

Supplementary file

Impregnation of activated carbon with ionic liquids for simultaneous removal of micropollutants and antibiotic-resistant bacteria from wastewater

Lucie Řepečká^{a,b,*}, Jan Bednárek^a, Tereza Stachurová^c, Anna Gavlová^a, Kateřina Smutná^a, Jana Vaštyl^a, Marek Šváb^d, Martina Vráblová^a

^a VSB-Technical University of Ostrava, CEET, Institute of Environmental Technology, 17. listopadu 15, Ostrava 70800, Czech Republic; jan.bednarek@vsb.cz, anna.gavlova@vsb.cz, katerina.smutna@vsb.cz, jana.vastyl@vsb.cz, martina.vrablova@vsb.cz

^b VSB-Technical University of Ostrava, Faculty of Materials Science & Technology, 17. listopadu 15, Ostrava 70800, Czech Republic

^c University of Ostrava, Faculty of Science, Department of Biology and Ecology, Chittussiho 10, 710 00, Ostrava, Czech Republic; tereza.stachurova@osu.cz

^d Dekonta, Dřetovice 109, 273 42 Stehelčevy, Czech Republic; marek.svab@deconta.cz

* Corresponding author. Email address: lucie.repecka@vsb.cz

S1 Materials and methods

S1.1 Nitrogen adsorption-desorption porosimetry

The textural properties of GAC and iGAC were measured with the aid of a commercial static adsorption set-up 3Flex (Micromeritics, USA). The samples were degassed at room temperature and 0.1 mbar for 48 hrs. The specific surface and pore volume was achieved with the use of Rouquerol Brunauer-Emmett-Teller theory, the micropore volume was calculated by the t-plot method, using the Carbon Black STSA standard isotherm, and the maximum pore volume was obtained by Horwath-Kawazoe method, assuming the slit pore geometry.

S1.2 Fourier transform infrared spectroscopy (FTIR)

The FTIR spectra of GAC and iGAC were measured by a Nicolet iS10 FTIR Spectrometer (Thermo Scientific, MA, USA). The measurements were carried out in the range 500-4000 cm^{-1} with a resolution of 2 cm^{-1} using transmission mode.

A small amount of sample was placed on the diamond measuring window on ATR attachment where the spectrum was collected. Each spectrum consists of at least 64 scans lasting 1 second. Before each measurement, the background is collected to eliminate apparatus and environmental effects. Each sample spectrum is ratioed.

S1.3 Scanning electron microscopy (SEM)

For microscopic investigations of GAC and iGAC scanning electron microscope Tescan Vega (Tescan, Czech Republic) with Tungsten cathode was used. Micrographs were obtained with using energy-dispersive X-ray spectroscopy (EDS) with an acceleration voltage of 15 keV. Samples before imaging were gold sputtered in order to ensure adequate electron conductivity. Photos were taken from random places of samples to ensure an unbiased examination of the samples (without focusing on unnecessary anomalies or artefacts).

Table S1. Textural properties of granular activated carbon Hydraxfin 30 N

Specific surface ($\text{m}^2 \cdot \text{g}^{-1}$)	Iodine value ($\text{mg} \cdot \text{g}^{-1}$)	Ash (%)	Skeletal density ($\text{g} \cdot \text{cm}^3$)	Specific surface of mesopores ($\text{cm}^3_{\text{liq}} \cdot \text{g}^{-1}$)	Specific surface of pores ($\text{cm}^3_{\text{liq}} \cdot \text{g}^{-1}$)	Specific surface of micropores ($\text{cm}^3_{\text{liq}} \cdot \text{g}^{-1}$)
959	1 001	12.9	1.92	0.07	0.51	0.44

