Occurrence of Antibiotics in Drinking Water

Zhengqi Ye, Howard S. Weinberg
Department of Environmental Sciences and Engineering
The University of North Carolina at Chapel Hill

Michael T. Meyer
U. S. Geological Survey, Kansas

Abstract

The occurrence of antibiotics in the aquatic environment has raised concern regarding their potential impact on drinking water quality. Analytical methods were developed for determination of a wide range of antibiotics in source and finished drinking waters using solid-phase extraction and liquid chromatography electrospray tandem mass spectrometry. The methods were applied to a preliminary occurrence study in source and finished drinking waters in North Carolina. A variety of antibiotics, including fluoroquinolones, sulfonamides, lincomycin, tetracyclines and macrolides were detected in source waters. Some of the antibiotic analytes persisted into finished drinking waters albeit at very much reduced levels.

Introduction

Antibiotics are widely used in human and veterinary medicines for disease treatment. They are also largely used in animal operations for growth promotion and for disease prophylaxis. They are often partially metabolized after administration and a significant portion of the antibiotic can be excreted as the parent compound or in conjugated forms that can be converted back to the parent antibiotic [1-3]. The residual antibiotics from human and animal use can enter the environment via various pathways, including wastewater effluent discharge, runoff from land to which agricultural or human waste has been applied, and leaching. A variety of antibiotics have been detected in wastewater effluents [4-10] and natural waters [4, 11-17] at ng/L to low µg/L levels.

As microcontaminants, antibiotics in the aquatic environment may persist and be transported to reservoirs supplying source water to drinking water treatment plants. The potential presence of antibiotics in source drinking water is of concern due to the unknown health effects of chronic low-level exposure to antibiotics over a lifetime if the antibiotics survive drinking water treatment and are present in consumer's drinking water. The objective of this study is to determine the occurrence levels of antibiotics in source and finished drinking waters.

Experimental

Chemicals

Tetracycline standards in their hydrochloride form, sulfonamides, macrolides, trimethoprim, lincomycin hydrochloride, and meclocycline sulfosalicylate salt were purchased from Sigma-Aldrich (St. Louis, MO). Ciprofloxacin hydrochloride was purchased from ICN Biochemicals, Inc. (Irvine, CA). Enrofloxacin hydrochloride (≥ 98%) was purchased from Fluka (Buchs, Switzerland). $^{12}C_6$-sulfamethazine was purchased from Cambridge Isotope Laboratories (Andover, MA). Simatone was purchased from Accustandards (New Haven, CT).

Sample collection

Source water samples were collected at drinking water treatment plant intakes prior to any water treatment process. Finished water samples were collected after the final treatment step and before entry into the

distribution system. The residual chlorine disinfectants in finished water were quenched by ascorbic acid at the time of sample collection. Grab samples were collected in acid-washed 1 L amber glass bottles, which were packed on ice and transported to the laboratory overnight. The samples were stored at 4˚C and extracted within 3 days of collection.

Analytical methods

250 mL of source water and 500 mL of finished water samples were extracted using 200 mg hydrophilic-lipophilic balance (HLB) cartridges (Waters, Milford, MA). The sample was spiked with surrogate standards ($^{13}C_6$-sulfamethazine and meclocycline). Na_2EDTA was added at 1 mg/L into sample to ensure that tetracycline antibiotics do not form complex with divalent ions and metals. The source water sample was adjusted to pH 6 while the pH of the finished water sample was adjusted to 3. The sample was then extracted through a HLB cartridge and subsequently eluted with acidified methanol. The extracts were reduced to a volume of 100 µL under a gentle stream of nitrogen, reconstituted up to 250 µL in a mixture of water/methanol (9:1), and spiked with the internal standard simatone. The extracts were filtered though 0.45 µm syringe filters (Waters, Milford, MA), transferred to amber autosampler vials, stored at -15ºC, and analyzed within one week.

