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## ANTIMICROBIAL PROFILE OF *SALMONELLA* SPECIES ISOLATED AT TERTIARY CARE HOSPITAL WESTERN INDIA

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**ABSTRACT: Background & Objective:** India is home to two infectious diseases caused by *Salmonella*: typhoid and paratyphoid fever. The medication of choice for *S. typhi* is ceftriaxone, a third-generation cephalosporin. Study was carried out on the Surveillance of Enteric Fever in India to ascertain the prevalence, clinical manifestation, and trajectory of antimicrobial resistance (AMR). This work aimed to examine the antibiogram profiles of *Salmonella typhi* found in patient blood cultures. Second, to record how often *Salmonella typhi* infections occur located in Vadodara, the Antibiotic Susceptibility Test is third. **Material and Methods:** The Retrospective study was carried out over a period of 12 months after obtaining ethical approval. The freshly drawn venous blood was placed in a blood culture vial. Following identification of pathogen isolates, Kirby-Bauer disc diffusion test was employed to determine antibiotic drug susceptibility as per CLSI rules. **Results:** Total 10,126 blood samples were collected. In 84 samples (0.83%), 83 instances of *Salmonella typhi* and 1 instance of *Para typhi* B. The ratio of males to females was 1:2. Children aged 0–14 years had greater incidence of enteric fever. From July to September, the most instances are solitary. The majority of the *Salmonella typhi* isolates showed resistance to Ceftriaxone but were sensitive to Ciprofloxacin, Azithromycin and Cotrimoxazole. **Conclusion:** It has been shown in this review that antibiotic resistance is on the rise among *Salmonella* species. The use of empirical treatment for enteric fevers should be avoided in favor of third generation cephalosporins, Cefepime, Carbapenem, and azithromycin.

**INTRODUCTION:** Typhoid fever is caused by the gram-negative rod-shaped Enterobacteriaceae family member *Salmonella typhi*, which can enter bloodstream and cause a potentially deadly illness. It has been a major contributor to illness and death in underdeveloped countries. Compared to South Asian and African nations with low or medium incomes, developed nations have a far lower typhoid incidence rate<sup>1</sup>. With an estimated 9.25 million cases in 2019, the

majority of the 110,000 fatalities caused by typhoid fever were in South Asia and Sub-Saharan Africa, according to the Global Burden of Disease Study<sup>2</sup>. *S. typhi* is a human-only pathogen in the wild, and contamination of food or water is the usual vector for its transmission. There were two deaths, and eighty-eight cases reported in the Malaysian province of Kelantan in 2005<sup>3</sup>.

Following consuming food or water contaminated with *S. typhi*, symptoms often appear 7–14 days following the asymptomatic phase. After the first asymptomatic period, patients may have gastrointestinal discomfort, nausea, vomiting, and diarrhea. The severity and quantity of mortality caused by several infectious illnesses have been greatly reduced thanks to antibiotics having been an essential weapon in the fight against typhoid<sup>4,5</sup>.

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The overuse and abuse of antibiotics in human therapy has unfortunately led to an increase in the resistance of *S. typhi* to these medications. On their own, mutations in *Salmonella typhi* can cause the development of AMR<sup>6, 7</sup>. Current antimicrobial resistance (AMR) data for *S. typhi* is essential for effective antibiotic stewardship and better patient outcomes, and this data should be accessible worldwide and in real-time. Consequently, this study used a systematic review and meta-analysis to assess the frequency of AMR in *S. typhi* isolated from people.

**MATERIALS AND METHODS:** “Researchers from Medical College Baroda’s Microbiology Department in Vadodara, Gujarat, carried out a retrospective study from January to December 2023. A venous blood sample was obtained from patients exhibiting typhoid-like symptoms, regardless of whether they were hospitalized or not (5 mL for adults and 2 mL for children). The venous blood samples that were taken were transferred using blood culture sterile vials, which can be either the classic or Bactec variety (Adult & Pediatric). The first culture is carried out in a standard blood culture bottle following a 24-hour incubation period in a carbon dioxide incubator. In addition, we accurately label the blood vial so that the Bactec machine can recognize the development.

