Untreated wastewater as a source of carbapenem-resistant bacteria to the riverine ecosystem

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ABSTRACT

Bacteriological pollution, especially that including clinically important bacteria, of the aquatic environment caused by anthropogenic pressure attracts much attention with regard to public health. Reports to date have not addressed the concentration of emerging carbapenem-resistant bacteria (CRB) in riverine ecosystems, and the source of this pollution is hard to track. We examined the impact of discharge of untreated wastewaters on the bacterial population in the riverine ecosystem, with emphasis on clinically important CRB using a small river in Croatia as a model. River sediments were analysed mineralogically and geochemically. Cultivation of CRB was performed at 37 and 42°C to distinguish the presumably environmental intrinsically resistant (CRB37) from the presumably clinically important acquired resistance (CRB42) species. The significantly positive correlation of CRB42 with CRB37 and total heterotrophs, but not with intestinal enterococci, suggests that entry of CRB42 in riverine ecosystem is not necessarily connected to faecal pollution. The numbers and prevalence of CRB42 are rather dependent on the type of pollution, and is connected to the discharge of wastewaters from different human and animal healthcare centres. Emerging hospital pathogens *Acinetobacter baumannii* and *Klebsiella pneumoniae* were exclusively isolated among CRB42 from river water after the discharge of wastewater of a general hospital. The CRB42, once discharged into the riverine ecosystem, behaves as part of the indigenous bacterial population, and could be spread through the natural water bodies or accumulate in river sediments. This implies the need for disinfection of hospital wastewater prior to its discharge into the natural environment in order to avoid the consequent public-health threat. The anthropogenic impact evidenced as bacteriological changes is accompanied by an increase in heavy metal concentrations in river sediments.

Keywords: bacteria, carbapenems, water, sediment, environment

INTRODUCTION

Water is the most important resource on the planet. With the constant growth and increasing needs of human populations, aquatic environments are under anthropogenic pressure. Wastewaters from industry and agriculture, together with hospital and domestic wastewaters, as well as changes in hydrogeomorphology, contribute to the contamination and degradation of aquatic environments (Lenart-Boroń et al., 2017). Some contaminants (solids, chemicals, microorganisms) are removed from sewage by wastewater treatment plants with varying efficiency. Moreover, not all wastewater is treated before discharge into the natural environment. Contamination of water bodies is dependent on the amount, type and degradation potential of contaminants, and the self-purification ability of the recipient waterbody (Ostroumov, 2006).

Human and animal pathogens are considered to be important water contaminants. Efficient microbial indicators of anthropogenic pressure are total coliforms, faecal coliforms, *Escherichia coli* and intestinal enterococci, as their populations are proportional to the amount of faeces deposited into the environment (Ashbolt et al., 2001; Páll et al., 2013). Furthermore, clinically important bacteria in aquatic environments that are resistant to multiple frequently used antibiotics are also of great importance. These bacteria could propagate the resistance genes in aquatic environments and cause community-acquired infections in both humans and animals (Dexter et al., 2015; Marathe et al., 2017).

Carbapenems are broad range β-lactam antibiotics used as a last resort in treating infections with multi-drug resistant bacteria. In the past few decades, the emergence of strains resistant to carbapenems has been a growing concern (Meletis, 2016). In February 2017, the World Health organization (WHO) published a list of the most dangerous pathogens for human health. At the top of that list are carbapenem-resistant *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and Enterobacteriaceae (WHO, 2017). In Croatia, carbapenem resistance of *A. baumannii* has increased from 10% in 2008 to 86% in 2016 (CAMS 2017). There have been several reports of bacteria with acquired carbapenem resistance isolated from hospital wastewaters (Ferreira et al., 2011; Zhang et al., 2012; Zhang et al., 2013; Chandran et al., 2014; Seruga Music et al., 2017), as well as raw and secondary treated municipal wastewaters (Hrenovic et al., 2016; Hrenovic et al., 2017a,b). Regarding the natural aquatic environment, in the Danube River during the Joint Danube Survey 3 in 2013 one carbapenem-resistant *Klebsiella pneumoniae* and two other Enterobacteriaceae were reported (Kittinger et al., 2016). Additionally, one carbapenem-resistant isolate of *A. baumannii* was found in the Seine River (Gihrlich et al., 2010) and four in the Sava River (Seruga Music et al., 2017). There have also been reports of carbapenem-resistant Enterobacteriaceae from rivers in the United States (Aubron et al., 2005), Tunisia (Chouchani et al., 2013), Switzerland (Zurfluh et al., 2013), Portugal (Kieffer et al., 2016), India (Akiba et al., 2016), and Spain (Piedra-Carrasco et al., 2017). These reports point to the growing occurrence of clinically

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important bacteria with acquired resistance to carbapenems in the aquatic environment. However, all of these studies report the finding of one or several isolates, and there are no data regarding the concentration of clinically important carbapenem-resistant bacteria in riverine ecosystems. Furthermore, studies demonstrating the anthropogenic impact on the riverine ecosystem in general have lacked information on the origin of the pollution.

