Occurrence of antibiotics in pharmaceutical industrial wastewater, wastewater treatment plant and sea waters in Tunisia

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ABSTRACT

Antibiotics are among the most commonly used group of pharmaceuticals in human medicine. They can therefore reach surface and groundwater bodies through different routes, such as wastewater treatment plant effluents, surface runoff, or infiltration of water used for agricultural purposes. It is well known that antibiotics pose a significant risk to environmental and human health, even at low concentrations. The aim of the present study was to evaluate the presence of aminoglycosides and phenicol antibiotics in municipal wastewaters, sea water and pharmaceutical effluents in Tunisia. All analysed water samples contained detectable levels of aminoglycoside and phenicol antibiotics. The highest concentrations in wastewater influents were observed for neomycin and kanamycin B (16.4ng mL^{-1} and 7.5 ng mL^{-1}, respectively). Chloramphenicol was found in wastewater influents up to 3 ng mL^{-1}. It was observed that the waste water treatment plants were not efficient in completely removing these antibiotics. Chloramphenicol and florfenicol were found in sea water samples near aquaculture sites at levels up to, respectively, 15.6 ng mL^{-1} and 18.4 ng mL^{-1}. Also aminoglycoside antibiotics were found near aquaculture sites with the highest concentration of 3.4 ng mL^{-1} for streptomycin. In pharmaceutical effluents, only gentamycin was found at concentrations up to 19 ng mL^{-1} over a sampling period of four months.

Key words | antibiotics contamination, marine pollution, pharmaceutical wastewater, wastewater treatment plant

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INTRODUCTION

During recent decades, research of environmental contaminants has expanded beyond classical contaminants like pesticides, biocides and dioxins, to personal care products and pharmaceuticals (Ternes *et al.* 2004). Antibiotic compounds are widely used in human and veterinary medicine as a preventive or curative treatment for bacterial infections or as growth promoters (Sapkota *et al.* 2008). After administration, these compounds are only partially metabolized and are usually excreted unchanged in the urine and faeces (Junker *et al.* 2006; Mitani & Kataoka 2006).

Wastewaters become one of the main routes for the introduction of antibiotics into the environment (Ericson 2002; Karthikeyan & Meyer 2006; Xu *et al.* 2007) since they are often not completely eliminated during waste water treatment (Fatta-Kassinos *et al.* 2011; Yan *et al.* 2013a, 2013b). Resistance to antibiotics constitutes a major threat to public health and ought to be recognized as such more widely than it is at present. Moreover, wildlife could be severely affected by the presence of antibiotics in the environment, even at trace levels. Therefore, the European Union (EU) recommends the prudent use of antimicrobial agents in medicine (Werner & Bronzwaer 2007; Torres *et al.* 2010).

Aminoglycoside antibiotics are widely used in hospitals for treatment of serious human infection by Gram-negative and Gram-positive bacteria (Löffler & Ternes 2003) and in

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veterinary medicine (Salisbury 1995). Gentamicin, spectinomycin and lincomycin are among the most commonly used aminoglycosides (Mitscher *et al.* 2008; Beale 2011; Vucicevic-Prceti *et al.* 2011). Chloramphenicol, thiamphenicol and florfenicol have been used in human and veterinary medicine since the 1950s. Although many countries have strictly banned the use of these antibiotics in food-producing animals (Gantverg *et al.* 2003; European Commission EEC 2377/90). Illegal use of chloramphenicol and thiamphenicol in livestock and aquaculture still exists in China due to their low prices and steady antibiosis effectiveness (Chen *et al.* 2009). However, few researches have examined the contamination of those compounds, especially in the aquatic environment (Yan *et al.* 2013b). In our study, we evaluate the presence of aminoglycosides and phenicol antibiotics in Tunisian waters and pharmaceutical effluents. Samples were analysed using ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS). To our best knowledge, this is the first study to determine those antibiotics in pharmaceutical effluents, waste waters and sea waters nearby aquaculture sites in North African countries.

MATERIALS AND METHODS

Water sampling

All samplings were performed in 2013. Influent and effluent waste water samples were obtained from four full-scale wastewater treatment plants (WWTPs) in the region of Grand-Tunis, a big metropolitan area in Tunisia, covering Tunis-nord, Charguia, Chotrana and Ben Arous.

The pharmaceutical effluents were collected at two discharge sites in the environment in the north of Tunisia (Zghouen and Ariana). At each sampling site, one monthly sampling was performed for four successive months (February–May).

