Phylogenetic diversity of quinolone resistant *Escherichia coli* isolated from wastewaters

Ana Rita Varela*,José Miguel Rebelo, Joaquim Azevedo, Célia M. Manaia

CBQF/Escola Superior de Biotecnologia, Universidade Católica Portuguesa, 4200-072 Porto, Portugal
*avarela@porto.ucp.pt

Scope and objectives

•*Escherichia coli* is an ubiquitous species which includes different phylogenetic lineages with distinct patterns of environmental distribution ;

•Previous studies showed antibiotic resistance increases significantly after biological wastewater treatment, suggesting wastewater treatment plants (WWTP) may represent important reservoirs of bacteria tolerating antibiotics[1].

•This study is based on the hypothesis that different different lineages may have distinct roles in the dissemination of quinolone resistance.

•The aim was to infer about the possible relationships between origin, phylogenetic lineage and quinolone resistance determinants in wastewater *E. coli* isolates.

Methodology

Isolation of *E. coli* from raw inflow and treated wastewater, and urban water streams in media supplemented or not with antibiotics.

Media	Supplements
PCA mFC BEA	4 mg/L ciprofloxacin, 32 mg/L amoxicillin, 16 mg/L tetracyclin or 350 mg/L sulphamethoxazole

Analyses

Identification by 16s rRNA PCR
Determination of phylogenetic group
(A, B1, B2 and D)

+

Multilocus Sequence Typing analysis[2]
(*adk, fumC, recA, icd, mdh, purA, gyrB)*

+

Antibiotic resistance phenotypes
(disk diffusion method)

+

Quinolone-resistance gene detection
(*qnrA, B, S, C, D, qepA, aac(6')-ib-cr*)

Results

•As previously demonstrated, the MLST analysis evidenced the misclassification of the phylogenetic groups of some isolates, mainly for groups A and B1;

•Isolates sharing the same ST for all the seven genes had origin in different geographic areas, suggesting their widespread distribution;

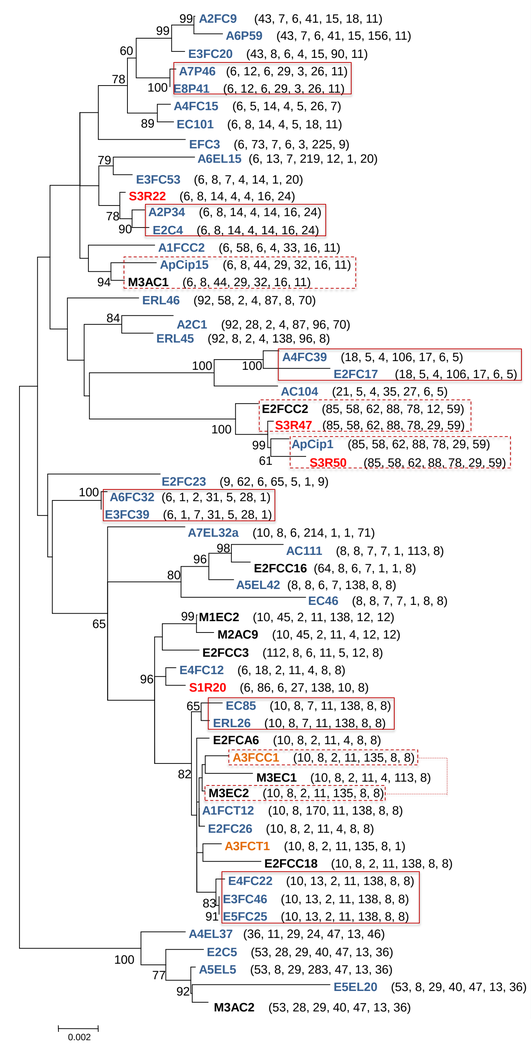
•Isolates sharing the same ST for all the seven genes were found in the raw and treated wastewater of MWWTP2, suggesting their survival during the process.

•It was not possible to establish a clear relationship between phylogenetic lineage and antibiotic resistance pattern;

•Neverthless, isolates with identical or very close MLST types, showed distinct resistance patterns suggesting antibiotic resistance acquisition;

• Quinolone resistance could be associated with *gyr*A and/or *par*C mutations, irrespective of the MLST type or origin:
• *gyrA* - TTG(Leu)[83] + AAC(Asn)[87] () - 74%-84% of the isolates;
• *parC* - ATC (III)[80] 46% of the isolates;

•Plasmid-mediated quinolone resistance was rare:
- genes *qnrS* and *aac(6')-ib-cr* detected in R3 isolates.