The aim of this study was to examine the anthropogenic impact on the viable bacterial population in the riverine ecosystem with emphasis on clinically important carbapenem-resistant bacteria using a small river as a model. The anthropogenic impact was additionally followed by mineralogical and geochemical analyses of river sediment.

MATERIALS AND METHODS

Site location and sampling

The examined area of the Krapinica and Krapina Rivers is shown in Fig. 1. Stations 1–3 represent the flow of Krapinica River, while Stations 4 and 5 were chosen to examine the

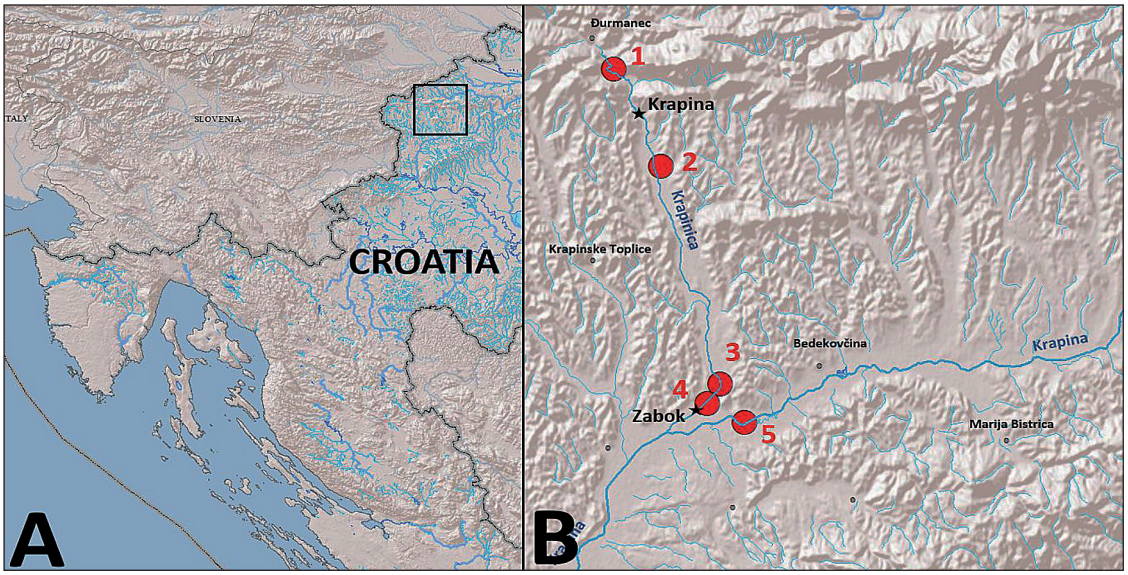

Figure 1
Location of the study site in Croatia (A, black rectangle) and position of sampling stations (B). For explanation see Table 1.

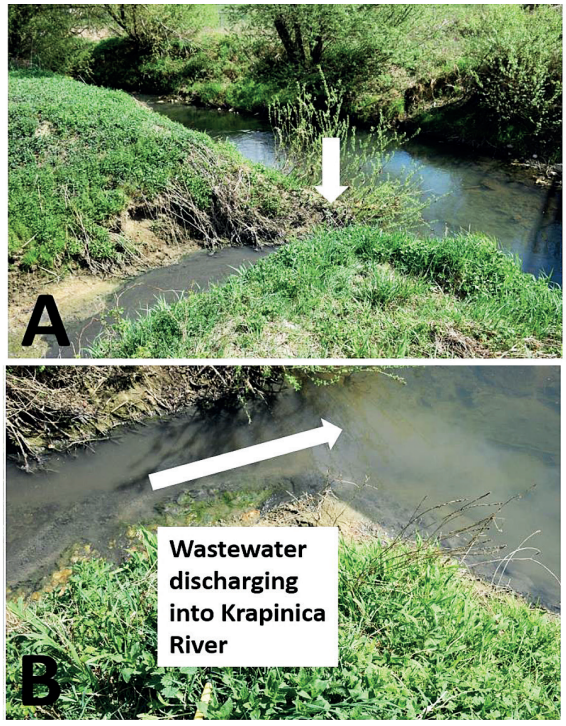

Figure 2
The mouth of Zabok Creek in the Krapinica River (north of Sampling Station 4). White arrow (A) points to the position of the discharge site (B).

