# FLUOROQUINOLONE RESISTANCE IN *MYCOBACTERIUM TUBERCULOSIS* STRAINS ISOLATED IN KENYA.

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## SUMMARY

*Background:* **Fluoroquinolones are key second-line anti-tuberculosis drugs usually used in the treatment of patients with Multi-Drug Resistant Tuberculosis (MDR-TB). Anti-TB fluoroquinolones include ciprofloxacin, moxifloxacin, gatifloxacin (Gat), ofloxacin and levofloxacin. Resistance to one fluoroquinolone usually translates to resistance to the others in the group.**

*Objective:* **To determine whether there is fluoroquinolone resistance in *Mycobacterium tuberculosis* strains isolated in Kenya.**

*Design:* **A retrospective descriptive study involving archived strains from previous studies carried out at the Centre for Respiratory Diseases Research (CRDR), Kenya Medical Research Institute (KEMRI) between 2002 and 2007.**

*Setting:* **CRDR, KEMRI.**

*Methods:* **A total of 216 first-line Drug Susceptibility Testing (DST) pre-tested MTB strains were used including 78 resistant to one or more drugs, and randomly selected 138 susceptible to all four drugs. Of the 78 resistant strains, 25 were MDR-TB. The strains were subjected to drug susceptibility testing to Gat among other second-line drugs.**

*Results:* **Of the 216 strains tested, 32 [32/216 (14.8%)] showed resistance to second-line drugs. Of these seven [7/32 (21.9%)] were fully resistant to Gat of which six [6/7 (85.7%)] were mono-resistant strains and one [1/7 (14.3%)] with combined resistance strain to Ethionamide. Four [4/25 (16%)] MDR-TB strains showed mono-resistance to Gat.**

*Conclusion:* **Presence of Gat resistance especially in MDR-TB patients may significantly contribute to Extensively Drug Resistance TB, a more difficult to treat strain than MDR-TB. Therefore strict drug adherence among MDR-TB patients and proper and appropriate use of flouroquinolones should be implemented in Kenya.**

**Key words:** Fluoroquinolones, MDR-TB, resistance.

## Introduction

Fluoroquinolones have become indispensable in the treatment of Multi-Drug Resistant Tuberculosis (MDR-TB)[1]. As measured by their *in vitro* activity against *Mycobacterium tuberculosis* (MTB), the most potent of the currently available fluoroquinolones are, in descending order, moxifloxacin (MXF), gatifloxacin (Gat), levofloxacin (Lev), ofloxacin (Ofl), and ciprofloxacin (CIP)[2]. The first three of these drugs are commonly known as anti-pneumococcal fluoroquinolones used in community-acquired pneumonia. MTB clinical isolates that demonstrate high-level phenotypic resistance to fluoroquinolones, which appears to be predominantly due to gyrA mutations, exhibit cross-resistance to all six important fluoroquinolones. Unfortunately, resistance to one fluoroquinolones usually means resistance to the others[3]. Fluoroquinolones act by inhibiting bacterial DNA gyrase. They are less effective than other first-line agents in treating TB and are mainly used for patients with MDR-TB. These are the most promising anti-TB drugs as they have the potential to shorten treatment to four months or less. This class includes: Gat, MXF, which have an anti-TB activity four times more potent than other fluoroquinolones. Older and cheaper fluoroquoinolones which are also used in the treatment of MDR-TB include Lev, Ofl and CIP[4].

Patients diagnosed with MDR-TB based on first-line Drug Susceptibility Testing (DST) results receive a standardised second-line regimen consisting of Gat, Cyc, ethionamide and amikacin. Ethambutol and pyrazinamide

are also added to the regimen based on first-line DST results [5, 6]. In Kenya flouroquinolones are used in the treatment of MDR-TB patients according to WHO standards and are also used in the treatment of other bacterial infections. Treatment of MDR-TB takes 18 to 24 months and must be done on the basis of susceptibility testing, it is impossible to treat such patients without this information. If treating a patient with suspected MDR-TB, the patient should be started on streptomycin, isoniazid, rifampicin, ethambutol, pyrazinamide, MXF and Cyc pending the result of laboratory sensitivity testing[7]. The current WHO recommendations for the drug management of patients suspected to have MDR-TB is the empiric MDR-TB regimen[6].

Fluoroquinolones have been widely promoted and used for the management of community acquired respiratory infections in Kenya including pneumonia. A proportion (not less than 10%) of patients with community acquired pneumonia, have TB and the use of fluoroquinolones in these patients may not only delay the diagnosis of TB but also stimulate the development of fluoroqunilones resistance in MTB. This study was done to check whether there is fluoroquinolone resistance in MTB strains isolated in Kenya.

