Emergence of Ciprofloxacin resistant *Salmonella enterica* serovar Typhi in Italy

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Introduction

Salmonella enterica serovar Typhi infection is a major cause of disease burden in developing countries. The typhoid fever is not an endemic Italian illness but is often associated to touristic travels to endemic places and to the immigration of foreign people from these areas. Due to the widespread emergence and spread of strains resistant to chloramphenicol, ampicillin, and trimethoprim, fluoroquinolones are considered the drugs of choice, however this choice should be reevaluated in the light of decreased ciprofloxacin susceptibility often occurring among *S.* Typhi.

Materials and Methods

In May and June 2013, two ciprofloxacin-resistant *S.* Typhi isolates were identified from two patients who had recently travelled to India. A retrospective analysis of the database of the laboratory surveillance of enteric pathogens (EnterNet Italia) was carried out from January 2011 to December 2013. 19 strains were selected in order to analyze the prevalence of *S.* Typhi strains with resistance or decreased susceptibility to this antimicrobial. The strains were isolated from blood, urine, or stool of patients aged 0 to 47 years. Patients were travelers coming back from India and Bangladesh, India and Africa natives. Susceptibilities to 16 antimicrobials were determined. Clonal relationships were assessed by pulse-field gel electrophoresis (PFGE) according to the PulseNet protocol (1). All the quinolone resistance-determining regions (QRDR) (2) and plasmid mediated quinolone resistance (PMQR) regions (*qnrA, qnrB, qnrC, qnrD, qnrS, aac(6')-Ib-cr, qepA, oqxAB*) were studied by PCR (3) and sequencing to analyze the quinolone resistance mechanisms.

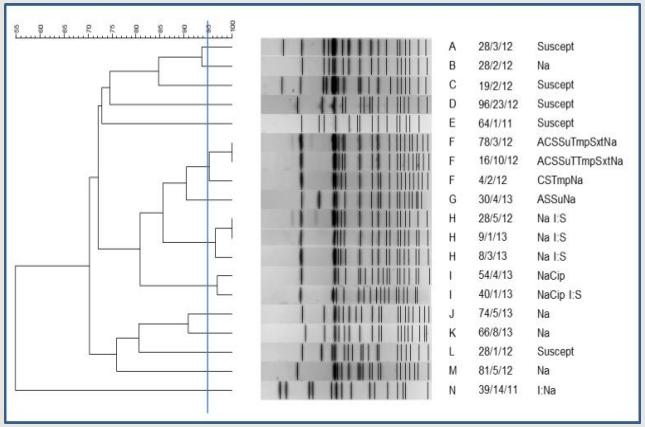
Results

A total of 19 *S.* Typhi strains were studied for the CIP resistance. Two strains were resistant to nalidixic acid (NAL) and highly resistance to ciprofloxacin (CIP) (CIP MIC 12 and 16 mg/L), 7 strains were NAL resistant and one NAL intermediate, all of them showed decreased susceptibility to CIP or low-level CIP resistance (CIP MIC >0.06 mg/L by EUCAST v.4.0). Four strains were multi-drug resistant (ASSuNA, CSTmpNa, and two ACSSuTmpSxtNa) with low-level CIP resistance (CIP MIC 0.25mg/L). One strain was only NAL resistant and 5 strains were susceptible to all the antimicrobials tested.

