ORIGINAL ARTICLE

Evaluation of the Current Trends in the Antimicrobial Susceptibility Patterns of *Typhoid Salmonellae*

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ABSTRACT

Background: Typhoid fever, being severe systemic illness is controlled in developed world due to enhanced preventive modalities like sanitation, while it continues to plague the South Asian countries. Salmonella typhi is the most widespread etiological agent of enteric fever, with a current rise in Salmonella paratyphiA strains as well.

Aims: To find outinvitro antimicrobial susceptibility patterns of Typhoid Salmonellae isolated from blood culture confirmed cases of typhoid fever to build an imminent approach to improvise empiric therapy.

Methods: Atotal of 141 Typhoid Salmonellae were isolated from 5115 blood cultures received from different departments of Pakistan Institute of Medical Sciences from July 2011 to October 2012. All isolates were further processed for identification using API & serological tests. Drug susceptibilities were performed as per CLSI 2012 criteria by Kirby-bauer disc diffusion and MIC's of selected isolates.

Results:S.typhi (71) &S.paratyphiA (70) were isolated in almost equal. Predominantly males between 11-30 years were affected. Patients between 1-20 years had S.typhi as the predominant organism. Combined susceptibilities of Typhoid Salmonellae showed Chloramphenicol (70.9%) as the most sensitive antibiotic, followed by Amoxicillin (70.2%) and SXT (65%). More than 90% of resistance was seen with Quinolones both in S.typhi and S.paratyphi A. Third generation Cephalosporins (Ceftriaxone) and Carbapenems revealed 100% sensitivity. Multi Drug Resistant isolates (21.2%) & Extreme Drug Resistant isolates (12.02%) were all S.typhi. 21% of isolates were β-lactamase producers whereas no ESBL production was seen. Azithromycin susceptibility was also done but could not be interpreted due to non-availability of interpretive guidelines.

Conclusions: Reversion of susceptibility pattern of first line anti-typhoid drugs noted, but still don't qualify as empirical drugs. At presentthird generation Cephalosporins and Carbapenems are the only empirical treatment options. Flouroquinolones is no more recommended for the treatment of typhoid fever. As no interpretive guidelines are available for Azithromycin, so it can be evaluated in future on the basis of its clinical efficacy in therapeutic trials and available guidelines.

Keywords: S.typhi, S.paratyphi A, Minimum Inhibitory Concentration.

INTRODUCTION

Typhoid fever is a grave, potentially lethal multisystem illness caused by *Salmonella typhi*, and less often by *S. paratyphi A, S. paratyphi B and S. paratyphi C*[1]. The protean manifestation of the diseases makes it a true diagnostic challenge. It is an primeval disease and is thought to be responsible for the Great Plague of Athens around 426-430 BC[2].

Due to the advances in public health, hygiene & antibiotic treatment, it is almost eradicated in developed world, but still remains endemic in most of the developing countries[3] especially the tropical world with 80%-90% of burden residing in South East Asian countries like China, India, Pakistan and Vietnam[4].

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According to WHO, global burden of the disease is 22 million new cases/year and 217,000 to 600,000 deaths/year[6]. *S. paratyphi A*, which previously was responsible for only about 15-20% cases of typhoid fever in Asia, is increasingly becoming a pathogen in India[7] probably due to non-availability of vaccination. Multi drug resistant (MDR) *S.typhi*has also become endemic, causing epidemics in many parts of Southeast Asia[8].

Appropriate antimicrobial therapy is known to reduce significantly in mortality from typhoid fever[9]. Chloramphenicol was introduced as an effective cure of enteric fever during 1948[10], followed by Trimethoprim-sulfamethoxazole (Co-trimoxazole) and Ampicillin in 1962[11]. Before 1970's typhoid fever strains were sensitive to all the first line drugs[12] but resistance started developing soon. By 1990, there were reports of MDR *S.typhi* strains from India & Pakistan, limiting the usefulness of conventional anti-typhoid drugs[13].Hence Fluoroquinolones were introduced for treatment of typhoid fever in 1990[14].By

the year 2000 high level of resistance to the second line drugs was seen in *S.typhi* strains, particularly due to indiscriminate use of antibiotics[15] rising up to 90% during the year 2005[6].With the development of Quinolone resistance, third-generation Cephalosporinswere introduced[16]but unfortunately there are already reports of high-level of resistance to Ceftriaxone in*S. typhi* and*S. paratyphi A,* leading to treatment failures.[17] In West a new class of antibiotic, Azithromycin (macrolide) is currently being used in children with typhoid fever, showing excellent therapeutic effects, but it is still under trials.[18] The emergence of such high level of resistance in anti-typhoid drugs and spread of broad-spectrum β-lactamase have greatly limited the therapeutic options, leaving expensive drugs like Carbapenems&Tigecycline as secondary antimicrobial drugs.

