Mechanisms of Quinolone Resistance in Enterotoxigenic *Escherichia coli* Isolates from Peruvian Children

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1.- BACKGROUND

► Enterotoxigenic *Escherichia coli* (ETEC) is one of the main causes of pediatric diarrhea in developing countries and in travelers´ diarrhea.

► Antibiotics may shorten the duration of diarrhea caused by ETEC, but the rapid emergence of resistant strains limits their use (Wenneas et al., 2004). Furthermore, the dissemination of antimicrobial resistance genes among bacteria is an increasingly serious problem throughout the world.

► Quinolones and azithromycin remain as drugs of choice for the treatment of ETEC infections, especially for traveler´s diarrhea in Peru.

OBJECTIVE

The aim of this study was to determine the mechanisms of quinolone resistance in ETEC strains isolated from Peruvian children.

2.- MATERIAL AND METHODS

► We analyzed a total of 205 ETEC strains previously isolated from two cohort studies in children <24 months of age in Lima, Peru. Cohort 1: Peri-urban community of the South Lima (District of Chorrillos), Peru (n=83); Cohort 2: Peri-urban community of the North Lima (District of Independencia), Peru (n=122).

Detection of ETEC by Real Time PCR.

► ETEC (*lt/st*) was detected using a multiplex real-time PCR method using previously validated specific primers for each diarrheagenic *Escherichia coli* (Guion et al., 2008).

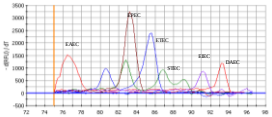


Figure 1. Multiplex Real Time PCR: Picks of Melting Tempeture (°C).

Determination of antimicrobial susceptibility and mechanisms of resistance.

► Susceptibility to nalidixic acid (Nal) and ciprofloxacin (Cip) was tested by disk diffusion.

► Thirty-one ETEC isolates exhibiting resistance or diminished susceptibility to Nal. Among this strains, the presence of target mutations and transferable quinolone-resistance mechanisms were determined by PCR and sequencing.

► Additionally, the role of Phe-Arg-β-Naphtylamyde (PAN) inhibitible efflux pumps were evaluated.

3.- RESULTS

► Sixty-eight percent (21/31) of the isolates were resistant to Nal and none to Cip. Nal^R-Cip^S strains was the most frequent resistant phenotype detected (17/31, 55%). Followed by Nal^I-Cip^S (8/31, 26%), Nal^R-Cip^I (4/31, 13%) and Nal^I-Cip^I (2/31, 6%).

► Among 31 ETEC isolates exhibiting resistance or diminished susceptibility to Nal, the most frequent transferable mechanisms of quinolone resistance (TMQR) genes detected were *aac(6')Ib-cr* gene (9/31, 29%), followed by *qnrS* gene (6/31, 19%).

Table 1. Quinolone resistance patterns associated with presence of transferable mechanisms of quinolone resistance, mutations in targets and MIC in presence or absence of efflux pump inhibitor (n=31).

Toxin	Phenotype		Sequencing	Genes			MIC	
type	Nal	Cip	gyrA	aac(6')Ib-cr	Qnr	qepA	Nal	Nal+PAN
LT	R	S	83 (s-L), 87 (D)	-	-	-	>256	16
LT	R	S	83 (s-L), 87 (D)	-	-	-	256	8
LT	R	I	83 (s-L), 87 (D)	-	-	-	128	8
LT	R	S	83 (s-L), 87 (D)	+	-	-	>256	8
LT	R	S	83 (s-L), 87 (D)	+	-	-	256	8
LT	R	S	wt	-	-	-	256	8
LT	R	I	wt	+	B	-	32	8
LT	I	S	wt	-	B	-	16	8
LT	I	S	wt	+	-	-	32	8
ST	R	S	83 (s-L), 87 (D)	-	-	-	256	8
ST	R	I	83 (s-L), 87 (D)	-	-	-	256	8
ST	R	S	83 (s-L), 87 (D)	-	-	-	256	8
ST	R	S	83 (s-L), 87 (D)	+	-	-	256	8
ST	R	S	wt	-	-	-	32	8
ST	I	S	83 (s-A), 87 (D)	-	-	-	64	8
ST	I	S	83 (s-A), 87 (D)	-	-	-	32	8
ST	I	S	wt	-	-	-	2	2
ST	I	I	wt	-	S	-	2	2
ST	I	S	wt	+	S	-	8	4
ST	I	S	wt	+	S	-	2	2
LT-ST	R	I	83 (s-L), 87 (D)	-	-	-	>256	8
LT-ST	R	S	83 (s-L), 87 (D)	-	-	-	256	16
LT-ST	R	S	83 (s-L), 87 (D)	-	-	-	256	8
LT-ST	R	S	83 (s-L), 87 (D)	-	-	-	256	8
LT-ST	R	S	83 (s-L), 87 (D)	-	-	-	256	8
LT-ST	R	S	83 (s-L), 87 (D)	-	-	-	128	8
LT-ST	R	S	83 (s-L), 87 (D)	-	-	+	256	8
LT-ST	R	S	83 (s-L), 87 (D)	+	-	-	>256	16
LT-ST	R	S	wt	-	S	-	256	16
LT-ST	I	I	83 (s-L), 87 (D)	+	S	-	16	8
LT-ST	I	S	wt	-	S	-	16	8

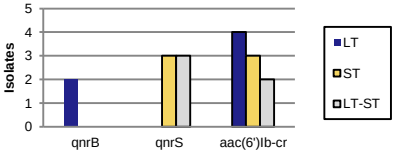
► Chromosomal mutations in drug target genes, such as *gyrA* gene (encoding DNA Gyrase) were detected in 65% (20/31) of the ETEC strains; and none presented substitutions in the *parC* gene (encoding Topoisomerase IV).

► Neither the *qnrC, qnrD, qnrVC, oqxAB* gene was detected.

► The unusual Nal^I or Nal^R phenotype without target mutations in *gyrA* and *parC* genes was observed, being detected in 11 isolates (35%).

► On the other hand, the MIC_{50} of Nal, when the efflux pump inhibitor was added to the media, a reduction in at least two-fold dilutions was found.

Figure 2. Distribution of transferable mechanisms of quinolone resistance in ETEC (n=31).



4.- CONCLUSIONS

► Nal^R-Cip^S strains was the most frequent resistant phenotype detected.

► The most frequent TMQR gene detected was the *aac(6')Ib-cr* gene.

► Two different point mutations in *gyrA* gene were detected.

► The efflux pumps has an important role in quinolone resistance acquisition.

SELECTED REFERENCES

1. Ochoa TJ, et al. Age-related susceptibility to infection with diarrheagenic *Escherichia coli* among infants from periurban areas in Lima, Peru. Clin Infect Dis 2009;49:1694–702.
2. Ochoa TJ, et al. Randomized double-blind controlled trial of bovine lactoferrin for prevention of diarrhea in children. J Pediatr. 2013 Feb;162(2):349-56.
3. Medina AM, et al. Comparative analysis of antimicrobial resistance in enterotoxigenic *Escherichia coli* isolates from two paediatric cohort studies in Lima, Peru. Trans R Soc Trop Med Hyg. 2015 Aug;109(8):493-502

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