



Salmonella Entericaserovars Showing Resistance to Azithromycin in A Tertiary Care Hospital From South India

Authors

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Introduction

Enteric fever is an important public health problem caused by salmonella species (typhoidal group) Salmonella enteric serovar typhi, Paratyphi A, Paratyphi B and Paratyphi C. WHO estimates 21.7 million cases every year with 217,000 deaths¹. Without treatment enteric fever has a mortality rate of 30% if appropriately treated with the sensitive antibiotic, the mortality can be reduced to below 0.5%¹. With the emergence of multi drug resistance Salmonella i.e Resistance to ampicillin, chloramphenicol and Co-trimoxazole, Fluroquinolones were being used (i.e Ciprofloxacin) or Ceftriaxone for the treatment of enteric fever^{1,2}. With the extensive use of Fluroquinolones, Salmonella species are less susceptible to Ciprofloxacin (with the MIC of Nalidixic acid ≥ 32 $\mu\text{g/ml}$ and that of ciprofloxacin ≤ 0.25 $\mu\text{g/ml}$) emerged. Some strains of Salmonella especially Salmonella enteric serovar para typhi A shows resistance to Ciprofloxacin with MIC of ≥ 1 $\mu\text{g/ml}$ also with the use of Ceftriaxone, it is found that slowly the time taken for fever defervesce is increasing.

Many clinical trials have shown the increased efficacy of Azithromycin compared to Fluroquinolones^{3,4}. Hence IAP (Indian

Association of Pediatrics) recommends the use of Azithromycin in the treatment of enteric fever on Outpatient basis if there are no indications for admission to hospital and after discharge from hospital. But of late resistance to Azithromycin has been encountered in India and other countries⁵. Hence, before the treatment, it is vital to know the susceptibility of Salmonella isolates to Azithromycin.

Although emergence of multidrug resistant Salmonella was a reality, with the decrease in the use of Ampicillin, Co-trimoxazole and Chloramphenicol, most of the Salmonella isolates have regained sensitivity to their antibiotics^{5,6}. Hence the study aims at knowing the current trend in the antibiogram of Salmonella enterica serovar isolated in a tertiary care hospital in South India.

Materials & Methods

All the clinical isolates of Salmonella enterica serovars form blood of patients attending tertiary care hospital in Mysore, South India from June 2014 to May 2015(1 year) the identity of the isolates were confirmed by standard biochemical tests and agglutination with specific antisera. Antibiotic susceptibility tests were put up by:

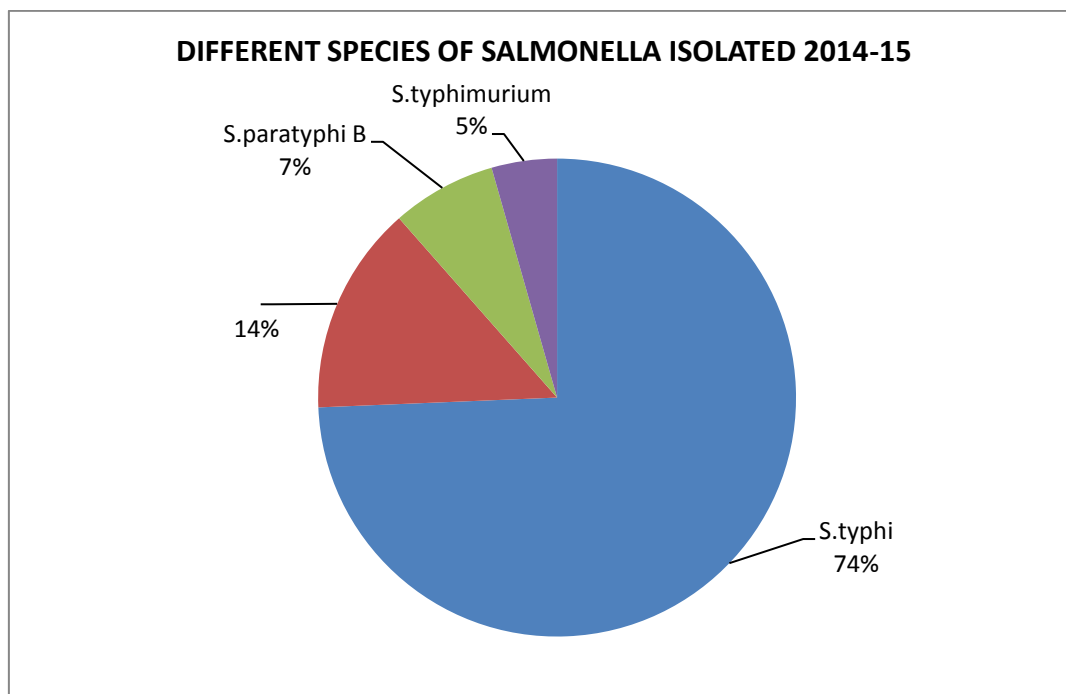
1. Vitek 2 automated culture ID and sensitivity system (organon technical) using N280 cartridges.
2. By manual Kirby-bauer disc diffusion technique following the CLSI guidelines. Following were the antibiotics tested:

BY VITEK 2 SYTEM	MANUAL DISC DIFFUSION (µg)
Ampicillin	Ampicillin (10mcg)
Amoxyclav	Amoxyclav (50/10mcg)
Piperacillintazobactam	Piperacillintazobactam (100/10mcg)
Cefuroxime	Ceftriaxone(30mcg)
Cefuroxime axetil	Cefaperazonesulbactam(30/10mcg)
Ceftriaxone	Cefepime (30mcg)
Cefaperazonesulbactam	Ertapenem (10mcg)
Cefepime	Meropenem(10mcg)
Ertapenem	Imipenem(10mcg)
Meropenem	Nalidixic acid(30mcg)
Imipenem	Ciprofloxacin(5mcg)
Gentamicin	Tigecycline(15mcg)
Amikacin	Colistin (10mcg)
Nalidixic acid	Co-trimoxazole(25mcg)
Ciprofloxacin	AZITHROMYCIN (15mcg)
Tigecycline	
Colistin	
Co-trimoxazole	

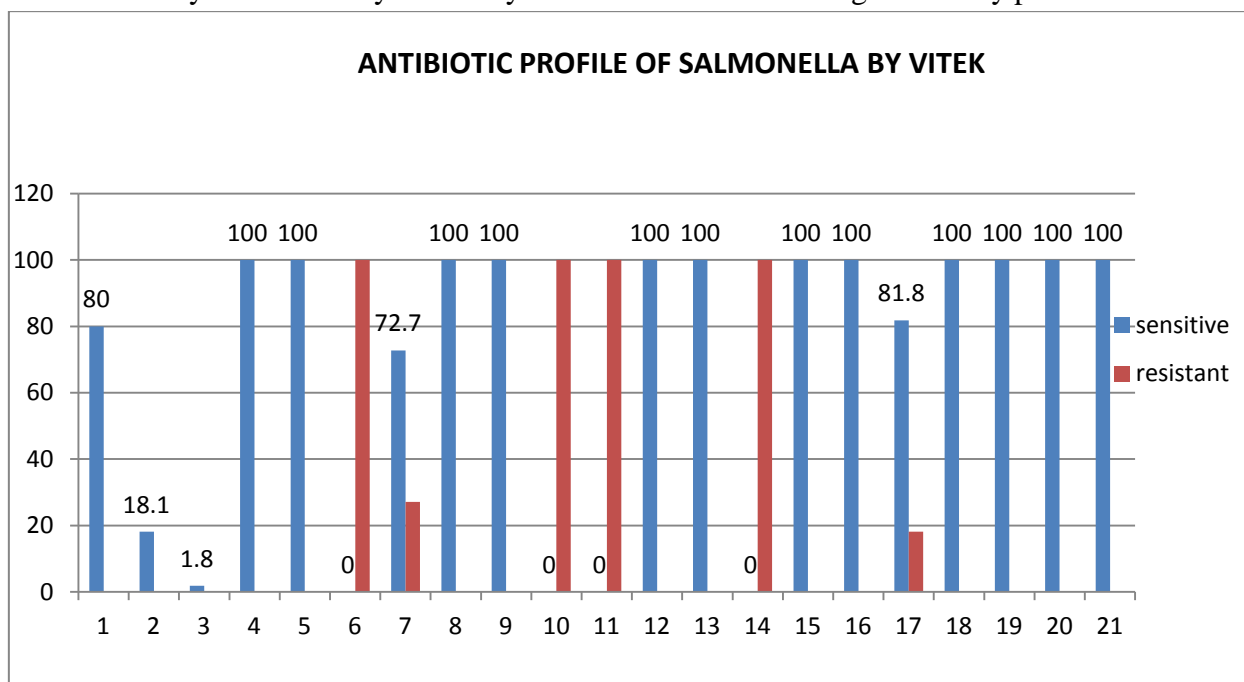
Azithromycin is not available in the Vitek 2 sensitivity cartridge (N280) for Enterobacteriaceae, hence 10µg disc of Azithromycin was used for sensitivity testing. A zone diameter of ≥13mm was considered as sensitive and <13mm resistant. The sensitivity test for Azithromycin was also confirmed by agar dilution method using a concentration of 2µg/ml,4,8,16,32 and 64µg/ml. results were noted down and compiled. MIC of ≤16µg/ml was considered sensitive and >16µg/ml was considered resistant.

