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Antimicrobial resistance of fecal indicators in municipal wastewater treatment plant

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ABSTRACT

Antimicrobial resistance of fecal coliforms ($n = 153$) and enterococci ($n = 199$) isolates was investigated in municipal wastewater treatment plant (WWTP) based on activated sludge system. The number of fecal indicators (in influent and effluent as well as in the aeration chamber and in return activated sludge mixture) was determined using selective media. Susceptibility of selected strains was tested against 19 (aminoglycosides, aztreonam, carbapenems, cephalosporins, β-lactam/β-lactamase inhibitors, fluoroquinolones, penicillines, tetracycline and trimethoprim/sulfamethoxazole) and 17 (high-level aminoglycosides, ampicillin, chloramphenicol, erythromycin, fluoroquinolones, glycopeptides, linezolid, lincosamides, nitrofuration, streptogramins, tetracycline) antimicrobial agents respectively. Among enterococci the predominant species were *Enterococcus faecium* (60.8%) and *Enterococcus faecalis* (22.1%), while remaining isolates belonged to *Enterococcus hirae* (12.1%), *Enterococcus casseliflavus/gallinarum* (4.5%), and *Enterococcus durans* (0.5%). Resistance to nitrofuration and erythromycin was common among enterococci (53% and 44%, respectively), and followed by resistance to ciprofloxacin (29%) and tetracycline (20%). The resistance phenotypes related to glycopeptides (up to 3.2%) and high-level aminoglycosides (up to 5.4%) were also observed. Most frequently, among *Escherichia coli* isolates the resistance patterns were found for ampicillin (34%), piperacillin (24%) and tetracycline (23%). Extended-spectrum β-lactamase producing *E. coli* was detected once, in the aeration chamber. In the study the applied wastewater treatment processes considerably reduced the number of fecal indicators. Nevertheless their number in the WWTP effluent was higher than 10^4 CFU per 100 ml and periodically contained 90% of bacteria with antimicrobial resistance patterns. The positive selection of isolates with antimicrobial resistance patterns was observed during the treatment processes. Substantial concern should be paid to the isolates resistant to 3 or more chemical classes of antimicrobials (MAR). In treated wastewater MAR *E. coli* and MAR enterococci constituted respectively 9% and 29% of tested isolates.

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1. Introduction

In Poland the antimicrobial agents' consumption varies from year to year, with the increasing trend observed especially in outpatient therapy. Daily identified doses per 1000 inhabitants per day (DID) increased from 16.56 DID in 1997 to 24.77 DID in 2001, and in 2001 this number was sixth in Europe (Ferech et al., 2006). According to the data collected in ESAC project (European Study on Antibiotic Consumption), the increase was observed in the classes of penicillin and fluoroquinolones, while the use of other antimicrobial agents stagnated or decreased (Elseviers et al., 2007; Ferech et al., 2006; Muller et al., 2007). Antimicrobial agents used in human therapy as well as their residues reach the sewage systems via urine and feces. Their concentrations in wastewater, although significantly lower than therapeutic dosages, are suspected to affect the susceptible bacteria and select resistant strains (Kim et al., 2007; Al-Ahmad et al., 1999; Backhaus and Grimme, 1999). The wastewater treatment processes remove the antimicrobial agents by sorption and/or by biodegradation (Kümmerer, 2009; Golet et al., 2003; Lindberg et al., 2006), thus their antimicrobial activity and fate in final effluents depends on their chemical properties. Growing evidences have additionally supported the thesis of horizontal genes transfer under wastewater treatment conditions (Soda et al., 2008; Marcinek et al., 1998). Biological processes based on the activated sludge, due to the high density of cells, are regarded as factors promoting the dissemination of resistance among bacteria (Soda et al., 2008; Marcinek et al., 1998). In the environmental risk assessment, the importance of continuous input of resistant bacteria versus continuous input of antimicrobial agents themselves requires investigations (Kümmerer, 2009).

