Surveillance of drug-resistant *Mycobacterium tuberculosis* in The Gambia

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SUMMARY

To determine the rates of drug-resistant tuberculosis in The Gambia, *Mycobacterium tuberculosis* isolates obtained from 225 patients during a nationwide survey were tested against isoniazid, rifampicin, ethambutol and streptomycin using the resistance ratio method. Only nine (4%) of the patients had strains that were resistant to one or more drugs. None of the patients with drug-resistant *M. tuberculosis* had previously been treated for tuberculosis. Drug-resistant tuberculosis is, as yet, not common in The Gambia. Periodic surveys for drug-resistant tuberculosis are recommended to monitor changes that may emerge over time.

KEY WORDS: drug resistance; *Mycobacterium tuberculosis*; The Gambia

THERE ARE REPORTS of increasing prevalence of drug-resistant *Mycobacterium tuberculosis* worldwide.[1] Resistance rates vary from country to country, but the general pattern is on the rise.[2] Patients with multidrug-resistant tuberculosis (MDR-TB), defined as resistance to at least isoniazid and rifampicin, are less likely to achieve cure,[3] and surveillance of drug resistance is important for monitoring the performance of tuberculosis (TB) control programmes.[4] The Gambia's National TB Control Programme (TBCP) has been in operation since 1986, and registers over 1000 new cases annually.[5] The TBCP activities are carried out by Leprosy/TB Inspectors (LTIs) throughout the country through nine divisional health centres and a TB sanatorium with facilities for smear microscopy for acid-fast bacilli (AFB), treatment and follow-up of patients. Pulmonary cases are confirmed by microscopy of sputum smears, and all cases are treated with a three times weekly directly observed short-course regimen consisting of 2 months of rifampicin (R), isoniazid (H), pyrazinamide (Z) and ethambutol (E), followed by 4 months of RH ($2R_3H_3Z_3E_3/4R_3H_3$).

The prevalence of drug-resistant *M. tuberculosis* has not previously been assessed in The Gambia. We undertook community-based surveillance of drug-resistant *M. tuberculosis* as a part of the WHO/IUATLD Global Project on Anti-Tuberculosis Drug Resistance.[6] The main objective of this study was to determine the prevalence of drug resistance in new patients with TB and patients with previously treated TB in The Gambia and to identify any associated risk factors.

MATERIALS AND METHODS

A nationwide survey was conducted by LTIs at the nine main health centres and the national sanatorium between June and December 1999. A pilot study was undertaken at one of the largest health centres to ensure that the survey protocol was understood by field and laboratory staff.

Survey subjects were all patients (aged ≥15 years) diagnosed with smear-positive pulmonary tuberculosis (PTB) using Ziehl-Neelsen (ZN) staining. During the course of the survey, enrolment was slowed down in two health centres (Serekunda and Brikama) that were recording large numbers of TB patients in order to include enrolment of registered TB patients from other health centres. Apart from the initial sputum specimen, each eligible patient provided two other spot specimens before treatment was commenced. A short questionnaire was used to obtain information on demographic details and possible risk factors from each patient. Previous TB treatment and outcome of chemotherapy were assessed by the history given by patients and available medical records at the health centre were extensively checked to determine whether the patient had previously been registered as a TB patient.

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Sputum specimens were collected without cetylpyridium bromide, stored at −20°C and transported on ice to the Medical Research Council (MRC) laboratory within 48 hours of collection for further analysis. Specimens collected in two nearby health centres and the sanatorium were taken to the MRC laboratory on the day of collection, while specimens from distant areas of the country were transferred to the laboratory by a field assistant within one day after the collection of the specimen.

Blood specimens were collected from 122 study participants from six health centres and the sanatorium after appropriate counselling for human immunodeficiency virus (HIV) testing. We were unable to collect blood from 103 patients due to unavailability of trained counsellors and staff at the time of enrolment.

