

## *Azithromycin: Is it a favorable alternative therapeutic option against salmonella species ?*

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### *Abstract*

**Background:** For decades chloramphenicol has been highly effective against *S. typhi* and *S.paratyphi* . However the known hematological side effects of chloramphenicol and the widespread emergence of multiple drug resistance (MDR) to *S. typhi* has necessitated the search for other therapeutic options .

**Aim of study:** To compare between azithromycin and chloramphenicol as antimicrobial drugs for isolated salmonella species

**Materials and Methods:** 65 isolates of *salmonella spp.* was collected by performing blood culture and stool culture for suspected patients with history of typhoid fever and diarrhea at Maternity and Child teaching Hospital in Diwaniyah city. For each isolates antimicrobial susceptibilities for azithromycin and chloramphenicol were determined by Disk diffusion methods. In addition, a broth dilution tests for azithromycin were performed by using serial dilution of concentrations ranging from 4- 32 mg\ liter to record the minimal inhibitory concentration (MIC) for azithromycin .

**Results:** The isolated salmonella species are high susceptible of to azithromycin in comparison to chloramphenicol with lower MIC.

**Conclusion:** though it needs further clinical studies, azithromycin seems to be a suitable therapeutic option for the treatment of typhoid fever in children as well as in adult.

### *Introduction*

*Salmonella* species(spp.), a highly evolved gram-negative bacterial parasite that infects humans<sup>(1)</sup>, and some times cause fatal infection in adults and children as a result of bacteremia and inflammatory destruction of the intestine and other organs ,it is endemic in most countries ,especially throughout Asia and Africa<sup>(2)</sup>. For decades chloramphenicol has been highly effective against *S. typhi* and *S.paratyphi* ,and it often remains the antibiotic of choice for the treatment<sup>(3,4)</sup>. However ,the widespread emergence of multiple drug resistance (MDR) to *S. typhi* has necessitated the search for therapeutic options. Fluoroquinolones have proven to be effective, however, to date, they are restricted from routine use in children , pregnancy and lactation<sup>5</sup>. The cephalosporins; cefotaxime ,ceftriaxone and cefixime are useful against MDR

typhoid fever, but the required intravenous route of administration render them impractical for some patients<sup>(5,6,7)</sup>.

Azithromycin is the first of a new class of broad spectrum antibiotics called azalides. Azithromycin, a 15-membered lactone macrolide ring compound, is derived from erythromycin by addition of methylated nitrogen into the lactone ring of erythromycin with better activity than erythromycin against gram-negative bacteria<sup>(8)</sup>. Azithromycin penetrates into most tissues (except cerebrospinal fluid) and phagocytic cell extremely well, with tissue concentrations exceeding serum concentrations by 10-100 fold. The drug is slowly released from tissues (tissue half-life of 2-4 days) to produce elimination half-life approaching 3 days. These unique properties permit once-daily dosing and shortening of the duration of the treatment in many cases. Because it has a 15-member lactone ring, azithromycin does not inactivate cytochrom P-450 enzymes and therefore is free of drug interaction that occurs with macrolides (9).

Aim of the present in vitro study is to compare the antimicrobial activity of azithromycin versus chloramphenicol for isolated *salmonella spp.* through disk diffusion test and minimal inhibitory concentration (MIC).

### *Materials and methods*

Over six month period, 65 isolates of *salmonella spp.* Was collected by performance blood culture and stool culture for suspected patients with history of typhoid fever and diarrhea, at Maternity and Child Teaching Hospital. Isolates confirmed by biochemical reactions and agglutination antisera as *S.typhi* and *S. paratyphi*. For each isolates antimicrobial susceptibilities were determined by Disk diffusion methods according to the procedure performed by NCCLS, National committee for clinical laboratory standards, 2000 by preparing a suspension of salmonella species colonial growth from an overnight culture on XLD (xylose lysine deoxycholate) agar in tube with 2ml of muller-Hinton broth and adjust to turbidity equivalent to McFarland standard equal to 0.5 ( $1.5 \times 10^8$  CFU/ml), and we inoculated the muller-Hinton plate evenly by streaking it with prepared suspension across its surface, antibiotic disks of azithromycin and chloramphenicol were applied within 3-5 minutes after inoculation, thereafter incubated at 35-36°C for 24 hours then we measure the inhibitor zone<sup>(10)</sup>.

Species were considered as susceptible when zone diameters for azithromycin disks containing 15ug were equal or more than 13 mm, and for chloramphenicol disk containing 30 ug were equal or more than 12mm, Finally a broth dilution tests for azithromycin were performed by using serial dilution of concentrations ranging from 4- 32 mg/liter, the lowest

antimicrobial concentration that completely inhibits bacterial growth is recorded as the minimal inhibitory concentration (MIC) for azithromycin.

