Croatian Science Foundation

Abundance of carbapenem-resistant bacteria in wastewater treatment plant

Tomislav Ivankovic, Faculty of Science, University of Zagreb, Croatia
Svjetlana Dekic, Faculty of Science, University of Zagreb, Croatia
Jasna Hrenovic, Faculty of Science, University of Zagreb, Croatia
Marin Ganjto, Zagreb Wastewater - Management and Operation Ltd., Zagreb, Croatia

Annual Conference 2017 Microbiology Society, Edinburgh, April 5. 2017

BACKGROUND

- From 2015 - 2019. project „Natural habitat of clinically important *Acinetobacter baumannii*" funded by Croatian Science Foundation

- *A. baumannii* is an emerging opportunistic pathogen causing hospital-acquired infections, multi-drug resistant, extensive-drug resistant and pan-drug resistant

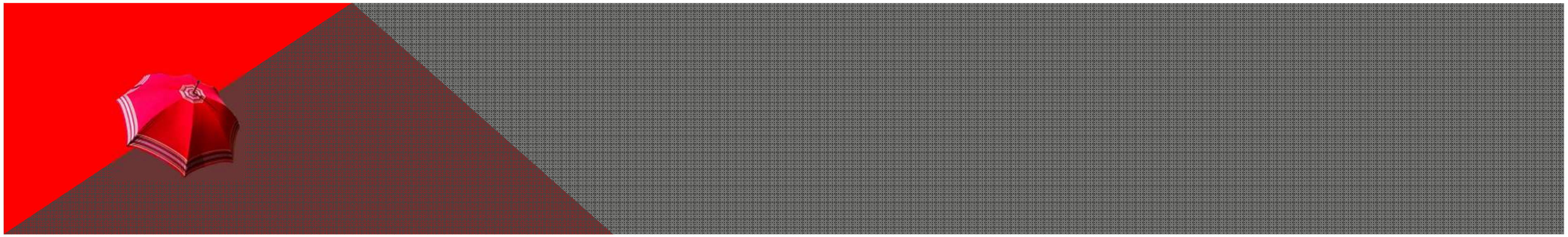
- carbapenem-resistance in clinical isolates of *Acinetobacter baumannii* rapidly increased from 10 % in 2008 to 87 % in 2015

BACKGROUND

- One of the tasks was monitoring of carbapenem-resistant bacteria in wastewater treatment plant (WWTP)

- Carbapenems are beta-lactam class antibiotics, used for treatment of MDR bacterial infections and are widely used in hospital patients

- Resistance to carbapenems has been worldwide confirmed in *Enterobacteriaceae* (*Klebsiella pneumoniae*), *Pseudomonas aeruginosa* and *Acinetobacter baumannii*



https://www.statnews.com/2017/02/27/who-list-bacteria-antibiotic-resistance/

Carbapenem - Wi... | WHO releases l... | Bakteriologija Pmf | Naturaci - Prirodn...

File Edit View Favorites Tools Help

STAT Sections Topics Multimedia Popular STAT Plus Job Board About Newsletters Log In / Subscribe

HEALTH

WHO releases list of world's most dangerous superbugs

By HELEN BRANSWELL @HelenBranswell

FEBRUARY 27, 2017

"Within a generation, without new antibiotics, deaths from drug-resistant infection could reach 10 million a year. Without new medicines to treat deadly infection, lifesaving treatments like chemotherapy and organ transplant, and routine operations like caesareans and hip replacements, will be potentially fatal."

The full list is:

Priority 1: Critical

1. Acinetobacter baumannii, carbapenem-resistant
2. Pseudomonas aeruginosa, carbapenem-resistant
3. Enterobacteriaceae, carbapenem-resistant, ESBL-producing

Priority 2: High

4. Enterococcus faecium, vancomycin-resistant
5. Staphylococcus aureus, methicillin-resistant, vancomycin-intermediate and resistant

EXPERIMENT

- Abundance of carbapenem-resistant bacterial population (CRBP) was monitored during 10 months in major parts of WWTP of city of Zagreb

