Research Article

Detection of chromosomal and plasmid mediated fluoroquinolone resistance among clinical isolates of *Salmonella* species from urban and peri-urban settings of South India.

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Abstract

Background: Enteric fever remains a major public health problem. Fluoroquinolones have became the drug of choice for the treatment of multidrug resistant *Salmonella* species. However, increased use of fluoroquinolone antibiotics leads to the emergence of *Salmonella* strains with reduced susceptibility to fluoroquinolones. This study was performed to detect chromosomal and plasmid mediated fluoroquinolone resistance among clinical isolates of *Salmonella* species from urban and periurban settings of South India.

Method: A total of 250 blood culture isolates of *Salmonella* spp. from patients with suspected enteric fever attending tertiary care hospitals in urban and peri-urban settings of South India were included. Antibiotic susceptibility testing was performed using disc diffusion and agar dilution methods. Genes encoding Qnr, QepA and AAC(6′)-Ib-cr proteins and mutations in quinolone resistance determining regions (QRDRs) of genes encoding DNA gyrase (*gyrA* and *gyrB*) and toposiomerase IV (*parC* and *parE*) were detected using PCR and nucleotide sequencing analysis.

Results: Most of the *Salmonella* isolates were intermediate resistant to ciprofloxacin. The majority of the isolates were sensitive to a third generation fluoroquinolone, levofloxacin. No mutation was detected in *Salmonella* isolates with ciprofloxacin MIC of 0.032 µg/ml whereas isolates with ciprofloxacin MIC of ≥4 µg/ml had triple mutation in QRDR. All the isolates were negative for plasmid encoded *qnr* genes, efflux pump (*qepA*) and fluoroquinolone acetylating aminoglycoside-(6)-N-acetyltransferase (*aac(6')-Ib-cr*) genes.

Conclusion: We have observed *Salmonella* strains with reduced susceptibility to fluoroquinolone. A lower proportion of MDR *Salmonella* isolates was detected in this study. Fluoroquinolone

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resistant isolates were found to have chromosomal mutations in *gyrA* and *parC* genes and all the isolates were negative for plasmid encoded fluoroquinolone resistance genes.

Keywords: Fluoroquinolones, ciprofloxacin, Salmonella, typhoid fever, mutation

Introduction

Enteric fever poses a global public health problem, mainly in developing countries. The causative organisms for this systemic diseases are *Salmonella enterica* serovars Typhi and *Salmonella enterica* serovars Paratyphi. Early diagnosis and appropriate antibiotic treatment reduces the morbidity and mortality of enteric fever.[1] Due to increased resistance to first line drugs such as ampicillin, chloramphenicol, trimethoprim-sulfamethoxazole (co-trimoxazole) and tetracycline, the fluoroquinolone antibiotic, ciprofloxacin has been used in the treatment of *Salmonella* infections in the late 1980s.[2]

Salmonella strains with reduced susceptibility to fluoroquinolones emerged during the 1990s and 2000s due to the increased use of fluoroquinolone antibiotics. *Salmonella* spp. resistant to the first line drugs and fluoroquinolones can be effectively treated with third generation cephalosporins or azithromycin. However, studies have reported the development of *Salmonella* strains resistant to cephalosporins and azithromycin, which further complicates the situation.[1] Cuypers *et al*. (2018) reported that there is a significant increase in the minimum inhibitory concentration (MIC) of ciprofloxacin[2] due to which fluoroquinolone resistant *Salmonella* spp. have been listed as priority pathogen by the World Health Organisation.[3]

Fluoroquinolones inhibit enzymes which regulate DNA supercoiling, i.e., DNA gyrase (encoded by *gyrA* and *gyrB* genes) and topoisomerase IV (encoded by *parC* and *parE* genes), resulting in the disruption of bacterial DNA replication.[2] The complete mechanism of fluoroquinolone resistance is not clearly understood but studies have documented that mutation in the quinolone resistance-determining regions (QRDRs) of *gyrA / gyrB* and *parC / parE* genes; plasmid encoded quinolone resistance mechanisms which includes *qnr* genes (*qnrA, qnrB, qnrS*); fluoroquinolone acetylating aminoglycoside-(6)-N-acetyltransferase (*aac(6')-Ib-cr*) genes and quinolone efflux protein system encoded by *qepA* were found to confer resistance to fluoroquinolones.[4-6] The present study aims to detect the mutations in the QRDR and plasmid mediated resistant genes among clinical isolates of *Salmonella* species with decreased susceptibility to fluoroquinolone antibiotics.

