

LETTER

Frequency of virulence and antibiotic resistance genes in *Escherichia coli* isolates in two rivers of central Italy

Frequenza di geni di virulenza e di resistenza agli antibiotici in ceppi di Escherichia coli isolati da due fiumi dell'Italia centrale

Dear Editor,

Antibiotic resistance is a growing global threat with serious health, political and economic implications (1). In river systems, human exposure might arise through different uses of water (2-3), and effluents from urban waste water treatment plants (WWTP) are known to increase the risk of antibiotic resistant (AR-) bacteria in the receiving water bodies (4). Here we briefly report on the presence of genes usually associated with antibiotic resistant and pathogenic phenotypes of *Escherichia coli* in the last tract of two rivers of Central Italy. This study was conducted in the frame of the collaborative international project TRACE (Water Challenges for a Changing World; Joint Programming Initiative). *E. coli* was chosen because it is a ubiquitous indicator of fecal pollution and several of its strains present pathogenic effect.

The Arrone and Tiber rivers are both located near the city of Rome and flow into the Mediterranean Sea, near recreational beaches that are very busy in Summer months. In each river, we collected water samples approximately 1 km upstream (UP) the WWTP discharge point, at the WWTP effluent (WWTP) and approximately 1 km downstream (DW) the discharge point. At each site, water was collected at a depth of 60 cm with a telescopic sampler using sterile 1 L borosilicate bottles in 3 dates (September 2016, December 2016, April 2017). In laboratory, water was first filtered through 0.45 µm nitrocellulose filters and then filters were placed on plates containing Tryptone Bile X-Glucuronide and incubated at 44°C for 24 h. For each sample, 10–30 grown pigmented colonies were picked and used to inoculate API-20E system according to manufacturer instructions to confirm their identification as *E. coli*. Isolates were tested for the presence of selected antibiotic resistance and virulence genes associated with *E. coli* diarrheagenic pathotypes by standard PCR assays (5).

The frequency of isolates carrying virulence and antibiotic resistance genes at each sampling site is reported in Table 1. Genetic markers of *E. coli* pathogenic strains *stx1*, *Lt*, *St*, *eaeA*, *bfp*, *cdjB*, *ipaH*, *aggR* and AR-genes *qnrA*, *bla_{SHV}* were not detected in any of the isolates. Considering both rivers, at least 1 virulence gene was detected in 20.7% of total *E. coli* isolates and at least 1 AR gene was detected in 50.6% of total *E. coli* isolates. In the Arrone, pathogenic strains were present also at the upstream site, possibly as consequence of agriculture or farm runoff, while in the Tiber they were mostly found downstream the WWTP effluent. Regarding the presence of selected antibiotic-resistance genes in *E. coli* isolates, we found presence of *bla_{TEM}* and *bla_{CTX-M}*, conferring resistance to beta-lactam antibiotics such as penicillins, cephalosporins, carbapenems, *sul1* and *sul2* conferring resistance to sulfonamides and *qnrB* and *qnrS* conferring resistance to quinolones. In both rivers, higher frequencies were found in the WWTP effluent and downstream. The results were consistent with those of many other studies conducted in freshwater ecosystems (6), as the increased availability of AR *E. coli* in WWTPs could facilitate their propagation and dissemination to the natural microbiota at downstream sites. Notably, at the downstream sites of both rivers we detected 1 isolate carrying both virulence and resistance genes.

Table 1 - Frequency of *E. coli* isolates carrying virulence or resistance genes in sites upstream (UP) and downstream (DW) the waste water treatment effluent (WWTP). Virulence genes *stx1*, *Lt*, *St*, *eaeA*, *bfp*, *cdfB*, *ipaH*, *aggR* and antibiotic resistance (AR-) genes *qnrA*, *bla_{SHV}* had null frequency in all isolates and are not shown in the table.

	Arrone				Tiber			
	UP	WWTP	DW	Sum	UP	WWTP	DW	Sum
Number of isolates tested	14	15	15	44	15	13	15	43
Virulence genes (pathotype)								
East1 (EAEC)	6	1	3	10	0	0	4	4
EhxA (EHEC)	0	0	2	2	0	0	0	0
Stx2 (EHEC)	0	0	0	0	0	1	1	2
At least 1 VF gene (%)	42.9	6.7	33.3	27.3	0.0	7.7	33.3	14.0
Resistance genes								
<i>bla_{TEM}</i>	0	0	0	0	1	0	0	1
<i>bla_{CTX-M}</i>	2	4	2	8	5	7	4	16
<i>qnrB</i>	1	0	0	1	0	0	0	0
<i>qnrS</i>	0	0	0	0	1	0	2	3
<i>sul1</i>	0	1	1	2	0	1	0	1
<i>sul2</i>	1	2	2	5	2	2	3	7
At least 1 AR-gene (%)	28.6	46.7	33.3	36.4	60.0	76.9	60.0	65.1

Given the potential adverse health effects of resistant bacteria, it is urgent to quantify the potential human exposure through different uses of river water such as irrigation or bathing. Although in our study most searched virulence genes were absent in *E. coli* isolates, genes indicative of potential presence of pathogenic strains EHEC and EAEC were detected not only in the WWTP but also in isolates sampled at downstream sites, not far from the nearby recreational beaches where the risk of gastrointestinal and other diseases for bathers might be not negligible (7-8). It should be noticed that AR and VF genes investigated here are only a (small) selection of the whole gene pool potentially conferring phenotypic antibiotic resistance and/or virulence to *E. coli* strains. Therefore, more extensive genomic analysis is needed to estimate the extent of resistance determinants for bacteria with clinical relevance present in rivers and the associated human risk.

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