Original Article

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# Rising Minimum Inhibitory Concentration of Azithromycin: A Therapeutic Challenge in Treating Enteric Fever

Microbiology Section

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## ABSTRACT

**Introduction:** Enteric fever continues to be endemic in the Indian subcontinent carrying with it significant morbidity, despite available antibiotics. With changing trends in antibiotic use, concern for emerging resistance to many common pathogens is very common. Taking enteric fever, as a case in point, there is evidence of increased use of azithromycin and third-generation cephalosporins. Documenting evidence of increasing concentrations of antibiotics, required to inhibit the organism, is necessary to alter the prescribing practice and to adopt course correction. This is required to modify antibiotic policies in health care setups both for the management of antibiotic susceptible and resistant cases of enteric fever.

**Aim:** To document the rising Minimum Inhibitory Concentration (MIC) of azithromycin among *Salmonella* isolates.

**Materials and Methods:** A cross-sectional study was conducted in Pondicherry Institute of Medical Sciences for a period of seven years (January 2014 to December 2020). A total of 168 clinical isolates from enteric fever cases were tested for drug resistance to azithromycin by disk diffusion as per Clinical and Laboratory Standards Institute (CLSI) guidelines. The MIC was estimated using the Epsilometer test. Results were interpreted as per CLSI 2020 guidelines. Spearman's rank correlation coefficient (r) and two-tailed p-values were estimated to note the trend.

**Results:** Out of 168 *Salmonella* isolates, 65 were *Salmonella* Typhi and 103 were *Salmonella* Paratyphi *A*. The MIC of these isolates ranged from 1.5-64 µg/mL and three isolates were resistant to azithromycin with MIC ≥32 µg/mL and nine isolates had a high level of MIC of 24 µg/mL. Disc diffusion test results were consistent with MIC of azithromycin against *Salmonella* isolates from enteric fever. Regression coefficient for MIC for the given value of zone diameter for 65 *Salmonella* Typhi isolates was -0.579 ($p<0.001$, considered highly significant) and -0.475 ($p<0.01$, considered as significant) for *Salmonella* Paratyphi *A* isolates. Rising MIC to azithromycin was observed among *Salmonella* isolates over a period of seven years.

**Conclusion:** There is a need to monitor the rising trend of MIC, which may pose a therapeutic challenge for treating enteric fever cases in near future. Regular MIC estimation can pre-empt overt resistance. Hence, MIC testing should be routinely done where facilities are available than doing only disk diffusion testing for enteric fever isolates.

**Keywords:** Antibiotic, Rising trend, *Salmonella*

## INTRODUCTION

Enteric fever is an important public health challenge globally, with a burden of approximately 12 million cases and about 1,30,000 deaths every year [1]. Mortality is as high as 30% in untreated or partially treated cases hence antibiotic therapy remains the mainstay of management; bringing down mortality to less than 1% with appropriate therapy is required [2]. Appropriate and timely antimicrobial therapy is a therapeutic challenge, with reports of rising Multidrug Resistant (MDR) strains leading to treatment failures [3].

Current practice of management of enteric fever includes azithromycin and ceftriaxone as front line antibiotics. These antibiotics with spectrum of activity extending to management of various life threatening infections like meningitis, pneumonia and recently Coronavirus Disease 2019 (COVID-19) respiratory complications; in addition to the antibiotics being costly. Additionally, treatment with ceftriaxone requires hospitalisation as it is administered intravenously, with prolonged period of defervescence [4]. However, use of azithromycin has increased in the recent years, as it is thought to be clinically efficacious in cases who do not respond to oral administration is an added advantage; making it a superior antibiotic for outpatient management. Recent reports of decreased susceptibility to these agents have led to the fear of re-emergence of untreatable enteric fever [5-7].

As azithromycin is a part of the regimen for treatment of enteric fever, either alone or in combination with ceftriaxone in this tertiary care hospital, continual monitoring mechanism should be in place to document emerging resistance among *Salmonella* isolates reported by the laboratory. Therefore, present study was done to find MIC of azithromycin among *Salmonella* isolates with the objective to detect discrepancy, if any between the results of azithromycin disk diffusion and MIC method for interpreting the susceptibility of the clinical isolates of *Salmonella*, as it would directly impact patient care.

