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Release of antibiotic resistant bacteria and genes in the effluent and biosolids of five wastewater utilities in Michigan

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ABSTRACT

The purpose of this study was to quantify the occurrence and release of antibiotic resistant genes (ARGs) and antibiotic resistant bacteria (ARB) into the environment through the effluent and biosolids of different wastewater treatment utilities including an MBR (Membrane Biological Reactor) utility, conventional utilities (Activated Sludge, Oxidative Ditch and Rotatory Biological Contactors-RBCs) and multiple sludge treatment processes (Dewatering, Gravity Thickening, Anaerobic Digestion and Lime Stabilization). Samples of raw wastewater, pre- and post-disinfected effluents, and biosolids were monitored for tetracycline resistant genes (*tetW* and *tetO*) and sulfonamide resistant gene (*Sul-I*) and tetracycline and sulfonamide resistant bacteria. ARGs and ARB concentrations in the final effluent were found to be in the range of ND(non-detectable)-2.33 $\times$ 10^6 copies/100 mL and 5.00 $\times$ 10^2–6.10 $\times$ 10^5 CFU/100 mL respectively. Concentrations of ARGs (*tetW* and *tetO*) and 16s rRNA gene in the MBR effluent were observed to be 1–3 log less, compared to conventional treatment utilities. Significantly higher removals of ARGs and ARB were observed in the MBR facility (range of removal: 2.57–7.06 logs) compared to that in conventional treatment plants (range of removal: 2.37–4.56 logs) ($p < 0.05$). Disinfection (Chlorination and UV) processes did not contribute in significant reduction of ARGs and ARB ($p > 0.05$). In biosolids, ARGs and ARB concentrations were found to be in the range of 5.61 $\times$ 10^6–4.32 $\times$ 10^9 copies/g and 3.17 $\times$ 10^4–1.85 $\times$ 10^9 CFU/g, respectively. Significant differences ($p < 0.05$) were observed in concentrations of ARGs (except *tetW*) and ARB between the advanced biosolid treatment methods (i.e., anaerobic digestion and lime stabilization) and the conventional dewatering and gravity thickening methods.

1. Introduction

The escalating problem of emergence of antibiotic resistant bacteria and their resistant genes is becoming a major global health issue (Levy, 2002; Chee-Sanford et al., 2001). The use of numerous antimicrobial agents as treatments in animal, human, and plant health maintenance, is a worldwide practice providing both desirable and undesirable consequences. Links have been found to exist between antibiotic use and the emergence of antibiotic resistant bacterial pathogens (Aminov et al., 2001; Levy, 2002; Peak et al., 2007; Séveno et al., 2002). Studies have proven increase in antibiotic resistance strains that belong to pathogenic bacteria (Blasco et al., 2008) and over the years, nearly every bacterial pathogen has developed resistance to one or more clinical antibiotics (Todar, 2008).

The general observation published in different studies is that the environmental compartments which are most directly impacted by human or agricultural activities showed higher

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Table 1a – Wastewater treatment characteristics.

	East Lansing	Imlay	Romeo	Traverse city	Lansing
Wastewater treatment process (Biological treatment)	Activated Sludge (AS)	Oxidation Ditch (OD)	Rotating Biological Contactors (RBCs)	Membrane Biological Reactor (MBR)	Activated Sludge (AS)
Capacity	18.8 MGD	0.9 MGD	2.1 MGD	17.0 MGD	37.0 MGD
Average flow	13.4 MGD	0.4 MGD	0.8 MGD	8.5 MGD	20.0 MGD
Discharge Rate	14.1 MGD	0.02 MGD	0.8 MGD	4.0 MGD	19.0 MGD
Disinfection	Chlorine (Cl)	Ultra-Violet (UV)	Chlorine (Cl)	Ultra-Violet (UV)	Ultra-Violet (UV)

MGD-Millions gallon per day.

