Antibiotic resistance of human pathogen bacteria and apathogenic environmental bacteria isolated from sewage and surface water

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Bacterial infectious diseases were thought to be vanquished with the discovery of antibiotics at the beginning of the last century, but resistant bacteria occurred shortly after the introduction of antibiotics to the market. Today multi-resistant pathogens related to heavy aetiopathology and hospitalisation cause particular problems. While environmental transmission within soil is well investigated, little is known about the importance of surface water in the spreading of resistant genes. In this study, the relevance of a municipal wastewater treatment plant and its effluent released into a river was examined on the basis of pathogens from sewage and apathogenic environmental bacteria. Rhodospirillaceae (non-sulfur purple bacteria) live ubiquitously in surface water and other humid environments and are known to be involved in horizontal gene transfer.

Study site: Catchment area of the river Swist, Germany.
Samples were taken regularly for the period of a hydrological year from the influent and effluent of a municipal sewage treatment plant with secondary treatment and from the receiving stream 80 m upstream and downstream from the discharge point.

Methods

✓Identification of *P. aeruginosa* (DIN EN 12780):
Cultivation on Cetrimid agar, oxidase activity, fluorescein and pyocyanin building, acetamide utilisation

✓Identification of *Campylobacter* sp. (ISO 17995) :
microaerobically enrichment in mCCD-Bouillon, oxidase and catalase activities, mobility, Gram reaction

✓Identification of Rhodospirillaceae (Hougardy et al. 2000):
anaerobically cultivation on RCV plates in the light, Gram reaction, morphology, biochemical characteristics

✓Antibiotic susceptibility testing of approved isolates:

Agar diffusion tests with discs containing:

piperacillin (PRL)	75 µg,	ampicillin/sulbactam (SAM)	20 µg,
imipenem (IPM)	10 µg,	meropenem (MEM)	10 µg,
cephazoline (KZ)	30 µg,	ceftazdime (CAZ)	30 µg,
ciprofloxacine (CIP)	5 µg,	ofloxacin (OFX)	5 µg.

✓The classification of resistance levels followed DIN 58940-3.

Table 1 Concentration of bacteria in wastewater and river water samples

Organism	water sample	Median	Range
P. aeruginosa [CFU/100 ml]	influent STP	1.5×10^5	10^5 - x 10^5
	effluent STP	5.0×10^1	ND - 10^2
	river upstream STP	1.2×10^1	ND - 10^2
	river downstream STP	6.0×10^0	ND - 10^2
Campylobacter sp. [MPN/100 ml]	influent STP	2.8×10^5	10^5 - 10^6
	effluent STP	4.3×10^3	10^3 - 10^4
	river upstream STP	4.3×10^2	10^2 - 10^4
	river downstream STP	9.3×10^2	10^3 - 10^4
Rhodospirillaceae [CFU/100 ml]	effluent STP	2.9×10^3	10^3 - 10^4
	river upstream STP	6.9×10^3	10^3 - 10^4
	river downstream STP	6.9×10^3	10^3 - 10^4

ND = not detected

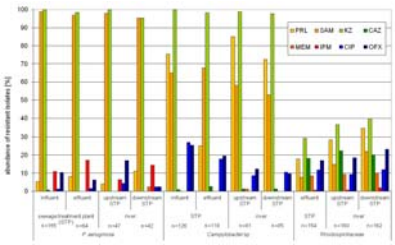

Figure 1: Abundance of antibiotic resistances in the bacteria isolates in wastewater and surface water

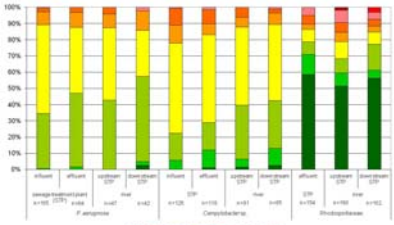

Figure 2: Multi-resistances of bacterial isolates from sewage and river water

Results

✓The reduction of pathogens during the sewage treatment process was median 3 log. The species' concentrations in the surface water upstream and downstream of the sewage treatment plant (STP) did not vary significantly (Table 1).

✓Seasonal variations could not be identified. Discrimination during the wastewater treatment process of pathogen bacteria with a certain resistance and of multi-resistant pathogens was not observed.

✓In total, 318 *P. aeruginosa*, 410 *Campylobacter* sp. and 476 Rhodospirillaceae isolates were tested for antibiotic susceptibility (Figure 1).

✓The number of multi-resistant isolates within one species and between the sampling sites was similar. Only 1% of all *P. aeruginosa* isolates showed maximum single resistance, but 5% showed minimum four resistances. *Campylobacter* sp. seemed to be more resistant. In the case of Rhodospirillaceae, 59% were free of any resistance tested, but 6% showed minimum sixfold resistance (Figure 2).

Conclusions

- ✓ Resistant pathogens were detectable in wastewater as well as in surface water. The low susceptibility of *P. aeruginosa* against OFX, IPM and PRL and *Campylobacter* sp. against SAM, KZ and PRL are remarkable.
- ✓ Non-pathogenic antibiotic resistant environmental bacteria were also detectable. In contrast to the pathogens reaction, a relative low sensitivity against CAZ and MEM of Rhodospirillecae was noticeable.
- ✓ The results could not clearly indicate influences on the resistance situation of the bacteria from surface water used as a discharge system.
- ✓ There was no direct hint for antibiotic resistance transfer between the different species, also it is known that Rhodospirillaceae are capable of horizontal gene transfer in general.
- ✓ Additional examinations in the upper reaches of the river system (which are definitely not influenced by settlements or wastewater) have to clarify whether the levels of antibiotic resistance found in the river water upstream of the STP are of natural origin.

Reference
Hougardy, A., Tindall, B. J., J.-H. Klemme (2000): Rhodopseudomonas rhenobacensis sp. nov., a new nitrate-reducing purple non-sulfur bacterium. *International Journal of Systematic and Evolutionary Microbiology* **50**, 985–992.