Antibiotic resistance genes in onsite wastewater treatment systems

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Introduction

Wastewater treatment systems are important point sources of micropollutants, such as antibiotics and heavy metals. Continuous exposure to these substances may cause microorganisms to develop antibiotic resistance, which can spread among bacterial pathogens with the help of mobile antibiotic resistance genes. Onsite wastewater treatment systems treating the wastewater of a single or a few households provide alternative solutions to centralized systems and are becoming increasingly popular. Domestic wastewater, however, may contain antibiotics and other drivers of antibiotic resistance in relatively high concentrations, and while little is known about performance, on-site treatment systems can essentially act as a source of antibiotic resistant genes and bacteria. In our study, we have analyzed the analytical and microbiological composition of raw and treated wastewaters from three identical on-site systems, in order to assess the presence of micropollutants and antibiotic resistance genes.

Hypotheses

- Domestic wastewater contains pharmaceuticals in higher concentrations than municipal wastewater.
- Onsite wastewater treatment systems cannot remove pharmaceuticals completely.
- Onsite wastewater treatment systems cannot effectively remove antibiotic resistance genes.
- Maintencance has an effect on removal efficiencies, which can be adressed using a questionnaire.

Methods

A questionnaire was developed to asses unit maintenance behaviour, water use, chemcial and pharmaceutical use of the owners. DNA extraction, fragment libraries were prepared, and sequenced by Illumina NextSeq and NovaSeq. Quality of the sequences were assessed using FastQC. Adapter sequences were trimmed using BBDuk. Kraken2, then Bracken with PlusPF database were applied for taxonomical classification. Data analysis was performed using RStudio. For resistance genes, reads were assembled to contigs using Megahit, and were analyzed using CARD-RGI.

Pharmaceuticals and antimicrobial agents

Table 1. Different pharmaceutical and antimicrobial agents identified at sampling locations, and their removal efficiencies.
R: Raw wastewater A: Post-settler of the treatment unit. B: Short-term storage tank. C: Long-term storage tank.

Components	Unit	1R	1A	Removal [%]	2R	2A	2B	Removal [%]	3R	3A	3B	3C	Removal [%]	Reference removal [%]
Azithromycin	µg/L	**37**	**15**	59	nd	nd	nd	-	4380	3340	4290	22400	24 / -411	-494 [2]
Ofloxacin	µg/L	nd	nd	-	**39**	61	88	-56 / -125	136	100	130	128	26 / 6	65 [3]
Diclofenac	µg/L	**29**	**35**	-20	4110	2350	237	43 / 94	**17**	**11**	**15**	62	35 / -264	55 [4, 5]
Ibuprofen	µg/L	nd	nd	-	1720	nd	nd	>94,2	nd	nd	nd	nd	-	76 [6]
Carbamazepine	µg/L	nd	nd	-	nd	nd	**29**	*	nd	nd	nd	nd	-	14 [5, 7]
Caffeine	µg/L	1470	509	65	121	215	71	-77 / 41	162	**66**	**30**	**32**	59 / 80	68 [8]
Triclosan	µg/L	**19**	**8**	57	**6**	nd	nd	-	55	**22**	**10**	**8**	60 / 85	95 [9]

nd: not detected
***bold** values are below the limit of quantification; *potentially persists in the short-term storage tank*

Removal efficiencies were calculated from the difference between the raw wastewater and post settler / last treatment step of a unit if available. Reference removal was calculated from the removal efficiencies measured in multiple centralized wastewater treatment plants, taken from the literature. A negative removal means there was an increase in the concentration. There is a huge difference between both concentrations in raw wastewater samples, and removal efficiencies. Centralized treatment is generally better at removal, however the values vary greatly. Concentration increased greatly for Azithromycin, and decreased for Diclofenac when adequately high values were measured.

Antibiotic Resistance Genes

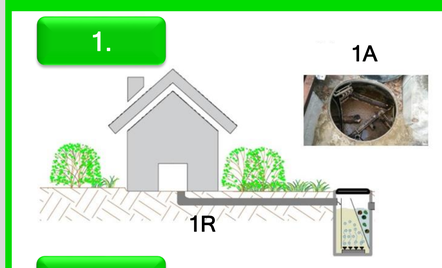
Table 2. ARGs identified in sampling locations. R: Raw wastewater A: Post-settler of the treatment unit. B: Short-term storage tank. C: Long-term storage tank.

ARG	1R	1A	2R	2A	2B	3R	3A	3B	3C
RCR-1			+	+	+	+	+	+	+
sul1	+	+	+	+	+	+		+	+
sul2		+	+	+	+				
sul4	+	+							
OXA-2	+								
OXA-4	+	+	+	+	+				
OXA-10							+	+	
OXA-21	+	+							
tet(C)			+	+	+				
mphA	+								
mphF	+	+							
msrE	+	+				+		+	+
mphE	+	+		+					+
qacEdelta1	+	+	+		+	+			
cmlA9						+			
aadS						+			
aadA5		+							

+: ARG is present at the sampling location.

Only perfect hits are shown in Table 2. Many different strict and loose hits were recorded at each sampling location, with multiple hits. The raw wastewater of unit 1 contained the most types of ARGs, and activated sludge treatment had little effect on their presence. Raw wastewater of unit 2 and 3 contained noticeably lower number of different ARGs. In case of unit 2, the treatment steps had little effect, however in unit 3, the perfect hits for ARGs increased through short-term and long-term storage.