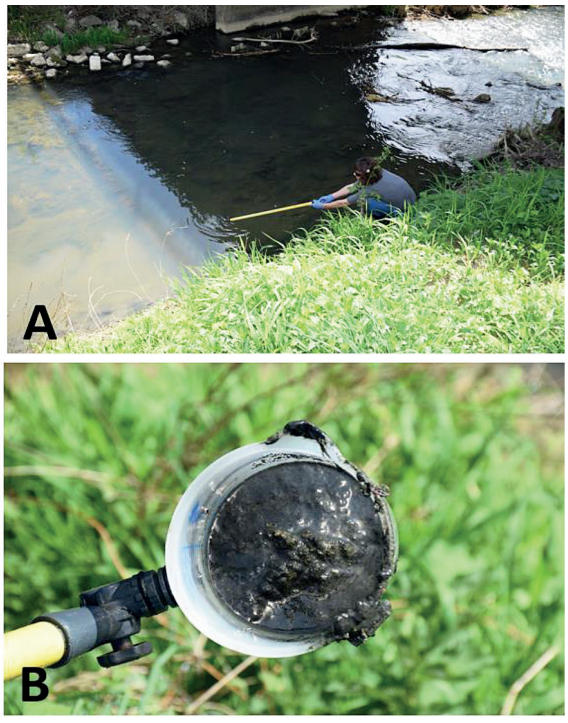

Figure 3
Sampling of water (A) and sediment (B) at Sampling Station 4

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TABLE 1 List and description of collected water and sediment samples		
Station number	**Description**	**pH value**
1	Water, upstream of town Krapina, village (1 000 residents)	7.76
	Sediment, upstream of town Krapina	7.27
2	Water, downstream of town Krapina (12 500 residents), 5 primary care stations and veterinary station	7.57
	Sediment, downstream of town Krapina, 5 primary care stations and veterinary station	6.88
3	Water, upstream of town Zabok, rehabilitation hospital	7.62
	Sediment, upstream of town Zabok, rehabilitation hospital	6.96
4	Water, downstream of town Zabok (9 000 residents), primary care station and veterinary station	7.62
	Sediment, downstream of town Zabok, primary care station and veterinary station	6.05
5	Water, downstream of Zabok General Hospital	7.72
	Sediment, downstream of Zabok General Hospital	6.69

separate input of urban (Zabok creek, Fig. 2) and hospital wastewater (Krapina River) on the ecosystem. In total, 5 samples of water and 5 samples of corresponding sediment were collected along the Krapinica and Krapina Rivers (Table 1). It is very common and constant in this region of Croatia to have untreated sewage released directly into the riverine ecosystem. Based on the distribution of settlements, all sampling locations were in the proximity of untreated sewage release points. Raw wastewaters entering the river consist of domestic wastewaters at Station 1, but at Stations 2–5, in addition the wastewaters from different human and animal healthcare centres (primary care and veterinary stations, rehabilitation and general hospital) are discharged.

For bacteriological analyses the water and river sediment samples were taken aseptically (Fig. 3) in sterile plastic bottles and processed in the laboratory within 4 h after collection. The pH value of water was measured in the original samples, while pH value of sediment was measured after suspension (1:2.5) of sediment in distilled water.

River sediment analyses

River sediments were sieved through a 2 mm sieve and the fraction < 2 mm was prepared for analyses. In general, the ≥ 2 mm fraction (gravel) was less than 5 wt%. Particle size distribution was determined by pipette-method, with wet sieving and sedimentation after dispersion with sodium-pyrophosphate ($Na_4P_2O_7$, c = 0.4M). The chemical composition (fraction < 2 mm) was determined by the commercial ACME Analytical Laboratory, Canada. Major oxides and several minor elements were determined by ICP-emission spectrometry following a lithium metaborate/tetraborate fusion and dilute nitric digestion. Refractory elements were determined by ICP mass spectrometry following a lithium metaborate/tetraborate fusion and nitric acid digestion. The mineral composition of < 2 mm and < 2 µm fractions of original samples (and samples after the carbonate mineral phases were removed) was determined by X-ray powder diffraction (XRD) using a Philips diffractometer (graphite monochromator, CuKµ radiation, proportional counter). To remove carbonates, samples were treated with a 1 M NaOAc solution buffered at pH 5 with HOAc (Jackson, 1979; Tassier et al., 1979). The < 2 µm fraction was separated by sedimentation in cylinders and quantitatively obtained after the appropriate settling time. The XRD patterns of clay fraction (non-oriented and oriented preparations) were obtained after the following treatments: air drying; Mg-saturation; K-saturation; K-saturation and ethylene glycol solvation; K-saturation and DMSO solvation; Mg-saturation and ethylene glycol solvation; and heating for 2 h at 350 and 550°C after K and Mg saturation. The identification of clay minerals was based on the methods outlined by Brindley and Brown (1980) and Moore and Reynolds (1989). The term 'illitic material' was used as defined by Środoń (1984) and Środoń and Eberl (1984). Semi-quantitative estimates of minerals were based on the relative intensities of characteristic X-ray peaks.