The aim of the present study was to evaluate the effects of wastewater treatment on the presence of antimicrobial resistance patterns among the indicator bacteria of wastewater origin. In the environmental studies *Escherichia coli* and enterococci are taken as the indicators of fecal contamination. Those bacteria are present in the intestinal tract of humans; however, they have been also frequently related to the human and animal diseases. Among humans some strains of *E. coli* are for instance one of the most important food-borne pathogens and the most frequent agent of urinary tract infections (Gupta et al., 2001). The emergence of pathogenic enterococci is connected with their high tenacious and intrinsic resistance to a broad range of antimicrobial agents. The resistance mechanisms, however, differ among different species (Murray, 1998). Besides of the exposition of pathogenic *E. coli* and *Enterococcus* spp. to antimicrobial agents, the selection of resistant strains may also occur among commensals as a result of bystander effect (Karami et al., 2006). In this study the prevalence of antimicrobial resistant fecal indicators was studied in the wastewater treatment plant (WWTP), which discharges the treated wastewater into the recreationally important coastal water of Gdańsk Bay.

2. Methods

The samples of raw and treated wastewater as well as wastewater from the aeration chamber and returned activated sludge were collected from WWTP 'Gdańsk–Wschód' (Northen Poland) working in modified UCT (University of Cape Town) – type system. The WWTP treats wastewater for the population of about 570,000 people in the area of Gdańsk, Sopot, Kolbudy, Pruszcz Gdański, Żukowo, and some local industry (5%) as well as it treats undisinfected (0.17%) hospital wastewater. Population equivalent estimated for the WWTP is equal to 700,000, the average daily flow is 96,000 m^3 per day with 24 h of retention time. The schematic diagram of the WWTP and sampling points are indicated in Fig. 1. During the study the average values of chemical and biological oxygen demand, total nitrogen and phosphorus as well as total suspended solids observed in treated wastewater were lower than those legally established for the examined WWTP 'Gdańsk–Wschód' (125 $mgO_2\ l^{-1}$, 15 $mgO_2\ l^{-1}$, 10 $mgN\ l^{-1}$, 1 $mgP\ l^{-1}$, 35 $mg\ l^{-1}$, respectively). Treated wastewater is pumped by the 2300 m collector into the coastal water of Gdańsk Bay.

2.1. Samples collection

The 24 h flow-proportional wastewater samples (5 l) were taken from the influent (INF) and effluent (EFF) as well as the mixed liquor from the aerobic chamber (BR) and return activated sludge (RAS) in the three following days of January and June (Fig. 1). The BR, RAS and EFF samples were taken

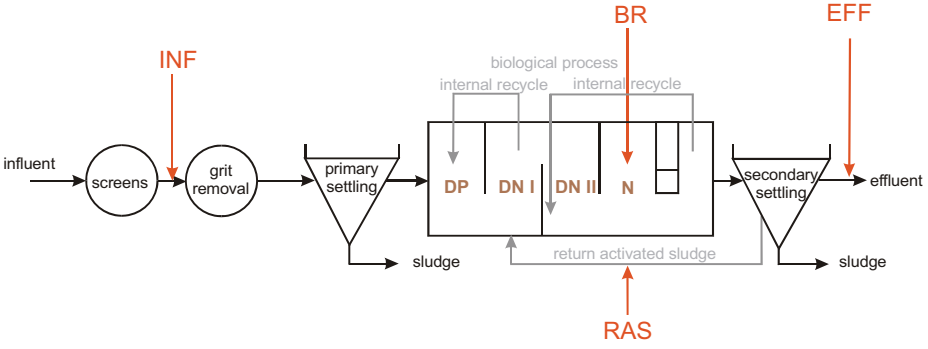

Fig. 1 – Scheme of the WWTP (DP – dephosphatation, DN – denitrification, N – nitrification) and the sampling points location (INF – influent, BR – aerobic chamber, RAS – return activated sludge, EFF – effluent).