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Changes in *Escherichia coli* resistance to co-trimoxazole in tuberculosis patients and in relation to co-trimoxazole prophylaxis in Thyolo, Malawi

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Abstract

In Thyolo district, Malawi, an operational research study is being conducted on the efficacy and feasibility of co-trimoxazole prophylaxis in preventing deaths in HIV-positive patients with tuberculosis (TB). A series of cross-sectional studies were carried out in 1999 and 2001 to determine (i) whether faecal *Escherichia coli* resistance to co-trimoxazole in TB patients changed with time, and (ii) whether the resistance pattern was different in HIV-positive TB patients who were taking co-trimoxazole prophylaxis. Co-trimoxazole resistance among *E. coli* isolates in TB patients at the time of registration was 60% in 1999 and 77% in 2001 ($P < 0.01$). Resistance was 89% among HIV-infected TB patients (receiving co-trimoxazole), while in HIV-negative patients (receiving anti-TB therapy alone) it was 62% ($P < 0.001$). The study shows a significant increase of *E. coli* resistance to co-trimoxazole in TB patients which is particularly prominent in HIV-infected patients on co-trimoxazole prophylaxis. Since a high degree of plasmid-mediated transfer of resistance exists between *E. coli* and the *Salmonella* species, these findings could herald limitations on the short- and long-term benefits to be expected from the use of co-trimoxazole prophylaxis in preventing non-typhoid *Salmonella* bacteraemia and enteritis in HIV-infected TB patients in Malawi.

Keywords: *Escherichia coli*, HIV infections, tuberculosis, chemoprophylaxis, co-trimoxazole, drug resistance, Malawi

Introduction

Non-typhoid *Salmonella* (NTS) bacteraemia particularly with *Salmonella typhimurium* and *S. enteritidis* is known to be among the leading causes of morbidity and mortality in human immunodeficiency virus (HIV)-infected patients with tuberculosis (TB) in Africa (GILKS *et al.*, 1990; BRINDLE *et al.*, 1993; FERNANDEZ GUERRERO *et al.*, 1997; GORDON *et al.*, 2001). Prophylaxis with sulphamethoxazole–trimethoprim (co-trimoxazole) has been shown to reduce mortality and morbidity among HIV-infected patients with sputum-positive TB in Abidjan (ANGLARET *et al.*, 1999; WIKTOR *et al.*, 1999). Protection from NTS bacteraemia and enteritis accounted for much of this beneficial effect, susceptibility of NTS to co-trimoxazole in Abidjan being very high (91%).

The National TB Control Programme of Malawi is currently testing the feasibility and efficacy of co-trimoxazole prophylaxis as an adjunct to anti-TB therapy in reducing overall mortality in HIV-positive TB patients within routine programme conditions in Malawi. The eventual short- and long-term efficacy of co-trimoxazole in reducing morbidity and mortality in Malawi would depend on the baseline levels of co-trimoxazole resistance and particularly the impact of co-trimoxazole prophylaxis on progressive resistance development among a spectrum of common target opportunistic pathogens such as NTS. High resistance to co-trimoxazole in NTS or other opportunistic pathogens could have implications on the potential benefits of co-trimoxazole prophylaxis in HIV-infected individuals and would therefore be important to monitor.

It is known that there is a high degree of rapid plasmid-mediated transfer of co-trimoxazole resistance between faecal *E. coli* and the *Salmonella* species as well as other Enterobacteriaceae (MARSIK *et al.*, 1975; MURRAY & RENSIMER, 1983; BALIS *et al.*, 1996). The level of faecal *E. coli* resistance to co-trimoxazole, which is relatively more simple to measure than that for *Salmonella* in a resource-poor setting such as Malawi, would therefore herald similar resistance patterns in NTS and other Gram-negative bacteria.

The objective of this study was to measure changes in faecal *E. coli* (bacterial flora) resistance to co-trimoxazole in TB patients with time and to determine whether this pattern was different in HIV-positive TB patients who were taking co-trimoxazole prophylaxis.

Materials and Methods

Study setting

The study was carried out in Thyolo district of southern Malawi, which is one of the districts in the country in which the National TB Control Programme of Malawi is currently testing the feasibility and efficacy of co-trimoxazole prophylaxis as an adjunct to anti-TB therapy in reducing overall mortality in HIV-infected TB patients. In this study of co-trimoxazole prophylaxis, which is still being implemented since July 1999, all registered TB patients are started on standardized anti-TB treatment according to National Guidelines (MOHP, 1999). Patients undergo voluntary counselling and HIV testing and are offered co-trimoxazole prophylaxis (800 mg of sulphamethoxazole and 160 mg of trimethoprim) if they test HIV-seropositive and if there are no contraindications to the medication.

