Original Article

# Antimicrobial Resistance Surveillance in Typhoidal *Salmonella* in Ahmedabad in an Era of Global Antimicrobial Resistance Surveillance Systems

**Veena Iyer, Anal Ravalia, Kankshi Bhavsar, Susanna Abraham Cottagiri, Ayushi Sharma, Mahendra Vegad[1], Parul Shah[2], Bhavini Shah[3], Bhavin Solanki[4], Sumeeta Soni[1], Dileep Mavalankar**

Indian Institute of Public Health, Public Health Foundation of India (PHFI), Gandhinagar, [1]Department of Microbiology, B. J. Medical College, New Civil Hospital, [2]Department of Microbiology, Smt NHL Municipal Medical College, [3]Department of Microbiology, Supratech Micropath Laboratory and Research Institute Private Limited, [4]Health Department, Ahmedabad Municipal Corporation, Ahmedabad, Gujarat, India

## Abstract

**Introduction:** India possibly carries the highest burden of antimicrobial resistant typhoidal salmonellae in the world. We report on the health-care ecosystem that produces data on antimicrobial resistance (AMR) testing and the resistance patterns of typhoidal *Salmonella* isolates in the city of Ahmedabad. **Materials and Methods:** Through municipality records and internet searches, we identified 1696 private and 83 public laboratories in the city; 4 medical colleges, 4 health-care institution attached laboratories, and 4 corporate laboratories (CLs) were performing culture and antibiotic sensitivity testing (AST), but only 2 medical colleges and 1 CL shared their data with us. There was considerable variation in culturing and sensitivity testing methodology across laboratories. **Results:** Out of 51,260 blood cultures, *Salmonella* isolates were detected in only 146 (0.28%). AST was conducted on 124 isolates, of which 67 (54%) were found resistant. Multidrug resistance was absent. Concurrent resistance to more than one antibiotic was very high, 88%, among the 67 resistant isolates. Ciprofloxacin resistance varied widely between the private and public sector laboratories. Notably, isolates from the private sector laboratory showed complete resistance to azithromycin. **Conclusions:** High resistance to ciprofloxacin and azithromycin observed in Ahmedabad may be due to the increased use of these two antibiotics in the public and private sectors, respectively. The need of the hour is to identify a representative sample of laboratories from both the public and the private sectors and encourage them to participate in the national AMR surveillance network.

**Keywords:** Ahmedabad, antimicrobial resistance, antimicrobial resistance in *Salmonella*, antimicrobial-resistance surveillance, regional antimicrobial-resistant pattern, typhoidal *Salmonella*

## Introduction

*Salmonella enterica* serovars *Typhi* and *Paratyphi* (known as typhoidal *Salmonella*) are rod-shaped, Gram-negative bacteria, which infect only humans and cause typhoid and paratyphoid fevers, together called enteric fever.[1]

A recent systematic review placed the incidence of typhoid in India at approximately 3.4 million cases, which would account for approximately 30% of the global burden.[2,3] Neighboring Nepal has reported a significant doubling of septicemia rates due to *Salmonella* spp. This high burden of enteric fever in the subcontinent is further compounded by a high prevalence of antimicrobial resistance (AMR) in typhoidal *Salmonella*.[4] In 1990–1992, around 65% of typhoidal *Salmonella* strains in India showed multi drug resistance (MDR)[5] (implying resistance to three early, commonly used antibiotics – chloramphenicol, ampicillin, and cotrimoxazole). As a result, ciprofloxacin became the drug of choice in the empirical treatment of enteric fever.[6] Its widespread use led to increased resistance

Access this article online

Quick Response Code:

Website: www.jgid.org

DOI: 10.4103/jgid.jgid_149_18

**Address for correspondence:** Dr. Veena Iyer, Indian Institute of Public Health, Gandhinagar, Public Health Foundation of India, Opposite Air Force HQ, Near Lekavada Bus Stop, CRPF P.O., Gandhinagar - 382 042, Gujarat, India.
E-mail: veenaiyer@iiphg.org



**How to cite this article:** Iyer V, Ravalia A, Bhavsar K, Cottagiri SA, Sharma A, Vegad M, *et al*. Antimicrobial resistance surveillance in typhoidal *Salmonella* in Ahmedabad in an era of global antimicrobial resistance surveillance systems. J Global Infect Dis 2019;11:153-9.