concentrations of antibiotic resistant bacteria and antibiotic resistant genes (Pruden et al., 2006; Chee-Sanford et al., 2001). Large amounts of antibiotics are released into municipal wastewater due to incomplete metabolism in humans or due to disposal of unused antibiotics (Nagulapally et al., 2009), which finally find their ways into different natural environmental compartments. Antibiotic resistant genes and antibiotic resistant bacteria have been detected in wastewater samples (Zhang et al., 2009a,b; Auerbach et al., 2007; Brooks et al., 2007; Pruden et al., 2006; Reinthaler et al., 2003). Also, the release of antibiotic resistant organisms through wastewater effluents into streams has been previously reported (Gallert et al., 2005; Iwane et al., 2001). Iwane and their colleagues reported approximately 8% and 6.7% of tetracycline resistant bacteria to be found in the pre- and post-chlorinated samples of a wastewater treatment plant respectively and then close to discharge location in the river water, similar percentages of bacteria were found to be resistant to tetracycline (Iwane et al., 2001). In addition, biosolids samples were reported to contain a high concentration of antibiotic resistant bacteria (Brooks et al., 2007). Also, the role of wastewater treatment plants in reducing the load of antibiotic resistant bacteria present in raw sewage is not well known (Rijal et al., 2009). However, it has been suggested that certain conditions within the wastewater treatment plants might increase the number of antibiotic resistant bacteria during the treatment process (Silva et al., 2006; Reinthaler et al., 2003). To the best of our knowledge, comparisons between different wastewater and biosolids treatment processes have not been studied so far.

The objective of this study was to quantify the release of antibiotic resistant genes (ARGs) and antibiotic resistant bacteria (ARB) in the effluent and biosolids of wastewater treatment plants (WWTPs). This is the first study that surveys the release of ARGs and ARB into the environment through the effluent and biosolids of different wastewater treatment utilities including an MBR (Membrane Biological Reactor), conventional wastewater utilities and multiple sludge treatment processes. This study has attempted to provide comparisons between different wastewater treatment processes and biosolid treatment processes along with the comparison of release loads of ARGs and ARB in the environment through the effluent and biosolids. In this study, samples of raw wastewater, effluent and biosolids were monitored for tetracycline and sulfonamide resistant bacteria, tetracycline resistant genes (*tetW* and *tetO*) and sulfonamide resistant gene (*SulI*) using quantitative polymerase chain reaction (qPCR) assays and conventional heterotrophic plate count methods. Tetracycline and sulfonamide resistance genes (*tetW*, *tetO* and *SulI*) were chosen in this study because tetracycline and sulfonamide are the most commonly used antibiotics in human and veterinary medicine (Boxall et al., 2003; Chopra and Roberts, 2001). In addition, quantitative detection systems already exist for this class of genes (Pei et al., 2006; Aminov et al., 2001). *TetW* and *tetO* genes are common in intestinal and rumen environments (Aminov et al., 2001) and have been cited as being promiscuous in their ability to spread among and across populations (Pei et al., 2006; Smith et al., 2004; Billington et al., 2002). *SulI* gene is also one of the most commonly detected sulfonamide resistant genes in the environment (Pei et al., 2006).

2. Materials and methods

2.1. *Sample collection*

Samples of raw wastewater, effluent prior to disinfection, and final effluent after disinfection were collected from five

Table 1b – Biosolid treatment characteristics.

	East Lansing	Imlay	Romeo	Traverse city	Lansing
Sludge treatment	Dewatering (No Anaerobic Digestion)	Gravity Thickening (No Anaerobic Digestion)	Anaerobic Digestion	Anaerobic Digestion	Lime Stabilization
Disposal of sludge	Landfill	Agricultural land	Agricultural land	Agricultural land	Agricultural land
Disposal rate (dry tons per year)	3596	118	125	850	4380
% solid	18.05%	1.49%	7.98%	4.85%	9.20%