## MATERIALS AND METHODS

The present cross-sectional study was conducted in Pondicherry Institute of Medical Sciences, Puducherry, India, after reviewed and cleared by the Institute Ethics Committee (IEC) for a waiver of consent (IEC No- RC/14/104). Consecutive sampling method was used for the study. As the work was on archived *Salmonella* isolates, waiver of consent was obtained.

**Inclusion criteria:** All consecutive (total of 168), *Salmonella* isolates from positive blood cultures with clinically suspected enteric fever cases, between January 2014 to December 2020 were included. Archived *Salmonella* isolates had been identified by the standard biochemical tests and serotyping [8].

**Exclusion criteria:** Repeat *Salmonella* isolates from the same patients were excluded from the study.

### Antibiotic Susceptibility Testing

Antimicrobial susceptibility tests of the isolates which had been determined by Kirby Bauer disc diffusion method were reconfirmed for the present analysis. The antimicrobial agents tested were ampicillin (10 µg), ciprofloxacin (5 µg), ceftriaxone (30 µg), cotrimoxazole (25 µg), chloramphenicol (30 µg) and azithromycin (15 µg). The MIC of azithromycin was determined by Epsilometer (E) strip test method (Himedia, Mumbai, India). *Escherichia coli* ATCC 25922 was used as control for both the disc diffusion testing and for MIC estimation.

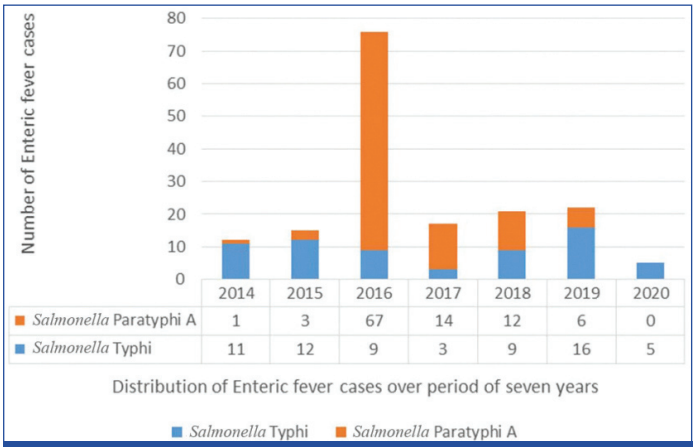
Results were interpreted as per CLSI 2020 and EUCAST 2020 guidelines [9,10]. As per CLSI 2020, the zone interpretative criteria for azithromycin susceptible isolates is ≤12 mm and resistant isolates is ≥13 mm and azithromycin MIC of ≥32 µg/mL is resistant and ≤16 µg/mL is considered as susceptible [9].

## STATISTICAL ANALYSIS

Data analysis was undertaken using the Microsoft Excel and Statistical Package for the Social Sciences (SPSS) software version 20.0. Spearman's rank correlation coefficient and regression coefficient (by linear regression) between disc diffusion and MIC for azithromycin was calculated, taking MIC as a dependent variable and zone diameter by disc diffusion as an independent variable. A p-value of 0.05 or below was considered significant. The p-value was calculated using Spearman's rho test for correlation and F test for regression analysis. All the tests were two-sided.

## RESULTS

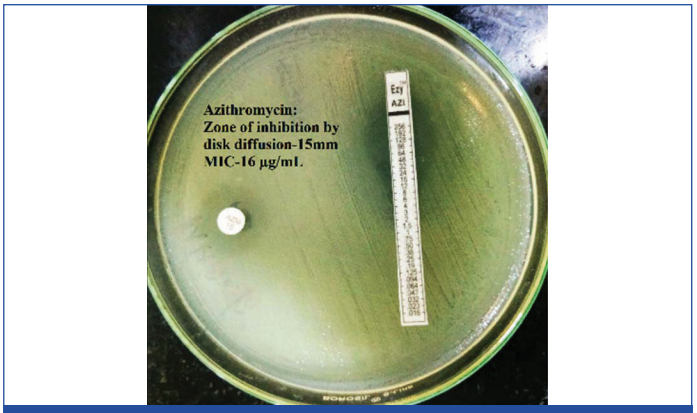
Of 168 *Salmonella* isolates from blood, 65 isolates (38.7%) were *Salmonella* Typhi and 103 were (61.3%) were *Salmonella* Paratyphi *A*. While majority of patients were males, 114 (68%), a male to female ratio of 2.1:1 was noted. Age of the patients ranged from 3-69 years, with majority of patients (76, 45.2%) of 21-30 years, followed by 47 (28%) in the age group of 31-40 years, 28 (16.6%) in 11-20 years, 6 (3.6%) each in the age group of ≤10 years and 41-50 years, 4 (2.4%) of 51-60 years and 1 (0.6%) patient of >60 years. Between 2016 to 2018; there was a surge in cases with *Salmonella* Paratyphi *A*. However, *Salmonella* Typhi remained the predominant isolate in rest of the years [Table/Fig-1].