Pakistan being endemic for the disease, hence this study was designed to evaluate the cases of typhoid fever in a tertiary care hospital, in order to see the changes in the susceptibility profiles and resistance patterns over a period of time, so as to find better and targeted treatment options.

Objectives

1. To isolate *TyphoidSalmonellae* from blood culture and find out thecurrentin vitro antimicrobial susceptibility patterns of clinical isolatesagainst the in use anti-typhoid drugs.
2. To detect phenotypically the mechanism of resistance to antimicrobials used in the treatment of enteric fever.

MATERIALS & METHODS

It was a prospective, non-randomized, descriptive, observational study conducted from July 2011 to October 2012 in a clinical setting. A structured questionnaire was designed to get relevant data in a uniform manner. The blood culture samples from out-patients, wards and emergency department cases at PIMS, Islamabad were collected under aseptic conditions in BactecPlus Aerobic/F Culture Vials (Bactec Dickinson, USA)and incubated in Bactec 9240 Automated Blood culture System. Duplicate samples from same patients were excluded from the study. All positive cultures were included in the study and processed for isolation of pathogens by sub-culturing on Blood agar and MacConkey agar (Oxoid, USA) and incubated aerobically at 35±2^{o}C for 18-24 hrs. Pathogens were identified on the basis of their colonial morphology, color imparting change in media, Gram staining, biochemical tests (API 20E) and serotyping (Remel Europe Ltd U.K) according to standard methods[19]. Antimicrobial disc testing, Minimum Inhibitory Concentration testing (MIC) and Extended Spectrum β-Lactamase (ESBL) screening were done as per CLSI 2012 recommendations.[20] Agar disc diffusion was performed by modified Kirby-Bauer method. MIC's of the selected isolates were recorded using E-strips(Liofilm). Screening of ESBL's was done by double disc diffusion method. Phenotypic detection of β-lactamase production was done by comparing the disc diffusion results of Amoxicillin and Amoxicillin-Clavulanic Acid. A Panel of 10 antibiotics was used to establish the antibiograms.Quality control was established by using *E.coli* 25922, which was within range. Recommendations for the zone sizes and MIC range for Azithromycin was taken from BSAC 2012[21], as there were no established breakpoints for Azithromycin in CLSI. Means, percentages and P value of the present research data were derived. The graphic representation and statistical analysis were determined by using SPSS version 16.

RESULTS

According to the inclusion criteria, a total of 141 clinical isolates of *TyphoidSalmonellae* were obtained of which 71 were *Salmonella typhi*& 70 *Salmonella paratyphi A.* Male to femaleratio was 1.8:1. Age ranged from 2-80 years, with a mean of 22 years. It was established that in age group of less than 20 years, 61% of isolates were *S.typhi*, contrasting with the older age group of 21-40 years where *S.paratyphiA* was the predominant organism (62.8%).

The combined susceptibility profile of *TyphoidSalmonellae* on disc diffusion showed that the first line drugs were up to 70.9% sensitive, and Chloramphenicol was the most sensitive of the three. The percentage sensitivity amongst the second line drugs i.e.,Nalidixic acid and Ciprofloxacin was only 7.1%. Third generation Cephalosporins and Carbapenems were 100% sensitive. Azithromycin was sensitive in 10.6% of cases only following the criteria of BSAC 2012 (Table I).

1.4-17 % of the *S. typhi* isolates were resistant to first line anti typhoid drugs, whereas 42-50% of the *S. paratyphi* isolates were resistant to the first line drugs. (Table: II). Multi drug resistance (MDR) was defined as isolates resistant to all three first line anti-typhoid drugs. (i.e., Amoxicillin, Chloramphenicol, Trimethoprim-sulfamethoxazole) andExtreme drug resistant (XDR) as isolates resistant to first line and second line anti typhoid drugs (6 fluorinated quinolone i.e., Ciprofloxacin). 21.2% MDR's & 12% XDR's were isolated and all were amongst *S.typhi*.

Minimum inhibitory concentration for all the antibiotics was performed on selected isolates according to the selection criteria given in the table II.I

MIC results along with the interpretive criteria are shown in table IV. P values were calculated by applying Pearson t-test and it was established that the MIC results were in concordance with the disc diffusion results with significant P value <0.00001, except for Azithromycin. (P value= 0.011). (Table: IV). All the isolates were studied for β-lactamase production.Out of 42 Amoxicillin resistant isolates, 29 were sensitive to Amocillin-Clavulanic acid combination, concluding that 20.56% of *TyphoidSalmonellae* had β-lactamase production as the mechanism of resistance to Amoxicillin. No isolate of Typhoid *Salmonellae* was ESBL producer.

