NDM-1 in Israel, Evidence of Traveling and Carriage of Carbapenem-Resistant Organisms

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ABSTRACT

Objectives: To investigate the carbapenem (CARB) resistance (R) mechanism in an *Escherichia coli* strain recovered from a rectal swab of a patient hospitalized in Israel that received medical care in India during a vacation accident. NDM-1 was detected in several countries harboured by various Gram-negative bacilli species and patient cases were closely linked to receipt of medical care in India or Pakistan.

Methods: Gram-negative isolates from wound cultures were susceptibility (S) tested by CLSI reference broth microdilution methods. Isolates displaying imipenem (IMI) and/or meropenem (MER) MIC at ≥2 mg/L were screened for carbapenemase production by Modified Hodge test (MHT) and PCR for bla_{IMP}, bla_{VIM}, bla_{KPC} and bla_{NDM-1}. Amplicons were sequenced. S1 nuclease digests were resolved in agarose and hybridized with bla_{NDM-1} probe. Transformation was performed by electroporation using *E. coli* DH5alfa and plating in selective media (4 mg/L of ceftazidime). Transformants were confirmed by PCR and S testing.

Results: A 52 year old female was transferred to Chaim Sheba Med Center (CSMC; Tel-Hashomer, Israel) with an infected fracture of the right ankle after a motorcycle accident followed by surgery while vacationing in India. At CSMC, the patient underwent various surgical procedures (five incisions and drainage). Three *Aeromonas hydrophilia/caviae,* two *K. pneumoniae* (one each of ESBL-positive and -negative) and a methicillin-S *S. aureus* were recovered from wound cultures. One *K. pneumoniae* was carbapenem-R (MIC, >8 mg/L for IMI and MER) and MHT and carbapenemase PCR negative. An *E. coli* R to carbapenems (MIC, >8 mg/L for IMI and MER) grew in rectal screen cultures. MHT was positive and initial PCR for bla_{KPC} was negative. PCR and sequencing confirmed the presence of bla_{NDM-1}. This MBL gene was carried on a 150-kb plasmid that was transformed into an *E. coli* host. Transformants displayed elevated MIC values for carbapenem (4 and 8 mg/L for IMI and MER, respectively) and aminoglycosides (>32 and >16 mg/L for amikacin and tobramycin, respectively).

Conclusions: The infection control challenges posed by globalization and easy travel are now being highlighted by clinical cases of infections caused by NDM-1-producing Enterobacteriaceae strains. NDM-1 appears to be easily transferred and well expressed in Enterobacteriaceae and elevated carbapenem MIC values were demonstrated in this Israeli clinical isolate and in our laboratory transformant strains.

INTRODUCTION

The metallo-β-lactamase (MβL) NDM-1 (New Delhi Metallo-β-lactamase) was initially reported from *Klebsiella pneumoniae* and *Escherichia coli* strains recovered from a Swedish diabetic patient of Indian origin that traveled to New Delhi and acquired a urinary tract infection. Like other MβLs, NDM-1 demonstrates a broad-spectrum of hydrolytic activity against β-lactam agents that includes the carbapenems. NDM-1 is not inhibited by β-lactamase inhibitors currently marketed or in development for clinical use. Furthermore, this enzyme displayed low homology when compared to other MβL groups, being only 32.4% similar to VIM-1/VIM-2.

After the initial report, NDM-1 was described by JMI Laboratories from isolates recovered in India as early as 2006 and this enzyme has also been detected in various Enterobacteriaceae species (*K. pneumoniae*, *E. coli*, *Enterobacter cloacae* and *Citrobacter freundii*) and *Acinetobacter* spp. in several countries. The detection of NDM-1-producing strains appears to be troublesome and cases of false-negative Modified Hodge Test (MHT) results and MIC values at or below the current carbapenem breakpoints have been described. More recently, NDM-2 a point mutant from its ancestor has been detected in *Acinetobacter baumannii* from Egypt.

In this study, we report an NDM-1-carrying *E. coli* isolate from a rectal swab culture from a patient hospitalized in Israel after experiencing a motorcycle accident followed by surgery, when vacationing in India.

MATERIALS AND METHODS

Bacterial isolates. Five Gram-negative bacilli collected from wound and rectal swab specimens from a patient hospitalized in Israel were received in our reference laboratory (JMI Laboratories, North Liberty, Iowa, USA). Isolates were identified using conventional biochemical methods and the Vitek 2 system (bioMérieux; Hazelwood, Missouri, USA).

Susceptibility testing. Isolates were susceptibility tested by reference broth microdilution (CLSI, M07-A8, 2009) using validated panels manufactured by TREK Diagnostics (Cleveland, Ohio, USA). Interpretations of susceptibility testing results were as described in M100-S21 (CLSI, 2011). *E. coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were concurrently tested for quality assurance; all the results were within published ranges.

