Resistance to antimicorbial agents in *Salmonella typhi* in Vietnam: clinical response to therapy and molecular mechanisms

VMB-5

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Abstrak

Pada tahun 1989 hanya 1% isolat S. typhi *di Vietnam yang resisten terhadap berbagai antibiotik* (multidrug resistant = *MDR) yaitu terhadap ampisilin, kloramfenikol, trimetoprim, tetrasiklin, sulfonamida; namun pada tahun 1993, sudah 85% isolat* S. typhi *adalah MDR. Sejak tahun 1992, fluorokuinolon digunakan secara luas, dan dengan cepat pula berkembang galur resisten kuinolon yang dilaporkan dari area Delta Mekong, Vietnam. Pada tahun 1997, isolat resisten terhadap kuinolon telah mencapai 20%. Pada suatu uji coba klinik yang dilakukan pada 150 penderita demam tifoid tanpa komplikasi dan diterapi dengan ofloksasin 10 mg/kg/hari selama 2-3 hari, berhasil ditemukan 18* S. typhi *yang resisten kuinolon (resisten asam nalidiksat, NA^R). Waktu yang dibutuhkan untuk menurunkan demam rata-rata 156 (30-366) jam bila penderita terinfeksi oleh* S. typhi *NA^R dan 84 (12-378) jam pada penderita terinfeksi* S. typhi *sensitif kuinolon (NA^R) ($p \leq 0,001$). Enam dari 18 penderita dengan* S. typhi *NA^R memerlukan terapi tambahan sedangkan hanya satu dari 132 penderita infeksi* S. typhi *NA^R memerlukan terapi serupa, dan hasil perhitungan resiko relatif (95% C1) 44 (5,6 - 345) ($p \leq 0,0001$). Perbedaan kemajuan pengobatan secara klinis tersebut sangat penting dibandingkan dengan bila hanya melihat angka KHM (konsentrasi hambat minimal =* MIC*) saja. Dengan teknik ekstraksi plasmid dan eksperimen transfer gen dapat terlihat bahwa MDR pada* S. typhi *di Vietnam dibawa oleh suatu plasmid yang mudah ditransfer dan mempunyai molekul yang besar. Pada tahun 1992, enam jenis plasmid yang berbeda berhasil diekstraksi dari 22 isolat* S. typhi. *Pada tahun 1996 hanya dua jenis plasmid ditemukan dari 19 galur* S. typhi. *Dengan teknik PCR dan SSCP pada galur resisten kuinolon ditemukan adanya dua mutasi titik* (point mutation) *baru pada gen* gyrA *pada 20* S. typhi *yang resisten kuinolon. Mutasi pertama terjadi pada nukleotida 97 menyebabkan berubahnya asam amino Asp menjadi Gly (n=17). Mutasi kedua terjadi pada nukleotida 83 yang menyebabkan berubahnya Ser menjadi Phe (n=3). Timbulnya galur* S. typhi *MDR selama tahun 90-an di delta Mekong. Vietnam adalah diperantarai oleh plasmi. Namun kewaspadaan harus pula ditujukan pada peningkatan MDR yang disebabkan oleh adanya perubahan kromosomal. Meskipun seringkali sesitivitas* in vitro *adalah sama, namun terdapat perbedaan yang besar pada hasil pengobatan antara infeksi NA^R dan NA^S. Bertahannya galur MDR serta munculnya* S. typhi *resisten kuinolon mungkin akan menyebabkan patogen yang penting ini di masa mendatang hanya akan dapat diobati dengan antibiotika sefalosporin generasi ketiga yang jauh lebih mahal.*

Abstract

In 1989 1% of isolates of S. typhi *(ST) in Vietnam were multi-drug resistant (MDR) (Amp. Chlor. Trim. Tet. Sulphon.), by 1993 85% of ST were resistant to these antibiotics. In 1992 fluoroquinolones uses became widespread, and rapidly quinolone resistance was reported from the Mekong Delta of Vietnam. By 1997 20% of isolates were resistant. In a clinical trial of 150 patients with uncomplicated typhoid randomised to 2 or 3 days ofloxacin (10mg/kg/day), 18 quinolone (nalidixic acid NA^R) resistant ST were isolated. The median (range) fever clearance time was 156 (30-366) hours in patients infected with NA^RST and 84 (12-378) hours in those infected with Na^S ST ($p \leq 0.001$). 6/18 patients with NA^R isolated required retreatment compared with 1/132 patients with NA^S ST, relative risk (95%Cl) 44 (5.6-345) ($p \leq 0.0001$). These important clinical differences between the NA^R and NA^S isolated were noted despite similar MIC's. By plasmid extraction and transfer experiments we have demonstrated that MDR in* S. typhi *in Vietnam is mediated by large molecular weight transferable plasmid. In 1992 there were six distinct plasmid patterns extracted from 22* S. typhi *isolates. In 1996 there were only 2 plasmid patterns seen in 19 ST strains. PCR and SSCP of the quinolone resistant determining gyrA gene of* S. typhi *from 20 NA^R isolates revealed two novel point mutations. The first, at nucleotide 97 caused an amino acid change Asp ⇒ Gly (n=17). The second, at nucleotide 83 caused a change Ser ⇒ Phe (n=3). There has been a rapid emergence of MDR ST strains during the 1990's in the Mekong delta of Vietnam. This is plasmid mediated. Of increasing concern is the development of chromosomally mediated quinolone resistance. There are important differences in the treatment response between NA^R and NA^s infections, despite similar* in vitro *sensitivities. The persistence of MDR strains and the emergence of quinolone resistant* S. typhi *may render this important pathogen treatable only with expensive third generation cephalosporins in the near future.*

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INTRODUCTION

Typhoid is a major cause of morbidity in tropical countries. The development of antibiotic resistance in *Salmonella typhi* poses the considerable threat of increased mortality and morbidity to many communities of the world. In southern Vietnam multidrug resistance (MDR); resistance to chloramphenicol, ampicillin and co-trimoxazole, in *S. typhi* had become established by late 1992 and early 1993[1]. The fluoroquinole antibiotics have been shown to be superior to the third generation cephalosporins[1] and effective in short courses[2]. Resistance to these compounds will therefore have serious public health consequences, particularly in endemic areas. We report the nature of plasmids involved with transmission of MDR and the emergence of nalidixic acid resistance in isolates of *S. typhi* in southern Vietnam.

