Original Article

Status of First-Line Anti-TB drugs: An Audit of 84 Clinical Sputum AFB Isolates in Karachi

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ABSTRACT

***Objective*:** To evaluate the current incidence of multidrug-resistant tuberculosis (MDR-TB) among positive sputum culture isolates in two commercial laboratories in Karachi, Pakistan.

***Methodology*:** In this laboratory-based study eighty four AFB smear-positive sputum culture isolates grown on routine Lowenstein-Jensen (LJ) medium in two separate diagnostic labs during 12 months ending August 2011 were identified and subjected to antimicrobial susceptibility testing using LJ medium and anti-TB First-Line drugs (FLD). MICs of the control H37RV-NCTC strain 7416 were compared with the test strains inoculated with the batch tested. Results were evaluated by the traditional resistance ratio method.

***Results*:** The sputa expectorated by 45 females (53.57%) and 39 males (46.43%) aged 15-58 years yielded 84 *M. tuberculosis* isolates. The percentages of FLD resistance were Rifampicin (48.8%), Streptomycin (28.57%), Ethambutol (7.14%), and Isoniazid (4.76%). Kanamycin (28.57%) was also tested. Only 12 (14.28%) of these were sensitive to all the FLDs, and 4 (4.76%) were MDR-TB strains (indifferent to Rifampicin and Isoniazid).

***Conclusion*:** The sparse labs in Karachi that do AFB-cultures should periodically assess and publicize the frequency of MDR-TB isolates to guide empirical therapy. Rifampicin, a consistent part of the initial drug regimen for empirical prescription, was currently least effective on the strains encountered.

KEY WORDS: TB Cultures, First-Line drugs, Drug resistance, MDR-TB.

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INTRODUCTION

A worldwide resurgence of TB is ongoing despite the fact that the WHO declared it a global infectious emergency in 1993. Pakistan alone accounts for 44% of the total TB burden in the Eastern Mediterranean Region comprising 23 countries[1] and the infection distressingly is the second leading cause of adult death in impoverished communities such as those of Karachi.[2]

To further complicate the situation, Pakistan has a high defaulter rate for the completion of TB treatment ranging from 79% in 1997 to 45% in 1999.[3] Indeed, the emergence and spread of MDR and extensively drug-resistant (XDR) TB are facilitated by inadequate detection and flawed treatment.[4]

The standard treatment regimen as recommended by the WHO includes four antibiotics (Isoniazid, Rifampicin, Pyrazinamide, Ethambutol or Streptomycin).[5] When a *M. tuberculosis* isolate is resistant to INH and Rifampicin (MDR-TB), the effectiveness of customary treatment is significantly reduced.[6] Recently, XDR strains of *M. tuberculosis* have emerged which are resistant to at least the two most potent

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anti-TB drugs, INH and Rifampicin, in addition to any fluoroquinolone and to at least one of the injectable second-line drugs, Amikacin, Kanamycin, Viomycin and Capreomycin.[7-9] The frequency of XDR-TB in Pakistan is reported to have increased from 1.5% in 2006 to 4.5% in 2009[9] underlining the extent of incidence and raising concerns despite the fact that the XDR rate is below the global average (6.6%-23.7%).[10]

Prescription errors in terms of dosage and duration, along with high defaulter rate for the completion of treatment are important factors leading to the rise in MDR-TB in Pakistan. In Karachi alone, the primary resistance rates for the 4 FLDs in 1993 were reported to be 11%, 2%, 3% and 9% for Isoniazid, Ethambutol, Rifampicin and Streptomycin respectively[11] which increased to 27%, 15%, 11% and 13% respectively in 1996.[12]

It was therefore considered beneficial to ascertain the current status of FLD prescription on sputa isolates cultured from a cross-section of Karachi TB patients. Indeed, contributing to the success of initial treatment is important in reducing the expansion of the grim infection, and possibly decreasing the progress of MDR-TB strains that is an ongoing challenge.

METHODOLOGY

ZNCF-stained smear-positive clinical sputa expectorated by 45 females (53.57%) and 39 males (46.43%) aged 15-58 years referred for AFB culture in two separate commercial diagnostic labs in Karachi, Pakistan, that yielded positive growth of *M. tuberculosis* (n=84) on LJ media (Oxoid, UK) slants during a 12-month period ending August 2011, were included in this study. The sputa for inoculation were subjected to liquefaction (using N-acetyl-cysteine), decontaminated (with 4% Sodium hydroxide) and concentrated using centrifugation (3,500 x g). Incubation was maintained for up to 12 weeks with regular examination of cultures. Identification of the isolates included colonial morphology (rough, buff-colored colonies), ZNCF smear, sensitivity to p-nitro-benzoic acid, and niacin (nicotinic acid) production.

