KPC-2 and OXA-48 carbapenemase-harbouring Enterobacteriaceae detected in an Austrian wastewater treatment plant

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Abstract

Multiresistant Enterobacteriaceae, like carbapenemase-producing strains, have their primary reservoir in medical institutions. They can also be found with increasing tendency in other reservoirs. One possible way for entrance of multiresistant Enterobacteriaceae into the environment is via waste water. The aim of the study was to screen isolates from a wastewater treatment plant for the presence of carbapenemase-producing Enterobacteriaceae. Three isolates harboured carbapenemase genes, one *Klebsiella pneumoniae* harboured KPC-2 and one *K. pneumoniae* and one *Escherichia coli* harboured OXA-48. This is the first report of carbapenemase-harbouring Enterobacteriaceae isolated outside medical institutions in Austria and the first detection of KPC-harbouring *K. pneumonia* MLST ST 1245.

Keywords: Carbapenemase, *Klebsiella*, KPC, OXA-48, waste water

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Multiresistant Enterobacteriaceae have become a relevant problem in human medicine.

In the evolution and spread of multiresistant Enterobacteriaceae, carbapenemases are the new threat, overcoming one of the last lines in antibiotic defence. The classic carbapenemases are metallo-β-lactamases (Ambler class B), first detected in the 1990s. Different metallo-β-lactamase gene families have their hotspots all over the world. The enzyme KPC is the most recent member in the carbapenemase family, first detected in Turkey in 2003. It is the clinically most relevant Ambler class A serin β-lactamase with worldwide dissemination in the last decade. KPC are mainly present in *Klebsiella* spp. but can also be found in other Enterobacteriaceae and also occur in other gram-negative bacteria. In contrast, OXA-48 and its derivatives (Ambler class D) are only harboured by Enterobacteriaceae [1–3].

In central Europe, carbapenemases are only sporadically detected but with increasing tendency [3]. This is reflected by Austrian studies that show NDM-1, KPC, VIM, IMP and OXA-48 in human isolates. Since 2010 a rapid increase of KPC-producing Enterobacteriaceae has been observed [4–7].

Multiresistant Enterobacteriaceae with different resistance mechanisms have their primary reservoir in medical institutions. They can also be found with increasing tendency in the general public, in companion, farm and wildlife animals and in environmental samples [8–10]. It seems only a matter of time until resistance mechanisms find their way out of medical institutions into the human community and the environment. One possible route for the entrance of multiresistant Enterobacteriaceae into the environment and so into the food chain is via waste water. Several studies demonstrate the presence and increase of (multi)resistant bacteria in the aquatic environment caused by human waste water [11–13]. Some of these studies indicate that carbapenemases will follow these footsteps and reports have been published of carbapenemase-producing Enterobacteriaceae in the aquatic environment [14,15]. In Austria, previous studies evaluating waste water or sewage sludge could not detect carbapenem-resistant Enterobacteriaceae [12,16,17].

The aim of the study was to screen activated sewage sludge from an Austrian wastewater treatment plant for the presence of carbapenemase-producing Enterobacteriaceae.

In the period between September 2011 and February 2012, activated sewage sludge samples were collected once a month from the basin of the incoming untreated waste water at a sewage treatment plant in the area of Graz, Styria/Austria [16]. Wastewater entry into this treatment plant (max. 500 000 population equivalent) is mainly domestic waste water, but also includes waste water from a hospital (1533 beds). Samples were mixed with sterile glycerine (50% glycerine by volume) and frozen at −80°C.

TABLE 1. Carbapenemase-producing Enterobacteriaceae isolated from activated sewage sludge

Isolate no.	Species	MLST	Isolation date	MIC (mg/L)			Carbapene mases	Other β-lactamases
				ETP	IMP	MEM		
SeS1	*Klebsiella pneumoniae*	ST 1245	September 2011	8	4	8	KPC-2	TEM-1, SHV-26, FOX
SeS2	*Escherichia coli*	ST 38	November 2011	4	2	0.5	OXA-48	TEM-1, CTX-M-24
SeS3	*Klebsiella pneumoniae*	ST 15	February 2012	2	2	1	OXA-48	TEM-1, SHV-1, CTX-M-15

ETP, ertapenem; IMP, imipenem, MEM, meropenem; MLST, multilocus sequence typing.

