International Journal of Research in Medical Sciences
Marathe A et al. Int J Res Med Sci. 2025 Dec;13(12):5318-5322
www.msjonline.org
pISSN 2320-6071 | eISSN 2320-6012
DOI: https://dx.doi.org/10.18203/2320-6012.ijrms20253957

Original Research Article

Phenotypic changes in *Salmonella typhi*: observations during the recent upsurge in typhoid cases in Vadodara, Gujarat

Anant Marathe*, Asmabanu Shaikh, Bhavita Prajapati

Department of Microbiology, Parul Institute of Medical Sciences and Research, Parul University, Waghodia, Vadodara, Gujarat, India

Received: 04 October 2025
Revised: 11 November 2025
Accepted: 19 November 2025

***Correspondence:**
Dr. Anant Marathe,
E-mail: dranantmarathe@hotmail.com

ABSTRACT

Background: *Salmonella enterica* serotype *typhi* is a causative agent of enteric fever (typhoid). The study was aimed at seeing why the *S. typhi* showing in vitro susceptibility to ceftriaxone required longer treatment for defervescence. We observed phenotypic change characterized by bipolar staining (safety-pin appearance) in the bacilli. We also observed bacilli forming elongated (filamentous forms). We hypothesize a possible association between this structural change in the cell-wall and the resistance pattern of *S. typhi*.
Methods: The study was conducted in a rural hospital with tertiary care facilities. Blood cultures were performed in cases of all the patients with suspected sepsis. The blood cultures were performed on an automated BACT/Alert 3D system. Identification and drug susceptibility of the bacterial isolates are done on VITEK 2. The study comprised 25 blood culture-positive typhoid cases in the last three months.
Results: We observed that gram-stained smears made from positive blood culture bottles revealed conspicuous changes in staining pattern of the bacilli. The bacilli showed Bipolar staining (safety pin appearance) of *Salmonella enterica* serotype *typhi* under the oil immersion objective.
Conclusion: This is the first observational study to investigate the phenotypic change in the staining properties of *S. typhi*. We hypothesize that this change in staining pattern is associated with structural changes in the bacterial cell wall, which is responsible for *S. typhi* altered response to antibiotic therapy.

Keywords: *S. typhi*, Phenotypic change, Safety pin appearance, Filamentous form

INTRODUCTION

Salmonella is a genus of gram-negative bacilli of the family Enterobacteriaceae. It has two *species Salmonella enterica* and *Salmonella bongarii*. *S. enterica* is further divided into six subspecies that contain over 2600 serotypes.[1,2] Salmonella species are non-spore-forming, mostly motile with peritrichous flagella, measuring 0.7-1.5 µm in diameter and 2-5 µm in length.[3] The Centers for Disease Control and Prevention (CDC) is currently using the Salmonella nomenclature system suggested by the World Health Organization (WHO) Collaborating Centre as a nomenclature system- species: *Salmonella enterica* serotype *typhi.*

The evolution of drug resistance in *Salmonella typhi* is a great public health concern. Multi-drug resistance (MDR) that emerged in 1990 is defined, in the case of *S. typhi*, as resistance to Chloramphenicol, Amoxicillin, and Co-cotrimoxazole.[4] This was followed by a decreased susceptibility to fluoroquinolones and high-level fluoroquinolone resistance emerged and spread throughout the world.[4]

Clinicians relied more on third-generation cephalosporins and Azithromycin. Recently there have been reports of Ceftriaxone resistant *S. typhi* isolates from different parts of the world. Ceftriaxone resistant in *S. typhi* is because of the acquisition of blaCTX-M.[5]

Clinicians in Vadodara, during recent upsurge of typhoid cases, experienced that even in cases of Ceftriaxone susceptible *S. typhi* infections the patients required prolonged treatment (up to two weeks) to achieve defervescence.

Gram stained smear made directly from positive blood culture bottle revealed a peculiar change in the staining pattern of *S. typhi*. Instead of uniform gram-negative bacilli, they appeared safety-pin like (exhibiting bi-polar staining). Additionally, some bacilli displayed elongated, filamentous forms. This observation raised our curiosity and the present work was carried out on subsequent blood cultures flagged positive in cases of suspected enteric fever.

METHODS

Study design

The prospective observational study was carried out at Parul Sevashram Hospital, a NABH-accredited tertiary care hospital in Vadodara, after obtaining ethical approval. Over a three-month period from June 2024 to August 2024, blood cultures were performed on patients suspected of having sepsis. A total of 1,582 blood culture bottles were processed at the central clinical laboratory, with 352 testing positive.

