ORIGINAL ARTICLE

In Vitro Efficacy of Third Generation Cephalosporins against Nalidixic Acid Resistant and Multi Drug Resistant *Salmonella Typhi* and *Paratyphi* via E-strip Method.

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Abstract

Background
Samonella typhi and *paratyphi* are the strains causing typhoid which carries significant mortality in our region. There have been reports of fluoroquinolone treatment failure due to resistance. After fluoroquinolones, cephalosporins have reliable efficacy against these bacteria. We aimed to find the minimum inhibitory concentrations of cefixime and ceftriaxone against nalidixic acid resistant and multi drug resistant *Salmonella typhi* and *paratyphi* in clinical isolates of a tertiary care setting to detect the rising minimum inhibitory concentrations.

Materials and methods
This prospective, cross-sectional study was carried out in Microbiology laboratory of Army Medical College, Rawalpindi from 2010-2011. Blood cultures were dealt via standard microbiological techniques for detection of *Salmonella typhi* and *paratyphi*. Isolates were then tested against antibiotic disks of nalidixic acid, chloramphenicol, ampicillin and co-trimoxazole. Minimum inhibitory concentration of cefixime and ceftriaxone were determined by E-strip method against multidrug resistant and nalidixic acid resistant *Salmonella typhi* and *paratyphi* according to CLSI guidelines. This is an ongoing study.

Results
In the first part of this study one hundred and fifty-six isolates of nalidixic acid resistant and multidrug resistant typhoidal salmonellae were included in our study. Among these eighty-one (51.92%) were *Salmonella typhi* and seventy-five (48.08%) were *Salmonella paratyphi A.* All the isolates had minimal inhibitory concentrations of cefixime and ceftriaxone in susceptible range.

Conclusion
Both ceftriaxone and cefixime prove very effective antibiotics against nalidixic acid resistant and multidrug resistant typhoidal Salmonellae. Ceftriaxone is the therapeutic agent of choice for inpatients. While cefixime, can be given on outpatient basis due to its convenient oral administration till full emergence of cephalosporin resistant typhoidal Salmonella.

Keywords
Cephalosporins, Multidrug resistant *Salmonella*, Nalidixic acid resistant *Salmonella*.

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Introduction

The oral fecal route of transmission of *Salmonella* makes it a public health threat. There is a rising trend in prevalence of infections caused by resistant *Salmonella* in humans.[1] Asian countries account for a very high incidence of typhoid fever compared to other countries. Mortality rate for typhoid fever without appropriate antimicrobial treatment is estimated to be around 30% which reduces to as low as 0.5% with effective therapy.[2]

The earliest report of successful treatment of *Salmonella* infection dates back in 1948 when Woodward and colleagues have successfully used chloramphenicol for treatment of typhoid fever. For the next two decades, chloramphenicol remained the drug of choice. In late 1970s, the first outbreak of infections caused by antibiotic resistant *Salmonella* appeared. Co-trimoxazole, ampicillin and chloramphenicol, were the antimicrobials used in the treatment of infections caused by *Salmonella* before the 1980s.[3] Multi drug resistant (MDR) typhoidal *Salmonellae* is defined as a strain of typhoidal *Salmonella* resistant to all three first line antibiotics i.e. chloramphenicol, ampicillin and trimethoprim-sulfamethoxazole. Many outbreaks of resistant strain infections have occurred in Asian-Pacific countries, Middle East and Africa.[4] This resistance usually results from dissemination of individual MDR strains or from transfer of R-Plasmid.[5] Fluoroquinolones are usually recommended as alternatives in such cases.[6, 7] But unfortunately, some strains of typhoidal *Salmonellae* have shown reduced susceptibility to fluoroquinoloes.[6,8,9] On disc diffusion testing with the recommended break-points, organisms which are labeled