Table-I: Susceptibility of isolates (n=84) against five anti-TB drugs.

Drug	*No. Resistant*	*Percentage*
Rifampicin	41	48.8%
Streptomycin	24	28.57%
Kanamycin	24	28.57%
Ethambutol	06	7.14%
Isoniazid	04	4.76%

The isolates were subjected to antimicrobial susceptibility testing by agar dilution method using LJ medium and first-line anti-TB drugs, along with kanamycin. MICs of the control strain (H37RV-NCTC strain 7416) were compared with the test strains inoculated along with the batch tested. Results were evaluated by the traditional resistance ratio method: a ratio of 4 was taken as probably sensitive and a ratio of <4 were considered as sensitive.

RESULTS

A total of 84 *M. tuberculosis* isolates were identified during the study period. Among the drugs tested, Rifampicin (RMP) was least effective (48.8%). Both Streptomycin and Kanamycin were equally ineffective with 24 isolates noted to be indifferent to these drugs. INH (4.76%) in particular, and Ethambutol (7.14%) were chiefly efficient.

Only four (4.76%) of the isolates expectorated in sputa by 4 females aged 45-58 years were MDR-TB strains resistant to both RMP and INH.

DISCUSSION

The number of AFB isolates from clinical sputa (n=84) were admittedly not substantial, but the observation that 41 of them (48.8%) were indifferent to Rifampicin, a regular drug in the initial FLD recipe, cannot be minimized. Indeed, Khan et al (1993) reported the primary resistance rate of Rifampicin to be a mere 3%,[11] which almost tripled within three years to 11% in 1996, according to the study by Hussain et al[12] thereby emphasizing the rising trend of resistance to an important treatment ingredient.

Additionally, in their studies in Karachi, the primary resistance rates for the other three first line drugs in 1993 were 11%, 2%, and 9% for Isoniazid, Ethambutol and Streptomycin respectively[11] which increased to 27%, 15% and 13% correspondingly in 1996.[12] In comparison, we noted the current resistance rates of these drugs to be 4.76%, 7.14% and 28.57% respectively, suggesting a mounting indifference to Streptomycin, but a simultaneous increasing sensitivity to Ethambutol and in particular, to Isoniazid. Both Streptomycin and Kanamycin were equally ineffective on 24 of the 84 isolates, suggesting a possible co-efficacy between the two aminoglycosides. However, Satti et al (2010) in their study reported that strains resistant to Streptomycin were sensitive to Amikacin, and that there is no co-resistance between Streptomycin

and other aminoglycosides.[8] Clearly Amikacin, a synthetic derivative of Kanamycin, is currently the most potent of the aminoglycosides, with a significantly broader spectrum of activity than other members of this antibiotic class.

Due to financial constraints drug susceptibility testing is currently still restricted to a small number of diagnostic labs in Pakistan, and which mostly quote the sensitivity profile of first-line drugs, if at all, against clinical isolates; further testing is normally performed in reference labs, which are also few in number. But with a rising trend in drug-resistant *M. tuberculosis* strains being reported,[4,5,9,11,13] the dire need for additional sensitivity coverage is evident, especially since there has unfortunately not been any satisfactory relevant new cost-effective drug discovery in the last four to five decades, although 1.7 million people around the globe are said to be dying of TB each year. Hence the possible efficacy of currently available drugs representing various classes of antibiotics should continue to be evaluated.

Indeed, efforts to this effect have been lately ongoing; among others, Satti et al (2010) have reported on the efficacy of Amikacin and Ciprofloxacin against clinical isolates of *M. tuberculosis*,[8] Ruiz-Serrano et al (2000) have earlier described the *in vitro* activities of 6 fluoroquinolones against 250 clinical isolates of the bacterium,[14] while Fattorini et al (1999) studied the effect of 16 antimicrobial agents on the organism,[15] paving the way for further survey.

However, sorely needed in supporting such studies for clinical trials is that *in vitro* laboratory observations should be backed by reasonable *in vivo* data. Certainly, new quinolone compounds and macrolides show promise in the treatment of some mycobacterial infections, but the prescription regimens have to be developed. This includes appropriate dosage, possible toxicity and synergistic combination with other drugs, affordable cost, and the appropriate duration when administered. Alangaden and Lemur (1997) for example did well in commenting on the clinical use of fluoroquinolones in the treatment of mycobacterial disease,[16] which is a step in the right direction and a desirable corollary to *in vitro* studies.

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