Article published online: 2020-04-06

Original Article

Access this article online

Quick Response Code:

Website: www.jlponline.org

DOI: 10.4103/JLP.JLP_99_18

Reconsidering azithromycin disc diffusion interpretive criteria for *Salmonellae* in view of azithromycin MIC creep among typhoidal and nontyphoidal salmonella

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Abstract:

PURPOSE: Enteric fever continues to be an important public health challenge for the developing world. With the emergence of fluoroquinolone resistance in *Salmonellae* spp. azithromycin is increasingly being used for oral treatment of enteric fever. We investigated the antibiotic susceptibility pattern of azithromycin in *Salmonellae* spp. isolates from a tertiary care hospital to detect emerging resistance.

METHODS: The study assessed the reliability of disc diffusion as a screening test to detect azithromycin resistance by comparing it with the minimum inhibitory concentrations (MICs) of the drug in 100 *Salmonellae* spp. strains. The strains of *Salmonellae* spp. showing resistance to azithromycin were further investigated for resistance markers – *mphA*, *mphB,* and *mef B* genes.

RESULTS: This study was conducted on 100 *Salmonella enterica* strains recovered from blood culture samples between 2013 and 2017. Among these isolates, 18 showed resistance to azithromycin by disc diffusion methodology with zones of inhibition <13 mm. MIC of 6 of these isolates were ≥32 mg/L. The mean MIC of azithromycin increased from 5 mg/L in 2013 to 24 mg/L in 2017. Azithromycin consumption as defined daily doses per 1000 patient days also showed an increase over the past 4 years.

CONCLUSION: Azithromycin disc diffusion diameter interpretations as recommended by Clinical and Laboratory Standards Institute can mislabel a few sensitive strains as resistant. Azithromycin resistance is emerging in typhoidal and nontyphoidal *Salmonella. MphA* gene is associated with high MICs in nontyphoidal *Salmonella* spp.

Key words:

Azithromycin disc diffusion, azithromycin resistant *Salmonella* spp., enteric fever, mphA gene, *Salmonella* spp.

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Submission: 24-07-2018
Accepted: 08-12-2018

Introduction

Enteric fever remains an important public health challenge for the developing world. Geographically, south central and southeast Asia have the highest incidence of typhoid fever with an estimated 100 cases per 100,000 person-year.[1] Risk factors commonly associated with a high incidence of typhoid fever include poor sanitation, limited access to clean drinking water and low socioeconomic status.[2] Changing susceptibility patterns of typhoidal *Salmonella* spp. has also added to the challenges faced by the treating physician. While multidrug-resistant *Salmonella* spp. has become a thing of the past in the Indian

How to cite this article: Khan S, Kurup P, Vinod V, Biswas R, Pillai GK, Kumar A. Reconsidering azithromycin disc diffusion interpretive criteria for *Salmonellae* in view of azithromycin MIC creep among typhoidal and nontyphoidal salmonella. J Lab Physicians 2019;11:39-44.

subcontinent, fluoroquinolone-resistant *Salmonellae* spp. have emerged as a formidable problem.[3,4] Consequently, intravenous ceftriaxone and oral azithromycin are increasingly being used in the empirical treatment of typhoid fever.[5] Although ceftriaxone continues to be effective against *Salmonella* spp., it is parenteral administration, cost and longer time for defervescence are factors that limit its use as an ideal antibiotic for enteric fever.[4]

Azithromycin has been shown to be equally effective, or in some cases, a superior treatment alternative to chloramphenicol or fluoroquinolones by several randomized control trials.[6,7] It has the ability to achieve intracellular concentrations which are 50–100 times higher than the serum level of the antibiotic.[7]

Guidelines for testing azithromycin susceptibility for *Salmonella* Typhi were released by Clinical and Laboratory Standards Institute (CLSI) in 2015.[8] EUCAST does not prescribe any clinical breakpoints for azithromycin testing but mentions that azithromycin has been used in the treatment of *S*. Typhi infections with minimum inhibitory concentration (MIC) ≤16 mg/L.[9] The emergence of azithromycin resistance in *Salmonellae* spp. has been documented by several studies.[10]

We investigated the antibiotic susceptibility pattern of azithromycin in *Salmonella* spp. isolates from a tertiary care hospital to detect emerging resistance and change in MICs over a 5-year period. The study also assessed the reliability of disc diffusion as a screening test to detect azithromycin resistance by comparing it with the MICs of the drug.