Sea water samples were taken at Tunis, Bizerte, Monastir, Sousse and Hergla. The five sampling sites were chosen because of their human activities (aquaculture and hotels).

All samples were collected in a 1L amber recipient. After filtration through a 1 μm membrane filter (Euro Scientific, Belgium), aliquots were taken and stored at $\leq -20\ ^{\circ}C$ until analysis.

Chemicals and reagents

All analytical standards, except chloramphenicol-d_5, were purchased from Sigma-Aldrich (Bornem, Belgium). Chloramphenicol-d_5 was purchased from Witega (Berlin, Germany). Separate stock solutions of each antibiotic were prepared in methanol ($1\ mg\ mL^{-1}$) and stored in amber glass vials at $\leq -20\ ^{\circ}C$. ULC-grade solvents were purchased from Biosolve (Valkenswaard, The Netherlands). All other chemicals for extraction and purification were of high-performance liquid chromatography-grade quality (HPLC-grade). HPLC-grade water was produced by a Milli-Q® Gradient A10 water purification system (Millipore, Bedford, MA, USA) Oasis® HLB (600 mg, 6 mL) and Oasis® CBA (500 mg, 3 mL) solid phase extraction (SPE) cartridges were purchased from Waters (Milford, MA, USA).

Sample analysis

Water filtrates (100 mL) were spiked with the internal standard (chloramphenicol-d_5 or tobramycin for phenicol or aminoglycosides respectively). The water samples were further purified by SPE using Oasis® HLB cartridges for phenicol antibiotics and Oasis® CBA cartridges for aminoglycoside antibiotics. After elution, evaporation to dryness and reconstitution, an 8 μL aliquot was injected on the analytical column. UPLC-MS/MS analysis was performed using a Waters Acquity UPLC® class system and an Acquity UPLC® BEH C18 column (100×2.1 mm) interfaced with a Xevo TQ-S tandem quadrupole mass spectrometer, all from Waters (Milford, MA, USA). The instrument was used in the positive electrospray ionization mode for the analysis of the aminoglycosides and in the negative mode for phenicols. The method was validated according to commission decision 2002/657/EC for linearity, specificity, sensitivity, accuracy and precision (European Commission 2002/657/EC).

RESULTS AND DISCUSSION

Two distinct analytical protocols were applied to extract the phenicols and the aminoglycosides in waste and sea water. After extraction and purification, the samples were analysed with UPLC-MS/MS within 14 min. The methods were fully

validated and the validation data (data not shown) were compliant with the acceptance criteria of the commission decision 2002/657/EC. The methods were able to detect and quantify all compounds below 0.5 ng mL^{-1}.

Waste water samples were taken at influent and effluent from four sewage treatment plants in Grand Tunis, i.e. Tunis-nord, Charguia, Chotrana and Ben Arous. The results are presented in Table 1. Florfenicol and neomycin were detected in all waste water samples. The highest concentrations were observed for neomycin and kanamycin B (16.4 ng mL^{-1} and 7.5 ng mL^{-1}, respectively). The most detected antibiotics were present in both treated and untreated municipal water samples. This might indicate that the wastewater treatment process in Tunisia, which consists of pretreatment, primary settling and biological treatment (activated sludge) (Saddoud *et al.* 2007), is not very efficient in completely removing aminoglycoside and phenicol antibiotics from wastewater.

The phenicol antibiotics chloramphenicol and florfenicol were most frequently found in Hergla and Monastir sea waters (Table 2). The highest concentrations were detected in Hergla at 15.6 ng mL^{-1} and 18.4 ng mL^{-1} for chloramphenicol and florfenicol, respectively. Chloramphenicol is not authorized for use in food-producing animals in the EU (European Commission EEC 2377/90). The presence of this antibiotic in the vicinity of aquaculture sites of Hergla and Monastir might suggest that chloramphenicol is likely to enter the Tunisian food chain.

In comparison, chloramphenicol was quantified up to 68 ng L^{-1} in sewage treatment plant effluent in the study of Alexy & Kümmerer (2006). Lin *et al.* (2008) quantified the chloramphenicol in hospital effluent at 1 ng L^{-1} and the thiamphenicol at 4 ng L^{-1}. In the study of Lopez-Serna *et al.* (2013), chloramphenicol was not found in the groundwater in Spain, however this antibiotic was detected in Victoria, Pearl River, Harbour and in sea water in China (Xu *et al.* 2007). Xu *et al.* (2007) reported chloramphenicol concentrations up to 266 ng L^{-1} in the Pearl River in China.

