International Journal of Mycobacteriology xxx (2016) xxx–xxx

Available at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/IJMYCO

Full Length Article

Fluoroquinolone consumption and -resistance trends in *Mycobacterium tuberculosis* and other respiratory pathogens: Ecological antibiotic pressure and consequences in Pakistan, 2009–2015

S. Shakoor [a,*], S. Tahseen [b], K. Jabeen [a], R. Fatima [c], F.R. Malik [a], A.H. Rizvi [b], R. Hasan [a]

[a] Aga Khan University, Karachi, Pakistan
[b] National Reference Laboratory (NRL), National TB Control Programme, Pakistan
[c] National TB Control Programme, Pakistan

ARTICLE INFO

Article history:
Received 19 July 2016
Accepted 26 July 2016
Available online xxxx

Keywords:
Consumption
Defined daily dose
Fluoroquinolone
Resistance
Selective pressure
Tuberculosis

ABSTRACT

Objective/background: Fluoroquinolones (FQs) are important anti-tuberculous drugs for the treatment of multidrug-resistant (MDR) tuberculosis. Resistance to FQs leads to fewer options for treatment of tuberculosis (TB), and infection with such strains may also require longer treatment duration. Trends of resistance in *Mycobacterium tuberculosis* (MTB) are indicators of MTB-resistance evolution. Drivers of such resistance need to be understood and studied to inform preventive strategies.

Methods: Here, we present FQ-resistance rates and trends in Pakistan from 2010 to 2015 and compare rates with FQ-consumption data and rates in other community pathogens.

Results: Our results reveal a recent decrease in FQ-resistance rates in MTB, but an increase in resistance for *Haemophilus influenzae* and *Shigella* spp. Correlation of FQ resistance with FQ consumption at the population level was weak for MTB, although strong associations were noted for *H. influenzae* and *Shigella* spp.

Conclusion: We discuss the possible reasons for the decrease in resistance rates in TB, putative drivers of resistance other than volume of FQ consumption, and the possible impact of the National Tuberculosis Programme and drug regulatory activities.

* Corresponding author at: Department of Pathology, Laboratory Medicine, and Paediatrics, Aga Khan University, Stadium Road, P.O. Box 3500, Karachi 74800, Pakistan.
E-mail address: sadia.shakoor@aku.edu (S. Shakoor).

Peer review under responsibility of Asian African Society for Mycobacteriology.
http://dx.doi.org/10.1016/j.ijmyco.2016.07.008

Please cite this article in press as: S Shakoor et al. Fluoroquinolone consumption and -resistance trends in Mycobacterium tuberculosis and other respiratory pathogens: Ecological antibiotic pressure and consequences in Pakistan, 2009–2015. Int. J. Mycobacteriol. (2016), http://dx.doi.org/10.1016/j.ijmyco.2016.07.008

Introduction

Fluoroquinolones (FQs) are essential drugs for the treatment of multidrug-resistant (MDR) tuberculosis (TB). Increasing rates of resistance worldwide have sparked concerns over the driving factors of FQ resistance *in vivo* and in the environment [1]. Environmental factors contributing to resistance include residual concentration of FQs in food, water, animal feed, and also subinhibitory concentrations of FQs used to treat various community acquired infections [2]. This phenomenon may be especially pronounced in countries with weak healthcare systems. Such environmental stressors have also led to increases in FQ-resistance rates in other community pathogens, such as *Salmonella* spp., *Shigella* spp., *Neisseria gonorrhoeae*, and *Streptococcus pneumoniae* [1]. A directly proportional relationship is expected between the volume of FQ use in the community at the population level (selective pressure in the environment) and emergence, maintenance, and rise in resistance rates of many organisms [3]. This was demonstrated for *S. pneumoniae* [4], but has not been studied in *Mycobacterium tuberculosis* (MTB).

We previously reported high and increasing FQ-resistance rates among MTB isolates from all over Pakistan from 2005 to 2009 [5,6]. Here, we report subsequent resistance data and correlate population-level FQ-consumption rates in Pakistan with resistance trends in MTB and other pathogens.