## Materials and Methods

*Samples*: A total of 216 pre-tested first-line MTB strains were used in this cumulative data design study. The strains were obtained from previous studies at CRDR, KEMRI. They included all first-line drug resistant and randomly selected first-line susceptible strains obtained between 2002 and 2007. The studies from which these strains were obtained had included a Quality Assurance (QA) using supranational reference laboratory in accordance to the WHO guidelines on DST procedures[8].

**Table 1: Spectrum of first-line strains used in the study.**

| First-line resistant strains (78) | First-line susceptible strains |
|---|---|
| Isoniazid related resistant strains (73) | 138 |
| Rifampicin related resistant strains (25) | |
| Streptomycin related resistant strains (24) | |
| Ethambutol related resistant strains (11) | |
| Multi-drug resistant strains (25) | |

*Laboratory procedures:* Subcultures were first done on Lowenstein Jensen (LJ) to obtain fresh strains. Drug susceptibility testing (DST) was performed on LJ media using proportion method. The drug that was used was Gat[8].

Drug containing LJ slopes were made by adding appropriate amounts of drugs aseptically to LJ media before inspissation. First a stock solution was prepared and the drug solution sterilised using a membrane filter with a size of 0.45µm so as to make the solutions aseptic. Suitable working concentrations of the drug were made using sterile distilled water and then added aseptically to the LJ media. Standard volume of the media was then dispensed into sterile universal bottles and then inspissated for 45 minutes to 1 hour at a temperature of 85°C (6). The final drug concentration used was 0.5 µg/ml critical concentration[8].

Each bacterial suspension was prepared by adding approximately 4 mg moist weight of the test sample of the bacterial mass visualized as 2/3 loopful of a 3mm internal diameter, 24 standard wire gauge wire loop into 1 ml of sterile distilled water in a 7 ml Bijou bottle containing three 3mm glass beads. This suspension was vortexed for 30 seconds to produce a uniform suspension of 1.0 McFarland turbidity standard ($10^7$ CFU/ml). A standardized inoculum of 0.1 ml of the bacterial suspension was inoculated onto drug-free and drug containing LJ slopes using a loopful of a 3 mm diameter, 27 standard wire gauge wire loop. These cultures were incubated at a temperature of 37°C for four weeks and then interpreted[8].

*Interpretation of results*: The results were interpreted as either fully resistant or susceptible according to standard methods each isolate was taken and the number of colonies resistant to each drug concentration was expressed as a percentage of the number of colonies growing on the drug-free slope. The critical proportion regarded as 1%[8].

*Quality control:* All manipulations were done in a class II biosafety conditions. Control strain MTB H37Rv and known resistance strains were included in each new batch of media. LJ media was prepared using fresh eggs (less than seven days old) and sterility check was carried out on all batches of media by incubating a few slopes of the LJ media randomly at 37°C for at least five days and checked daily to ensure that there was no contamination. All batches of media for DST were stored at 4°C for not longer than four weeks from date of preparation. Preparation of suspensions from each strain was done using individual sterile wire loops per strain to avoid cross-contamination between the strains[9].

*Ethical considerations:* This study was approved by both the Kenya Medical Research Institute (KEMRI) Scientific Steering Committee (SSC) and the National Ethical Review Committee (ERC).

*Data analysis:* Using S.P.S.S. computer data analysis programme, data was analysed using chi-square to compare resistance and susceptibility among the drugs and to compare resistance and susceptibility between the first-line susceptible and resistant strains[10]. The data was presented in form of tables.

## Results
Of the 216 strains tested, 32 [32/216 (14.8%)] showed resistance to second-line drugs. Of these 7 [7/32 (21.9%)] were fully resistant to Gat. Of the 25 MDR-TB strains, 4 (16%) were fully resistant to Gat and 21 (84%) were sensitive to it (Table 2). Of the 7 strains, 6 (41.2%) were mono-resistant to Gat and one combined resistance with ethionamide.

Of the 25 rifampicin resistant strains tested, 21 (84%) were found to be sensitive and 4 (6%) were fully resistant to Gat. Of the 73 isoniazid resistant strains, 67 (91.8%) were sensitive and 6 (8.2%) were fully resistant to Eth. Of the 11 ethambutol resistant strains, 9 (81.8%) were sensitive and 2 (18.2%) fully resistant to Eth. Of the 24 streptomycin resistant strains, 22 (91.7%) were sensitive and 2 (8.3%) were fully resistant to Eth (Table 3).