MATERIALS AND METHODS

Prospective diagnostic and treatment studies in typhoid fever have been in progress at the Centre for Tropical Disease, a 500 bed referral centre for patients with infectious diseases, Ho Chi Minh City, since 1992[1,2,4,5]. Adults received either ofloxacin 10 mg/kg body weight in two daily divided doses for three days or 15 mg/kg body weight in two daily divided doses for two days. Children received 15 mg/kg body weight in two daily divided doses for either three days or for two days. A clinical failure was defined as failure to attain fever clearance 7 days after the start of treatment, associated with lack of resolution of symptoms and signs, or the development of severe of complicated enteric fever. Repeat blood cultures were performed 48 hours after the last dose of ofloxacin and on day 7 if the patient was still febrile on that day. Patients who failed clinically were retreated with a further course of ofloxacin at the discretion of the attending physician or paediatrician. Each study was approved by the Scientific and Ethical committee of the Centre for Tropical Diseases.

All isolates of *S. typhi* were from blood and were identified by specific antiserum (Wellcome Diagnostics, UK) and with standard biochemical tests. Antibiotic disc susceptibility was determined at the time of isolation by the modified Kirby-Bauer method with discs containing ampicillin (10μg), chloramphenicol (30μg), trimethoprim-sulphamethoxazole (1.25/23.75μg), ceftriaxone (30μg) and ofloxacin (5μg). Twenty NA^R and 3 NA^S isolates of *S. typhi* were studied. DNA prepared from bacterial strains[6] was amplified by PCR using the oligonucleotide primers P1 (3'-TGTCCGAGATGGCCTGAAGC-5') and biotinylated P2BIO (5'-TACCGTCATAGT-TATCCACG-3'). The amplified products were stored at 4°C until used for SSCP or DNA sequencing. SSCP was performed using MDE high resolution gel according to the manufacturers instructions (FMC, Rockland, USA) PCR products were purified by binding to streptavidin coated magnetic beads (Dynal, Oslo, Norway) for direct sequencing. Plasmid DNA was extracted by the method of Kado and Lieu.

The clinical features and response to treatment of patients with NA^S and NA^R isolates and laboratory features of the isolates were compared by Student's t test or analysis of variance. The Mann Whitney U test was used for non normally distributed data. Proportions were compared by survival analysis using the Kaplan-Meier plot and logrank test and relative risk (RR) calculated. The statistical package SPSS for Windows (V6.1.1) was used for these analyses.

RESULTS AND DISCUSSION

During the treatment trials there were 13 clinical failures, 9 amongst the 18 patients with NA^R isolates, i.e. an overall failure rate in these patients of 50%, and 3.1% (4/132) in the patients with NA^S strain; RR (95% CI) 16.5 (5.7 to 48.1) ($P<0.0001$). Only one of the clinical failures was also a microbiological failure and this was in the NA^R group. Retreatment of these clinical failures with a further course of ofloxacin was considered necessary by the attending physician in one (0.8%) of the 132 patients with an NA^S isolate and 6 (33.3%) of the 18 patients with a NA^R isolate, RR (95% CI) 44 (5.6 to 345), ($P<0.0001$). All *S. typhi* isolates, tested at the time of isolation by disc diffusion, were found to be susceptible to ofloxacin. Of the 41 isolates of *S. typhi* isolated in the first study between December 1992 and June 1993[2], 26 (63%) were multidrug resistnt (MDR) but none were nalidixic acid resistant. In the short course treatment studies between October 1993 and December 1994 [5,16] there were 150 *S. typhi* isolates including 117 (78%) MDR strains of which 18 (15%) were also nalidixic acid resistant. The first NA^R isolate was detected in October 1993. During 1995 there were 4,946 patients investigated by blood culture on admission to The CTD. *S. typhi* was isolated from 720 (14.5%) cultures of which 662 (92%) were MDR, and 14 (2.1%) of these were also NA^R. The MIC values of ofloxacin for the NA^R isolates were generally two or three doubling dilution's higher than the NA^S isolates although there is some overlap between the

two groups. Of 51 isolates with an ofloxacin zone ≤ 26 mm 14 (27%) were nalidixic acid-resistant whereas none of 343 isolates with a ofloxacin zone > 26 mm were nalidixic acid resistant. Isolates from Vietnam sensitive to nalidixic acid gave the same SSCP pattern as the control *S. typhi* Type A. None of the 20 nalidixic acid resistant isolates had the same pattern as the sensitive strains. There were two novel patterns, designated II (n=17) and III (n=3). The 218 nucleotide fragment of *gyrA* of the type A control strain of *S. typhi* gave the same sequence for this region of the QRDR of *gyrA* as the nalidixic acid sensitive strain of *S.typhi* from Vietnam TY84. Two isolates that gave SSCP pattern II (TY66 and CT48) and two isolates that gave pattern III (5182 and 5214) were chosen for DNA sequencing. Pattern II isolates had a point mutation at codon 83 (C to T), substituting phenylalanine for serine.

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