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Wastewater treatment plants as a hub between clinical and environmental antibiotic resistance

Oczyszczanie ścieków z punktu widzenia klinicznej i środowiskowej oporności na antybiotyki

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ABSTRACT

Antibiotics (AB) are among the most important pharmaceuticals applied in both, human and veterinarian medicine. As long as their use, concerns about an increase in resistance (ABR) of originally targeted organisms exist. Within the last few years the significance of wastewater treatment plants and the release of antibiotic resistant bacteria (ARB) or genes (ARG) by discharged effluents got into the focus of scientific research. Within a wastewater treatment plant there are two specific conditions that could induce transfer of ARGs and selection of ARBs:

- Low concentrations of antibiotics that are far below a therapeutically dose and can act as selection parameter for ABR
- Living or dead resistant clinically relevant bacteria that can transfer their ARGs via different means to environmental bacteria that are adapted to usual environmental conditions and therefore can transfer ARGs to the gene pool of the aquatic environment

Both aspects can be demonstrated by applying the concept of the "mutant selection window" to wastewater treatment plants that implies that between a miniumum inhibitory concentration (MIC) and a higher mutant prevention concentration (MPC) there is a concentration range where an antibiotic has an effect on the selection of ARBs. The pathway of resistance back from the environment to the human is not clear now and hardly investigated. As awareness ARBs and ARGs by wastewater treatment plants is there now, engineers are about to adopt existing treatment technologies for decreasing the release and are designing new technologies that even are able to inhibit their release to the aquatic environment.

Key words: wastewater plants, antibiotic resistance, environment

STRESZCZENIE

Antybiotyki (AB) są zaliczane do najważniejszych farmaceutyków używanych zarówno u ludzi jak i u zwierząt. Od początku ich stosowania były obawy o możliwość powstawania na nie oporności (ABR). W czasie kilku ostatnich lat znaczenie oczyszczalni ścieków i wydalanie bakterii opornych na antybiotyki (ARB) albo genów (ARG) w wodach ściekowych stało się przedmiotem szczególnych badań naukowych. W zakładzie oczyszczanie ścieków odgrywają rolę dwie specyficzne okoliczności, które mogą indukować transfer ARG i selekcję ARB:

- niskie stężenie antybiotyków znacznie poniżej stężenia terapeutycznego, które działać może jako parametr selekcyjny dla ABR
- obecność żywych lub martwych opornych bakterii o znaczeniu klinicznym, które mogą transferować swoje ARG na różne sposoby do bakterii zaadaptowanych do zwykłych swoich warunków środowiskowych i które mogą przenosić ARG do puli genów środowiska wodnego.

Oba czynniki mogą być wykazane stosując koncepcję „okienka selekcji mutantów" w odniesieniu do oczyszczalni ścieków, która zakłada, że między najniższym stężeniem hamującym (MIC) a wyższym stężeniem zapobiegającym mutacji (*mutant prevention concentration*, MPC) znajduje się przedział koncentracji, gdzie antybiotyk wywiera wpływ na selekcję ARB. Droga powrotu oporności z powrotem ze środowiska do człowieka nie jest jeszcze wyjaśniona i jest usilnie badana. Stan obecnej wiedzy co do ARB i ARG przenoszonych w oczyszczalniach ścieków pozwala na to, żeby inżynierowie utrzymywali dotychczasowe technologie obniżające ich wydalanie i projektowali nowe, które będą zdolne nawet do całkowitego zahamowania wydalania czynników oporności bakterii do środowiska wodnego.

Słowa kluczowe: oczyszczalnie ścieków, antybiotykooporność, środowisko

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Antibiotics (AB) are among the most important pharmaceuticals applied in both, human and veterinarian medicine. As long as their use, concerns about an increase in resistance (ABR) of originally targeted organisms exist. Those concerns are supported by increasing observations of a continuous spreading of even multi-drug resistant organisms that are a serious threat to public health. The reasons for the spreading of antibiotic resistance are agreed to be multifactorial with overuse, unnecessary and careless prescriptions [1] being only some of possible reasons. Within the last few years the significance of wastewater treatment plants and the release of antibiotic resistant bacteria (ARB) or genes (ARG) by discharged effluents of even tertiary treated wastewater got into the focus of corresponding scientific research. The number of peer reviewed papers in that context rose from about 35 in 1994 to 100 in 2004 and already is exceeding 700 published 2014. The common agreement in all of the papers is, that wastewater treatment plants are a significant source for the emission of ARBs and ARGs into the environment either via effluents into the aquatic compartment or via application of biosolids in agriculture.