Sputum smears were prepared and stained with auramine-phenol;[7] positive smears were confirmed by ZN after initial examination by fluorescence microscopy. Decontamination of specimens was performed by the NaOH-NALC method.[8] Decontaminated specimens were inoculated onto one slope each of Löwenstein-Jensen medium (L-J) containing glycerol and sodium pyruvate, respectively, and one vial of BACTEC 9000 MB medium for isolation of *M. tuberculosis*. All mycobacterial cultures were identified and confirmed as *M. tuberculosis* using standard procedures. All cultures were tested for drug susceptibility against H, R, E and streptomycin (S) (Sigma Chemical Co. St. Louis, MO, USA) by the resistance ratio method.[7] All resistant isolates and 10% of all susceptible isolates were sent for concordance analysis to the Supra-National Reference Laboratory (SNL), at the Public Health Laboratory Service Mycobacterium Reference Unit in London, UK. Quality control samples of drug-susceptible and drug-resistant isolates were received from the SNL as required by the WHO guidelines for the programme.[9]

Data were recorded and analysed using Epi Info (version 6.04d, CDC, Atlanta, GA, 2001).

The study was approved by the MRC Scientific Co-ordinating Committee and The Gambia Government/MRC joint ethics committee.

RESULTS

A total of 474 smear-positive PTB cases were registered in The Gambia during the survey period, of whom 225 were enrolled into the study; 129 (57.3%) were from Serekunda health centre, a major urban clinic where most of the TB patients are seen in The Gambia. The other patients were enrolled at the following health centres: Brikama (28), Farafenni (20), Basse (11), Bansang (8), Kuntaur (7), Polyclinic Banjul (5), Esau (3), Mansakonko (1) and the sanatorium (13). Enrolment rates varied greatly in different parts of the country, and some time was required to achieve nationwide sampling.

All patients enrolled had their smear-positive status confirmed at the MRC laboratory. Only three of 450 (0.7%) sputum samples cultured from the 225 study participants did not grow mycobacteria at the MRC laboratory. In all three samples growth of *M. tuberculosis* was obtained in the second pair of specimens.

Table 1 Characteristics of patients with *Mycobacterium tuberculosis* strains resistant to one or more drugs

Patient	Age (years)	Sex (M/F)	HIV (+/−/ND*)	Previous treatment (Y/N)	Strain resistance
1	18	F	−	N	S
2	20	M	−	N	H
3	24	M	−	N	S
4	24	M	ND	N	H
5	26	F	−	N	H
6	26	M	−	N	H
7	29	M	+	N	R
8	30	M	+	N	S
9	78	F	ND	N	H, R

M = male; F = female; HIV = human immunodeficiency virus; + = positive; − = negative; ND = not done; Y = yes; N = no; H = isoniazid; R = rifampicin; E = ethambutol; S = streptomycin.

Of the 225 patients with sputum cultures positive for *M. tuberculosis*, 172 (76%) were males and 53 (24%) were females, giving a male:female ratio of 3.2:1. The mean age was 35 years (median 31 years, range 14–80). Eleven (9%) of the 122 tested were HIV-positive. Of those enrolled, 15 (7%) had a history of TB treatment.

Of the 210 new TB cases, resistance to at least one anti-tuberculosis drug was identified in nine patients (Table 1), giving a primary resistance prevalence of 4.3%. Their mean age was 31 years, the male:female ratio was 2:1, and none had previously been treated for TB. The prevalence of primary resistance for H was 2.4% (5/210), for S it was 1.4% (3/210) and for R it was 1.0% (2/210). The prevalence of primary monoresistance was 3.8% (8/210) and primary multidrug resistance was 0.5% (1/210). No cases of drug resistance were identified among the 15 previously treated TB patients, giving a prevalence of acquired resistance of 0%.

Of 23 isolates found to be fully susceptible to all four drugs in The Gambia, concordance with the results for each drug from the SNL in London was 91% (H), 100% (R), 100% (E) and 96% (S). Of the nine strains considered to be resistant to one or more drugs, one isolate found to be resistant to H was contaminated and not recoverable at the SNL. Concordance rates were 4/4 (100%) for H, 1/2 (50%) for R, and 2/4 (50%) for S, but the numbers were small (Table 2). The only isolate that was found to be multidrug-resistant in The Gambia was also resistant to S and E at the SNL. The overall concordance rate for E was therefore 30/31 (97%).