Results

A total of 1250 blood samples collected from patients with suspected enteric fever yielded growth of Salmonella enterica serovars in 113 samples. Out of this salmonella enterica serovar typhi were 84(74.33%), salmonella enterica serovar Paratyphi A were 16(14.15%), salmonella enterica Paratyphi B were 8(7.07%) and Salmonella enterica Typhi murium were 5(4.24%).



Antibiotic sensitivity carried out by Vitek2 system showed the following sensitivity pattern.



Total no: of Salmonella entericaserovars isolated= 113

DRUGS	SENSITIVE	RESISTANCE	INTERMEDIATE
AMPICILLIN	100%	0%	0%
AMOXYCLAV/CLAVALONATE	100%	0%	0%
AMIKACIN	0%	100%	0%
CIPROFLOXACIN	72.7%	27.2%	0%
CEFTRIAZONE	100%	0%	0%
CEFUROXAME	0%	100%	0%
CEFUROXAME.AXETIL	0%	100%	0%
ERTAPENEM	100%	0%	0%
CEFEPIME	100%	0%	0%
GENTAMYCIN	0%	100%	0%
IMIPENEM	100%	0%	0%
MEROPENEM	100%	0%	0%
NALADIXIC ACID	0%	100%	0%
COTRIMOXAZOLE	100%	0%	0%
TEGICYCLINE	100%	0%	0%
PIPERACILLIN/TAZOBACTAM	100%	0%	0%
CEFAPERAZONE/SULBACTAM	100%	0%	0%

