexpected, as resistance is a man-made problem and management of patients with MDR-TB is at present not following recommended PMDT guidelines in Mali. We, as the whole management team of MDR-TB patients, failed in multiple steps after the first identification of their extensive resistance, and thus we hope that the measures to be taken will be sufficient to contain resistance TB and prevent its further spread. The lessons learned in Mali may serve as an example to other countries that have not yet identified XDR-TB patients.

Abbreviations

MDR: multidrug resistant; XDR: extensively drug resistance; WHO: World Health Organization; DST: drug susceptibility testing; RR: resistance to rifampicin; UCRC: University Clinical Research Center; BSL-3: biosafety level 3; UTH: University Teaching Hospital of Point-G; ITM: Institute of Tropical Medicine; DR-TB: drug resistant-tuberculosis; USTTB: University of Sciences, Techniques and Technologies of Bamako; TB: tuberculosis; R: rifampicin; H: isoniazid; Z: pyrazinamide; E: ethambutol; S: streptomycin; K: kanamycin; O: ofloxacin; Et: ethionamide; HIV: human immunodeficiency virus; NTM: non-tuberculous mycobacteria; Mtbc: *Mycobacterium tuberculosis* complex; AFB: acid fast bacilli; MTB T1/T4: *Mycobacterium tuberculosis* T family 1 and 4; MIRU-VNTR: mycobacterial interspersed repetitive units, variable number of tandem repeats; WT: wild type; NTP: National TB Program; DGF: Global Drug Facility; Bdq: bedaquiline; Cfz: clofazimine; Dlm: delamanid; Lzd: linezolid; PAS: para acid salicylic; MUT: mutation; MGIT: mycobacterium growth incubator tube; BD: Becton–Dickinson; MD: Maryland; NIAID/NIH: National Institutes of Allergy and Infectious Diseases, National Institutes of Health; USA: United States of America; AST: antimycobacterial susceptibility testing; SIRE: streptomycin, isoniazid, rifampicin, ethambutol; PMDT: programmatic management of drug-resistant tuberculosis; LPA: Line Probe Assay; NRL: national reference laboratory; IRB: institutional review board.

Authors' contributions

BD, YT, BK, BdJ and SD3, conceived and developed the study. MS, ACGT, BB, DG, FC, GB, DS, MM, SD1, YSS, MB and SO, helped in the protocol development. SS, SD2, SD1, SD3, and RLM, led the study. BD, MS, FC, ACGT, BB, GB, DS and YSS, were instrumental in data generation and laboratory assays. BD and BdJ, collated, reviewed and interpreted data and contributed to the writing of the manuscript. BD wrote the first draft. MS, DG, BB, SS, SD1, SD3, RLM, and BdJ, YT, BK, reviewed the drafts. SS, SD3, SD1, SD2, RLM, and BdJ provided mentoring for this work. All authors had access to the data and reviewed the manuscript. All authors read and approved the final manuscript.

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Competing interests
The authors declare that they have no competing interests.

Availability of data and materials
All the data supporting our findings can be found within the manuscript.

Consent to publish
Written informed consent from each patient's family member was attained before samples were taken, and written informed consent was obtained from both patients for publication of this case report and any accompanying images.

Ethics approval and consent to participate
The study protocol was approved by the ethic committee of the faculty of medicine, pharmacy and dentistry of Bamako. The ethical committee also approved the storage and testing of samples collected as part of the approved protocol. Signed informed consent was obtained from both patients.

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