Materials and Methods

Bacterial strains

The study was conducted in a 1200 bed tertiary care hospital in Southern India between January 2013 and December 2017. A total of 100 *Salmonella enterica* strains recovered from blood samples were included in the study. Isolates were identified by standard biochemical reactions and VITEK 2 compact system (bioMérieux). The confirmation of identification was done by agglutination with specific antisera (Denka Seiken).

Azithromycin susceptibility testing

Antibiotic susceptibility testing was performed by Kirby Bauer disc diffusion method using azithromycin discs (15 μg). Azithromycin discs from two different manufacturers were used for disc diffusion (HiMedia and BD BBL sensi disc). Testing was done in triplicate for each isolate and disc. Disc diffusion diameters were recorded as mean of three readings. MIC of azithromycin was determined using Etest (bioMérieux). CLSI (2015–2017) guidelines were used to interpret azithromycin susceptibility-sensitive ≥13 mm and ≤16 mg/L and resistant ≤12 mm and ≥32 mg/L. Since CLSI mentions azithromycin breakpoints for *S*. Typhi only, these were used for interpreting *Salmonella* Paratyphi A and Group B *Salmonella* spp. susceptibility also. MIC50 and MIC90 of the isolates were determined. *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923 were used as the control strain for all susceptibility testing.

Errors in susceptibility were defined as follows:

- Very major error: False susceptible result by disc diffusion compared to the MIC value
- Major error: False resistant result produced by disc diffusion compared to MIC value
- Minor errors: A difference of >2 mm in disc diffusion diameters while using two different discs.

Synergy testing

The evaluation of 10 strains of *Salmonella* Typhi for *in vitro* synergy between azithromycin and ceftriaxone was performed. Mueller-Hinton agar plates were inoculated with the suspension of the study strains grown to an optical density of 0.5 McFarland units. For each isolate, MIC of azithromycin and ceftriaxone was determined individually and in combination (AB Biodisk Etest Customer Information Sheet EAS023). For combination testing, E strip A (azithromycin) was put on the inoculated MHA plate and left for 1 h at room temperature. After an hour, this strip was removed after marking its outline, was washed with alcohol and stored as MIC reading scale. E strip B (ceftriaxone) was placed on the imprint of E strip A immediately and incubated at 35°C for 18 h. MIC scales were used to read the combination MIC gradients.

Fractional inhibitory concentration index (FIC index) calculations were made according to the formula:

$$\text{FIC index} = MIC_{AB}/MIC_{A} + MIC_{BA}/MIC_{B}$$

Synergy was defined as MIC of combination ≥2 dilutions lower than MIC of the most active drug alone or FIC index ≤0.5.

Polymerase chain reaction

Mechanism responsible for azithromycin resistance was studied by amplifying *mphA*, *mphB*, and *mefB* gene using previously published primers.[11] The thermal method of DNA extraction from *Salmonellae* spp., described by Gibson and McKee, was used with minor modifications.[12] Briefly, isolates were grown on brain heart infusion agar plates for 18–24 h. 2–3 colonies were scooped using an inoculation loop and suspended in 100 μl of sterile distilled water. The suspension was heated in a dry bath at 95°C for 15 min. After

centrifugation the 1 µl of supernatant solution was used as DNA template for polymerase chain reaction (PCR).

PCR reactions were done in 25 µl volumes using EmeraldAmp® MAX PCR Master Mix, 100 ng of each primer and the extracted whole DNA as the template. The PCR was carried out in Primus 25 thermal cycler (Peqlab) starting with an initial denaturation at 95°C for 3 min. This was followed by 30 cycles of denaturation at 95°C for 30 s, annealing at 60°C for 30 s, and elongation at 72°C for 30 s. A final elongation step was run at 72°C for 10 min. The PCR products were subjected to 2% agarose gel electrophoresis stained using ethidium bromide solution and visualized under trans-UV illumination.

Results

This study was conducted on 100 *Salmonella enterica* strains which included *S.* Typhi (*n*=46), *S.* Paratyphi A (*n* = 12), and Group B *Salmonella* spp. (*n* = 42) recovered from blood culture samples between 2013 and 2017.

Among these isolates, 18 showed resistance to azithromycin by disc diffusion methodology with zones of inhibition <13 mm. Of these 18 isolates, 10 were *S.* Typhi, three were *S.* Paratyphi A and five were Group B *Salmonella* spp. MICs of six of these isolates were ≥32 mg/L [Table 1].

