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High diversity of ESBL-producing *Escherichia coli* in the Danube river and tributary streams at Budapest, Hungary

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Background: The spread of multidrug resistant *Enterobacteriaceae* (MDRE) outside the clinical setting is currently observed. The surface waters likely play a role in this spread, as they serve both as habitats and as transport systems for microorganisms. Currently very limited data on occurrence of MDRE in Danube are available. In a survey performed in 2010-2011, MDRE were not found in surface water at Budapest, the capital of Hungary. In our study we focused on treated sewage emission and sewage-influenced tributaries at the Budapest section of Danube to survey the presence of MDRE.

Material/methods: The samples were taken in summer 2016 from the Danube 500 m before and after the main sewage treatment plant of Budapest, and from two streams. Bacteria were isolated from water samples by membrane filtration using antibiotic supplemented media: ESBL-screen agar (bioMérieux) and Tergitol TTC agar (TA) (10 mg/l ceftriaxone). For the most probable number method, 100 ml samples were mixed with Colilert broth (CB) (10 mg/l ceftriaxone) (Table 1). The colonies were identified using MALDI-TOF and biochemical tests. The antimicrobial susceptibility testing was performed by disk diffusion method and interpreted using EUCAST guidelines. Acquired cephalosporinase production was tested by modified double-disk synergy test. The presence of different extended-spectrum (*bla*_{SHV}, *bla*_{TEM}, *bla*_{CTX-M}) and acquired AmpC-type β -lactamase encoding genes was verified using conventional PCRs. The possible clonal relationships were investigated by PFGE and ST131-specific PCR.

Results: Overall 27 MDRE isolates were found: 25 *Escherichia coli*, one *Klebsiella pneumoniae* and one *Raoultella ornithinolytica*. The *E. coli* isolates were assigned to 23 diverse pulsotypes (Table 1)

where two of isolates belonged to the ST131 international clone and 21 were not detected from human sources in Hungary so far. All but two isolates were CTX-M-type ESBL-producers, one was SHV-ESBL producer, and one was only acquired AmpC-producer. All investigated isolates proved wild type to carbapenems, 24% were resistant to at least one aminoglycoside, 28% to ciprofloxacin, 44% to trimethoprim/sulfamethoxazole and 100% to cephalosporins.

Conclusions: Our finding suggests that MDRE are present with high diversity in aquatic ecosystem of the Danube at Budapest and may facilitate the horizontal transmission of resistance genes. Sewage-influenced tributaries were shown to be the major source of MDRE. The Danube is used as a source of drinking water (by bank filtration) in Budapest, and also for recreational purposes, thus the presence of MDRE may pose a risk to human health.

Table 1. MDR *Escherichia coli* at the investigated water sites

Sampling site by river flow direction	Colony-forming unit/100 ml			No of different pulsotypes
	TA	CB	ESBL-screen	
Mouth of stream Aranyhegyi	13	1	7	5
Mouth of stream Rákos	4400	>200	1700	10
Before the sewage work	14	3	3	5
After the sewage work	4	2	2	4