Blood culture sample collection

Blood samples were collected in specialized (FA+/ PF+) aerobic blood culture bottles, with volumes of 7-10 ml for adult patients and 2-3 ml for pediatric patients. The automated BACT/ALERT 3D system processed the blood culture bottles over five days.

Procedure

All blood culture-positive bottles were sub-cultured on MacConkey agar, blood agar, and nutrient agar plates. For clinically suspected typhoid cases with high-grade fever, abdominal discomfort with a change in bowel habits, and a negative malaria blood smear, additional cultures were performed on SS agar and XLD agar, followed by incubation at 37°C.

Morphological analysis

A smear was prepared from each positive blood culture bottle, and gram staining was performed. The slides were examined under oil immersion light microscopy (1000x magnification) by two independent experienced microbiologists.

Observations regarding morphology, gram reaction, and the presence of characteristic staining patterns (e.g., bipolar appearance) were recorded for each isolate.

Identification of bacteria

Colony morphology was analyzed after an 18-24 hours incubation period. The VITEK 2 system was used for identification of bacterial isolates and antimicrobial susceptibility testing, utilizing the GN ID card for identification and the AST405 card for susceptibility testing. Antibiotic susceptibility reports were interpreted based on CLSI guidelines.

All the blood culture positive *S. typhi* isolates were further confirmed by antisera.

RESULTS

Over the course of three months, a total of 1,582 blood culture bottles were collected at the central clinical laboratory. Of these, 352 (22.2%) tested positive. Notably, 25 (7.1% of positive cultures) of these positive blood cultures were identified as *S. typhi*. Recently, our city (Vadodara) experienced a sudden upsurge in the cases of typhoid and the cases were more severe requiring prolonged antibiotic treatment. All confirmed typhoid cases were aged between 18-25 years and from Vadodara (Table 1).

Table 1: Demographic data of patients.

Characteristics	N
Patient age (years)	
16-20	11
21-25	14
Gender	
Male	17
Female	8

The susceptibility pattern of all the isolates is shown in Table 2.

The antibiotic susceptibility pattern revealed that only 1 out 25 strains was resistant to Ceftriaxone while rest were all susceptible.

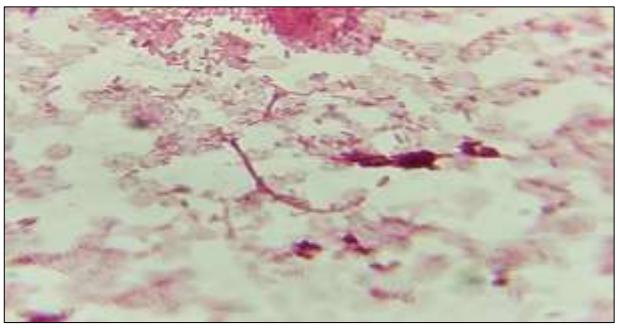

Figure 1: Gram-stained smear from positive blood culture of *S. typhi*, showing bipolar (safety-pin) appearance and filamentous forms in oil immersion field (100X).

We observed that gram's-stained smears prepared from blood culture positive bottles, revealed conspicuous changes in the morphology of bacilli. All *S. typhi* bacilli showed bipolar staining (safety pin appearance) under the oil immersion objective (Figure 1) and some isolates presented with filamentous forms (Figure 2).

Table 2: Antimicrobial resistance pattern of S. *typhi* isolated from blood cultures.

Antimicrobial agents	Sensitive (%)	Resistance (%)
Amoxicillin-clavulanic acid	100	0
Amikacin	100	0
Ciprofloxacin	75	25
Ceftriaxone	95	5
Colistin	100	0
Cefuroxime	91	9
Cefuroxime axetile	91	9
Ertapenem	100	0
Cefepime	97	3
Fosfomycin	100	0
Gentamicin	100	0
Imipenem	100	0
Meropenem	100	0
Cefoperazone-salbactum	100	0
Trimethoprim/ Sulfamethoxazole	100	0
Tigecycline	100	0
Piperacillin-Tazobactum	100	0

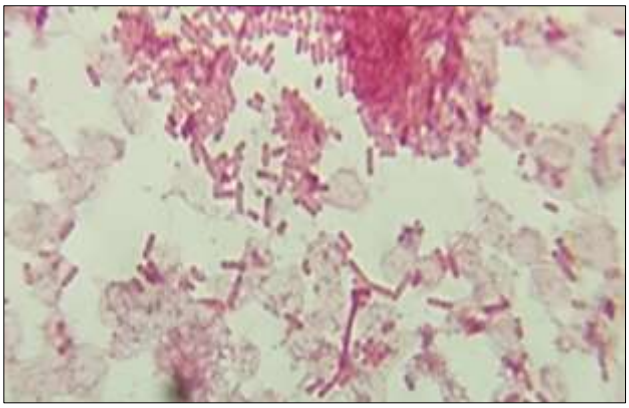

Figure 2: Enlarged figure of *S. typhi* revealing Bipolar staining.