**Received:** 24 October 2018 **Revised:** 11 January 2019
**Accepted:** 28 February 2019 **Published:** 26 November 2019

to ciprofloxacin and a reduction in MDR at the turn of the century. Newer antibiotics such as azithromycin began to be recommended for the treatment of enteric fever in the last decade.[7] However, recent reports of resistance to these antibiotics from across the country have again raised a challenge to effective control and treatment efforts.[8]

Since *Salmonella typhi* and *Salmonella paratyphi* exclusively infect humans, resistance mechanisms in these organisms are more likely to be driven by antibiotic consumption by patients, rather than environmental transfer.[9] Recent studies from the Wellcome Institute carry alarming reports of newer mechanisms and characteristics of resistance in *S. typhi*.[10]

The traditional strategy to both combat and delay the development of resistance in all pathogenic bacteriae has been to combine and cycle (rotate) antimicrobials based on the AMR pattern of the organism.[11] Until rapid diagnostics become available to detect the AMR pattern of an infecting organism at the bedside, health-care providers in all countries will need information about regional susceptibility to guide empirical therapy of *S. typhi*.[12] For this, it is essential to map resistance patterns and genetic mechanisms at regional levels, particularly in regions endemic for resistant *S. typhi* and *S. paratyphi*.

AMR studies have been regularly published from select, public sector tertiary care centers in India over the last many decades and recently from private centers too.[7,8] These studies reveal the magnitude of AMR only in single tertiary centers and reflect the spatial fluctuations in AMR patterns across the country. There have not been any reports of AMR patterns at a city level, even in large cities such as Delhi and Mumbai. Relating AMR reports from the private and public sector assumes importance, given the possibility that different antibiotic prescription and consumption patterns in the private and public sector could result in different resistance patterns among typhoidal salmonellae from within the same city.[13] It is vital that we document resistance patterns in contiguous geographic areas so that regionally appropriate prescription guidelines may be formulated.[14]

In recognition of rising AMR as a global problem, the World Health Assembly adopted the global action plan on AMR in May 2015. The Global AMR Surveillance System is presently gathering data on resistance in eight priority bacteria, including *Salmonella* spp. Integrating private laboratories into national AMR surveillance in all regions of the globe is a recognized priority area.[15]

Given this backdrop, the purpose of this study is to document the health-care ecosystem that produces data on AMR in typhoidal salmonellae in Ahmedabad, the seventh largest city in India.[16] This study reports the antibiotic resistance testing methodology used and resistance pattern recorded in the city in the past 5 years.

## Materials and Methods

We followed three filtering steps (as described below) to narrow down our search for facilities which perform culture and antibiotic sensitivity testing (AST) for *Salmonella* [Figure 1].

### Filtering steps

#### *Listing*

The public sector in the city consists of five tertiary level

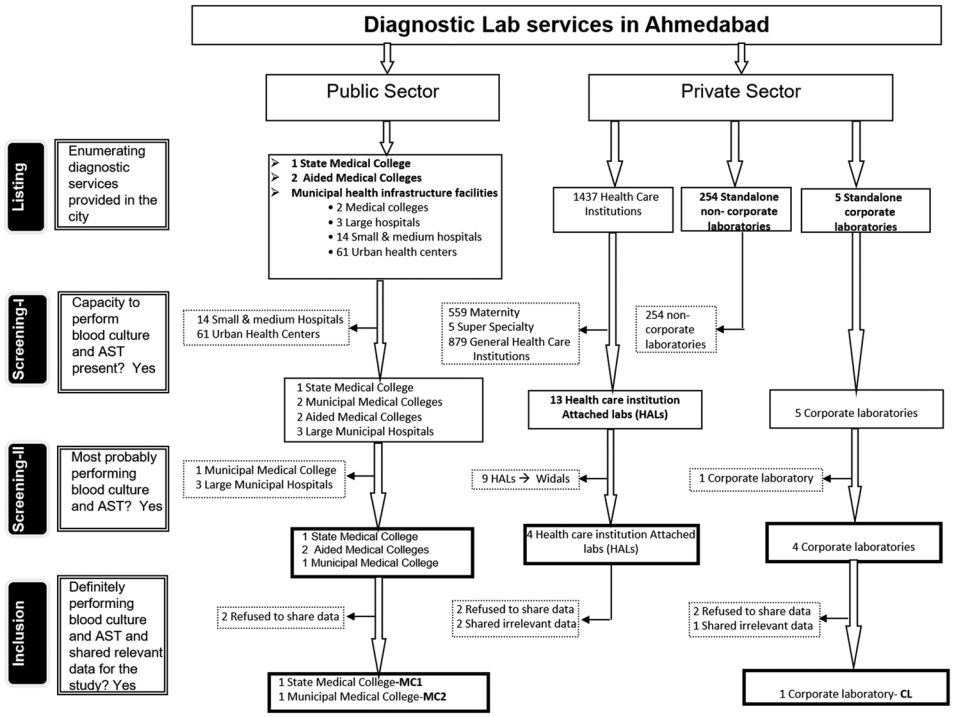


**Figure 1:** Filtering of diagnostic laboratory services in Ahmedabad to detect facilities with culture and antibiotic sensitivity testing capacity