Methods

Salmonella isolates and serotyping

This study included 250 *Salmonella* isolates (collected from May 2012 to September 2016) from blood samples of patients with suspected enteric fever attending tertiary care hospitals in urban and peri-urban settings of South India were included. The isolates were characterized and identified using standard biochemical tests and serotyping tests[7] and maintained at -80 ºC for further analysis.

Antibiotic susceptibility testing

Disc diffusion method

Antibiotic susceptibility testing was done on all the isolates using the list of antibiotics provided in Table 1. As we planned to analyse molecular level characterisation of fluoroquinolone resistant *Salmonella* isolates, we did not include pefloxacin in our study. Results were interpreted as per the guidelines of CLSI.[8] The CLSI guidelines did not provide zone diameter breakpoints of levofloxacin, ofloxacin, ceftazidime and cefepime for *Salmonella* species which were interpreted as per EUCAST guidelines.[9]

Minimum inhibitory concentration (MIC)

The agar dilution method was used to determine MICs of ampicillin, chloramphenicol, tetracycline, cefotaxime and ciprofloxacin as per CLSI guidelines.[8] A dilution range of 0.016 µg/ml to 32 µg/ml was used for ciprofloxacin.

Nucleotide sequence analysis of *gyrA, gyrB, parC,* and *parE* genes

Amplification of QRDRs of *gyrA, gyrB, parC,* and *parE* genes were carried out using forward and reverse primer pairs[10] (Table 1). The *gyrA* primers used in this study covers the nucleotides from codon Lys65 to Met178 with an amplicon size of 344 bp. The *gyrB* primers covers the nucleotides from codon Glu359 to Ile506 with an amplicon size of 446 bp. The *parC* primers cover the nucleotides from codon Lys39 to Thr169 with an amplicon size of 395 bp. The *parE* primers cover the nucleotides from codon Ser393 to Gly546 with an amplicon size of 465 bp.[10] The amplicons were sequenced and then analysed using Basic Local Alignment Search Tool (BLAST) and ExPASy (Expert Protein Analysis System). [14,15]

Table 1: **Primers used in this study**

Gene(s)	Oligonucleotide sequence (5' to 3')	Annealing Temperature (°C)	Amplicon size (bp)	Reference
gyrA	F: AAATCTGCCCGTGTCGTTGGT	58 °C	344	[10]
	R: GCCATACCTACTGCGATACC			
gyrB	F: GAATACCTGCTGGAAAACCCAT	57 °C	446	
	R: CGGATGTGCGAGCCGTCGACGTCCGC			
parC	F: AAGCCGGTACAGCGCCGCATC	57 °C	395	
	R: GTGGTGCCGTTCAGCAGG			
parE	F: CCTGCGGCCCGGCGTTGCCGGGG	62 °C	465	
	R: CGCCCGCCTTCTCTTCTTCCGTCAGCGCG			
qnrA	F: ATTTCTCACGCCAGGATTTG	53 °C	516	[11]
	R: GATCGGCAAAGGTTAGGTCA			
qnrB	F: GATCGTGAAAGCCAGAAAGG	53 °C	469	
	R: ACGATGCCTGGTAGTTGTCC			
qnrS	F: ACGACATTCGTCAACTGCAA	53 °C	417	
	R: TAAATTGGCACCCTGTAGGC			
qepA	F: GCAGGTCCAGCAGCGGGTAG	60 °C	199	[12]
	R: CTTCCTGCCCGAGTATCGTG			
aac-(6')-Ib-cr	F: GCAACGCAAAAACAAAGTTAGG	50 °C	561	[13]
	R: GTGTTTGAACCATGTACA			

Amplification of plasmid mediated quinolone resistance genes

Salmonella isolates (n = 250) were analysed for genes encoding Qnr[11], QepA[12] and AAC(6′)-Ib-cr proteins[13] (Table 1).

