JIDC | THE JOURNAL OF INFECTION IN DEVELOPING COUNTRIES

Brief Original Article

Unusual antibiotic resistance pattern among blood culture isolates of *Salmonella* Paratyphi A

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Abstract

Introduction: With increasing fluoroquinolone resistance, extended spectrum cephalosporins are recommended for the treatment of invasive *Salmonella* infections. However, Extended spectrum beta-lactamases (ESBL) producing *Salmonella* Paratyphi A causing enteric fever is on the rise and constitutes a major therapeutic challenge. Hence, we aimed to assess the incidence of ESBL production, fluoroquinolone resistance in *S.* Paratyphi A and to compare the fluoroquinolone resistance detection methods.

Methodology: Seventeen blood-culture isolates of *S.* Paratyphi A were tested for susceptibility to ampicillin, chloramphenicol, co-trimoxazole, streptomycin and tetracycline (ACCuST), fluoroquinolones, azithromycin and ceftriaxone by disk diffusion method. We compared and correlated between disk diffusion of ciprofloxacin and pefloxacin with ciprofloxacin MIC. Combined disk test was employed to determine ESBL production.

Results: In this study, 13(76.5%) isolates were nalidixic acid resistant (NAR), 16 (94.1%) were pefloxacin resistant, while 7 (41.2%), 9 (52.9%) exhibited resistance and intermediate susceptibility to ciprofloxacin respectively. The MIC50, MIC90 of ciprofloxacin was 1 µg/mL, 2 µg/mL respectively. Among the NAR, 76.92% were DSC (MIC 0.5-1 µg/mL) and 23.08% had an MIC of 2-4 µg/mL. Of note, 4 isolates with DSC were NAS. Of the 17 *S.* Paratyphi A isolates, 14 (82.4%) were ESBL producers and 11 (64.7%) isolates were ceftriaxone susceptible.

Conclusions: Multidrug resistant ($Amp^RChl^RSxt^R$) *S.* Paratyphi A with combined resistance to fluoroquinolones and ESBL production is a cause of concern. We found *S.* Paratyphi A isolates with a relatively unusual phenotype: nalidixic acid susceptible but exhibited DSC; pefloxacin susceptible but ciprofloxacin resistant. Of note one multidrug resistant ($Amp^RChl^RSxt^R$) isolate, an ESBL producer exhibited resistance to azithromycin, cephalosporins and fluoroquinolones but was susceptible to carbapenems and streptomycin.

Key words: DSC; ESBL; enteric fever; NAR; NAS; pefloxacin; *Salmonella* Paratyphi A.

J Infect Dev Ctries 2021; 15(4):579-583. doi:10.3855/jidc.12336

(Received 23 December 2019 – Accepted 31 August 2020)

Introduction

Enteric fever is endemic in India and is a major public health concern. Despite the availability of vaccines and antibiotics, enteric fever especially those associated with drug resistant strains is a major cause of morbidity and mortality. *Salmonella* Typhi is the most common cause of enteric fever, nevertheless, there is an increasing incidence of *S.* Paratyphi A cases in the Indian subcontinent especially in paediatric population [1,2]. Though *S.* Paratyphi A fever is often milder with shorter incubation time and a lesser case fatality rate than *S.* Typhi, nevertheless the antibiotic resistance profile of *S.* Paratyphi A is often worrisome [3,4]. With increasing fluoroquinolone resistance, ceftriaxone is recommended for enteric fever treatment. Nevertheless, ESBL production in different serovars of *S.* enterica is increasingly reported in several countries with the first Indian case of ESBL producing *S.* Paratyphi A reported in 2013 [5].

The emergence of multidrug resistant (MDR) typhoidal Salmonellae that exhibit resistance to the first line drugs, ampicillin, chloramphenicol and trimethoprim-sulfamethoxazole had promoted the use of fluoroquinolones since the last few decades. Nevertheless, treatment failures have been associated with infections with nalidixic acid resistant strains of *S.* Paratyphi A that exhibit reduced susceptibility to ciprofloxacin (MIC, 0.25 -1 µg/mL) [6]. In recent years, there is a declining trend in MDR and an increasing level of fluoroquinolone resistance among typhoidal *Salmonella* [7,8]. Hence, this study was undertaken to assess the incidence of ESBL production, fluoroquinolone resistance and to compare the methods to detect fluoroquinolone resistance among *S.* Paratyphi

A causing enteric fever among the paediatric population.