Bacteriological analyses

The water and sediment samples were analysed in triplicate after its suspension and dilution in sterile peptone water. Aerobically grown total heterotrophic bacteria were determined on Nutrient agar (Biolife) after incubation at 22°C/72 h (APHA et al., 2005). The intestinal enterococci were determined according to HRN ISO 7899-2 (2000). Membrane filters were incubated on Slanetz Bartley agar (Biolife) at 37°C/72 h and subsequent confirmation of intestinal enterococci was done on Bile esculin azide agar (Sigma-Aldrich) after incubation at 44°C/4 h. Carbapenem-resistant bacteria were determined on CHROMagar Acinetobacter supplemented with CR102 (CHROMagar), intended for the cultivation of clinically relevant carbapenem-resistant bacteria, after incubation at both 37°C/72 h and 42°C/48 h (CRB37 and CRB42, respectively). Cultivation of carbapenem-resistant bacteria was performed at 42°C to suppress the growth of environmental autochthonous species with intrinsic resistance to carbapenems, which grows only at 37°C (Hrenovic et al., 2016; Hrenovic et al., 2017a,b). The numbers of total heterotrophic bacteria, intestinal enterococci and carbapenem-resistant bacteria were determined as colony forming units (CFU), logarithmically transformed, and expressed as log CFU per 1 mL of water or 1 g of wet sediment.

The prevalence of carbapenem-resistant bacteria among total heterotrophic bacteria was calculated as a percentage ratio of absolute numbers ($CFU_{CRB}/CFU_{heterotrophic}$) x 100. The correlations between variables were estimated with Spearman's rank correlation by the Statistica 13 software (StatSoft, Inc., Tulsa, USA). A p-value of < 0.05 was considered significant.

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Identification and characterization of carbapenem-resistant bacteria

Identification of morphologically different colonies from plates intended for cultivation of CRB grown at 37°C and 42°C was performed by routine bacteriological techniques and matrix-assisted laser desorption ionization–time of flight mass spectrometry – MALDI-TOF MS (software version 3.0, Microflex LT, Bruker Daltonics) on cell extracts (Sousa et al., 2014). For the carbapenem-resistant bacteria from critical list of WHO (2107) (*A. baumannii* and Enterobacteriaceae) the confirmation of resistance to carbapenems (meropenem, imipenem) was performed. The antibiotic susceptibility was firstly assessed by disk diffusion method and confirmed according to MICs values obtained by Vitek2 system (Biomerieux). MICs values were interpreted according to EUCAST breakpoints (2017). In order to assess the genetic relationship of *A. baumannii* isolate with described clinical and environmental isolates the multi-locus sequence typing (MLST) of fragments of 7 traditional housekeeping genes (*gltA, gyrB, gdhB, recA*, *cpn60, gpi* and *rpoD*) were performed (Seruga Music et al., 2017). The sequence type (ST) was retrieved from the *A. baumannii* MLST website (http://pubmlst.org/abaumannii/).

RESULTS

Grain size, geochemistry and mineral composition of river sediments

River sediments from 5 sampling locations were analysed. Samples from Stations 1, 2 and 3 are composed dominantly of sand sized particles and represent sand, sandy loam and loamy sand, respectively (Fig. 4, Soil Science Division Staff, 2017). Samples from Stations 4 and 5 contain a higher amount of silt and clay than sand sized particles and represent loams (Fig. 4). Higher content of silt and clay fraction is accompanied