Study Sites[1]



- 6 PE unit, a family of 4
- soil infiltration
- conscious water use
- yearly check-ups
- no maintenance

Figure 2. Ökotech AB Clear wastewater treatment system

- 6 PE unit, a family of 3
- 2 m^3 storage tank → soil infiltration
- conscious water use
- regular maintenance
- weekly check-ups, monthly cleaning
- unit works well

- 6 PE unit, a family of 4
- 3 m^3 and 49 m^3 storage tank
- conscious water use
- regular maintenance
- monthly check-ups,
- cleaning every 3 months
- unit works well

Figure 1. Domestic activated sludge systems used for samplings: units 1, 2, and 3. R: Raw wastewater A: Post-settler of the treatment unit. B: Short-term storage tank. C: Long-term storage tank. Treated wastewater flows from the post-settler of the treatment unit (A) either to a soil infiltration unit or to a short-term storage tank (B) then into a long-term storage tank. (C).

Microbial composition

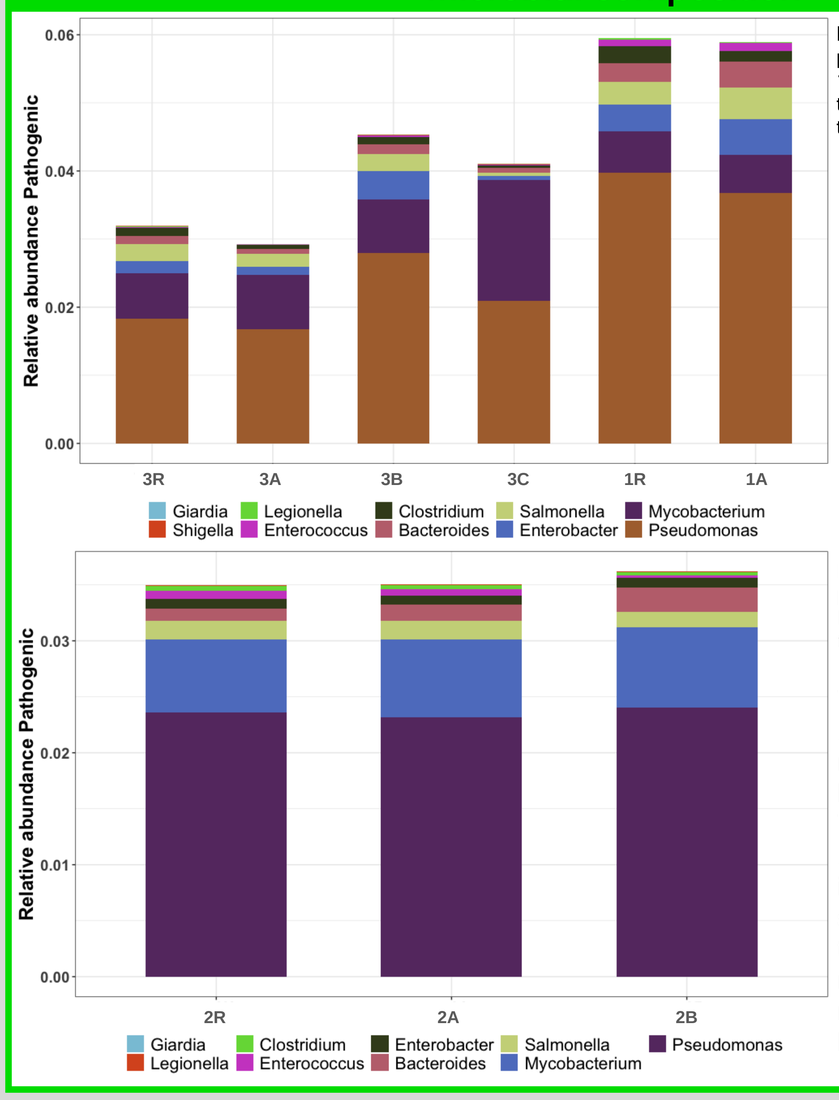


Figure 3. Relative abundances of pathogenic genus identified in units 3 and 1. R: Raw wastewater A: Post-settler of the treatment unit. B: Short-term storage tank. C: Long-term storage tank.

The *Pseudomonas* and *Mycobacterium* genus are the most abundant in all sampling locations of both units.
The relative abundances of all pathogenic genus increased during short-term storage of unit 3, but decreased during the long-term storage. Long-term storage had the highest effect on microbial composition and relative abundances.

Figure 4. Relative abundances of pathogenic genus identified in unit 2. R: Raw wastewater A: Post-settler of the treatment unit. B: Short-term storage tank.

The *Pseudomonas* and *Mycobacterium* genus are the most abundant in all sampling locations of unit 2.
The relative abundance of the *Bacteroides* genera increased in the post-settler and during short-term storage, however, there is no significant change in microbial composition regarding pathogenic genus between the treatment steps.

Conclusion

- ✓ The removal efficiencies of pharmaceuticals and antimicrobial agents fluctuate between OWTSs, and are generally lower than in centralized wastewater treatment plants
- ✓ Antibiotic resistance genes are present in wastewater, and are not removed during treatment
- ✓ Apart from long-term storage, treatment steps had little to no effect on the microbial composition
- ✓ More studies are needed to asses the effect of storage on pharmaceuticals and ARGs
- ✓ The monitoring of ARGs and pharmaceuticals besides routine measurements is crucial in OWTSs

References:

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