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KLİNİK ÇALIŞMA
RESEARCH ARTICLE

In vitro effects of ciprofloxacin, levofloxacin and moxifloxacin on *Mycobacterium tuberculosis* isolates

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SUMMARY

In vitro effects of ciprofloxacin, levofloxacin and Moxifloxacin on *Mycobacterium tuberculosis* isolates

Introduction: *Increased tuberculosis prevalence, and isolation of multidrug resistant (MDR) Mycobacterium tuberculosis strains frequently as causative organisms from tuberculosis infections are resulted in increasing need of new anti-tuberculosis drugs. Nowadays, fluoroquinolones known to have fewer side effects than the other drugs used in treatment of tuberculosis are sometimes assessed even as first-line anti-tuberculosis drugs due to their in vitro and in vivo strong activity. It was aimed in this study to investigate phenotypically the fluoroquinolone susceptibility in MDR and non-MDR M. tuberculosis isolates.*

Materials and Methods: *A total of 126 MDR and non-MDR M. tuberculosis isolates from mycobacteriology laboratory of two hospitals in the Aegean Region of Turkey were included in the study. Ciprofloxacin (CIP), levofloxacin (LEV) and moxifloxacin (MXF) susceptibilities were assessed by agar proportion method according to the Clinical and Laboratory Standards Institute (CLSI) recommendations.*

Results: *Twelve (15.2%), 5 (6.3%) and 4 (5.1%) of the MDR M. tuberculosis strains were resistant to CIP, LEV, MXF, respectively [resistance breakpoints (µg/mL); CIP (> 2), LEV (> 1), MXF (> 0.5)] while non-MDR strains were susceptible to CIP, LEV, MXF.*

Conclusion: *Consequently, although high fluoroquinolone susceptibilities were evaluated as a pleasing data in this study, to preserve their efficiency for many years steadily, quinolone usage and resistance increment in MDR M. tuberculosis isolates should be monitored elaborately.*

Key words: *Mycobacterium tuberculosis, fluoroquinolones, multidrug resistance (MDR)*

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ÖZET

***Mycobacterium tuberculosis* kökenleri üzerine siprofloksasin, levofloksasin ve moksifloksasinin in vitro etkinliği**

Giriş: *Tüberküloz prevalansındaki artış ve çok ilaca dirençli (ÇİD) Mycobacterium tuberculosis suşlarının tüberküloz infeksiyonlarından sıklıkla etken organizmalar olarak izole edilmesi, yeni anti-tüberküloz ilaçlara duyulan ihtiyacın artmasına neden olmaktadır. Günümüzde, tüberküloz tedavisinde kullanılan ve diğer ilaçlardan daha az yan etkisi olduğu bilinen florokinolonlar, in vitro ve in vivo güçlü aktiviteleri nedeniyle bazen birinci basamak anti-tüberküloz ilaçlar olarak bile değerlendirilmektedir. Bu çalışmada, ÇİD ve ÇİD olmayan M. tuberculosis izolatlarında florokinolon duyarlılığının fenotipik olarak araştırılması amaçlanmıştır.*

Materyal ve Metod: *Türkiye'de Ege Bölgesi'ndeki iki hastanenin mikobakteriyoloji laboratuvarından ÇİD ve ÇİD olmayan toplam 126 M. tuberculosis izolatı çalışmaya dahil edildi. Siprofloksasin (CIP), levofloksasin (LEV) ve moksifloksasin (MXF) duyarlılıkları, "Clinical and Laboratory Standards Institute (CLSI)" önerilerine göre agar proporsiyon yöntemi ile değerlendirildi.*

Bulgular: *Çok ilaca dirençli M. tuberculosis izolatlarının 12 (%15.2)'si CIP, 5'i (%6.3) LEV ve 4 (%5.1)'ü MXF dirençliydi [direnç sınırları (µg/mL); CIP, LEV (> 1), MXF (> 0.5)]. Çok ilaca dirençli olmayan suşlar ise CIP, LEV, MXF'ye duyarlıydı.*

Sonuç: *Yüksek florokinolon duyarlılıkları bu çalışma için memnuniyet verici bir veri olarak değerlendirilse de uzun yıllar florokinolonların etkinliğini korumak için kinolon kullanımı ve ÇİD M. tuberculosis izolatlarına karşı direnç artışı dikkatle izlenmelidir.*

Anahtar kelimeler: *Mycobacterium tuberculosis, kinolon direnci, çok ilaca direnç (ÇİD)*

