Supplementary Material

Fecal Carriage of Extended-Spectrum Beta-Lactamase-Producing Enterobacterales in Healthy Spanish Schoolchildren

Mireia López-Siles[1,2,*], Zaira Moure[3,4], Aly Salimo Muadica[5,6], Sergio Sánchez[7], Raquel Cruces[1], Alicia Ávila[3], Noelia Lara [3], Pamela Carolina Köster[5], Alejandro Dashti[5], Jesús Oteo-Iglesias[3,8,9,†], David Carmena[5,9, †], Michael J. McConnell[1, †]

[1]Intrahospital Infections Unit, Reference and Research Laboratory in Resistance to Antibiotics and Infections related to Healthcare, National Centre for Microbiology, Instituto de Salud Carlos III (ISCIII), Madrid, Spain

[2]Serra Húnter Fellow. Microbiology of Intestinal Diseases, Biology Department, Universitat de Girona, Girona, Spain

[3]Reference and Research Laboratory in Resistance to Antibiotics and Infections related to Healthcare, National Centre for Microbiology, Instituto de Salud Carlos III, Majadahonda, Madrid, Spain

[4]Servicio de Microbiología, Hospital Universitario Marqués de Valdecilla, Instituto de Investigación Sanitaria, (IDIVAL), Santander, Spain.

[5]Parasitology Reference and Research Laboratory, National Centre for Microbiology, Instituto de Salud Carlos III, Majadahonda, Madrid, Spain

[6]Departamento de Ciências e Tecnologia, Universidade Licungo, Quelimane, Zambézia, Mozambique

[7]Reference and Research Laboratory of Food and Waterborne Bacterial Infections, National Center for Microbiology, Instituto de Salud Carlos III, Majadahonda, Madrid, Spain

[8]Spanish Network for Research in Infectious Diseases (REIPI RD16/0016), Instituto de Salud Carlos III, Madrid, Spain

[9]Centro de Investigación Biomédica en Red de Enfermedades Infecciosas (CIBERINFEC), Instituto de Salud Carlos III, Madrid, Spain

[†] These authors contributed equally to this work and share senior authorship.

1 Supplementary Tables

Supplementary Table 1. Primers used in this study and PCR conditions.

Target	Primer name	Sequence (5'-3')	Use	Cycles*	Denaturing T[a] (ºC); t (sec)	Annealing T[a] (ºC), t (sec)	Extension T[a] (ºC), t (sec)	Ref
16S rRNA	Bac27F	AGAGTTTGATCCTGGCTCAG	Species identification	30	95; 45	55; 45	72; 45	[1]
	Uni1492R	ACGGTTACCTTGTTACGACTT						
bla$_{SHV}$ group	SHVM-F	AACGGAACTGAATGAGGCG	ESBL gene identification	25	95; 45	65; 45	72; 45	This study
	SHVM-R	TCCACCATCCACTGCAGCAGCT						
bla$_{CTX-M-9}$ group	CTXM14M-F	TACCGCAGATAATACGCAGGTG	ESBL gene identification	25	95; 45	65; 45	72; 45	
	CTXM14M-R	CAGCGTAGGTTCAGTGCGATCC						
bla$_{CTX-M-1}$ group	CTXM15M-F	AATCACTGCGCCAGTTCACGCT	ESBL gene identification	25	95; 45	65; 45	72; 45	
	CTXM15M-R	GAACGTTTCGTCTCCCAGCTGT						
adk	AdkF	ATTCTGCTTGGCGCTCCGGG	MLST	30	95; 45	54; 45	72; 120	[2]
	AdkR	CCGTCAACTTTCGCGTATTT						
fumC	FumCF	TCACAGGTCGCCAGCGCTTC	MLST	30	95; 45	54; 45	72; 120	
	FumCR	GTACGCAGCGAAAAAGATTC						
gyrB	GyrBF	TCGGCGACACGGATGACGGC	MLST	30	95; 60	60; 60	72; 120	
	GyrBR	ATCAGGCCTTCACGCGCATC						
icd	IcdF	ATGGAAAGTAAAGTAGTTGTTCCGGCACA	MLST	30	95; 45	54; 45	72; 120	
	IcdR	GGACGCAGCAGGATCTGTT						
mdh	MdhF	ATGAAAGTCGCAGTCCTCGGCGCTGCTGGCGG	MLST	30	95; 60	60; 60	72; 120	
	MdhR	TTAACGAACTCCTGCCCCAGAGCGATATCTTTCTT						
purA	PurAF1	TCGTAACGGTGTTGTGCTG	MLST	30	95; 45	54; 45	72; 120	
	PurAR	CATACGGTAAGCCACGCAGA						
recA	RecF1	AGCGTGAAGGTAAAACCTGTG	MLST	30	95; 60	58; 60	72; 120	
	RecR1	ACCTTTGTAGCTGTACCACG						

* All PCR programs included an initial denaturing step at 95ºC for 5 min and a final elongation step at 72ºC for 10 min.