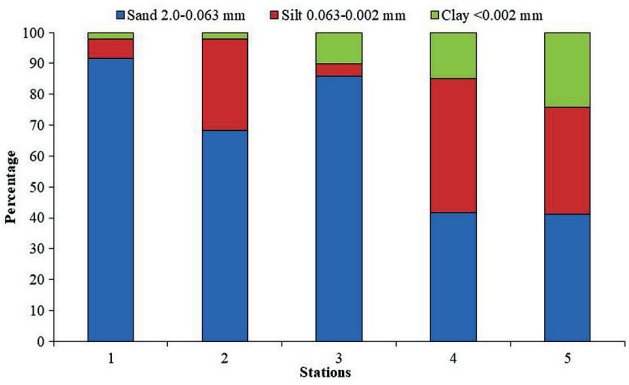

Figure 4
Particle size distribution of the investigated sediments

by higher LOI, content of Al_2O_3, Fe_2O_3, MgO, Na_2O, K_2O and TiO_2, but lower content of SiO_2 as compared to sand-enriched samples (Table 2). Samples containing a higher content of silt and clay fraction are also enriched in As, Ba, Cd, Co, Cs, Cu, Hg, Mo, Ni, Pb, Ta, U, V and Zn (Table 3). This indicates that silt and clay fractions of analysed sediments are probably the main scavengers of heavy metals (Salomons and Förstner, 1984). Analyses of < 2 mm and < 2 µm fractions showed that all samples are composed of quartz as the predominant mineral phase, following calcite, dolomite, plagioclase, K-feldspar, goethite, micaceous clay minerals (mica and illitic material), kaolinites, mixed-layer clay minerals, while 14 Å clay minerals (smectite and/or vermiculite) and XRD-amorphous compounds are detected only in the clay fraction of Samples 3–5. Although the decrease in particle size of downstream sediments is a natural phenomenon, the increased LOI, heavy metal concentration and both 14 Å clay minerals and amorphous matter contents suggest that Sediment Samples 4 and 5 are under high anthropogenic pressure.

TABLE 2
Chemical composition (in wt%) of the investigated sediments

Station number	SiO_2	Al_2O_3	Fe_2O_3	MgO	CaO	Na_2O	K_2O	TiO_2	P_2O_5	LOI[a]	Sum
1	84.11	3.98	2.03	0.86	2.95	0.56	1.20	0.13	0.06	4.1	99.98
2	85.61	3.94	1.69	1.04	2.11	0.56	1.25	0.15	0.06	3.5	99.91
3	80.19	5.06	2.85	0.93	2.11	0.60	1.30	0.23	0.15	6.5	99.92
4	65.17	10.33	4.55	2.14	3.24	0.86	1.94	0.57	0.28	10.7	99.78
5	67.71	12.42	4.84	1.56	2.00	1.03	1.89	0.86	0.13	7.2	99.64

[a]*loss on ignition (1 000°C).*

TABLE 3
Content of sulfur and selected trace elements in the investigated sediments

Station number	S	As	Ba	Cd	Co	Cs	Cu	Hg	Mo	Ni	Pb	Ta	U	V	Zn
1	0.02	3.1	188	< 0.1	3.5	1.1	3.2	0.08	0.2	5.8	5.6	0.2	0.8	16	21
2	0.04	1.8	182	< 0.1	3.2	1.1	4.9	0.16	0.1	6.5	12.1	0.3	0.7	17	26
3	0.08	9.3	229	< 0.1	6.7	1.8	11.3	0.07	0.3	12.7	16.4	0.3	1.1	26	41
4	0.08	7.2	336	0.2	11.1	4.4	29.5	0.34	0.4	27.7	24.5	0.7	2.0	71	83
5	< 0.02	9.3	380	0.3	15.4	4.9	23.4	0.11	0.5	39.8	16.3	1.1	2.6	95	65

S in wt%, As to Zn in ppm

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Bacteriological analyses

The pH values of water and sediment samples averaged 7.34, with a significant drop for sediment at Station 4 (Table 1). However, all pH values were within the range that supports the survival of neutrophilic bacteria. Numbers of bacteria in river water and sediment at 5 stations along the river are shown in Fig. 5. Bacterial counts at all stations were generally present in descending order: total heterotrophs, intestinal enterococci, CBR37, and CRB42. The numbers of all monitored bacteria were higher in sediment as compared to the corresponding water sample. The numbers of total heterotrophic bacteria and intestinal enterococci averaged 6.7 ± 0.9 and 2.2 ± 1.1 log CFU per 1 mL of water or 1 g of wet sediment, respectively, in all analysed samples. A substantial increase with increasing distance downstream (Stations 1–3) was evidenced only for CRB42 (Fig. 5), i.e., no CRB42 were isolated at Station 1 (detection limit 50 mL of water or 1 g of sediment), while they were present at Stations 2 and 3. The highest increase of both CBR37 and CRB42 was evidenced in sediment at Stations 4 and 5, which receive the input of urban and hospital wastewater. The prevalence of CRB37 ranged from 0.0002–0.001% in water and 0.001–1.1% in sediment samples. The prevalence of CRB42 was lower and averaged from 0–0.0001% in water and 0–0.001% in sediment samples (Fig. 5). The prevalence as well as the absolute numbers of CRB42 were always lower