Methodology

A total of 17 non-repetitive blood culture isolates of *S.* Paratyphi A from paediatric patients with clinical signs of enteric fever attending a tertiary care hospital in Chennai, South India from 2015 -2017 were included in the study. Blood culture was performed using BacT /Alert® PF plus (Aerobic Paediatric) (Biomerieux Inc, Durham, USA). Positive blood culture samples were subcultured on MacConkey agar, Deoxycholate citrate agar and Xylose Lysine Deoxycholate agar. Non-lactose fermenting gram negative bacilli were provisionally identified as *S.* Paratyphi A using standard biochemical tests. The serotype of the isolates was confirmed using group-specific and type-specific antisera at Central research institute, Kasauli, Himachal Pradesh, India.

Antibiotic susceptibility profile of the *S.* Paratyphi A isolates was determined by Kirby Bauer disk diffusion method as per CLSI guidelines, 2018 [9]. Isolates were tested for susceptibility to antimicrobials, ampicillin, chloramphenicol, co-trimoxazole, streptomycin and tetracycline (ACCuST), fluroquinolones (nalidixic acid, ciprofloxacin, pefloxacin), azithromycin and ceftriaxone. Agar dilution method was employed to determine the minimum inhibitory concentration (MIC) of ciprofloxacin only. Nalidixic acid resistance was used as an indicator for reduced/decreased susceptibility to ciprofloxacin (DSC). The isolates were also screened for the presence of plasmid mediated quinolone resistance genes, *qnrA*, *qnrB*, *qnrS* [10].

Combined disk test (ceftazidime(CAZ: 30 µg): ceftazidime-clavulanic acid (CAC: 30µg/10µg) and Cefotaxime (CTX: 30 µg):cefotaxime-clavulanic acid (CEC: 30 µg/10 µg) (Himedia Laboratories Pvt Ltd, Mumbai, India) was employed to determine Extended spectrum beta-lactamases (ESBL) production. *Klebsiella pneumoniae* ATCC 700603 was included as the control. Carbapenamase production was assessed using ertapenem (10 µg), meropenem (10 µg), imipenem (10 µg) and doripenem (10 µg) disks (HiMedia Laboratories Pvt Ltd, Mumbai, India).

The test isolates that exhibited an increase in zone diameter for either antibiotic tested in combination with clavulanic acid vs the zone diameter for the antibiotic tested alone was (CAZ:CAC ≥ 5 mm, CTX:CEC ≥ 5 mm) confirmed as an ESBL producer. Plasmid mediated AmpC production was detected using AmpC disk test [11]. Further the presence of genes coding for beta-lactamases, $bla_{CTX\text{-}M\ group\ 1}$, $bla_{CTX\text{-}M}$, bla_{SHV}, bla_{TEM} were analysed by PCR as previously described [12,13]. *Klebsiella pneumoniae* BAA2146 was included as the positive control strain for the PCR.

Results

In our study, 13 (76.5%) isolates were found to be Nalidixic acid resistant (NAR). Screening for susceptibility to fluoroquinolones showed that majority of the isolates (16/17, 94.1%) were resistant to pefloxacin, while 7/17 (41.2%) and 9/17 (52.9%) exhibited resistance and intermediate susceptibility to ciprofloxacin respectively (Table 1). The MIC_{50} and MIC_{90} of ciprofloxacin was found to be 1 µg/mL and 2 µg/mL respectively. Among the NAR, 10/13 (76.92%) had an MIC of 0.5-1 µg/mL i.e. they were scored as DSC and 3/13 (23.08%) had an MIC of 2-4 µg/mL (Table 2). Of note, 4 isolates with DSC were susceptible to nalidixic acid (NAS phenotype).

Of the 17 *S.* Paratyphi A isolates, 11 (64.7%) isolates were susceptible to ceftriaxone. Nevertheless, 14 (82.4%) were ESBL producers, of which 2/14 (14.3%) were resistant to cefoxitin indicating AmpC production. However, screening for AmpC production by AmpC disk test revealed that AmpC resistance was not plasmid mediated in both the isolates. None of the isolates exhibited resistance to the carbapenems tested. Nine (52.9%) isolates were susceptible to azithromycin.

Plasmid mediated quinolone resistance genes, *qnrA*, *qnrB*, *qnrS* were not detected in the any of the study isolates. Screening for genes encoding for beta-lactamases, revealed that only 1 isolate harboured

Table 1. Antibiotic resistance among the *S.* Paratyphi A isolates.