- WWTP – 1 200 000 PE, secondary type treatment (activated sludge)
- combined sewage consisted of domestic, industrial, hospital and storm wastewaters

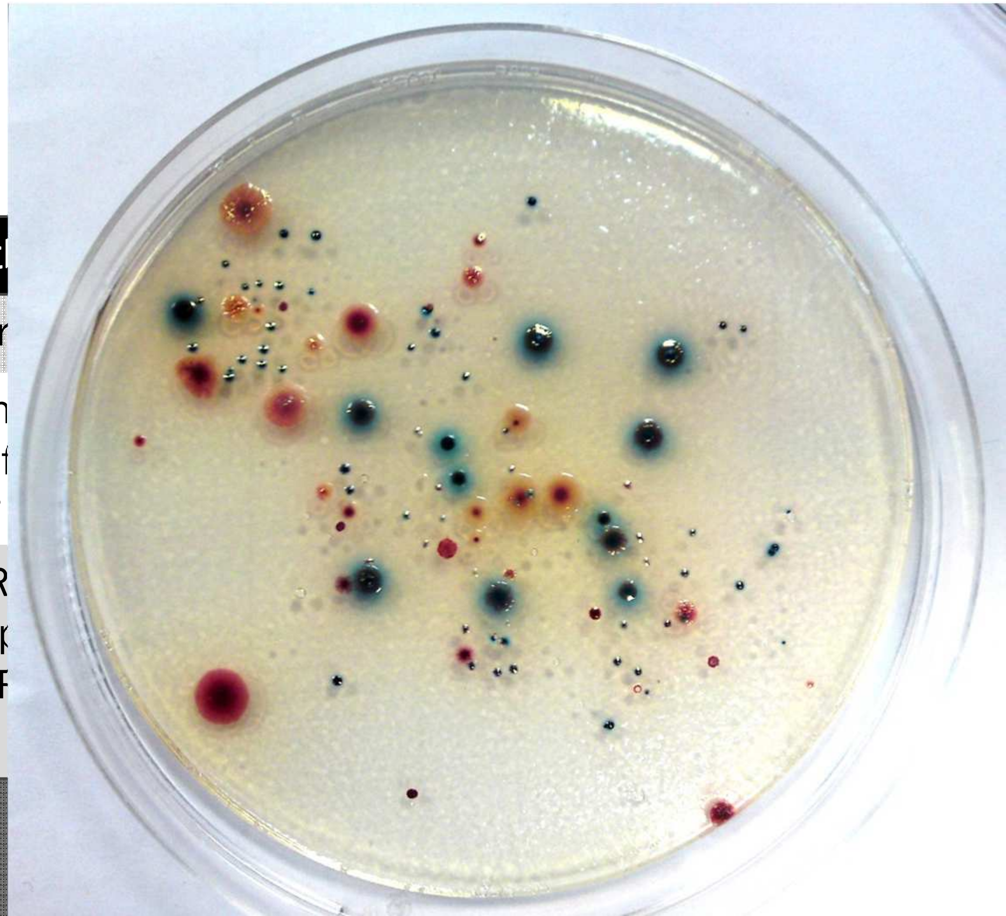
- 20 samplings in total

  September 2015. – June 2016.

