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Drug Resistance in *Salmonella* Typhi with special reference to Ciprofloxacin

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Abstract: A study was conducted to evaluate the resistance pattern and MIC of *Salmonella* typhi isolated from blood of patients suffering from enteric fever, Approximately 50 isolates were taken for the studies. Which were isolated from collected clinical samples within a time span of 3 month. The isolates were biochemically analyzed for confirmation of their identity. The MIC and sensitivity patterns of all the isolates were studied using fourteen antibiotics that usually represented most of the available antimicrobial groups. Resistance to several drugs, amoxicillin (18%), gentamicin (16%), nalidixic acid (22%), sulfonamides (24%), and tetracycline (24%), has been observed. However among all the antibiotics experimented, it was observed that all the isolates sensitive for ciprofloxacin. MIC of ciprofloxacin was observed to be 4µg/ml and 8µg/ml in *Salmonella* Typhi isolates collected.

The resistance of ciprofloxacin is chromosomal mediated. When plasmid of resistance ciprofloxacin insert in to the E.coli, the transformed strain could not be show the resistance. This conform that resistance to Ciprofloxacin was chromosomal.

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1. Introduction

Typhoid fever is a life-threatening infection caused by the bacterium Salmonella enterica subspecies enteric serotype Typhi. It is usually spread through contaminated food or water. Once *Salmonella* Typhi bacteria are eaten or drunk, they multiply and spread into the bloodstream. An estimated 11–20 million people get sick from typhoid and between 128000 and 161000 people die from it every year (1). Typhoid fever can be treated with antibiotics although increasing resistance to different types of antibiotics is making treatment more complicated. In addition, increasing resistance to antibiotic treatment is making it easier for typhoid to spread through overcrowded populations in cities and inadequate and/or flooded water and sanitation systems.

Typhoid risk is higher in populations that lack access to safe water and adequate sanitation. Poor communities and vulnerable groups including children are at highest risk. The emergence of multidrug-resistant (MDR) S. Typhi (resistant to ampicillin chloramphenicol and co-trimoxazole) led to the introduction of fluoroquinolones (FQs) as the first-line drug during the last two decades of the 20th century (2). However, frequent treatment failures along with an increase in the minimum inhibitory concentration (MIC) of ciprofloxacin (CIP) is great concern. This study was therefore planned to review the drug resistance patterns in S. Typhi strains isolated over a period of time.

2. Material and Methods

It was a prospective study which was conducted in Mudrakshi Histology Centre, Daily routine samples were collected and were inoculated in Blood culture bottle and after positive indication, it was inoculated onto blood and MacConkey agar plates and incubated aerobically at 37°C for 18 hrs. Growth was observed and the isolates were identified using standard protocols.

Preserved strains on a peptone stab agar containing a subinhibitory concentration of antibiotic to avoid loss of plasmids were revived and subjected to purification and identification. Antibiotic susceptibility was determined by the disk diffusion method using a modified Kirby–Bauer technique as well as by MIC determination.

by the agar dilution method according to the Clinical and Laboratory Standards Institute (CLSI) (3). Antibiotic disks used for susceptibility testing included amoxicillin (30 mcg), chloramphenicol (30 mcg), ciprofloxacin (5 mcg), amikacin.

(30 mcg), Gentamicin (10 mcg), netilmicin (30 mcg) (Hi-Media, Mumbai, India).

MIC determination

For each strain, two independent cultures were grown for 25 h at 37°C in LB (Luria-Bertani) containing no antibiotic. From each culture, □10^4 CFU were spotted in duplicate onto LB agar containing ciprofloxacin at 0, 0.5, 1, 2, 4 and 8µg/ml. After 24 h of incubation at 37°C, the MIC was determined to be the concentration at which no visible growth was observed. Single colonies were not counted as growth and represent ciprofloxacin-resistant mutants. (4)

Plasmid extraction and transfer

Plasmid DNA extraction was performed using the alkaline lysis method (5). The plasmids isolated from S. Typhi clinical isolates were used to transform E. coli DH5K competent cells using CaCl2(6), with selection on LB agar plates containing 5 µg/ml of ciprofloxacin (Hi-Media, Mumbai, India).

3. Results

S. Typhi isolates studied, were isolated from blood were characterized by resistance most of the primary drugs used for the treatment (table-1). Demonstrated increased MICs for 2 S Typhi stain show great concern. Transformation attempts failed to detect a plasmid-encoded resistance, indicating a chromosomal location of the resistance gene.

4. Discussions

Prevalence of fluoroquinolone-resistant isolates and increasing prevalence of multidrug-resistant isolates have been shown. Hence, early detection of resistance mechanisms and their sensitivity patterns will help in formulation of antibiogram and infection control policies, thus reducing morbidity, mortality and prevent emergence of drug resistant strains.

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