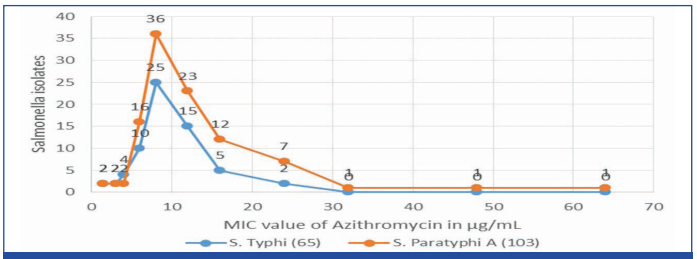

**[Table/Fig-1]:** Distribution of *Salmonella* Typhi and *Salmonella* Paratyphi *A* isolates over the period of seven years.

All *Salmonella* isolates (n=168) were uniformly susceptible to ampicillin, cotrimoxazole, chloramphenicol, and ceftriaxone; whereas 38 isolates were resistant and 127 were intermediately susceptible to ciprofloxacin. Only three isolates were susceptible to ciprofloxacin by disk diffusion method. Azithromycin resistance was detected in three isolates of *Salmonella* Paratyphi *A* while remaining 165 isolates were uniformly susceptible (with varying zones of inhibition). The MIC of azithromycin among these isolates ranged from 1.5-64 µg/mL. Maximum number of isolates 125/168 (74%) had MIC ranging between 6-12 µg/mL. Three isolates were resistant to azithromycin with MIC ≥32 µg/mL and nine isolates had high level of MIC of 24 µg/mL. Disk diffusion testing (zone of inhibition-15 mm) and MIC (16 µg/mL) of azithromycin for one *Salmonella* Typhi isolate is shown in [Table/Fig-2]. The MIC distribution of azithromycin among 168 *Salmonella* isolates is shown in [Table/Fig-3,4].

The MIC for azithromycin among *Salmonella* Typhi isolates (n=65) ranged from 1.5-24 µg/mL and corresponding zone standardised regression coefficient (Beta) for MIC for given value of zone diameter was -0.579 (p<0.001, considered significant). If zone of inhibition is reduced by 1 mm, then corresponding MIC also increases for




**[Table/Fig-2]:** Disk diffusion testing (zone of inhibition-15 mm) and MIC (16 µg/mL) of azithromycin for one *Salmonella* Typhi isolate.



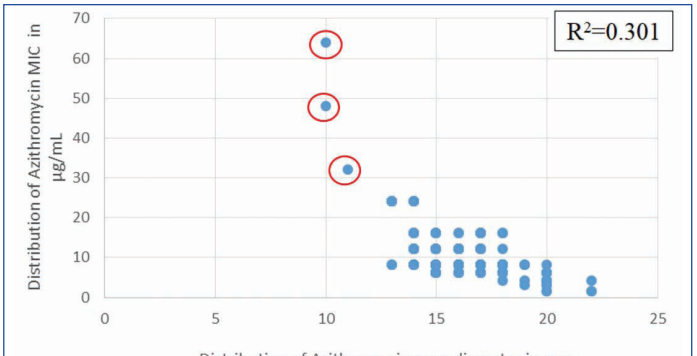

**[Table/Fig-3]:** Distribution of MIC of azithromycin among 168 *Salmonella* isolates (as per CLSI- azithromycin MIC of ≥32 µg/mL is resistant and ≤16 µg/mL is susceptible).

| Year | No. of isolates (*Salmonella* Typhi and *Salmonella* Paratyphi *A*) | MIC range (µg/mL) |
|---|---|---|
| 2014 | 12 | 1.5-8 |
| 2015 | 15 | 1.5-12 |
| 2016 | 76 | 1.5-64 |
| 2017 | 17 | 1.5-48 |
| 2018 | 21 | 3-24 |
| 2019 | 22 | 4-16 |
| 2020 | 5 | 4-16 |

**[Table/Fig-4]:** Distribution of MIC of azithromycin among 168 *Salmonella* isolates over the period of seven years.

azithromycin. However, MIC and zone diameters for azithromycin had significant negative correlation (r=-0.549; $R^2$=0.301, p<0.01, which was significant).