INTRODUCTION

Mycobacterium tuberculosis that causes tuberculosis (TB) is the world's most widespread and oldest public health problem, especially in Asian and African countries. It is reported that currently about one third of the world is infected with *M. tuberculosis*. Resistance and high HIV prevalence, mostly due to the treatment of TB with medication, are the most important problems in controlling TB. Drug resistance rates and TB incidence are the most needed data to provide control of this disease (1-5). Isoniazid (INH), rifampicin (RIF), ethambutol (ETB) and pyrazinamide (PZ) are the first choice drugs in TB treatment. *M. tuberculosis* isolates that are "least resistant to INH and RIF" are defined as multi-drug resistant (MDR). The emergence of MDRs is an important problem that makes TB treatment difficult. Depends on the increase in MDR-TB cases and the difficulties in treatment, second-line medications were needed. Due to the irregular and inappropriate use of the drugs in the treatment, extended drug resistant (XDR) *M. tuberculosis* isolates, which are resistant to one of quinolone and parenterally used medicines (kanamycin, capreomycin, amikacin) besides INH, RIF resistance, have begun to be observed as well as the MDR isolates (3,6-8).

Fluoroquinolones (FQs) are a widely-favoured broad-spectrum group used for the treatment of respiratory, gastrointestinal and urinary tract infections, osteomyelitis and sexually transmitted infections, especially. Demonstration of in vitro and in vivo efficacy of FQs against *M. tuberculosis* ensured that these drugs were also included in the treatment of TB. FQs rarely cause serious side effects than other drugs used for the treatment of TB, they are suitable to provide the appropriate dosage range and is also advantageous because of high bioavailability. Ciprofloxacin (CIP) and ofloxacin (OFX) is at least effective against TB bacilli, levofloxacin (LEV), gatifloxacin (GTX), moxifloxacin (MXF), and sparfloxacin (SPX), which is reported as the most effective FQs. It is also stated that powerful FQs such as MXF and LEV may be considered as first-line drugs to shorten the duration of treatment in susceptible cases (6,9-12). FQs exhibit bactericidal effects against *M. tuberculosis* via the DNA gyrase enzyme, a Type II topoisomerase encoded by gyrA and gyrB genes. FQ resistance in *M. tuberculosis* has been observed to be a consequence of the presence of mutations, low cell wall permeability, and efflux pump systems in or around the "quinolone resistance determining region (QRDR)" on gyrA/gyrB in general (6,9-14).

In this study, it was aimed to investigate phenotypically the resistance against FQ antibiotics CIP, LEV and MOX by agar proportion method in MDR and non-MDR *M. tuberculosis* isolates from various clinical specimens in the Aegean region of Turkey.

MATERIALS and METHODS

A total of 126 *M. tuberculosis* isolates between 1999-2008 from Ege University Faculty of Medicine, Department of Medical Microbiology, Mycobacteriology Laboratory and between 2006-2010 from Dr. Suat Seren Chest Diseases and Thoracic Surgery Training and

Research Hospital, Medical Microbiology Laboratory were studied. Seventy nine (62.7%) of these isolates were MDR. FQ susceptibilities in the isolates were phenotypically determined by the agar proportion method in accordance with the Clinical and Laboratory Standards Institute (CLSI) recommendations (15). Stok solutions of CIP (Koçak Farma, Turkey), LEV (Sanofi Aventis, Turkey) and MXF (Bayer AG, Turkey) were prepared and sterilised by filtration. Middlebrook (MB) 7H10 agar (BD & Difco) supplemented with 10% oleic acid, albumin, dextrose and catalase (OADC) was used as a growth medium. The dehydrated medium was prepared and autoclaved according to the manufacturer's recommendations. The OADC solution and two-fold dilution series of antibiotic solutions were added to the sterilized medium, kept at 50-55°C in a water bath, and allowed to solidify after poured into separated plates. Antibiotic-free medium were also prepared for growth control. The isolates were adjusted to a McFarland No: 1, and then diluted to 10^{-2} ve 10^{-4}. Antibiotic-free (control) and antibiotic media were inoculated with 100 µL of dilutions of bacterial suspensions. Plates were wrapped in parafilm and incubated for three weeks in an incubator with a CO_2 ratio of 5% at 37°C. The concentration of antibiotic in the plate, which inhibited more than 99% of the growth compared to the control, was accepted as the minimal inhibitor concentration (MIC) for that strain [Breakpoints: CIP (> 2 µg/mL), LEV (> 1 µg/mL), MXF (> 0.5 µg/mL)]. *M. tuberculosis* H37Rv (ATCC 27294) was used as a control strain in the study.