Study population and specimen collection

To determine whether there were changes in co-trimoxazole resistance with time, 2 serial cross-sectional resistance studies were conducted in 1999 and in 2001 using stool specimens collected from cohorts of new TB cases selected randomly before starting anti-TB therapy. In these 2 studies, stool specimens were collected immediately after registration and before start of anti-TB therapy. None of the patients was taking co-trimoxazole prophylaxis.

In order to compare resistance patterns with patients who were taking co-trimoxazole, another study was carried out in 2001 on a cohort of TB patients selected randomly while visiting health facilities for monthly drug collection. This cohort of patients was receiving anti-TB therapy and included a proportion of HIV-seropositive patients that were receiving adjunctive co-trimoxazole over 6–8 months.

HIV status was not known for the first serial resistance study in 1999 as HIV testing and counselling

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were not available until July 1999. In the other 2 subsequent studies, HIV-status results were available. A structured questionnaire was used to gather information on basic socio-demographic data. All patients were residents of the rural district, and stool specimens were collected after obtaining voluntary informed consent, confidentiality of results being respected.

Laboratory methods

Initial inoculation of stool specimens for culture was done on MacConkey 3 (Oxoid) agar plates. Suspect colonies were subcultured for purity and identified by colony morphology and biochemical tests. Isolates were confirmed to be *E. coli* using the API 20E identification system (API-Biomérieux, Basingstoke, England, UK).

Anti-microbial susceptibility testing to co-trimoxazole was done on modified Mueller–Hinton (Oxoid) agar plates. The Kirby–Bauer disk-diffusion technique was used and inhibition-zone sizes (IZS, nearest whole millimetre) for *E. coli* were read according to National Committee for Clinical Laboratory Standards guidelines (NCCLS, 1998). Interpretation of IZS for co-trimoxazole (23·75/1·25 μg) was as follows: 16 mm, susceptible; 11–15 mm, intermediate; 10 mm, resistant. Antibiograms were validated using standardized control strains of *E. coli* ATCC No. 25922 (American Type Culture Collection, Rockville, MD, USA) on a regular basis. Independent control testing was done on specimens that were transported using microbank tubes (Pro-lab Diagnostics, Neston, Wirral, UK) to the National Infectious Diseases Reference Laboratory in Luxembourg. HIV testing was performed using a combination of the Capillus (Cambridge Diagnostics Ltd, Galway, Ireland) and HIV Spot (Genelabs Diagnostics Pte Ltd, 85 Science park Drive, Singapore) tests.

Statistical analysis

The EpiInfo software (Centers for Disease Control, Atlanta, USA) was used for data analysis. The level of significance was set at 0·05 and 95% confidence intervals (CI) were used throughout in expressing differences in proportions. All laboratory results of resistance levels are expressed to the nearest whole millimetre.

Results

Characteristics of the study population

A total of 443 registered TB cases were studied; 16 patients could not produce stool specimens at the time of specimen collection and were excluded. Data are therefore available for 427 TB patients.

Of the 427 patients there were 210 male and 217 (51%) female patients, the mean age being 33 years. Over half (240 patients) of the study group had smear-positive pulmonary TB (PTB), 91 had smear-negative PTB and 96 had extrapulmonary TB. The overall HIV seroprevalence (all TB types) in all those that had undergone HIV testing in 2001 ($n = 286$) was 75%.

Co-trimoxazole resistance

Of the 427 stool specimens that were collected, 406 (95%) grew *E. coli* on culture. In the 2 serial studies conducted on newly registered TB patients (before starting anti-TB therapy or co-trimoxazole) co-trimoxazole resistance among isolates was 60% in 1999 ($n = 118$), and increased significantly ($P < 0·01$) to 77·1% ($n = 144$) in the similar cohort in 2001 (Table). There was no significant difference in co-trimoxazole resistance between HIV-positive (79·0%) and HIV-negative (68·8%) patients in 2001 ($P = 0·23$). Overall co-trimoxazole resistance in patients who had received anti-TB therapy (HIV positive and HIV negative) for 6–8 months (with or without adjunctive co-trimoxazole) was 82·6% in 2001 (Table). In this cohort, resistance in HIV-infected patients receiving adjunctive co-trimoxazole ($n = 113$) was 89·4% (95% CI 82·2–94·4) while in HIV-negative patients ($n = 26$) receiving anti-TB therapy alone it was 61·5% (95% CI 40·6–79·8), the difference being highly significant ($P < 0·001$) (Table). This resistance was also higher when compared to HIV-positive TB cases that were on neither anti-TB therapy nor co-trimoxazole in 2001 ($P = 0·03$).

