EFFICACY OF AMIKACIN AND CIPROFLOXACIN AGAINST CLINICAL ISOLATES OF MYCOBACTERIUM TUBERCULOSIS

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Background: Tuberculosis was a leading cause of death at the turn of the 20th century and continues to be one of the medical scourges of mankind. Before the availability of antimicrobial drugs the cornerstone of treatment was rest in the open air in sanatoria. The major breakthrough in treatment of tuberculosis came with the discovery of Streptomycin. Later, INH, Ethambutol, Pyrazinamide, Rifampicin were added to the arsenal. Objective of this study was to determine the sensitivity of clinical isolates of Mycobacterium tuberculosis against two second-line anti-tuberculosis drugs, Amikacin and Ciprofloxacin. **Methods:** This cross-sectional study was conducted at Department of Microbiology, Armed Forces Institute of Pathology (AFIP) Rawalpindi. All routine clinical samples received for acid fast bacilli (AFB) in the Department of Microbiology, AFIP, Rawalpindi were processed by modified Petroff's technique and inoculated on Lowenstein Jensen (LJ) medium and Bactec 460 Mycobacterium tuberculosis culture system. After identification of M. tuberculosis sensitivity was performed against first-line anti-tuberculosis drugs. Then susceptibility of M. tuberculosis isolates against Amikacin and Ciprofloxacin was performed on LJ medium. H37Rv was used as control strain. **Results:** Results were interpreted using resistance ratio method. Out of 100 M. tuberculosis isolates, 98% were sensitive to Amikacin and 97% to Ciprofloxacin. **Conclusion:** Amikacin and Ciprofloxacin are very effective 2nd line anti-tuberculosis drugs against tuberculosis isolates in our set-up.

Keywords: Tuberculosis, MDR-tuberculosis, susceptibility, second-line anti-tuberculosis drugs

INTRODUCTION

Tuberculosis was a leading cause of death at the turn of the 20th century and continues to be one of the medical scourges of mankind.[1] Before the availability of antimicrobial drugs the cornerstone of treatment was rest in the open air in sanatoria. The major breakthrough in treatment of tuberculosis came with the discovery of Streptomycin. Later, INH, Ethambutol, Pyrazinamide, and Rifampicin were added to the arsenal.[2]

Patients who take the prescribed drugs irregularly have an increased chance of developing acquired drug resistant tuberculosis.[3] The regions where tuberculosis is more prevalent, lack the resources to implement appropriate measures to control the disease. Due to financial constraints drug susceptibility testing is still restricted to a few centres in our setup. However advanced centres in march against tuberculosis have also incorporated molecular techniques in diagnosis and susceptibility testing to save the turnaround time.[4,5] There has been resurgence of tuberculosis in both developed and developing countries since early 1980s.[6] The standard treatment as recommended by the World Health Organization (WHO) is multidrug regimen that includes four antibiotics (Rifampicin, INH, Pyrazinamide, Ethambutol or Streptomycin).[7] When a M. tuberculosis strain is resistant to INH and Rifampicin (Multi-drug resistant tuberculosis, MDR-TB), the effectiveness of standard treatment is diminished by 15–77%.[8] Recently a new strain of M. tuberculosis has emerged called as extensive drug resistant tuberculosis M. tuberculosis (XDR-TB). This type of tuberculosis is caused by a strain of Mycobacterium tuberculosis resistant to INH, Rifampicin, and in addition, to any fluoroquinolone and at least 1 of 3 injectable drugs, Capreomycin, Kanamycin and Amikacin.[9]

In the light of this situation this study was organised to find the sensitivity of M. tuberculosis isolated from various specimens against Amikacin and Ciprofloxacin the suggest second-line drugs in case of resistance to first-line anti-tuberculosis drugs.

MATERIAL AND METHODS

The study was carried out at Microbiology Department, Armed Forces Institute of Pathology (AFIP) Rawalpindi. All routine clinical samples received for acid fast bacillus culture and yielding positive growth on LJ media were included in the study. The isolates were from sputum (n=71), bronchoalveolar lavage (n=9), fine needle aspiration (n=6), lymph nodes (n=6), pleural fluid (n=4), endometrium (n=4), a total of 100.

The M. tuberculosis isolates after sensitivity to first-line anti-tuberculosis drugs were selected and grouped based on the following criteria:

Group-1: Isolates which were susceptible to all first-line anti-tuberculosis drugs (n=34)

Group-II: Isolates which were resistant to one or more anti-tuberculosis drugs but not both INH and Rifampicin (n=40)

Group-III: Multidrug resistant (MDR) isolates, which were resistant to both INH and Rifampicin simultaneously (n=26)

Antimicrobial susceptibility testing was done by agar dilution method using LJ medium.[10] Stock solutions of antibiotics were prepared and sterilised by passing through a filter (0.22 µm). The stock solutions were stored at 4 ºC. Antibiotics were used in different strengths.[11,12] The concentrations of Amikacin and Ciprofloxacin used were 1, 2, 4, 8, 16, and 32 µg/ml.

