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U.S. nationwide reconnaissance of ten infrequently monitored antibiotics in municipal biosolids

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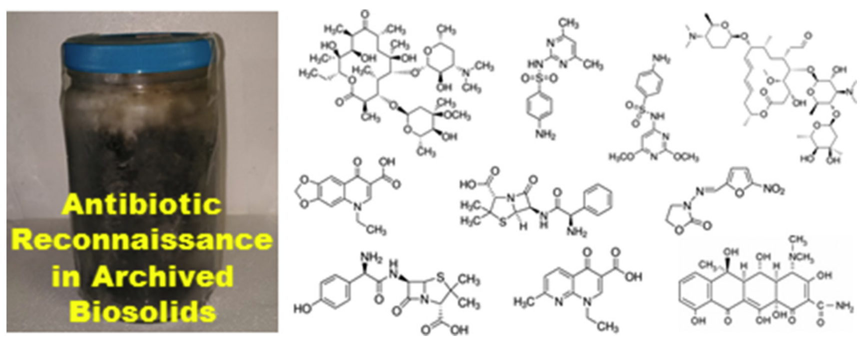
HIGHLIGHTS

- Six of 10 antibiotics tested were found in U.S. sewage sludges.
- First data on spiramycin, NP-AOZ, and nalidixic acid in U.S. sludge
- Detected concentrations were in the range of 0.1 to 46.5 ng/g dry weight.
- Analysis of archived sludges can reveal antibiotic uses from the past.
- Percent losses of 5 antibiotics during freezing storage are reported.

GRAPHICAL ABSTRACT



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ABSTRACT

Ten infrequently monitored antibiotics in biosolids were examined in archived American sewage sludges ($n = 79$) collected as part of the 2006/2007 U.S. Environmental Protection Agency (EPA) Targeted National Sewage Sludge Survey. This study inspected the occurrence of amoxicillin, ampicillin, erythromycin, furazolidone [proxy metabolite: 3-(2-nitrobenzylidenamino)-2-oxazolidinone (NP-AOZ)], nalidixic acid, oxolinic acid, oxytetracycline, spiramycin, sulfadimidine, and sulfadimethoxine in sewage sludges after nearly a decade in frozen storage. Six antibiotics were detected at the following average concentrations (ng/g dry weight): amoxicillin (1.0), nalidixic acid (19.1), oxolinic acid (2.7), erythromycin (0.6), oxytetracycline (4.5), and ampicillin (14.8). The remaining four were not detected in any samples (<method detection limit, ng/g dry weight): sulfadimethoxine (<0.5), sulfadimidine (<1.0), spiramycin (<2.0), and NP-AOZ (<20.0). This study provides the first data on spiramycin, NP-AOZ, and nalidixic acid in U.S. sewage sludges. This study also provides new data on the losses of 5 antibiotics during long term frozen storage (−20 °C) in comparison to the 2006/2007 U.S. EPA Targeted National Sewage Sludge Survey.

1. Introduction

Antibiotics are used in human medicine, in companion animals, as well as in agriculture and aquaculture to treat various bacterial infections (Done et al., 2015). Recent research has shown that wastewater

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treatment plants (WWTPs) represent an important route of dispersal into the water environment of antibiotics, their transformation products, drug-resistant bacteria, and drug-resistance genes (Pruden et al., 2013). Treated effluent dominates antibiotic inputs into aquatic environments, and disposal of sewage sludge is a major release route of antibiotics to terrestrial environments (McClellan and Halden, 2010). The U.S. Environmental Protection Agency (EPA) defines biosolids as treated solids produced from wastewater treatment that are nutrient-rich and can be safely recycled and applied as fertilizer upon appropriate treatment (U.S. Environmental Protection Agency, 2016). It is estimated that approximately 6.5 million metric tons of municipal sludge are generated in the United States each year (Venkatesan and Halden, 2016). About 55% of this mass is applied on land for beneficial purposes, and the remainder is either incinerated or placed in landfills (Kinney et al., 2006; U.S. Environmental Protection Agency, 2016). It has been established that antibiotic occurrence influences the development of antibiotic resistance genes and furthermore, land application of biosolids has been associated with the occurrence of antibiotic resistance genes in soils (as reviewed by (McLain et al., 2017)). Several recent studies have highlighted the abundance of antibiotic resistance genes in soils applied with manure (Li et al., 2017; McKinney et al., 2018; Wallace et al., 2018), biosolids (Guo et al., 2017; Yang et al., 2018), or both (Rogers et al., 2018). Other recent studies have examined municipal waste treatment methods and the effect of removal of antibiotic resistance genes on biosolid land application (Burch et al., 2017; Lau et al., 2017; Liao et al., 2018).

The present study explored frozen, archived biosolids for the presence and abundance of erythromycin (ERY), oxolinic acid (OXA), oxytetracycline (OXY), sulfadimethoxine (SUL), sulfadimidine (synonym: sulfamethazine, SDD), ampicillin (AMP), amoxicillin (AMX), nalidixic acid (NDA), spiramycin (SPI), and furazolidone (FUR) as its metabolite 3-(2-nitrobenzylidenamino)-2-oxazolidinone (NP-AOZ). ERY has been used in both human and animal care (Alexander and Schoch, 1954; Forbes, 1954; Gilchrist et al., 2007; Urteaga et al., 1955) as well as OXY (Duff and Galyean, 2007; Linsell and Fletcher, 1950; Rigos and Smith, 2015). In the U.S. OXA, SUL, and SDD have primarily been used in veterinary applications (Baroni et al., 2008; Knox et al., 2011; Wells, 1994). Ampicillin (AMP) and amoxicillin (AMX) are widely used in the U.S. for human and veterinary applications (Chang et al., 2016; Elizalde-Velázquez et al., 2016; Gozlan et al., 2013; Hof et al., 1997; Knox et al., 2011). Nalidixic acid (NDA), spiramycin (SPI), and furazolidone (FUR) are three antibiotics that have never before been monitored in U.S. biosolids. The use of NDA was discontinued in the U.S. in 1992 due to human toxicity and carcinogenic concerns (Emmerson and Jones, 2003), but there are NDA derivatives (quinolones) in use today (Hicks et al., 2015). SPI was discovered in the 1950s (Knox et al., 2011), but it has not been subjected to the stringent testing required by the U.S. Food and Drug Administration (FDA) for use in the U.S. (Kuhlmann and Fleckenstein, 2017). The U.S. FDA banned FUR from use in animals in 2002 and humans in 2005 due to tumorigenic properties of the compound (Francesco et al., 2009).

