Typhoid fever due to multiresistant *Salmonella enterica serovar typhi* having reduced susceptibility to ciprofloxacin and nalidixic acid resistance

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Salmonella typhi (*S.typhi*), the etiological agent of typhoid fever is pathogenic only to humans. It is endemic in developing countries, where around 33 million cases occur every year, leading to high morbidity and mortality. Early effective antimicrobial therapy of typhoid fever, without waiting for the blood culture results is necessary for the prevention of complications and to avoid mortality. Chloramphenicol remained the drug of choice for the treatment of typhoid fever until 1972, when extensive outbreaks of chloramphenicol resistant *S.typhi* occurred in India and Mexico.[1] Until the middle of 1980 to 1990, ampicillin and trimethoprim-sulfamethoxazole were the alternative drugs for treatment of typhoid fever and resistance to these drugs appeared along with resistance to chloramphenicol in the strains causing outbreaks in Indian subcontinent and Vietnam.[2] Fluoroquinolones have become the first line drugs for treatment of multi drug resistant typhoid fever for their proven efficacy. Subsequently resistance to these drugs was observed in strains of *S.typhi*.[3] The nalidixic acid resistant *S.typhi* strains with reduced susceptibility to ciprofloxacin is an emerging problem in the developing countries. Limited reports are available from the Indian subcontinent and some of the Asian countries where such strains were sensitive to ciprofloxacin by the disc diffusion test but clinically there was treatment failure, leading to complications and longer period of hospitalization.[4-5] This study presents a report of a self-limiting outbreak of typhoid fever due to multi drug resistant strains of *S.typhi* with nalidixic acid resistance and reduced susceptibility to ciprofloxacin, from the Al-Hasa region of the Kingdom of Saudi Arabia (KSA).

The study was conducted at a 500-bed, King Fahad Hospital and Tertiary Care Center, Al-Hofuf, an Ancient City and oasis of the Eastern Province of KSA, with native population of approximately 1 million. Indoor admissions in the medical wards during September 2003 with clinical suspicion based on the symptoms and signs of typhoid fever and confirmed through blood culture were enrolled for the study. A detailed history, findings on clinical examination, the progress and a close follow-up was recorded for each patient. Blood samples were collected in Bactec blood culture bottles and processed in Bactec 9240 blood culture system (Becton Dickinson Company, United States of America (USA)). *Salmonella typhi* strains were biochemically identified by API20E identification system (Bio Merieux SA France) and serotyped using Salmonella O9, Vi, d-H antisera (Difco Laboratories, USA). Antibiotic susceptibility was performed by disc diffusion technique according to the criteria of the National Committee for Clinical Laboratory Standards. Following concentrations of the antibiotics µg/disc (Becton Dickinson Company, USA) was used for the susceptibility test, ampicillin-25, amoxicillin/clavulanic acid 20/10, cefoxitin-30, cephalothin-30, cefotaxime-30, ceftriaxone-30, cefepime-30, chloramphenicol-30, trimethoprim/sulfamethoxazole 1.25/23.75, tetracycline-30, streptomycim-10, gentamicin-10, amikacin-30, imipenem-10, aztreonam-30, piperacillin-100, nalidixic acid-30 and ciprofloxacin-5. Minimum inhibitory concentration (MIC) to nalidixic acid and ciprofloxacin was performed by agar dilution method.

The study involved 12 patients admitted within a short span of 15 days with clinical symptoms of typhoid fever and confirmed by positive blood culture for *S.typhi*. All these patients were native from the Al-Hasa region and none of them were expatriates. Of these, 10 patients were directly admitted to this tertiary care center and 2 patients were admitted to the peripheral hospital and subsequently transferred to this center for treatment. Blood culture of these 2 patients were positive in the peripheral hospitals. All these patients were admitted with the history of fever and dry cough of 5-10 days duration. Mean age of the patients was 24.5 years (range 5-46 years), 5 of them were male and 7 female. Diarrhea, abdominal pain and fever were the main presenting symptoms in 8 patients. Laboratory investigations revealed mean white blood cell (WBC) count of $4.3x10^3$/µl (range 3.1-7.6), platelet count mean $131x10^3$/µl (range 57-189), AST mean 180 U/L (range 32-376) and ALT mean 116 U/L (range 40-330). *Salmonella typhi* strains isolated from the blood of all these patients were resistant to ampicillin, amoxicillin/clavulanic acid, chloramphenicol, trimethoprim/sulfamethoxazole, tetracycline, streptomycin, piperacillin and nalidixic acid. While all were sensitive by disc diffusion test to ciprofloxacin, cefotaxime, ceftriaxone, cefepime,