The antibiotic solution of the required concentration was added to LJ medium and set in the slopes and inspissated at 80 ºC. The growth of the Mycobacterium tuberculosis was scraped from fresh LJ slants and suspended in 5 ml of distilled water. It was homogenised with glass beads by vortexing and turbidity was adjusted to Macfarland standard 1 with distilled water. Three drops of this suspension were added to the drug containing bottles. The bottles were incubated at 37 ºC and were aerated twice a week for 3 weeks. Control strain used was H37Rv, which was inoculated on a parallel set of slopes.[10]

Minimum inhibitory concentration (MIC) was defined as the lowest concentration of the antibiotic that inhibited the growth. Growth was considered to be inhibited if less than 20 colonies appeared on the LJ slope.[10] Minimum inhibitory concentration (MICs) of the organisms were noted. MICs of the test strains were compared with the control strains inoculated along with the batch tested. Results were evaluated by resistance ratio method, (MIC of test/MIC of control). A ratio of ≤2 was considered as sensitive. A ratio of ≥4 was considered as resistant.[10]

RESULTS

The entire Group-I and Group-II isolates were sensitive to Amikacin with a resistance ratio of 2 (n=63), and 1 (n=13). In Group-III, 25 isolates were sensitive while 1 isolate was resistant. Mean percentage sensitivity of 3 groups in combination against Amikacin was 98%, (Table-1).

The entire Group-I and Group-II M. tuberculosis isolates were sensitive to Ciprofloxacin while 2 MDR M. tuberculosis isolates were resistant to Ciprofloxacin, (Table-1). In combination, mean percentage sensitivity of the isolates to Ciprofloxacin was 97.4%.

Table-1: Susceptibility of M. tuberculosis isolates against 2nd line anti-TB drugs separately and in combination

Drug	Percentage Sensitivity			
	Group-I (n=34)	Group-II (n=40)	Group-III (n=26)	Total (n=100)
Amikacin	100%	100%	96%	98.7%
Ciprofloxacin	100%	100%	92.3%	97.4%
Amk+Cip	100%	100%	100%	100%

DISCUSSION

Prevention of MDR- tuberculosis is very important as it is not only beneficial to the patients but also it is cost effective. Tuberculosis and especially MDR-TB is an ongoing challenge. A concerted effort by the family, physician, microbiologist, Health Department, and volunteers is required.

In the last decade there has been renewed interest in infections caused by M. tuberculosis. This has been due to resurgence of tuberculosis cases globally. Tuberculosis caused by drug resistant strains of M. tuberculosis is a therapeutic challenge for the clinician.

In our study two antimicrobial compound were investigated for sensitivity testing of M. tuberculosis. Ninety-seven percent of M. tuberculosis isolates were sensitive to ciprofloxacin regardless of susceptibility to first-line anti-tuberculosis drugs. Minimum inhibitory concentrations (MICs) of susceptible M. tuberculosis strains were lower than drug resistant M. tuberculosis strains. Two of the isolates resistant to Ciprofloxacin were from MDR-TB group. This is in agreement with another study by Serrano *et al*.[11] There was no relationship between level of resistance to first-line anti-TB drugs and the activity of Ciprofloxacin against M. tuberculosis. It has also been demonstrated in a study by Tomioka *et al*.[13] In a study by Rastogi *et al*, Sparfloxacin demonstrated superior activity as compared to Ofloxacin and Ciprofloxacin (MIC 0.5–1 µg/ml).[12] Ciprofloxacin has been shown to have early bacteriocidal activity against M. tuberculosis, a characteristic shared only with isoniazid. Fattorini *et al* found 20% resistance to fluoroquinolones among drug resistant strains of M. tuberculosis which is quite high as compared to others and our study.[14] In a study carried out by Tomioka *et al*[13] MICs of Ciprofloxacin were higher for Rifampicin resistant isolates of M. tuberculosis which is in agreement with our study. This may be due to use of agar medium which may cause binding of fluoroquinolones to proteins. Hoffner *et al* [15] reported all M. tuberculosis isolates except two MDR strains sensitive to Ciprofloxacin. The MDR strains exhibited higher MICs (4 µg/ml). Various studies have demonstrated resistance to fluoroquinolones in isolates recovered from samples after treatment from patients who did not respond or relapse.[16] In our study resistance to Ciprofloxacin is low at present but it can increase in future if fluoroquinolones are used inadvertently.