There were no significant differences in co-trimoxazole resistance with regards to different age-groups, gender and TB type. Independent quality control testing conducted on 103 isolates transported to Luxembourg showed conformity of laboratory results.

Discussion

This study shows that co-trimoxazole resistance in *E. coli* among TB patients has significantly increased in a 2-year period in a rural district of Malawi. In a resource-poor country such as Malawi where effective and affordable interventions to limit morbidity and mortality in people living with HIV are limited, there has been a growing market demand for co-trimoxazole following its introduction in Thyolo district for HIV-infected TB patients in July 1999. Co-trimoxazole, being a relatively cheap antibiotic, which is easy to administer, has since 1999 become readily available in public pharmacies, from private drug vendors and even at some grocery stores, and it can be purchased without any formal prescription. The uncontrolled availability and widespread use of co-trimoxazole in the Thyolo

Table. Resistance to co-trimoxazole in faecal *Escherichia coli* isolates from tuberculosis (TB) patients in Thyolo, Malawi

Patient group	Resistant/total isolates	%	*P* value[c]
TB patients on registration in 1999[a]	71/118	60·2	–
TB patients on registration in 2001[a]	111/144	77·1	
HIV positive	83/105	79·0	0·23
HIV negative	22/32	68·8	–
TB patients on follow-up in 2001[b]	119/144	82·6	
HIV positive (receiving co-trimoxazole)	101/113	89·4	0·001
HIV negative (not receiving co-trimoxazole)	16/26	61·5	–

[a]Specimens collected during TB registration, patients being on neither anti-TB therapy nor co-trimoxazole. HIV status was not known at the time of registration in 1999 and for 7 patients on registration in 2001.
[b]Specimens collected during 6–8-month follow-up visit. HIV-positive patients had received anti-TB therapy and adjunctive co-trimoxazole while HIV-negative patients had received anti-TB therapy alone. HIV status was not known for 5 patients in this group.
[c]*P* values are for HIV-positive as compared to HIV-negative patients.

community could explain the relatively rapid increase in co-trimoxazole resistance in *E. coli* between 1999 and 2001. In Malawi, sulfadoxine-containing antimalarials (Fansidar) and co-trimoxazole are the first-line drugs recommended for malaria and respiratory tract infections in children respectively, and this might also contribute to a high baseline resistance to co-trimoxazole.

Co-trimoxazole resistance in *E. coli* isolates from HIV-infected TB patients who had been receiving adjunctive co-trimoxazole was significantly higher than in HIV-negative TB patients (on anti-TB therapy alone) and is most likely linked to the prophylactic use of co-trimoxazole in these patients (MURRAY *et al.*, 1982; MARTIN *et al.*, 1999).

These findings of high *E. coli* resistance to co-trimoxazole may also herald high levels of resistance in NTS and other Gram-negative bacteria and would limit the protective effect of co-trimoxazole against these Enterobacteriaceae. As the beneficial effects on morbidity and mortality of co-trimoxazole prophylaxis in HIV-infected TB patients in Côte d'Ivoire were mainly linked to protection from NTS bacteraemia and enteritis, limited benefits of adjunctive co-trimoxazole might be expected in this regard in Malawi. However, co-trimoxazole prophylaxis provides a protective effect against other HIV-related opportunistic pathogens, such as *Pneumocystis carinii*, *Isospora belli* and *Toxoplasma gondii*, and might therefore still be effective in reducing overall mortality in TB patients within our context.

In the co-trimoxazole operational research study which is still being implemented in Thyolo, over 1000 TB patients have been recruited of whom nearly 700 have been placed on co-trimoxazole prophylaxis. End of treatment outcomes, compared to historical controls, will help the National Tuberculosis Control Programme of Malawi make a decision about the efficacy (or lack thereof) of this intervention in reducing death rates.

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