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Detection of beta-lactamase resistance genes in a hospital chlorinated wastewater treatment system

Claudia G. Țugui[1], Ioana Vlădăreanu[1], Andreea Baricz[2] and Cristian Coman[2] ✉

SUMMARY. In this study, by amplification of several specific resistance genes we aimed at evaluating the presence of beta-lactamase resistance genes in the influent and effluent of a hospital chlorinated wastewater treatment system. Four types of beta-lactamase resistance genes (blaPER, blaVIM, blaNDM-1 and SHV) were detected in the influent water sample, two of which (blaVIM and SHV) being detected in the effluent as well. Our results indicate the reduced effectiveness of wastewater treatment, as several resistance genes can be found in the water discharged after the chlorination treatment.

Keywords: Antibiotic resistance genes, beta-lactam antibiotics, chlorination, hospital wastewater

Introduction

Beta-lactam antibiotics are active agents against many Gram-positive as well as Gram-negative microorganisms (Thomson and Bonomo, 2005). These antimicrobials are widely used to treat bacterial infections in humans, thus, in most countries they are the largest group of antibiotics used by hospitals (ECDC 2013) potentiating the emergence of resistance strains able to withstand high concentrations of antibiotics. Clinical settings can be a potential source for spread and development of antibiotic resistance (Pauwels and Verstraete, 2006) due to the dispersal of antibiotic resistance genes by vertical or horizontal gene transfer to bacteria that are related or unrelated evolutionary and ecologically (Gomes *et al*., 2013). Chlorination is a widely used disinfection method, nonetheless recent studies indicated that chlorination can enhance changes in ARG (antibiotic resistance genes) abundance and diversity (Jia *et al.*, 2015). The aims of this study were to evaluate and compare the presence of beta-lactamase resistance genes in a hospital wastewater collected from a public hospital in Romania, before (influent water) and after the treatment by chlorination steps (effluent wastewater).

[1] *Faculty of Biology and Geology, Babeș-Bolyai University, Cluj-Napoca, Romania*
[2] *National Institute of Research and Development for Biological Sciences, Cluj-Napoca, Romania*
✉ ***Corresponding author**: **Cristian Coman**, Institute of Biological Research, Cluj-Napoca, Romania*
E-mail: cristian.coman@icbcluj.ro

Material and methods

The water samples were collected from the influent and effluent of a chlorination wastewater treatment system from a public hospital in Cluj-Napoca, Romania, in sterile 1 L containers and transported on ice to the laboratory, for further analyses. To concentrate the microbial biomass, the water was filtered onto MCE filtration membranes (47 mm in diameter and 0.2 μm pore size). Genomic DNA was extracted from the membranes by using ZR Soil Microbe DNA Miniprep (ZymoResearch, USA) according to the manufacturer's protocol. Beta lactamase specific genes were amplified by PCR using several primer pairs (Table 1). PCR mixture contained 2 μl genomic DNA, 0.25 μM of each primer, 5 μl of 5x MyTaq Reaction Buffer, 1.5 U of MyTaq Red DNA Polymerase, and water to a final volume of 25 μl. The amplification protocol included a initial denaturation step of 5 min at 95 ^{o}C, followed by 35 cycles of denaturation 30 sec at 95 ^{o}C, primer annealing at 56-58 ^{o}C (specific for each primer pair), and extension for 30 sec at 72 ^{o}C, and a final extension step of 10 min at 72 ^{o}C. PCR products of the expected size were verified by 2 % agarose gel electrophoresis and were visualized under UV after ethidium bromide staining.

Table 1.

Primers used for amplification of Beta-lactamase resistance genes.
Forward and reverse primer sequences are given.

Gene	Gene classification (Beta-lactamase Classes)	Sequence of forward primer (5'-3')	Sequence of reverse primer (5'-3')	References
blaPER	A	TGCTGGTTGCTGTTTTTGTGA	CCTGCGCAATGATAGCTTCAT	Jiang *et al*., 2013
blaPSE	A	TTGTGACCTATTCCCCTGTAATAGAA	TGCGAAGCACGCATCATC	Zhu *et al*., 2013
blaCTX-M	A	GGAGGCGTGACGGCTTTT	TTCAGTGCGATCCAGACGAA	Zhu *et al*., 2013
blaIMP	B	AACACGGTTTGGTGGTTCTTGTA	GCGCTCCACAAACCAATTG	Zhu *et al*., 2013
blaOXA-10	D	CGCAATTATCGGCCTAGAAACT	TTGGCTTTCCGTCCCATTT	Zhu *et al*., 2013
blaVIM	B	GCACTTCTCGCGGAGATTG	CGACGGTGATGCGTACGTT	Zhu *et al*., 2013
blaOXA-48	D	GTAGCAAAGGAATGGCAA	CCTTGCTGCTTATTGTCA	Naas *et al*., 2012
blaKPC	A	GATACCACGTTCCGTCTGG	GCAGGTTCCGGTTTTGTCTC	Hindiyeh *et al*., 2008
blaNDM-1	B	ATTAGCCGCTGCATTGAT	CATGTCGAGATAGGAAGTG	Naas *et al*., 2011
SHV	A	GCGAAAGCCAGCTGTCGGGC	ATTGGCGGCGCTGTTATCGC	Jiang *et al*., 2013
ampC	C	CAGCCGCTGATGAAAAAATATG	CAGCGAGCCCACTTCGA	Zhu *et al*., 2013