MIC 50 and MIC 90 of azithromycin for *Salmonella* spp. was found to be 4 mg/L and 12 mg/L, respectively. Errors in susceptibility were evaluated when disc diffusion was compared with MIC values. Major error was seen in 12 isolates which were labeled as resistant by disc diffusion and showed MICs in the susceptible zone [Table 2].

Synergy testing was inconclusive. MIC of azithromycin showed reduction when combined with ceftriaxone. However, the FIC indices did not show values supporting synergy [Supplemental Table 1].

Isolates which showed resistance by disc diffusion and MIC were investigated for *mphA* gene. A single isolate of Group B *Salmonella* spp. was positive for *mphA* gene. All the isolates were negative for *mphB* and *mef B* gene.

Mean MICs of azithromycin of the *Salmonella* spp. isolates from 2013 to 2017 were calculated and the mean MIC increased from 5 mg/L in 2013 to 24 mg/L in 2017. Azithromycin consumption as defined daily doses per 1000 patient days also showed an increase over the past 4 years [Figure 1]. Mean MIC of ceftriaxone was also calculated and the ceftriaxone consumption was compared from 2013 to 2017. Mean MIC of ceftriaxone over the 4 years were around 0.13 mg/L.

Table 1: Minimum inhibitory concentration values of Salmonella spp. showing azithromycin resistance by disc diffusion

Identification	Disc diffusion[a] (mm)	Disc diffusion[b] (mm)	MIC (mg/Ll)
S. Typhi	12	12	4
S. Typhi	12	11	12
S. Typhi	9	8	16
S. Typhi	12	12	24
S. Typhi	6	6	32
S. Typhi	6	6	32
S. Typhi	6	6	32
S. Typhi	6	6	96
S. Typhi	12	11	16
S. Typhi	6	6	32
S. Paratyphi A	6	6	8
S. Paratyphi A	6	8	8
S. Paratyphi A	10	11	24
S. Paratyphi B	10	11	12
Salmonella spp.	10	12	12
Salmonella spp.	10	12	12
Salmonella spp.	12	12	12
Salmonella spp.	8	10	64

Disc diffusion[a]: HiMedia; Disc diffusion[b]: BD BBL Sensi disc. *S.*Typhi = *Salmonella* Typhi, *S.* Paratyphi = *Salmonella* Paratyphi, MIC = Minimum inhibitory concentration

Table 2: Error classification of *Salmonella* spp. showing discordant results by disc diffusion and minimum inhibitory concentration

Identification	Very major error	Major error	Minor error
S. Typhi	0	5	0
S. Paratyphi A	0	3	0
Salmonella Group B	0	1	0
Salmonella species	0	3	0
Total	0	12	0

S. Typhi = *Salmonella* Typhi, *S.* Paratyphi = *Salmonella* Paratyphi

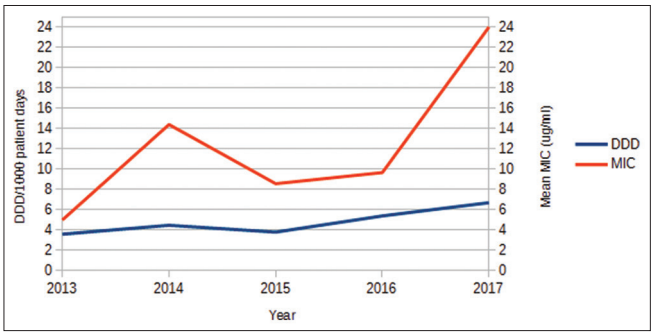

Figure 1: Azithromycin consumption trends in the tertiary care hospital from 2013 to 2017 and mean minimum inhibitory concentration of *Salmonellae* spp. during that period

Ceftriaxone consumption also remained stable around 0.74 DDD/1000 patient days.

The clinical outcomes of the patients harboring six strains showing true resistance were analyzed. All the patients recovered with ceftriaxone treatment. Two patients received prolonged ceftriaxone therapy (14and 28 days).

One patient gave a history of gall bladder polyps and repeated episodes of enteric fever [Table 3].