Screening for carbapenemases. Isolates displaying reduced susceptibility to imipenem or meropenem (MIC, ≥2 mg/L) were screened for production of carbapenemases. Modified Hodge Test (MHT) was performed to detect carbapenemase production using imipenem and meropenem as substrates.

Isolates were also evaluated for the presence of bla_{IMP}, bla_{VIM}, bla_{NDM-1}, bla_{KPC}, bla_{SME}, bla_{IMI}, bla_{NMC-A}, bla_{GES} and bla_{OXA-48} by PCR. Amplicons were sequenced on both strands and nucleotide sequences obtained were analyzed using Lasergene® software package (DNAStar; Madison, Wisconsin, USA) and compared to available sequences via NCBI BLAST search (http://www.ncbi.nlm.nih.gov/blast/).

Gene location analysis. Total cellular DNA embedded in 1% agarose plugs was subjected to partial digestion with S1 nuclease. Plasmids were resolved by electrophoresis performed on the CHEF-DR II (BioRad, Richmond, CA), with the following conditions: 0.5 X TBE, 1% agarose, 13°C, 200V, for 6 hours with switch time ramping from 5 to 25 seconds and 14 hours with the switch time from 30 - 45 seconds. ICeuI digested genomic DNA was also resolved on PFGE as described previously. DNA gels were transferred to nylon membranes by southern blotting and hybridized with a digoxigenin labeled (Roche Diagnostics GmbH, Mannheim, Germany) bla_{NDM-1}-specific probe.

Plasmid transfer. Plasmid preparations (QIAprep Spin Mini prep kit, Qiagen, Hilden, Germany) were electroporated into *E. coli* DH5α. Electroporation parameters were 2.5 kV, 25 µF and 400 Ω using the Bio-Rad Gene Pulser apparatus (BioRad, Richmond, California, USA). Selection was performed in agar plates containing 4 mg/L of ceftazidime.

Screening for resistance genes associated with bla_{NDM-1}. Specific primers were used to screen NDM-1-producing *E. coli* and transformant strain for genes encoding plasmid mediated AmpC, ESBLs (TEM, SHV, GES, PER, VEB, CTX-M, OXA-2, -10, -1/-30, -18/-45) and 16S rRNA methylases (*armA*, *rmtA-D* and *npmA*).

CLINICAL CASE

A 52 year old female was transferred to Chaim Sheba Medical Center (CSMC; Tel-Hashomer, Israel) on August 27, 2010 with an infected right ankle fracture after a motorcycle accident followed by surgery. The patient sustained bilateral ankle fractures in the accident (August 21, 2010) while on holiday in India. Her left ankle underwent surgery in India with no complications. The right ankle underwent open reduction and fixation. She subsequently has undergone eight surgical procedures, five of them incision and drainage. Numerous organisms were isolated from her surgical wound cultures, including three *Aeromonas hydrophilia/caviae,* two *K. pneumoniae* (one each of ESBL-positive and -negative) and a methicillin-susceptible *Staphylococcus aureus*. Given the patient's medical history, a rectal screen for carbapenem-resistant organisms was performed. Two carbapenem-resistant organisms grew in rectal screen cultures, an *E. coli* carrying bla_{NDM-1} and a *K. pneumoniae* that was negative by MHT and PCR for carbapenemase-encoding genes.

RESULTS

- Table 1 summarizes the susceptibility results and carbapenemase screening tests for three *A. hydrophilia/caviae* recovered from wound specimens and one each of *E. coli* and *K. pneumoniae* from a rectal swab culture.
- All five Gram-negative bacilli were highly resistant to β-lactam agents, including the four carbapenems tested (MIC, ≥8 mg/L). *A. hydrophila/caviae* isolates were susceptible to cefepime (MIC range, 0.5 to 4 mg/L) and aztreonam (0.25-1 mg/L; Table 1).
- Isolates were also resistant to amikacin, tobramycin and fluoroquinolones. All strains were susceptible to tigecycline (MIC range, 0.12-0.5 mg/L; Table 1).
- Positive MHT results were observed for the *A. hydrophila/caviae* strains from wound specimens and for the *E. coli* recovered from the rectal swab (Table 1).
- PCR and sequencing confirmed the presence of bla_{NDM-1} in the *E. coli* strain. Screening for acquired carbapenemase genes was negative for the *A. hydrophila/caviae* and *K. pneumoniae*, including for bla_{KPC} primers (Table 1).
- S1 nuclease experiments demonstrated that bla_{NDM-1} was carried in a 150-kb plasmid that was transferred by electroporation to an *E. coli* host. Transformants displayed elevated MIC values for carbapenem (4 and 8 mg/L for imipenem and meropenem, respectively) and aminoglycosides (>32 and >16 mg/L for amikacin and tobramycin, respectively; Table 2).
- Detection tests for 16S rRNA methylases demonstrated the presence of *armA* and *rmtC* in the clinical strains, but only *rmtC* was transfered to the *E. coli* host (Table 2).
- ESBL and plasmidic AmpC genetic screening showed that the NDM-1-producing clinical strains also carried $bla_{CTX-M-15}$-like; however, negative results for the transformant *E. coli* suggested that this gene was not embedded in the same plasmid (Table 2).