Strain	Region	Date	Sample	Gender	Age	Origin	Travel	Resistance pattern	Cip MIC(µg/ml)	gyrA	gyrB	parC	parE	PMQR	PFGE
28/1/12	CAMPANIA	2011	nn	nn	nn	nn	nn	Suscept	0,008	wt	wt	wt	wt	neg	L
28/4/12	CAMPANIA	24/03/2012	faeces	nn	nn	Italian	nn	Suscept	0,008	wt	wt	wt	wt	neg	C
28/3/12	CAMPANIA	27/07/2011	faeces	nn		Italian	nn	Suscept	0,008	wt	wt	wt	wt	neg	A
96/23/12	CAMPANIA	2012	nn	nn	nn	nn	nn	Suscept	0,015	wt	wt	wt	wt	neg	D
64/1/11	FRIULI VENEZIA GIULIA	22/10/2011	urine	M	20	African	nn	Suscept	0,015	wt	wt	wt	wt	neg	E
28/2/12	CAMPANIA	2011	nn	nn	nn	nn	nn	Na	0,032	D82N	wt	wt	wt	neg	B
39/14/11	MARCHE	21/06/2011	blood	M	0	Indian	nn	I:Na (Cip)	0,125	wt	wt	T57S	wt	neg	N
81/5/12	LAZIO	12/10/2012	faeces	M	14	Bangladesh	nn	Na (Cip)	0,15	S83F	G435A	wt	wt	neg	M
66/8/13	LAZIO	20/08/2013	blood	F	13	Bangladesh	nn	Na (Cip)	0,19	D87N	G435E	wt	wt	neg	K
30/4/13	PIEMONTE	2013	nn	nn	nn	nn	nn	ASSuNa (Cip)	0,25	S83F	wt	wt	wt	neg	G
4/2/12	TRENTO	25/10/2011	blood	F	16	Bangladesh	nn	CSTmpNa (Cip)	0,25	S83F	wt	wt	wt	neg	F
78/3/12	PIEMONTE	08/07/2012	faeces	F	nn	Bangladesh	Bangladesh	ACSSuTmpSxtNa (Cip)	0,25	S83F	G435E	wt	wt	neg	F
16/10/12	LAZIO	28/02/2012	faeces	M	nn	Bangladesh	nn	ACSSuTmpSxtNa (Cip)	0,25	S83F	wt	wt	wt	neg	F
28/5/12	CAMPANIA	09/04/2012	blood	F	33	Indian	nn	Na I:S (Cip)	0,25	S83F	wt	wt	wt	neg	H
8/3/13	TRENTO	16/10/2012	blood	M	3	Bangladesh	nn	Na I:S (Cip)	0,25	S83F	wt	wt	wt	neg	H
9/1/13	CAMPANIA	26/01/2013	blood	M	29	Italian	India	Na I:S (Cip)	0,38	S83Y	wt	wt	S493F	neg	H
74/5/13	MARCHE	07/01/2013	faeces	M	32	Indian	nn	Na (Cip)	0,5	S83F	G435V	wt	wt	neg	J
40/1/13	PIEMONTE	24/05/2013	faeces	F	40	Italian	India	NaCip I:S	12	S83F,D87N	G435E	S80I	wt	neg	I
54/4/13	PIEMONTE	04/06/2013	blood	F	47	Italian	India	NaCip	16	S83F,D87N	wt	S80I	wt	neg	I

Results

Multiple point mutations in the QRDRs were identified. The strains with a CIP MIC 12 and 16 mg/L both presented three point mutation, two in the *gyrA* (S83F and D87N) and one in the *parC* gene (S80I). The strains with low-level CIP resistance to CIP presented one point mutation in *gyrA* (D82N, S83F, S83Y or D87N) or in *parC* gene (T57S or S80I), with or without additional point mutations in *gyrB* (G435A, G435E or G435V) or *parE* (S493F). No PMQR determinants were found.



PFGE revealed a total of 14 patterns among the 19 isolates, with a maximum of three isolates sharing the same pulsotype. The same pulsotypes were found in people native from Bangladesh and India. Resistance patterns were correlated to the different pulsotypes.

Conclusions

We described the first ciprofloxacin-resistant *S.* Typhi strains isolated in Italy. Further investigations showed that these two patients have travelled together in India. As previously reported (4) we have observed that the mutations in *gyrA* and *parC* genes are the main responsible to the NAL and CIP resistance or the low-level CIP resistance in *S.* Typhi. No PMQR determinants that can spread by horizontal transmission were found. The emergence and spread of antimicrobial-resistance in *S.* Typhi as a result of the increase of populations of expatriate employees and overseas travelers from endemic countries may become a threat to public health. Clinicians should be well alerted on the opportunity to choose fluoroquinolones or other antimicrobial possibilities as initial therapy for people presenting enteric fever and that are coming from endemic areas such as Indian subcontinent.

References

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