Discussion

Enteric fever due to nalidixic acid resistant strains of typhoidal *Salmonellae* spp. requires ceftriaxone or azithromycin for its treatment.[5,13] While resistance to extended spectrum cephalosporins is uncommon, the need for parenteral therapy limits their use as a preferred first line treatment.[14] Azithromycin appears to be an attractive oral alternative for treatment of uncomplicated enteric fever. However, as cautioned by Misra and Prasad, irrational antibiotic therapy and easy over the counter availability of azithromycin could contribute to its emerging resistance and subsequent treatment failure in *Salmonellae* spp.[14]

Our study was conducted on 58 typhoidal *Salmonella* spp. and 42 nontyphoidal *Salmonella* spp. Among these isolates, 18% showed resistance to azithromycin if disc diffusion interpretive criteria were used to determine resistance. However, only 6% of the isolates showed true resistance as their MICs were ≥32 mg/L. An analysis of the discordant disc diffusion diameters showed that of the 12 isolates, eight (66.7%) had diameters between 10–12 mm. Of the 12 isolates showing discordant results between disc diffusion and MIC of azithromycin-five were *S.* Typhi, three *Salmonella* Paratyphi A and four *Salmonella* spp. As CLSI interpretive criteria were specific for *S.* Typhi, our study had extrapolated these criteria for *S.* Paratyphi A and *Salmonellae* spp. also. While this can be a major reason for the large number of discordant results, the presence of a considerable number of *S.* Typhi showing discordance is a matter of concern. Zone diameters of 10–12 mm can therefore be considered as grey areas for determining azithromycin resistance for *Salmonella* spp. Therefore, unlike other studies which found good correlation between disc diffusion diameters and MIC values, our study found disc diffusion diameters for determining azithromycin resistance as unreliable.[14,15] The results of this study indicate that isolates with diameters between 10–12 mm should be reconfirmed with respective MIC values before arriving at any conclusion.

Six isolates in this study showed true resistance to azithromycin. These isolates were studied for genes of azithromycin resistance - *mphA, mphB*, and *mefB*. The *mphA* gene was detected in a single isolate of non typhoidal *Salmonella* spp. Five *S.* Typhi isolates were negative for *mphA* gene while none of the isolates showed *mphB* and *mefB* genes. Azithromycin resistance in non typhoidal *Salmonellae* spp. has been reported from several centers.[10,16] Elevated MICs of typhoidal *Salmonella* spp. have been reported occasionally.[17] The plasmid borne

Table 3: Clinical details of patients with azithromycin resistant salmonella infection

Patient	Gender/ age	Isolate from blood	Provisional diagnosis	Associated comorbidities	Clinical features	Ceftriaxone susceptibility	Ciprofloxacin susceptibility	Treatment	Outcome
1	Female/18 years	*S.* Typhi	Enteric fever	None	Low grade fever with mild chills, no rigor (1 weeks duration) No history of abdominal pain, loose stools	S	R	Ceftriaxone for 7 days, Cefixime for 7 days	Recovered
2	Male/57 years	*S.* Typhi	PUO	Hypertension Coronary Artery Disease Mixed airway disease Gall Bladder polyp	Low grade fever loss of appetite and weight loss Treated for enteric fever 1 week back	S	R	Ceftriaxone 28 days Azithromycin for 7 days	Recovered
3	Male/55 years	*S.* Typhi	Enteric fever	None	Low grade fever loss of appetite and weight loss stools; 2-3 episodes of vomiting	S	R	Ceftriaxone for 14 days	Recovered
4	Female/17 years	*S.* Typhi	PUO	None	High grade fever for 10 days, nausea, vomiting loose stools	S	R	Ceftriaxone for 14 days and Azithromycin for 5 days	Recovered
5	Male/30 years	*S.* Typhi	Enteric fever	None	Fever for 7 days with headache, 1 episode of vomiting	S	R	Ceftriaxone for 14 days	Recovered
6	Female/70 years	Group B Salmonella	Retroperitoneal liposarcoma	Diabetes mellitus Hypertension Splenectomy	Neuropathic pain	S	S	-	-

S. Typhi: *Salmonella* Typhi, *S.* Paratyphi = *Salmonella* Paratyphi

mphA gene has been reported as one of the reasons for azithromycin resistance.[18] Association of a chromosomal macrolide inactivation gene cluster *mphA-mrx-mphr(A)* has also been associated with azithromycin resistance in non typhoidal *Salmonella*e spp.[16] In the present study as well, a single high-level azithromycin-resistant isolate (MIC 64 mg/L) harbored the *mphA* gene, while other 5 (MIC: 16–32 mg/L) did not. These results indicate that the *mphA* gene may mediate a high level of resistance to azithromycin in *Salmonella*, as described previously in studies.[10,16] In addition, azithromycin resistance could arise from other probable mechanisms such as mutations in the *rlpV* and *rlpD* genes.[19] Currently, few studies have investigated azithromycin resistance mechanisms in *Salmonella*, specifically, typhoidal salmonella.