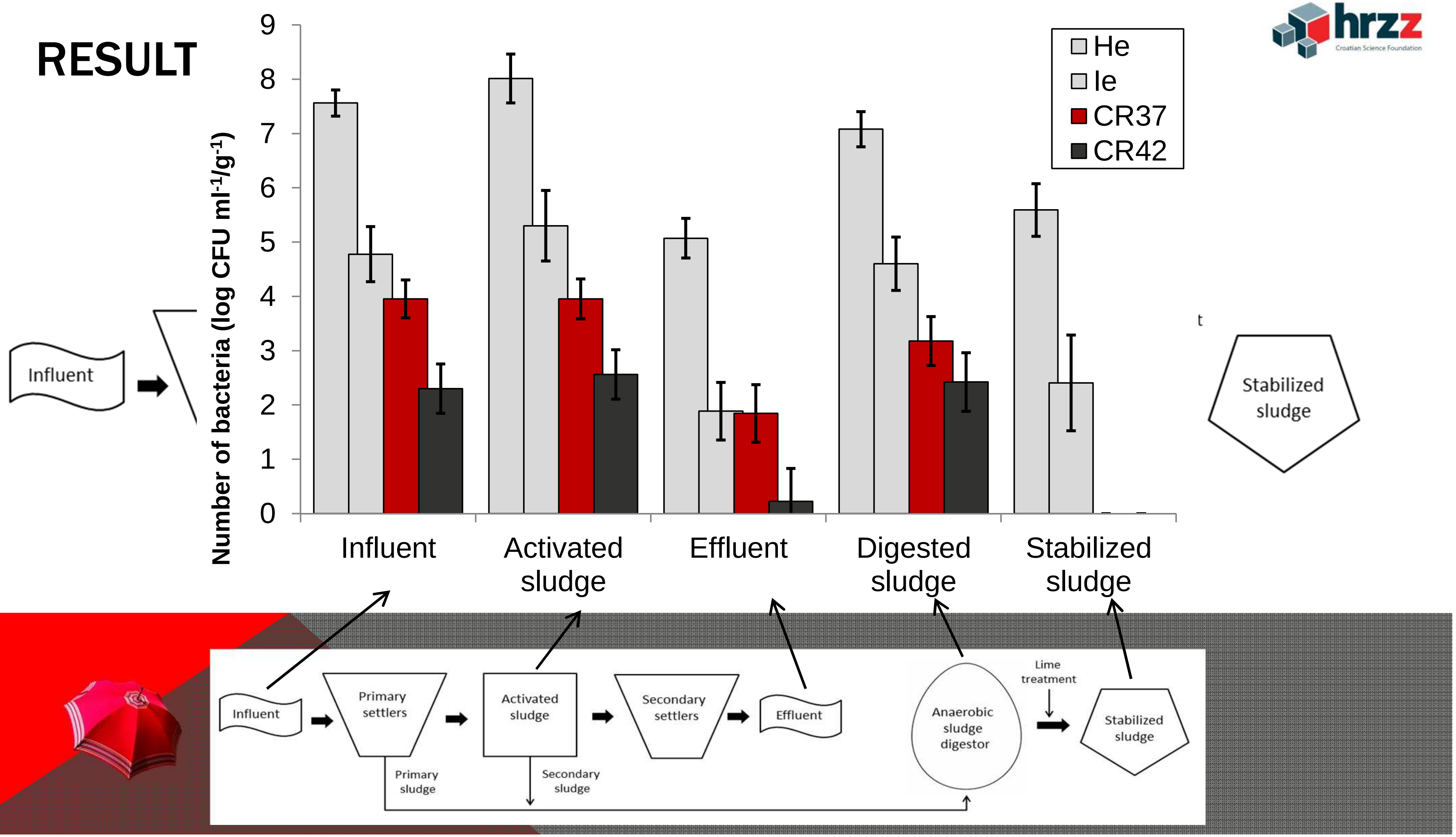
EXPERIMENT

- Bacteriological analyses:

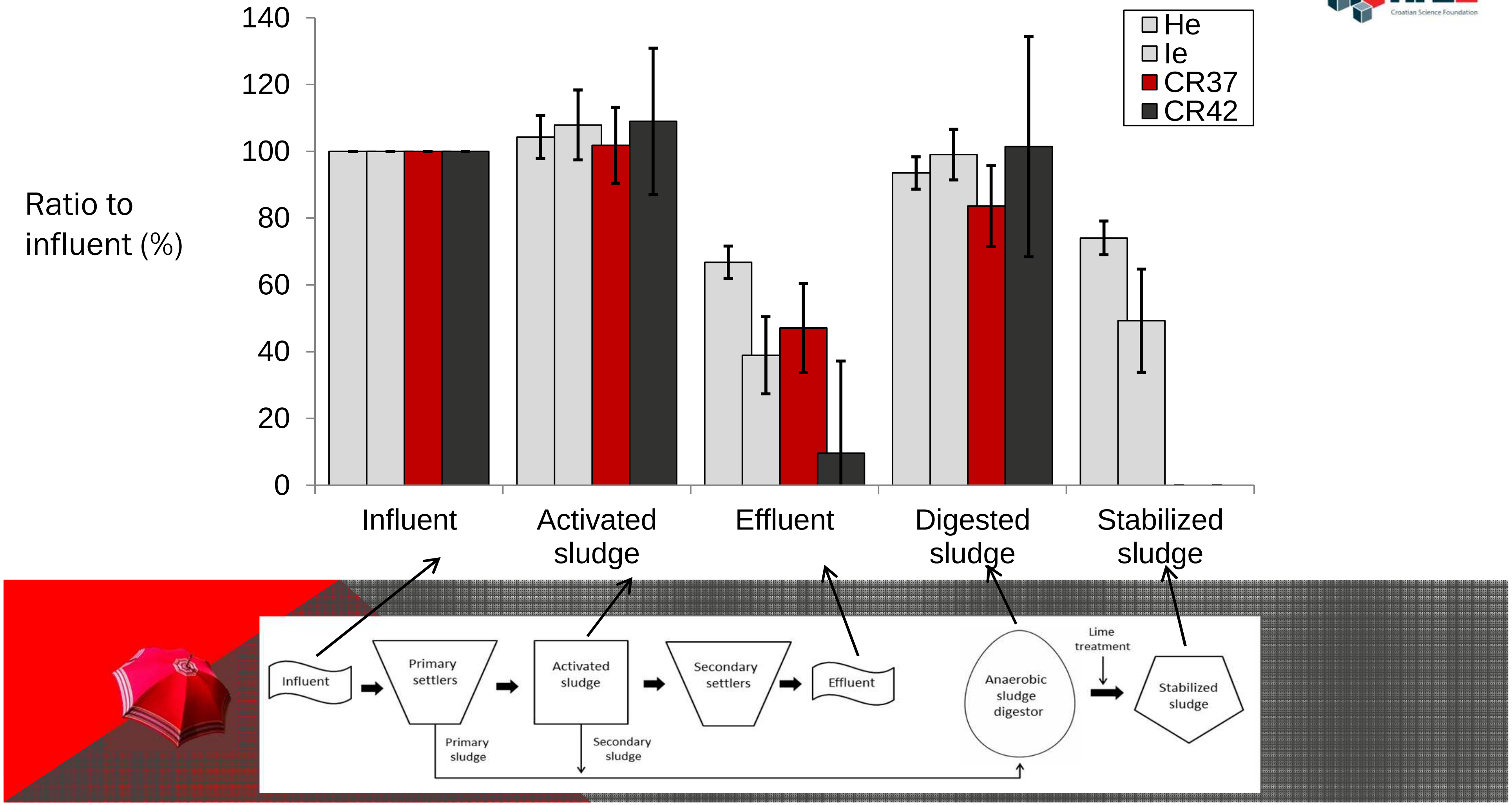
Bacteria	Met
Total heterotrophic bacteria	Nutr
Intestinal enterococci	Slan Conf 44°
CRBP grown at 37°C CRBP grown at 42°C	CHR supp (CHF and