susceptible to fluoroquinolones, show poor clinical response, when treated with these agents. However, these isolates are resistant to nalidixic acid via disk diffusion method. So, in other words resistance to nalidixic acid can predict therapeutic failure of fluoroquinolones and can be used to guide antimicrobial treatment.[2] However CLSI has introduced updated breakpoints of fluoroquinolones in 2012 against typhoidal Salmonella and this has precluded the use of Nalidixic acid to screen for fluoroquinolones resistance. Azithromycin is used as an oral alternative, but resistance to this antibiotic is also emerging in different parts of the world. In this scenario, we are left with very limited options and the third generation cephalosporins prove to be a promising alternative till full emergence of cephalosporin resistant typhoidal Salmonella.[8] Keeping in view the emergence of Ceftriaxone resistant typhoidal Salmonella, the objective of our study is to determine the minimum inhibitory concentrations (MIC) of ceftriaxone and cefixime against nalidixic acid and multidrug resistant typhoidal *Salmonellae* from clinical isolates in a tertiary care setting ten years back and now compare it with current MIC of ceftriaxone and cefixime against typhoidal Salmonella.

Materials and methods

First part of this prospective cross sectional research was performed from 2010-2011 in Microbiology laboratory, Army Medical College, Rawalpindi. Proper approval was obtained from the ethical committee of the institute. One hundred and fifty-six isolates of nalidixic acid resistant (NAR) and multidrug resistant (MDR) typhoidal *Salmonella* isolated from blood of patients admitted to the Military Hospital Rawalpindi, Pakistan with a strong clinical suspicion of typhoid fever were included in our study. Total 5ml venous blood sample was drawn under aseptic precautions from adult patients and inoculated in 50ml of Brain heart infusion (BHI) broth. For children, 3 ml of venous blood was inoculated in 30ml of BHI, so that blood to broth ratio 1:10 was maintained. The cultures were incubated for 7 days at 37ºC before being reported as negative. All culture bottles were examined daily. If the bottle showed any visible sign of growth, subculture was done on blood agar and MacConkey's agar plate at day 1, 2, 5 and 7. The plates were then incubated at 37ºC for 24 hours. The typhoidal Salmonellae were identified by adopting the standard microbiological procedures which included colony morphology, gram staining results and biochemical reactions such as catalase, oxidase, urease, motility, Voges Proskauer test, citrate agar slant test, methyl red test and triple sugar iron agar test. The isolates of typhoidal Salmonellae were further confirmed by type-specific anti-sera. Further antibiotics susceptibility test of all the isolates were performed by Kirby-Bauer disc diffusion technique.[10] The Mueller Hinton agar plates with antibiotic discs were incubated at 37ºC and zone of inhibition around the antibiotics were measured after 18 hours and 24 hours of incubation. The routinely used antibiotics were amoxicillin, azithromycin, aztreonam, cefixime, ceftriaxone, ciprofloxacin, co-trimoxazole, nalidixic acid. All NAR and MDR typhoidal salmonellae were tested for the determination of minimum inhibitory concentrations (MICs) of cefixime and ceftriaxone by using E strips. A well isolated colony from an overnight agar plate was emulsified in a suitable suspension medium to achieve inoculum turbidity comparing to 0.5 McFarland turbidity standards. Inoculum was applied with a sterile swab on sensitivity agar. Swab was streaked on agar plate thrice to evenly distribute the inoculum. After the agar had dried, E-test strips were applied to the inoculated agar surface with a pair of sterile forceps. After the required incubation period and only when an even lawn of growth was distinctly visible, the MIC values were read and were interpreted according to the criteria set by CLSI.[10] MIC 90 and MIC 50 were calculated.

Results

One Hundred and fifty-six NAR and MDR typhoidal salmonellae were included in our study. Among these eighty-one (51.92%) were *Salmonella typhi* and seventy-five (48.08%) were *Salmonella paratyphi A.* Minimum inhibitory concentrations of both ceftriaxone and cefixime were insusceptible range. MIC results of ceftraixone and cefixime are shown in table 1. Table 2 and Fig 1 show the range, MIC 50 and MIC 90 of ceftriaxone and cefixime against MDR and NAR typhoidal Salmonellae.

Unpaired t test was applied on MIC of Ceftriaxone and Cefixime against Salmonella Typhi and Paratyphi and no statistically

Table 1. Minimum inhibitory concentrations of cefixime and ceftriaxone against MDR and NAR typhoidal Salmonellae (n=100)

Antimicrobial agent	% of isolates susceptible at MIC (µg/ml)										
	0.016	0.023	0.032	0.047	0.064	0.094	0.125	0.19	0.25	0.38	0.50
Ceftriaxone	0	3	3	20	52	10	9	3	0	0	0
Cefixime	0	2	2	4	18	51	14	7	2	0	0

Table 2. Range, MIC 50 and MIC 90 of cefixime and ceftriaxone against MDR and NAR typhoidal *Salmonellae*.