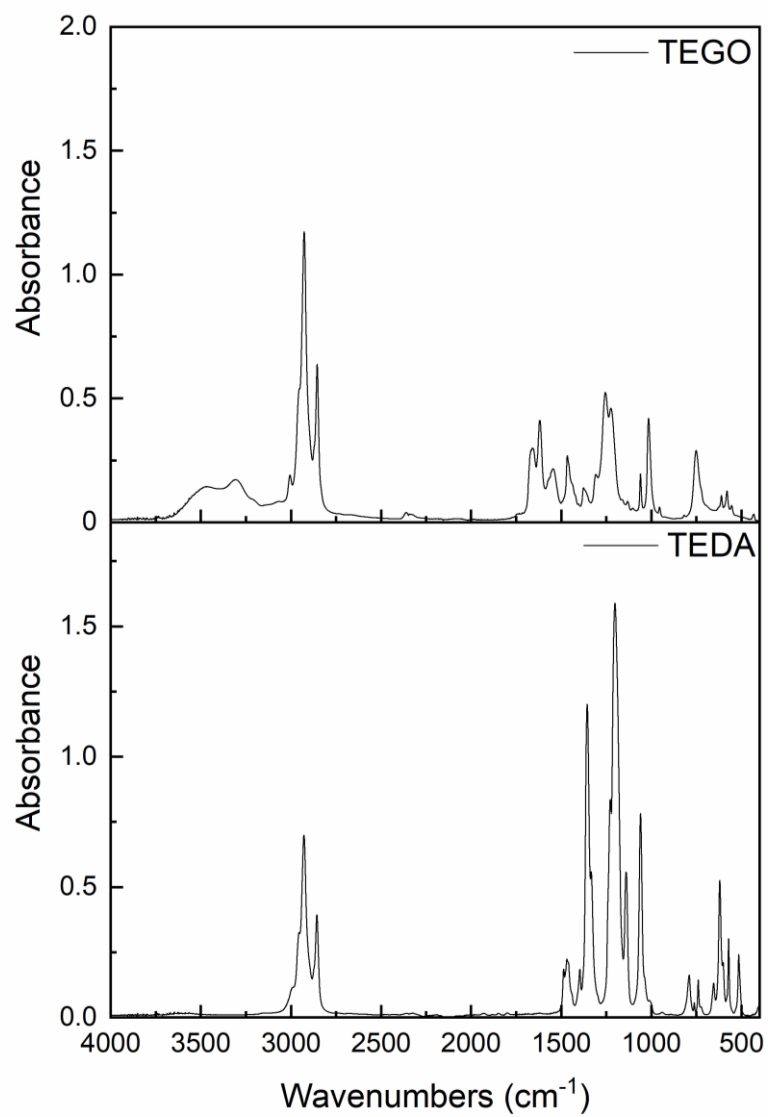
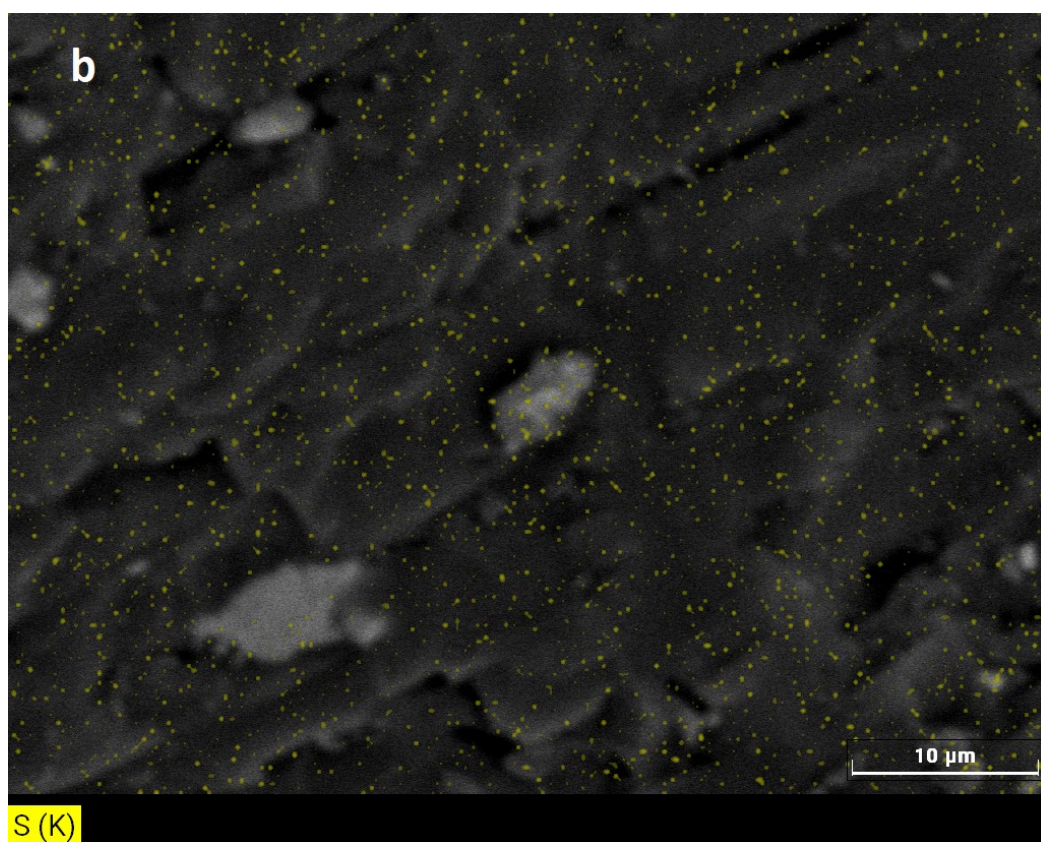
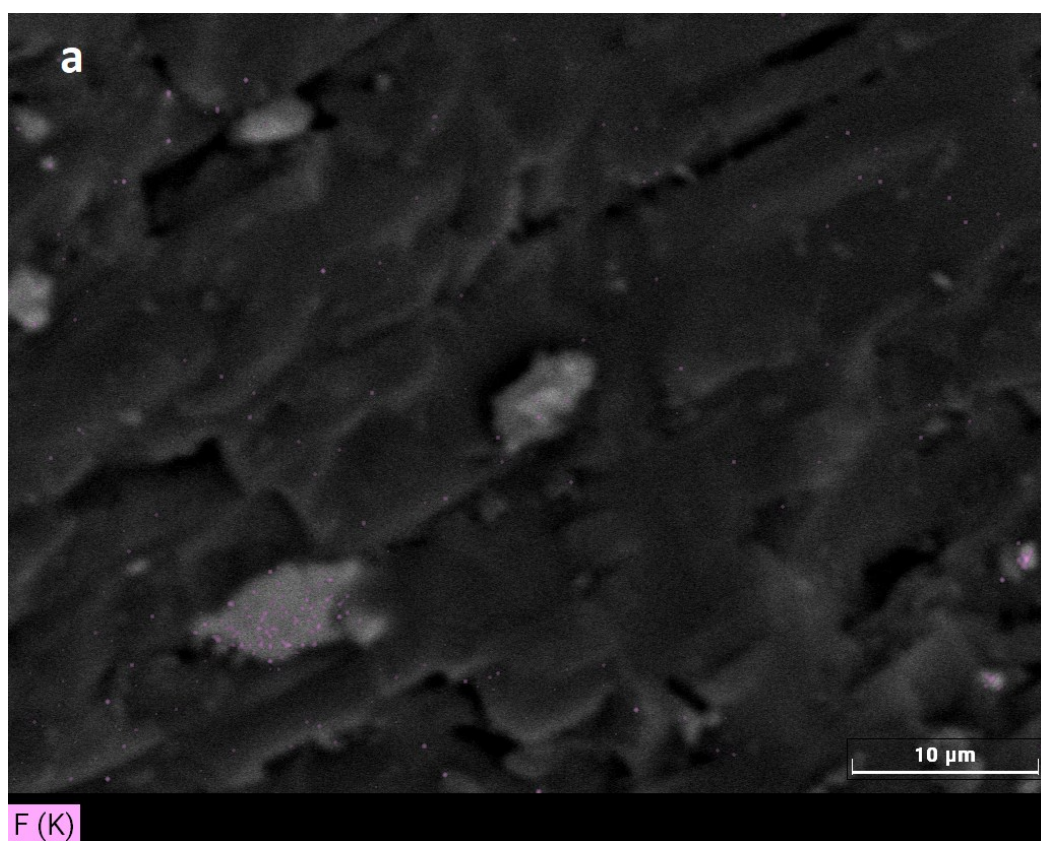