All our strains of M. tuberculosis were susceptible to Amikacin with MICs of 2 µg/ml equivalent to resistance ratio of 1 or 2. Only 1 MDR-TB isolate was resistant. Other investigators have reported similar results.[12] In our study strains resistant to Streptomycin were sensitive to Amikacin. This was in accordance with other studies that there is no co-resistance between Streptomycin and other aminoglycosides.[17] Pfyffer *et al* have suggested breakpoint for Amikacin of 1 µg/ml for Bactec and 4 µg/ml for Middlebrook 7H10 agar. Amikacin has demonstrated superior bactericidal activity against M.

tuberculosis with MICs well below maximum concentration (C_{max}) and low MIC/MBC ratio. It has shown low MIC as compared to other aminoglycosides.[18]

In our study we only tested Amikacin which is less toxic as compared to other aminoglycosides like Kanamycin. Its blood levels can be monitored easily. Testing and reporting results of Amikacin favours our physicians who are more conversant with its use.

RECOMMENDATIONS

It is recommended to test resistant M. tuberculosis strains against a broad range of anti-mycobacterial agents, so that it may be possible to design an appropriate treatment regimen. As a fringe benefit of our study we can suggest Amikacin and Ciprofloxacin in treating cases of MDR-TB.

REFERENCES

1. Kochi A. The global tuberculosis situation and the newer control strategy of the world health organization. Tubercle 1991;72:1–6. Reproduced: Bull World Health Organization 2001;79(1):71–5.
2. Haas DW, Prez RMD. Mycobacterial Diseases. In: Mandell GL, Benett JE, Dolin R. (eds). Mandell Douglas and Benett's Principle and Practice of infectious diseases, 6th ed. New York: Churchill Livingstone;2005.p.2213–43.
3. Salfinger. HM, Hale YM, Driscoll JR. Diagnostic tools in tuberculosis. Respiration 1998;65:63–170.
4. Doren GV. Diagnostic mycobacteriology; where are we today? J Clin Microbiol 1996;34:1873–6.
5. Boehme CC, Nabeta P, Hillemann D, Nicol MP, Shenai S, Krapp F, *et al*. Rapid molecular detection of tuberculosis and rifampin resistance. NEJM 2010;363:1005–15.
6. Croften SJ, Chaulet P, Maher D. Guidelines for the management of drug resistant tuberculosis. World Health Organization, Geneva 1997;WHO/TB/96.210:1–47.
7. Karamat KA, Hayat S, Butt T, Abbasi S. Multidrug resistant tuberculosis. Pak Armed F Med J 2000;50(2):114–6.
8. Heymann SJ, Brewer TF, Wilson ME, Fineberg HV. The need for global action against multidrug resistant tuberculosis. JAMA 1999;281:2138–41.
9. Raviglione MC, Smith IM. XDR tuberculosis–Implications for global public health. N Engl J Med 2007;356:656–9.
10. Laidlaw M. Mycobacterium tubercle bacilli. In: Collee JG, Duguid JP, Frazer AG, Marmion BP (eds). Mackie & Mc Cartney Practical Medical Microbiology. 14th ed. London: Churchill Livingstone;1989.p.399–424.
11. Ruiz-Serrano MJ, Alcalá L, Martínez L, Díaz M, Marín M, González-Abad MJ, *et al*. *In vitro* activities of six fluoroquinolones against 250 clinical isolates of M. tuberculosis susceptible or resistant to first line antituberculosis drugs. Antimicrobe Agents Chemother 2000;44:2567–8.
12. Rastogi N, Labrousse V, Goh KS. *In vitro* activities of fourteen antimicrobial agents against drug susceptible and resistant clinical isolates of M. tuberculosis and comparative intracellular activities against the virulent H37Rv strain in human macrophages. Curr Microbiol 1996;33:167–75.
13. Tomioka H, Sato K, Kajitani H, Akaki T, Shishido S. Comparative antimicrobial activity of the newly synthesized quinolones WQ-3034, levofloxacin,. sparfloxacin and ciprofloxacin against Mycobacterium tuberculosis and Mycobacterium avium complex. Antimicrobial Agents Chemother 2000;44:283–6.
14. Fattorini L, Iona E, Ricci ML, Thoresen OF, Orru G, Oggioni MR, *et al.* Activity of 16 antimicrobial agents against drug-resistant strains of Mycobacterium tuberculosis. Microb Drug Resist 1999;5:265–70.
15. Hoffner SE, Gezelius L, Liljequist BO. *In vitro* activity of fluorinated quinolones and macrolides against drug resistant M. tuberculosis. J Antimicrobiol Chemother 1997;40:885–88.
16. Alangaden GJ, Lerner SA. The clinical use of fluoroquinolones for the treatment of mycobacterial disease. Clin Infec Dis 1997;25:1213–21.
17. Hoffner SE, Kallenius G. Susceptibility of streptomycin resistant M. tuberculosis strains to amikacin. Eur J Clin Microbiol Infec Dis 1988;7:188–90.
18. Pfyffer GE, BonatoDA, Ebrahimzadeh A, Gross W, Hotaling J, Kornblum J, *et al*. Multicentre laboratory validation of susceptibility testing of M. tuberculosis against classical second-line and newer antimicrobial drugs by using the radiometric Bactec 460 technique and the proportion method with solid media. J Clin Microbiol 1999;37:3179–86.

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