Antimicrobial agent	Range	MIC 50	MIC 90
Ceftriaxone	0.023-0.19	0.064	0.125
Cefixime	0.023-0.25	0.094	0.125

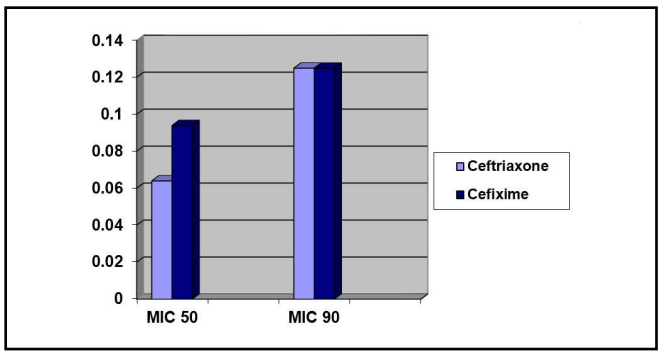

Fig 1. Graph showing comparison of MIC 50 and MIC 90 of cefixime and ceftriaxone.

significant difference between the MIC of two groups was found and thus Ceftriaxone and Cefixime may either be used in these isolates till full emergence of ceftriaxone resistance.

Discussion

Our results show that ceftriaxone and cefixime have same in vitro efficacy against NAR and MDR typhoidal salmonellae. These microbiological results supplement the available data, which showed cefixime as an efficacious and suitable oral alternative, even in cases of MDR *S. typhi*. Our results are similar to a study carried out in Bangladesh in year 2008 which also showed 100% sensitivity of typhoidal *Salmonellae* against ceftriaxone and cefixime.[11] A study done in Egypt also concluded that ceftriaxone was most effective for admitted patients, because of rapid clinical cure, and cefixime was the most effective for oral therapy for outpatients.[12] All the typhoidal *Salmonella* strains were uniformly susceptible to ceftriaxone, cefpodoxime and cefixime in a study carried out in India in 2008.[13] A study done in Bangladesh, Indonesia, Taiwan and Vietman has revealed that not a single isolate was resistant to ceftriaxone.[14] In medical literature, a few case reports have been noted reporting isolates of typhoidal *Salmonella* resistant to third generation cephalosporins. Such cases are reported sporadically including Pakistan.[15, 16, 17] However, recent studies on large sample sizes conducted in big cities of Pakistan, have shown that the resistance to these agents is still low.[18-21] Resistance to ceftriaxone varies from 0% to 3.3% and for cefixime ranges between 0% to 13.3%.

Clinical studies have shown excellent in vivo efficacy of cefixime and ceftriaxone against enteric fever.[22] Cefixime is the first extended spectrum cephalosporin which is available as oral formulation. This antibiotic has a strong activity against *Salmonella* strains causing typhoid (MIC^{90} of 0.25 µg/ml), and its clinical usefulness has also been proven in many studies. This activity is comparable to sister drug ceftriaxone as well.[23]

If the cost of oral cefixime is to be compared with ciprofloxacin then there is difference of only around 187 PKR or 1.7 USD, so we still have an alternative still affordable and with the same ease of oral administration till full emergence of ceftriaxone resistant typhoidal Salmonella. To conclude, both ceftriaxone and cefixime prove very effective antibiotics NAR and MDR typhoidal Salmonellae. Ceftriaxone is the therapeutic agent of choice for inpatients. While cefixime, can be given on outpatient basis, due to its convenient administration.

Financial Disclosure

Financial aid was provided by National University of Sciences and Technology and Army Medical College, Pakistan.

Acknowledgements

We acknowledge financial assistance of National University of Sciences and Technology, Pakistan.

Limitations of study

Limitations are the isolates are from year 2010-2011 but this data will help us compare the recent rising trend of ceftraixone and cefixime resistance in Typhoidal Salmonella in our region.

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