Aminoglycoside antibiotics were only detected at low levels in all sea water samples. On the other hand, almost every sampling point was contaminated with these antibiotics. The highest concentration was found for streptomycin in sea water of Hergla at 3.4 ng mL^{-1}. Results of sea water samples are presented in Table 2.

Table 1 | Antibiotic concentrations detected in wastewater samples (ng mL^{-1}) using UPLC-MS/MS analysis

	Sampling sites							
	Tunis-nord		Charguia		Chotrana		Ben Arous	
Compound	I	E	I	E	I	E	I	E
Chloramphenicol	3.3	1.1	nd	nd	0.5	0.3	nd	nd
Thiamphenicol	1.2	nd	nd	nd	nd	nd	nd	nd
Florfenicol	3.3	0.8	1.2	0.8	1.4	0.6	0.9	0.1
Paromycin	4.2	1.3	nd	nd	nd	nd	1.2	0.9
Dihydrostreptomycin	0.9	0.4	nd	nd	nd	nd	nd	nd
Kanamycin B	7.5	5.4	1.2	0.7	1.0	0.7	0.5	nd
Aparamycin	1.5	0.5	1.2	0.4	nd	nd	0.7	nd
Streptomycin	nd	nd	2.7	1.2	1.6	0.8	nd	nd
Amikacin	2.1	1.8	nd	nd	nd	nd	2.3	1.0
Sisomycin	6.7	3.9	nd	nd	2.3	1.0	4.1	2.0
Neomycin	12	4.6	16.4	11.2	6.2	2.1	1.8	0.4
Gentamycin c1a	1.6	0.6	0.5	0.2	nd	nd	nd	nd
Gentamycin c1	0.8	0.3	0.8	0.4	nd	nd	0.6	nd
Gentamycin c2	1.0	0.3	nd	nd	nd	nd	nd	nd

nd: not detected.
I: influent water. E: effluent water.

Table 2 | Antibiotic concentrations in sea water samples (ng mL^{-1}) using UPLC-MS/MS analysis

	Sampling sites				
Compound	**Tunis**	**Bizerte**	**Monastir**	**Sousse**	**Hergla**
Chloramphenicol	0.4	nd	3.5	0.2	15.6
Florfenicol	nd	nd	2.9	nd	18.4
Paromycin	nd	nd	nd	nd	nd
Dihydrostreptomycin	0.7	0.3	0.9	0.3	1.9
Kanamycin B	0.1	0.2	0.5	0.6	1.5
Aparamycin	0.2	0.4	0.9	1.8	0.4
Streptomycin	nd	0.7	1.4	0.7	3.4
Amikacin	0.1	0.3	0.1	0.8	1.2
Sisomycin	nd	nd	0.4	nd	0.4
Neomycin	nd	nd	0.1	nd	0.7
Gentamycin c1a	nd	nd	0.8	0.2	1.4
Gentamycin c1	nd	nd	nd	nd	nd
Gentamycin c2	nd	nd	nd	nd	nd

nd: not detected.

Aminoglycosides are mostly non-metabolized after administration and excreted unchanged via the urine (Marzo & Dal Bo 1998). The analysis of hospital wastewater in Germany revealed that the concentration of gentamycin was between 0.4 and 7.6 μg mL^{-1} (Löffler & Ternes 2003).

Table 3 | Antibiotic concentrations determined in a pharmaceutical effluent (ng mL^{-1}) over a period of 4 months (in 2013) in Zghouen, North-Tunisia using UPLC-MS/MS analysis

Compound	**February**	**March**	**April**	**May**
Chloramphenicol	nd	nd	nd	nd
Florfenicol	nd	nd	nd	nd
Paromycin	nd	nd	nd	nd
Dihydrostreptomycin	nd	nd	nd	nd
Kanamycin B	nd	nd	nd	nd
Aparamycin	nd	nd	nd	nd
Streptomycin	nd	nd	nd	nd
Amikacin	nd	nd	nd	nd
Sisomycin	nd	nd	nd	nd
Neomycin	nd	nd	nd	nd
Gentamycin c1a	15.2	19.1	10.8	14.4
Gentamycin c1	nd	nd	nd	nd
Gentamycin c2	nd	nd	nd	nd

nd: not detected.