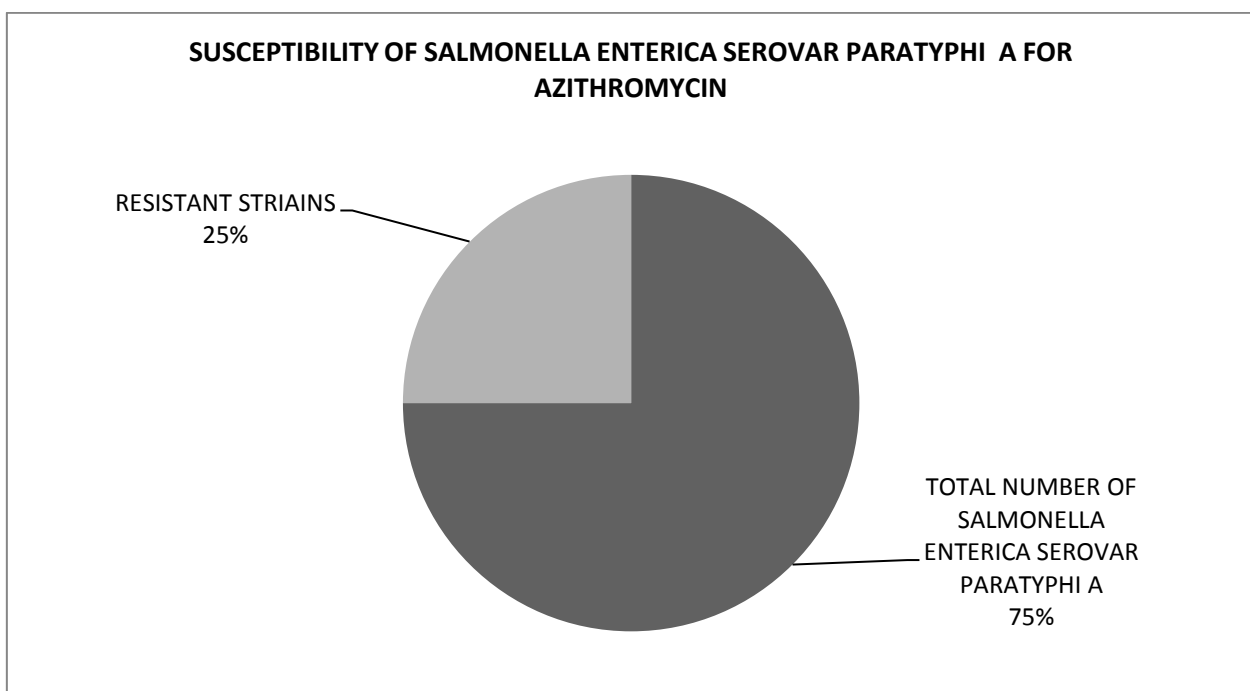
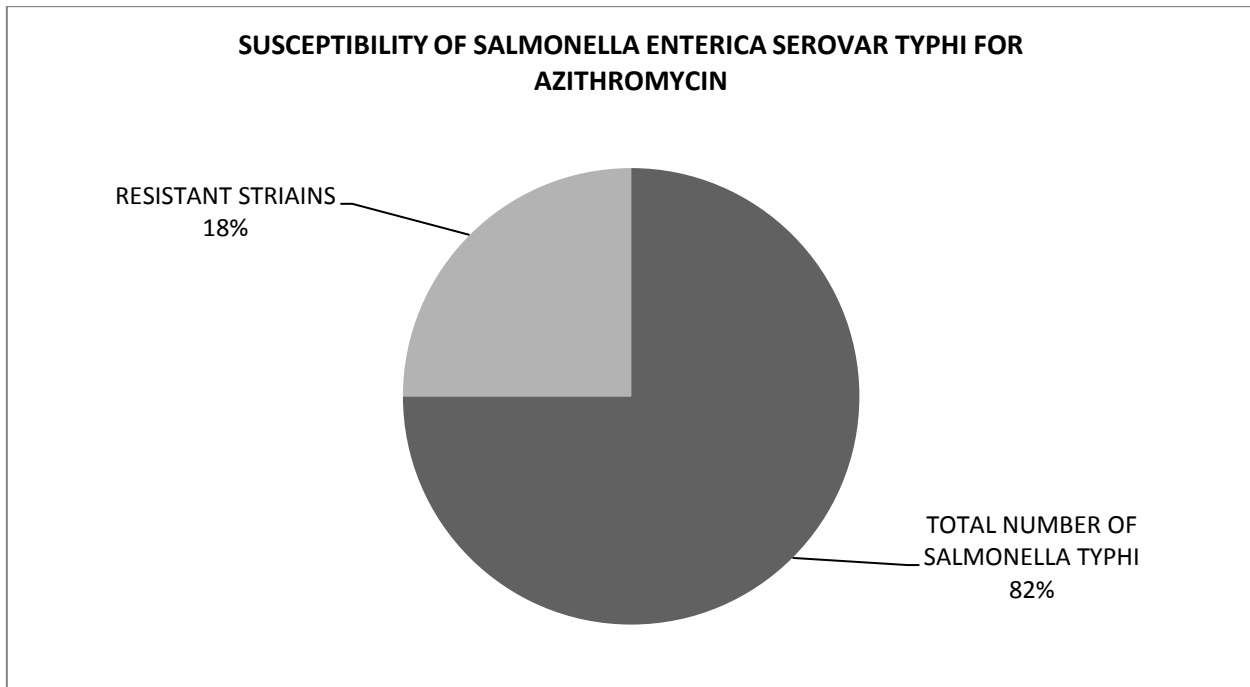
As Salmonella serovarenterica are intrinsically resistant to first and second generation cephalosporins and Aminoglycosides, Cefuroxime, Cefuroxme-axetil, Gentamicin and Amikacin discs were not tested for susceptibility by Kirby-bauer disc diffusion method with all other