Annual consumption of azithromycin in the hospital was tracked from 2014 to 2017. Azithromycin showed an increased consumption from 4.4DDD per 1000 patient days to 6.7 DDD per 1000 patient days. The mean MIC of *Salmonella* isolates also increased from 5 mg/L in 2013 to 24 mg/L in 2017 showing the MIC creep over 5 years. Therefore, the increasing utilization of this antibiotic probably had a considerable role to play in the development of resistance in these organisms. The easy over the counter availability of azithromycin and its widespread use for treating upper respiratory infections may have an important role to play in the emerging drug resistance among Salmonella species in our population. A comparison with the utilization of ceftriaxone in the hospital showed that the DDD s of ceftriaxone remained constant over the past 4 years. Therefore, our study reinforces that ceftriaxone can be used as an effective therapeutic option for culture proven enteric fever cases as well as empirical therapy for suspected cases of enteric fever.

The clinical outcome of six patients who harbored azithromycin resistant strains was analyzed. All patients were treated with injection ceftriaxone for 14 days. One patient showed gall bladder polyps and received an extended treatment with injection ceftriaxone for 28 days. All the patients growing typhoidal salmonella recovered with appropriate therapy. One patient received injection ceftriaxone and azithromycin in the initial course of therapy. Salmonella isolates with elevated azithromycin MICs have been isolated and reported from travellers from India and also from the Indian population. The clinical relevance of elevated MICs remains unknown. Clinically and microbiologically correlated breakpoints by Parry *et al*. indicates that azithromycin MIC of <16 mg/L likely predicts a favorable clinical outcome.[13] Azithromycin reaches 50–100 fold concentration within the macrophages and polymorphonuclear cells which is crucial in treating intracellular organisms of enteric fever; nevertheless, it achieves very low plasma levels. Hence, MIC might not be a good indicator of decreased susceptibility to azithromycin.[20]

Conclusion

This study has several limitations which include a small sample size, being a single center study, use of Estrip for MIC and use of interpretive criteria of *S.* Typhi for *S.* Paratyphi A and nontyphoidal salmonella. However, azithromycin disc diffusion diameter interpretations as recommended by CLSI can mislabel a few sensitive strains as resistant. Caution needs to be exercised when using disc diffusion breakpoints for *S.* Typhi to interpret nontyphoidal Salmonella breakpoints. *MphA* gene is seen in resistant nontyphoidal salmonella with high azithromycin MICs. It is not seen in isolates with lower MICs and in typhoidal *Salmonella.* While, further studies to understand the mechanism of resistance to azithromycin among Salmonella spp. is required, ceftriaxone continues to be a reliable drug for empirical treatment of enteric fever.

Acknowledgment

We would like to thank Dr Vrinda Nampoothiri, Clinical Pharmacist, Amrita Antimicrobial Stewardship Programme for her invaluable help in determining azithromycin consumption for the study duration.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. 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.
2. Sur D, Ali M, von Seidlein L, Manna B, Deen JL, Acosta CJ, *et al.* Comparisons of predictors for typhoid and paratyphoid fever in Kolkata, India. BMC Public Health 2007;7:289.
3. 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 2014;32:149-52.
4. Menezes GA, Harish BN, Khan MA, Goessens WH, Hays JP. Antimicrobial resistance trends in blood culture positive *Salmonella* typhi isolates from Pondicherry, India, 2005-2009. Clin Microbiol Infect 2012;18:239-45.
5. Crump JA, Sjölund-Karlsson M, Gordon MA, Parry CM. Epidemiology, clinical presentation, laboratory diagnosis, antimicrobial resistance, and antimicrobial management of invasive *Salmonella* infections. Clin Microbiol Rev 2015;28:901-37.
6. Frenck RW Jr., Nakhla I, Sultan Y, Bassily SB, Girgis YF, David J, *et al.* Azithromycin versus ceftriaxone for the treatment of uncomplicated typhoid fever in children. Clin Infect Dis 2000;31:1134-8.
7. Frenck RW Jr., Mansour A, Nakhla I, Sultan Y, Putnam S,