	AMP	CHL	SXT	STREP	TET	NA	CIP	PEF	CX	CAZ	CTX	AZT	CTR	IMP	MER
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
R	13 (76.5)	1 (5.9)	2 (11.8)	3 (17.6)	5 (29.4)	13 (76.5)	7 (41.2)	16 (94.1)	2 (11.8)	6 (35.3)	11 (64.7)	8 (47.1)	4 (23.5)	0 (0)	0 (0)
I	0 (0)	0 (0)	0 (0)	2 (11.8)	4 (23.5)	0 (0)	9 (52.9)	0 (0)	6 (35.3)	1 (5.9)	4 (23.5)	0 (0)	2 (11.8)	0 (0)	0 (0)
S	4 (23.5)	16 (94.1)	15 (88.2)	12 (70.6)	8 (47.1)	4 (23.5)	1 (5.9)	1 (5.9)	9 (52.9)	10 (58.8)	2 (11.8)	9 (52.9)	11 (64.7)	17 (100)	17 (100)

R: resistant; I: intermediate; S: susceptible; AMP: ampicillin; CHL: chloramphenicol; SXT: cotrimoxazole; STREP: streptomycin; TET: tetracycline; NA: nalidixic acid; CIP: ciprofloxacin; PEF: pefloxacin; CX: cefoxitin; CAZ: ceftazidime; CTX: cefotaxime; AZT: azithromycin; CTR: ceftriaxone; IMP: imipenem; MER: meropenem.

Table 2. Correlation between disk diffusion of ciprofloxacin and pefloxacin with MIC of ciprofloxacin among the nalidixic acid resistant and susceptible isolates.

Disk Diffusion	Disk Diffusion			MIC by Agar dilution			Disk Diffusion	
	CipR	CipI	CipS	CipR	CipI	CipS	PefR	PefS
NARs (n=13)	6 (46.2%)	6 (46.2%)	1 (7.6%)	13 (100%) [MIC (0.5 µg/ml, n=1); (1 µg/ml, n=9); (2µg/ml, n=1); (> 4 µg/ml, n=2)]	0	0	12 (92.31%)	1 (7.69%)
NASs (n= 4)	1 (25%)	3 (75%)	0 (0%)	4 (100%) [MIC (0.5 µg/ml, n=1); (1 µg/ml, n=3)]	0	0	4 (100%)	0 (0%)

NAR: nalidixic acid resistant; NAS: nalidixic acid susceptible; Cip: ciprofloxacin; Pef: pefloxacin.

bla$_{TEM}$ while, none of isolates possessed *bla*$_{CTX\text{-}M\ group\ 1}$, *bla*$_{CTX\text{-}M}$, *bla*$_{SHV}$. This *bla*$_{TEM}$ positive isolate belonged to the NAR phenotype and exhibited resistance to ciprofloxacin, azithromycin and ceftriaxone.

Of the 17 *S.* Paratyphi A isolates screened, only 1 isolate exhibited multidrug resistance (MDR) resistant to Amp^{R}Chl^{R}Sxt^{R}. Of note this isolate was an ESBL producer, resistant to azithromycin and all the cephalosporins, fluoroquinolones tested, however was susceptible to carbapenems and streptomycin (Table 3).

Discussion

Interestingly, in our study, we observed a lower incidence of MDR strains (5.88%) and a higher incidence of NAR (76.5%) suggesting the re-emergence of susceptibility to first line antibiotics (chloramphenicol and streptomycin) and hence the possibility of antibiotic cycling. Incidence of MDR and NAR in our study is in concordance with other Indian reports [14-16]. A five year (2005-2009) study from Pondicherry (South India) had reported an MDR incidence of 1.7% and a higher incidence (98.9%) of NAR among *S.* Paratyphi A [4]. Another South Indian report (2007- 2009, 2011-2012) by Elumalai *et al*. had showed a higher (100%) incidence of NAR but complete absence (0%) of MDR among the *S.* Paratyphi A isolates studied [17]. This decline in the NAR incidence possibly reflects the change in antibiotic prescribing practice in our region.

According to the CLSI, ciprofloxacin MIC is the preferred test for assessing ciprofloxacin susceptibility [9]. Considering the CLSI breakpoints for ciprofloxacin resistance (≤ 1 µg/mL), all the 17 isolates were found to be resistant (MIC range: 1 to > 4 µg/mL). However, none of the isolates were found to harbor *qnr* genes, *qnrA*, *qnrB*, *qnrS* and therefore resistance is likely due to gene mutation(s) in the quinolone resistance determining region (QRDR): *gyrA*, *gyrB*, *parC* and *parE* genes [18].

Nalidixic acid susceptibility was previously used as the first line screen for the detection of ciprofloxacin. Also, a positive correlation is being observed between

Table 3. Antibiotic resistance profile of the *S.* Paratyphi A isolates.