DISCUSSION

The first drug susceptibility results of *M. tuberculosis* isolates reported from The Gambia show high con-

Table 2 Quality assurance drug sensitivity tests at the Supranational Reference Laboratory in Dulwich

Drug	H	R	E	S
Strains considered susceptible at MRC, The Gambia				
Number tested at Dulwich	23	23	23	23
Number reported susceptible	21	23	23	22
Number reported resistant	2	0	0	1
Concordance (%) with MRC	91	100	100	96
Strains considered resistant at MRC, The Gambia				
Number tested at Dulwich	4	2	NA	4
Number reported susceptible	0	1	NA	2
Number reported resistant	4	1	NA	2
Concordance (%) with MRC	100	50	NA	50

H = isoniazid; R = rifampicin; E = ethambutol; S = streptomycin; MRC = Medical Research Council, The Gambia; NA = not applicable.

cordance with the results from the SNL in London. Despite the presence of a TBCP since 1986, only 4.3% of *M. tuberculosis* isolates in new patients were resistant to one or more drugs, and only one patient had a multidrug-resistant isolate. No resistance was detected in previously treated patients, although there were only 15 such patients. It is certainly of interest that resistance was so low in a country where treatment completion rates have consistently been below 80%.[5]

Similar low levels of initial drug resistance in TB isolates have been reported elsewhere in Africa, despite the increasing prevalence of both tuberculosis and HIV-1 infection.[10] It is also of note that none of those with a history of previous tuberculosis treatment had a resistant isolate. It is possible that these patients had new, as opposed to recrudescent, infections.

With so few resistant isolates it is not possible to speculate with respect to possible risk factors for resistance in our population. In The Gambia, all antituberculosis medicines are under the control of the TBCP, and are not available to private practitioners. Medicines are donated regularly by the Royal Netherlands Tuberculosis Association (KNCV), so patients seldom receive less than their correct combinations of medicines, thus reducing the chances of monotherapy. Furthermore, single drug preventive therapy is not standard practice in The Gambia, again reducing the chances of inducing drug resistance. Although the prevalence of resistance is low, its presence at all is a matter of concern, and care must be taken to ensure that non-adherence and other factors associated with its development are kept to a minimum. Monotherapy of patients with a high bacterial load must be avoided. If one of the standard drugs is unavailable it would be preferable not to administer any drugs until correct multidrug treatment is provided.

The male to female ratio of patients in this study (3.2:1) was higher than that documented by the TBCP, which has reported a relatively constant rate of 2.5:1 for several years.[5] The reasons for this discrepancy are not clear. We believe it is unlikely that this would have introduced a bias of relevance to the levels of drug resistance in TB isolates. The mean age of the patients in this study (31 years) is similar to that of all cases registered in The Gambia (32 years).

Drug-resistant tuberculosis is not yet a problem in The Gambia. This report provides the baseline for comparison with future surveys. Periodic surveys for drug-resistant tuberculosis are recommended to monitor any changes that may emerge over time.

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RÉSUMÉ

Pour déterminer les taux de tuberculose à germes résistants aux médicaments en Gambie, des isolats de *Mycobacterium tuberculosis* obtenus chez 225 patients au cours d'une enquête nationale ont été testés à l'égard de l'isoniazide, la rifampicine, l'éthambutol et la streptomycine par la méthode des ratios de résistance. Une résistance à l'égard d'un ou de plusieurs médicaments n'a été observée que dans les souches de 4% de patients. Aucun des patients où *M. tuberculosis* résistant aux médicaments a été isolé n'avait été traité antérieurement pour tuberculose. Jusqu'à présent, la tuberculose à germes résistants aux médicaments n'est pas encore courante en Gambie. Des enquêtes périodiques au sujet de la tuberculose à germes résistants aux médicaments sont recommandées pour suivre les modifications qui pourraient survenir avec le temps.

RESUMEN

Se realizaron tests de sensibilidad a la isoniacida, rifampicina, etambutol y estreptomicina utilizando el método de los coeficientes de resistencia, en aislados de *Mycobacterium tuberculosis* obtenidos de 225 pacientes, durante un estudio a escala nacional, para determinar las tasas de tuberculosis resistente en Gambia. Sólo nueve (4%) de los pacientes tenían cepas resistentes a uno o más medicamentos. Ninguno de los pacientes con *M. tuberculosis* resistente a los medicamentos había sido tratado previamente por tuberculosis. Hasta ahora, la tuberculosis resistente a los medicamentos no es frecuente en Gambia. Se recomienda realizar encuestas periódicas con respecto a la tuberculosis resistente a los medicamentos a fin de controlar los cambios que podrían ocurrir con el tiempo.