cefoxitin, cephalothin, gentamicin, amikacin, aztreonam, imipenem. Minimum inhibitory concentration of all the strains to nalidixic acid was >256 µg/ml and all the strains had reduced susceptibility to ciprofloxacin (MIC 0.20-0.25 µg/ml). Each patient was treated with ceftriaxone 80mg/kg body weight for 7 days. All became afebrile after average of 4.8 days of ceftriaxone therapy. Mean period of hospital stay of these patients was 16.5 days (**Table 1**). Patients who remained afebrile for 6 consecutive days following ceftriaxone therapy and had negative stool culture for *S.typhi* were discharged from the hospital. On the other hand, 2 patients (aged 13 and 15 years) transferred from the peripheral hospital who received initial ciprofloxacin therapy for 6 days with out any clinical improvement also became afebrile 4 days following commencement of ceftriaxone therapy. Only 6 patients had 4 folds rise in antibody titer by Widal test (Initial titer of 1:80 increased to 1:320 in 2 patients and from 1:40 to 1:160 in 4 patients). The endemicity of typhoid fever and appearance of multi drug resistance in *S.typhi* to the cost effective drugs such as chloramphenicol, trimethoprim-sulfamethoxazole has added to the burden of already overstretched health budget of developing countries. These multi resistant strains are responsible for massive outbreaks in the countries with scarce resources. An outbreak of typhoid fever with such strains in Tajikistan, in 1997 affected more than 8000 people causing 150 deaths.[6] The potential spread of such strains of *S.typhi* to other areas of the world through international travel is of serious concern. In the United Kingdom (UK) multi drug resistant strains of *S.typhi* with nalidixic acid resistance and reduced susceptibility to ciprofloxacin, increased from 3% in 1995 and 23% in 1999. Most of the patients from whom such strains were isolated had recently traveled to Indian subcontinent.[5] In the USA, most of the identified ciprofloxacin resistant *Salmonella* infections were acquired from outside. In the present study none of the affected patients traveled to any of the country which is endemic for multi drug resistant and nalidixic acid resistant *S.typhi* during last one year. Drinking water in this region is safe and is procured by the consumer from the reverse osmosis units. These units use chlorinated under ground water for desalination by the reverse osmosis process. The typhoid cases were distributed in the wider urban and rural area of the region; the drinking water was not procured from one particular source. High quality hygiene standards are maintained by the restaurants and food stores. All the food handlers are regularly screened for carriage of *salmonella*. There was no history of taking food or eatables from one particular source among these patients and there was no other common factor among these patients admitted with typhoid fever. All the patients were native Saudis and belonged to different areas of this region (both rural and urban) and there was no concentration of cases to specific area. There are many people from Indian subcontinent and Far East (where such strains are prevalent) working in this region, the possibility of introduction of this *S.typhi* strain through

Table 1 - Patient characteristics admitted with typhoid fever.

Sex	age	Duration of stay (days)	Afebrile after CRO (days)	Diarrhea	WBC count x103/µl	Platelet count x103/µl	AST U/L	ALT U/L	Widal test
F	28	20	10	+	3.7	187	162	134	+
M	16	18	5	-	2.8	156	81	122	-
M	28	10	5	-	4.4	143	51	77	-
F	30	20	4	+	3.5	128	62	78	+
M	32	20	5	+	3.8	112	122	130	+
M	22	22	4	+	4.2	128	82	110	-
M	37	7	3	+	3.2	90	376	330	+
F	5	21	4	+	5.6	57	217	78	-
F	13	20	4	+	7.6	74	32	34	-
F	23	13	6	-	6.2	158	32	40	-
F	15	10	4	-	3.1	139	172	115	+
F	46	17	4	+	4.2	189	61	60	+
Mean	24.5	16.5	4.8	8	4.3	130	118	109	6

F - female, M - male, CRO - ceftriaxone, WBC - white blood cell, AST - alanine aminotransferase, ALT - antilymphocyte serum

asymptomatic carrier in this region cannot be completely ruled out. However distribution of the cases to a wider area does not support this possibility. During last 4 years, no expatriate patient having typhoid fever with multi drug resistant strain was admitted to this hospital and such multi drug resistant *S.typhi* strains with resistance to nalidixic acid and reduced susceptibility to ciprofloxacin were isolated for the first time in this region. All the affected patients were native, possibly this was an autochthonous infection leading to a short self limiting outbreak, the source of which could not be traced. Appearance of fluroquinolone resistance in non typhoidal *salmonella* in the UK and the USA has been linked to use of fluoroquinolones in the poultry and animal feed. *Salmonella typhi* is pathogenic to human only, the source of selective pressure leading to development of fluoroquinolone resistance is not certain, possibly the widespread use of quinolone in treatment of diarrheal disease could be a source of selective pressure.