referral hospitals with attached medical colleges as well as the municipality's network of hospitals and health centers. Private diagnostic laboratories in Indian cities are of three types – health-care institution attached laboratories (HALs), standalone noncorporate laboratories (NCLs), and stand-alone corporate laboratories (CLs).[17] We obtained a record of 1437 HALs registered by the municipality in 2015–2016 and a list of 254 NCLs. We searched for CLs in the city through internet searches and interviews with colleagues in the public and private systems. We identified five functioning CLs in the city.

### *Screening*

#### First phase: Telephonic screening

Medical colleges have full-fledged microbiology departments and carry out culture and AST. Therefore, five of these directly entered our second phase of screening. We interviewed key health officials of the municipality regarding the capacity for AST at their health facilities. We retained the three large municipal hospitals in our list for the next stage of physical screening along with the five medical colleges.

We sorted the list of 1437 HALs by specialties and range of bed sizes. One-third (559) were purely maternity, and five were purely superspecialty hospitals (cardiac, bariatric, knee replacement, and cancer) and would not carry out any investigations for *Salmonella*. The remaining 879 facilities had mean and median bed sizes of 13.3 and 10 (range: 1–310 beds; interquartile range: 5–15 beds). We used a cutoff bed size of 15–75 beds and randomly sampled 10% of the 161 medium-sized facilities (18 facilities) and telephoned them. All of them reported that they did not possess laboratory facilities. We assumed that small-sized hospitals too would not possess laboratory facilities. We retained all the 13 HALs with >75 beds in Ahmedabad for detailed screening in the next phase.

Similarly, we randomly sampled 10% of the 254 NCLs (25 laboratories) for a telephonic survey regarding the type of enteric fever testing offered by them. All of them performed Widal tests, but none of them performed culture and AST. They reported that they sent such samples or patients to CLs. We thus identified 8 public facilities, 13 HALs, and 5 CLs that needed to be physically verified for the actual performance of culture and AST in the next phase.

#### Second phase: Physical screening

To screen the public facilities, we visited microbiology departments of all five medical colleges. All of them conducted culture and AST, but one had begun just 3 months previously and did not have any past data to share. The three large municipality hospitals reported that they had no AST facility and referred suspected patients of enteric fever to the medical colleges. Of the 13 HALs, 6 were charitable and 7 were for-profit hospitals. Only four of them, two charitable and two for-profit reported that they conducted culture and AST. The rest sent their samples to CLs. We contacted local city offices of five CLs. Four claimed that they performed culture and AST. At the end of the screening, we confirmed that four medical colleges, four HALs (two charitable and two for-profit), and four CLs were performing culture and AST. We formally requested these institutions to share their data.

### *Inclusion*

Two of the four medical colleges (MC1 and MC2) shared culture and AST data of 4½ and 2 years, respectively. The third did not respond to our requests but has recently published AMR data in their pediatric patients during the same period.[18] The fourth had begun performing culture and AST just 3 months previously and did not have any past data to share.

Among the four CLs, one shared data of 2 years (2014–2015) which we have used in this study. One shared data that were poorly labeled and did not contain culture and AST information and two refused to share data with us. One of the latter laboratories has recently published AMR data in typhoidal *Salmonella* of 6-month period in gray literature.[19]

One for-profit and one charitable HAL shared their data. The former shared data of only the past few months and less than 6 cultures had been performed. The latter reported only Widal test reports and had no data on culture and AST. The other for-profit HAL did not respond to our repeated requests and charitable HAL refused to share any data saying that the microbiologist wished to publish the same.