Phylogenetic group	Resistance phenotype: Tetracyclines TET	Aminoglycosides GEN	Aminoglycosides STR	Sulphonamides SXT	Sulphonamides RL	β-lactams AML	β-lactams TIC	β-lactams CP	β-lactams CEF	R≥3	Quinolone resistant genes	Mutations parC	Mutations gyrA
B1	S	S	R	R	R	R	R	R	S	+	-	AGA (Arg)[80]	TTG (Leu)[83]
B1	R	S	I	S	R	R	R	S	S	+	-	AGA (Arg)[80]	TTG (Leu)[83], AAC (Asn)[87]
B1	R	S	R	R	R	R	R	R	S	+	-	ATC (III)[80]	TTG (Leu)[83], AAC (Asn)[87]
B1	R	S	I	S	R	R	R	S	S	+	-	n/d	n/d
B1	R	S	I	S	R	R	R	R	S	+	-	n/d	n/d
B1	S	S	R	S	R	S	I	S	S		-	n/d	n/d
B1	R	S	S	S	R	R	R	S	S	+	-	ATC (III)[80]	TTG (Leu)[83], AAC (Asn)[87]
B1	R	S	R	R	R	R	R	R	S	+	-	ATC (III)[80]	TTG (Leu)[83], AAC (Asn)[87]
A	S	S	S	I	R	S	S	S	S		-	n/d	n/d
B1	R	S	R	R	R	R	R	R	S	+	-	ATC (III)[80]	TTG (Leu)[83], AAC (Asn)[87]
B1	R	S	R	R	R	R	R	S	S	+	-	ATC (III)[80]	TTG (Leu)[83], AAC (Asn)[87]
B1	R	S	I	R	R	R	R	S	S	+	-	ATC (III)[80]	TTG (Leu)[83], AAC (Asn)[87]
B2	S	S	R	S	R	I	S	I	S		-	ATC (III)[80]	TTG (Leu)[83], AAC (Asn)[87]
B1	S	S	R	S	R	R	R	I	S	+	*qnrS, aac(6)-ib-cr*	ATC (III)[80]	TTG (Leu)[83]
B1	S	S	R	R	R	R	R	R	S	+	-	ATC (III)[80]	TTG (Leu)[83], AAC (Asn)[87]
B1	R	S	I	R	R	R	R	R	S	+	-	ATC (III)[80]	TTG (Leu)[83], AAC (Asn)[87]
D	R	S	I	S	R	R	R	R	S	+	-	ATC (III)[80]	TTG (Leu)[83], AAC (Asn)[87]
D	R	S	R	S	R	R	R	R	R	+	-	ATC (III)[80]	TTG (Leu)[83], AAC (Asn)[87]
D	R	S	R	S	R	R	R	R	S	+	-	ATC (III)[80]	TTG (Leu)[83], AAC (Asn)[87]
D	R	S	R	R	R	R	R	S	S	+	-	ATC (III)[80]	TTG (Leu)[83], AAC (Asn)[87]
D	R	S	R	R	R	R	R	S	S	+	-	ATC (III)[80]	TTG (Leu)[83], AAC (Asn)[87]
D	S	S	S	S	I	R	R	R	S		-	ATC (III)[80]	TTG (Leu)[83], AAC (Asn)[87]
D	R	R	R	R	R	R	R	R	S	+	-	ATC (III)[80]	TTG (Leu)[83], AAC (Asn)[87]
D	R	R	I	I	S	S	S	R	S		-	ATT (III)[80]	TTG (Leu)[83], AAC (Asn)[87]
D	R	S	R	R	R	R	R	R	S	+	-	ATC (III)[80], GGA (Gly)[84]	TTG (Leu)[83], AAC (Asn)[87]
D	R	R	R	S	S	S	S	R	S	+	-	ATT (III)[80]	TTG (Leu)[83], AAC (Asn)[87]
B1	R	R	R	S	R	R	R	S	S	+	-	ATC (III)[80]	TTG (Leu)[83], AAC (Asn)[87]
D	R	S	R	R	R	R	R	S	S	+	-	-	TTG (Leu)[83], AAC (Asn)[87]
D	R	S	R	S	R	S	S	S	S	+	-	ATC (III)[80], GGA (Gly)[84]	TTG (Leu)[83], AAC (Asn)[87]
A	S	S	S	S	S	S	S	R	S	+	-	n/d	n/d
D	S	S	R	S	R	S	S	S	S		-	ATC (III)[80]	TTG (Leu)[83], GGC (Gly)[87]
A	R	I	R	R	R	S	S	S	S	+	-	ATC (III)[80]	TTG (Leu)[83], AAC (Asn)[87]
A	S	S	S	S	R	R	R	R	S	+	-	n/d	n/d
B2	S	S	I	R	R	I	S	S	S		-	ATC (III)[80]	TTG (Leu)[83], AAC (Asn)[87]
B2	R	R	R	R	R	R	R	R	S	+	-	ATC (III)[80]	TTG (Leu)[83], AAC (Asn)[87]
B2	R	R	R	R	R	R	R	R	S	+	-	ATC (III)[80]	TTG (Leu)[83], AAC (Asn)[87]
B2	R	S	I	S	I	R	R	I	S		-	ATC (III)[80]	TTG (Leu)[83], AAC (Asn)[87]