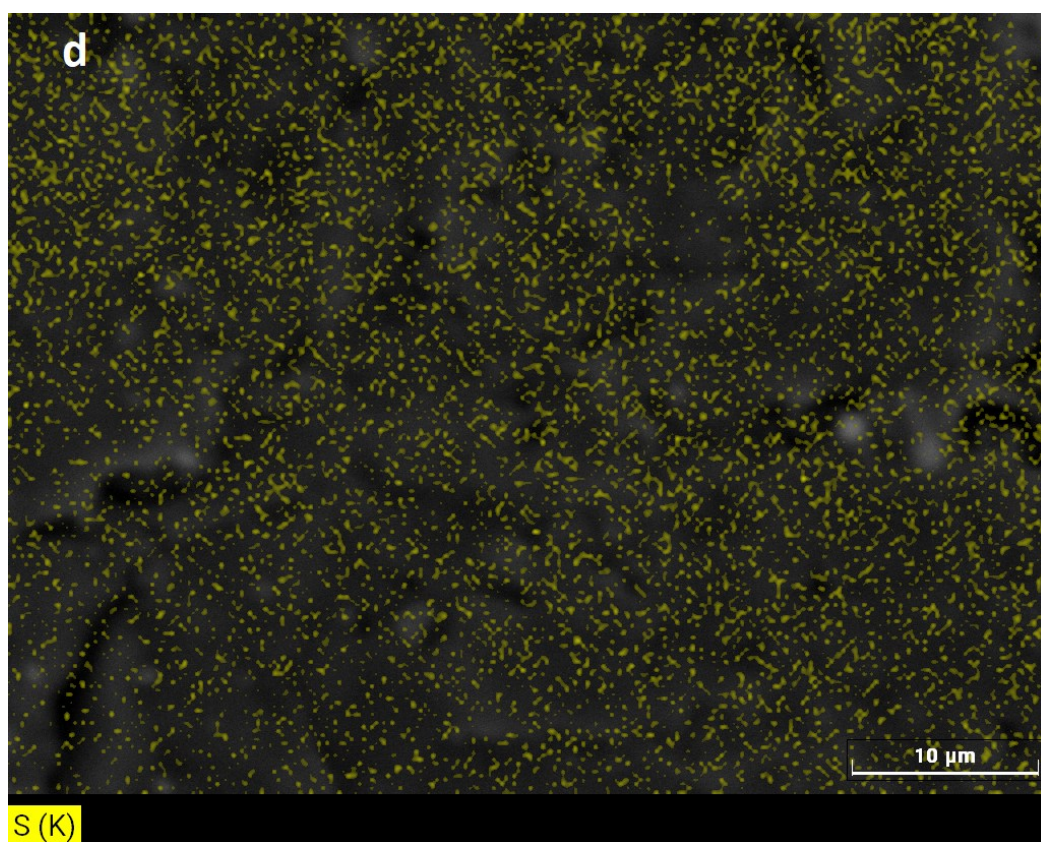
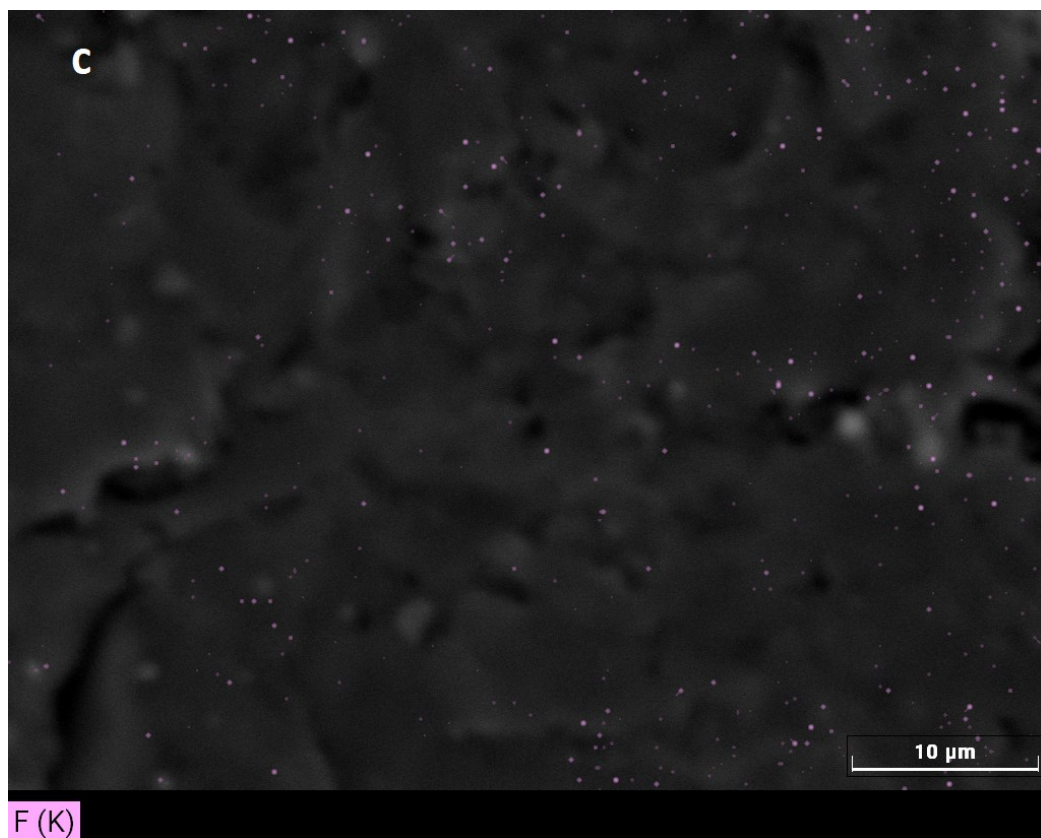


