NDM-1-producing Enterobacter aerogenes isolated from a patient with a JJ ureteric stent in situ

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CASE REPORT

NDM-1-producing *Enterobacter aerogenes* isolated from a patient with a JJ ureteric stent in situ

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Abstract

Urinary tract infections after JJ stent insertion are among the most common complications, and the associated microorganisms carry more antibiotic resistance determinants than those found in urine prior to stent insertion. In line with the trends in healthcare epidemiology which implicate multi-resistant microorganisms in a plethora of healthcare-associated infections, prosthetic stent material also represents an ideal milieu for biofilm formation and subsequent infection development with resistant bacterial agents. Here we describe a case of a 73-year-old Caucasian woman presenting with urinary tract infection after JJ ureteric stent insertion due to ureteric obstruction and hydronephrosis of her left kidney. Extensive microbiological work-up and comprehensive molecular analysis identified the putative microorganism as carbapenem-resistant *Enterobacter aerogenes* carrying New Delhi metallo-beta-lactamase 1 (NDM-1). This is a first literature report implicating such extensively resistant strain of this species in early indwelling ureteric stent complications, and also the first report of NDM-1 in *Enterobacter aerogenes* in Croatia and Europe.

Keywords *Enterobacter aerogenes* · NDM-1 · Antimicrobial resistance · JJ stent · Ureteric obstruction

Introduction

The JJ ureteric stents (also known as double-J stents) are an indispensable part of modern urological treatment armamentarium [1]. Although originally used to treat fistulas or ureteric obstructions, the indications have expanded substantially. However, different complications following JJ stent insertion may ensue, and urinary tract infections are on the forefront as the most common complication in females (and among the most common in men) [2].

Generally, bacteria isolated from urine following stent insertion and directly from the stents demonstrate more antibiotic resistance than those cultured from urine prior to stent insertion [3]. Albeit literature descriptions of exact pathogens causing these infections are scarce, the value of exact microbiological characterization (even on the molecular level) primarily lies in the adequate treatment decision making [4]. Moreover, certain microorganisms may prompt the formation of biofilms and encrustations on inserted stents, resulting in bacteriuria and urinary tract infection [5].

This is the first literature report of carbapenemase-producing organism originating from the JJ stent, pointing to the significance of artificial, prosthetic material as a source of extensively drug-resistant bacteria, and also the first report of New Delhi metallo-β-lactamase 1 (NDM-1) producing *Enterobacter aerogenes* (*E. aerogenes*) in Croatia and (to our knowledge) in Europe.

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Case report

Due to the obstruction of the upper urinary tract caused by ureteroliths (i.e. ureteral calculi) and hydronephrosis of the left kidney, a 73-year-old Caucasian female was subjected to the indwelling JJ ureteric stent insertion in October 2017 to re-establish the patency of the ureter. There was no known immunodeficiency or risk factors in her medical history. Due to intermittent fever and increased inflammatory markers (CRP of 192.5 mg/dL, total leukocyte count of 19.8×10^9/L, as well as 81% of segmented neutrophils in the differential blood count), the patient was treated with ciprofloxacin 15 days prior to the procedure (followed by the improvement in the aforementioned parameters), while ceftriaxone was prescribed as a peri-interventional antimicrobial coverage. Shortly after stent placement a purulent content was observed in her left ureter, and 15 days later a ureteral calculus (approximately 8 mm in diameter) was identified adjacent to the inserted JJ stent. Additionally, an increased number of polymorphonuclear leukocytes in urinary sediment prompted a detailed urine investigation.

Urine culture revealed a pure growth of Gram-negative bacterium identified as *E. aerogenes* by conventional biochemical testing and matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry. The antimicrobial susceptibility to a wide range of antibiotics was determined by disk diffusion and broth microdilution method. Phenotypic methods were used to detect extended spectrum β-lactamases (ESBLs), plasmid-mediated AmpC β-lactamases and carbapenemases. The transferability of meropenem resistance was determined by conjugation (broth mating method) employing *Escherichia coli* (*E. coli*) A15 R^- strain resistant to rifampicin and *E. coli* J65 resistant to sodium azide [6].

The genes conferring resistance to β-lactams including broad spectrum and extended-spectrum β-lactamases (bla_{SHV}, bla_{TEM}, bla_{CTX-M} and bla_{PER-1}), plasmid-mediated AmpC β-lactamases, class A carbapenemases (bla_{KPC}, bla_{SME}, bla_{IMI}, bla_{NMC},), class B metallo-β-lactamases ($bla_{VIM,}$ bla_{IMP} and bla_{NDM}), carbapenem hydrolyzing oxacillinases (bla_{OXA-48}) and fluoroquinolones (*qnr*A, *qnr*B, *qnr*S) were determined by polymerase chain reaction (PCR) using protocols and conditions as described previously [7]. Amplicons were column-purified with Qiagen DNA purification kit (Inel, Zagreb, Croatia) and sequenced in Eurofins sequencing services (Graz, Austria). PCR-based replicon typing was applied to type the resistance plasmids carrying carbapenemase genes [8]. Plasmid extractions from transconjugant strains were subjected to PCR for carbapenemase detection (MBL and OXA-48) to determine the location of bla_{CARB} genes.

The isolate was resistant to β-lactam antibiotics and gentamicin, but susceptible to colistin and fosfomycin (Table 1). It was classified as extensively drug resistant according to Magiorakos et al. [9]. Double disk synergy test and combined disk test with clavulanic acid were positive, suggesting the production of an ESBL. Combined disk test with EDTA was positive, confirming the MBL production. The isolate transferred meropenem resistance to *E. coli* recipient strains with the frequency of 10^{-3}. Resistance to gentamicin, sulphamethoxazole and tetracycline has been co-transferred alongside with meropenem resistance. PCR was positive with primers specific for NDM and TEM β-lactamases. Sequencing of the PCR products of the clinical isolate and the respective transconjugant experiment revealed TEM-1 and NDM-1. The transconjugant exhibited slightly lower minimum inhibitory concentration (MICs) of carbapenems and expanded-spectrum cephalosporins compared to the clinical isolate. The plasmid extraction from donor and recipient strain was positive for HI2 incompatibility group of plasmids.