Supplementary Table 2. Association between clonal complex, ST, *bla* family and gene variant in relation to sociodemographic data recruited from ESBL-E carriers. Numbers in cells represent *p*-value.

	Clonal complex	ST	*bla* family	*bla* gene
Age	0.58	0.56	0.625	0.924
Gender	0.481	0.655	0.159	0.232
School	0.768	0.469	0.212	0.303
Bristol scale	0.697	0.463	0.383	0.376
WHO region of origin	0.733	0.921	0.349	0.737
Number of siblings	0.525	0.734	0.368	0.639
Diarrhoea the past 7 days	0.548	0.858	0.565	0.223
Family member with diarrhoea the past 7 days	0.846	0.479	0.164	0.063
Pets' owner	0.35	0.192	0.123	0.145
Source of drinking water	0.895	0.781	0.059	0.341
Hands washing	0.185	0.349	0.712	0.933
Vegetables washing	0.502	0.434	0.768	0.594
Travel abroad in the past 6 months	0.282	0.107	0.462	0.089
Travel to EU in the past 6 months	□	0.223	0.386	0.223
Enteric protozoa	0.709	0.539	0.537	0.583
Clonal complex	□	□	0.277	0.105
ST	□	□	0.391	0.016

Legend

$p \leq 0.05$

$0.05 < p \leq 0.100$

$p > 0.100$

Supplementary Table 3. Minimum inhibitory concentration of antimicrobials for the 24 ESBL-E isolates. EUCAST guidelines were used for interpretation of susceptibility/ resistance breakpoints in all cases except for cefoxitin, where CLSI guideline was used for interpretation [3]. Cells are colored in green, red or yellow, according with susceptible, resistant and intermediate phenotype, respectively. No colored cells correspond to antimicrobials not included in any of the two guidelines.

