Is nalidixic acid resistance linked to clinical virulence in *Salmonella enterica* serovar Typhi infections?

Fluoroquinolones are the drugs of choice for the treatment of typhoid fever caused by multidrug-resistant *Salmonella enterica* serovar Typhi (MDR-ST) infections. Decreased susceptibility of *S. Typhi* isolates to fluoroquinolones is emerging as an important problem in South Asian countries such as India, Vietnam, and elsewhere (Parry, 2004; Rahman *et al.*, 2006; Renuka *et al.*, 2005). Nalidixic acid resistance is useful as a surrogate marker of decreased susceptibility to fluoroquinolones (Hakanen *et al.*, 1999). We have previously reported the association of nalidixic acid-resistant *S. Typhi* (NARST) infection with prolonged duration of illness at presentation, increased clinical severity of illness and complications (Kadhiravan *et al.*, 2005). Here, we present the results of the exploratory analysis of the data regarding the association of drug resistance with fever clearance time.

The study group comprised 60 consecutive patients, including children, with blood culture-confirmed typhoid fever, treated at a tertiary care hospital in northern India, followed up prospectively. The patient characteristics and study methods have been described previously (Kadhiravan *et al.*, 2005). Fever clearance time was defined as the time from the initiation of antibiotic therapy to the first instance when body temperature fell below 37.5 °C and remained so continuously for at least 48 h. In the present analysis, 51 patients in whom the fever clearance time could be assessed are considered. The effect of infection with NARST and MDR-ST on fever clearance time was assessed by Kaplan–Meier survival analysis, to display the fever clearance distributions of the groups (Bland & Altman, 1998). The log-rank test was applied to evaluate the equality of the fever clearance distributions (Bland & Altman, 2004). Further, a multivariable Cox proportional-hazards regression model was used to evaluate the relationship between NARST infection and

the fever clearance time, adjusting for other potential confounding variables. All tests were two-sided, and a $P < 0.05$ was considered statistically significant. All analyses were performed using a statistical software package (SPSS for Windows, version 10.0.1).

The mean age of the study group was 16 ± 9 years, and one-third of the patients were female [17 (33%) patients]. The isolate was resistant to nalidixic acid in 41 (80%) patients, and 18 (35%) patients had MDR-ST infection. The median time to fever clearance was 3 days [interquartile range (IQR) 2.75–5 days] in patients with nalidixic acid-susceptible *S. Typhi* (NASST) isolates and was 5 days (IQR 3.9–6.1 days) in patients infected with NARST. The difference between the NARST and NASST groups in the distribution of fever clearance time was statistically significant (Fig. 1a; $P = 0.028$). At any given point of time, fever clearance was about twice more likely to occur in patients with NASST as compared to those with NARST infection (unadjusted hazard ratio for NASST vs NARST infection, 2.19; 95% confidence interval 1.05–4.61). However, there was no significant difference in the distribution of the fever clearance time between patients with MDR-ST infection and the rest of the study group (Fig. 1b; $P = 0.948$).

In 12 (24%) of the 51 patients, the initial antibiotic regimen included a fluoroquinolone (alone or in combination with a cephalosporin), and 8 (16%) of them had NARST infection. In the multivariable Cox proportional-hazards regression model, with fever clearance as the outcome event, NARST infection, age, duration of fever at presentation and use of fluoroquinolone in the initial antibiotic regimen were included as covariates. The effect size of the association between NARST infection and prolonged fever clearance remained the same, even after adjusting for these potential confounders

[adjusted hazard ratio 2.36 (1.03–5.41); $P = 0.042$].

We observed an independent association between NARST infection and prolonged time to fever clearance in patients with typhoid fever. The overall distribution of fever clearance time between NARST and NASST groups was significantly different. Apart from nalidixic acid resistance, there are two other factors which could have been potentially responsible for the observed difference in fever clearance distribution between the NARST and NASST groups. First, it is well known that fever clearance occurs significantly earlier in patients treated with fluoroquinolones than in patients treated with ceftriaxone (Parry *et al.*, 2002). Second, in view of the significantly longer duration of illness at presentation among patients with NARST infection as reported earlier, it is quite possible that patients with NARST infection had a relatively more severe form of disease which could have possibly led to slower fever clearance. Apart from these two potential confounding factors, we also included the age of the patient as a covariate in the Cox regression model, since patients with NARST infection were significantly younger than those with NASST infection (Kadhiravan *et al.*, 2005).

A significant association between NARST infection and prolonged fever clearance time, even after adjusting for these potential confounders, suggests an independent effect of NARST infection on the fever clearance time. However, it needs to be stressed that finding an independent statistical association does not necessarily establish a biological cause–effect relationship. Nonetheless, the possibility of NARST strains being inherently more virulent than their nalidixic acid-susceptible counterparts needs to be considered. An earlier study had found that patients infected with drug-resistant *S. Typhi* had significantly higher blood bacterial counts (Wain *et al.*, 1998).

Fig. 1. Kaplan–Meier survival plots of time to fever clearance according to the drug susceptibility of the isolate in 51 patients with blood culture-confirmed typhoid fever. (a) NARST versus NASST; (b) MDR-ST versus non-MDR-ST. The y-axis represents the cumulative probability of event-free survival – i.e. remain febrile. NARST, nalidixic acid-resistant *S. Typhi*; NASST, nalidixic acid-susceptible *S. Typhi*; MDR-ST, multidrug-resistant *S. Typhi*.

A similar phenomenon is possible in the case of NARST infection also. It is likely that the drug-resistance phenotype and the factors that determine clinical virulence of the strain are genetically linked.

Further, in this study group, the majority of the patients were treated with ceftriaxone (alone or in combination with another antibiotic) (Kadhiravan *et al.*, 2005). Both NARST and NASST are equally susceptible to ceftriaxone, and hence one would expect not to find any significant difference in fever clearance time. The observation to the contrary, of prolonged fever clearance time among patients with NARST infection, lends further support to the hypothesis that NARST strains might be associated with increased clinical virulence.

The relatively smaller size of the study group and the *post hoc* nature of the analyses are possible limitations of the present study. However, systematic studies on the therapeutic response in patients with typhoid fever caused by NARST infection are limited, and to the best of our knowledge, the possible association of NARST infection with increased clinical virulence has not been reported previously. The hypothesis put forward here needs to be tested and confirmed by further larger studies employing quantitative blood

culture techniques, preferably performed in the community setting. Prolongation of fever clearance time would clinically translate into increased morbidity and loss of productivity in patients with typhoid fever in settings where NARST infection is widely prevalent. Moreover, typhoid fever caused by NARST infection has already been reported from Africa and North America (Nkemngu *et al.*, 2005; Slinger *et al.*, 2004), and typhoid fever is a common cause of febrile illness in international travellers (Freedman *et al.*, 2006). Thus the increased clinical virulence of NARST will have implications for countries outside South Asia also.

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