Results

Salmonella isolates and serotyping

Of the 250 *Salmonella* isolates, 78.8% were *Salmonella enterica* serovar Typhi (n=197) and 21.2% *Salmonella enterica* serovar Paratyphi A (n=53).

Antibiotic susceptibility testing

Ninety six percent of *Salmonella* isolates were susceptible to the ampicillin, chloramphenicol, co-trimoxazole, and tetracycline. Ten of the 250 isolates were multidrug resistant (4%). All the *Salmonella* isolates were sensitive to the cephalosporins and carbapenems tested in this study. Of the 250 study isolates, 240 (96%) were sensitive to the third generation fluoroquinolone, levofloxacin and 200 (80%) sensitive to ofloxacin (Table 2).

Table 2: Antibiotic susceptibility pattern of the *S*. Typhi and *S. Paratyphi A isolates* (n=250)

Antibiotics	Sensitive		Intermediate		Resistant	
	n	%	n	%	n	%
Ampicillin	240	96	0	0	10	4
Chloramphenicol	240	96	0	0	10	4
Co-trimoxazole	240	96	0	0	10	4
Tetracycline	240	96	0	0	10	4
Cefotaxime	250	100	0	0	0	0
Ceftazidime	250	100	0	0	0	0
Ceftriaxone	250	100	0	0	0	0
Cefepime	250	100	0	0	0	0
Ciprofloxacin	4	1.6	235	94	11	4.4
Ofloxacin	200	80	34	13.6	16	6.4
Levofloxacin	240	96	9	3.6	1	0.4
Imipenem	250	100	0	0	0	0

Nine of 250 ciprofloxacin sensitive isolates had MIC value of ≤ 0.06 µg/ml; 87.2% (218/250) isolates were intermediate resistant to ciprofloxacin (MIC 0.12 to 0.5 µg/ml) and 9.2% (23/250) were resistant to ciprofloxacin with the MIC $\geq$ 1µg/ml. Intermediate resistance to ciprofloxacin was observed in most of the *Salmonella* isolates (218/250, 87.2%) (Table 3). Of the 23 ciprofloxacin resistant isolates, 21 were *S.* Typhi (nine had MIC of 8 µg/ml; two had MIC of 4 µg/ml and 2 µg/ml each; ten had MIC of 1 µg/ml) and 2 were *S.* Paratyphi A (MIC of 1 µg/ml).

Table 3: MICs of various antibiotics for the *Salmonella* isolates (n=250)

MIC (µg/ml)	*S*. Typhi (n = 197)					*S*. Paratyphi A (n = 53)				
	Amp	**Cam**	**Tet**	**Ctx**	**Cip**	**Amp**	**Cam**	**Tet**	**Ctx**	**Cip**
≤ 0.032	-	-	-	-	1	-	-	-	3	-
0.064	-	-	-	15	7	-	-	-	3	1
0.125	-	-	-	98	8	-	-	-	27	-
0.25	-	-	-	62	99	-	-	-	18*	9
0.5	-	-	-	22*	61	-	-	-	2	41*
1	108	-	15	-	10*	13	-	3	-	2
2	61	1	81	-	1	32	-	18	-	-
4	17*	73	91*	-	1	8*	6	32*	-	-
8	1	113*	-	-	9	-	47*	-	-	-
16	-	-	-	-	-	-	-	-	-	-
≥ 32	10	10	10	-	-	0	0	0	-	-
Total	197	197	197	197	197	53	53	53	53	53

Amp (ampicillin); Cam (chloramphenicol); Tet (tetracycline); Ctx (cefotaxime); Cip (ciprofloxacin)
*MIC_{90} values; -, No growth

Nucleotide sequence analysis of *gyrA, gyrB, parC,* and *parE* genes

QRDRs of DNA gyrase and topoisomerase IV encoding genes of 20 representative *Salmonella* isolates (selected based on sensitive to resistant range for the ciprofloxacin antibiotic (MICs of 0.032 to 8 µg/ml)) were amplified and the nucleotide sequences were analysed for mutation (Table 4).