Bacteria that demonstrated growth were then transferred to several medium, including MacConkey agar, xylose lysine deoxycholate (XLD) agar, and *Salmonella-shigella* (SS), after being incubated at 37°C for 18-20 hours. The isolates were characterized using colony morphology and biochemical tests. The use of antisera allowed for the confirmation of *S. typhi* or *Para typhi* instances. Antibiotic susceptibility testing, which included the modified Kirby-Bauer disc diffusion method, was conducted in accordance with CLSI criteria<sup>8</sup>. Equally spaced were the Mueller-Hinton agar plates that had been impregnated. The following antimicrobial discs were applied to the plates using sterile forceps: the following dosages of antibiotics: ampicillin (10 micrograms), ceftriaxone (30 micrograms), azithromycin (15 micrograms), doxycycline (30 micrograms), imipenem (30 micrograms), ciprofloxacin (5 micrograms), chloramphenicol (30 micrograms), and co-trimoxazole (1.25/23.75 micrograms). The discs were 6 mm from the edge and 10-15 mm apart. For the purpose of quality control, we utilized ATCC strains (ATCC-27853 for *Pseudomonas aeruginosa*, ATCC-25923 for *Staphylococcus aureus*, and ATCC-25922 for *Escherichia coli*).



FIG. 1: MALONATE TEST

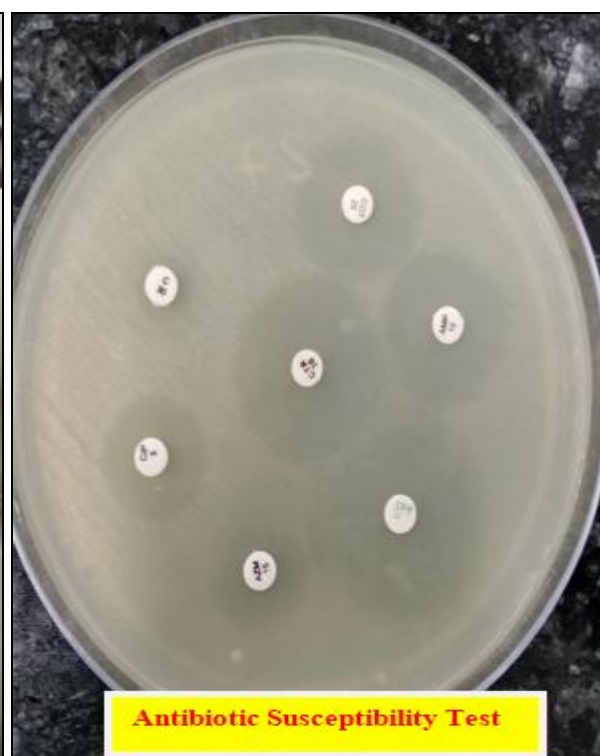


FIG. 2: ANTIBIOTIC SUSCEPTIBILITY TEST



FIG. 3: SALMONELLA ANTISERA TEST

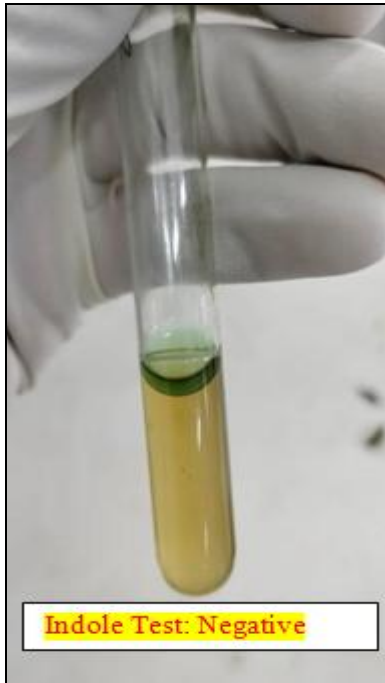


FIG. 4: INDOLE TEST

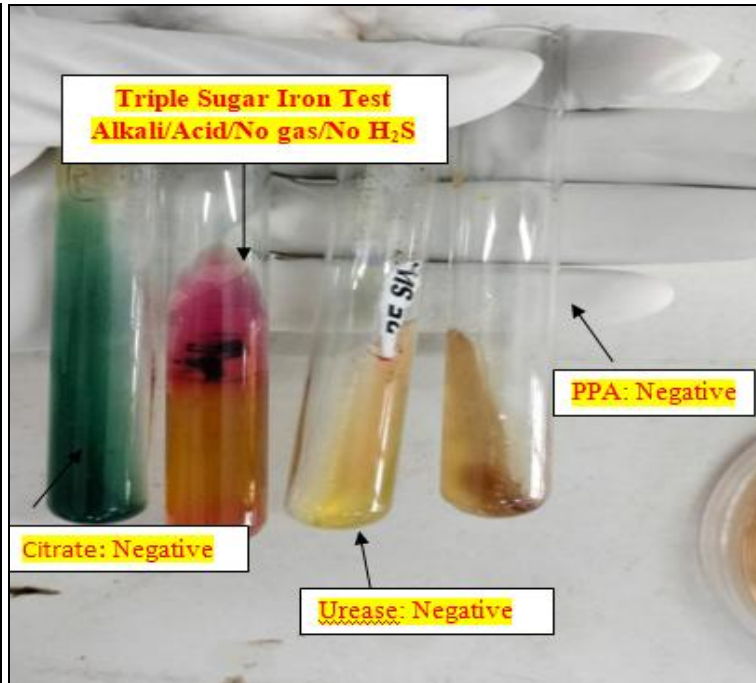


FIG. 5: TRIPLE SUGAR IRON TEST

**Inclusion Criteria:** If a patient has septicemia symptoms such as a high temperature, shortness of breath, low blood pressure, rapid heart rate, mental disorientation, or decreased urine output, medical attention is needed immediately.