A	S	S	I	S	R	R	R	R	S		-	n/d	n/d
A	S	S	I	S	I	S	I	S	S		-	AGA (Arg)[80]	TTG (Leu)[83]
A	S	S	R	S	R	S	S	S	S		-	ATC (III)[80]	TTG (Leu)[83], AAC (Asn)[87]
A	R	S	R	R	R	R	R	I	S	+	-	ATC (III)[80]	TTG (Leu)[83], AAC (Asn)[87]
B1	R	R	R	S	R	R	R	R	R	+	-	ATC (III)[80]	TTG (Leu)[83], AAC (Asn)[87]
A	R	S	I	R	R	S	S	S	S		-	ATC (III)[80]	TTG (Leu)[83], AAC (Asn)[87]
A	R	R	R	S	R	R	R	R	I	+	*qnrA*	ATC (III)[80]	TTG (Leu)[83], AAC (Asn)[87]
A	R	S	I	R	R	S	S	S	S		-	ATC (III)[80]	TTG (Leu)[83], AAC (Asn)[87]
A	R	R	R	S	R	R	R	R	I	+	-	ATC (III)[80]	TTG (Leu)[83], AAC (Asn)[87]
A	S	S	R	R	R	R	R	S	S	+	-	n/d	n/d
B1	R	S	R	R	R	S	S	S	S	+	-	ATC (III)[80]	TTG (Leu)[83], AAC (Asn)[87]
A	R	R	R	S	R	R	R	R	I	+	-	ATC (III)[80]	TTG (Leu)[83], AAC (Asn)[87]
A	R	S	R	R	R	R	R	R	S	+	-	ATC (III)[80]	TTG (Leu)[83], AAC (Asn)[87]
A	R	S	R	S	R	R	R	R	S	+	-	ATC (III)[80]	TTG (Leu)[83], AAC (Asn)[87]
A	R	S	R	R	R	R	R	R	S	+	-	ATC (III)[80]	TTG (Leu)[83], AAC (Asn)[87]
A	S	S	R	S	I	R	R	S	S		-	n/d	n/d
B2	R	S	R	R	R	S	S	I	S	+	-	n/d	TTG (Leu)[83], AAC (Asn)[87]
B2	R	R	R	R	R	R	R	R	S	+	-	ATT (III)[80], GTA (His)[84]	TTG (Leu)[83], AAC (Asn)[87]
B2	R	R	R	R	R	R	R	R	S	+	-	n/d	TTG (Leu)[83], AAC (Asn)[87]
B2	S	S	I	S	R	S	S	I	S		-	n/d	TTG (Leu)[83], AAC (Asn)[87]

Figure 1 - Neighbour-joining dendrogram based on the *adk, fumC, gyrB, icd, mdh, purA and recA* concatenated nucleotide sequences. Bootstrap values (≥60%) generated from 1000 replicates are indicated at branch points. Strains colour code and designation: **black**, MWWTP1, **blue**, MWWTP2, **orange**, MWWTP3, **red**, urban water streams. A/M3A, raw MWW; E/M3E, treated MWW; S, urban water streams . The Sequence Type (ST) determined[2] for each isolate/gene is indicated in brackets (order: *adk, purA, recA, fumC, gyrB, icd, mdh*). Outline: solid, same origin; dashed, different origins. Antibiotic resistance phenotype, shadowing: black, resistant; grey, intermediary; white, susceptible.; n/d, not determined

Conclusions

The results obtained in this study suggest that quinolone resistance in environmental *E.coli* are mainly due to gene mutation, which occur frequently and most of the times with the same substitution in the genes *gyr*A and *par*C.

The high diversity of phylogenetic lineages versus the frequent occurrence and stability of *gyr*A and *par*C mutations suggest a process of convergent evolution, which mechanisms deserve further studies.

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2. **MLST Databases at the ERI, University College Cork, http://mlst.ucc.ie/mlst/dbs/Ecoli**