International Journal of
Environmental Research and Public Health

MDPI

Supporting Text

Antibiotic Concentrations Decrease during Wastewater Treatment but Persist at Low Levels in Reclaimed Water

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Detailed description of all sampling sites included in the study

All sites were chosen based on the willingness of the site operator to participate. Mid-Atlantic WWTP1 is an urban tertiary wastewater treatment plant processing 681,390 m^3 of wastewater per day with a peak capacity of 1.51 x 10^6 m^3/d[1,2]. The influent includes domestic and hospital wastewater[1,2]. Treatment steps at this plant are screens, primary clarifier, activated sludge reactors, secondary clarifier, sand filters, chlorination (dose of 2 mg/L to 3 mg/L), de-chlorination (with sodium bisulfite) and effluent discharge (chlorine residual of <0.1 mg/L)[1,2]. Effluent from this plant is piped to a landscaping site (Mid-Atlantic SI1) for reuse in spray irrigation[2,3]. Mid-Atlantic SI1 performs on-site treatment and storage prior to spray irrigation[2,3]. On-site treatment includes screening (double-walled aluminum screen) and ultraviolet (UV) disinfection (minimum of 30,000 $\mu W/cm^2$ with 254 nm wavelength UV bulbs)[2,3]. The UV treated reclaimed water is then pumped to an open air storage pond (peak capacity 15,142 m^3) at a rate of 3.29 m^3/d[2,3]. Water from the storage pond is then pumped to spray heads based on irrigation needs[2,3]. Site employees use backpack sprayers to apply reclaimed water to locations not reached by spray heads[3].

Mid-Atlantic WWTP2 is a suburban tertiary treatment plant processing 7,570 m^3 of wastewater per day with a peak capacity of 45,425 m^3/d[1,2]. The influent includes domestic and hospital wastewater[1,2]. Treatment steps at this plant are screens, primary clarifier, primary aeration tank, secondary aeration tank, secondary clarifier, multimedia filter, chlorination (dose of 2 mg/L to 3 mg/L), de-chlorination (with sodium bisulfite) and effluent discharge (chlorine residual of <0.1 mg/L)[1,2]. Effluent from this plant is transported to a landscaping site for reuse via spray irrigation[1,2].

Midwest WWTP1 is a rural tertiary treatment plant processing 1,363 m^3 of wastewater per day with a peak capacity of 10,978 m^3/d[1,2]. The influent at this plant includes domestic wastewater and agriculturally influenced stormwater[1,2]. Treatment steps at this plant are screens, activated sludge lagoons, clarifiers, seasonal chlorination (in June, July and August; dose of 4 mg/L) and de-chlorination, and effluent discharge (chlorine residual of 0 mg/L)[1,2]. Effluent from this plant is transported to a landscaping site for reuse via spray irrigation[1,2].

Midwest WWTP2 is a rural tertiary treatment plant processing 1,439 m^3 of wastewater per day with a peak capacity of 7,571 m^3/d[1,2]. The influent includes domestic, food production and agriculturally influenced wastewater[1,2]. Treatment steps at this plant are screens, sequencing batch reactor, lagoon cell A, lagoon cell B, lagoon cell C, lagoon cell D, lagoon cell E and effluent discharge[1,2]. There is no on-site disinfection and unchlorinated effluent from this plant is transported to an agricultural site for irrigation of animal feed crops[1,2].

Supporting Figures and Tables

Table S1: A list of the nine antibiotics analyzed with the corresponding mass-charge ratios (*m*/*z*) of their parent and daughter ions and limit of detection (LOD) values (ng/mL)

Figure S1: Differences in antibiotic concentrations (ng/mL) between influent samples collected from Mid-Atlantic versus Midwest wastewater treatment plants (WWTPs)

Figure S2: Differences in antibiotic concentrations (ng/mL) between effluent samples collected from Mid-Atlantic versus Midwest wastewater treatment plants (WWTPs)

Table S1: A list of the nine antibiotics analyzed with the corresponding mass-charge ratios (*m*/*z*) of their parent and daughter ions and their limit of detection (LOD) values (ng/mL)

Antibiotic	Parent Ion (m/z)[a]	Daughter Ion (m/z)[a]	LOD (ng/mL)
Ampicillin (AMP)	366.7	206.9	0.0242
Azithromycin (AZI)	375.0	113.1	0.0092
Ciprofloxacin (CIP)	331.5	287.4	0.0131
Linezolid (LIN)	337.5	295.4	0.0217
Oxacillin (OXA)	402.0	158.2	0.0201
Oxolinic Acid (OXO)	261.1	243.0	0.0213
Penicillin G (PEN)	334.6	158.2	0.0308
Pipemidic Acid (PIP)	303.4	215.9	0.0279
Tetracycline (TET)	445.0	409.9	0.0107

