

## THE PREVALENCE AND CIPROFLOXACIN SUSCEPTIBILITY PATTERN OF TYPHOIDAL SALMONELLA IN A TERTIARY CARE HOSPITAL OF NORTH INDIA

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### ABSTRACT

#### BACKGROUND

Typhoid fever, also called enteric fever, is an acute, potentially life-threatening febrile illness caused by *Salmonella typhi* (*S. typhi*). It is a bacterial infection of the intestinal tract, and the blood stream. *S. enterica* serovar Paratyphi A, B, or C is estimated to cause 5.5 million cases of enteric fever each year. However, in some regions, notably, south Asia, the proportion of cases of disease due to *S. Paratyphi A, B, or C* strains is likely much. The emergence of antimicrobial resistance in *Salmonella* isolates from food potentially compromise the treatment of these infections.

The aim is to study the prevalence and ciprofloxacin susceptibility pattern of typhoidal salmonella in a tertiary care centre hospital of North India.

#### MATERIALS AND METHODS

In this retrospective study, a total of 733 diagnosed cases of *Salmonella* patients (550 male and 183 female) with appropriate symptoms were tested in Department of Microbiology, CMC & H, Ludhiana from January 2011 to December 2016.

#### RESULTS

Out of 733 *Salmonella* isolates, 594 (81.03%) were *Salmonella Typhi* and 139 (18.96%) were *Salmonella Paratyphi A*. There were 732 (99.86%) Nalidixic Acid resistant strains and ciprofloxacin resistant strains using the CLSI 2014 guidelines, which has shown same susceptibility pattern in the past years for CLSI 2011 and CLSI 2012 guidelines.

#### CONCLUSION

While first-line antimicrobials may still have a role to play in the treatment of enteric fever, ceftriaxone remains the sole defence against ciprofloxacin-resistant *Salmonella enterica* serotype Typhi and Paratyphi A.

#### KEYWORDS

*Salmonella*, Ciprofloxacin, CLSI.

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#### BACKGROUND

Enteric fever is an important cause of human morbidity and mortality in developing nations like India. This acute systemic infection is caused mainly by *Salmonella enterica* serotype Typhi and Paratyphi A, rarely by *S. paratyphi B* and C. The World Health Organization in 2006 reported an incidence of up to 33 million cases of enteric fever occurring globally per year, with 500,000 to 600,000 deaths and 1.5% - 3.8% case fatality rate.<sup>[1]</sup>

In most cases, salmonellosis is caused by contaminated food products, particularly those of animal origin such as poultry, eggs, beef and pork. Fruits and vegetables also have been reported as vehicles in *Salmonella* transmission, and contamination can occur at multiple steps along the food chain.<sup>[2]</sup>

Antimicrobial agents are currently used for three main reasons-

(1) To treat infections in humans, animals, and plants; (2) prophylactically in humans, animals, and plants; and (3) subtherapeutically in food animals as growth promoters and for feed conversion. When antibiotic use became the norm in both human and animal medicine, selection pressure increased the bacterial advantage of maintaining and developing new resistance genes that could be shared among bacterial populations.<sup>[3]</sup> Chloramphenicol was introduced in 1948 as the first effective antibiotic in the treatment of typhoid fever. Even though resistance started to develop within two years of its introduction, it did not emerge as a major problem until 1972. Chloramphenicol resistance was associated with high molecular weight, self-transferable, H incompatibility group (InCHI) plasmids. Amoxicillin and co-trimoxazole were the effective alternatives till the development of 'multidrug resistant (MDR) strains' (resistant to ampicillin, chloramphenicol and co-trimoxazole - ACCo) towards the end of 1980s and 1990s.<sup>[4]</sup>

In addition to high frequency and easy transmission; *Salmonella* outbreaks also accompany the threat of multidrug resistance. There are many reports of multidrug resistant *S. typhi* enteric fever epidemics from Indian subcontinent as has