RESULTS

Among the *M. tuberculosis* strains, 47 non-MDR isolates were all sensitive to CIP, LEV and MXF. On the other hand, among the 79 MDRs, 12 (15.2%) isolates were found to be resistant to CIP, 5 (6.3%) isolates resistant to LEV and 4 (5.1%) isolates resistant to MXF. CIP, LEV and MXF sensitivity ratios, MIC ranges (µg/mL), MIC_{50} and MIC_{90} values for *M. tuberculosis* strains were given in Table 1.

DISCUSSION

Tuberculosis, mainly due to migration from endemic regions, HIV epidemics and the presence of MDR *M. tuberculosis* isolates, has increased again since the 1980s. Today, it continues to be important as an infectious disease that has problems in control and results in high mortality, despite the improvement of diagnosis and treatment methods. The recognition of the treatment and resistance mechanisms of MDR-TB patients is accepted as a fundamental component of TB control. In countries with high TB incidence, high rates of resistance also indicate a relationship between incidence and resistance (2,14,16). In recent years, about 2-64% of MDR *M. tuberculosis* has been reported between 2000 and 2014 in different countries of the world and in different regions of our country. It is also stated that the rate of MDRs is low in new cases but it can be increased because of the use of antibiotics in patients who have already received TB treatment (1,2, 4,5,16-19).

Resistance in *M. tuberculosis* causes from natural resistance genes or spontaneous mutations. It is not possible to transfer the resistance genes or plasmids among these bacteria (2). FQ resistance in *M. tuberculosis* is thought to be led to primarily by gyrA mutations, then by drug permeability reduction and by efflux pump systems. Cross resistance against FQs and sometimes different group antibiotics can be seen in *M. tuberculosis* in consequence on common resistance mecha-

Table 1. Fluoroquinolone (FQ) susceptibilities in MDRs and non-MDRs

Isolates (n)	FQs	Susceptibility % (n)	MIC_{50} (µg/mL)	MIC_{90} (µg/mL)	MIC range (µg/mL)
MDRs (79)	CIP	84.8 (67)	1	2	0.25-2 <
	LEV	93.7 (74)	0.5	1	0.125-1 <
	MXF	94.9 (75)	0.25	0.5	< 0.06-0.5 <
Non-MDRs (47)	CIP	100 (47)	1	1	< 0.25-1
	LEV	100 (47)	0.5	1	< 0.125-1
	MXF	100 (47)	0.25	0.5	< 0.06-0.5

MIC: Minimum inhibitory concentration, MDR: Multi-drug resistant, CIP: Ciprofloxacin, LEV: Levofloxacin, MXF: Moxifloxacin, MIC_{50} and MIC_{90}: The antibiotic concentration inhibiting 50% and 90% of the bacteria, respectively, n: Number of isolates.

nisms (9,18,20-22). In a study comparing the efficacy of MXF, GTX and OFX on *M. tuberculosis*, MFX and GTX were found to be more effective than OFX, and cross resistance against these antibiotics were determined. It was stated that an isolate resistant to the all three FQs with no mutation in the gyrA or B, probably had different resistance mechanisms (22). Escribano et al. (2007) showed efflux pump systems effective for both FQ and linezolid resistance in *M. tuberculosis (20)*. Since FQs are commonly used against resistant *M. tuberculosis*, acquired FQ resistance is often associated with resistance to RIF and other first-line drugs. A study in Taiwan found that there was an association between resistance to FQ and resistance to any of the first-generation TB medicines, except streptomycin, and that this association was stronger in MDRs. The rate of FQ resistance was reported to be 3.3% when 420 *M. tuberculosis* isolates selected from the 2004-2005 period were considered and 19% when only the MDR cases were evaluated. Susceptibilities of CIP, LEV, MXF of the isolates were indicated as 98.6%; 98.6%; 97.6%, respectively. In another study, the rate of OFX resistance in the isolates from 640 patients in the USA between 2002 and 2006 was 2.5%. In addition, it has been reported that the use of FQ for more than 10 days, especially 60 days before the diagnosis of TB for any reason, may cause FQ resistance in *M. tuberculosis* (21). In a study about MDR *M. tuberculosis* in Pakistan, FQ resistance was tested using CIP between the isolates from 2005-2008, and OFX beginning from 2009. The reported rates of FQ resistance were increased from about 17.41% to 42.92%, and it was declared that this increase might be due to uncontrolled and irregular use of FQ for the treatment of TB or other infections (24). As opposed to the studies which claim that previously usage of FQs or longer exposure due to any infection causes resistance in *M. tuberculosis*, Wang et al. (2007) (18) stated in their study these factors did not affect FQ susceptibility. Patients whose strains in our study have been isolated have not been able to obtain any information on FQ exposure and duration in previous periods. However, according to our data, whereas there is no FQ resistance in non-MDRs, its presence in MDRs, supports the idea that to being MDR would be a factor that increases the tendency to FQ resistance.