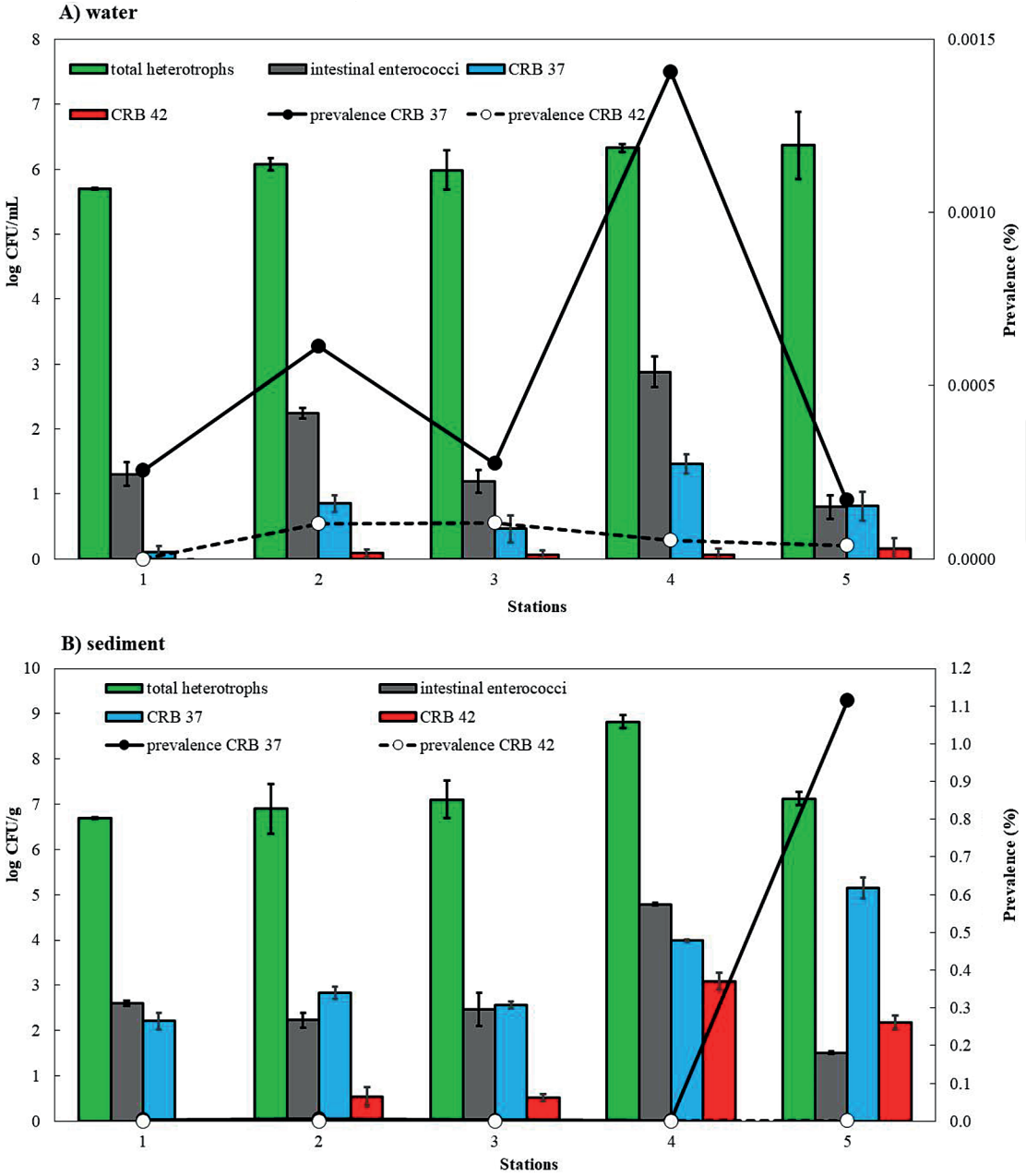

Figure 5

Numbers of bacteria per 1 mL of water (A) or 1 g of wet sediment (B) at stations along the river. Mean values of triplicate measurements and standard deviations are presented. CRB37, CRB42 - carbapenem-resistant bacteria grown at 37 and 42°C, respectively. Prevalence of CRB among total heterotrophic bacteria was calculated as (CFU_{CRB} / $CFU_{heterotrophs}$) x 100.