We extracted data from the three available and functional sources, MC1, MC2, and CL, into Microsoft Excel 2013 (v15.0) sheets and anonymized it for further analysis. MC1 and CL have national accreditation.[20]

## Method adopted by laboratories to perform blood culture and antibiotic sensitivity testing

### *Blood culture*

MC1 and CL used Becton Dickinson Bactec Instrumented blood culture systems to detect microbial growth in blood specimens. MC2 performed this task manually using conventional blood culture bottles. The subculture of *S. typhi*/*S. paratyphi*-positive samples was done manually on MacConkey, blood, and nutrient agars at all three laboratories.

### *Antibiotic sensitivity testing*

AST was performed in accordance with the Clinical and Laboratory Standards Institute (CLSI AST-M100) guidelines by the Kirby–Bauer disc diffusion method by all three laboratories. MC1 had tested for 42 antibiotics. While CL tested all isolates for resistance to all 58 antibiotics, MC1 and MC2 tested varying numbers of isolates to their set of antibiotics. Minimum inhibitory concentration was not determined at any of the laboratories.

Ethical approval for this study was obtained from the Institutional Ethics Committee of Indian Institute of Public Health, Gandhinagar (TRC-IEC No: 5/2015).

## Results

The three reporting laboratories had conducted 51,620 cultures

**Table 1: Overview of resistance**

| Laboratories | Period | Number of blood samples cultured | *Salmonella* isolates | AST done | | | Resistant isolates (%) | Resistance to 1 antibiotic | | Resistance to >1 antibiotic | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Total | *S.typhi* | *S. paratyphi* | | *S.typhi* | *S. paratyphi* | *S. typhi* | *S. paratyphi* |
| MC1 | May 11-October 15 | 34,748 | 42 | 40 | 32 | 8 | 19 (48) | 6 | 0 | 10 | 3 |
| MC2 | January 14-December 15 | 10,232 | 55 | 40 | 36 | 4 | 4 (10) | 2 | 0 | 1 | 1 |
| CL | January 14-December 15 | 6640 | 44 | 44 | 40 | 4 | 44 (100) | 0 | 0 | 40** | 4** |
| Total | 55 months | 51,620 | 141 | 124 | 108 | 16 | 67 (54) | 8 | 0 | 51 | 8 |

**All 44 isolates from private CL and one each from MC1 and MC2 were resistant to at least 18 antibiotics, *S. typhi: Salmonella typhi S. paratyphi: Salmonella paratyphi*, CL: Corporate laboratory, AST: Antibiotic sensitivity testing

during 2011–2016 [Table 1]. *Salmonella* isolation rates from these cultures were 0.12%, 0.53%, and 0.66% for MC1, MC2, and CL, respectively. Out of total 141 *Salmonella*-positive cultures, 121 (85%) were *S. typhi* and 18 (13%) were *S. paratyphi*. Species of two isolates remained unidentified. While the CL tested all their isolates against all antibiotics, MC1 and MC2 tested antibiotics inconsistently across their isolates.

MC1 had performed the largest number of blood cultures. However, there was no mention of the number of cultures that yielded *Salmonella*. Therefore, we assumed that the 42 blood cultures, which had been tested for sensitivity, were the total yield of *Salmonella* isolates. MC2 provided AST data for 40 out of the 55 *Salmonella* isolates, while CL provided AST data on all 44 isolates [Table 1].

Thus, the three laboratories provided 124 AST reports for 55 months. Resistance varied from 10% to 100% across the three laboratories. More than half of these reports, i.e. 67 (54%) were resistant to one or more antibiotics. The majority of this resistance came from the CL isolates, all 44 of which showed resistance. Eighty-eight percent of resistant isolates were concurrently resistant to more than one antibiotic.

### Antibiotic resistance pattern

The city's isolates showed the lowest resistance for ampicillin, co-amoxiclav, and co-trimoxazole (<8% of tested isolates) and highest for nalidixic acid and azithromycin (95% of tested isolates).

MDR – MC1 and MC2 tested only 2/40 and 31/40 isolates, respectively, for MDR while CL tested all isolates. However, MDR was not detected in any of the laboratories.

Quinolones and fluoroquinolones – nalidixic acid resistance was almost complete in all isolates tested at CL (*S. typhi* – 97% and *S. paratyphi* – 100%), whereas it was hardly tested at MC1 and MC2. Ciprofloxacin resistance was found at all laboratories although it varied – 50% in MC1 but only 8%–25% in CL. There was minimal resistance to the third and above generation of fluoroquinolones.

Cephalosporin – minimal resistance to third-generation cephalosporins was detected at all three laboratories.