Table 2. Characteristics of NDM-1-producing *E. coli* from rectal swab cultures of a patient hospitalized at an Israeli hospital and the *E. coli* DH5α carrying the bla_{NDM-1} plasmid.

Parameter	*E. coli* 8802J	*E. coli* DH5α (p8802J)
MIC (mg/L)		
Ampicillin	>8	>16
Piperacillin/tazobactam	>64	64
Cefoxitin	>16	>16
Ceftazidime	>32	>16
Ceftriaxone	>8	>32
Cefepime	>16	16
Aztreonam	>16	≤0.12
Ertapenem	>8	4
Doripenem	>8	4
Imipenem	16	4
Meropenem	64	4
Amikacin	>1024	1024
Gentamicin	>512	128
Tobramycin	>512	128
Arbekacin	>1024	256
Apramycin	8	2
Neomycin	32	≤0.5
Ciprofloxacin	>4	≤0.5
Levofloxacin	>4	≤0.5
Tetracycline	>8	≤2
Trimethoprim/sulfamethoxazole	>4	≤0.5
MHT[a]	pos	pos
bla_{NDM-1}	pos	pos
CTX-M-15-like	pos	neg
pAmpC[b]	neg	neg
rmtA	neg	neg
rmtB	neg	neg
rmtC	pos	pos
rmtD	neg	neg
armA	pos	neg
npmA	neg	neg

a. MHT = Modified Hodge Test.
b. pAmpC multiplex target genes encoding CMY-like, DHA-like, MOX-like, FOX-like, ACC-like and ACT-like.

Table 1. Susceptibility profiles and carbapenemase screening results of Gram-negative bacilli received from a patient hospitalized at an Israeli hospital.

Antimicrobial agent / Carbapenemase screening test	MIC results (mg/L)				
	A. hydrophilia/caviae 8799J	*A. hydrophilia/caviae* 8800J	*A. hydrophilia/caviae* 8801J	*E. coli* 8802J	*K. pneumoniae* 8803J
Ampicillin	>8	>8	>8	>8	>8
Ampicillin/Sulbactam	>32	>32	>32	>32	>32
Piperacillin	>128	>128	>128	>128	>128
Piperacillin/tazobactam	>64	>64	>64	>64	>64
Cefoxitin	>16	>16	>16	>16	>16
Ceftazidime	16	16	16	>32	>32
Ceftriaxone	>8	>8	>8	>8	>8
Cefepime	1	0.5	0.25	>16	>16
Aztreonam	4	2	0.5	>16	>16
Ertapenem	>8	>8	8	>8	>8
Doripenem	>8	>8	4	>8	4
Imipenem	>8	>8	8	8	2
Meropenem	>8	8	2	>8	8
Amikacin	32	32	32	>32	>32
Gentamicin	4	8	8	>8	>8
Tobramycin	16	>16	16	>16	>16
Ciprofloxacin	4	4	2	>4	>4
Levofloxacin	4	2	2	>4	>4
Tetracycline	1	0.5	0.5	>8	8
Tigecycline	0.25	0.5	0.5	0.12	0.5
Trimethoprim/sulfametoxazole	≤0.5	≤0.5	≤0.5	>4	>4
Colistin	>4	>4	>4	≤0.5	≤0.5
MHT[a]					
bla_{NDM-1}	neg	neg	neg	pos	neg
bla_{KPC}-like	neg	neg	neg	neg	neg
Other carbapenemases[b]	neg	neg	neg	neg	neg

a. MHT = Modified Hodge Test.
b. PCR for other carbapenemases included: bla_{IMP}, bla_{VIM}, bla_{SME}, bla_{IMI}, bla_{NMC-A}, bla_{GES} and bla_{OXA-48}.

CONCLUSIONS

- The association of bla_{NDM-1} and genes encoding other resistance mechanisms, including β-lactamases and rRNA methylases has been previously reported. Our findings emphasize the growing problem of the dissemination of plasmids carrying multiple resistance genes encoding pan-resistant phenotypes.
- This is the first report of a NDM-1-carrying strain in Israel. This MβL enzyme seems to be rapidly spreading worldwide in communities and hospital settings via worldwide travel (from India to Israel).
- Expanded studies are required to prevent dissemination and promote appropriate treatment options for NDM-1-producing strains.

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