The detection methods for antibiotics were developed on a Varian model 1200L triple quadrupole mass spectrometer (Varian, Walnut Creek, CA). All the analytes were separated using a Pursuit C-18 column (150 × 2 mm, 3 µm, Varian, Walnut Creek, CA) using gradient elution at a mobile phase flow rate of 0.2 mL/min. Mobile phase A was 0.1% formic acid (v/v) in water and phase B was 100% acetonitrile. 20 µL of sample was injected. All the analytes were analyzed using electrospray ionization in positive mode. Two major fragment ions of each analyte were monitored in MS/MS detection. Detection was based on the identification of the most intense product ion for each analytes. Quantitation was based on the ratio of peak area of the most intense product ion of the analyte to that of the product ion of internal standard. The method of standard addition was used for quantitation. The raw water samples were spiked with analytes at 20 to 100 ng/L, while the finished water samples were spiked with analytes at 10 to 75 ng/L.

Results and Discussion

Samples were collected from five drinking water treatment plants (WTP) that use conventional treatment processes. Antibiotics were detected in source and finished water samples (Tables 1 and 2). The values in parentheses indicate positive identification of the species but at concentrations below the lowest spiked level.

WTP1 showed the largest number of antibiotics in its source water that is downstream of multiple wastewater plants and agricultural non-point discharge. Overall, fluoroquinolones were the most frequently detected antibiotics in source waters followed by sulfonamides, lincomycin, tetracyclines and macrolides. Although the detection of ciprofloxacin, which is only used in human medication, seems to indicate the impact of wastewater discharge, the possibility of contamination from animal source should not be precluded as the veterinary fluoroquinolone enrofloxacin can, under certain conditions, be metabolized to ciprofloxacin [3]. The occurrence levels of antibiotics obtained in this study are generally lower than the data included in the U.S. Geological Survey's national reconnaissance survey [13] in which the selection of sampling streams was biased towards those susceptible to wastewater contamination. Although the occurrence level of individual antibiotics is low, the presence of a broad range of antibiotics in some source waters suggests that the overall effect of the antibiotics as a contaminant group should not be underestimated.

Most of the antibiotics detected in source water are not detected in finished water or are present at a much lower levels if detected, indicating their partial removal during conventional treatment processes or chemical transformation during disinfection. The incomplete removal of some antibiotics, such as ciprofloxacin, is of concern due to their unknown health effects if they do persist in finished waters even at ng/L levels. Future research will be focused on how these compounds persist and transform during drinking water treatment processes.

Table 1. Occurrence of Antibiotics in Source Drinking Water (ng/L)

	WTP1	WTP2	WTP3	WTP4	WTP5
Ciprofloxacin	70	68	55	(17)	(8.3)

Norfloxacin	64	37	38	< 20	< 20
Enrofloxacin	43	24	20	49	< 20
Sarafloxacin	(15)	< 20	< 20	< 20	< 20
Lincomycin	(8.0)	< 20	(14)	(7.0)	< 20
Sulfamethoxazole	62	< 20	< 20	< 20	< 20
Sulfadimethoxine	(15)	< 20	< 20	< 20	< 20
Sulfathiazole	< 20	< 20	< 20	< 20	< 20
Sulfamerazine	< 20	< 20	< 20	< 20	< 20
Sulfamethazine	< 20	< 20	< 20	< 20	< 20
Sulfamethizole	< 20	< 20	< 20	< 20	< 20
Sulfachlorpyridazine	< 20	< 20	< 20	< 20	< 20
Trimethoprim	< 20	< 20	< 20	< 20	< 20
Oxytetracycline	(15)	< 20	< 20	< 20	< 20
Tetracycline	25	(13)	< 20	< 20	< 20
Doxycycline	< 20	22	< 20	< 20	< 20
Chlortetracycline	< 20	< 20	< 20	< 20	< 20
Demeclocycline	< 20	< 20	< 20	< 20	< 20
Minocycline	< 100	< 100	< 100	< 100	< 100
Tylosin	21	(12)	< 20	< 20	< 20
Erythromycin	(6.7)	< 20	< 20	< 20	< 20
Roxithromycin	< 20	< 20	< 20	< 20	< 20