Methods

For resistance rates and trends, laboratory records were examined from the Aga Khan University (AKU) clinical microbiology laboratory from 2010 to 2015 for *S. pneumoniae* and *Haemophilus influenzae* among respiratory pathogens, *N. gonorrhoeae* (a venereal disease pathogen), *Salmonella* Typhi, *Salmonella* Paratyphi, *Shigella* spp., and *Vibrio cholerae* among gastrointestinal pathogens, where the use of FQ is common, and for the MTB complex. The AKU clinical laboratories received samples on physician request from all over Pakistan, with more than 200 collection units located throughout the country. Moreover, the mycobacteriology laboratory served as the WHO Supranational Laboratory. Following inception in 2009 of the National TB-reference Laboratory (NRL), and later in 2013 as a result of its expansion to perform culture and drug-susceptibility testing services for clinical management of the patients, the number of samples received and processed at the NRL increased substantially. Therefore, from 2013 to 2015, data from the NRL was also included for MTB.

Laboratory records were retrieved and duplicates removed for FQ resistance reported among pathogens of interest for the years 2010–2015. FQ-susceptibility testing for MTB was performed with the proportion method using ofloxacin (2 μg/mL) against MTB as recommended by the Clinical Laboratory Institute Standards (CLSI) [7]. MTB H37Rv was used as a control for each batch of susceptibility testing. Susceptibility testing of community acquired bacterial pathogens was performed in accordance with CLSI guidelines by disk diffusion (the Kirby-Bauer method) [8]. *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 25922 were used as control organisms.

FQ-sales data from Pakistan in total number of grams sold per year was obtained from IMS Health, Pakistan (IMS Health Inc., Karachi, Pakistan). The IMS Health database is a multi-sourced national database that compiles sales data from registered pharmacies, prescribing physicians, manufacturers, and wholesalers.

Data were analyzed and bar charts created in MS Excel (Microsoft Corp., Redmond, WA, USA). Coefficient-of-regression (r^2) values for trends were calculated in MS Excel (Microsoft Corp.), with an $r^2 \geqslant 0.65$ representing a significant trend [9]. Resistance trends were then correlated with FQ-sales data for MTB and for community pathogens to show a significant FQ-resistance trend.

To correlate resistance rates with FQ sales, we converted sales data (from grams sold annually) to defined daily dose (DDD) per 1000 inhabitants for each year from 2009 to 2014. DDD conversion was based on 2015 WHO guidelines for anatomical therapeutic chemical (ATC) classification [10] and DDD assignment for ciprofloxacin (oral and parenteral), levofloxacin (oral and parenteral), moxifloxacin (oral and parenteral), ofloxacin (oral and parenteral), enoxacin (oral), sparfloxacin (oral), norfloxacin (oral), pefloxacin (oral), and gatifloxacin (oral and parenteral). To convert to 1000 inhabitants per year, estimates for the population of Pakistan for each year were obtained from the World Bank. We also converted resistance data to the logarithm of the odds of resistance (odds = proportion of resistance organisms $(p)/1 - p$; odds converted to the natural logarithm of the odds). DDD-log odds resistant pairs were examined with 1-year gaps (e.g., 2009 DDDs correlated with 2010 resistance rate), given that previous population-level data showed that the effect of change in consumption was observed with a lag of $\geqslant 1$ year [9]. Linear regression (least squares) was then applied to obtain coefficients of determination (R^2) and p values.

The study was exempted from ethical review by the AKU Ethical Review Committee.

Results

Resistance rates in MTB

During the study period, 18,776 MTB strains were isolated, including 8492 (45.2%) that were MDR. The trend in MDR cases showed a non-significant increase ($R^2 = 0.4$) from 2010 to 2015; however, a decrease in MDR rates was observed in 2015 (Fig. S1).

FQ-resistance trends in MTB indicated variable rates (an increase from 2010 to 2011, followed by a consistent rate and a recent decrease in 2015; all MTB, $R^2 = 0.1$; Fig. 1). Among non-MDR MTB strains, FQ resistance increased from 10.3% ($n = 214/2059$) in 2010 to 16.8% ($n = 250/1487$) in 2015 (linear $R^2 = 0.1$), while an insignificant decrease in FQ resistance in MDR TB was observed from 54.6% ($n = 691/1266$) in 2010 to 52.3% ($n = 591/1129$) in 2015 (linear $R^2 = 0.5$).

Resistance rates in community pathogens

Over the 6-year study period, FQ-resistance rates remained consistently high in *Salmonella* Typhi and Paratyphi and *N.*

Please cite this article in press as: S Shakoor et al. Fluoroquinolone consumption and -resistance trends in Mycobacterium tuberculosis and other respiratory pathogens: Ecological antibiotic pressure and consequences in Pakistan, 2009–2015. Int. J. Mycobacteriol. (2016), http://dx.doi.org/10.1016/j.ijmyco.2016.07.008