No mutation was detected in one *Salmonella* isolate (ST1) with ciprofloxacin MIC of 0.032 µg/ml. Three *Salmonella* isolates (ST2, ST3, ST4) with ciprofloxacin MICs between 0.064 to 0.25 µg/ml had single *gyrA* mutation at Ser83-Phe, except ST3, which had Ser83-Tyr mutation.

S. Typhi isolates with ciprofloxacin MIC of 0.5 to 2 µg/ml (ST5-ST9) had mutation, either at position 84 (Glu84-Gly or Lys) or 80 (Ser80-Ile) of ParC in addition to the single mutation in position 83 of GyrA. Two *S*. Paratyphi A (SPA1 and SPA2) isolates with ciprofloxacin MIC of 0.5 and 1 µg/ml had a silent mutation at 167 position of ParC in addition to *gyrA* mutation at Ser83-Phe

An additional mutation in *gyrA* gene at Asp87-Asn was detected among *S*. Typhi isolates (ST10-ST18), which had ciprofloxacin MIC of > 2 µg/ml. In addition to double mutations at positions 83 and 87 of GyrA, these isolates had single *parC* mutation at Ser80-Ile (Genbank accession numbers PP824720 and PP824721).

Amplification of plasmid mediated quinolone resistance genes

All the isolates (n = 250) were negative for quinolone resistance protein (Qnr) encoding genes (*qnrA*, *qnrB* and *qnrS*), quinolone efflux pump encoding gene *qepA* and AAC(6′)-Ib-cr encoding gene *aac-(6')-Ib-cr*.

Table 4: MICs of strains and mutations detected within the QRDRs

Strain	Serovar	MIC (µg/ml)[a]	Amino acid substitution[b,c]				
		Cip	GyrA		GyrB	ParC	ParE
ST1	*S.* Typhi	0.032	--	--	--	--	--
ST2	*S.* Typhi	0.064	Ser83-Phe	--	--	--	--
ST3	*S.* Typhi	0.125	Ser83-Tyr	--	--	--	--
ST4	*S.* Typhi	0.25	Ser83-Phe	--	--	--	--
ST5	*S.* Typhi	0.5	Ser83-Phe	--	--	Glu84-Gly	--
ST6	*S.* Typhi	1	Ser83-Phe	--	--	Glu84-Gly	--
ST7	*S.* Typhi	1	Ser83-Phe	--	--	Glu84-Gly	--
ST8	*S.* Typhi	1	Ser83-Phe	--	--	Glu84-Lys	--
ST9	*S.* Typhi	2	Ser83-Phe	--	--	Ser80-Ile	--
ST10	*S.* Typhi	4	Ser83-Phe	Asp87-Asn	--	Ser80-Ile	--
ST11	*S.* Typhi	8	Ser83-Phe	Asp87-Asn	--	Ser80-Ile	--
ST12	*S.* Typhi	8	Ser83-Phe	Asp87-Asn	--	Ser80-Ile	--
ST13	*S.* Typhi	8	Ser83-Phe	Asp87-Asn	--	Ser80-Ile	--
ST14	*S.* Typhi	8	Ser83-Phe	Asp87-Asn	--	Ser80-Ile	--
ST15	*S.* Typhi	8	Ser83-Phe	Asp87-Asn	--	Ser80-Ile	--
ST16	*S.* Typhi	8	Ser83-Phe	Asp87-Asn	--	Ser80-Ile	--
ST17	*S.* Typhi	8	Ser83-Phe	Asp87-Asn	--	Ser80-Ile	--
ST18	*S.* Typhi	8	Ser83-Phe	Asp87-Asn	--	Ser80-Ile	--
SPA1	*S.* Paratyphi A	0.5	Ser83-Phe	--	--	Asn167-Asn[d]	--
SPA2	*S.* Paratyphi A	1	Ser83-Phe	--	--	Asn167-Asn[d]	--

[a]Cip, ciprofloxacin. [b]--, no mutation found. [c]Ser, serine; Phe, phenylalanine; Tyr, tyrosine; Asp, aspartic acid; Asn, asparagine; Glu, glutamic acid; Gly, glycine; Lys, lysine; Ile, isoleucine. [d]Silent mutation (AAT-AAC).