All isolates in CL showed complete resistance to gentamicin, amikacin, and azithromycin. In MC1 and MC2, a quarter of the isolates showed resistance to gentamicin and amikacin, while azithromycin was hardly tested. Less than a quarter of isolates at MC1 and MC2 presented resistance to tetracycline [Table 2].

## Discussion

We found very low *Salmonella* isolation rates; only 141 out of 52,000 blood cultures (0.28%) had yielded *Salmonella.* Half of these isolates showed some resistance. *Salmonella* isolation rates, antibiotics tested, and antibiotic resistance patterns showed considerable variation across the three facilities. The noteworthy resistance pattern was the absence of MDR, the emergence of resistance to third-generation cephalosporins, and a high degree of resistance to azithromycin. Among isolates that were resistant, resistance to multiple antibiotics was high.

*Salmonella* isolation rate in Ahmedabad of 0.28% was much lower than in four other Indian studies, which had reported *Salmonella* positivity in 1.2%–4.3% of samples against the 12,940–135,000 blood samples; they had cultured in their laboratories.[13,21-23] However, the ratio of *S. typhi* to *S. paratyphi* isolates (85:15) in our study was within the range seen in most sites in the last decade (65:35–90:10).[13,24] The extremely low isolation rates in our three facilities may be indicative of lower referral rates of suspected patients for blood culture or, possibly, lower incidence of *Salmonella* infection in Ahmedabad. Population-linked laboratory studies are needed in the city to understand the actual burden of cases and devise antibiotic strategies. Unless practitioners are guided by regional data, uninformed prescription practices will worsen existing AMR.

The Indian Council of Medical Research (ICMR) and the National Centre for Disease Control have recently published antibiotic regimens for a range of infectious syndromes with the objective of curtailing AMR in the country.[25,26] Both recommend ceftriaxone/cefixime and azithromycin as the empirical first line of treatment for enteric fever. ICMR also recommends re-introduction of cotrimoxazole as a first-line

**Table 2: Antibiotic resistance pattern**

| Antibiotics *r/n* (%) | MC1 | | MC2 | | CL | | Total | |
|---|---|---|---|---|---|---|---|---|
| | *S. typhi* | *S. paratyphi* | *S. typhi* | *S. paratyphi* | *S. typhi* | *S. paratyphi* | Total *S. typhi* | Total *S. paratyphi* |
| Total isolates (*N*) | 32 | 8 | 36 | 4 | 40 | 4 | 108 | 16 |
| Ampicillin | 1/16 (6.25) | 0/3 (0) | 1/30 (3) | 0/4 (0) | 4/33 (12) | 0/4 (0) | 6/79 (7.59) | 0/11 (0) |
| Chloramphenicol | 3/12 (25) | 0/5 (0) | 1/36 (3) | 0/1 (0) | 0/40 (0) | 0/4 (0) | 4/88 (4.54) | 0/10 (0) |
| Nalidixic acid | 0/1 (0) | - | - | - | 38/39 (97) | 4/4 (100) | 38/40 (95) | 4/4 (100) |
| Ciprofloxacin | 8/16 (50) | 0/4 (0) | 1/3 (33) | - | 3/40 (8) | 1/4 (25) | 12/59 (20.33) | 1/8 (12.5) |
| Ceftriaxone | 1/6 (17) | 0/3 (0) | 1/36 (3) | 0/4 (0) | 1/40 (3) | 0/4 (0) | 3/82 (3.65) | 0/11 (0) |
| Cefixime | - | - | 0/3 (0) | - | 1/40 (3) | 0/4 (0) | 1/43 (2.32) | 0/4 (0) |
| Azithromycin | 0/1 (0) | - | - | - | 39/40 (98) | 4/4 (100) | 39/41 (95.12) | 4/4 (100) |

*r*: Number of isolates resistant, *n*: Number of isolates tested, *S. typhi: Salmonella typhi, S. paratyphi: Salmonella paratyphi*, CL: Corporate laboratory

drug and chloramphenicol and ciprofloxacin as second-line drugs.

Resistance to ceftriaxone/cefixime was very minimal (3/93, 3.2%) in our study. Other studies from India also suggest that there is very minimal or no resistance to cephalosporins, indicating recent initiation of resistance to these drugs. However, few South Asian countries, Bangladesh, Pakistan, and Philippines are reporting considerable resistance to third-generation cephalosporins.[16,27,28]

However, the 100% resistance to azithromycin seen in our CL sample (43/45) is a significant finding, especially since high resistance to azithromycin (30%) has also been reported in another clinical study of pediatric enteric fever in Ahmedabad.[18] Some consider that high *in vitro* resistance of *Salmonella* to azithromycin may not be predictive of low clinical efficacy since this antibiotic is known to achieve very high intracellular concentrations.[29] However, clinical nonresponse to azithromycin has been recorded in northern India in 2012.[30] These experts argued that azithromycin has proved only marginally better than ciprofloxacin at fever clearance and recommended that large-scale randomized control trials are needed before azithromycin can be used in the Indian subcontinent.