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been previously notified in Mumbai Garrison, in Nagpur, in a rural area of North-east Karnataka and many other areas.<sup>[5,6]</sup>

The emergence of MDR Salmonella isolates led to the use of fluoroquinolones (Ciprofloxacin and ofloxacin) as the first-line drugs for its treatment. Fluoroquinolones have good in vitro and clinical activity against salmonellae and became the treatment of choice in cases of MDR Salmonellosis.<sup>[7]</sup> Isolates with low-level resistance (MIC  $\geq 0.25$   $\mu\text{g/mL}$  but  $< 4$   $\mu\text{g/mL}$ ) to fluoroquinolones appeared within a few years of this change.<sup>[8,9]</sup> Quinolone resistance is frequently mediated by single-point mutations in the quinolone resistance determining region of the gyr A gene, characteristically occurring at position 83 of the DNA gyrase enzyme (changing serine to phenylalanine) and position 87 (changing aspartate to tyrosine or glycine).<sup>[10]</sup>

### Aim

To study the prevalence and ciprofloxacin susceptibility pattern of typhoidal salmonella in a tertiary care centre hospital of North India.

### MATERIALS AND METHODS

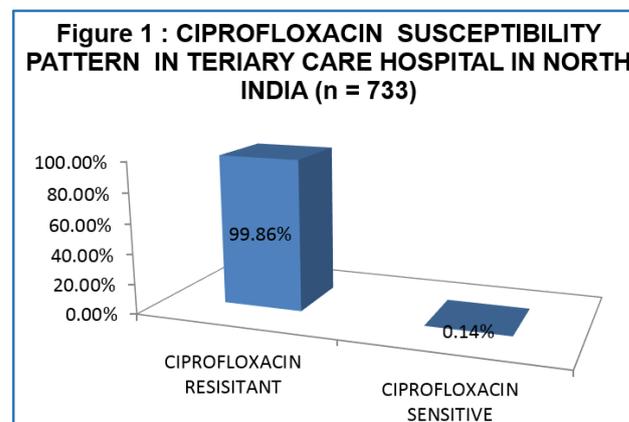
In this retrospective study, a total of 733 diagnosed cases of Salmonella patients (550 male and 183 female) with appropriate symptoms were tested in Department of Microbiology, CMC&H, Ludhiana by Kirby Bauer Disc Diffusion Method and MicroScan Walk Away 96 plus from different blood, stool and pus samples received in the microbiology laboratory over a period of 6 years from January 2011 to December 2016. Out of 733 isolates, 691 (94.27%) were isolated from blood, 41 (5.59%) from stool, and 1 (0.14%) from pus.

The Salmonella isolates were tested for susceptibility to antimicrobials (Ampicillin, chloramphenicol, cotrimoxazole, amikacin, gentamycin, ceftazidime, cefotaxime, ceftriaxone, ceftazidime, ciprofloxacin, nalidixic acid, piperacillin-tazobactam, cefoperazone-sulbactam, azithromycin) on Mueller-Hinton agar following the disc diffusion method and MicroScan Walk Away 96 plus according to the guidelines by Clinical and Laboratory Standards Institute (CLSI).

### RESULTS

Out of 733 Salmonella isolates, 594 (81.03%) were Salmonella Typhi and 139 (18.96%) were Salmonella Paratyphi A. There is 98% susceptibility to ampicillin and 100% susceptibility to chloramphenicol, cotrimoxazole, amikacin, gentamycin, ceftazidime, cefotaxime, ceftriaxone, ceftazidime, piperacillin-tazobactam, cefoperazone-sulbactam, azithromycin. There were 732 (99.86%) Nalidixic Acid resistant strains and ciprofloxacin resistant strains using the CLSI 2014 guidelines, which has shown same susceptibility pattern in the past years for CLSI 2011 and CLSI 2012 guidelines. MIC pattern among the Nalidixic Acid and Ciprofloxacin resistant isolates were interpreted with MIC  $\geq 32$  and  $\geq 4$  and disc diameters of  $< 13$  mm and  $< 20$  mm and among sensitive strain with MIC of  $\leq 16$  and  $\leq 1$  along with zone diameter of  $> 19$  mm and  $> 31$  mm respectively according to CLSI guidelines 2014. A positive correlation was observed between reduced ciprofloxacin susceptibility and Nalidixic Acid resistance in all the isolates.