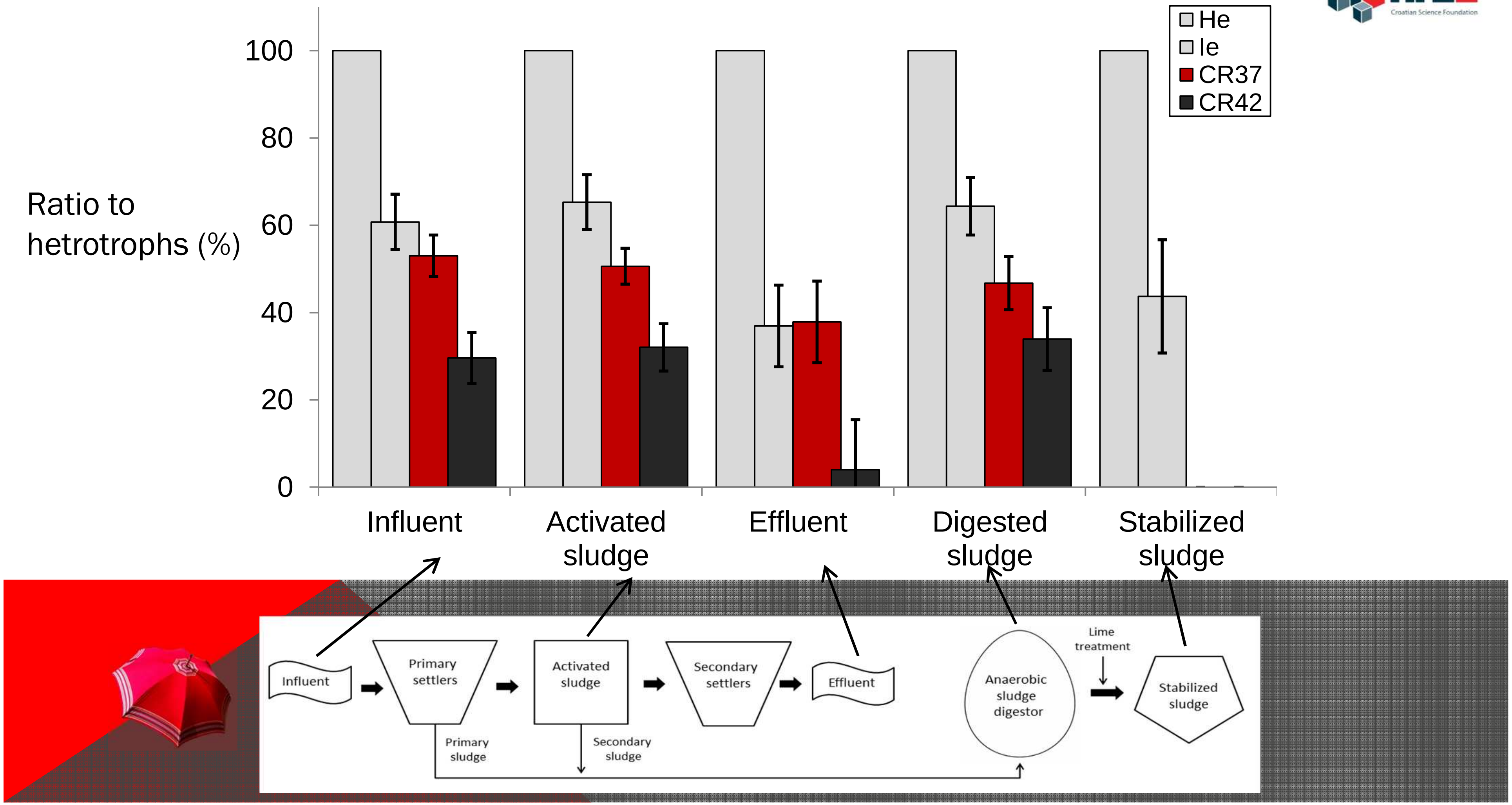


RESULT



Number of bacteria (log CFU ml^{-1}/g^{-1})
0
1
2
3
4
5
6
7
8
9
He
Ie
CR37
CR42
Influent
Activated sludge
Effluent
Digested sludge
Stabilized sludge
Influent
Stabilized sludge
Influent
Primary settlers
Activated sludge
Secondary settlers
Effluent
Primary sludge
Secondary sludge
Anaerobic sludge digestor
Lime treatment
Stabilized sludge


Ratio to influent (%)
140
120
100
80
60
40
20
0
He
Ie
CR37
CR42
Influent
Activated sludge
Effluent
Digested sludge
Stabilized sludge
Influent
Primary settlers
Activated sludge
Secondary settlers
Effluent
Primary sludge
Secondary sludge
Anaerobic sludge digestor
Lime treatment
Stabilized sludge



Ratio to hetrotrophs (%)
100
80
60
40
20
0
He
Ie
CR37
CR42
Influent
Activated sludge
Effluent
Digested sludge
Stabilized sludge
Influent
Primary settlers
Activated sludge
Secondary settlers
Effluent
Anaerobic sludge digestor
Lime treatment
Stabilized sludge
Primary sludge
Secondary sludge