The MIC for azithromycin among *Salmonella* Paratyphi *A* isolates (n=103) ranged from 1.5-64 µg/mL and corresponding zone standardised regression coefficient (Beta) for MIC for given value of zone diameter was -0.646 (p<0.001, considered significant). However, MIC and zone diameters for azithromycin had significant negative correlation (r=-0.475; p<0.01, considered as significant) in case of *Salmonella* Paratyphi *A*. Overall comparison of MIC with zone diameter by disk diffusion of azithromycin among *Salmonella* isolates (n=168) is shown in [Table/Fig-5].




**[Table/Fig-5]:** Overall comparison of MIC with disk diffusion zone diameter of azithromycin among *Salmonella* isolates (n=168) O=*Salmonella* Paratyphi *A*. The MIC of the control strain ATTC Escherichia coli 25922 was 1.5 µg/mL for azithromycin and zone of inhibition was 15 mm

## DISCUSSION

Antimicrobial susceptibility reports serve as a guide to clinicians for selecting most appropriate therapeutic agent. In the present study, all the *Salmonella* isolated over a period of seven years were found to be sensitive to chloramphenicol, cotrimoxazole, ampicillin, ceftriaxone and another welcome finding was that none were MDR. These findings were consistent with findings of Srirangaraj S et al., and Garg A et al., [4,6]. A previous study by Bhat KS et al., also correlated well the present findings [7]. On the contrary a study conducted by Menezes GA et al., from Pondicherry in 2011 showed a high level (22%) of *Salmonella* Typhi isolates being MDR [11].

In the last two decades, emergence of MDR strains of *Salmonella*, worldwide has led to withdrawal of ampicillin, cotrimoxazole and chloramphenicol from the therapeutic regimen for enteric fever and has been replaced by ciprofloxacin, ceftriaxone and azithromycin. This probably has led to re-emergence of susceptibility to the earlier antibiotics, while emerging resistance with increase in the MIC of ciprofloxacin/ceftriaxone and azithromycin.

Azithromycin, till date has been an effective and suitable alternative to treat mild-to-moderate enteric fever. Due to optimum serum availability leading to high intracellular concentrations achieved by azithromycin early fever clearance, as well as lower rates of relapse has been documented following a course of 5-7 days of treatment with the drug [12]. In a study conducted by Parry CM et al., *Salmonella* Typhi isolates with azithromycin MIC ranged between 4-16 µg/mL, showed very low clinical failure amounting to 8-11% [3]. As MIC value of an antibiotic is a good predictor of in-vivo efficacy, it is thought to be a superior indicator for defervescence of fever than the disk diffusion method. A report relying on the latter may result in delayed therapeutic response probably leading to serious complications [12,13].

The CLSI 2020 and EUCAST 2020 guidelines, give interpretation breakpoints by disk diffusion and by MIC for azithromycin susceptibility only for *Salmonella* Typhi but not for *Salmonella* Paratyphi *A* [9,10]. However, in present study *Salmonella* Typhi breakpoints was used to interpret the results for *Salmonella* Paratyphi *A* isolates also. In this study, only three isolates (*Salmonella* Paratyphi *A*) were resistant with MIC ≥32 µg/mL of azithromycin while the rest (165 isolates) were uniformly susceptible, with varying concentrations of inhibition ranging from 1.5-24 µg/mL. In the present study rising trend in the MIC distribution was observed (ranging from 1.5-24 µg/mL) over a period of first six years (from 2014 to 2019) among *Salmonella* isolates. Similar observations have been made by Parry CM et al., Das S et al., Bhat KS et al., and Archana M et al., [3,5,7,14]. However, in the year 2020, due to COVID-19 pandemic, only five *Salmonella* isolates were found. So rising trend in the azithromycin was not appreciated and this may expand the possible therapeutic options to treat MDR infections, eradicate the errors in forecasting therapeutic success in antibiotic susceptibility [15].