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as compared to corresponding CRB37, suggesting selection by an elevated temperature of incubation. CRB42 showed a significantly positive correlation with CRB37 ($r = 0.821$) and total heterotrophs ($r = 0.782$), but statistically insignificant correlation with intestinal enterococci ($r = 0.322$).

The list of CRB37 and CRB42 identified by MALDI-TOF MS is given in Table 4. The CRB37 were represented by intrinsically resistant bacterial genera with only the genus *Pseudomonas* having acquired resistance to carbapenems (EUCAST, 2017). Among CRB42, dominance by genera with acquired resistance to carbapenems was found, with the exception of one *Cupriavidus* sp. and one *Acidovorax temperans* isolate. The cultivation at 42°C allowed the isolation of 9 carbapenem-resistant bacteria from the critical list by WHO (2107) (*A. baumannii, K. pneumoniae* and *Klebsiella* sp.), for which the confirmation of resistance to carbapenems was performed. All isolates were confirmed resistant to meropenem and imipenem according to EUCAST (2017) breakpoints for clinical isolates, with MIC values ≥ 16 mg/L (data not shown). Carbapenem-resistant *A. baumannii* and *K. pneumoniae* were isolated only from water at Station 5, after discharge of wastewater from a general hospital. The MLST of carbapenem-resistant *A. baumannii* isolate showed that it belongs to the international clonal lineage 2 (IC2) within the ST-195 by the Oxford scheme or ST-2 by the Pasteur scheme (data not shown).

Species with acquired resistance isolated from both water and sediment comprised 46/63 (73%) after cultivation at 37°C, and 35/37 (95%) after cultivation at 42°C. No bacterium was isolated at 42°C (CRB 42) from 50 mL of water or 1 g of sediment at Station 1.

DISCUSSION

The CRB37 were recently detected in river water in Portugal (Tacao et al., 2015), where the number of imipenem-resistant bacteria averaged 2.24 CFU/mL. This concentration is comparable to the concentrations of CRB37 (1.29–7.37 CFU/mL) in the water flow of Krapinica River (Stations 1–3) and Krapina River receiving the general hospital wastewaters (Station 5). However, a much higher concentration of CRB37 (30 CFU/mL) was found in the creek water receiving the urban wastewater of the town Zabok (Station 4). This suggests that the concentration of CRB37 in river water is dependent on the proportion of wastewater that is discharged into the natural recipient.

The prevalence of CRB37 among total heterotrophic bacteria in analysed water samples (0.0002– 0.001%) was much lower than that reported (0.02–15.9%) for untreated drinking water in Portugal (Henriques et al., 2012). The difference in the representation of CRB37 probably arises from different cultivation techniques used in analyses. In the study

TABLE 4
List of bacteria with intrinsic and acquired* resistance to carbapenems according to EUCAST criteria isolated after cultivation at 37°C and 42°C

Isolate	N isolated	Cultivation (°C)	Water or sediment
Acidovorax sp.	1/1	37	Water/sediment
Chryseobacterium sp.	1	37	Water
Elizabethkingia sp.	1	37	Water
*Pseudomonas monteilii**	7	37	Sediment
*Pseudomonas oleovorans**	1	37	Sediment
*Pseudomonas putida**	17	37	Sediment
*Pseudomonas veronii**	1	37	Water
Pseudomonas sp.*	20	37	Sediment
Stenotrophomonas sp.	2/4	37	Water/sediment
Wautersiella sp.	1/4	37	Water/sediment
Wautersiella falsenii	2	37	Sediment
Acidovorax temperans	1	42	Sediment
*Acinetobacter baumannii**	1	42	Water
*Burkholderia cenocepacia**	2	42	Water
*Burkholderia cepacia**	1	42	Water
*Burkholderia pyrrocinia**	1	42	Water
Burkholderia sp.*	10/2	42	Water/sediment
Cupriavidus sp.	1	42	Water
*Enterococcus faecium**	5	42	Water
*Klebsiella pneumoniae**	6/1	42	Water/sediment
Klebsiella sp.*	1	42	Water
Ochrobactrum sp.*	1	42	Sediment
*Ochrobactrum intermedium**	2	42	Sediment
*Pseudomonas otitidis**	2	42	Sediment

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of Henriques et al. (2012) plates supplemented with 5 mg/L of imipenem were used, while isolates in this study had the MIC of imipenem above 16 mg/L. The prevalence of CRB42 was always lower as compared to corresponding CRB37, but reached 0.0001% in water and 0.001% in sediment samples. This prevalence of clinically important CRB42 is considered to be very high, since a prevalence of 0.0006% is reported for urban sewage (Hrenovic et al., 2017b).