RESULTS

Sample	Q ($m^3\ d^{-1}$)	BOD ($mg\ L^{-1}$)	COD ($mg\ L^{-1}$)
Influent			
MIN	2.6×10^5	73.0	109.0
MAX	5.7×10^5	291.0	421.0
Median	3.2×10^5	170.0	352.5
SD	9.0×10^4	57.2	88.5
Effluent			
MIN	2.5×10^5	2.0	16.0
MAX	5.6×10^5	6.2	32.0
Median	3.1×10^5	3.2	25.0
SD	8.9×10^4	1.4	4.1
He	0.048	0.750	0.799
Ie	0.061	0.781	0.817
CR37	0.026	0.738	0.756
CR42	0.057	0.779	0.788

		Imipenem	Meropenem	Meropenem metabolite
Influent				
MIN		198.4	20.0	48.6
MAX		4958.5	720.8	2348.6
Median		2011.8	169.2	156.5
SD		1550.9	261.2	730.6
Effluent				
MIN		78.4	6.4	6.5
MAX		1137.9	823.2	549.4
Median		256.1	260.2	37.4
SD		347.0	271.0	139.1
Imipenem		1.000	0.054	0.526
Meropenem			1.000	-0.248
Meropenem metabolite				1.000
He		0.781	0.084	0.566
Ie		0.682	0.105	0.659
CR37		0.609	-0.138	0.667
CR42		0.586	0.149	0.516

°C)	O_2 ($mg\ L^{-1}$)	He	Ie	CR37	CR42
.5	0.0	7.2	4.0	3.2	1.5
.8	6.1	8.0	5.8	4.3	3.1
.5	3.4	7.6	4.6	4.0	2.3
.5	1.9	0.2	0.5	0.3	0.4
.7	7.7	4.4	0.7	0.7	-0.9
.6	10.0	5.5	2.7	2.9	1.2
.1	8.7	5.1	1.9	1.8	0.2
.6	0.7	0.4	0.5	0.5	0.6
223	-0.768	1.000			
407	-0.691	0.804	1.000		
039	-0.811	0.803	0.734	1.000	
192	-0.721	0.818	0.777	0.801	1.000

DISCUSSION

- **WWTP are hotspots for proliferation of drug-resistant bacteria ?**

- conditions in WWTP were considered favourable for proliferation of antibiotic-resistant bacteria and resistance gene transfer (Kim et al. 2007; Huang et al. 2012; Davies 2012; Bouki et al. 2013, Rizzo et al. 2013)

- In here presented research the **CRBP** were not favoured in relation to total heterotrophic population or intestinal enterococci during the WWTP process

- Simillar conclusions to here presented research were reported in Ahmad et al. 2009; Munck et al. 2015; Bengtsson-Palme et al. 2016

Kim S, Jensen JN, Aga SD, Weber SA. 2007. Tetracycline as a selector for resistant bacteria in activated sludge. Chemosphere 66:1643–1651
Huang JJ, Hu HY, Lu SQ, Li Y, Tang F, Lu Y, Wei B. 2012. Monitoring and evaluation of antibiotic-resistant bacteria at a municipal wastewater treatment plant in China. Environ. Int. 42:31–36
Davies J. 2012. Sanitation: sewage recycles antibiotic resistance. Nature 487:302.
Bouki C, Venieri D, Diamadopoulos E. 2013. Detection and fate of antibiotic resistant bacteria in wastewater treatment plants: A review. Ecotox. Environ. Saf. 91:1-9.
Rizzo L, Manaia C, Merlin C, Schwartz T, Dagot C, Ploy MC, Michael I, Fatta-Kassinos D. 2013. Urban wastewater treatment plants as hotspots for antibiotic re-sistant bacteria and genes spread in to the environment: a review. Sci. Total Environ. 447:345–360.
Al-Ahmad A, Hai A,Unger J,Brunswick-Tietze A,Wiethan J, Kummerer K. 2009. Effects of a realistic mixture of antibiotics on resistant and non-resistant sewage sludge bacteria in laboratory-scale treatment plants. Arch. Environ. Contam. Toxicol. 57:264–273.
Munck C, Albertsen M, Telke A, Ellaban M, Nielsen PH, Sommer MOA. 2015. Limited dissemination of the wastewater treatment plant core resistome. Nature Comms. 6:8452.
Bengtsson-Palme J, Hammaren R, Pal C, Ostman M, Bjorlenius B, Flach CF, Fick J, Kristiansson E, Tysklind M, Larsson DGJ. 2016. Elucidating selection processes for antibiotic resistance in sewage treatment plants using metagenomics. Sci. Total Environ. 572:697-712.