The reasons for the release of ABs, ARBs and ARGs via wastewater treatment plants is obvious. After application and consumption of antibiotics they are excreted from humans as well as animals, collected in sewerage and transported to the wastewater treatment plants. The same is valid for ARBs and subsequently ARGs too as they are widely spread in the population and nowadays with the tendency for short hospital stays and ambulant treatments not necessarily are concentrated at health care centers or similar institutions. Not all of the collected wastewater is treated in wastewater treatment plants, but in the case of combined sewer systems (joint transport of fecal waters and rainwater from surface run offs) and heavy rains, a part of the wastewater directly is discharged into receiving waters by stormwater overflows. There is still no sufficient information in literature on the relevance and contribution of stormwater overflows for the prevalence of ARBs and ARGs in the environment.

The treatment processes in wastewater treatment plants are based on biological (mainly bacterial) processes for carbon and nitrogen removal. The "pollution" in the wastewater described e.g. as amount of total organic carbon (TOC) is used to about the same extend for assimilatory and dissimilatory processes. Due to kinetic consideration for bacterial growth (Monod kinetics), the bacteria are not able to utilize substrate completely and residual amounts of the substrate is released to the effluents. Additionally not all substances in wastewater can be utilized as substrate in a direct metabolic reaction. Some substances are used as co-metabolits and others cannot be used at all. Beside biological degradation of substances, adsorption to the biofilm of activated sludge is a relevant removal pathway from the aqueous phase too for hydrophobic substances. The consequence of all processes occurring are, that e.g. antibiotic are only removed to some extent, depending on their inflow concentration, structure and chemical properties resulting in (usually low) residual concentration in the effluent of conventional plants. A second important aspect in wastewater treatment is that the biocenosis involved in the biological treatment process are a mixture of bacteria originating from human and animal intestine and environmental bacteria, both "meeting" at that specific hub. Within the app. 6 to 24 hours of hydraulic retention time in the plants bacteria from human origin die off under the abiotic conditions of wastewater treatment plants quite fast [2], but some of them as enterococci are more persistent and survive the process despite the fact that their number is reduced by some log units [e.g. 3]. ARBs that die off nevertheless carry ARGs that can be incorporated by different means by other to that point not resistant bacteria.

Summing up within a wastewater treatment plant there are two specific conditions that could induce transfer of ARGs and selection of ARBs:

- Low concentrations of antibiotics that are far below a therapeutically dose and can act as selection parameter for ABR
- Living or dead resistant clinically relevant bacteria that can transfer their ARGs via different means to environmental bacteria that are adapted to usual environmental conditions and therefore can transfer ARGs to the gene pool of the aquatic environment

The importance of both aspect can be demonstrated by applying the concept of the "mutant selection window" [4] to wastewater treatment plants (figure 1).

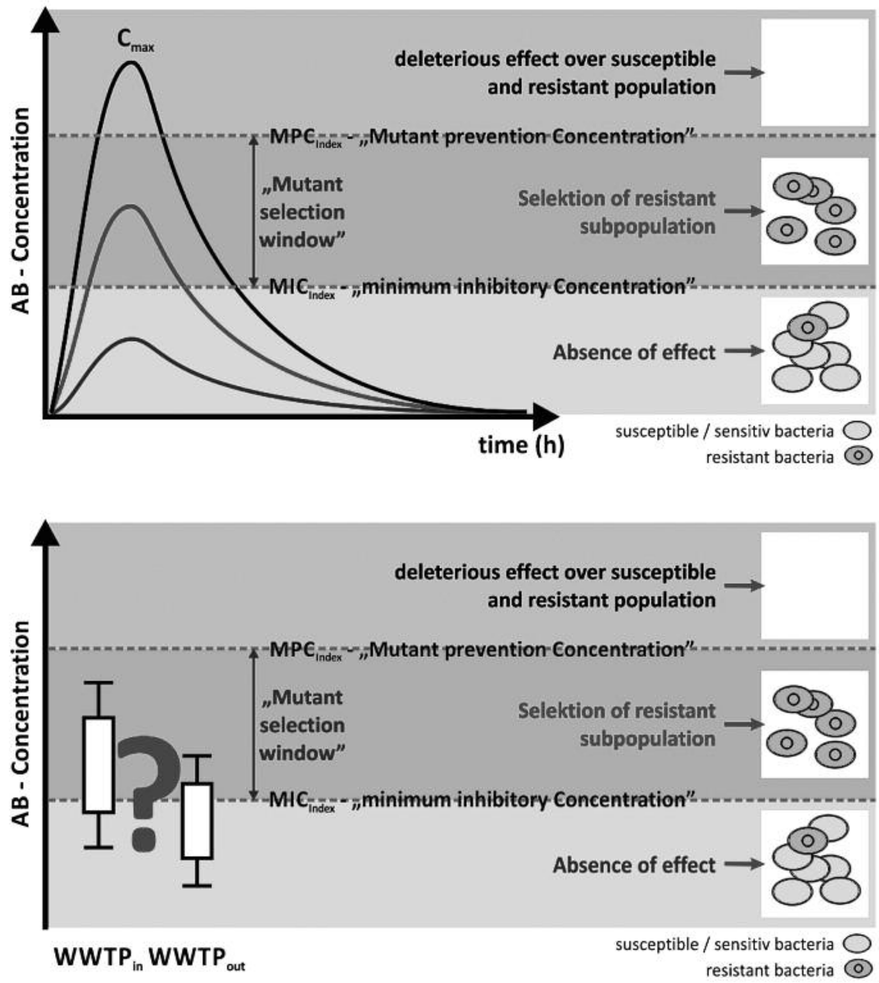

Fig. 1. „Mutant Selection Window" and wastewater treatment. Modified after [4]
Ryc. 1. „Okienko selekcji mutantów" a oczyszczanie ścieków. Zmodyfikowano wg [4]