In our study, ciprofloxacin resistance was much higher in the public (50%) than the private (8%) sample [Table 2], but this was within the range seen in other studies.[7] This may be indicative of more usage of the relatively cheaper ciprofloxacin among public hospital clientele.

MDR was completely absent. Although reducing trend of MDR has been reported from across the country, the complete absence of MDR has not been reported to date in any other study from India.[25]

Only two studies from India have reported concurrent resistance to two or more antibiotics.[31,32] In our study, this concurrent resistance was largely driven by the private sample. This may be reflective of the difference in antibiotic consumption patterns between public and private sector clientele, as is our finding of increased azithromycin resistance in the private samples and ciprofloxacin resistance in the public samples. The possibility of such an evolution of antibiotic resistance in the subcontinent as has been hypothesized in a recent publication needs further exploration.[33]

## Antimicrobial resistance surveillance in Ahmedabad in Indian and global context

The difference in the proportion of AMR isolates reported in our public and private samples, 30% versus 100%, as well as high ciprofloxacin resistance in public and high azithromycin resistance in private samples are important findings because it may be indicative of differential AMR patterns in the private and public sectors. Although all facilities followed CLSI guidelines, total antibiotics tested at each facility varied. Furthermore, public sector laboratories did not test all isolates against all antibiotics on their list. This probably reflects the inconsistent availability of Kirby–Bauer discs at these laboratories. Further, minimum inhibitory concentration to assess the extent of resistance was not tested in any of the laboratories. This patchy performance of AST and lack of standard procedures result in poor surveillance due to incomparability of sensitivity across laboratories.[34] For individual laboratories, both private and public, there is no inherent incentive to detect city-level AMR patterns or molecular-level mechanisms of resistance transmission. This lack of enthusiasm among microbiologists to further process their samples through more specialized laboratory testing and analysis is an issue in other parts of the world too.[15,35] Probably, they are unable to fully value the public health benefits that could be derived from AMR surveillance.[33]

The antibiotic regimen currently recommended by ICMR for the treatment of enteric fever in the entire country is based on 209 *Salmonella* isolates from only four public institutes.[25] Across India's cities and towns, there are several hundreds of public and private hospitals and laboratories undertaking ASTs, just like the ones in Ahmedabad presented in this study.[33] Drawing these varied facilities or at least a representative sample of them into a cohesive network is essential for surveillance of AMR in all major bacterial pathogens, particularly so for typhoidal *Salmonella* which is endemic in our part of the world, and is most exposed to antibiotics consumed by humans. Only a representative network of laboratories will provide the contextualized and stratified data necessary for the development of the most accurate strategy to formulate regional prescription guidelines. However, this is an

enormous challenge in our setting. Motivated and persistent teams would be needed to ensure the intensive networking activities necessary between all the actors.[36]

### Limitations

Out of approximately 1750 big and small, public and private facilities in Ahmedabad, we identified only 12 facilities with the ability to report AMR in typhoidal *Salmonella*. In spite of our efforts over 1 year, only three shared their data. Two of the facilities which refused to share data with us have recently described resistance patterns in typhoidal *Salmonella* isolates from the same study period. One aided medical college has reported 185 isolates from children below 14 years from 2014 to 2016. However, the denominator number of blood cultures performed, and thus, the *Salmonella* isolation rate has not been reported. Resistance to more than one antibiotic also has not been reported.[25] One of the CLs has also reported 54 isolates from 1211 blood cultures between November 2013 and April 2014 in gray literature, an isolation rate of 4.45%. This is much above the isolation rate of the CL that shared data with us.[19,29] Based on the quality and quantity of data shared by three HALs, – less than10 records of culture from one and no cultures from the other two – we believe that *Salmonella* isolation in the remaining seven HALs may be nonexistent or negligible. It is important to summarize that many facilities refused to share data; those that did had preserved records of a very short period and had very low isolation rates. Due to this incomplete data sharing, we are unable to estimate the true size of *Salmonella* positivity against total blood cultures in our city. Although the laboratories in our city are not equipped to report emerging AMR, as are those in the Western Pacific or European regions, they still hesitate to share their basic AMR data in the expectation that they will publish it in some form.[15] Thus, very varied results are being published from different laboratories, and there is a lack of consistency in methods adopted by them.