### Results

Sixty- Five isolates of *salmonella* species confirmed by biochemical reactions and agglutination antisera as *S.typhi* in 45 case and *S.paratyphi* in 20 case ,and by using zone diameter equal or more than 13 mm for azithromycin, susceptibility to azithromycin was reported in 51(78.4%) from 65 isolates , and by using zone diameter equal or more than 12mm for chloramphenicol ,susceptibility was reported in 50(76.9%) from 65 isolates table (2). Azithromycin MICs test were in range of 4-38 mg /l ,table(3). The isolates of *S .typhi* had lower MICs than isolates of *S. paratyphi* ,and most of *S. typhi* inhibited by azithromycin 4 mg/l , while most of *S. paratyphi* inhibited by azithromycin 16 mg/l .also there are 2 isolates of *S.typhi* was more resistance in repeated testing than other MIC equal or more than 32 mg /l.

Table (1): Characteristic of 65 cases with culture positive salmonella spp.

Parameter	Number(NO.)
Age(years) (2-10)	65
Sex	
Mal	43
Female	22
Patients with positive :	
- blood culture with <i>S. typhi</i>	20
- Stool culture with <i>S.typhi</i>	25
-Blood culture with <i>S. paratyphi</i>	8
-Stool culture with <i>S. paratyphi</i>	12
Total	65

Table(2):In vitro susceptibilities of 65 isolate of salmonella spp. for azithromycine versus chloramphenicol.

Disk diffusion	NO .of isolates(%)
Salmonella typhi (n=45)	
-Azithromycin susceptible or intermediate	33
-Azithromycine resistance.	12
-Chlormphenichol susceptible or ntermediate.	30
-Chloramphenicole resistance.	15
Salmonella paratyphi( n=20)	18
-Azithromycin susceptible or intermediate.	2
-Azithromycin resistance.	20
-Chloramphenicol susceptible or intermediate.	0
-Chloramphenicol resistance.	5
Total Salmonella spp. Sensitive to azithromycin	(78.4%)
Total Salmonella spp. Sensitive to chloramphenicol	50(76.9%)
<b>Total NO. of isolates</b>	<b>65</b>

Table(3): MICs in mg/L of azithromucin for 65 isolate of salmonella spp..

Concentration for S. typhi	NO. of isolates
-4	30
-8	12
-16	1
->32	2
Concentration for S.paratyphi	NO. of isolates
-4	0
-8	3
-16	17
>32	0

## Discussion

Chloramphenicol has been the drug of choice for typhoid fever for more many decades in the regions of the world where salmonella typhi remains susceptible to the drug .However the known hematological side effects of chloramphenicol and the multiple drug resistance to ampicilline, chloramphenicol, trimethoprim-sulphamethaxazol in Salmonella typhi that emerged in many countries of Asia and Afarica limit the usefulness of these drugs(11). Fluoroquinolones , ciprofloxacin and ofloxacin have proven to be effective, however, to date, they are generally not approved for use in children , pregnancy and lactation because of the potential for these drugs to damage cartilage in growing bones<sup>(12)</sup>. The cephalosporins; cefotaxime ,ceftriaxone and cefixime are useful against MDR typhoid fever , but the required intravenous route of administration render them impractical for some patients<sup>(7)</sup>.The availability of pediatric suspension of azithromycin, the drug ability to achieve intracellular concentration and the long half-life are all encouraging features to use azithromycin in salmonella typhi diseases.

In the current study we observed high percentage of resistance to chloramphenicol .These results was expected because of the high prevalence of multi-drug resistance to chloramphenicol ,ampicillin, co-trimoxazole and ciprofloxacin<sup>(5)</sup>.

The results of the present in vitro study indicated that azithromycin active against strains of *S.typhi* and *S.para typhi* ,regardless of whether they are multiple–drug resistance or susceptible to other drugs such as ampicillin and chloramphenicol. These findings are similar to that by Butler T et al 1999 (13). The present study showed that out of 45 *S.typhi* isolates , 43 are susceptible to azithromycin .Resistance was not expected because this drug has not been used extensively in our country. When 45 isolates of *S.typhi* were tested for azithromycin MICs, all isolates except 2 susceptible ,with MICs of 4-8mg/l ,this difference between results of susceptibility testing by disk zone diameter and MICs suggests that there was less azithromycin resistance in our isolates of *S.typhi* than was reported initially from results of disk diffusion. Our results were agree with recent study conducted by Egyptian Ministry Health which proved that oral azithromycin administered once daily appears to be effective for the treatment of uncomplicated typhoid fever in children<sup>(9)</sup>.

The availability of pediatric suspension of azithromycin, the drug ability to achieve intracellular concentration , the long half-life and large safety margin are all encouraging features to use azithromycin in salmonella typhi diseases in pediatric age group. The place of azithromycin in the treatment of typhoid fever needs to be defined by further clinical studies with adults and children.

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