**Table 2: Susceptibility profile of gatifloxacin**

|  | Sensitive n (%) | Fully resistant n (%) | Total n (%) |
|---|---|---|---|
| Susceptibility profile of the 216 MTB strains to Gat. | 209 (96.76) | 7 (3.24) | 216 (100) |
| Susceptibility profile of the 138 first-line sensitive MTB isolates to Gat | 137 (99.27) | 1 (0.72) | 138 (100) |
| Susceptibility profile of the 78 first-line resistant MTB isolates to Gat | 72 (92.3) | 6 (7.69) | 78 (100) |
| Susceptibility profile of the 25 MDR–TB strains | 21 (84) | 4 (16) | 25 (100) |

**Table 3: Susceptibility pattern of first-line resistant isolates resistant to gatifloxacin**

| First-line anti-TB drugs (n) | Sensitive n (%) | Fully resistant n (%) | P-value |
|---|---|---|---|
| Rifampicin (25) | 21 (84) | 4 (6) | 0.004 (*) |
| Isoniazid (73) | 67 (91.8) | 6 (8.2) | 0.007 (NS) |
| Ethambutol (11) | 9 (81.8) | 2 (18.2) | 0.016 (NS) |
| Streptomycin (24) | 22 (91.7) | 2 (8.3) | 0.176 (NS) |

*- statistically significant, NS-not statistically significant.

## Discussion
Fluoroquinolones have broad-spectrum antimicrobial activity and so are widely used for the treatment of bacterial infections of the respiratory, gastrointestinal and urinary tracts, as well as sexually transmitted diseases and chronic osteomyelitis[11, 12]. They have been demonstrated to be active in vitro and in vivo against mycobacteria species and effective in the treatment of infections caused by MTB, *M. leprae* and atypical mycobacteria such as *M. fortuitum*[13]. They exhibit very good bactericidal activity against mycobacteria and act by binding to complexes of bacterial DNA and DNA gyrases, thereby interfering with DNA replication[14].

This study on MTB fluoroquinolone resistance is the first of its kind in Kenya. Resistance was found in MTB strains isolated in Kenya. This resistance may jeopardize the potential for these drugs as a second-line anti-TB agents in the programmatic management of drug-resistant TB and creating incurable TB strains. Researchers have identified an MTB protein that confers fluoroquinolone resistance via a novel mechanism[14].

Fluoroquinolone resistance in MTB can develop after as little as 13 days of fluoroquinolone therapy[22]. Kenya's pharmaceutical industry is highly liberalized and there is hardly any pharmcovigilance for quality of drugs circulating in the Kenyan market. The presence of fluoroquinolone counterfeit drugs or drugs of inferior or

unknown quality could also stimulate the development of drug resistance even if those drugs are used rationally. Fluoroquinolone resistance occurred in both MDR-TB and Non-MDR-TB strains in this study. MDR-TB strains showing in vitro resistance to fluoroquinolones be a pre-requisite for extensively drug-resistant (XDR-TB) strains which is defined as MDR-TB strain that is resistant to an injectable second-line aminoglycoside (e.g. kanamycin) and one fluoroquinolone. It has been documented before that fluoroquinolone resistance in MTB seems to develop very rapidly[15].

This study showed one strain that was susceptible to all first-line drugs but was resistant to Gat. In Kenya fluoroquinolones are mainly used in the treatment of urinary tract infections, gonorrhea and drug-resistant TB. Patients with prior exposure to any of the fluoroquinolones are likely to develop resistance to other fluoroquinolones. Being broad spectrum antibacterial agents, their widespread and indiscriminate use, often in sub-therapeutic doses, is likely to rapidly enhance fluoroquinolone-resistant organisms, including mycobacteria[16]. Widespread use of these antibiotics for the treatment of other bacterial infections may select for fluoroquinolone-resistant MTB strains[17], but the extent to which this use can have an impact on the selection of fluoroquinolone- resistant mutants of MTB is not known[18]. Recent studies showed that previous fluoroquinolone use and MDR-TB were associated with its resistance in MTB strains[19, 20]. There is also possible danger of development of fluoroquinolone resistance if a patient co-infected with MTB is treated with a fluoroquinolone for another infection, pharmacovigilance is required[17].

In a study done be Peng Xu et al, fluoroquinolone resistance was independently associated with resistance to at least one first-line drug and prior TB treatment[21]. In this study, resistance to Gat was also statistically associated with first-line anti-TB resistance.

## Conclusions

Presence of Gat resistance especially in MDR-TB patients may trigger XDR-TB. Therefore strict drug adherence among MDR-TB should be implemented. Overuse and inappropriate use of fluoroquinolones may also lead to the loss of these drugs as effective medications against MDR-TB or other diseases. Fluoroquinolones resistance was correlated with first-line drug resistance statistically, suggesting the need for routine fluoroquinolones susceptibility testing in patients with these characteristics.

## Recommendations

Presence of fluoroquinolone-resistant TB in Kenya may call for DST to second-line drugs for MDR-TB patients before start of therapy since fluroquinolones are key drugs. There should also be restriction on the use of fluoroquinolones so as to preserve them for future use in MDR-TB treatment.

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