[a]mass-charge ratio

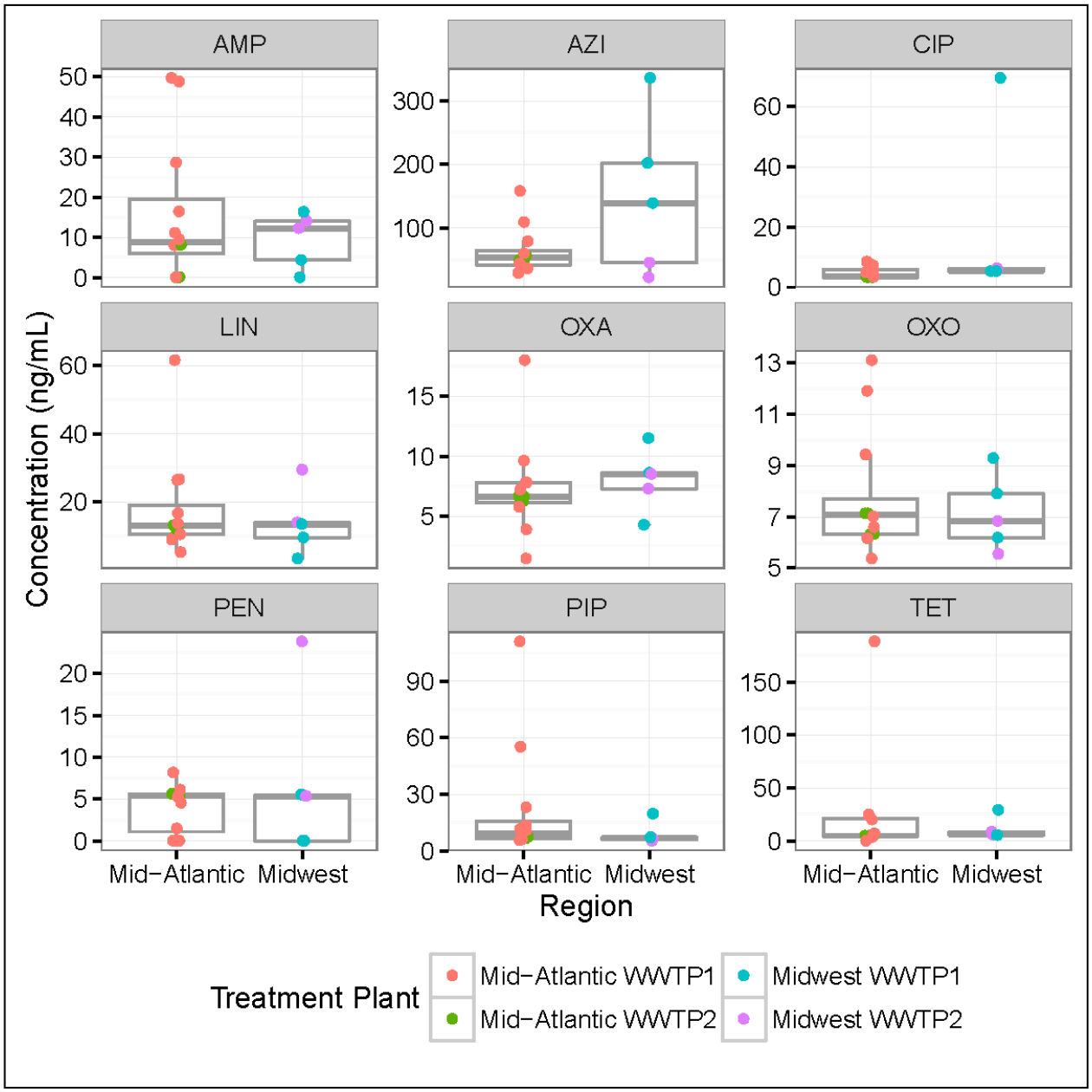

Figure S1: Differences in antibiotic concentrations (ng/mL) between influent samples collected from Mid-Atlantic versus Midwest wastewater treatment plants (WWTPs)

AMP = Ampicillin; AZI = Azithromycin; CIP = Ciprofloxacin; LIN = Linezolid;

OXA = Oxacillin; OXO = Oxolinic Acid; PEN = Penicillin; PIP = Pipemidic Acid;