Figure 1 showing the susceptibility pattern of ciprofloxacin in a tertiary care hospital of North India.



### DISCUSSION

In developed countries, typhoid fever is often associated with persons who travel to endemic areas or immigrate from them. Resistance to ciprofloxacin by this Salmonella serovar represents an emerging public health issue.<sup>[11]</sup> There are many studies reporting high level ciprofloxacin resistance in *S. Typhi* (MIC  $\geq 32$   $\mu\text{g/mL}$ ) from various parts of India including Kolkata, Pondicherry and Delhi.<sup>[12]</sup> In Qatar, over one half of the isolates demonstrated RSC (54%), while only 14% were MDR. This may be attributed to the importation of *S. Typhi* strains from the Indian sub-continent, where it is endemic, and the Far East; it has been estimated that expatriate workers constitute 30–40% of the population in the Gulf States. In our study, Ciprofloxacin resistant isolates were interpreted with MIC  $> 4$   $\mu\text{g/mL}$  and disc diameter of  $< 20$  mm among sensitive strain with MIC  $\leq 1$   $\mu\text{g/mL}$  along with zone diameter of  $> 31$  mm respectively according to CLSI guidelines 2014.

The difference in the study of susceptibility may be due to a different geographical area or the genetic variability of the population. The resistant strains of ciprofloxacin, levofloxacin, ofloxacin, pefloxacin, or nalidixic acid may be associated with continuation of clinical symptoms or delayed response to therapy in fluoroquinolone-treated patients with salmonellosis. If a ciprofloxacin, levofloxacin, or ofloxacin MIC test cannot be done, pefloxacin disk diffusion may be used as a surrogate test.<sup>[13]</sup>

There has been reports about the isolation of Salmonella enterica serotype Typhi strains showing high-level resistance to ciprofloxacin. The exact mechanism of fluoroquinolone resistance in Salmonella enterica serotype Typhi and Salmonella enterica serotype Paratyphi A is not fully understood. Various studies have found that a single mutation in the gyr A gene is sufficient to confer resistance to Nalidixic Acid and reduced susceptibility to fluoroquinolones, and a second mutation leads to high-level fluoroquinolone resistance.<sup>[14]</sup> The prevalence of paratyphoid fever, caused by *S. Paratyphi A*, has been increasing worldwide; with studies from India and Nepal suggesting that in some settings, *S. Paratyphi A* can contribute up to half of all cases of enteric fever.<sup>[15]</sup> Antibiotic resistance in *S. Paratyphi A* isolates is an emerging problem. For example, though a 1996 outbreak of paratyphoid fever in India demonstrated that isolates were susceptible to all antibiotics including chloramphenicol, amoxicillin, cotrimoxazole, gentamicin, ciprofloxacin and ceftriaxone, 2 years later in New Delhi, the prevalence of

resistance to ciprofloxacin had increased to 24% with 32% of isolates exhibiting decreased ciprofloxacin susceptibility.<sup>[16]</sup>

### CONCLUSION

While first-line antimicrobials may still have a role to play in the treatment of enteric fever, ceftriaxone remains the sole defence against ciprofloxacin - resistant *Salmonella enterica* serotype Typhi and Paratyphi A. The usage of this drug in the empiric therapy should be discouraged. Farmers and their veterinarians should be responsible for judicious use of antibiotics in the animal industry just as physicians should be judicious in their use of antibiotics in human medicine.

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