Molecular detection of ciprofloxacine resistance of Salmonella typhi

Hazim A. N. Alhadrawi*, Mahdi Hussain Al-Ammar , Haider Laitef

Abstract—The study was conducted to susceptibility to antibiotics and molecular level analysis of the cause of reduced sensitivity of *Salmonella typhi* isolates from patients in Babylon . Out of 50 blood cultures obtained during the study, 12 (14.0%) showed positive blood cultures were due to *S. typhi* and rests were mostly of *S. paratyphi* A. The prevalence was highest between the age group 3 - 15 year.. Among all *S. typhi* isolates, 41.4% were sensitive to ampicillin, cotrimoxazol and chloramphenicol, respectively. All isolates were sensitive to ceftriaxon and ceftazidim; 5 isolates were ciprofloxacin resistant, others were moderate to highly sensitive; whereas, only 2.2% isolates were sensitive and almost all (97.8%) were found resistant to nalidixic acid. The E-strip test among isolates showed the MIC value nearer to the sensitive between 0.125-0.5 and rest other isolates showed from > 2.0 µg/ml to very highly resistant. VNTR pattern of all ciprofloxacine resistant *S. typhi* was also same. Restriction fragment analysis of *gyrase-A* gene indicated point mutations in different loci that bear the cause of being resistant to ciprofloxacin.

Index Terms— Salmonella entericaserovarParatyphi A, DNA gyrase, Typhoid fever, *Salmonella typhi*, Fluoroquinolone resistance.

1 INTRODUCTION

Typhoid fever is a major cause of morbidity and mortality with anestimated global incidence in world.1 Salmonella enterica-serovar Typhi (*S. Typhi*) is responsible for the majority of cas-esfollowed by *S. Paratyphi* A(1) . In the lasttime, the world-wideemergence of multidrug-resistant strains of Salmonella has led tovirtual withdrawal of chloramphenicol and its replacement withfluoroquinolones and third-generation cephalosporins(.2,3) Nalidixic-acid-resistant strains exhibiting reduced susceptibilityto ciprofloxacin (MICs 0.125–1 mg/L) (4).Clinical treatment fail-ures after the administration of ciprofloxacinand other fluoro-quinolones to patients with typhoid feverattributable to these strains have been reported.(4,5).

Recent reports ofinfections because of strains of *S. Paratyphi A* with high-levelresistance to fluoroquinolones are therefore par-ticularlyworrying.(5,6) The targets of fluoroquinolones are the two enzymes.

DNA gyrase and topoisomerase IV, whose subunits are encod-edrespectively by gyrA and gyrB and the parC and parE genes. Thealteration caused by single point mutations within the quin-oloneresistance-determining region (QRDR) of the DNA gyrase subunitgyrA gene leads to quinolone resistance.(7) In Salmonella, the most commonresidues associated with mutation leading to quinolone resistancehave been Ser-83 and Asp-87 in the gyrA gene, either alone ortogether.(4,7)Additional mutations may be required to attainhigh-level fluoroquinolone resistance.(10,8,9).

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Complete fluoroquinoloneresistance in the Enterobacteriaceae usually results from twoor more point mutations within the QRDRs of the DNA gyraseand topoisomerase IV genes(9,10)

To our knowledge this is the first report in Iraq of molecular-characterization of *S. Typhi* showing a full fluoroquinoloner-esistance phenotype causing enteric fever. The molecularcharacter-istics of ciprofloxacin-resistant isolates of *S. Typhi* were com-pared with those of strains fully susceptibleto ciprofloxacin and with reduced susceptibility to ciprofloxacin.(11,12).

2.2 Materials and Methods

2.1. Bacterial Strains

A total of 12 isolates, which included *S. Typhi* strains isolated from blood cultures of patients suffering from enteric fever, were studied .The blood samples were collected from unvaccinated patients who were clinically diagnosed and admitted to Babylon hospital. Blood cultures were made using standard methodology on Blood-Agar, Chocolate-Agar and MacConkey-Agar medi-um,these included five ciprofloxacin-resistant strains, were iden-tified by standard biochemical tests and agglutination using spe-cific antisera (Murex com.).stored at -20 °C.. The isolates of *S. typhi* were confirmed by standard biochemical (API-20E system, BioMeriux,) (13)c compared withstandard results of*S. Typhi* was used as control.

2.2. Antimicrobial Susceptibility Testing

Antibiotic sensitivity and minimum inhibitory concentration for fluoroquinolone (ciprofloxacin) were performed on Mueller Hinton agar using disc diffusionmethod in accordance with Na-tional Committee forClinical Laboratory standards (NCCLS) (14).The antibiotics tested were gentamicin, amika-cin,piperacillin, ciprofloxacin, ceftazidime, piperacil-lin,ceftriaxone and ceftizoxime (Hi-media Laboratories). MICs of ciprofloxacin were determined by agar dilution and final analysis was done using an E-test kit.