Table I: Susceptibility Profile of *TyphoidSalmonellae* on disc diffusionn= 141(*S.typhi*=71 &*S.paratyphi A*=70)

Antibiotics	Sensitive	Intermediate	Resistant
Amoxicillin	99 (70.2)	11 (7.9)	31(21.9)
Amoxicillin-Clavulanic acid	128 (90.8)	5 (3.5)	8 (5.7)
Trimethoprim-sulfamethoxazole	92 (65.2)	2 (1.5)	47 (33.3)
Chloramphenicol	100 (70.9)	7 (4.9)	34 (24.2)
Nalidixic acid	10 (7.1)	0 (0)	131 (92.9)
Ciprofloxacin	10 (7.1)	57 (40.4)	74 (52.4)
Ceftazidime	141 (100)	0 (0)	0 (0)
Ceftriaxone	141 (100)	0 (0)	0 (0)
Imipenem	14 (100)	0 (0)	0 (0)
Azithromycin	15 (10.6)	0 (0)	126 (89.3)

Table II: Percentage resistance in *TyphoidSalmonellae* by Disc Diffusion(n=141)

Antimicrobial agent	S.typhi (n=71)		S.paratyphi A (n=70)	
Amoxicillin	30	42.2%	1	1.4%
Amoxicillin-Clavulanic acid	8	11.2%	0	0%
Trimethoprim-Sulfamethoxazole	35	49.2%	12	17.1%
Chloramphenicol	34	47.9%	0	0%
Nalidixic acid	64	90.2%	67	95.8%
Ciprofloxacin	36	50.7%	38	54.3%
Ceftazidime	0	0%	0	0%
Ceftriaxone	0	0%	0	0%
Imipenem	0	0%	0	0%
Azithromycin	60	84.5%	66	94.3%

Table III: Selection Criteria for MIC Testing

Amoxicillin	Isolates sensitive to Amoxicillin & resistant to Chloramphenicol and Trimethoprim-sulfamethoxazole on disc diffusion.
Chloram-phenicol	Isolates sensitive to Chloramphenicol & resistant to Amoxicillin and Trimethoprim-sulfamethoxazole on disc diffusion.
Trimetho-prim-sulfamethoxazole	Isolates sensitive to Trimethoprim-sulfamethoxazole& resistant to Amoxicillin and Chloramphenicol on disc diffusion.
Nalidixic acid	All the Nalidixic acid and Ciprofloxacin sensitive isolates on disc diffusion.

Table IV: Comparison of Disc Diffusion and MIC Results of Selected Isolates

Drugs (MIC range of the E- strip) No. of Isolates	Isolates no (MIC range)µg/ml	MIC 50	MIC 90	Interpretation	P-value
Amoxicillin Sensitive on disc diffusion (0.016-256ug/ml) 9	*S.typhi =03(0.125-0.19)*	0.125	0.19	S	<0.00001
	S.paratyphi A = 06(0.5-1)	0.75	1		
Chloramphenicol Sensitive on disc diffusion (0.016-256ug/ml) 3	*S.typhi = 02(1.5-4)*	1.5	4	S	<0.00001
	S.paratyphi A = 01(4)	4	4		
Trimethoprim-Sulfamethoxazole Sensitive on disc diffusion (0.002-32ug/ml) 2	*S.typhi = 01(0.032)*	0.032	0.032	S	<0.00001
	S.paratyphi A = 01(0.012)	0.012	0.012	S	<0.00001
NA Sensitive on disc diffusion (0.016-256ug/ml) 8	*S.typhi = 07(2-6)*	3	6	S	<0.00001
	S.paratyphi A = 01(3)	3	3		
Ciprofloxacin sensitive isolates on disc diffusion (0.016-256µg/ml) 8	*S.typhi = 7 (0.008-0.047)*	0.016	0.047	S	<0.00001
	S.paratyphi A=01(0.032)	0.032	0.032		
Ciprofloxacin intermediate isolates on disc diffusion (0.002-32ug/ml) 9	*S.typhi =7 (0.25-0.5)*	0.25	0.5	I	
	S.paratyphi A=2 (0.25-0.5)	0.25	0.5		
Ceftriaxone Sensitive on disc diffusion(0.002-32ug/ml) 17	*S.typhi = 11(0.023-0.125)*	0.047	0.064	S	<0.00001
	S.paratyphi A=6(0.047-0.067)	0.047	0.064		
Imipenem Sensitive on disc diffusion (0.002-32ug/ml) 11	*S.typhi = 10(0.032-0.094)*	0.064	0.064	S	< 0.00001
	S.paratyphi A = 1(0.094)	0.094	0.094		
Azithromycin Sensitive on disc diffusion (0.016-256ug/ml) 10	*S.typhi = 07(1.5-8)*	4	6	S	0.011
	S.paratyphi A = 3(3-6)	6	6		

CONCLUSIONS

The study is clear evidence to the fact that the percentage of resistance among *Typhoid Salmonellae* and the mechanism behind them, diverge among different population groups both in Pakistan and at international level. A lot of work has been done on this subject previously, but as typhoid is a disease endemic in this region, so this subject needs continuous work and surveillance in order to look in to the varying pathogenicity amongst the typhoid bacilli, so as to formulate new and up dated guidelines for management.

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