Isolate	AMP	CHL	COT	STREP	TET	NA	CIP	PF	CX	CAZ	CTX	AZT	CTR	IMP	MER
PSPA1	R	S	S	I	S	S	I	R	I	S	R	S	I	S	S
PSPA2	R	S	S	S	S	R	I	R	S	S	R	R	I	S	S
PSPA3	R	S	S	S	S	R	S	R	S	S	I	S	S	S	S
PSPA4	S	S	S	S	I	R	I	S	I	I	S	S	S	S	S
PSPA5	R	S	S	S	S	R	R	R	I	R	R	R	R	S	S
PSPA6	R	S	S	S	S	R	R	R	I	R	I	R	R	S	S
PSPA7	R	S	S	S	S	R	I	R	I	S	R	R	S	S	S
PSPA8	R	S	S	I	R	R	I	R	S	S	R	R	S	S	S
PSPA9	S	S	R	S	R	R	R	R	I	R	R	S	S	S	S
PSPA10	R	R	R	S	R	R	R	R	S	R	R	R	R	S	S
PSPA11	R	S	S	R	I	R	R	R	R	R	R	R	R	S	S
PSPA12	R	S	S	S	I	S	I	R	S	S	I	S	S	S	S
PSPA13	R	S	S	S	I	R	R	R	S	S	S	S	S	S	S
PSPA14	S	S	S	S	R	S	R	R	S	R	R	R	S	S	S
PSPA15	R	S	S	R	R	R	I	R	R	S	R	S	S	S	S
PSPA16	R	S	S	R	S	S	I	R	S	S	R	S	S	S	S
PSPA17	S	S	S	S	S	R	I	R	S	S	I	S	S	S	S

R: resistant; I: intermediate; S: susceptible; AMP: ampicillin; CHL: chloramphenicol; SXT: cotrimoxazole; STREP: streptomycin; TET: tetracycline; NA: nalidixic acid; CIP: ciprofloxacin; PEF: pefloxacin; CX: cefoxitin; CAZ: ceftazidime; CTX: cefotaxime; AZT: azithromycin; CTR: ceftriaxone; IMP: imipenem; MER: meropenem.

decreased ciprofloxacin susceptibility and NAR phenotype. Of special mention, four *S.* Paratyphi A isolates (n = 4) exhibited decreased ciprofloxacin susceptibility (cipro MIC 0.5 µg/mL (n = 1), 1 µg/mL (n = 3)) but were susceptible to nalidixic acid. This has been rarely reported in our country. Our result is in concurrence with a report from European study that reported 2% of *S.* Paratyphi A that exhibited low level resistance to ciprofloxacin but were susceptible to Nalidixic acid [6]. A study in our neighboring region, Pondicherry had reported *S.* Typhi isolates that exhibit DCS phenotype but were susceptible to nalidixic acid [19]. Though, pefloxacin susceptibility testing is considered surrogate for ciprofloxacin resistance [20-22], one of our *S.* Paratyphi A isolate that was found susceptible to pefloxacin, exhibited resistance to nalidixic acid and intermediate susceptibility to ciprofloxacin. A report on similar resistance pattern (pefloxacin susceptible but intermediate resistance to ciprofloxacin) among 3 *Salmonella* isolates had been published in India [22]. This resistance pattern could possibly be attributed to a plasmid (*aac(6')-lb-cr*) mediated resistance mechanism specific for fluoroquinolones possessing a piperazinyl secondary amine (ciprofloxacin and norfloxacin but not pefloxacin) [23].

With increasing fluoroquinolone resistance among *Salmonella* spp, extended spectrum cephalosporins, ceftriaxone is currently used in the treatment of invasive infections. However, previous reports have emphasized that ceftriaxone needs to be instituted only in cases that are non-responsive to ciprofloxacin not empirically [19]. Since the first identification of ESBL production among *S.* Paratyphi A in Nepal in 2006 [24], ESBL producing *S.* Paratyphi A is increasingly being reported in several countries including India [5,25-27]. Emergence of ESBL producing *Salmonella* strains constitutes a major challenge in the therapeutic management of enteric fever.

Though Extended Spectrum beta-lactamase producing *S.* Paratyphi A have been reported earlier, this paper highlights the following:

- One *S.* Paratyphi A isolate was found to be an MDR as well as exhibited combined resistance to fluoroquinolones and ESBL production thereby limiting therapeutic options.
- We report from South India *S.* Paratyphi A isolates with a rare phenotype- NAS but exhibit DSC; pefloxacin susceptible but ciprofloxacin resistant.

Our results suggest that for routine disk antibiotic susceptibility testing,

i) using pefloxacin disk alone would not detect plasmid (*aac(6')-lb-cr*) mediated fluoroquinolone resistance.
ii) using nalidixic acid disk alone would not detect NAS strains with DSC phenotype.
iii) using ciprofloxacin disk alone cannot be preferred as no single test detects resistance resulting from all possible fluoroquinolone resistance mechanisms elaborated in *Salmonella* species.

Hence, it would be better to screen for susceptibility to nalidixic acid, ciprofloxacin and pefloxacin.

Acknowledgements

We would like to thank the Department of Science & Technology, India for the instrumentation facility provided through Fund for Improvement of Science & Technology (FIST)(SR/FST/College -2017/23).

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Conflict of interests: No conflict of interests is declared.