**RESULTS:** The study included 84 patients, with 39 being males and 45 being females, for a male to female ratio of approximately 1:1.2. The age range of 0-14 years comprised the majority of the Salmonella cases (n=54). Below, you can find the

age distribution of the Salmonella cases in Fig. 6 and the gender distribution in chart 2. As per Fig. 7 of Age wise distribution of salmonella showed that in children under 14 year (n=54) old age group a greater number of salmonella were isolated. One third number of Salmonella were isolated in more than 14 year of age (n=30) children and adult. As per above chart 2 of Gender wise distribution of salmonella shown that there is no major difference between Male and Female Gender.

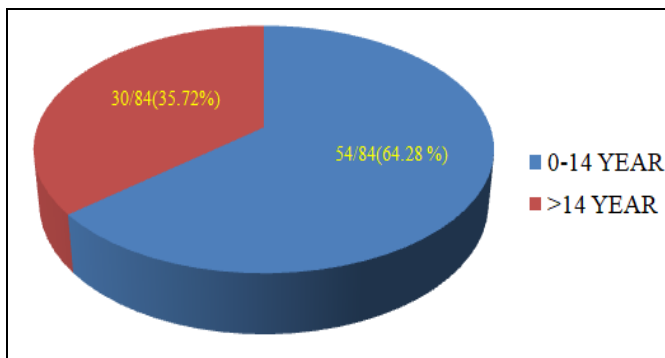


FIG. 6: AGE WISE DISTRIBUTION

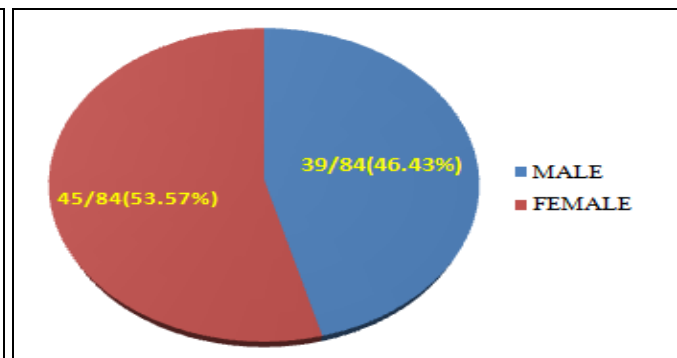


FIG. 7: GENDERWISE DISTRIBUTION

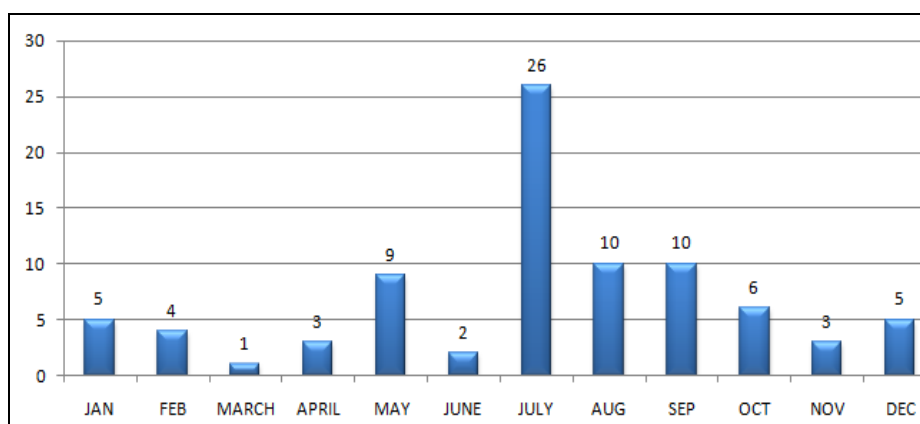


FIG. 8: MONTHWISE DISTRIBUTION

In this study, the rate of typhoid fever was varied during the year time. In this study as shown in Table no 1 highest number of cases were isolated from July (26 cases) followed by August &

September (10 cases). Also 1 cases of *Salmonella Para typhi B* was isolated in October. A total of 57 cases were isolated in summer, monsoon and post monsoon season.