In this study, quite good susceptibility results were determined for CIP nearly over 80%, and for LEV and MXF over 90%. Similarly, Özkütük et al. (2008), in their study which was detected the efficacy of second-line drugs against MDR *M. tuberculosis* isolates from our region; found a high susceptibility rate of 95% for OFX used as representative of FQs (8). The tests used to detect resistance to second-line TB drugs are mostly difficult, time consuming, expensive, not fully standardized methods, and are generally indicated that to be appropriate evaluating by experienced laboratories. For this reason, although there are numerous studies on the susceptibility of *M. tuberculosis* to first-line drugs, limited research on susceptibility to FQs and other second-line drugs has been found. In recent years, in addition to standard and reference method "agar proportion method", also used in this study, a number of new phenotypic and genotypic tests that are advantageous in terms of speed, time to completion, cost and ease of use aiming diagnosis of MDR-TB and XDR-TB have been developed. In other respects, although genotypic tests that determine the mutations responsible for resistance have yielded fairly fast and reliable results, these tests have not always been able to identify the phenotypic resistance due to mutations that have not yet been discovered and some different resistance mechanisms (6,17,25).

Currently, FQs are indicated as an alternative to first-line anti-tuberculosis drugs, especially in resistant cases. When the results of this study are evaluated, high FQ susceptibility rates in MDRs and non-MDRs are pleasing, but the resistance rates are also expected to rise in time depend on increased use. For this reason, it is thought that the spread of resistant strains would be controlled rapidly by monitoring the resistance particularly in MDR *M. tuberculosis* isolates against FQs and second-line alternative drugs, and also developing and acquiring the related rapid, easy, safe methods to a large number of laboratories.