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2.3. S Typhi DNA Extraction

DNA was extracted from pure culture of *Salmonella typhi* on MacConkey-agar plate by heat-lysis method. Molecular typing was done by PCR using primers as described by Liu *et al* (1995). The PCR amplified product of *gyraseA* gene was confirmed by running it through gelelectrophoresis. (15,16).

3. Results and Discussion

Out of the 50 blood cultures obtained during the study period, 12 (14.0%) yielded significant growth were *S. typhi*. The isolation rate from boys and girls was apparently similar .The present laboratory - based study, showed the prevalence of *S. typhi* among the age group between 2 and 10 years. . In addition, the isolation rate was highest in the summer and monsoon season, with peaks in September and was relatively low from November to March. Among all *Salmonella typhi* isolates studied, 42.6% of the total isolates were sensitive to ampicillin, whereas 57.4% resistant; 42% of the total isolates were sensitive to cotrimoxazol, whereas 57.4% are resistant. On the other hand, all isolates were sensitive to ceftriaxon and ceftazidim; 41.4% of the total isolates were sensitive to chloramphenicol and 58.6% are resistant; 5 isolates collected were ciprofloxacin resistant, others were moderate to highly sensitive; however, only 2.2% isolates were nalidixic acid sensitive and almost all (97.8%) were found resistant (Fig. 1).

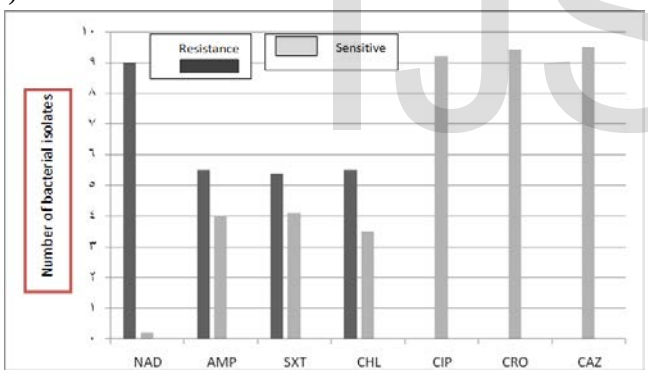

Fig. 1. *Antibiogram profile of Salmonella typhi to different antibiotics*

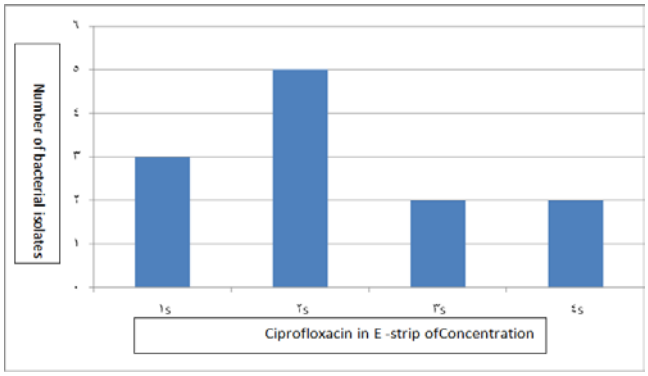

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Fig. 2. *Status of susceptibility of S. typhi isolates to Fluoroquinolone. (S1 is < 0.125, S2 is in between 0.125 and 0.5, S3 is in between 0.5 and 2.0 and S4 is > 2.0 µg/ml, respectively.).*

Present study shows an increase in the reduced fluoroquinolone susceptibility from 3.9 to 23.5% among all the *Salmonella* serovar *typhi* . Among all 12 *S. typhi* isolates studied, E-strip test was performed for isolates of which 3 showed the MIC value nearer to the very sensitive (< 0.125 µg/ml), 5 showed between 0.125 to 0.5 µg/ml, 2 showed between 0.5 to 2.0 µg/ml and rest other 2 isolates showed from > 2.0 µg/ml to vary highly resistant e.g. 512 µg/mlfigure .2.

All the ciprofloxacine resistant isolates were also highly resistant to ampicillin (> 256 µg/ml), cotrimoxazole (> 32 µg/ml), chloramphenicol (> 256 µg/ml), ciprofloxacin (> 32 µg/ml) and nalidixic acid (> 256 µg/ml), and were susceptible to ceftriaxone (0.094 µg/ml) according to their MIC of respective antibiotics. All isolates were found to be identical by Api 20E) (Fig. 3).