For screening, 200 μL of the complete thawed samples was plated on chromID® CARBA (bioMérieux, Craponne, France) screening agar plates.

For pure cultures, colonies growing on CARBA agar were transferred to blood agar and Endo agar (24 h, 37°C). Identification was performed using the matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS; Axima™ Assurance Shimadzu, Kyoto, Japan) system. Identified Enterobacteriaceae were characterized for their resistance pattern by susceptibility testing according to EUCAST 2012 (listed in the supplementary data). The MICs for three carbapenems were tested (ertapenem, imipenem and meropenem) with E-test® (bioMérieux).

Isolates showing resistance to at least one of the tested carbapenems were screened with a Checkpoint MDR 103 kit (Check-Points, Wageningen, The Netherlands) according to the protocol http://www.check-points.com/support/manuals/.

Sequencing of the detected carbapenemases and of the members of the extended-spectrum β-lactamase (ESBL) gene family was performed as previously described [17–19]. All isolates were also typed by multilocus sequence typing, as previously described. [20,21].

Altogether nine Enterobacteriaceae could be detected on the carbapenem screening plates (three *Escherichia coli*, four *Klebsiella* spp., two *Enterobacter* spp.).

Three isolates—two *Klebsiella pneumoniae* and one *E. coli*—out of three different samples, showed resistance to at least one of the tested carbapenems. Two OXA-48 genes and one KPC-2 gene were detected.

Isolate SeS1 was a *K. pneumoniae* harbouring a KPC-2 gene and in addition the non-ESBL genes SHV-26 and TEM-1. Additionally, a FOX gene for AmpC β-lactamase was detected. Co-resistance was found for ciprofloxacin and moxifloxacin. Multi-locus sequence typing assigned the isolate to a novel sequence type ST1245. The *E. coli* isolate (SeS2, ST38) was harbouring an OXA-48 gene and in addition an ESBL CTX-M-14 gene and TEM-1 gene. In addition, this isolate showed resistance for gentamicin and trimethoprim-sulfamethoxazole. The third isolate (SeS3, ST15), a *K. pneumoniae* harboured the genes OXA-48, CTX-M-15 and non-ESBLs TEM-1 and SHV-1. Co-resistance was found for gentamicin and trimethoprim-sulfamethoxazole (Table 1).

Carbapenemase-producing Enterobacteriaceae in the samples are only represented by isolates expressing serin β-lactamases. KPC was first detected in Austria in 2010 with a growing number of isolates, including nosocomial outbreaks. In Europe, KPC is endemic in some countries such as Italy or Greece [6,22]. There are also some reports of KPC-producing Enterobacteriaceae from wastewater and river-water samples from Europe and other parts of the world [14,23].

OXA-48 seems to be not very common in Austria, but there are only a few studies and all of them only have local data settings [4,5,7]. Also, publications from neighbouring regions suggest that OXA-48 is more common in Austria than the literature would suggest [6]. Another characteristic, which may lead to an underestimation of OXA-48 in Austrian clinical samples, is the reduced carbapenem resistance that can be observed in OXA-48-harbouring strains [1]. Enterobacteriaceae harbouring metallo-β-lactamases have been known in Austria since 2005 [4] but no positive isolates were found in this study. It is known from other studies that metallo-β-lactamase carbapenemase has the ability to spread into the environment [24]. This may be a reflection of a more dramatic spread of KPC and OXA-48 in the human and clinical settings, but may be a result of the low number of samples used.

This is the first report of KPC- and OXA-48-producing Enterobacteriaceae isolated outside medical institutions in Austria. The presence of these strains in waste water is very low but this is only a first investigation. Further studies have to be conducted to evaluate the presence of these strains in other environmental samples, such as river water or animals. Until now, even in the medical setting there is a lack in screening and reporting carbapenemase-producing Enterobacteriaceae.

These findings are worrying because they document the first step of these genes out of the clinical setting into the environment and from there, by using water from river and sewage sludge for agriculture, back into the human community.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Data S1. Supplemental Methods.

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