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Original Article

Rationale of azithromycin prescribing practices for enteric fever in India

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Abstract

Purpose: The present study was performed to assess the current susceptibility pattern of blood isolates of *Salmonella* spp from a super specialty hospital in North India against nalidixic acid, ciprofloxacin and azithromycin and compare the *in vitro* and *in vivo* response against azithromycin. **Materials and Methods:** We evaluated the minimum inhibitory concentration's (MIC's) of 107 blood isolates of *Salmonella* spp against nalidixic acid, azithromycin and ciprofloxacin and correlated *in vitro* and *in vivo* response of azithromycin from the treatment and discharge summaries from the Hospital Information System (HIS) software. **Results:** Among the 107 isolates evaluated, 94 (87.8%) were nalidixic acid-resistant (NAR) *Salmonella* and 36 were resistant to azithromycin by MIC testing. The MIC_{90} value for azithromycin was 24 µg/mL. Among the 57 treatment histories evaluated using the HIS software, 19 (33%) patients had documented clinical non-response to azithromycin which required change of therapy. **Conclusions:** The present study observed a higher MIC_{90} values for azithromycin compared to *Salmonella* isolates from Western studies. There was also a documented clinical non-response against azithromycin. The *in vitro* and *in vivo* findings in this study suggest a guarded use of azithromycin for cases of enteric fever in India. The study also augments the reversal of resistance pattern in favour of chloramphenicol, ampicillin and trimethoprim – sulfamethoxazole.

Key words: *Azithromycin, multidrug resistant Salmonella Typhi, nalidixic acid, Salmonella*

Introduction

Enteric fever caused by *Salmonella* spp is one of the most common causes of systemic infections in India and is one of the common causes of travel associated illnesses.[1] Drug resistance in *Salmonella* has been on the rise in India with emergence of nalidixic acid-resistant (NAR) *Salmonella* and an increasing clinical non-response to fluoroquinolones.[2-6] Treatment options are getting limited with emergence of resistance to third- and fourth-generation cephalosporins.[7-9] A reversal of resistance pattern in favour of chloramphenicol has been observed and with the rising resistance to third-generation cephalosporins, continuous dynamism has been observed in antibiogram patterns worldwide. The Western studies have favoured azithromycin as the potential drug that produces good clinical response.[10] However, due to the lack of breakpoint concentrations in various international guidelines, its *in vitro* interpretation has often been difficult for *Salmonella*. In the Western literature, treatment has heavily banked upon the use of azithromycin due to its high intracellular concentration and good clinical response. Clinical trials advent the use of 20 mg/kg per day with a maximum dose of 1000 mg/day for five to seven days for complete cure.[10,11] Limited role of azithromycin has been suggested by a study where the *in vitro* MIC range was between 4 and 16 µg/mL.[12] . Randomized trials have suggested similar efficacy of azithromycin and ciprofloxacin, both clinically and *in vitro* studies, against enteric fever caused by sensitive as well as MDR *Salmonella* Typhi isolates.[13] Non-availability of breakpoint concentrations of azithromycin for *Salmonella* in most standard antibiotic guidelines makes the laboratory interpretation difficult.

The purpose of the present study was to assess the current susceptibility pattern of blood isolates of *Salmonella*, correlate with other Indian studies and to evaluate and compare the pivotal role of azithromycin prescribing practices for enteric fever in India and Western countries.

Materials and Methods

The present study was performed on 107 non-repeat isolates of *Salmonella* spp. isolated from blood samples from 2005 to 2008 in a tertiary care Super Specialty hospital of North India. Identification of fresh growth of the isolates was done by routine biochemical tests followed by serotyping with standard specific antisera (Denka Seiken Co

