

**Research Article**

## **Comparative prevalence of MDR *S. typhi* in Northern and Southern parts of Karnataka, South India**

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### **Abstract**

Typhoid fever due to infection of MDR *Salmonella enterica* serovar *typhi* resistance to Chloramphenicol, ampicillin and cotrimoxazole has posed a serious therapeutic challenge in many parts of the world, including South India. Till recently ciprofloxacin was the drug of choice for the treatment for the MDR *S.typhi*. However treatment failure to ciprofloxacin has been recorded with decreased susceptibility to nalidixic acid, which is the marker for ciprofloxacin resistance in *S.typhi*. Alternative drugs for the treatment of MDR *S. typhi* the cephalosporins; however, emergence of cephalosporins resistance is a matter of concern. This prompted us to study drug resistance patterns among *S. typhi* isolates from North Karnataka, which is endemic for typhoid fever and compared with South Karnataka which is non endemic region and formulate the appropriate drugs for the management of enteric fever.

**Keywords:** Typhoid fever, salmonella typhi, antibiogram, ESBL

### **1. Introduction**

Typhoid fever caused by *Salmonella enterica* serovar *typhi* continues to remain a public health problem in many countries. An estimated 21.7 million illnesses and 2,17,000 deaths are caused worldwide<sup>1</sup>. Incidence of Typhoid in Asia has been reported to be 274 per 1, 00,000 persons<sup>1,2,4</sup>. *Salmonella enterica* serovar *typhi* and *Salmonella enterica* serovar *paratyphi A* are the main etiological agents of enteric fever in India and *S.typhi* is predominant<sup>3</sup>. Many outbreaks have been reported in the developing countries<sup>4</sup>.

The emergence of multidrug resistance in *S.typhi* has further complicated the treatment and management of enteric fever, mainly resistance to ampicillin, chloramphenicol and cotrimoxazole which are the first line drugs for the treatment of enteric fever. In India multidrug resistant *S. typhi* (MDRST) was first reported from Calicut<sup>5</sup>. Since then a steady increase in the MDRST over the next two decades has been recorded. There was a decline in MDR *S.typhi* with the introduction of nalidixic acid. Strains with decreased susceptibility to fluoroquinolones and increased incidence of nalidixic acid resistant *S.typhi* (NAST), especially that exhibit decreased susceptibility to most recent fluoroquinolones used for the treatment of Typhoid fever are reported<sup>7,8</sup>. Interestingly there are reports on the gradual decrease in MDR *S.typhi* and increased sensitivity to the chloramphenicol<sup>9</sup>. There are also reports on the third generation cephalosporin resistant *S.typhi* and ESBL *S.typhi* in India and various parts of the world<sup>10</sup>.

The above background prompted us to determine the antibiotic resistance pattern of outbreak causing as well as sporadic isolates of *S.typhi* from two geographically different regions namely Northern and Southern parts of Karnataka in India, and the Northern part of Karnataka is the endemic for *S.typhi*.

### **2. Methodology**

The study was carried during April 2010 to October 2012 in Gulbarga and Raichur Districts in North Karnataka and from May 2011 to Sept 2012 in Bangalore, South Karnataka. The study included a total of 105 *S.typhi* isolates from blood samples of patients suffering from suspected typhoid fever who attended the outpatient clinics or were admitted in the private and government hospitals of these regions. A total of 1500 blood samples, 1200 from Northern part of Karnataka and 300 from Southern part of Karnataka were included in the study.

#### **2.1 Isolation and Characterisation**

Blood samples from the febrile patients 5 to 10 ml were collected by vein puncture and inoculated directly in to blood culture bottles containing 50 ml of brain heart infusion broth<sup>11</sup>. The enriched samples after visible turbidity were streaked on Mac-Conkey Agar and Wilson Blair Agar plates<sup>12</sup>. The isolates producing characteristic colonies were identified by standard biochemical tests and confirmed by agglutination with salmonella O9 vi specific and Hd antisera (Kings Institute of Preventive Medicine Guindy, Chennai)

#### **2.2 Antimicrobial susceptibility testing:**

Antimicrobial susceptibility of the isolates was determined by the Kirby Bauer disc diffusion method according to CLSI guidelines using the following antibiotics (Hi media)<sup>13</sup>; ampicillin (10µg), amoxycylav (30µg), (amoxicillin 20µg and clavulanic acid 10µg), Chloramphenicol (30µg), ciprofloxacin (5 µg), nalidixic acid (30 µg), cotrimoxazole (35 µg), imipenem (10 µg), ceftazidime (30 µg), ceftriaxone (30 µg), cefotaxime (30µg), cefoxitine (30 µg), cefexime (30 µg), cefotaxime (30 µg), cefeprozone (30 µg) and sulbactam (30 µg). *E. coli* ATCC 25922 was used as a control strain<sup>14</sup>.