DISCUSSION

Salmonella, a genus within the family Enterobacteriaceae, comprises gram-negative, rod-shaped bacteria. Blood cultures are generally performed on indoor patients who are suspected to have sepsis. Typhoid fever is one of the few causes of septicemia for which blood cultures are recommended, even for outpatients. In the first week of fever, blood culture yield is best for the diagnosis of typhoid. When the blood culture instrument flags for positive blood culture, the blood culture bottle is taken out and a few drops of the blood in inoculated on culture media and incubated. The Identification and the susceptibility of the isolate in done next day after the growth on the culture media. A smear is also made from the positive blood culture bottle and stained with Gram stain. The bacterial morphology is read to get the probable pathogen identification. We observed the change in the appearance of the bacilli in stained smear; we conducted this study on subsequent positive cultures. We hypothesize a link between emerging phenotypic changes in bacilli and their evolving tolerance to antibiotics, which necessitates prolonged antibiotic therapy.

Recent trends in S. typhi bacteremia

The temporal change in trends of antimicrobial resistance in *S. typhi* bacteremia has been observed in the state of Gujarat in the last few months. Cephalosporins have been the mainstay of management of MDR *S. typhi* infections for the past several years.[6] Perhaps cephalosporins failed as empirical therapy in a substantial number of cases as we observed a rise in ESBL XDR *S. typhi* infections in recent outbreaks.

Also, especially the children required longer hospital stays due to delayed fever defervescence. Average days for fever defervescence were observed to be 6-8 days of intravenous therapy. Prolonged fever in most children was observed to be due to immune dysregulation or exaggerated immune response as we ruled out other causes, such as persistent bacteremia, deep-seated abscesses, or secondary infections. Culture-proven *S. typhi* bacteremia was also observed in children who were vaccinated with 2 doses of typhoid conjugate vaccine. Relapse and complications were observed in those who were treated with inappropriate antibiotics and for inadequate duration. Distant complications such as osteomyelitis and spondylodiscitis were also observed in children treated for enteric fever.

Animal experiments have proved that antibiotic therapy sometimes is not enough to eliminate some bacterial population refractory to antibiotics.[7,8] Such bacterial population shows phenotypic change that enables then survive in the host tissue and cause relapses and chronic infections. Mechanism of this is still not clearly understood. Multiple mechanisms can induce antimicrobial tolerance in vitro, but the *in vivo* relevance remains unclear.[8]

Any changes in the outer membrane of gram-negative bacteria like changes in the hydrophobic properties or changes in the porins or any other change leads to antibiotic resistance.[6] This property of gram-negative bacteria, which is lacked by Gram positive bacteria, has made gram negative bacteria showing more resistance to antibiotics.[9,10]

Traditionally, a bipolar staining pattern was used for probable identification of *Burkholderia* species especially pseudomallei in blood culture in patients with community-acquired pneumonia or *B. cepacia* in case of VAP.[11] The other bacilli having similar staining patterns include *Yersinia pestis* and *Fransisella tularences*. Research has shown that bacteria can acquire antibiotic resistance through alterations in cell shape alone.[12,13]

Furthermore, a study by Yang et al revealed that beta-lactam antibiotics trigger a four-stage process of cell lysis in *E. coli*, consisting of elongation, bulge formation, bulge stagnation, and ultimately, cell lysis.[14] The occurrence of *Salmonella typhimurium* L-forms, often detected in patients following β-lactam antibiotic treatment, is associated with chronic infections. Due to their cell wall deficiencies, these L-forms display increased tolerance to cephalosporins, which normally target bacterial cell walls, thereby complicating treatment efforts.[14]

Since the 1990s, it has been well-established that a crucial aspect of the pathogenesis of *Burkholderia pseudomallei* is its remarkable ability to persist and thrive within both phagocytic and non-phagocytic cells. This intracellular survival is a significant factor contributing to its virulence and the complexity of infections it causes.[15] Our recent observations suggest a compelling association between this phenotypic characteristic and the acquisition of specific virulence factors by *S. typhi*.