In our study, the presence of antibiotics in sea water can be explained by the discharges of municipal treated water and wastewaters from hotels in the sea around the populous region of Sousse and Tunis. Regarding sea waters, and as expected, concentrations found were lower than the ones in waste water samples. This could indicate that a dilution factor occurs once antibiotics enter the sea.

In pharmaceutical effluents, gentamycin c1a was detected at one sampling point (Zghouen) on each monthly taken sample. The average concentration found was 14.8 ng mL^{-1} (Table 3). It seems that this pharmaceutical company continuously releases these antibiotics into the environment.

The widespread occurrence of antibiotics in the environment may cause deleterious health effects. The transfer of resistant bacteria to humans via water and food or to other pathogenic bacteria is an important health issue (Fatta-Kassinos *et al.* 2011). In addition to the risk of development and spread of resistant bacteria, the antibiotics may be toxic for human health (anaemia and hypersensitivity) (Roybal 1998).

CONCLUSION

To the best of our knowledge, this is the first time that antibiotics were monitored in Tunisian waste and sea waters.

All analysed water samples contained detectable levels of aminoglycoside and phenicol antibiotics. Influent water of Tunis-Nord WWTP was heavily contaminated and contained not less than 14 antibiotics at concentrations ranging from 0.9 to 12 ng mL^{-1}. The highest concentrations in wastewater were observed for neomycin. It was further observed that the WWTPs were not efficient in removing the antibiotics.

Florfenicol and the EU-forbidden chloramphenicol antibiotic were found in sea water samples near aquaculture sites at levels up to 18.4 ng mL^{-1} and 15.6 ng mL^{-1}, respectively. Also aminoglycoside antibiotics were found nearby aquaculture sites with the highest concentration of 3.4 ng mL^{-1} streptomycin. The measured antibiotics are quite ubiquitous pollutants in the Tunisian region. The concentrations found in the waste and sea water samples might pose environmental and health risks.

REFERENCES

Alexy, R. & Kümmerer, K. 2006 Antibiotics for human use. In: *Organic Pollutants in the Water Cycle: Properties, Occurrence, Analysis and Environmental Relevance of Polar Compounds* (T. Reemtsma & M. Jekel, eds). Wiley-VCH, Weinheim, Germany, pp. 65–85.

Beale Jr., J. H. 2011 Antibacterial antibiotics. In: *Wilson and Gisvold's Textbook of Organic Medicinal and Pharmaceutical Chemistry*, 12th edn (J. M. Beale & J. H. Block Jr, eds). Lippincott Williams & Wilkins Publishers, Philadelphia, USA, pp. 294–300.

Chen, H. X., Chen, H., Ying, J., Huang, J. L. & Liao, L. 2009 Dispersive liquid-liquid microextraction followed by high-performance liquid chromatography as an efficient and sensitive technique for simultaneous determination of chloramphenicol and thiamphenicol in honey. *Anal. Chim. Acta* **632**, 80–85.

Ericson, B. E. 2002 Analyzing the ignored environmental contaminants. *Environ. Sci. Technol.* **36**, 140A–145A.

European Commission (2002/657/EC) of 12 August 2002, Implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results, Official Journal of the European Commission M221, 8–36.

European Commission (2377/90) of 26 June 1990, Laying down a Community procedure for the establishment of maximum residue limits of veterinary medicinal products in food stuffs of animal origin, Official Journal of the European Commission L244, 1–8.

Fatta-Kassinos, D., Meric, S. & Nikolaou, A. 2011 Pharmaceutical residues in environmental waters and wastewater: current state of knowledge and future research. *Anal. Chem.* **399**, 251–275.

Gantverg, A., Shishani, I. & Hoffman, M. 2003 Determination of chloramphenicol in animal tissues and urine liquid chromatography-tandem mass spectrometry versus gas chromatography-mass spectrometry. *Anal. Chim. Acta* **483**, 125–135.

Junker, T., Alexy, R., Knacker, T. & Kummerer, K. 2006 Biodegradability of 14 C-labeled antibiotics in a modified laboratory scale sewage treatment plant at environmentally relevant concentrations. *Environ. Sci. Technol.* **40**, 318–324.

Karthikeyan, K. G. & Meyer, M. T. 2006 Occurrence of antibiotics in wastewater treatment facilities in Wisconsin – USA. *Sci. Total Environ.* **361**, 196–207.