Isolate	ID child	Antimicrobials resistance*																														
		AMP	TIC	P	MEC	AMC	P/T	FOX	CRM	CFE	FOT	FOTC	CPD	CAZ	CAZC	FEP	AZT	ERT	IMI	MER	AK	GEM	TOB	NAL	NXN	CIP	LEV	NIT	FOS	COL	TGC	TRI	TRS
1	1057	>8	>16	>16	>8	8	<4	<8	>8	>1	>32	<0.5	>1	2	<0.25	>4	>4	<0.12	<1	<0.12	<8	>4	>4	>16	>1	>1	>1	<64	<32	<2	<0.5	>4	>4
2	1119	>8	>16	>16	<2	8	<4	<8	>8	>1	>32	<0.5	>1	<0.5	<0.25	>4	4	<0.12	<1	<0.12	<8	<2	<2	<16	<0.5	<0.25	<0.5	<64	<32	<2	<0.5	>4	>4
3	1129	>8	>16	>16	<2	16	<4	<8	>8	>1	>32	<0.5	>1	1	0.5	>4	4	<0.12	<1	<0.12	<8	<2	<2	<16	<0.5	<0.25	<0.5	<64	<32	<2	<0.5	<2	<2
4	1134	>8	>16	>16	<2	<4	<4	<8	>8	>1	>32	<0.5	>1	16	<0.25	>4	>4	<0.12	<1	<0.12	<8	<2	<2	<16	0.5	0.5	<0.5	<64	<32	<2	1	>4	>4
5	1202	>8	>16	>16	<2	<4	<4	<8	>8	>1	32	<0.5	>1	>32	<0.25	>4	>4	<0.12	<1	<0.12	<8	<2	<2	<16	<0.5	<0.25	<0.5	<64	<32	<2	<0.5	<2	<2
6	1251	>8	>16	>16	<2	<4	<4	<8	<4	>1	2	<0.5	>1	8	<0.25	<1	>4	<0.12	<1	<0.12	<8	<2	<2	<16	<0.5	<0.25	<0.5	<64	<32	<2	<0.5	>4	>4
7	1289	>8	>16	>16	<2	8	<4	<8	>8	>1	>32	<0.5	>1	16	<0.25	>4	>4	<0.12	<1	<0.12	<8	>4	>4	>16	1	<0.25	<0.5	>64	<32	<2	<0.5	>4	>4
8	1311	>8	>16	>16	<2	<4	<4	<8	>8	>1	>32	<0.5	>1	1	<0.25	>4	4	<0.12	<1	<0.12	<8	<2	<2	<16	<0.5	<0.25	<0.5	<64	<32	<2	<0.5	<2	<2
9	1345	>8	>16	>16	<2	<4	<4	<8	>8	>1	>32	<0.5	>1	32	<0.25	>4	>4	<0.12	<1	<0.12	<8	<2	<2	>16	<0.5	<0.25	<0.5	<64	<32	<2	<0.5	<2	<2
10	1428	>8	>16	>16	<2	<4	<4	<8	>8	>1	>32	<0.5	>1	16	<0.25	>4	>4	<0.12	<1	<0.12	<8	<2	<2	<16	<0.5	<0.25	<0.5	<64	<32	<2	<0.5	<2	<2
11	1446	>8	>16	>16	<2	8	<4	<8	>8	>1	>32	<0.5	>1	1	<0.25	>4	4	<0.12	<1	<0.12	16	<2	<2	<16	<0.5	<0.25	<0.5	<64	<32	4	<0.5	<2	<2
12	1530	>8	>16	>16	<2	8	<4	<8	>8	1	>32	<0.5	>1	<0.5	<0.25	2	2	<0.12	<1	<0.12	<8	<2	<2	<16	<0.5	<0.25	<0.5	<64	<32	<2	<0.5	>4	>4
13	1546	>8	>16	>16	8	<4	<4	<8	>8	>1	>32	<0.5	>1	>32	<0.25	>4	>4	<0.12	<1	<0.12	<8	<2	<2	>16	>1	>1	>1	<64	<32	<2	<0.5	<2	<2
14	1546	>8	>16	>16	<2	<4	<4	<8	>8	>1	4	<0.5	>1	16	<0.25	<1	>4	<0.12	<1	<0.12	<8	<2	<2	<16	<0.5	<0.25	<0.5	<64	<32	<2	<0.5	<2	<2
15	1592	>8	>16	>16	<2	<4	<4	<8	8	<0.5	2	<0.5	>1	1	<0.25	<1	2	<0.12	<1	<0.12	<8	<2	<2	>16	>1	>1	>1	<64	<32	<2	<0.5	<2	<2
16	1622	>8	>16	>16	<2	16	<4	<8	>8	>1	16	<0.5	>1	1	<0.25	<1	4	<0.12	<1	<0.12	<8	<2	>4	>16	>1	>1	>1	<64	<32	<2	<0.5	>4	>4
17	1648	>8	>16	>16	<2	<4	<4	<8	>8	>1	>32	<0.5	>1	2	<0.25	>4	>4	<0.12	<1	<0.12	<8	<2	<2	>16	1	<0.25	<0.5	<64	<32	<2	<0.5	<2	<2
18	1690	>8	>16	>16	<2	16	8	<8	>8	>1	>32	<0.5	>1	4	<0.25	<1	2	<0.12	<1	<0.12	16	<2	>4	>16	>1	>1	>1	<64	<32	<2	<0.5	>4	>4
19	1706	>8	>16	>16	<2	<4	<4	<8	>8	>1	>32	<0.5	>1	16	<0.25	>4	>4	<0.12	<1	<0.12	<8	<2	<2	<16	<0.5	<0.25	<0.5	<64	<32	<2	<0.5	<2	<2
20	1806	>8	>16	>16	<2	8	<4	<8	>8	>1	>32	<0.5	>1	16	<0.25	>4	>4	<0.12	<1	<0.12	<8	<2	>4	>16	>1	>1	>1	<64	<32	<2	<0.5	>4	>4