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REFERENCES

1. *Alışkan HE, Bostanoğlu E, Turunç T, Çolakoğlu Ş, Demiroğlu YZ, Kurşun E, et al. Retrospektif olarak tüberküloz laboratuvarının altı yıllık sonuçları ve antimikobakteriyel ilaçlara direnç oranları. Turk Toraks Derg 2013;14:53-8.*
2. *Bozdağ İ, Coşar AD, Uysal EB, Özer A. Klinik örneklerden izole edilen Mycobacterium tuberculosis kompleks suşlarının antibiyotiklere direnç oranları. Tıp Araş Derg 2015;13(1):6-10.*
3. *Cesur S, Şimşek H. Çoklu ilaca ve yaygın ilaca dirençli tüberküloz için hızlı tanı yöntemleri. Ortadoğu Med J 2015;7(2):85-90.*
4. *Öz Y, Aslan M, Akşit F, Durmaz G, Kiraz N. Mycobacterium tuberculosis kompleks izolatlarının primer antitüberküloz ilaçlara duyarlılığının değerlendirilmesi. ANKEM Derg 2012;26(1):20-4.*
5. *Uysal EB, Kaya H. Klinik örneklerden izole edilen Mycobacterium tuberculosis kompleks suşlarının major anti-tüberküloz ilaçlara duyarlılıkları. Tıp Araş Derg 2014;12(2):67-70.*
6. *Eldin AS, Mostafa NM, Mostafa SI. Detection of fluoroquinolone resistance in Mycobacterium tuberculosis clinical isolates as determined by gyrA/B gene mutation by using PCR technique. Egyptian J Chest Dis Tub 2012;61:349-53.*
7. *Özkara Ş. Yaygın ilaç dirençli tüberküloz (YİD-TB). Sol Hast 2007;18:88-92.*
8. *Özkütük N, Sürücüğlu S, Gazi H, Coşkun M, Özkütük A, Özbakkaloğlu B. Second-line drug susceptibilities of multidrug-resistant Mycobacterium tuberculosis isolates in Aegean Region-Turkey. Turk J Med Sci 2008;38(3):245-50.*
9. *Mdluli K, Ma Z. Mycobacterium tuberculosis DNA gyrase as a target for drug discovery. Inf Disorders - Drug Targets 2007;7(2):159-68.*
10. *Malik S, Willby M, Sikes D, Tsodikov OV, Posey JE. New insights into fluoroquinolone resistance in Mycobacterium tuberculosis: functional genetic analysis of gyrA and gyrB mutations. PLoS ONE 2012;7(6):e39754.*
11. *Mayer C, Takiff H. The molecular genetics of fluoroquinolone resistance in Mycobacterium tuberculosis. Microbiol Spectrum 2014;2(4):MGM2-0009-2013.*
12. *Rubinstein E, Keynan Y. Quinolones for mycobacterial infections. Int J Antimic Agents 2013;42(1):1-4.*
13. *Evranos-Aksöz B. Tüberküloz tedavisinde yeni ilaç adayları. Türk Hij Den Biyol Derg 2014;71(4):207-20.*
14. *Kaya NM, Sarıbaş Z. Mikobakterilerde dışa atım pompaları ve ilaç direnci. Türk Mikrobiyol Cem Derg 2012;42(3):81-4.*
15. *Clinical and Laboratory Standards Institute (CLSI). Susceptibility testing of mycobacteria, nocardiae, and other aerobic actinomycetes; Approved standard-2nd ed. CLSI Document M24-A2. 2011, Wayne, PA.*
16. *Perincek G, Tabakoğlu E, Otkun M, Özdemir L, Özdemir B. Mycobacterium tuberculosis üremesi saptanan akciğer tüberkülozlu hastaların antitüberküloz ilaçlara direnç oranları. Turk Toraks Der 2011;12:111-3.*
17. *Baykal ES, Güdücüoğlu H, Yaman G, Berktaş M. Van yöresinde izole edilen Mycobacterium tuberculosis suşlarının dört farklı yöntemle antimikobakteriyel ajanlara duyarlılık tespiti. Tuberk Toraks 2014;62(2):122-30.*
18. *Wang J-Y, Lee L-N, Lai H-C, Wang S-K, Jan I-S, Yu C-J et al. Fluoroquinolone resistance in Mycobacterium tuberculosis isolates: associated genetic mutations and relationship to antimicrobial exposure. J Antimicrob Chemother 2007;59:860-5.*
19. *Agarwal M, Gunal S, Durmaz R, Yang Z. Integration of Mycobacterium tuberculosis drug susceptibility testing and genotyping with epidemiological data analysis to gain insight into the epidemiology of drug-resistant tuberculosis in Malatya, Turkey. J Clin Microbiol 2010;48(9):3301-5.*
20. *Escribano I, Rodriguez JC, Llorca B, Garcia-Pachon E, Ruiz M, Royo G. Importance of the efflux pump systems in the resistance of Mycobacterium tuberculosis to fluoroquinolones and linezolid. Chemother 2007;53:397-401.*
21. *Devasia RA, Blackman A, Gebretsadik T, Li H, Maruri F, Shintani A, et al. Fluoroquinolone resistance in Mycobacterium tuberculosis, the effect of duration and timing of fluoroquinolone exposure. Am J Crit Care Med 2009;180:365-70.*
22. *Groll AV, Martin A, Jureen P, Hoffner S, Vandamme P, Francoise P, et al. Fluoroquinolone resistance in Mycobacterium tuberculosis and mutations in gyrA and gyrB. Antimicrob Agents Chemother 2009;53(10):4498-500.*
23. *Devasia R, Blackman A, Eden S, Griffin M, Shintani A, May C et al. High proportion of fluoroquinolone-resistant Mycobacterium tuberculosis isolates with novel gyrase polymorphisms and a gyrA region associated with fluoroquinolone susceptibility. J Clin Microbiol 2012;50(4):1390-6.*
24. *Jabeen K, Shakoor S, Chishti S, Ayaz A, Hasan R. Fluoroquinolone resistant Mycobacterium tuberculosis, Pakistan, 2005-2009. Emerg Infect Dis 2011;17(3):564-6.*
25. *Kiet VS, Lan NTN, An DD. Evaluation of the MTBDRsl test for detection of second-line-drug resistance in Mycobacterium tuberculosis. J Clin Microbiol 2010;48(8):2934-9.*