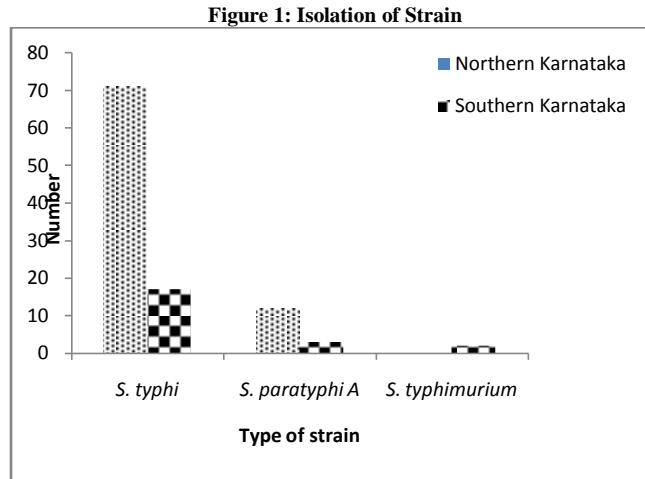
#### **2.3 ESBL detection**

The isolates that were resistant to the third generation cephalosporins by disc diffusion method were selected for ESBL screening by double disc synergy test (DDST)<sup>15</sup>. A disc of amoxycylav (30 µg) was placed on the centre of Mueller Hinton Agar plate. Each disc of cefetazidime, cefoxitine, cefotaxime, and ceftriaxone was placed around the amoxycylav disc, 20 mm apart and incubated for 24 hrs at 37°C. A perfect formation of edge of the inhibition zone of any of the antibiotics towards the disc containing amoxycylav was interpreted as positive for ESBL production.

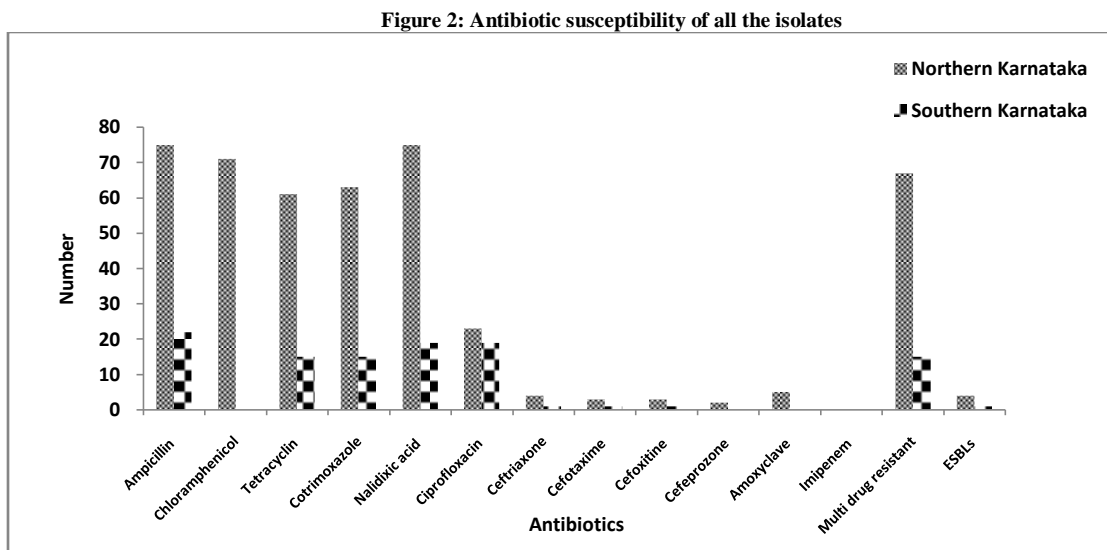
Confirmation of ESBL producers was done by phenotypic confirmatory disc diffusion method using cefotaxime with and without clavulanic acid<sup>13</sup>. A clear zone difference of ≥5mm with antibiotic + clavulanic acid disc when compared to antibiotic alone was considered confirmatory. Strains showing resistance to more than three different groups of antimicrobials as quinolones, tetracycline, cephalosporin's etc. were grouped as multi drug resistant strains<sup>14</sup>.

3. Results

The study included a total of 1500 blood samples. A total of 105 *S.typhi* were isolated from the samples showing an isolation rate of 7%, which was slightly higher (7.3%) in South Karnataka as compared to North Karnataka (6.9%). Among the 83 isolates from North Karnataka, 71 were *S.typhi* and 12 were *S.paartyphi A*, whereas in South Karnataka among the 22 isolates, 17 were *S.typhi*, 3 were *S.paartyphi A* and 2 were *S.typhimurium* (fig-1).