Lin, A. Y., Yu, T. & Lin, C. 2008 Pharmaceutical contamination in residential, industrial and agricultural waste streams; risk to aqueous environments in Taiwan. *Chemosphere* **74**, 131–141.

Löffler, D. & Ternes, T. A. 2003 Analytical methods for the determination of aminoglycoside gentamicin in hospital wastewater via liquid chromatography-electrospray-tandem mass spectrometry. *J. Chromatogr. A* **1000**, 583–588.

López-Serna, B., Kasprzyk-Hodern, B., Petrović, M. & Barceló, D. 2013 Multi-residue enantiomeric analysis of pharmaceuticals and their active metabolites in the Guadalquivir River basin (South Spain) by chiral liquid chromatography coupled with tandem mass spectrometry. *Anal. Bioanal. Chem.* **405**, 5859–5873.

Marzo, A. & Dal Bo, L. 1998 Chromatography as an analytical tool for selected antibiotic classes: a reappraisal addressed to pharmacokinetic applications. *J. Chromatogr. A* **812**, 17–34.

Mitani, K. & Kataoka, H. 2006 Determination of fluoroquinolones in environmental waters by in-tube solid-phase microextraction coupled with liquid chromatography–tandem mass spectrometry. *Anal. Chim. Acta* **562**, 16–22.

Mitscher, L. A., Lemke, T. L. & Gentry, E. J. 2008 Antibiotics and antimicrobial agents. In: *Foye's Principles of Medicinal Chemistry*, 6th edn (D. A. Williams & T. L. Lemke, eds). Lippincott Williams & Wilkins Publishers, Philadelphia, USA, pp. 1028–1083.

Roybal, J. E. 1998 Chloramphenicol and related drugs. In: *Analytical Procedures for Drug Residues in Food of Animal Origin* (S. B. Turnipseed & A. R. Long, eds). Science Technology System, West Sacramento, CA, USA, pp. 227–260.

Saddoud, A., Ellouze, M., Dhouid, A. & Sayadi, S. 2007 Anaerobic membrane bioreactor treatment of domestic waste water in Tunisia. *Desalination* **207**, 205–215.

Salisbury, C. D. C. 1995 Chemical analysis aminoglycoside antibiotics. In: *Chemical Analysis for Antibiotics Used in Agriculture* (H. Oka, H. Nakazawa, K. K. E. Harada & J. D. MacNeil, eds). AOAC International, Arlington, USA.

Sapkota, A., Sapkota, A. R., Kucharski, M., Burke, J., McKenzie, S., Walker, P. & Lawrence, R. 2008 Aquaculture practices and potential human health risks: current knowledge and future priorities. *Environ. Int.* **34**, 1215–1226.

Ternes, T. A., Joss, A. & Siegrist, H. 2004 Scrutinizing pharmaceuticals and personal care products in wastewater treatment. *Environ. Sci. Technol.* **38**, 392–399.

Torres, C., Moreno, M. A. & Zarazaga, M. 2010 Prudent use of antimicrobial agents: not just for humans. *Enfer. Infecc. Microbiol. Clin.* **28**, 669–671.

Vucicevic-Prceti, K., Cservenak, R. & Radulovic, N. 2011 Development and validation of liquid chromatography tandem mass spectrometry methods for the determination of gentamicin, lincomycin, and spectinomycin in the presence of their impurities in pharmaceutical formulations. *J. Pharmaceut. Biomed.* **56**, 736–742.

Werner, G. & Bronzwaer, S. 2007 Ensuring prudent use of antimicrobials in human medicine in the European Union. *Eurosurveillance* **12**, 64–67.

Xu, W., Zhang, G., Zou, S., Li, X. & Liu, Y. 2007 Determination of selected antibiotics in the Victoria Harbour and the Pearl River, South China using high-performance liquid chromatography-electrospray ionization tandem mass spectrometry. *Environ. Pollut.* **145**, 672–679.

Yan, C., Dinh, Q. T., Chevreuil, M., Garnier, J., Roose-Amsaleg, C., Labadie, P. & Laverman, A. 2013a The effect of environmental and therapeutic concentrations of antibiotics on nitrate reduction rates in river sediment. *Water Res.* **47**, 3654–3662.

Yan, C., Yang, Y., Zhou, J., Liu, M., Nie, M., Shi, H. & Gu, L. 2013b Antibiotics in the surface water of the Yangtze Estuary: occurrence, distribution and risk assessment. *Environ. Pollut.* **175**, 22–29.

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