TABLE 1: ANTIBIOGRAM OF *SALMONELLA TYPHI* (N=83)

Antibiotic	Susceptible	Resistant	Intermediate
Ampicillin (10 µg)	67 % (n=54)	32% (n=26)	1% (n=3)
Ceftriaxone (30 µg)	79% (n=66)	17 % (n=14)	4% (n=3)
Azithromycin (15 ug)	71.08% (n=59)	28.92 % (n=24)	-
Doxycycline (30 µg)	65.06% (n=54)	30.12 % (n=25)	4.81 (n=4)
Ciprofloxacin (5 µg)	10 % (n=9)	71 % (n=59)	19% (n=15)
Co-Trimoxazole (1.25/23.75 µg)	70 % (n=59)	25 % (n=21)	5% (n=3)

In present study 79% susceptibility to ceftriaxone was found followed by, 71.08% to Azithromycin, 70% to cotrimoxazole, 67% to ampicillin, 65.06% to doxycycline and 10% to ciprofloxacin. 14 cases resistance to third-generation cephalosporins (Ceftriaxone 30 µg) is the most worrisome finding in this study. Imipenem susceptibility testing is done by disc diffusion method in isolates were observed Ceftriaxone resistant. One case of *Salmonella Para typhi B* was isolated and it was fully susceptible to all antibiotic drugs.

**DISCUSSION:** Enteric fever is primarily caused by *Salmonella typhi*. Antimicrobial susceptibility patterns have been found to be changing throughout several regions of India, highlighting the need for ongoing evaluation and monitoring<sup>9</sup>.

In the present study, out of the total 84cases of typhoid and paratyphoid cases are observed with majority almost 98% being typhoid fever. These findings were similar to study done by Patil *et al*<sup>10</sup>, Mishra *et al*<sup>11</sup> and Khadaka *et al*<sup>12</sup> all reported that *S. typhi* is major isolates followed by *S. Para typhi A*. Patients in rural areas were more likely to have poor faeco-oral hygiene and water sanitation,

which is why the majority of *Salmonella* isolates came from those areas. Patients in rural areas were more likely to have poor faeco-oral hygiene and water sanitation, which is why the majority of *Salmonella* isolates came from those areas<sup>9</sup>.

Typhoid fever cases highest number in below 14 year of age group which is differ from Al-Roubaea *et al.*<sup>13</sup> study which represent maximum number of salmonella cases isolates in 21-30 year. The reason for which may be because enteric fever is endemic in India.

There was no significant difference between the sexes when looking at the gender distribution of *Salmonella* spp. This result agreed with what Jaafar *et al.* found in their previous research<sup>14</sup>.

In this study that the highest number of cases with enteric fever (54.76%) are in monsoon months like July, August and September which is similar to Al khafaji *et al*<sup>15</sup> study that observed highest number (65.53%) of patients with typhoid fever in Summer and monsoon months. Consistent with previous research, the current study found that nearly all *Salmonella typhi* isolates were sensitive to

cephalosporins, with a particularly high percentage (79%) to Ceftriaxone Patil *et al*<sup>10</sup> is completely susceptible to ceftriaxone, as has been observed in comparable studies.

Present study has 71% susceptibility to Azithromycin and similar findings are observed by Chayani *et al*<sup>16</sup> 83% and different findings were noted by Patel SR *et al*<sup>17</sup> and Jain *et al*<sup>18</sup> study that 21% & 7% susceptibility to Azithromycin respectively (2011-2013).

Jain *et al*<sup>18</sup> study has noted the 19.1% susceptibility to cotrimoxazole which was lower than present study (70%).

Medella *et al*<sup>19</sup> showed decreased susceptibility towards the FQs mainly ciprofloxacin that was 0.6% that was similar to present study susceptibility that was 10%

In this present study shown that total 14 cases resistance to third-generation Cephalosporins (Ceftriaxone 30 µg) which like as Farah Naz Qamar *et al*<sup>20</sup>. Study in which two cases of resistance to third-generation Cephalosporins Ceftriaxone. For all isolated, that is Ceftriaxone resistant, Imipenem susceptibility testing is done by disc diffusion method and all the isolates were observed to susceptible<sup>21</sup>.

So, Evaluation of susceptibility data of various antibiotic drug is made there is constant increase in resistance of ceftriaxone pattern. Therefore, it is important to monitor the changes in susceptibility pattern over the years.

**Limitation:** Although Quality Control was done regularly, AMR pattern of *S. typhi* is done by disc diffusion method, for confirmation of Ceftriaxone resistance better method like Broth Microdilution or Gene Sequencing is required.

Treatment given & Outcome of the patients was not captured in this study.

**CONCLUSION:** It has been shown in this review that antibiotic resistance is on the rise among *Salmonella* species. The use of empirical treatment for enteric fevers should be avoided in favour of third generation cephalosporins, Cefepime, Carbapenem, and azithromycin.

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