Isolate	ID child	Antimicrobials resistance*																														
		AMP	TIC	P	MEC	AMC	P/T	FOX	CRM	CFE	FOT	FOTC	CPD	CAZ	CAZC	FEP	AZT	ERT	IMI	MER	AK	GEM	TOB	NAL	NXN	CIP	LEV	NIT	FOS	COL	TGC	TRI	TRS
21	1939	>8	>16	>16	8	8	<4	16	>8	>1	>32	<0.5	>1	8	<0.25	>4	>4	<0.12	<1	<0.12	<8	<2	<2	>16	>1	>1	>1	<64	<32	<2	<0.5	>4	>4
22	2032	>8	>16	>16	<2	<4	<4	<8	>8	>1	>32	<0.5	>1	<0.5	<0.25	>4	2	<0.12	<1	<0.12	<8	<2	<2	>16	<0.5	<0.25	<0.5	<64	<32	<2	<0.5	<2	<2
23	2048	>8	>16	>16	<2	<4	<4	<8	>8	>1	>32	<0.5	>1	4	<0.25	4	>4	<0.12	<1	<0.12	<8	<2	<2	>16	>1	>1	>1	<64	<32	<2	<0.5	>4	>4
24	2058	>8	>16	>16	<2	<4	<4	<8	>8	>1	>32	<0.5	>1	16	<0.25	>4	>4	<0.12	<1	<0.12	<8	<2	<2	<16	<0.5	<0.25	<0.5	<64	<32	<2	<0.5	<2	<2

* Compounds abbreviations: AMP, Ampicillin; TIC, Ticarcillin; P, Piperacillin; MEC, Mecillinam; AMC, Amoxicillin-Clavulanic; P/T, Piperacillin-Tazobactam; FOX, Cefoxitin; CRM, Cerfurixime; CFE, Cefixime; FOT, Cefotaxime; FOTC, Cefotaxime-Clavulanic; CPD, Cefpodoxime; CAZ, Ceftazidime; CAZC, Ceftazidime-Clavulanic; FEP, Cefepime; AZT, Aztreonam; ERT, Ertapenem; IMI, Imipenem; MER, Meropenem; AK, Amikacin; GEM, Gentamicin; TOB, Tobramycin; NAL, Nalidixic acid; NXN, Norfloxacin; CIP, Ciprofloxacin; LEV, Levofloxacin; NIT, Nitrofurantoin; FOS, Fosfomycin; COL, Colistin; TGC, Tigecycline; TRI, Trimethoprim; TRS, Trimethoprim-Sulfamethoxazol.

Supplementary Table 4. Association between antimicrobials resistance of ESBL-E isolates and clonal complex, ST, *bla* family and gene variant. Numbers in cells represent *p*-value.

Antibiotic*	Clonal complex	ST	*bla* family	*bla* gene
Ampicillin-Clavulanic	0.070	0.523	0.585	0.766
Cefoxitin	0.516	0.893	0.540	0.199
Ceftazidime	0.235	0.088	0.073	0.019
Aztreonam	0.690	0.954	0.924	0.822
Amikacin	0.628	0.194	0.724	0.805
Gentamicin	0.842	0.194	0.275	0.009
Tobramycin	0.110	0.046	0.258	0.027
Norfloxacin	0.040	0.033	0.938	0.060
Ciprofloxacin	0.058	0.099	0.842	0.07
Levofloxacin	0.058	0.099	0.842	0.07
Nitrofurantoin	0.516	0.893	0.540	0.199
Trimethoprim	0.136	0.225	0.420	0.038
Trimethoprim-Sulfamethoxazol	0.136	0.225	0.420	0.038

Legend	
$p \leq 0.05$	
$0.05 < p \leq 0.100$	
$p > 0.100$	

* Analysis for ampicillin, ticarcillin, piperacillin, piperacillin-tazobactam, cefotaxime, cefpodoxime, fosfomycin, colistin and tigecycline was not possible to calculate because all isolates presented similar susceptibility to these antibiotics.

2 Supplementary References

1. Lane, D. J. 1991. 16S/23S rRNA sequencing, p.115–148. In E. Stackebrandt and M. Goodfellow (ed.), Nucleic acid techniques inbacterial systematics. John Wiley and Sons, New York, N.Y.

2. Wirth, T., et al., Sex and virulence in *Escherichia coli*: an evolutionary perspective. Mol Microbiol, 2006. 60(5): p. 1136-51. [available at https://enterobase.readthedocs.io/en/latest/mlst/mlst-legacy-info-ecoli.html]

3. Wayne, PA: Clinical and Laboratory Standards Institute (CLSI). Performance Standards for Antimicrobial Susceptibility Testing. 30th ed. CLSI supplement M100. Wayne, P. C. a. L. S. I. & 2020.