In the present study apart from dry cough and fever, 8 patients had diarrhea, this was an unusual finding, as constipation has been commonly described in typhoid fever. The majority of the patients had thrombocytopenia, leucopenia and altered liver enzymes, which improved with ceftriaxone therapy. Most of the patients became afebrile after 4.8 days of ceftriaxone therapy and none of them had treatment failure or relapse, where as fever clearance time of 6.1 days with ceftriaxone therapy has been reported.[7,8] Ceftriaxone therapy for the treatment of typhoid fever with multi resistant nalidixic acid resistant strains of *S.typhi* appears to be safe and an effective alternative. Two patients who had treatment failure with the ciprofloxacin therapy, responded to ceftriaxone therapy and both of them became afebrile 4 days after treatment. Widal test had 4 folds rise in titer only in 6 patients indicating the poor reliability of the test. Incidentally this is the only cost effective diagnostic test available in the developing countries, which needs to be replaced by a more sensitive test.

Resistance to quinolone is mediated by point mutation in the quinolone resistant determining region of gyrA gene at position 83 of the deoxyribonucleic acid gyrase enzyme. In the present study, all the nalidixic acid resistant strains of *S.typhi* had reduced susceptibility to ciprofloxacin (MIC 0.20-0.25 µg/ml) although they were sensitive (zone diameter >21 mm) to this drug by disc diffusion test. The routine disc diffusion test with ciprofloxacin cannot detect the reduced susceptibility and can lead to treatment failure if typhoid fever due to *S.typhi* strain having reduced susceptibility is treated with this drug. Sensitivity to nalidixic acid should be carried out routinely for the *S.typhi* strains and nalidixic acid resistance should be taken as an indicator of reduced susceptibility to ciprofloxacin. As first line of treatment of typhoid fever ciprofloxacin should be avoided to prevent the treatment failure, in the areas where nalidixic acid resistant strains of *S.typhi* are prevalent or the patient has recently visited the country known to have the prevalence of such strains. Ceftriaxone therapy for 7 days appears to be an effective alternative for typhoid fever due to multi drug resistant *S.typhi* with nalidixic acid resistance and reduced susceptibility to ciprofloxacin.

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References

1. Paniker CKJ, Vimla KN. Transferable chloramphenicol resistance in Salmonella typhi. ***Nature*** 1972; 239: 109-110.
2. Hoa NT, Diep TS, Wain J, Parry CM, Hein TT, Smith MD, et al. Community acquired septicemia in southern Vietnam: The importance of multi drug resistant S.typhi. ***Trans R Soc Trop Med Hyg*** 1998; 92: 503-508.
3. Threlfall EJ, Ward LR, Skinner JA, Smith HR, Lacy S. Ciprofloxacin resistant Salmonella typhi and treatment failure. ***Lancet*** 1999; 353: 1590-1591.
4. Asna SMZH, Haq JA, Rahman MM. Nalidixic resistant Salmonella enterica serovar typhi with reduced susceptibility to ciprofloxacin caused treatment failure: report from Bangladesh. ***Jpn J Infect Dis*** 2003; 56: 32-33.
5. Threlfall EJ, Ward LR. Decreased susceptibility to ciprofloxacin in Salmonella enterica serotype typhi, United Kingdom. ***Emerg Infect Dis*** 2001; 7: 448-450.
6. Hermin JH, Villar R, Carpenter J, Robert L, Sanaridden A, Gasanova L, et al. A massive epidemic of multi drug resistant typhoid fever in Tajikistan associated with consumption of municipal water. ***J Infect Dis*** 1999; 179: 1416-1422.
7. Mitra DP, Dutta S, De A, Chatterjee MK, Bhattacharya SK. Ceftriaxone therapy in ciprofloxacin treatment failure typhoid fever in children. ***Indian J Med Res*** 2001; 113: 210-213.
8. Parry CM, Hien TT, Cougan G, White NJ, Farrar JJ. Typhoid fever. ***N Engl J Med*** 2002; 347: 1770-1782.