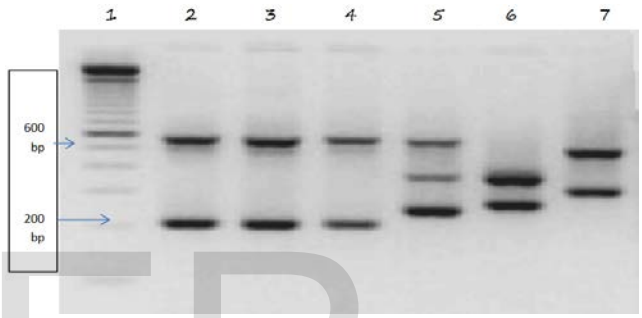

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Fig. 3. *VNTR pattern of ciprofloxacin resistant S. typhi isolates with that of comparative sensitive isolates (Lane 1: 100 bp marker; Lane 2,3,4: Ciprofloxacin resistant isolates, Lane 5,7: Ciprofloxacin sensitive isolates.*

Transferable, mutational resistance, and clonal spread are reasons for the rapidly increased quinolone resistance in *Salmonella typhi* isolates,(5) As far as we know, however, transferable fluoroquinolone resistance appears to be rare in bacteria *in vivo*. Thus, either clonal spread or resistance due to mutations in chromosomal genes remains the potential mechanism accounting for the high level of reduced fluoroquinolone susceptibility. In Clonal spread as a major contributing factor was excluded by identification of 5 serotypes among the quinolone-resistant isolates. In addition, some of these serotypes contained different antimicrobial resistance patterns along with their different VNTR patterns. Based on these data, it could bewe can conclude that the reduced fluoroquinolone susceptibility of *Salmonella typhi* in Iraq primarily involves mutations in the chromosomal genes. This concept is identify with our experiment and finally proved by sequencing data where all *Salmonella typhi*isolates with reduced fluoroquinolone susceptibility were analyzed in gel electrophoresis have shown point mutation leading to nucleotides change in their QRDR of the *gyraseA* gene.

Taq-DNA polymerase successfully amplified the 195 bps *gyraseA* gene from the genomic DNA of ciprofloxacin sensitive and ciprofloxacin resistant *Salmonella* serover *typhi* using primer of *gyraseA* (Fig. 4).

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Fig. 4. *Amplified gyraseA gene using PCR.*

The increase in the incidence of fluoroquinolone resistance in *Salmonella* and other enteric bacteria, especially *Campylobacter* sp. and *E. coli*(6-16,), drives a situation that impede the effectiveness of this antimicrobial group. The significantly common multi drug resistance observed here among the fluoroquinolone-resistant *Salmonella* compared with the susceptible population (47.4% vs. 11.5%) is also a matter of concern. This finding suggests that the abrupt use of fluoroquinolone for multidrug resistant *Salmonella* give proven of whether the same could happen to other bacterial species. Collectively, these data indicate that safe use of the fluoroquinolone antimicrobial group is warranted to prevent further development of resistance and to preserve the usefulness of this valuable drug.(17,18,19).

In conclusion, it has been shown that reduced susceptibility of *Salmonella* to the fluoroquinolone group was significantly associated with multidrug resistance. Moreover, all quinolone-resistant *Salmonella* isolates had undergone a point mutation in the QRDR of the *gyrA* gene. In contrast to previous reports on quinolone resistance in a specific clone or in a few *Salmonella* serotypes, the reduced fluoroquinolone susceptibility of our isolates was nonclonal(20).These data give more evidence of the rapid spread of multidrug-resistant pathogens from one country to another. The result of antimicrobial resistant pathogen in any city of the world may have universal finding and is therefore a general concern.

In Gram-negative bacteriathe primary target of fluoroquinolones is gyrase rather thantopoisomerase IV, hence gyrAmutations precede those of Styphi. Since eachmutation in gyrAwas associated with differentciprofloxacin MICs, further studies on other resistance mechanisms,such as alterations in membrane permeability and changesin efflux and influx, are required to evaluate the contribution ofparCmutations to fluoroquinolone resistance in S. typhi and are presently under investigation(1).

This study suggests that isolates with reduced susceptibility tofluoroquinolones might be important in clinical development ofresistance as they could become highly resistant upon sequentialacquisition of resistance. Double mutations in gyrA, along with asingle mutation in parC, have also been reported in in vitroselected ciprofloxacin-resistant mutants of S. Paratyphi A,strongly suggesting that such triple mutation is important for thedevelopment of high-level fluoroquinolone resistance(3).

All ciprofloxacin-resistant S. typhi isolates demonstratedan identical PFGE pattern and mutations in DNA gyraseand topoisomerase IV as did the S. Paratyphi A isolates. Thepatients infected with these resistant isolates did not give a historyof prior treatment with fluoroquinolones. This is the first report in Iraq country suggesting the spread and the infection by a circulating resistantstrain rather than the emergence of resistance during treatment. There is widespread availability and uncontrolled use of antibioticsincluding quinolones. Therefore, there is selective pressure on alarge bacterial population of endemic Salmonella spp. Reducingthe exposure to fluoroquinolones would definitely lessen thelikelihood of selecting mutants. As isolates with reducedsusceptibility to fluoroquinolones could become highly resistantupon sequential accumulation of mutations in topoisomerasegenes, the use of fluoroquinolones as first-line drugs formanagement of enteric fever in areas where these strains areendemic, therefore, requires urgent review.Continuous surveillance of the plasmid and chromosome ofS. typhi is essential to alter treatmentstrategies aimed at maintaining the useful life of the fewremaining antimicrobials available to treat enteric fever(4,5).

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