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Ltd, Japan). All isolates were subjected to susceptibility against chloramphenicol (30 µg), nalidixic acid (30 µg), ampicillin (10 µg), trimethoprim-sulphamethoxazole (1.25/23.75 µg), ceftriaxone (30 µg), cefepime (30 µg), ciprofloxacin (5 µg), gatifloxacin (5 µg), tetracycline (30 µg), amoxicillin–clavulanic acid (20/10 µg) and ofloxacin (5 µg) (Oxoid, Basingstoke, UK) by disc diffusion as per Clinical and Laboratory Standards Institute (CLSI) guidelines.[14] *Escherichia coli* ATCC 25922 was used as a quality control strain. The minimum inhibitory concentration (MIC) was determined against nalidixic acid, azithromycin and ciprofloxacin by E – Test® (AB Biomerieux, Solna, Sweden). The MIC breakpoints were interpreted according to CLSI guidelines. International guidelines on antimicrobial susceptibility testing do not mention a value for MIC breakpoint for azithromycin against *Salmonella*. The British Society of Antimicrobial Chemotherapy (BSAC) guidelines however suggest clinical susceptibility to azithromycin in isolates with MIC values of ≤ 16 µg/mL.[15] Therefore, an MIC breakpoint of ≤ 16 µg/mL was considered as sensitive and > 16 µg/mL as resistant. The MIC_{90} and MIC_{50} values were calculated for nalidixic acid, azithromycin and ciprofloxacin. MIC_{90} values of ciprofloxacin were assessed for NAR *Salmonella* isolates. MIC_{90} values were also assessed for patients who did not respond to azithromycin therapy.

Available treatment history and hospital discharge summary of patients from whom these isolates were recovered was retrospectively analysed using their central registration numbers on the Hospital Information System (HIS) software and correlated with the susceptibility findings. Strains resistant to third-generation cephalosporins were investigated for production of extended spectrum beta-lactamase (ESBL) using cefotaxime, cefotaxime/clavulanate and ceftazdime, ceftazidime clavulanate discs as per CLSI guidelines.[14]

Results

Of the 107 blood isolates of *Salmonella* [Table 1], 80 were serotype Typhi, 21 Paratyphi A and 6 belonged to other serotypes (3 Typhimurium, 2 Senftenberg and 1 Enteritidis). Among these, 94 (87.8%) were nalidixic acid-resistant (NAR) *Salmonella* and remaining 13 were nalidixic acid-sensitive (NAS) *Salmonella*. Among the 80 strains of serotype Typhi, 70 (87.5%) were NAR *Salmonella* and remaining 10 (12.5%) were NAS *Salmonella*. As per definition, only four (3.7%) of the *Salmonella* isolates were multi drug resistant (MDR) i.e., resistant to ampicillin, chloramphenicol and cotrimoxazole.[7] All of the MDR isolates were serotype Typhi and were NAR *Salmonella*. All of the 21 strains of serotype Paratyphi A were also NAR *Salmonella*.

Isolation of non-typhoidal *salmonellae* from blood though common in Sub-Saharan Africa is a rare finding in India.[16] Such strains are usually isolated from wound and blood samples of hospitalized patients and are likely to be potential sources of hospital outbreaks.[17] All Senftenberg and Enteritidis isolates were NAR and ESBL producers while all three Typhimurium strains were NAS and sensitive to third-generation cephalosporins. As previously mentioned, the MIC interpretative breakpoint of >16 µg/mL was considered resistant for azithromycin.[15] Taking this into consideration, 36 (33.64%) of all *Salmonella* isolates were resistant to azithromycin. Even among the 13 NAS *Salmonella* (10 Typhi and three Typhimurium), 5 had azithromycin MIC's ≥ 24 µg/mL. Sensitivity to chloramphenicol was observed in 102 (95.32%) of all the isolates thereby showing a reversal of the susceptibility pattern. This was in correlation with another Indian study favouring reuse of chloramphenicol.[18] Among remaining five isolates which were resistant to chloramphenicol, four were MDR. There was also an increase in the prevalence of Paratyphi A isolates from a single isolate in the year 2005 to 4 in 2006, 5 in 2007 and 11 isolates in 2008. The MIC_{90} values for nalidixic acid, azithromycin and ciprofloxacin were >256, 24 and 0.75 µg/mL while MIC_{50} values were >256, 12 and 0.38 µg/mL, respectively. Of the total NAR isolates, the MIC_{90} value for ciprofloxacin was 0.75 µg/mL

Table 1: Details of resistance pattern of *Salmonella* isolates from blood during 2005 to 2008

Different serovars of *Salmonella (n)*	NAR n (%)	NAS n (%)	ESBL n (%)	MDR n (%)	Azithromycin MIC ≥16 µg/ml n (%)	Chloramphenicol resistance n (%)
Typhi (80)	70/80 (87.5)	10/80 (12.5)	Nil	4/80 (5)	27/80 (33.75)	5/80 (four were NARS) (6.25)
Paratyphi A (21)	21/21 (100)	Nil	Nil	Nil	8/21 (38.09)	Nil
Senftenberg (2)	2/2 (100)	Nil	2/2 (100)	Nil	1/2 (50)	Nil
Typhimurium (3)	Nil	3/3 (100)	Nil	Nil	Nil	Nil
Enteritidis (1)	1/1 (100)	Nil	1/1 (100)	Nil	Nil	Nil
Total (107)	94/107 (87.85)	13/107 (12.14)	3/107 (2.80)	4/107 (3.78)	36/107 (33.64)	5/107 (4.67)