The positive correlation of CRB42 with CRB37 and total heterotrophs, but not with intestinal enterococci, suggests that entry of CRB42 into the riverine ecosystem is not necessarily connected to faecal pollution. The number of CRB42 is rather dependent on the type of pollution, and is associated with the discharge of wastewaters from different human and animal healthcare centres. Once in the environment, CRB42 behave as a part of the indigenous bacterial population. Similar behaviour of CRB42 has been reported for different stages of a secondary wastewater treatment plant (Hrenovic et al., 2017b).

A variety of environmental bacterial species of Proteobacteria, Firmicutes and Bacteroidetes possess intrinsic resistance to carbapenem (EFSA Panel on Biological Hazards, 2013). However, intrinsic resistance to carbapenems is not common among clinically important bacteria (Meletis, 2016). Clinical isolates of carbapenem-resistant bacteria possess acquired resistance and represent a global healthcare problem (Meletis, 2016). Previous studies (Hrenovic et al., 2016; Hrenovic et al., 2017a,b) have shown that cultivation of carbapenem-resistant bacteria at 37 and 42°C is useful for distinguishing the presumably environmental (CRB37) from the presumably clinically important (CRB42) bacteria from environmental samples. In this study the presented diversity of species from river water and sediment confirmed that elevated temperature favours the isolation of clinically important CRB42.

The CRB37 were dominated by intrinsically resistant bacteria, while only *Pseudomonas* spp. had acquired resistance, which is in agreement with the diversity of carbapenem-resistant bacteria from river water (Tacao et al., 2015) and untreated drinking water in Portugal (Henriques et al., 2012). Therefore, the CRB37 could generally be considered as part of the indigenous bacterial population of the riverine ecosystem.

The cultivation at 42°C allowed the isolation of a higher diversity of carbapenem-resistant bacteria, together with *A. baumannii* and Enterobacteriaceae, which are listed by WHO (2107). Carbapenem-resistant *A. baumannii* and *K. pneumoniae* were exclusively isolated from river water at Station 5 after discharge of the wastewater of the general hospital. The molecular characterization of carbapenem-resistant *A. baumannii* isolate classified it as ST-195 inside IC2. This clonal lineage was also found to be predominant among clinical isolates of *A. baumannii* in European hospitals, including those of Croatia (Higgins et al., 2010; Seruga-Music et al., 2017). This indicates that the above-mentioned emerging hospital pathogens isolated from river water are presumably of clinical origin. They are discharged via untreated hospital wastewaters into natural water bodies. In natural water, *A. baumannii* is able to multiply and survive for more than 50 days (Hrenovic et al., 2016). Moreover, the statistical analyses showed that CRB42 behave in the environment as part of the indigenous bacterial population. This implies the need for disinfection of hospital wastewater prior to its discharge into the receiving natural water body, in order to avoid the consequent public-health threat.

Anthropogenic pressure in the form of discharge of untreated wastewaters causes bacteriological changes in the riverine ecosystem. The bacteriological changes are accompanied by an increase of silt and clay fractions, together with the concentration of heavy metals in sediments. The most worrying bacteriological change is the input of anthropogenically derived CRB42 from urban and especially hospital wastewaters. Dilution and auto-purification potential of the river was not evidenced through the river flow. This suggests that CRB42, once discharged in a riverine ecosystem, could be spread through the natural water bodies or accumulate in river sediments. The contaminated river water and sediment thus represent a potential source of clinically important CRB42.

CONCLUSIONS

Anthropogenic pressure, in the form of discharge of untreated wastewaters, cause the bacteriological changes of the riverine ecosystem. The bacteriological changes are accompanied by an increase in silt and clay fractions together with increased concentration of heavy metals in sediments. The input of wastewaters from human and animal healthcare centres results in the appearance of clinically important CRB42 in both river water and sediment. Emerging hospital pathogens *A. baumannii* and *K. pneumoniae* were confirmed among CRB42 only in river water sampled after the discharge of wastewater from a general hospital. Disinfection of hospital wastewater prior to its discharge into the natural environment should be performed in order to avoid both the propagation of CRB42 in the environment and consequent public-health threat.

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