In the present study, resistance of azithromycin by disk diffusion correlated well with the MIC by E test for both the species showing significant negative correlation ($r=-0.549$, $p<0.01$ for *Salmonella* Typhi and $r=-0.475$; $p<0.01$ for *Salmonella* Paratyphi *A*).These results were consistent with other studies by Parry CM et al., Srirangaraj S et al., and Garg A et al., [3,4,16]. Detecting the MIC routinely, has following advantages- first it has direct therapeutic impact, while Kirby Bauer method of reporting S, I, R may not actually reflect the exact inhibitory dose required for disease defervescence. Secondly, by mapping the MIC regularly would help in detecting rising trends through the drug concentrations creeping upwards. In the present study, as well as several others, *Salmonella* Paratyphi A is emerging as leading cause of enteric fever [3,17,18]. As reported by Bhat KS et al., continuous monitoring of azithromycin MIC is important for early recognition of any emergence of resistance, and such cases can be treated by alternate options, to prevent treatment failures [7]. In the absence of CLSI guidelines for cut-offs for *Salmonella* Paratyphi A, multicentric studies are required to establish uniform performance standards for both disk diffusion and MIC breakpoints. This will help to establish Indian standards to interpret azithromycin susceptibility against *Salmonella* Paratyphi A. Enteric fever continues to be a problem of the developing nations [2,17,18]. Standards and interpretative criteria need to be formulated to suit local isolates and needs. This would help to improve consistency in reporting between laboratories in the region.

### Limitation(s)

In the present study, small sample size has limited further analysis on rising trend of azithromycin MIC. There is need for more multicentric studies to establish uniform performance standards for azithromycin disk susceptibility testing and MIC estimation among *Salmonella* isolates, especially for *Salmonella* Paratyphi *A*. This would help to improve consistency between reporting laboratories and therapeutic utility of azithromycin for enteric fever cases.

## CONCLUSION(S)

Although a large proportion of *Salmonella* isolates from patients with enteric fever still continue to remain susceptible to azithromycin and ceftriaxone, there is a need to watch out for rising trend of MIC of azithromycin, which may pose a threat to future treatment of enteric fever. It is hoped that MIC estimation adopted as a routine antibiotic susceptibility test, will pre-empt overt resistance by implementing alternate therapeutic options. May be the time is now appropriate to recycle first-line anti-*Salmonella* antibiotics like ampicillin, cotrimoxazole and chloramphenicol to treat cases with enteric fever. As *Salmonella* Paratyphi A is emerging as a frequent cause of enteric fever, it is necessary to develop defined breakpoints for azithromycin for optimum therapeutic utility.

## REFERENCES


[1] Muhammad R, Ali Z, Mehmood K, Ziauddin, Afridi AR, Bari F. Antibiotic sensitivity pattern of *Salmonella* serotypes in patients with enteric fever in a teaching hospital. J Postgrad Med Inst. 2013;27(4):397-402.

[2] Singhal L, Gupta PK, Kale P, Gautam V, Ray P. Trends in antimicrobial susceptibility of *Salmonella* Typhi from North India (2001-2012). Indian J Med Microbiol [Internet]. 2014;32(2):149-52. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24713900.

[3] Parry CM, Thieu NTV, Dolecek C, Karkey A, Gupta R, Turner P, et al. Clinically and microbiologically derived azithromycin susceptibility breakpoints for *Salmonella enterica* serovars Typhi and Paratyphi A. Antimicrob Agents Chemother [Internet]. 2015;59(5):2756-64. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25733500.

[4] Srirangaraj S, Kali A, Charles MVP. A study of antibiogram of *Salmonella enterica* serovar Typhi isolates from Pondicherry, India. Australas Med J [Internet]. 2014;7(4):185. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4009880/pdf/AMJ-07-185.pdf.

[5] Das S, Ray U, Dutta S. Revisit of fluoroquinolone and azithromycin susceptibility breakpoints for *Salmonella enterica* serovar Typhi. J Med Microbiol. 2016;65(7):632-40. Doi: 10.1099/jmm.0.000288.