NAR: Nalidixic acid resistant , NAS: Nalidixic acid sensitive , ESBL: Extended spectrum beta lactamase, MDR: Multidrug resistant

which falls in the decreased susceptibility range (0.125–1 μg/mL) for ciprofloxacin.[19]

Of the 107 isolates evaluated, treatment history was retrieved from discharge summaries and clinical notes for 57 patients from the Hospital Information System database. The signatory clinicians of the discharge and treatment summaries were contacted where changes in therapy was made from azithromycin to other drugs. The description of clinical non-response was not uniform and varied from 2 to 5 days after intake of first dose of azithromycin with no significant improvement in patient's symptoms. Among these, 36 patients were given azithromycin therapy varying from 0.5 to 1 g PO per day for 3 to 5 days. There was a change made in the therapy of 28 patients from azithromycin to oral third-generation cephalosporins or amoxicillin as per standard recommendations. This was done on the basis of non responsiveness to azithromycin therapy as mentioned in 19 of the 28 patients. Reason for changes made in the remaining nine patients was not mentioned, but another probability could have been the non-availability of azithromycin sensitivity from the bacteriology laboratory. Among these 19 isolates, all were NAR *Salmonella* Typhi (NARST). MIC range for azithromycin in these isolates ranged from 6 to 64 μg/mL with a MIC_{90} value of 24 μg/mL.

Discussion

The prevalence of NAR *Salmonella* (NARS) has been increasing in India with reports rising from 51% in 2006[8] to as high as 87.8% in the present study and even higher in other recent Indian studies.[20-22] In contrast, the literature has cited a fall in the prevalence of MDR isolates of *Salmonella*, being as high as 94% in 1989–91,[23] to 92.3% in 1994,[24] 61.4% in 1996,[25] 39% in 2006[26] to as low as 3.7 % in the present study. In another Indian study done on 305 *Salmonella* isolates, only one was MDR[20] while a study from South India reported the MDR prevalence to be 12%.[21] While studies in India have correlated susceptibility of fluoroquinolones in NARS isolates[27] and their molecular epidemiology,[28] the role of azithromycin and its clinical response has not been correlated. Another prospective study from Pondicherry, from 2005 to 2009 demonstrated high sensitivity to ampicillin, chloramphenicol and cotrimoxazole of 66% and just 22% multidrug resistant salmonella typhi (MDRST) indicating a steady fall and rise of MDRST and NARS isolates.[6]

With respect to prescribing azithromycin, most of the antimicrobial susceptibility standards do not mention the MIC breakpoints of azithromycin for *Salmonella*. However, it is still being prescribed worldwide with many clinical trials suggesting its superior clinical efficacy.[29] From a microbiological point of view, this is a questionable practice. Secondly, the MIC_{90} values for azithromycin against *Salmonella* isolates from India do not coincide with the strains isolated from the Western countries. In the present study, MIC_{90} for azithromycin was 24 μg/mL, which was the same as mentioned in another study from India.[30] MIC_{90} of *Salmonella* isolates studied in the Western countries have values as low as 4 or 8 μg/mL.[31,12] A review done on the role of azithromycin in enteric fever indicated that fever clearance time with the use of azithromycin was not different from any other drug and that it was only marginally better than fluoroquinolones in terms of reducing clinical failure.[32] The present study augments this finding as 19 patients had documented clinical non-response to azithromycin. This information may become important especially for patients who acquire enteric fever in India and get azithromycin therapy when they reach back to their native Western countries. In developing countries like India, ciprofloxacin continues to be the mainstay for the treatment of enteric fever as it is orally effective and economical[33] and also probably the clinicians are not aware of the clinical implications of a NARS isolate. With the rise of NARS and fall in MDR isolates,[34] one may look at recycling of chloramphenicol, cotrimoxazole and ampicillin instead of azithromycin. Large-scale randomized control trials with follow up and laboratory correlation need to be done for azithromycin usage in the Indian subcontinent before incorporating its Western prescribing practices.

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