DISCUSSION

- The presence of antibiotics in wastewaters selects in favour of antibiotic resistant bacteria?

- resistant bacterial strains have selective advantage over susceptible strains even at antibiotic concentrations way lower than respective minimal inhibitory concentration (sub-MIC) (Liu et al. 2011; Gullberg et al. 2011; Gullberg et al. 2014)

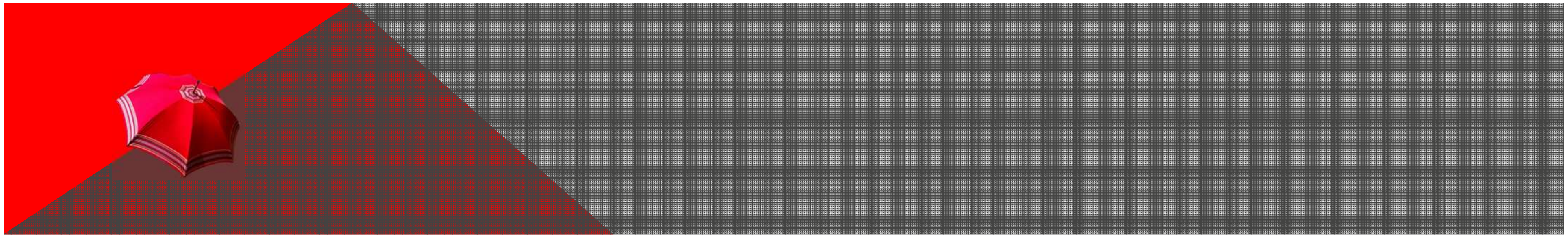
- Even though imipenem and meropenem concentrations were above the theoretical selective concentration (PNEC) **no selective advantage** for carbapenem-resistant bacteria was recorded in any part of full-scale WWTP treatment process in here presented research

Liu A, Fong A, Becket E, Yuan J, Tamae C, Medrano L, Maiz M, Wahba C, Lee C, Lee K, Tran KP, Yang H, Hoffman RM, Salih A, Miller JH. 2011. Selective advantage of resistant strains at trace levels of antibiotics: a simple and ultrasensitive color test for detection of antibiotics and genotoxic agents. Antimicrob. Agents. Chemother. 55: 1204–1210.
Gullberg E, Albrecht LM, Karlsson C, Sandegren L, Andersson DI. 2014. Selection of a multidrug resistance plasmid by sublethal levels of antibiotics and heavy metals. mBio 5:e01918-14.
Gullberg E, Cao S, Berg OG, Ilbäck C, Sandegren L, Hughes D, Andersson DI. 2011. Selection of resistant bacteria at very low antibiotic concentrations. PLoS Pathog. 7:e1002158.

CONCLUSIONS

- The CRBP was found in all parts of Zagreb WWTP except lime treated stabilized sludge

- Relative abundance of CRBP when compared to total bacterial count was very similar in influent, activated and digested sludge, and even lowered in effluent
- No significant correlation was found between any physico-chemical parameter of wastewater or sludge nor carbapenem concentrations in wastewater that would influence CRBP in particular



CONCLUSIONS

- There was no evidence that Zagreb WWTP selects for CRBP; resistant bacteria were “behaving” as a part of regular microbial flora through all the parts of treatment process

- 10 months of monitoring showed the importance of effluent and sludge disinfection in preventing dissemination of carbapenem-resistant bacteria to the environment

THANK YOU FOR YOUR ATTENTION!

from Croatia with love!