Table 2. Occurrence of Antibiotics in Finished Drinking Water (ng/L)

	WTP1	WTP2	WTP3	WTP4	WTP5
Ciprofloxacin	(18)	(13)	(7.6)	< 10	< 10
Norfloxacin	< 20	5.5	< 75	< 10	< 20
Enrofloxacin	< 20	< 20	< 75	< 10	< 10
Sarafloxacin	< 20	< 20	< 75	< 10	< 10
Lincomycin	(9.6)	< 20	< 75	<10	< 10
Sulfamethoxazole	< 20	< 20	< 75	< 20	< 10
Sulfadimethoxine	< 20	< 20	< 75	< 10	< 10
Sulfathiazole	< 20	< 20	< 75	< 20	< 20
Sulfamerazine	< 20	< 20	< 75	< 20	< 10
Sulfamethazine	< 20	< 20	< 75	< 10	< 10
Sulfamethizole	< 20	< 20	< 75	< 20	< 20
Sulfachlorpyridazine	< 20	< 20	< 75	< 10	< 10
Trimethoprim	< 20	< 20	< 75	< 10	< 10
Oxytetracycline	< 20	< 20	< 75	< 10	< 10
Tetracycline	< 20	< 20	< 75	< 10	< 10
Doxycycline	< 20	(4.5)	< 75	< 10	< 10
Chlortetracycline	< 20	< 20	< 75	< 10	< 10
Demeclocycline	< 20	< 20	< 75	< 10	< 10
Minocycline	< 75	< 75	< 75	< 50	< 50
Tylosin	< 20	(12)	< 75	< 10	< 10
Erythromycin	< 20	< 20	< 75	< 10	< 10
Roxithromycin	< 20	< 20	< 75	< 10	< 10

Acknowledgements

This work was funded by the North Carolina Urban Water Consortium through the Water Resource Research Institute of the University of North Carolina. Additional support was provided through the Science to Achieve Results (STAR) program of the U. S. Environmental Protection Agency (Project No. R829014).

References

1. Physician's Desk Reference, 2004, Thomson PDR, Montvale, NJ

2. McEvoy, G. K (editor), 2004, *AHFS Drug Information*, the American Society of Health-System Pharmacists, Inc.

3. USP Veterinary Pharmaceutical Information Monographs – Antibiotics, 2003, Journal of Veterinary Pharmacology and Therapeutics, 26, supplement

4. Hirsch, R., Ternes, T., Haberer, K., Kratz, K. 1999, *Occurrence of antibiotics in the aquatic environment.* Science of the Total Environment, 225(1, 2), 109-118

5. Hartig, C., Storm, T., Jekel, M. 1999, *Detection and identification of sulphonamide drugs in municipal waste water by liquid chromatography coupled with electrospray ionisation tandem mass spectrometry*. Journal of Chromatography A, 854, 163-173

6. Golet, E. M., Alder, A. C., Hartmann, A., Ternes, T. A., Giger, W. 2001, *Trace determination of Fluoroquinolone antibacterial agents in urban wastewater by solid-phase extraction and liquid chromatography with fluorescence detection.* Analytical Chemistry, 73, 3632-3638

7. McArdell, C. S., Molnar, E., Suter, M. J.-F., Giger, W. 2003, *Occurrence and fate of macrolide antibiotics in wastewater treatment plants and in the Glatt Valley watershed, Switzerland*. Environmental Science and Technology, 37(24), 5479-5486

8. Miao, X., Bishay, F., Chen, M., Metcalfe, C. D. 2004, *Occurrence of antimicrobials in the final effluents of wastewater treatment plants in Canada*. Environmental Science and Technology, in press

9. Renew, J., Huang, C.-H. 2004, *Simultaneous determination of fluoroquinolone, sulfonamide, and trimethoprim antibiotics in wastewater using tandem solid phase extraction and liquid chromatography-electrospray mass spectrometry*. Journal of Chromatography A. in press

