Ciprofloxacin Resistant *Shigella flexneri* in India– A New Therapeutic Challenge

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ABSTRACT

Background & Objective: Fluoroquinlones (FQ) have been highly effective drugs for treatment of shigellosis all over the world. Ciprofloxacin resistance in *Shigella flexneri* has emerged as a therapeutic challenge in our region. Here, we report clinical presentation of patients in whom *S. flexneri* was isolated from stool specimens as well as trends of ciprofloxacin susceptibility for the period 2000-2005.

Material and Methods: Stool samples were cultured and *shigella spp.* were identified using standard methods Antibiotic susceptibility was performed in accordance with Clinical Laboratory Standards (CLSI). Minimum inhibitory concentration (MIC) studies were performed by using the agar dilution technique of CLSI and E test. Patients' clinical details and response to therapy were noted. Plasmid profile of ciprofloxacin resistant strains was performed by the rapid alkaline lysis method. Conjugation experiments were done to determine whether quinolone resistance was transferable to *E .coli* J 53 Rif R.

Results : From 995 stool samples submitted from 1st Jan 2005 to 31st Dec 2005, 53 *shigellae* were isolated. *S. flexneri* (31 isolates, 60.7%) was the predominant isolate, followed by *S. dysenteriae* (7), *S .sonnei* and *S. boydii* (6 each) and 3 (nonagglutinable). Isolates from 23 out of 28 patients (82%) with *S.flexneri* shigellosis showed ciprofloxacin resistance (MIC>4). Seven patients infected with *S.flexneri* did not show any response to either ciprofloxacin/ ofloxacin, but 3 patients responded to ceftriaxone & 4 patients respond to combination of amikacin & ciprofloxacin. Three patients showed a partial response, 2 relapsed after an initial response. Over a period of five years, a trend towards increasing MIC was noticed. Though the increase in MIC values appears gradual for MIC <4, a sharp peak is noticed for MIC>4 in 2005. Though plasmids of 2, 4 and 1.8 Kb were transferred to *E coli*, the *E coli* conjugants were susceptible to ciprofloxacin, thereby confirming that ciprofloxacin resistance was not plasmid mediated

Interpretation & Conclusion: There is a great immediate need for an effective oral agent that can be safely used for treatment of children with shigellosis along with continued surveillance required at regional and national level.

Keywords : *Shigella flexneri*, Ciprofloxacin resistance, India

INTRODUCTION

Fluoroquinlones (FQ) have been highly effective drugs for treatment of shigellosis all over the world. Ciprofloxacin resistance in *Shigella flexneri* is sporadic & uncommon, although resistance to cotrimoxazole & ampicillin is common and in some areas resistance to nalidixic acid has also emerged.[1-4] At our tertiary care referral centre in Chandigarh, northern India, which caters to a large population of 5 neighboring states, antibiotic resistance in *Shigella* is being constantly monitored. In 2003, there was an outbreak of ciprofloxacin resistant *S. dysenteriae serotype* 1[5] which re-emerged as the predominant serotype after a gap of nearly a decade. There was a serogroup shift in 2004 when *S. flexneri* again became the predominant isolate. Ciprofloxacin resistance in *S. flexneri* has emerged as a

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therapeutic challenge in our region. Here, we report the clinical presentation of patients in whom *S. flexneri* was isolated from stool specimens as well as trends of ciprofloxacin susceptibility for the period 2000-2005.

MATERIAL AND METHODS

Stool samples were obtained, at the discretion of the provider, from hospitalized patients with symptoms of diarrhea. They were cultured and *shigellae* were identified using standard methods.[6] *Shigellae* were confirmed by serotyping using antisera from Denka-Seiken (Japan). Antibiotic susceptibility was performed in accordance with Clinical Laboratory Standards (CLSI).[7] Minimum inhibitory concentration (MIC) studies were performed by using agar dilution technique of CLSI[7] and E test. Patients' clinical details and response to therapy were noted. We also performed MIC studies of the available *S. flexneri* strains (n=116) isolated from 2000 to 2005 for ciprofloxacin. Plasmid profile of ciprofloxacin resistant strains was performed by the rapid alkaline lysis method.[5] Conjugation experiments were done to determine whether quinolone resistance was transferable to *E. coli* J 53 Rif R.[8] Transconjugants were selected on Mueller- Hinton agar plates containing rifampicin (100mg/l) alone and with ciprofloxacin (8 mg/l).