During 2006 and 2007, the U.S. EPA conducted a biosolid sampling campaign at 74 U.S. WWTPs and released the results in 2009 as the Targeted National Sewage Sludge Survey (TNSSS) (U.S. Environmental Protection Agency, 2009). 44 antibiotics were screened in this study with concentrations detected in the ng/g to μg/g dry weight (DW) range (U.S. Environmental Protection Agency, 2009). Our lab procured these samples and maintains them in frozen storage as a part of the National Sewage Sludge Repository (NSSR) at Arizona State University (Venkatesan et al., 2015). In order to determine the longevity of antibiotics in frozen storage, the TNSSS samples were re-examined after nearly a decade in storage for the presence of ERY, OXA, OXY, SUL, and SDD. AMP and AMX were added to the present analysis because of their high use in the U.S. in both humans and animals, but they were absent from the 2009 EPA study. NDA, SPI, and FUR (as NP-AOZ) were banned for use in the U.S. at the time of sampling. Sources of these compounds may be making it into the U.S. and finding their way into wastewater as highlighted in a 2006 Australian study which found antibiotic resistance genes present in aquaculture despite no antibiotics approved for aquaculture use (Akinbowale et al., 2006). Understanding the long term efficacy of antibiotics in storage can shed light on the persistence of antibiotics in the environment and highlight the need for continued monitoring for approved and unapproved antibiotics.

2. Materials and methods

2.1. Samples

Biosolids grab samples were collected by the EPA as described previously (U.S. Environmental Protection Agency, 2009; Venkatesan et al., 2014). Briefly, sampling personnel were provided with sampling instructions and received hands-on training prior to field work. Biosolid samples were collected from multiple areas of large piles, for example, a compost pile or a drying bed, or multiple grabs from a continuous process such as a belt press or filter press. Several kilograms of sample were collected, mixed, and stored in amber glass jars with a PTFE lid liner without the addition of preservatives. Samples were placed on ice for transport and were stored at −11 °C upon arrival at the EPA.

A total of 83 samples were collected from 74 publically owned U.S. WWTPs, but 4 samples were compromised during shipping and storage resulting in 79 samples for analysis (U.S. Environmental Protection Agency, 2009; Venkatesan et al., 2014). Four of these samples are duplicate samples from WWTPs that utilize 2 different forms of treatment and 6 samples are duplicate samples from WWTPs to account for variation in the sampling procedure. Details on the wastewater treatment type used at the WWTPs were not made available. The collected samples were categorized into four regions of the U.S. (Northeast $n = 20$ states, South $n = 26$, Midwest $n = 21$, West $n = 12$; Fig. 1). See Supplemental material for a complete list of contributing cities (Table 1). The U.S. regions were previously determined by the EPA during sampling in 2006/2007. In this present study, four regional composites of all samples from one of the four regions were used for method development, and for the determination of analyte recovery and method detection limit (MDL). Samples ($n = 79$) from the previous EPA study were individually extracted and analyzed in here (U.S. Environmental Protection Agency, 2009; Venkatesan et al., 2014). Samples were obtained by our lab and have been stored at −20 °C in freezers monitored by a Rees Scientific Centron System (Trenton, NJ, USA). These samples are included in the National Sewage Sludge Repository (NSSR) and Human Health Observatory (HHO) at Arizona State University (Venkatesan et al., 2015).

2.2. Materials

Analytical standards of antibiotics were purchased from Sigma-Aldrich (St. Louis, MO): AMP, AMX, ERY, NDA, OXA, OXY, SDD, SPI (≥80.0% SPI I, ≤5.0% SPI II, and ≤10.0% SPI III), SUL, and NP-AOZ (for determination of FUR as presented by Vass et al. (2005)). The isotopically-labeled analogs erythromycin-(*N,N-dimethyl-*$^{13}C_2$), [ERY-$^{13}C_2$]; 3-(2-nitrobenzylidenamino-)-2-oxazolidinone-d_4, [NP-AOZ-d_4]; and sulfadimethoxine-(*phenyl-*$^{13}C_6$), [SUL-$^{13}C_6$]; were purchased from Sigma-Aldrich (St. Louis, MO). LC-MS grade acetonitrile (ACN), water, acetic acid, and methanol (MeOH) were also purchased from Sigma-Aldrich (St. Louis, MO). Ortho-phosphoric acid (85%) was purchased from Fisher Scientific (Pittsburgh, PA, USA). Sodium hydroxide pellets (NaOH; J.T. Baker brand) were purchased from VWR (Radnor, PA, USA). Ultrapure water (18.3 Ohm) was provided by a NANOpure water system (Elga; Woodridge, IL, USA). Stock solutions of 1.0 mg/mL in methanol were created for each analyte, with the exception of ERY, which was purchased at a concentration of 1.0 g/L in water and NDA, which required the addition of 0.1 M NaOH to increase solubility (Dinh et al., 2011). Standard mixtures of all antibiotics were