Figure S1. FTIR analysis of ionic liquids used for the impregnation of GAC





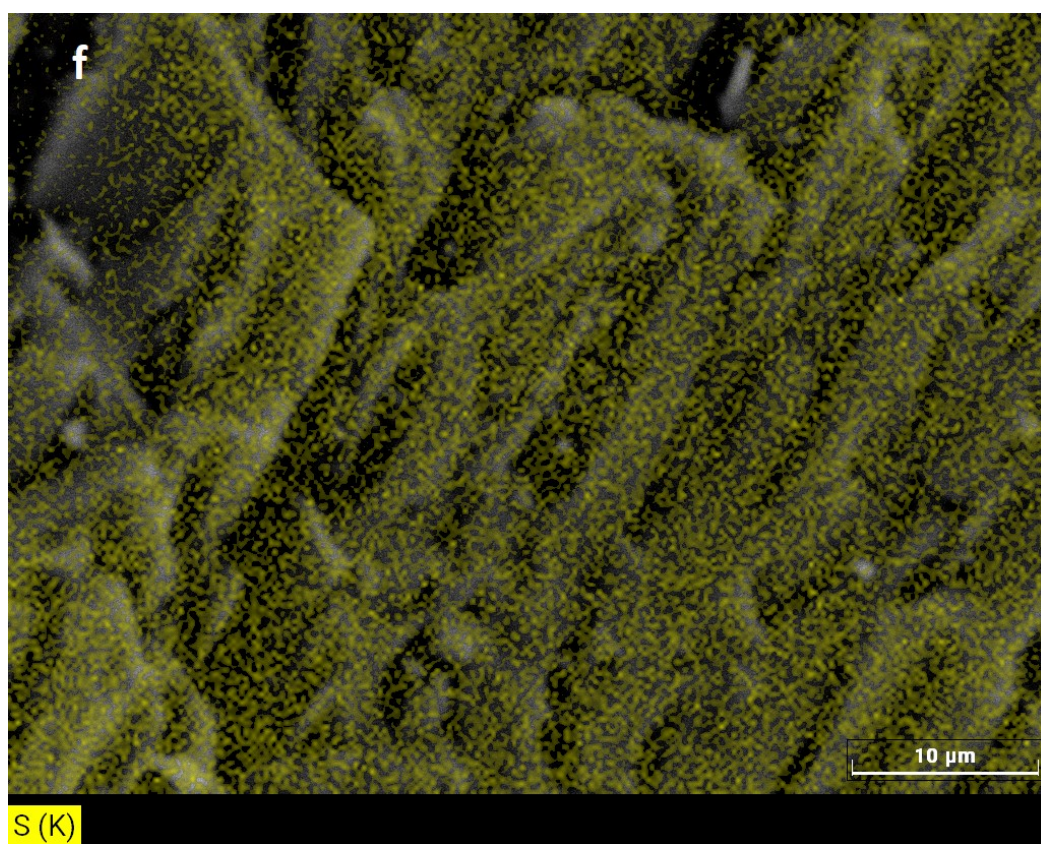
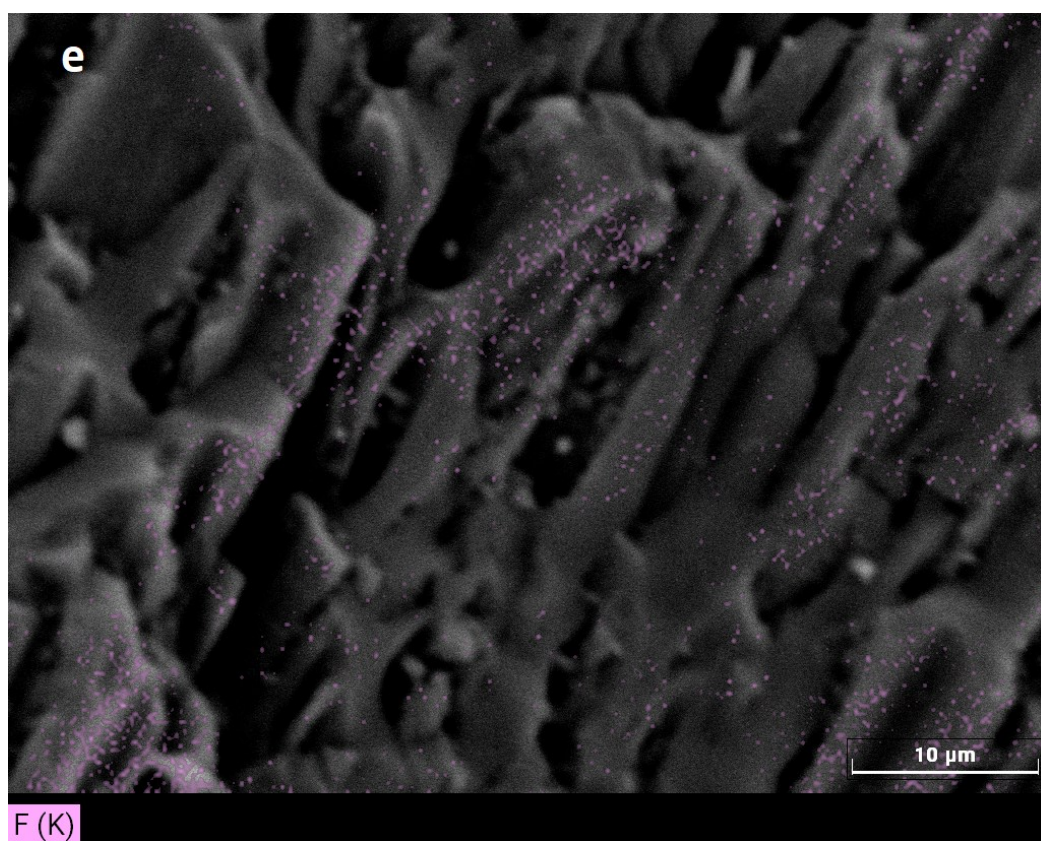


Figure S2. SEM/EDS of the presence of sulphur (yellow) and fluorine (violet) on the surfaces of GAC (a, b), GAC+TEGO (c, d) and GAC+TEDA (e, f)

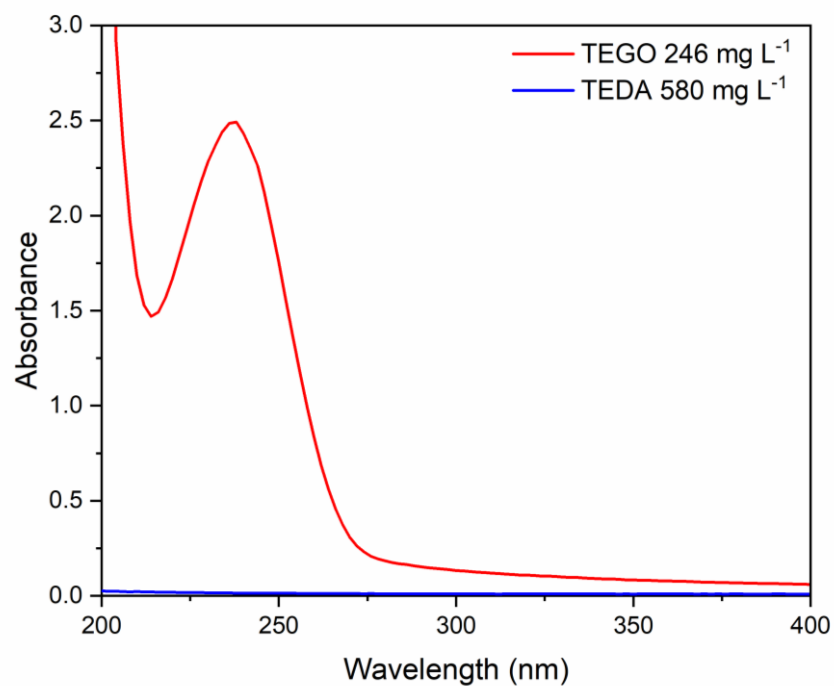


Figure S3. Determination of ionic liquids in water-IL mixtures with UV-VIS spectroscopy