RESULTS

From the 995 stool samples submitted from 1st Jan 2005 to 31 Dec 2005, 53 *shigellae* were isolated. *S. flexneri* (31 isolates, 60.7%) was the predominant isolate, followed by *S. dysenteriae* (7), *S. sonnei* and. *S. boydii* (6 each) and 3 (nonagglutinable). Thirty-one *S.flexneri* were isolated from 28 patients. On presentation, duration of illness ranged from 2 days to 9 months, (median 7 days and average 28 days). The clinical presentation was as follows –acute dysentery 19, acute diarrhea 5, chronic diarrhea 1, chronic dysentery 1 and pseudo-membraneous colitis 1. Age of patients (male 20, female 8; 10 adults and 18 children) ranged from 3 months to 60 years. All children were below 5 years of age and 11 were below 2 years. All patients were treated with ciprofloxacin / ofloxacin. Seven patients did not show any response to either ciprofloxacin/ ofloxacin, but 3 patients responded to ceftriaxone & 4 patients respond to combination of amikacin & ciprofloxacin. Three patients showed a partial response (responded to prolonged treatment with 10 days of fluroquinolone), 2 relapsed after an initial response. Sixteen patients were from Chandigarh and rest 12 were from the neighboring regions, 344 kilometers across in length, showing widespread distribution of resistance to ciprofloxacin.

The MIC values to ciprofloxacin were as follows: one strain each with MIC values (µg/ml) of 0.064, 0.125, 0.5 and 4 respectively; two strains each with MIC values of 0.25 , 6 and 32 respectively; seven strains with MIC values of 16 and 14 strains with MIC of 8. Isolates from 23 out of 28 patients (82%) with *S.flexneri* shigellosis showed ciprofloxacin resistance (MIC>4). Thirteen resistance patterns were obtained out of which 15 isolates had resistance to amoxicillin, nalidixic acid, cotrimoxazole, ciprofloxacin & norfloxacin pattern. By disc diffusion susceptibility testing the following resistance was observed: amoxicillin (69%), nalidixic acid (96.7%), cotrimoxazole 27/31, norfloxacin (80.6%), ciprofloxacin (19.3%) and chloramphenicol (12.9%). Ciprofloxacin resistance was not adequately detected by disc diffusion. In fact norfloxacin disc diffusion testing was a more accurate predictor for high MIC values against ciprofloxacin. Over a period of five years, a trend towards increasing MIC was noticed. Though the increase in MIC values appears gradual for MIC <4, a sharp peak was noticed for MIC>4 in 2005.The other notable downward trend is seen in MIC values <0.0325 (Fig I).

DISCUSSION

Fluoroquinolones are extensively used and misused for many other illnesses in our region . Quinolone resistance is linked mainly to mutations located in the quinolone

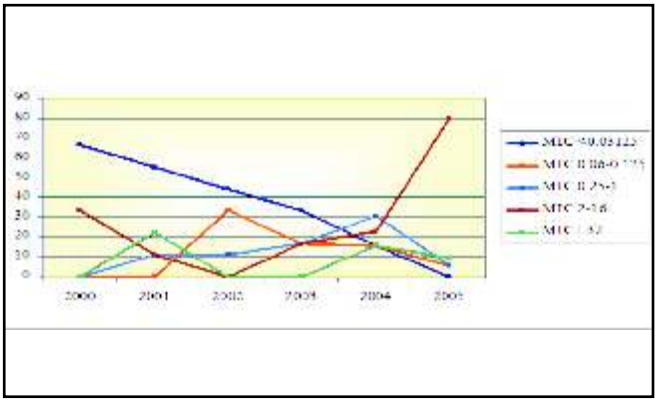

Fig. I: Decreasing susceptibility to ciprofloxacin in S. flexneri from 2000 to 2005

resistance- determining regions (QRDRS) of DNA gyrase (gyrA and gyrB) and topoisomerase IV (par C and Par E).[9] Recently, plasmid mediated fluoroquinolone resistance has been reported in Shigella.[10] Though plasmids of 2, 4 and 1.8 Kb were transferred to *E coli*, the *E coli* conjugants were susceptible to ciprofloxacin, thereby confirming that ciprofloxacin resistance was not plasmid mediated. Treatment failures were earlier reported with ciprofloxacin resistant strains of *S. dysenteriae* 1 at our centre.[5] Nalidixic acid resistance has been used as a marker for reduced susceptibility to ciprofloxacin in *Salmonellae* indicating possible first step gyr A mutations.[11] However, as our strains were uniformly resistant to nalidixic acid , this disc could not be used to predict ciprofloxacin resistance. Disc diffusion susceptibility has limitations and therefore it is important to determine MIC. In our case, we found that resistance to norfloxacin disc could accurately detect ciprofloxacin resistance.

Unlike *S. dysenteriae* 1 which carries the maximum potential for epidemic spread, *S. flexneri* causes endemic shigellosis. Our past experience with *S. flexneri* has shown that >60 % cases presented with diarrhea and not dysentery. This time 68% of patients presented with frank dysentery. With drug resistant organisms there would be a delay in response and frank dysentery would develop later. But this alone cannot explain all the cases. Could these strains be more invasive? Could these strains be clonal? Antibiotic resistance has shown 15 strains belonging to one antibiogram. Plasmid profile of ciprofloxacin resistant strains showed eleven different patterns with common plasmids being of 2, 1.8, 1.2 and 4 kb sizes. Does this resistance have anything to do with ciprofloxacin resistance in *S. dysenteriae* 1 which occurred in 2003. Answering all these questions will require studies at molecular level. Also there is an immediate need for an effective oral agent that can be safely used for treatment of children with shigellosis along with continued surveillance required at regional and national level.

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