## Conclusions

AMR patterns in typhoidal *Salmonella* from Ahmedabad suggest that they may be resistant to azithromycin and to a lesser degree to ciprofloxacin. However, they are in need of further molecular characterization.

Clinical microbiological methods lack uniformity and laboratory referral networks are not developed even in large cities of India. Although some useful data are produced by a few individual laboratories, the crucial exercise of meaningful networking for effective surveillance remains. As we enter an era of internationally linked AMR surveillance systems, the biggest challenge lies in selecting performing laboratories and inducing them to integrate with it.

### Acknowledgments

We are grateful to the medical record department and HMIS software experts of laboratories for cooperation during data collection.

### Financial support and sponsorship

The research reported in this manuscript has been funded by the Public Health Research Initiative (PHRI) Research grant awarded by PHFI with the financial support of Department of Science and Technology.

### Conflicts of interest

There are no conflicts of interest.

## References

1. Baron S, editor. Medical Microbiology. 4th edition., Ch. 21 Salmonella; Galveston (TX): University of Texas Medical Branch at Galveston; 1996. Available from: https://www.ncbi.nlm.nih.gov/books/NBK7627
2. John J, Van Aart CJ, Grassly NC. The burden of typhoid and paratyphoid in India: Systematic review and meta-analysis. PLoS Negl Trop Dis 2016;10:e0004616.
3. GBD 2016 Disease and Injury Incidence and Prevalence Collaborators. Global, regional, and national incidence, prevalence, and years lived with disability for 328 diseases and injuries for 195 countries, 1990-2016: A systematic analysis for the global burden of disease study 2016. Lancet 2017;390:1211-59.
4. Maskey AP, Basnyat B, Thwaites GE, Campbell JI, Farrar JJ, Zimmerman MD, *et al.* Emerging trends in enteric fever in Nepal: 9124 cases confirmed by blood culture 1993-2003. Trans R Soc Trop Med Hyg 2008;102:91-5.
5. Pillai PK, Prakash K. Current status of drug resistance & phage types of *Salmonella* typhi in India. Indian J Med Res 1993;97:154-8.
6. Liberti A, Loiacono L. Ciprofloxacin versus chloramphenicol in the treatment of *Salmonella* infection. Int J Antimicrob Agents 2000;16:347-8.
7. Madhulika U, Harish BN, Parija SC. Current pattern in antimicrobial susceptibility of *Salmonella* typhi isolates in pondicherry. Indian J Med Res 2004;120:111-4.
8. Harish BN, Menezes GA. Antimicrobial resistance in typhoidal salmonellae. Indian J Med Microbiol 2011;29:223-9.
9. Threlfall EJ. Antimicrobial drug resistance in *Salmonella*: Problems and perspectives in food- and water-borne infections. FEMS Microbiol Rev 2002;26:141-8.
10. Phan MD, Wain J. IncHI plasmids, a dynamic link between resistance and pathogenicity. J Infect Dev Ctries 2008;2:272-8.
11. Davies J, Davies D. Origins and evolution of antibiotic resistance. Microbiol Mol Biol Rev 2010;74:417-33.
12. Wain J, Hendriksen RS, Mikoleit ML, Keddy KH, Ochiai RL. Typhoid fever. Lancet 2015;385:1136-45.
13. Gandra S, Mojica N, Klein EY, Ashok A, Nerurkar V, Kumari M, *et al.* Trends in antibiotic resistance among major bacterial pathogens isolated from blood cultures tested at a large private laboratory network in India, 2008-2014. Int J Infect Dis 2016;50:75-82.
14. Kiffer CR, Camargo EC, Shimakura SE, Ribeiro PJ Jr., Bailey TC, Pignatari AC, *et al.* A spatial approach for the epidemiology of antibiotic use and resistance in community-based studies: The emergence of urban clusters of *Escherichia coli* quinolone resistance in Sao Paulo, Brasil. Int J Health Geogr 2011;10:17.
15. World Health Organization: Global AMR Surveillance System. 2nd High Level Technical Meeting on Surveillance of Antimicrobial Resistance for Local and Global Action: Meeting Report. World Health Organization; 2017.
16. Al Naiemi N, Zwart B, Rijnsburger MC, Roosendaal R, Debets-Ossenkopp YJ, Mulder JA, *et al.* Extended-spectrum-beta-lactamase production in a *Salmonella enterica* serotype typhi strain from the philippines. J Clin Microbiol 2008;46:2794-5.
17. Rao B, Jain R. Medical diagnostic laboratories provisioning of services in India. CHRISMED J Health Res 2015;2:19.
18. Jeeyani HN, Mod HK, Tolani JN. Current perspectives of enteric fever: A hospital based study of 185 culture positive cases from Ahmedabad, India. Int J Contemp Pediatr. 2017;4 (3):4-9.
19. Arya M, Patel D. Current Pattern of Antimicrobial Susceptibility of