Discussion

Fluoroquinolones are considered to be the most effective antibiotics to treat typhoid and paratyphoid fever for all age groups with short courses of treatment.[16] Infections due to MDR *Salmonella* can be treated with a fluoroquinolone.[2]

The development of *Salmonella* isolates with fluoroquinolone resistance phenotype has been reported in Europe and Asian countries including India.[5,17] The majority of isolates in our study showed intermediate resistance to fluoroquinolones. Better sensistivity was observed for the third generation fluoroquinolone (levofloxacin) than the second generation fluoroquinolones (ciprofloxacin and ofloxacin). Studies have documented that ciprofloxacin and ofloxacin are effective against DNA gyrase alone, whereas levofloxacin has increased activity against both topoisomerase IV and DNA gyrase. Therefore, mutations only in DNA gyrase encoding gene, *gyrA* has less of an effect on the activity of third generation fluoroquinolones than second-generation fluoroquinolones.[1]

All ciprofloxacin resistant *Salmonella* isolates (23/250) with the MIC of ≥ 1µg/ml were sensitive to cephalosporins (cefotaxime, ceftazidime, ceftriaxone, cefixime and cefepime). Of the 23

ciprofloxacin resistant *Salmonella* isolates, 22 were susceptible to ampicillin, chloramphenicol, co-trimoxazole and tetracycline. The *S*. Typhi isolate with a ciprofloxacin MIC of 8 µg/ml was resistant to these four antibiotics.

Fluroquinolone resistance is often reported to occur due to mutations in *gyrA*/*gyrB* genes and *parC* / *parE* genes which results in the modification of DNA gyrase and topoisomerase IV enzymes, respectively.[1,18] In this study, no mutation was observed in the *S*. Typhi isolate with ciprofloxacin MIC of 0.032 µg/ml. A single *gyrA* mutation at Ser83-Phe was detected in an isolate with ciprofloxacin MIC of 0.064 µg/ml. *gyrA* gene with a single mutation has been documented to confer decreased sensitivity to fluoroquinolones (i.e. MIC ranging from 0.125 to 0.25 µg/ml)[1,19], which correlates with our study results. In our study, a single *gyrA* mutation at Ser83 was present in *Salmonella* isolates with decreased sensitivity or complete resistance to ciprofloxacin.

S. Typhi isolates with double mutations (substitution in GyrA subunit at Ser83 and in ParC subunit at Glu84 or Ser80) had a ciprofloxacin MIC of ≥ 0.5 to 2 µg/ml, which is in accordance with other study findings.[20] We observed that *S*. Typhi isolates with ciprofloxacin MIC ≥4 µg/ml had triple mutations (i.e., substitution in GyrA subunit at Ser83 and Asp87 along with substitution in ParC subunit at Ser80). Reports from other studies also document similar triple point mutations in *S*. Typhi isolates with high level ciprofloxacin resistance (MIC >4 µg/ml).[21,22] *S*. Typhi isolates with this triple point mutations (substitution at Ser83 and Asp87 in GyrA subunit along with substitution at Ser80 in ParC subunit) was found to have high level resistance to the tested fluoroquinolones, which suggests that substitution of amino acid may shield the GyrA and ParC subunits from a higher fluoroquinolone dose.

Though previous studies describe the association of fluoroquinolone resistance in *Salmonella* serotypes carrying mutations in *gyrB* and *parE* [4,23], none of the *Salmonella* isolates in our study were found to have mutations in *gyrB* and *parE* genes.

Though plasmid mediated quinolone resistance has been documented in a study from South India[24], all the isolates in our study were negative for *qnr*, *qepA* and *aac(6')-Ib-cr* genes. These findings correlate well with findings from Hassing *et al*. (2011)[19] that the increase in MIC levels of fluoroquinolone in *Salmonella* species are mainly due to mutations in genes encoding DNA gyrase and topoisomerase IV enzymes.