Antibiotic susceptibility of all the isolates is depicted in fig 2. A total of 67 (80.7%) isolates were MDR in Northern Karnataka and 15 (68.18%) in southern Karnataka. All the isolates of Southern Karnataka were sensitive to chloramphenicol and 71 (86.02%) were resistant to chloramphenicol in Northern region is one of the clear difference in drug resistance pattern between the two different geographical regions of Karnataka.



Out of the 83 isolates from North Karnataka, 4 (4.8%) were ESBL producers while only one isolate (4.5%) from South Karnataka was producing ESBL (Table-2). A comparative account of the antibiogram of the ESBL producers and ESBL non-producers from North Karnataka and South Karnataka is given in Table-3, which shows that the frequency of multidrug resistance is higher among ESBL producers than non ESBL producers from both the regions.

Table: 3. Antibiotic resistance among non-ESBL and ESBL producing *Salmonella* isolates

Antibiotics used	non ESBL <i>Salmonella</i> (n=100)		ESBL producing <i>Salmonella</i> (n=5)	
	North Karnataka (n=79)	South Karnataka (n=21)	North Karnataka (n=4)	South Karnataka (n=1)
Ampicillin	94.93%	100%	100%	100%
Chloramphenicol	89.87%	00%	75%	00%
Tetracyclin	89.87%	68.18%	75%	100%
Cotrimoxazole	79.74%	68.18	75%	100%
Nalidixic acid	94.93%	90.4%	100%	100%
Ciprofloxacin	29.11%	90.4%	75%	100%
Ceftriaxone	5.06%	4.7%	100%	100%
Cefotaxim	3.79%	4.7%	100%	100%
Cefoxitin	3.79%	4.7%	75%	100%
Cefoperzone	3.79%	00%	50%	00%
Amoxyclave	3.79%	00%	75%	100%
Imipenem	00%	00%	00%	100%
Multi drug resistant	84.81%	68.18%	100%	100%

#### 4. Discussion

Enteric fever caused by the *S.typhi* continues to be the global burden causing considerable morbidity and mortality and 80% of the global burden of *S.typhi* is mainly seen in the Asian and African countries<sup>4</sup>. It is a serious problem in endemic countries and travelers to these areas. It may be due to a combination of factors including poor sanitation and health care infrastructure<sup>16</sup>.

In the present study, the antimicrobial susceptibility of *typhoidal Salmonellae* isolated from two geographically distinct regions of Karnataka Southern India is reported. Northern part of Karnataka is endemic for typhoid fever and Southern parts mainly Bangalore city which is densely populated and a site of travelers from various regions.

The study highlighted the predominance of multi drug resistance among *S. typhi* isolates from both geographic areas. Further, increased nalidixic acid resistance is of concern. However, there is a decreased susceptibility to fluoroquinolones especially to ciprofloxacin<sup>17</sup> which is the choice of drug for the treatment of MDR typhoid fever. Study also shows the increased drug resistance to first line drugs namely ampicillin, chloramphenicol and cotrimoxazole. A remarkable difference in chloramphenicol resistance has been observed among these two geographical areas. All the 22 isolates from South Karnataka region are susceptible to chloramphenicol where as 71 (86.6%) isolates from Northern Karnataka are resistant to chloramphenicol<sup>17</sup>. This shows a change in the drug resistance pattern between two geographical regions. Increase in the ciprofloxacin resistance has been recorded in both regions from North Karnataka, 27.7% and South Karnataka 86%, when compared with previous reports of 8%<sup>16</sup>, which may be attributed to the increased nalidixic acid resistance. Previous studies have indicated that increased nalidixic acid resistance results in the gradual increase in ciprofloxacin MIC ( $\leq 0.125\mu\text{g/L}$  >  $1\mu\text{g/L}$ ). This indicates that there has been a situation to relook at the break points and the zone diameters for reporting ciprofloxacin resistance for *Salmonella*<sup>17</sup>. In the present study 4.7% of third generation cephalosporin resistant *S.typhi* have been isolated, which is a matter of concern<sup>18</sup>.

We have screened for the ESBL producers among the third generation cephalosporin resistant strains by DDST phenotypic method. A total of 5 ESBL isolates have been recorded which is alarming in both the regions. And all the organisms from both the regions are susceptible to imipenem which is promising.

To conclude this study demonstrates the increased MDR *S.typhi* in both the regions and difference in the susceptibility to the chloramphenicol stresses the need for a regular and regional monitoring of the antibiotic susceptibility and check the empirical use of antibiotics which is one of the causes for development of multi drug resistance. Third generation cephalosporin resistant and ESBL *S.typhi* are alarming which further stresses the necessity for rapid and economical techniques to detect and monitor the ESBL producers.

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