Salmonella enterica Serovar Typhi and Paratyphi A Isolated from Blood Cultures [Internet]. [Last accessed on 2017 Nov 28].
20. The National Accreditation Board for Testing and Calibration Laboratories. National Accreditation for Testing and Calibration Laboratories: Accredited Laboratories. The National Accreditation Board for Testing and Calibration Laboratories; 2017. [Last accessed on 2017 Nov 29]. Available from: http://www.nabl-india.org/nabl/index.php?c=search
21. Gupta V, Singla N, Bansal N, Kaistha N, Chander J. Trends in the antibiotic resistance patterns of enteric fever isolates – A three year report from a tertiary care centre. Malays J Med Sci 2013;20:71-5.
22. Raveendran R, Datta S, Wattal C. Drug resistance in *Salmonella enterica* serotype typhi and paratyphi A. J Int Med Sci Acad 2010;23:21-4.
23. Sudeepa Kumar M, Vijaykumar GS, Prakash R, Prashanth HV, Raveesh PM, Nagaraj ER. Comparison of *Salmonella* typhi and paratyphi A occurrence in a tertiary care hospital. J Clin Diagnostic Res 2013;7:2724-6.
24. Tewari R, Jamal S, Dudeja M. Antimicrobial resistance pattern of *Salmonella enterica* servars in Southern Delhi. Int J Community Med Public Health 2015;2:254-8.
25. Indian Council of Medical Research. Treatment Guidelines for Antimicrobial Use in Common Syndromes Indian Council of Medical Research Department of Health Research New Delhi, India. Indian Council of Medical Research; 2017.
26. National Centre for Disease Control. National Treatment Guidelines for Antimicrobial Use in Infectious Diseases. National Centre for Disease Control; 2016. p. 1-64.
27. Afzal A, Sarwar Y, Ali A, Haque A. Current status of fluoroquinolone and cephalosporin resistance in *Salmonella enterica* serovar typhi isolates from Faisalabad, Pakistan. Pak J Med Sci 2012;28:602-7.
28. Saha SK, Talukder SY, Islam M, Saha S. A highly ceftriaxone-resistant *Salmonella* typhi in Bangladesh. Pediatr Infect Dis J 1999;18:387.
29. Molloy A, Nair S, Cooke FJ, Wain J, Farrington M, Lehner PJ, *et al.* First report of *Salmonella enterica* serotype paratyphi A azithromycin resistance leading to treatment failure. J Clin Microbiol 2010;48:4655-7.
30. Rai S, Jain S, Prasad KN, Ghoshal U, Dhole TN. Rationale of azithromycin prescribing practices for enteric fever in India. Indian J Med Microbiol 2012;30:30-3.
31. Krishnan P, Stalin M, Balasubramanian S. Changing trends in antimicrobial resistance of *Salmonella enterica* serovar typhi and *Salmonella enterica* serovar paratyphi A in Chennai. Indian J Pathol Microbiol 2009;52:505-8.
32. Achla P, Grover SS, Bhatia R, Khare S. Sensitivity index of antimicrobial agents as a simple solution for multidrug resistance in *Salmonella* typhi. Indian J Med Res 2005;121:185-93.
33. Gandra S, Merchant AT, Laxminarayan R. A role for private sector laboratories in public health surveillance of antimicrobial resistance. Future Microbiol 2016;11:709-12.
34. Masterton RG. Surveillance studies: How can they help the management of infection? J Antimicrob Chemother 2000;46:53-8.
35. Petti CA, Polage CR, Quinn TC, Ronald AR, Sande MA. Laboratory medicine in africa: A barrier to effective health care. Clin Infect Dis 2006;42:377-82.
36. Kirk CJ, Shult PA. Developing laboratory networks: A practical guide and application. Public Health Rep 2010;125 Suppl 2:102-9.