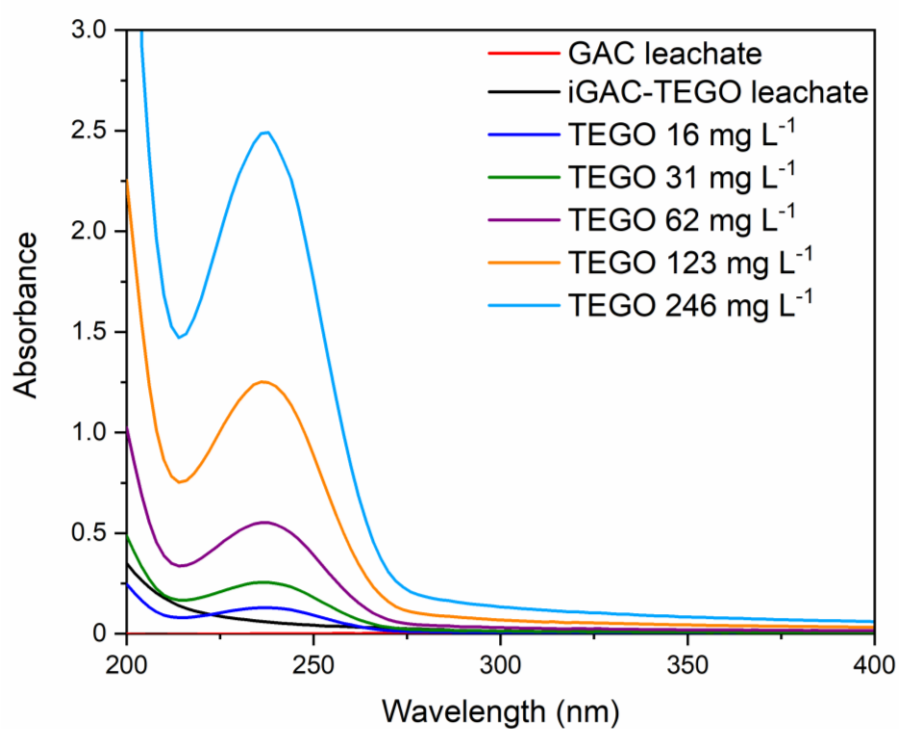


Figure S4. Calibration of TEGO in water-TEGO mixture and determination of the rest IL concentration in GAC and iGAC-TEGO leachates.

Table S2. Primers and PCR conditions used for detection of resistance genes in bacterial isolates from wastewater

Gene	Primer	5'-3' sequence	Standard PCR conditions	Reference
16S rRNA	984F	GAACGCGAAGAACCTTAC	95°C 2min, 35x(95°C 45s, 55°C 30s, 72°C 60 s), 72°C 10min	Heuer et al. 1997
	1378R	CGGTGTGTACAAGGCCGGAACG		
<i>bla</i> TEM	bla-TEM-RX	CTTTATCCGCCTCCATCCAGTCTA	94°C 2min, 40x(94°C 15s, 60°C 30s, 72°C 45s), 72°C 10min	Marti et al. 2013
	bla-TEM-FX	GCKGCCAACTTACTTCTGACAACG		
<i>bla</i> NDM-1	NDM-Rm	CGGAATGGCTCATCACGATC	94°C 2min, 40x(94°C 15s, 53°C 30s, 72°C 45s), 72°C 10min	Poirel et al. 2011
	NDM-Fm	GGTTTGGCGATCTGGTTTTTC		
<i>bla</i> OXA-48	bla-OXA-48-R	GAGCACTTCTTTTGTGATGGC	95°C 5min, 35 (95°C 60s, 56°C 60s, 72°C 60s), 72°C 5min	Mlynarcik et al. 2016
	bla-OXA-48-F	TTGGTGGCATCGATTATCGG		
<i>bla</i> KPC	bla-KPC-R	TTACTGCCCCGTTGA CGCCC	94°C 3min, 30 (94°C 60s, 55°C 60s, 72°C 60s), 72°C 5min	Ribeiro et al. 2016
	bla-KPC-F	ATGTCACTGTATCGCCGTCT		
<i>mecA</i>	mecA-LP	GATAGCAGTTATATTCTA	95°C 5min, 35x(94°C 15s, 48°C 60s, 72°C 80s), 72°C 4min	Colomer-Lluch et al. 2011
	mecA-UP	ATACTTAGTTCTTTAGCGAT		
<i>tetW</i>	tet(W)-RV	GGGCGTATCCACAATGTTAAC	94°C 2min, 40x(94°C 15s, 60°C 30s, 72°C 45s), 72°C 10min	Marti et al. 2013
	tet(W)-FW	GAGAGCCTGCTATATGCCAGC		
<i>sulI</i>	sulI-RV	AAAAATCCCATCCCCGGRTC	94°C 2min, 40x(94°C 15s, 65°C 30s, 72°C 45s), 72°C 10min	Heuer and Smalla, 2007
	sulI-FW	CTGAACGATATCCAAGGATTYCC		
<i>vanA</i>	vanA-R	GATTCCGTA CTGCAGCCTGATT	94°C 3min, 40x(94°C 15s, 60°C 30s, 72°C 60s), 72°C 10min	Rathnayake et al. 2012
	vanA-F	TGTGCGGTATTGGGAAACAG		