The concept of the „mutant selection window" implies that between a miniumum inhibitory concentration (MIC) and a higher mutant prevention concentration (MPC) there is a concentration range where an antibiotic has an effect on the selection of ARBs. Therapeutical dose as well as the duration of an application should be in a way that there is minimum risk for the selction of mutants during medical application. Due to dilution of AB concentrations and partial degradation the concentrations observed in wastewater treatment plants are far below the therapeutical dose and the MPC. It is assumed, that concentrations are slightly above or below the MIC, but not solid scientific information is available on that asumption. It only is clear that the AB concentrations in the plants do not effect the biological treatment processes.

In two review articles by Michael et al. [5] and Rizzo et al. [6] the scientific data on occurence of ABs, ARBs and ARGs are summarized:

Table I. Examples for concentrations of antibiotics in the effluent of wastewater treatment plants. Modified from [5]
Tabela I. Przykłady stężenia antybiotyków z odpływów oczyszczalni ścieków. Zmodyfikowano wg [5]

Antibiotics	Effluent concentrations [ng L^{-1}]
Penicilline	up to 200
Makrolide	up to 700
Erythromycine	up to 287
Clarithromycine	up to 328
Roxithromycine	up to 68
Fluorchinolone	up to 100
Ofoxacine	up to 82
Sulfonamide	up to 1.000
Sulfamethoxazole	up to 370
Tetracycline	up to 20
Trimethoprim	up to 38
Chloramphenicol	up to 68
Clindamycin	up tp 110

Table II. Examples for ARGs in wastewater treatment plants. Modified from [6]
Tabela II. Przykłady stwierdzenia ARG z odpływów oczyszczalni ścieków. Zmodyfikowano wg [6]

Antibiotics group	ABGs found in wwtps
Aminoglycosides	aad(A1, A2, A13, B) aph(A, A-3, A-6, 2); str(A, B), strB
Beta-lactams	Class A: CTX, GES, NPS, PER, SHV, TEM,TLA, VEB Class B: IMP, VIM Class C: ampC, CMY Class D: OXA mecA
Glycopeptides	vanA
Macrolides	Mel, ereA2, ermB, erm(B, F) mph(A, B), aacA6-ib-cr
Quinolones	aacA6-ib-cr qnr(A3, B1, B2, B4, B5, S2)
Sulfonamides	qnrVC; qnrS sul2; sul(1, 2, 3)
Tetracyclines	tetU, tetA tet(A, B, D, G, H, Y, 31, 35, 36, 39) tetM, tet(M, S), tetX
Trimethoprim	dfr(A1, A12, 18) dfr (II, V, VII, XII, 13, 16, 17, A19, B2, D) dhfr (I, VIII, XV)
Multidrug	acr(B, D), mex(B, D, F, I, Y)

One of the few studies proving the horizontal transfer of ARGs from clinical to environmental bacteria was published by Yang et al. [7] using a metagenomics approach. In that comprehensive study, 271 ARGs belonging to 18 types of ABR were identified in different compartments (inflow; effluent; activated sludge and anaerobic digested sludge) of a wastewater treatment plants. Even the total removal of ARGs was determined with 99.82%, 78 of the ARGs persisted throughout the treatment processes. The aerobic treatment process was found to be more efficient compared to the anaerobic step that shows environmental conditions very similar to those in human intestines (37°C; anaerobic). Some of the ARGs were related to bacteria species that were not found in the inflow of the plants but are commonly found in biological wastewater treatment plants, so it was concluded, that horizontal gene transfer occurred from clinical to environmental bacteria

Despite the fact, that a lot of scientific papers prove the significance of wastewater treatment plants as a significant source for the release of AB, ARBs and ARGs to the environment and even the transfer from clinical relevant bacteria to environmental bacteria, the pathway of resistance back from the environment to the human is not clear now and hardly investigated. Application of sewage sludge as fertilizer or reuse of treated wastewater for irrigation in agriculture as well as direct uptake in recreational waters may be possibilities for closing the loop. As awareness for the potential impacts of the release of ARBs and ARGs by wastewater treatment plants is there now, engineers are about to adopt existing treatment technologies for decreasing the release and are designing new technologies that even are able to inhibit their release to the aquatic environment.

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