10. Gobel, A., McArdell, C. S., Suter, M. J.-F., Giger, W. 2004, *Trace determination of macrolide and sulfonamide antimicrobials, a human sulfonamide metabolite, and trimethoprim in wastewater using liquid chromatography coupled to electrospray tandem mass spectrometry*. Analytical Chemistry, in press

11. Lindsey, M. E., Meyer, M. T., Thurman, E. M. 2001, *Analysis of trace levels of sulfonamide and tetracycline antimicrobials in groundwater and surface water using solid-phase extraction and liquid-chromatography/mass spectrometry*, Analytical Chemistry, 73, 4640-4646

12. Sacher, F., Lange, F. T., Brauch, H-J., Blankenhorn, I. 2001, *Pharmaceuticals in groundwaters Analytical methods and results of a monitoring program in Baden-Württemberg, Germany*. Journal of Chromatography A, 938, 199-210

13. Kolpin, D. W., Furlong, E. T., Meyer, M. T., Thurman, E. M., Zaugg, S. D., Barber, L. B., Buxton, H. T. 2002, *Parmaceuticals, hormones, and other organic wastewater contaminants in U.S. streams 1999-2000: A national reconnaissance*, Environmental Science and Technology, 36, 1202-1211

14. Hilton, M. J., Thomas, K. V. 2003, *Determination of selected human pharmaceutical compounds in effluent and surface water samples by high-performance liquid chromatography-electrospray tandem mass spectrometry*. Journal of Chromatography A, 1015(1-2), 129-141

15. Reverté, S., Borrull, F., Pocurull, E., Marcé, R. M. 2003, *Determination of antibiotic compounds in water by solid-phase extraction-high-performance liquid chromatography-(electrospray) mass spectrometry*. Journal of Chromatography A, 1010(2), 225-232

16. Vanderford, B. J., Pearson, R. A., Rexing, D. J., Snyder, S. A. 2003, *Analysis of endocrine disruptors, pharmaceuticals, and personal care products in water using liquid chromatography/tandem mass spectrometry*. Analytical Chemistry, 75(22), 6265-6574

17. Yang, S., Carlson, K. 2003, *Evolution of antibiotic occurrence in a river through pristine, urban and agricultural landscapes*. Water Research, **3**7 (19), 4645-4656

Biographical Sketches

Zhengqi Ye
Department of Environmental Sciences and Engineering
The University of North Carolina at Chapel Hill
Chapel Hill, NC 27599
Phone: 919-966-0520
Fax: 919-966-7911
E-mail: yzq@email.unc.edu

Zhengqi Ye is a Ph.D. candidate in Environmental Sciences and Engineering at The University of North Carolina at Chapel Hill. She received her B.S. and M.S. degrees in Environmental Chemistry from Nankai University in China. Her research interest is in the analysis of organic contaminants in drinking water and their fate in water treatment.

Howard S. Weinberg
Department of Environmental Sciences and Engineering
The University of North Carolina at Chapel Hill
Chapel Hill, NC 27599
Phone: 919-966-3859
Fax: 919-966-7911
E-mail: howard_weinberg@unc.edu

Dr. Howard Weinberg is an assistant professor in the Department of Environmental Sciences and Engineering at the University of North Carolina at Chapel Hill, U.S.A. As an environmental and analytical chemist Dr Weinberg has, over the past 15 years, developed many new analytical methods for byproducts of disinfection and other micropollutants in drinking water.

Michael T. Meyer
U.S. Geological Survey
4821 Quail Crest Place
Lawrence, KS 66049
Phone: 785-832-3544
Fax: 785-832-3500
E-mail: mmeyer@usgs.gov

Dr. Michael Meyer has been a Research Geochemist for the U.S. Geological Survey (USGS) since 1988. He received his Ph.D. in Geology from the University of Kansas in 1994. His research has focused on the development of methods for the analysis of emerging organic contaminants (e.g. herbicide metabolites and pharmaceuticals) and determining their occurrence, fate, and geochemical transport in the environment. Currently he is Director of the USGS, Kansas District, Organic Geochemistry Research Laboratory.