S1.4 LC-MS Methods

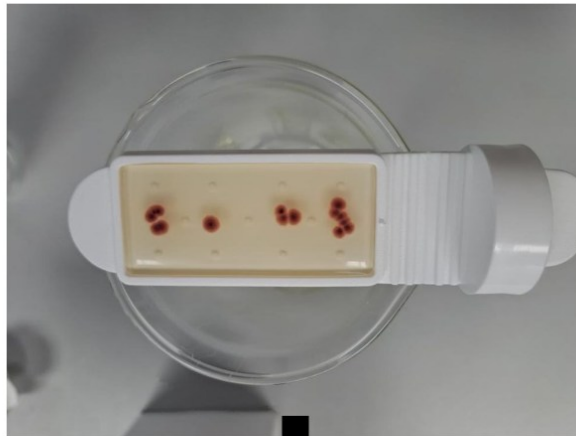
For the chromatographic separation, Kinetex 2.6 μm XB-C18 100 Å, 100 x 2.1 mm analytical column guarded with a C18 guard column (both Phenomenex, Torrance, CA, USA) was used along with the binary mobile phase (MP), which consisted of ultrapure water (MP A) and mixture of acetonitrile and methanol (1:1; v:v) (MP B), both acidified with formic acid to obtain 0.1 % solution. The analysis was 15 minutes long and the applied gradient was as follows: 0 – 1 min, 5 % B; 1 – 6 min, 5-75 % B; 6 – 9 min, 75-98 % B; 9 – 10.5 min, 98 % B; 10.5 - 11 min, 98 – 5 % B, 11 – 15 min, 5 % B; the flow rate was 0.3 ml min⁻¹ and the injection volume was 10 μL . For the mass-spectrometric detection, multiple reaction monitoring (MRM) type of scan was used. Two MRM transitions were used for each analyte and internal standard (IS), one for quantification and one for qualification. The MRM transitions were measured in positive mode as well as in negative mode with electrospray used as an ionization source. MRM transitions and retention times of analytes and internal standards are all listed in Tab S3. The MS operational settings were: capillary voltage 5.5 kV, capillary temperature 450 °C, the nebulizer gas pressure 50 psi, the heater gas pressure 60 psi. The quantification was done using the IS method, a six-point calibration curve (0.1 $\mu\text{g L}^{-1}$ - 200 $\mu\text{g L}^{-1}$) spiked with IS solution was used for this purpose. For the quality control check of each batch run, two QC standards (on 10 $\mu\text{g L}^{-1}$ and 100 $\mu\text{g L}^{-1}$ level) were measured. If needed, the samples were diluted with ultrapure water to fit the calibration range.

Table S3. Overview of analytes and internal standards, their MRM transitions, mass spectrometric parameters and retention times.

Analyte/Internal standard	Retention time (min)	MRM transitions		Entrance Potential (kV)	Collision Energy (kV)	Declustering Potential (kV)	Collision Cell Exit Potential (kV)
		Q1 (<i>m/z</i>)	Q2 (<i>m/z</i>)				
Metformin	0.78	130.11	70.90	10	29	1	28
		130.11	67.70	10	31	1	16
Nicotine	0.86	163.12	129.93	10	27	36	14
		163.12	131.96	10	21	36	14
Cotinine	1.16	177.09	79.88	10	31	71	10
		177.09	98.02	10	27	71	12
Theobromine	3.31	181.03	137.30	10	25	51	18
		181.03	162.90	10	25	51	8
Paracetamol	3.34	152.07	110.00	10	21	51	12
		152.07	92.60	10	31	51	10
Paraxantine	3.71	181.22	123.70	10	25	116	16
		181.22	68.80	10	39	116	8
Theophylline	3.90	181.02	123.89	10	25	76	14
		181.02	95.91	10	31	76	12
Lamotrigine	4.38	255.91	171.69	10	37	86	22
		255.91	157.10	10	43	86	14
Metoprolol	4.45	268.28	115.8	10	23	46	54
		268.28	132.8	10	22	46	32
Caffeine	4.19	195.05	137.91	10	27	76	16
		195.05	109.86	10	31	76	12
Bisoprolol	5.03	326.30	115.80	10	23	6	14
		326.30	55.90	10	57	6	14
1H-benzotriazol	4.46	120.24	65.00	10	27	66	8
		120.24	92.00	10	23	66	12
Telmisartan	6.25	515.14	497.1	10	45	136	16
		515.14	276.1	10	63	136	14
Sum of 5- and 4-methyl-1H-benzotriazol	5.25	134.21	77.00	10	33	66	10
		134.21	78.90	10	25	66	10
DEET	6.87	192.18	118.60	10	23	91	14

		192.10	90.80	10	35	91	14
Irbesartan	6.63	429.09	207.10	10	31	86	24
		429.09	205.10	10	75	86	24
Losartan	6.77	423.08	405.09	10	17	26	20
		423.08	207.03	10	31	26	12
Candesartan	6.86	441.08	423.07	10	15	51	12
		441.08	263.08	10	17	51	10
Diclofenac	7.91	295.98	215.00	10	27	20	14
		295.98	213.90	10	43	20	12
Sulfamethoxazole	5.11	254.08	107.80	10	23	43	14
		254.08	155.80	10	31	43	10
Furosemide	6.18	328.90	284.80	-10	-20	-5	-15
		328.90	204.80	-10	-30	-5	-23
Ibuprofen	12.44	204.97	161.00	-10	-10	-5	-7
		204.97	159.00	-10	-8	-5	-11
Acesulfam K ⁺	3.54	161.96	81.80	-10	-18	-30	-35
		161.96	77.80	-10	-48	-30	-33
Saccharine	4.30	181.92	41.90	-10	-52	-35	-19
		181.92	106.00	-10	-24	-35	-11
Sucralose	4.63	394.89	34.00	-10	-56	-50	-15
		394.89	358.60	-10	-16	-50	-29
Nicotine-d ₄	0.86	167.12	133.93	10	27	36	14
		167.12	135.96	10	21	36	14
Paracetamol-d ₃	3.34	155.02	110.90	10	23	81	12
		155.02	92.90	10	31	81	10
Theophylline-d ₆	3.90	189.03	127.80	10	27	51	16
		189.03	99.80	10	31	51	12
Metoprolol-d ₇	4.45	275.24	122.80	10	21	1	16
		275.24	104.70	10	31	1	42
Telmisartan-d ₃	6.25	518.15	500.20	10	47	40	24
		518.15	279.00	10	63	40	28
Saccharine-d ₄	4.30	185.93	41.90	-10	-58	-30	-19
		185.93	105.70	-10	-26	-30	-49