Conclusion

We have observed better sensitivity for third generation cephalosporins and carbapenems than for other classes of antibiotics studied. Similar to recent reports from India, a lower percentage of MDR *Salmonella* isolates (4%) was detected in this study. Our study results confirmed the prevalence of *Salmonella* strains with reduced susceptibility to fluoroquinolone. We found that mutations in *gyrA* and/or *parC* genes are primarily associated with fluoroquinolone resistance in *Salmonella* isolates. We recommend continuous surveillance of antibiotic resistance and constant re-evaluation of empiric antimicrobial therapy to control the emergence and spread of antibiotic resistant pathogens.

Declarations

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Author contributions:
SE: Conceptualization, Methodology, Investigation, Formal analysis, Writing – Original draft preparation.
EMS: Resources.
SS: Supervision, Writing – Reviewing and Editing.

References

1. Khadka S, Shrestha B, Pokhrel A *et al*. Antimicrobial resistance in *Salmonella* Typhi isolated from a referral hospital of Kathmandu, Nepal. *Microbiology Insights*. 2021; 14:11786361211056350. *doi: https://doi.org/10.1177/11786361211056350.*
2. Cuypers WL, Jacobs J, Wong V *et al*. Fluoroquinolone resistance in *Salmonella*: insights by whole-genome sequencing. *Microbial Genomics*. 2018; 4(7):e000195. *doi: https://doi.org/10.1099/mgen.0.000195* .
3. Tacconelli E, Carrara E, Savoldi A *et al*. WHO Pathogens Priority List Working Group. Discovery, research, and development of new antibiotics: the WHO priority list of antibiotic-resistant bacteria and tuberculosis. *Lancet Infectious Diseases*. 2018; 18(3):318-327. *doi: https://doi.org/10.1016/s1473-3099(17)30753-3.*
4. Eaves DJ, Randall L, Gray DT *et al*. Prevalence of mutations within the quinolone resistance determining region of *gyrA, gyrB, parC*, and *parE* and association with antibiotic resistance in quinolone-resistant *Salmonella enterica*. *Antimicrobial Agents and Chemotherapy*. 2004: 48(10):4012-4015. *doi: https://doi.org/10.1128/aac.48.10.4012-4015.2004.*
5. Robicsek A, Jacoby GA, Hooper DC. The worldwide emergence of plasmid mediated quinolone resistance. *Lancet Infectious Diseases*. 2006; 6(10):629–640. *doi: https://doi.org/10.1016/s1473-3099(06)70599-0.*
6. Yamane K, Wachino J, Suzuki S *et al*. New plasmid-mediated fluoroquinolone efflux pump, QepA, found in an *Escherichia coli* clinical isolate. *Antimicrobial Agents and Chemotherapy*. 2007; 51:3354-3360. *doi: https://doi.org/10.1128%2FAAC.00339-07.*
7. Old DC. *Salmonella*. In: Collee JG, Fraser AG, Marmion BP and Simmons A (eds). Mackie and McCartney Practical Medical Microbiology, 14th edition, Edinburgh: Churchill Livingstone, 1996:pp 385–404.
8. Clinical and Laboratory Standards Institute (CLSI), 2024. Performance Standards for Antimicrobial Susceptibility Testing: Thirty Fourth Informational Supplement M100-S34. CLSI, Wayne, PA, USA.
9. The European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters. Version 14.0, 2024. http://www.eucast.org.
10. Fàbrega A, du Merle L, Le Bouguénec C *et al*. Repression of invasion genes and decreased invasion in a high-level fluoroquinolone-resistant *Salmonella typhimurium* mutant. *PLoS One*. 2009; 4(11):e8029. *doi: https://doi.org/10.1371/journal.pone.0008029.*
11. Robicsek A, Strahilevitz J, Sahm DF *et al*. *qnr* prevalence in ceftazidime-resistant *Enterobacteriaceae* isolates from the United States. *Antimicrobial Agents and Chemotherapy.* 2006; 50:2872-2874. *doi: https://doi.org/10.1128%2FAAC.01647-05.*
12. Yamane K, Wachino J, Suzuki S *et al*. Plasmid-mediated *qepA* gene among *Escherichia coli* clinical isolates from Japan. *Antimicrobial Agents and Chemotherapy*. 2008; 52:1564-1566. *doi: https://doi.org/10.1128%2FAAC.01137-07.*