TET = Tetracycline

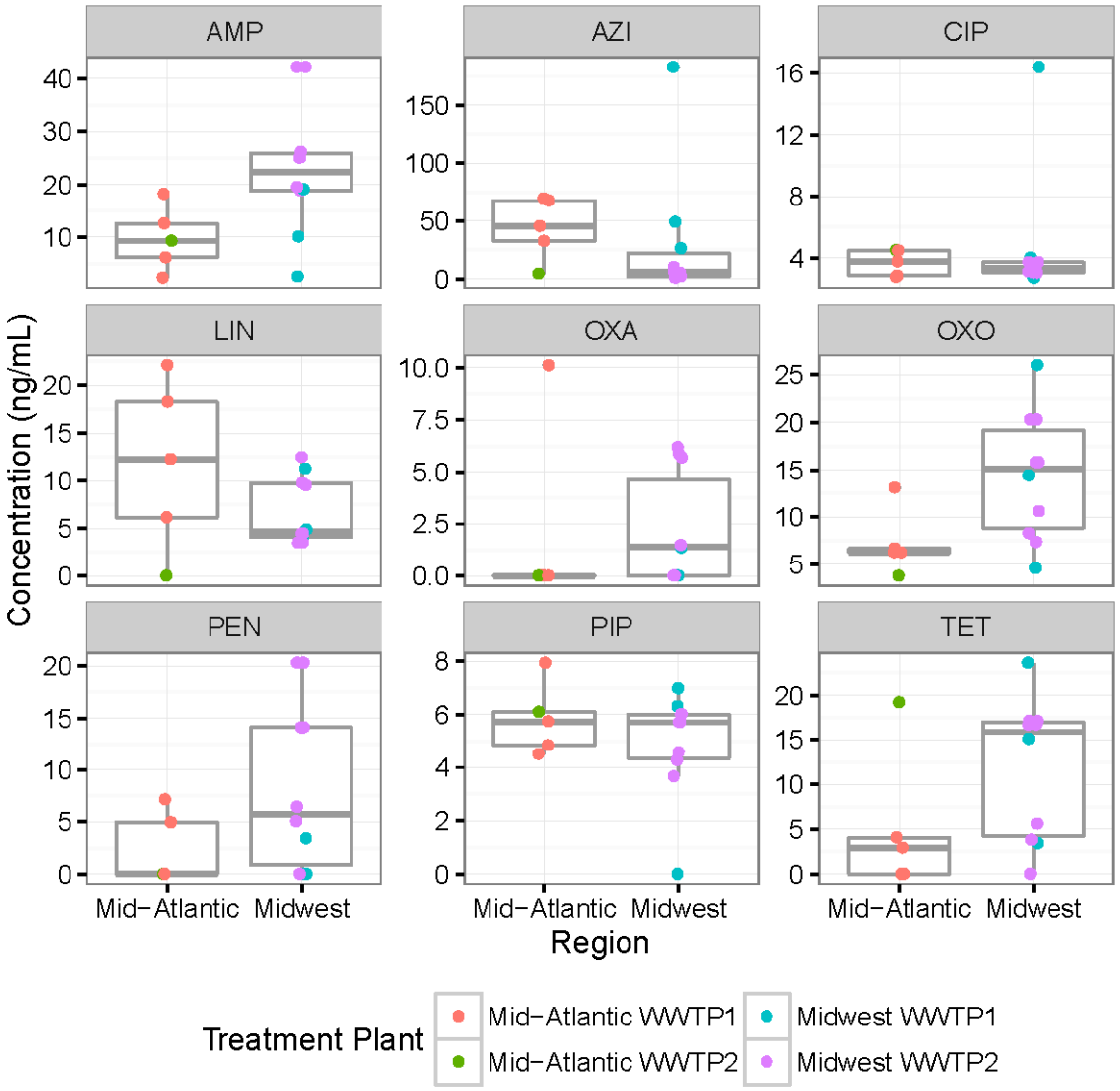

Figure S2: Differences in antibiotic concentrations (ng/mL) between effluent samples collected from Mid-Atlantic versus Midwest wastewater treatment plants (WWTPs)

AMP = Ampicillin; AZI = Azithromycin; CIP = Ciprofloxacin; LIN = Linezolid;

OXA = Oxacillin; OXO = Oxolinic Acid; PEN = Penicillin; PIP = Pipemidic Acid;

TET = Tetracycline

References

1. Rosenberg Goldstein RE, Micallef SA, Gibbs SG, et al. Methicillin-resistant Staphylococcus aureus (MRSA) detected at four U.S. wastewater treatment plants. *Environ Health Perspect*. 2012;120(11):1551-1558. doi:10.1289/ehp.1205436.
2. Rosenberg Goldstein RE, Micallef SA, Gibbs SG, et al. Detection of vancomycin-resistant enterococci (VRE) at four U.S. wastewater treatment plants that provide effluent for reuse. *Sci Total Environ*. 2014;466-467:404-411. doi:10.1016/j.scitotenv.2013.07.039.
3. Rosenberg Goldstein RE, Micallef SA, Gibbs SG, et al. Occupational exposure to Staphylococcus aureus and Enterococcus spp. among spray irrigation workers using reclaimed water. *Int J*

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