antibiotics tested, the results of disc diffusion concurred with that of Vitek 2 system.

Important observations note worthy are:

1. 100% of the isolates are sensitive to Ampicillin, Chloramphenicol and Co-trimoxazole.

2. 100% of the isolates were sensitive to Ceftriaxone, their MIC was $\leq 1\mu\text{g/ml}$.
3. 100% of isolates were resistant to Nalidixic acid with a MIC of $\geq 32\mu\text{g/ml}$.
4. Most of the Salmonella entericaserovartyphi (number74% and 14.15 %) showed a MIC value of $<0.25\mu\text{g/ml}$, these isolates are to be considered intermediate to Ciprofloxacin (CLSI version ----).
5. Salmonella enterica serovar Paratyphi A showed MIC of $>1\mu\text{g}$ to ciprofloxacin indicating resistance to quinolones.
6. Out of 113 number isolates of Salmonella enterica serovars,19 (16.81%) showed resistance to Azithromycin (S.enterica serovar typhi 15 (17.85%)and S.enetrica serovar para typhi A 4(25%).



Discussion

Although emergence of MDR salmonella (R to A, COT and C) posed a great challenge to the treating physician, 100% of S.serovar Typhi and Paratyphi A were susceptible to A, C and COT. This could be due to the non use of these drugs recently for the treatment of enteric fever. Similar results were also reported by Ashwinichowdary et.al.⁵

Although S.enetrica serovar Typhi were all (100%) resistant to Nalidixic acid (MIC >32µg/ml), the MIC for ciprofloxacin was observed in the sensitive range (<0.25µg/ml). As Nalidixic acid is a surrogate marker for quinolones, Ciprofloxacin was reported as intermediate as per CLSI guidelines 2014⁹.but many isolates (No = 16 & 100 %) of S.enetrica serovarpara typhi A had a higher MIC of >1µg/ml for ciprofloxacin and hence were reported as resistant. Similar results were noted by Marcus Ho Yin Wong et al, Meiying⁷.

But MDR Salmonella is still a problem in Southern Indian countries (Indian J Med Res 137, April 2013, pp 800-802)⁵.

Resistant to Azithromycin was noted in 17.85% % of S.enterica serovar typhi and 25 % of S.enterica serovarpara typhi A. resistant to Azithromycin was also encountered by Marcus Ho Yin Wong et.al⁷ and Erika R.Vliegheetal⁸

Summary & Conclusion

All the isolates of Salmonella are sensitive to (100%) Ampicillin, Chloramphenicol & Cotrimoxazole. With the drying up of pipeline of antibiotics, recycling of antibiotics becomes the order of the day. Emergence of resistance to quinolones is due to overuse or misuse of antibiotics directing the rational use of antibiotics. Gradual increase in the time of fever defervesce noted clinically by treatment with Ceftriaxone predicts the development of resistance to 3rd generation cephalosporins with the emergence of resistance to Azithromycin. It should not be used in the treatment of enteric fever unless the sensitivity report shows sensitive result.

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