This discovery not only sheds light on the mechanisms underlying its survival strategies but also opens up exciting new avenues for genotypic studies. Such research aims to uncover the precise factors that enable these strains to survive and propagate within host cells, ultimately enhancing our understanding of their pathogenic potential and informing future therapeutic approaches.

CONCLUSION

This is the first observational study regarding the phenotypic change in the staining property of *S. typhi*. We hypothesize an association between the phenotypic change and change in the behaviour of typhoid infections. We suggest further studies in *S. typhi* antibiotic resistance pattern *in vivo* due to some configurational changes in the cell-wall and to make necessary changes in the guidelines for the treatment of typhoid to tackle the menace.

ACKNOWLEDGEMENTS

Authors would like to thank Parul Institute of Medical Sciences and Research, Parul University for providing necessary resources and support for this research.

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

REFERENCES

1. Su LH, Chiu CH, Chu C, Wang MH, Chia JH, Wu TL. In vivo acquisition of ceftriaxone resistance in Salmonella enterica serotype Anatum. Antimicrob Agents Chemother. 2003;47(2):563-7.
2. Gal-Mor O, Boyle EC, Grassl GA. Same species, different diseases: how and why typhoidal and non-typhoidal Salmonella enterica serovars differ. Front Microbiol. 2014;5:1-10.
3. Fàbrega A, Vila J. Salmonella enterica serovar *Typhi*murium skills to succeed in the host: virulence and regulation. Clin Microbiol Rev. 2013;26(2):308-41.
4. Kariuki S, Gordon MA, Feasey N, Parry CM. Antimicrobial resistance and management of invasive Salmonella disease. Vaccine. 2015;33(3):C21-9.
5. Thirumoorthy TP, Jacob JJ, Velmurugan A, Teekaraman MP, Shah B, Iyer V, et al. Recent emergence of cephalosporin-resistant Salmonella *Typhi* in India due to the endemic clone acquiring IncFIB(K) plasmid encoding blaCTX-M-15 gene. Microbiol Spectr. 2025;13(5):e0087524.
6. Veeraraghavan B, Pragasam AK, Ray P, Kapil A, Nagaraj S, Perumal SPB, et al. Evaluation of antimicrobial susceptibility profile in Salmonella *Typhi* and Salmonella Para*typhi* A: presenting the current scenario in India and strategy for future management. J Infect Dis. 2021;224(5):S502-16.
7. Abshire KZ, Neidhardt FC. Growth rate paradox of Salmonella *typhi*murium within host macrophages. J Bacteriol. 1993;175(12):3744-8.
8. Claudi B, Spröte P, Chirkova A, Personnic N, Zankl J, Schürmann N, et al. Phenotypic variation of Salmonella in host tissues delays eradication by antimicrobial chemotherapy. Cell. 2014;158(4):722-33.
9. Exner M, Bhattacharya S, Christiansen B, Gebel J, Goroncy-Bermes P, Hartemann P, et al. Antibiotic resistance: what is so special about multidrug-resistant Gram-negative bacteria? GMS Hyg Infect Control. 2017;12:Doc05.
10. Miller SI. Antibiotic resistance and regulation of the Gram-negative bacterial outer membrane barrier by host innate immune molecules. mBio. 2016;7(5):e01541.
11. Pongsunk S, Thirawattanasuk N, Piyasangthong N, Ekpo P. Rapid identification of Burkholderia pseudomallei in blood cultures by a monoclonal antibody assay. J Clin Microbiol. 1999;37(11):3662-7.
12. Cylke C, Si F, Banerjee S. Effects of antibiotics on bacterial cell morphology and their physiological origins. Biochem Soc Trans. 2022;50(5):1269-79.
13. Ojkic N, Serbanescu D, Banerjee S. Antibiotic resistance via bacterial cell shape-shifting. mBio. 2022;13(3):e00603-22.
14. Yang C, Li H, Zhang T, Chu Y, Zuo J, Chen D. Study on antibiotic susceptibility of Salmonella

*typhi*murium L forms to the third and fourth generation cephalosporins. Sci Rep. 2020;10(1):1-6.
15. Jones AL, Beveridge TJ, Woods DE. Intracellular survival of Burkholderia pseudomallei. Infect Immun. 1996;64(3):782-90.

Cite this article as: Marathe A, Shaikh A, Prajapati B. Phenotypic changes in *Salmonella typhi*: observations during the recent upsurge in typhoid cases in Vadodara, Gujarat. Int J Res Med Sci 2025;13:5318-22.