Evaluation of the antimicrobial tests



8 bit

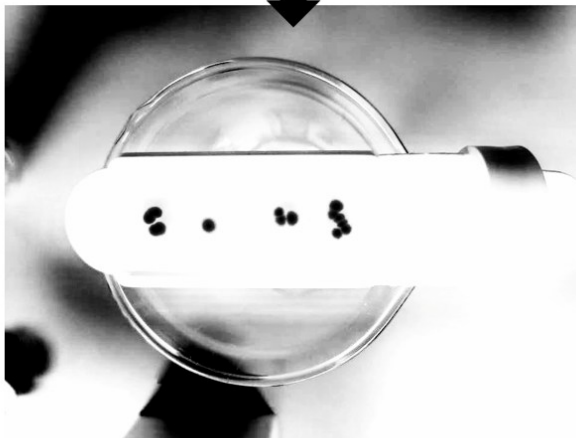
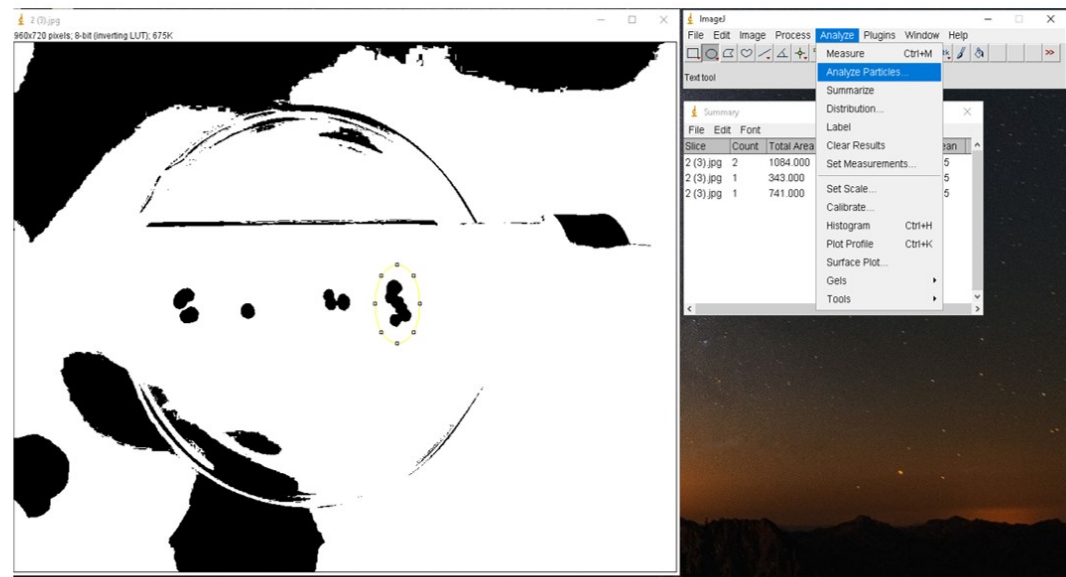
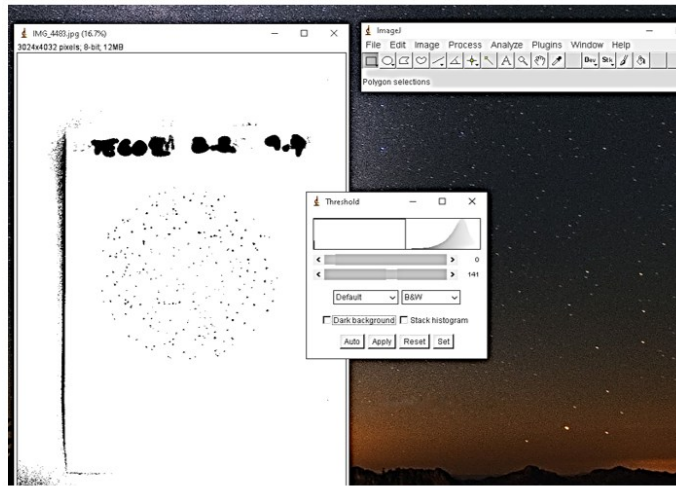


IMAGE J

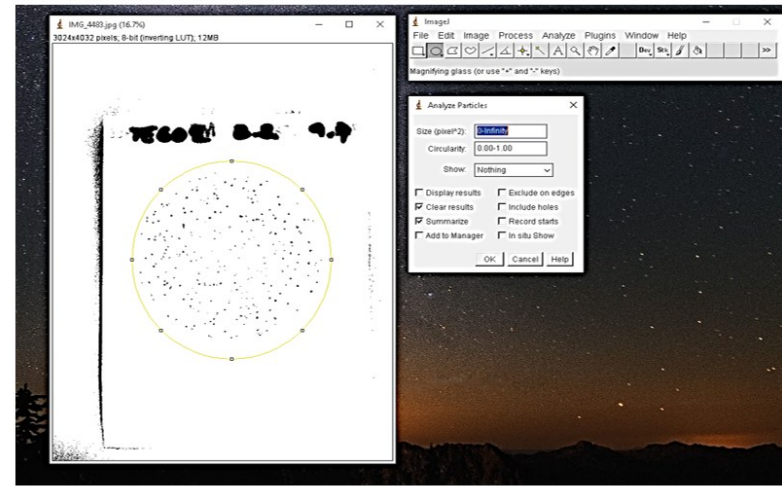
- 8-bit format
- Process – Binary – Make Binary
- Image – Adjust – Threshold – Apply
- Select area – Analyze – Analyze Particles



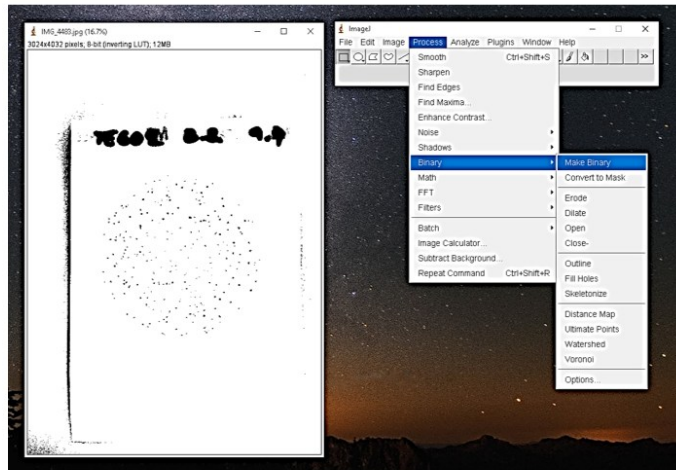
Step 1:



Step 3:



Step 2:



Result:

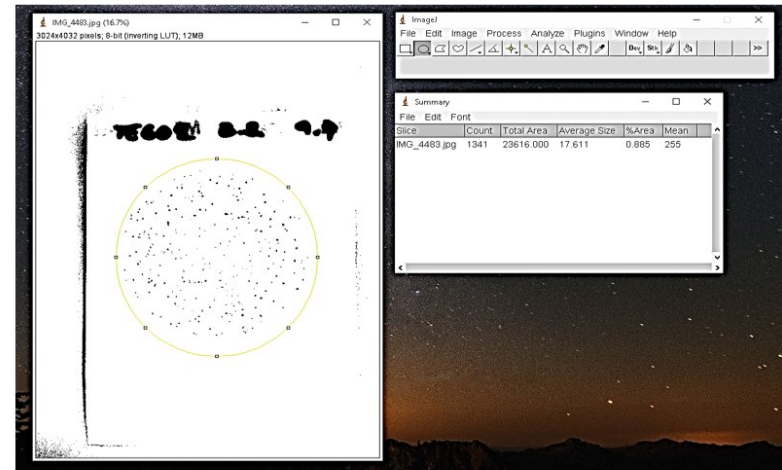


Figure S5. Evaluation of the antimicrobial tests using ImageJ software

Table S4. Ecotoxicity effects of the IL TEGO Dispers 662 C taken from safety data sheet of the manufacturer Evonik Tego Chemie GmbH, Germany. Lethal concentration LC50 results in a 50 percent mortality relative to the control; effective concentration EC50 results in a 50 percent biological response relative to the control; ErC50 and ErC10 concentrations result in a 50 and 10 percent reduction in growth rate relative to the control, respectively.

Parameter	Species	Ecotoxicity effect	Dose (mg L ⁻¹)	Exposure time (h)	Method
Toxicity to fish	<i>Leuciscus idus</i>	LC50	1.8	96	OECD 203
Toxicity to bacteria	activated sludge	EC50	564	3	OECD 209
Toxicity to daphniae	<i>Daphnia magna</i>	EC50	0.065	48	OECD 202
Toxicity to algae	<i>Pseudokirchneriella</i>	ErC50	>0.4	72	OECD 201
	<i>subcapitata</i>	ErC10	0.28		

Table S5. Intensity and inhibition of *V. fischeri* luminescence in aqueous leachates of GAC, iGAC-TEGO, and iGAC-TEDA

Sample	Luminescence intensity			Luminescence inhibition (%)	
	Time 0 min	Time 15 min	Time 30 min	Time 15 min	Time 30 min
GAC	418.1	460.5	440.4	-2.71	-1.43
GAC	498.5	503.0	492.7	5.90	4.83
iGAC-TEGO	493.4	448.2	465.1	15.29	9.23
iGAC-TEGO	473.8	439.3	440.9	13.54	10.39
iGAC-TEDA	495.5	488.1	509.2	8.14	1.05
iGAC-TEDA	486.1	472.7	499.9	9.32	0.97
Blank	461.6	522.7	508.8		
Blank	479.6	485.5	467.5		

References

- Colomer-Lluch M, Jofre J, Muniesa M. Antibiotic resistance genes in the bacteriophage DNA fraction of environmental samples. *PLoS One*. 2011 Mar 3;6(3):e17549. doi: 10.1371/journal.pone.0017549. PMID: 21390233; PMCID: PMC3048399.
- Heuer H, Krsek M, Baker P, Smalla K, Wellington EM. Analysis of actinomycete communities by specific amplification of genes encoding 16S rRNA and gel-electrophoretic separation in denaturing gradients. *Appl Environ Microbiol*. 1997 Aug;63(8):3233-41. doi: 10.1128/aem.63.8.3233-3241.1997. PMID: 9251210; PMCID: PMC168621.
- Heuer H, Smalla K. Manure and sulfadiazine synergistically increased bacterial antibiotic resistance in soil over at least two months. *Environ Microbiol*. 2007 Mar;9(3):657-66. doi: 10.1111/j.1462-2920.2006.01185.x. PMID: 17298366.
- Marti E, Jofre J, Balcazar JL. Prevalence of antibiotic resistance genes and bacterial community composition in a river influenced by a wastewater treatment plant. *PLoS One*. 2013 Oct 25;8(10):e78906. doi: 10.1371/journal.pone.0078906. PMID: 24205347; PMCID: PMC3808343.
- Mlynarcik P, Roderova M, Kolar M. Primer Evaluation for PCR and its Application for Detection of Carbapenemases in Enterobacteriaceae. *Jundishapur J Microbiol*. 2016 Jan 2;9(1):e29314. doi: 10.5812/jjm.29314. PMID: 27099689; PMCID: PMC4834133.
- Poirel L, Walsh TR, Cuvillier V, Nordmann P. Multiplex PCR for detection of acquired carbapenemase genes. *Diagn Microbiol Infect Dis*. 2011 May;70(1):119-23. doi: 10.1016/j.diagmicrobio.2010.12.002. Epub 2011 Mar 12. PMID: 21398074.
- Rathnayake IU, Hargreaves M, Huygens F. Antibiotic resistance and virulence traits in clinical and environmental *Enterococcus faecalis* and *Enterococcus faecium* isolates. *Syst Appl Microbiol*. 2012 Jul;35(5):326-33. doi: 10.1016/j.syapm.2012.05.004. Epub 2012 Jun 26. PMID: 22742879.
- Ribeiro PC, Monteiro AS, Marques SG, Monteiro SG, Monteiro-Neto V, Coqueiro MM, Marques AC, de Jesus Gomes Turri R, Santos SG, Bomfim MR. Phenotypic and molecular detection of the bla_{KPC} gene in clinical isolates from inpatients at hospitals in São Luis, MA, Brazil. *BMC Infect Dis*. 2016 Dec 7;16(1):737. doi: 10.1186/s12879-016-2072-3. PMID: 27927163; PMCID: PMC5142414.