Results and discussion

By PCR amplification using primer pairs specific for beta-lactamase resistance genes we detected four beta-lactamase resistance genes blaVIM, blaNDM-1, SHV and blaPER, in the influent sample, whereas SHV and blaVIM genes were found in the effluent sample (Table 2). Over the past few years metallo-beta-lactamase (MBL) producing isolates have emerged worldwide and are associated with outbreaks in healthcare settings. They cause serious infections such as bacteremia and ventilator associated pneumonia, particularly in patients admitted to the ICU (De *et al*., 2010).

Table 2.

Detection of beta-lactamase resistance genes in the influent and effluent of the hospital chlorinated wastewater treatment system.

Resistance gene	Influent	Effluent
blaPER	+	-
blaPSE	-	-
blaCTX-M	-	-
blaIMP	-	-
blaOXA-10	-	-
blaVIM	+	+
blaOXA-48	-	-
blaKPC	-	-
blaNDM-1	+	-
SHV	+	+
ampC	-	-

blaVIM and blaNDM are the most common MBL genes, encoded by integron borne mobile gene cassettes. The VIM-1 enzyme has very broad substrate specificity, presence of blaVIM conferring resistance to broad array of beta-lactams (ampicillin, carbenicillin, piperacillin, mezlocillin, cefotaxime, cefoxitin, ceftazidime, cefoperazone, cefepime, and carbapenems). Microorganisms expressing NDM-1 (New Delhi metallo-β-lactamase) are mostly multi-drug resistant. The NDM-1 gene confers resistance to beta-lactam antibiotics including last-resort carbapenem antibiotics. Lateral transfer of the plasmid-associated gene, blaNDM, has allowed it to be passed between *Enterobacteriaceae* genera commonly found in the human microbiome, including *Escherichia coli*,

Enterobacter cloacae, and *Klebsiella pneumoniae*. blaPER encodes an extended-spectrum β-lactamase (ESBL), conferring resistance to penicillins, cefotaxime, ceftibuten, ceftazidime, and the monobactam aztreonam but sparing resistance to carbapenems and cephamycins and has been found in *Aeromonas* spp., *Acinetobacter baumannii*, *Alcaligenes faecalis*, *Pseudomonas aeruginosa* and the *Enterobacteriaceae* in Asia and Europe (Poirel *et al*., 2005). Another extended-spectrum β-lactamase encoding gene identified both in the influent and effluent samples from the hospital wastewater treatment was SHV gene. The SHV enzymes are named after the sulfhydryl variable active site and are commonly associated with *K. pneumoniae*. Initially, these bacteria contained a single ESBL gene, but now multiple ESBL genes are commonly present in a single strain, further complicating the process of detection (Samaha-Kfoury and Araj, 2003).

Our results indicated the presence of beta-lactam resistance genes blaVIM and SHV in the effluent of the wastewater treatment system suggesting that microorganisms possessing beta-lactamase resistance genes might be present in spite of the chlorination step. It is hypothesized that although chlorination is used on a large scale for disinfection, it can significantly alter the antibiotic resistome. Although the total relative abundance of antibiotic resistant bacteria is reduced, antibiotic resistance genes increase in abundance (Jia *et al*., 2015).

This study is a first step in a more complex experiment regarding screening of influent and effluent hospital wastewater for different groups of antibiotic resistance genes and the effect of wastewater treatment and chlorination of the fate of antibiotic resistance bacteria and ARG.

Conclusion

Several resistance genes have been detected by molecular methods in the effluent of a hospital wastewater treatment system, suggesting microorganisms possessing beta-lactamase resistance genes could withstand treatment and chlorine disinfection. The persistence of antibiotic resistant microbes in the hospital wastewater could negatively impact the environment as it discharges in the municipal sewage system.

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