Wierzba T, *et al.* Short-course azithromycin for the treatment of uncomplicated typhoid fever in children and adolescents. Clin Infect Dis 2004;38:951-7.
8. Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing: Twenty Fourth Informational Supplement. M100–S24. Wayne, PA: Clinical and Laboratory Standards Institute; 2015.
9. EUCAST. Breakpoint Tables for Interpretation of MICs and Zone Diameters. Version 8.0; 2018. Available from: http://www.eucast.org. [Last accesses on 2018 Jul 01].
10. Wang J, Li Y, Xu X, Liang B, Wu F, Yang X, *et al.* Antimicrobial resistance of *Salmonella enterica* serovar typhimurium in Shanghai, China. Front Microbiol 2017;8:510.
11. Phuc Nguyen MC, Woerther PL, Bouvet M, Andremont A, Leclercq R, Canu A, *et al. Escherichia coli* as reservoir for macrolide resistance genes. Emerg Infect Dis 2009;15:1648-50.
12. Gibson JR, McKee RA. PCR products generated from unpurified *Salmonella* DNA are degraded by thermostable nuclease activity. Lett Appl Microbiol 1993;16:59-61.
13. Parry CM, Basnyat B, Crump JA. The management of antimicrobial-resistant enteric fever. Expert Rev Anti Infect Ther 2013;11:1259-61.
14. Misra R, Prasad KN. Antimicrobial susceptibility to azithromycin among *Salmonella enterica* typhi and paratyphi A isolates from India. J Med Microbiol 2016;65:1536-9.
15. Sharma P, Dahiya S, Manral N, Kumari B, Kumar S, Pandey S, *et al.* Changing trends of culture-positive typhoid fever and antimicrobial susceptibility in a tertiary care North Indian hospital over the last decade. Indian J Med Microbiol 2018;36:70-6.
16. Nair S, Ashton P, Doumith M, Connell S, Painset A, Mwaigwisya S, *et al.* WGS for surveillance of antimicrobial resistance: A pilot study to detect the prevalence and mechanism of resistance to azithromycin in a UK population of non-typhoidal *Salmonella*. J Antimicrob Chemother 2016;71:3400-8.
17. Sjölund-Karlsson M, Joyce K, Blickenstaff K, Ball T, Haro J, Medalla FM, *et al.* Antimicrobial susceptibility to azithromycin among *Salmonella enterica* isolates from the United States. Antimicrob Agents Chemother 2011;55:3985-9.
18. Glynn MK, Bopp C, Dewitt W, Dabney P, Mokhtar M, Angulo FJ, *et al.* Emergence of multidrug-resistant *Salmonella enterica* serotype typhimurium DT104 infections in the United States. N Engl J Med 1998;338:1333-8.
19. Roberts MC. Update on macrolide-lincosamide-streptogramin, ketolide, and oxazolidinone resistance genes. FEMS Microbiol Lett 2008;282:147-59.
20. Manesh A, Balaji V, Kumar DR, Rupali P. A case of clinical and microbiological failure of azithromycin therapy in *Salmonella enterica* serotype typhi despite low azithromycin MIC. Int J Infect Dis 2017;54:62-3.

Supplemental Table

Supplemental Table 1: Synergy testing of Ceftriaxone and Azithromycin

MIC_a	MIC_b	MIC_{ab}	MIC_{ba}	FIC Index
24	0.125	0.25	0.25	2.01
32	0.125	0.25	0.25	2.007
8	0.125	0.25	0.25	2.03
16	0.047	0.125	0.125	2.607
12	0.25	0.25	0.25	1.02
4	0.125	0.125	0.125	1.02
8	0.047	0.25	0.25	5.33
32	0.125	0.25	0.25	2.007
8	0.125	0.25	0.25	2.03
32	0.125	0.25	0.25	2.007

MIC_a: MIC of azithromycin; MIC_b: MIC of ceftriaxone; MIC_{ab}: MIC of the combination when ceftriaxone overlays azithromycin strip; MIC_{ba}: MIC of the combination when azithromycin overlays ceftriaxone strip; FIC Index=MIC_{ab}/MIC_a+MIC_{ba}/MIC_b