Such extensive microbiological work-up showed that this isolate was carbapenem-resistant *E. aerogenes* carrying NDM-1 resistance determinant. The treatment was prescribed according to the respective sensitivity pattern (with subsequent negative urine culture results) and the patient was scheduled for extracorporeal shockwave lithotripsy (ESWL).

Table 1 Minimal inhibitory concentrations (MICs) of various antimicrobial agents tested against isolated *Enterobacter aerogenes* strain and against transconjugant A15 R^- *E. coli* strain

Antimicrobial agent	MIC (mg/L) *E. aerogenes* 8020	MIC (mg/L) transconjugant *E. coli* A15 R^-
Amoxicillin	> 128	> 128
Amoxicillin/clavulanate	> 128	32
Piperacillin	> 128	> 128
Piperacillin/tazobactam	> 128	64
Cefazolin	> 128	> 128
Cefuroxime	> 128	64
Ceftazidime	> 128	32
Cefotaxime	> 128	64
Ceftriaxone	> 128	64
Cefepime	> 128	16
Ertapenem	> 128	32
Imipenem	> 128	16
Meropenem	> 128	16
Gentamicin	32	8
Ciprofloxacin	2	0.25
Colistin	0.12	0.06
Fosfomycin	64	8

Discussion

NDM-1-producing *E. aerogenes* is extremely rare and, to our knowledge, has not been described in Europe thus far. This extensively resistant isolate was previously found in Asian countries (more precisely in India, China and Vietnam), where it was responsible for different types of infections [10–12]. Although there are studies involving NDM-1-producing *Enterobacteriaceae* in urinary pathology and even urosepsis [13], this is the first report linking NDM-1-producing *E. aerogenes* to urinary tract infection after JJ stent insertion, with substantial peri-interventional and post-interventional drug selection ramifications.

Most patients usually receive one dose of gentamicin or ceftriaxone as prophylaxis prior to stenting procedure [2]; in this case, the latter was prescribed as peri-interventional antimicrobial coverage. Other authors recommend fluoroquinolones for that purpose [3]. However, all those recommendations would have to be reconsidered in case of increased encounters with carbapenemase-producing microorganisms related to JJ stenting, as described here.

Moreover, this type of pathogen-stent correlation could be also considered a biofilm infection, complicating treatment attempts even further. The surface of ureteral stents represents an ideal milieu for biofilm formation, which in turn provides nutrients to implicated microorganisms and protects them from phagocytes and antibiotics [5]. Since biofilms are generally seen as a major limiting factor for the long-term usage of ureteral stents [5], many research endeavours have focused on its pathogenesis and prevention; still, studies concentrating on potential inducing microorganisms are lacking. Hence the role of *E. aerogenes* as a potential biofilm inducer in ureteral stents warrants further research.

The prevalence of the carbapenemase-producing *Enterobacteriaceae* is highly variable across Europe, with high prevalence found in Greece, Israel, Italy and Turkey, and low prevalence noted in Nordic countries, Germany, Switzerland, and the Czech Republic [14]. *Enterobacter aerogenes* has been touted as an important opportunistic and multi-resistant bacterial pathogen for humans in hospital wards, largely described during several outbreaks of hospital-acquired infections in Europe [15]. NDM-1 producers have been identified on all continents with a direct link to Indian subcontinent in a majority of cases; furthermore, it has been shown that the Middle East and the South European states (including Croatia) may act as secondary reservoirs of NDM-1 producers [15].

Regarding the local situation in Croatia, the first carbapenem-resistant *Enterobacteriaceae* family member was NDM-1 producing *Klebsiella pneumoniae* (*K. pneumoniae*) [16]. Shortly after this description an increase of carbapenem-resistant microorganisms was observed, when a multicentre study on carbapenem resistance in *Enterobacteriaceae* revealed the predominance of VIM-1 β-lactamase in two large Croatian hospital centres, as well as sporadic occurrence of NDM-1 [17]. A study conducted 2 years later demonstrated an increase of NDM-1-producing *Enterobacteriaceae*, but predominantly *Enterobacter cloacae* and *K. pneumoniae* [18].

The isolate described here was phenotypically positive for ESBL; however, sequencing of TEM product identified TEM-1 which is a broad spectrum and not extended-spectrum β-lactamase. Such false-positive results in phenotypic tests can be attributed to hyperproduction of TEM-1 β-lactamase. In conclusion, exact pathogen identification with molecular determination of resistance determinants is pivotal when addressing infective sequelae after the insertion of JJ stent or other prosthetic devices.

Author contributions IF, BB, NB, ALG and GZ conceived and planned the experiments pertinent for this case description. IF, BB, NB and GZ carried out the experiments. IF, BB, ALG, LB and GZ contributed to sample preparation. BB, NB, SM, LB and TM contributed to the interpretation of the results. IF, BB and TM took the lead in writing the manuscript. All authors provided critical feedback and helped shape the research, analysis and final version of the manuscript.

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Compliance with ethical standards

Conflict of interest All authors have declared that no conflict of interest exists. No funding or financial support was received.

Ethical approval The study describes clinical and diagnostic procedures of a specific case. All procedures performed were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki declaration and its later amendments.

Informed consent Informed consent has been obtained from the patient presented in this paper.

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