13. Pazhani GP, Chakraborty S, Fujihara K *et al*. QRDR mutations, efflux system & antimicrobial resistance genes in enterotoxigenic *Escherichia coli* isolated from an outbreak of diarrhoea in Ahmedabad, India. *Indian Journal of Medical Research*. 2011;134**:**214-223. *PMID: 21911975* .
14. Elumalai S, Muthu G, Selvam EM *et al*. Detection of multidrug resistance and associated genes among *Salmonella* species from enteric fever cases. *Sri Lankan Journal of Infectious Diseases*. 2022; 12(2):E16 1-9. *doi: https://doi.org/10.4038/sljid.v12i2.8436.*
15. Gasteiger E, Gattiker A, Hoogland C *et al*. ExPASy: The proteomics server for in-depth protein knowledge and analysis. *Nucleic Acids Research*. 2003; 31(13):3784-8. *doi: https://doi.org/10.1093/nar/gkg563*.
16. Thaver D, Zaidi AK, Critchley J *et al*. A comparison of fluoroquinolones versus other antibiotics for treating enteric fever: meta-analysis. *British Medical Journal*. 2009; 338:b1865. *doi: https://doi.org/10.1136/bmj.b1865.*
17. Crump JA, Sjölund-Karlsson M, Gordon MA *et al*. Epidemiology, clinical presentation, laboratory diagnosis, antimicrobial resistance, and antimicrobial management of invasive *Salmonella* infections. *Clinical Microbiology Reviews*. 2015; 28:901-937. *doi: https://doi.org/10.1128/cmr.00002-15.*
18. Hawkey PM. Mechanisms of quinolone action and microbial response. *The Journal of Antimicrobial Chemotherapy*. 2003; 51:29-35. *doi: https://doi.org/10.1093/jac/dkg207*.
19. Hassing RJ, Menezes GA, van Pelt W *et al*. Analysis of mechanisms involved in reduced susceptibility to ciprofloxacin in *Salmonella enterica* serotypes Typhi and Paratyphi A isolates from travellers to Southeast Asia. *International Journal of Antimicrobial Agents*. 2011; 37:240-243. *doi: https://doi.org/10.1016/j.ijantimicag.2010.10.026.*
20. Nathania I, Nainggolan IM, Yasmon A *et al*. Hotspots sequences of *gyrA, gyrB, parC,* and *parE* genes encoded for fluoroquinolones resistance from local *Salmonella* Typhi strains in Jakarta. *BMC Microbiology*. 2022; 22(1):250. *doi*: *https://doi.org/10.1186/s12866-022-02666-z.*
21. Nüesch-Inderbinen M, Abgottspon H, Sägesser G *et al*. Antimicrobial susceptibility of travel-related *Salmonella enterica* serovar Typhi isolates detected in Switzerland (2002-2013) and molecular characterization of quinolone resistant isolates. *BMC Infectious Disease*. 2015; 15:212. *doi*: *https://doi.org/10.1186/s12879-015-0948-2.*
22. García-Fernández A, Gallina S, Owczarek S *et al*. Emergence of ciprofloxacin-resistant *Salmonella enterica* serovar Typhi in Italy. *PLoS One*. 2015; 10(6):e0132065. *doi*: *https://doi.org/10.1371/journal.pone.0132065.*
23. Gensberg K, Jin YF, Piddock LJ. A novel *gyrB* mutation in a fluoroquinolone-resistant clinical isolate of *Salmonella typhimurium*. *FEMS Microbiology Letters*. 1995; 132(1-2):57-60. *doi*: *https://doi.org/10.1111/j.1574-6968.1995.tb07810.x.*
24. Geetha VK, Yugendran T, Srinivasan R *et al*. Plasmid-mediated quinolone resistance in typhoidal *Salmonellae*: A preliminary report from South India. *Indian Journal of Medical Microbiology*.2014; 32(1):31-34. *doi: https://doi.org/10.4103/0255-0857.124292.*