[6] Garg A, Verma S, Kanga AK, Mokta K, Singh D, Singh B. MIC & zone diameter correlation *Salmonella*: Interpretive guidelines need of the hour. Int J Infect Dis [Internet]. 2014;21:101. Available from: http://ovidsp.ovid.com/ovidweb.cgi?T=JS&CSC=Y&NEWS=N&PAGE=fulltext&D=emed12&AN=71633976%5Cnhttp://wa4py6yj8t.search.serialssolutions.com/?url_ver=Z39.88-2004&rft_val_fmt=info:ofi/fmt:kev:mtx:journal&rfr_id=info:sid/Ovid:emed12&rft.genre=article&rft_id=inf.

[7] Bhat KS, Anandhalakshmi S, Rasitha D, Venkataraman S, Kanungo R. High-level resistance to ciprofloxacin and rising MIC to ceftriaxone and azithromycin among enteric fever isolates from a Tertiary Care Center, Puducherry, India. Int J Curr Microbiol App Sci. [Internet]. 2017;6(12):729-36. Available from: https://doi.org/10.20546/ijcmas.2017.612.076.

[8] Procop Gary W KEW. Koneman's Color Atlas and Textbook of Diagnostic ... [Internet]. Wolters Kluwer. 2016. Available from: https://shop.lww.com/Koneman-s-Color-Atlas-and-Textbook-of-Diagnostic-Microbiology/p/9781451116595.

[9] CLSI. PerforCLSI. (2018). Performance Standards for Antimicrobial Susceptibility Testing. Clinical and Laboratory Standards Institute. Retrieved from www.clsi.orgmance Standards for Antimicrobial Susceptibility Testing. [Internet]. Performance Standards for Antimicrobial Susceptibility Testing. 2018. 1-296 p. Available from: www.clsi.org.

[10] EUCAST 2018. In: European committee on antimicrobial susceptibility testing [Internet]. 2018. Available from: http://www.eucast.org.

[11] Menezes GA, Harish BN, Khan MA, Goessens WHF, Hays JP. Antimicrobial resistance trends in blood culture positive *Salmonella* Typhi isolates from Pondicherry, India, 2005–2009. Clin Microbiol Infect [Internet]. 2012;18(3):239-45. Available from: http://linkinghub.elsevier.com/retrieve/pii/S1198743X14616281.

[12] Veeraraghavan B, Pragasam AK, Bakthavatchalam YD, Ralph R. Typhoid fever: Issues in laboratory detection, treatment options & concerns in management in developing countries [Internet]. Future Sci OA. 2018;4(6):FSO312. Doi: 10.4155/fsoa-2018-0003. Available from: /pmc/articles/PMC6060388/.
[13] Rai S, Jain S, Prasad KN, Ghoshal U, Dhole TN. Rationale of azithromycin prescribing practices for enteric fever in India. Indian J Med Microbiol [Internet]. 2012;30(1):30-33. Available from: https://pubmed.ncbi.nlm.nih.gov/22361757/.
[14] Archana M, Shenoy B, Adhikary R, Malavalli BV. Study of azithromycin sensitivity in enteric fever in pediatric population. Indian J Child Heal. 2018;5(5):324-28.
[15] Meerwein M, Tarnutzer A, Böni M, Van Bambeke F, Hombach M, Zinkernagel AS. Increased azithromycin susceptibility of multidrug-resistant gram-negative bacteria on RPMI-1640 agar assessed by disk diffusion testing. Antibiotics (Basel). 2020;9(5):218.
[16] Garg A, Verma S, Kanga A, Singh D, Singh B. Antimicrobial resistance pattern and in vivo activity of azithromycin in *Salmonella* isolates. Indian J Med Microbiol [Internet]. 2013;31(3):287. Available from: http://www.ijmm.org/text.asp?2013/31/3/287/115641.
[17] Bhat SK, Venkataraman S, Kanungo R. Upsurge of *Salmonella* Paratyphi a infection in and around Puducherry following the torrential rains of December 2015. Int J Med Microbiol Trop Dis [Internet]. 2017;3(4):155-59. Available from: https://www.innovativepublication.com/journal-article-file/5393.
[18] John J, Van Aart CJC, Grassly NC. The burden of typhoid and paratyphoid in india: systematic review and meta-analysis. PLoS Negl Trop Dis [Internet]. 2016 Apr [cited 2018 Oct 15];10(4):e0004616. Available from: http://www.ncbi.nlm.nih.gov/pubmed/27082958.

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